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ATTI

Mercoledì, 16 ottobre 2019

Sala 500 – 12:30-17:00

PATOLOGIA PLEUROPOLMONARE

Slide Seminar

Moderatori: P. Graziano, O. Nappi

CYSTIC LUNG DISEASE

F. Barbisan

According to the Glossary of Terms for Thoracic Imaging of the Fleischner Society (Hansell et al, Radiology 2008; 246:697-722), a cyst appears as a round parenchymal lucency or low-attenuating area with a well-defined interface with normal lung. Cysts have variable wall thickness but are usually thin-walled (<2 mm) and occur without associated pulmonary emphysema. When they are solitary and in asymptomatic patients, they can represent an incidental finding, particularly in the elderly (>75 years) or as a result of a trauma or an acute pneumonia. On the contrary, when they are multiple, they can represent a diagnostic challenge, since an increasing number of uncommon systemic or genetic disorders can produce such a presentation. High-resolution computed tomography of the chest represents the starting point for the diagnostic evaluation, because it's necessary to detect the disease and to define the morphological aspects, the distribution and the associated findings, such as nodules or ground-glass opacities. Furthermore, it is mandatory to correlate the imaging appearance with the patient's clinical history, extrapulmonary manifestations, physical examination, laboratory findings, with the purpose to achieve a diagnosis or to narrow the differential diagnosis. The pathologist could be called at the end of the diagnostic workflow to confirm a diagnosis, to discern among different possible entities or to rule out the coexistence of a neoplastic process.
The main diseases in the group of the cystic lung disease are: lymphangioleiomyomatosis (LAM), Birt-Hogg-Dubé syndrome (folliculin gene-associated syndrome), pulmonary Langerhans cell histiocytosis, lymphocytic interstitial pneumonia/follicular bronchiolitis (LIP/FB); other rarer causes are infections, amyloidosis, light chain deposition disease, neoplastic disease, desquamative interstitial pneumonia (DIP) or hypersensitivity pneumonitis (HP).

Mercoledì, 16 ottobre 2019

Sala Londra – 12:30-17:00

PATOLOGIA GINECOLOGICA

Slide Seminar

Aspetti inusuali nella patologia ginecologica

Moderatori: G. De Rosa, G.L. Taddei

UNUSUAL ASPECTS OF ENDOMETRIAL CARCINOMA

L. Resta, L. Duda, T. Lettini, G. Serio, A. Marzullo

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Endometrioid carcinoma is considered the most frequent type of the endometrial carcinoma, being present in more than 60% of cases. But in this group of neoplasms are included several subtypes or variants. The mucinous and the secretory variants have to be identified and differentiated from the more aggressive clear cell carcinoma. The mucinous variant produces glycoproteins (diastase resistant), while the secretory and the clear cell ones accumulate glycogen (diastasi sensitive). The clear cell carcinoma, unlike secretory variant, has nuclear pleomorphism, nucleoli, and a more o less portion of papillary architecture. The G3 endometrioid carcinoma usually is not isolated, but associated with more differentiated areas. Cases with well differentiated tumor which presents an abrupt area of poor differentiated often with spindle cells, are termed "dedifferentiated" adenocarcinoma. Some Author considers that situation as suggestive for a Lynch syndrome, but the opinion is still controversial.
Neuroendocrine carcinoma of endometrium is extremely rare. The clinical history off the cases reports a rapid course of the disease. Histology is not different from that of similar tumors of other organs: large cell type is also described, but the survival rate is not different from the small cell variant. Immunohistochemistry shows the expression (not in all cases) of chromogranin, synaptophysin, and CD 56; in some cases the positivity to CD10 antigen may offer a confusion with a stromal tumor.
Malignant mullerian mixed tumors or carcinosarcoma of the uterus are well known. The more infrequent adenosarcoma is a condition characterized by a benign proliferative epithelium and a low aggressive malignant mesemchymal component. The tumor shows often the same cytology and immunohistochemistry of low grade stromal tumor and the polypoid adenomyoma. The diagnosis is based on an accurate cytological observation and the mitotic count. The tumor borders (invasive or not invasive) and the sarcomatous overgrowth are considered the most important features for the prognosis, but in hysteroscopic samples the judgment may be impossible.
Dysontogenetic tumors, other than rhabdomyosarcomas, are exceedingly rare in the uterus. Ewing's sarcoma aris-

es in young female and simulates stromal and smooth muscle cell tumors, which are more frequent. The neuroectodermal structures (neural tubes) are the diagnostic key. Immunohistochemistry positive for CD99, neural markers and genetic findings arealso usefull.

ASPETTI INUSUALI NELLA PATOLOGIA DELLA CERVICE

E. Bragantini, G. Negri

La cervice uterina è sede di patologia primitiva e secondaria, che talvolta può mostrare pattern inusuali di presentazione.
È importante, di fronte a una lesione non facilmente inquadrabile da un punto di vista morfologico, che si possano ipotizzare delle diagnosi differenziali, che seguano un criterio identificativo per pattern, in modo tale da impostare una corretta richiesta di indagini di immunoistochimica.
In tali casi inoltre è importante il confronto con il ginecologo, con richiesta di indicazioni sulla storia clinica della paziente, sul quadro colposcopico ed eventualmente radiologico.
Importante è inoltre avere materiale adeguato per la diagnosi, in quanto talvolta alcuni pattern tra lesioni benigne e maligne possono sovrapporsi.
Una patologia che talvolta crea difficoltà diagnostiche è l'endometriosi polipoide.
Clinicamente si può presentare come una lesione esofitica e sanguinante, ponendo il sospetto di una neoplasia maligna.
Da un punto di vista istologico la complessità può essere data dai fenomeni erosivi ed emorragici o da fenomeni iperplastici legati alla proliferazione stromale e ghiandolare.
Si possono osservare inoltre modificazioni metaplastiche e possono essere presenti atipie citologiche che possono creare difficoltà diagnostiche.
È da tenere in considerazione, inoltre, la possibilità di insorgenza di lesioni maligne associate ad endometriosi come il carcinoma endometrioide, il carcinoma a cellule chiare, l'adenosarcoma o il sarcoma stromale di basso grado. Pertanto è importante esaminare accuratamente ed estesamente il materiale inviato, soprattutto se le lesioni presentano ampie dimensioni per escludere foci di malignità associati alle lesioni endometriosica.

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ENDOMETRIAL MALAKOPLAKIA AFTER ABORTIOUS AND ENDOMETRIAL ABLATION

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Background Malakoplakia is a rare, chronical inflammatory disease with female to male ratio of 2:1 characterized by a tissue infiltration of large granular macrophages containing distinctive intracytoplasmic inclusions called "Michaelis-Gutman body". The name derives from the Greek "*malakos*" meaning "*soft*" and "*plakos*" meaning "*plaque*", describing its usual macroscopic presentation as a friable yellow soft plaque. It was first described by von Hansemann in 1901 and in 1902 by Michaelis and Gutman. Since then, more than 200 papers were published. Malakoplakia is currently believed to present an acquired defect in the lysosomal activity of monocytes and defective bacterial digestion. The urinary system is the most commonly involved site, followed by the gastrointestinal tract, skin, tongue, lungs, central nervous system and female tract. Symptoms reflect the organ involvement. Treatment is mostly medical with surgical intervention sometimes being necessary. There are no widely established guidelines for the medical treatment of malakoplakia, but most approaches involve antibiotics which work intracellularly. Prognosis is usually good. However, recurrences may occur.
Case report. In this paper, we present a case concerning a 40-year-old female patient admitted for conspicuous vaginal serohematic secretions. Anamnestic information asserts that the patient previously received endometrial revision due to the retention of placental rests after an abortion. Histologic diagnosis showed the regression of chorionic villus and the decidualization of the endometrium. The patient received two further endometrial revisions with the same histologic diagnosis. After being admitted for conspicuous vaginal serohematic secretions, the patient received a further endometrial ablation in order to check the retention of placental rests. The histologic exam didn't show any retention of chorionic rests, but an endometrial and myometrial infiltration by cellular elements with large granular cytoplasm and some acidophilic body in a chronical inflammatory background. Those elements were negative to CK-pool and strongly positive to CD68. Basing on that histological and immunophenotypical evidence, the histological diagnosis of malakoplakia was effectuated. Due to the symptomatic persistency, the patient received two further endometrial ablation with the same histological diagnosis.
Conclusions. Malakoplakia of endometrium is an extremely rare condition, affecting only 4 cases in the whole international literature. In this paper, we present the fifth and unique case associated to an abortion followed by many endometrial ablations. That condition should be attentively examined, considering differential diagnosis of uterine epithelioid leiomyomas and leiomyosarcomas or physiological conditions such as cumulus of foamy macrophages in the endometrium.

Mercoledì, 16 ottobre 2019

Sala Madrid – 12:30-17:00

PATOLOGIA TESTACOLLO

Neoplasie neuroendocrine di testa-collo

Moderatori: V. Canzonieri, A. Franchi

CRITERI DI LETTURA E INTERPRETAZIONE MORFOLOGICA ED IMMUNOISTOCHIMICA DELLE NEOPLASIE NEUROENDOCRINE DEL TRATTO NASO-SINUSALE.

M.P. Foschini, V.P. Fabbri, S. Asioli

Anatomia Patologica Ospedale Bellaria, Dipartimento di Scienze Biomediche e Neuromotorie, Università di Bologna

Il distretto testa-collo è sede di un ampio spettro di neoplasie neuroendocrine. In particolare a livello naso-sinusale possiamo riscontrare carcinomi neuroendocrini e il neuroblastoma olfattorio.
Il gruppo dei carcinomi neuroendocrini (CNE) che insorgono livello naso-sinusale è molto ampio e comprende tutte le forme che possono essere riscontrate a livello polmonare. Prevalgono le forme ad elevato potenziale di malignità, mentre le forme a basso grado di malignità sono rare e la conoscenza che abbiamo è limitata a descrizione di casi singoli. I casi di CNE possono essere suddivisi in CNE a piccole cellule ed in CNE a grandi cellule [1]. I CNE colpiscono in eguale misura maschi e femmine, in un ampio spettro di età, con un picco in 6 decade [1].

Il neuroblastoma olfattorio (NBO) colpisce prevalentemente soggetti con ampio range di età compreso tra i 2 ed i 90 anni, con un leggero incremento nella 5-6 decade di vita [1]. Non c'è predilezione di sesso. A livello istologico NBO può presentare aspetti che hanno un impatto prognostico. Infatti il grading secondo Hyams è un parametro prognostico indipendente.
A livello immunoistochimico NBO è caratterizzato da positività per marcatori neuroendocrini ed in genere è negativo per citocheratina. La negatività per citocheratina è utile nella diagnosi differenziale tra CNE e NBO. Purtroppo un terzo circa dei NBO presenta una focale positività per citocheratine [1]. Le citocheratine 8\18 rivestono particolare importanza nella diagnosi differenziale tra CNE e NBO [2]. In una revisione casistica multi-istituzionale, si è visto che l'introduzione delle citocheratine 8\18 nel pannello immunoistochimico, hanno portato a modificare la diagnosi in 8\98 (8.2%) casi [2].
La diagnosi differenziale corretta tra queste neoplasie è molto importante per una corretta pianificazione terapeutica e prognostica [3].

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Tab. I. Marcatori immunoistochimici nelle neoplasie neuroendocrine del tratto naso-sinusale.

Marcatore	Carcinoma Neuroendocrino	Neuroblastoma olfattorio	Carcinoma indifferenziato
Citocheratina 8\18	++	-	+
Citocheratina AE1/3	+	+\-	+
Cromogranina	++	++	-
Sinaptofisina	++	++	-
CD56			
NSE			
Proteina S-100	+ (cellsustentacolari)	+\- (cellsustentacolari)	
calretinina	+	-	
P16	+	-	
P63			
P40			

Mercoledì, 16 ottobre 2019

Sala Lisbona – 12:30-14:00

"SESSIONE SPECIALE" SIAPEC INCONTRA AIRTUM

NAP aiuta i patologi o solo i Registri Tumori?

Moderatori: A. Bartolazzi - G. Mazzoleni - M. Rugge

VENETO TUMOUR REGISTRY: IMPLEMENTING NAP-CODING IN CANCER REGISTRATION

S. Guzzinati[1], M. Zorzi[1], M. Baracco[1], S. Baracco[1], E. Bovo[1], C. Busato[1], E. Carpin[1], E. Chinellato[1], A. Dal Cin[1], A.R. Fiore[1], A. Greco[1], G. Martin[1], L. Memo[1], D. Monetti[1], S. Rizzato[1], A. Rosano[1], C. Stocco[1], S. Zamberlan[1], M. Rugge[1,2]

[1] Veneto Tumour Registry, Azienda Zero; [2] Department of Medicine (DIMED), University of Padua

Objectives. Critical assessment of the current experience of the Veneto cancer Registry (RTV) on the regional dataflow of neoplastic pathology records (ANAPAT), based on the NAP-coding.
Materials and methods. In the Veneto Public Heath Care System, cancer registration is regulated by a regional law (3/2013), which formally establishes an "information debt" of all the regional (both private, and public) healthcare institutions involved in the histological cancer assessment (i.e.: network of the Regional Pathology Departments [NRPD]). Cancer registration is basically settled on the collection of the pathology reports, as produced by the NRPD. The pathology reports are automatically transmitted (every six months) from the NRPD to a Regional Center of data collection (DWH). The collected data (always including Topography and Morphology) are coded according to NAP (*Nomenclatore Anatomia Patologica*), which has been implemented (year 2017) in all the Pathology Departments of the Regional Heath Care System. Each report includes 3 sections: i) demographics; ii) cancer biology/profile (topography/morphology); iii) textual health data. The NAP-coding includes about 25000 codes: 6185 topographic codes (based on the third SNOMED-edition; 3885 neoplastic morphologies (based on the 2005 edition of ICD-O-3, with multiple equivalent lexical variants); 490 procedure codes; 3171 qualifiers (including the TNM staging codes).
Results. Twice a year, the pathology reports are (automatically) uploaded in a regional portal for management of health sources (DWH), where a pre-fixed series of logical-formal checks is automatically performed (e.g.: presence of the required variables, values' consistency, keys' integrity). The regional-DWH automatically produces checking-reports of any occurring error/discrepancy; this first-level report is automatically delivered to the sending Health-care Institution. After data amendment, the "cleansed-files" are resubmitted to DWH. In a subsequent step, data are checked /managed by the Regional Cancer Registry (RTV). A second-level-checking is performed by the RTV-staff, focusing on both qualitative (e.g.: distribution of exams by year, month and diagnostic type) and quantitative (e.g.: misalignments and/or inconsistencies between Topography and Morphology; generic NAS codes; records without topographic code, records with non-tumour morphology [not M8-M9], etc). In a final step, any inconsistent record is returned to NRPD, for a critical conclusive reassessment and a conclusive download in the regional portal.
Conclusions. In 2017, about 55% of the reports entered into the RTV having the data transcoded from NAP to ICDO-3 and ICD-9; the remaining cases mainly consist of non-malignant pathology (e.g.: lipomas, nevi or polyps). Currently, the lines of further development on the ANAPAT dataflow are: i) anonymization and archiving in the regional DWH; ii) developing more extensive interaction with other regional Institutions involved in the clinical organization, monitoring, and in the efficiency/efficacy improvement of the Veneto Oncology network (calculation of indicators of diagnostic-therapeutic pathways); iii) implementation of the "text mining" for clinical variables (cancer stage, molecular profiling).

ESPERIENZE DI CODIFICA IN NAP REGISTRO TUMORI PIEMONTE

S. Rosso

SSD Registro Tumori Piemonte. A.O.U. Città della Salute e della Scienza di Torino

Il Registro Tumori Piemonte (RTP) raccoglie le informazioni contenuti nei referti dei Servizi di Anatomia Patologica (SAP) del Piemonte. Tali informazioni, essenziali per la definizione corretta, in positivo od in negativo, di caso tumorale incidente, includono, oltre ai dati anagrafici, anche le diagnosi in chiaro e le codifiche nosologiche utilizzate dai servizi. I testi di diagnosi, incluse le notizie cliniche e le descrizioni micro e macroscopiche, vengono trattate da algoritmi che restituiscono codifiche nosologiche per il 70% dei referti. I referti non codificati mediante algoritmi vengono esaminati manualmente dagli operatori, insieme ad una quota di referti su cui va posta particolare attenzione: in generale circa il 50% delle codifiche vengono riviste manualmente.
In questo contesto, è quindi possibile confrontare le codifiche originali con le codifiche ottenute dal lavoro del RTP. Va tuttavia premesso che gli scopi della codifica nosologica possono essere divergenti in alcuni casi (che verranno trattati in dettaglio nella presentazione) e quindi un certo grado di divergenza è insito nei metodi.
Attualmente la registrazione del RTP si effettua sui 29 SAP del Piemonte e sta trattando i referti degli anni dal 2015 al 2018. In questo periodo non tutti i SAP avevano effettuato la conversione delle codifiche in NAP e quindi i risultati della presente analisi vanno considerati come parziali ed in via di evoluzione.
Dai dati a disposizioni emerge un trend in crescita delle codifiche nosografiche che passa da poco più del 50% nel 2015 a circa l'80% nel 2018 su circa 400000 referti all'anno. Tuttavia, selezionando solo la patologia tumorale, le percentuali di codifica aumentano sensibilmente dal 60% nel 2015 al 73% nel 2017 e 87% nel 2018 (stima provvisoria). La codifica morfologica del tumore

è leggermente inferiore a quella topografica e passa dal 43% nel 2015 al 62% nel 2017. Questo implica che spesso non vengano definite direttamente le caratteristiche di invasività della lesione tumorale, costringendo a ricorrere agli algoritmi di codifica oppure alla tradizionale ricodifica manuale sulla base testuale.
Per quanto riguarda invece la congruenza con le codifiche finali del RTP, che, ricordiamo, possono differire da quella originale senza che alla base ci sia necessariamente un errore di codifica, gli indici di concordanza per il totale delle codifiche è del 60% (indice K), ma si registra una alta variabilità per tipo tumorale con la più alta variabilità e minore concordanza per le neoplasie ematologiche, dove il RTP codifica a più larghe maglie, e la definizione della sede nel caso di neoplasie addominali, dove prevale la sede del prelievo, piuttosto che l'origine della neoplasia.

Mercoledì, 16 ottobre 2019

Sala Istanbul – 12:30-17:00

EMATOPATOLOGIA

II SESSIONE

Moderatori: M. Ponzoni - A. Ramponi

CUTANEOUS LYMPHOID ATYPICAL INFILTRATE

M. Mascolo

Department of Advanced Biomedical Sciences, Pathology Unit, University "Federico II" of Naples, Italy

Cutaneous lymphoid infiltrate is common in daily practice and may represent a significant challenge for the pathologist. Many inflammatory dermatoses, as well as primitive cutaneous lymphomas or secondary lymphomatous disseminations to the skin, may present histologically as lymphoid infiltrate. Usually, histopathology is sufficient for a correct diagnosis. However, cutaneous lymphomas and some inflammatory dermatoses may present overlapping histopathologic features and, at times, be indistinguishable. In the majority of these case, a precise classification may be made through the close correlation of histopathology with accurate and complete clinical and, if necessary, molecular data. Nonetheless, in some cases, it is not possible to achieve a definite diagnosis. Cases that cannot be classified univocally as cutaneous lymphoma or pseudolymphoma are named atypical lymphoid infiltrate of the skin. It should be emphasized that this group does not include cases of cutaneous lymphoma non-otherwise specified. Cutaneous lymphoid atypical infiltrate may present as any of the following patterns: I) superficial lymphoid infiltrate with epidermotropism, II) infiltrate centered upon the dermis, with the formation of lymphoid follicles, III) infiltrate centered upon the dermis, without the formation of lymphoid follicles, IV) infiltrate primarily affecting the subcutaneous fat. Besides the close contact between the dermatologist and hematologist with the pathologist, a second opinion of an expert external pathologist may be particularly useful; this may reduce the number of unclassified cases. The knowledge of the wide spectrum of clinical and histologic presentation of inflammatory dermatoses and cutaneous lymphoproliferative disorders is necessary to prevent misdiagnosis that may be followed with needless excessive treatment and following consequences for the patients.

References

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PROLIFERAZIONI A CELLULE DENDRITICHE PLASMOCITOIDI MATURE E BLASTICHE

F. Facchetti

Plasmacytoid dendritic cell (PDC) neoplasms manifest in two clinically and pathologically distinct forms [1]. The first variant is represented by aggregates (often nodular) of clonally expanded PDC especially found in lymph nodes, skin, and bone marrow. This condition is rare, although likely underestimated in incidence and it is invariably associated with a myeloid neoplasm, especially chronic myelomonocytic leukemia. Due to lack of significant cytological atypia and expression of markers usually positive in normal PDC (with few exceptions) this condition has been defined as "Mature PDC proliferation associated with myeloid neoplasms" [2]. Its clonal nature and relatedness to the associated myeloid neoplasia have been definitely established [2]. The prognosis is usually dismal, but reflects mainly the evolution of the associated myeloid leukemia rather than progressive expansion of PDC; cases with long lasting remission have been reported. The second form of PDC tumor is represented by "Blastic plasmacytoid dendritic cell neoplasm" (BPDCN), a poorly differentiated ("blastic") proliferation of cells derived from PDC precursors [1 3]. It is included in the recently revised WHO classification of hematolymphoid neoplasia as a distinctive entity [4]. BPDCN is characterized by a common cutaneous and bone marrow tropism, with proliferation of small-medium size blastic cells whose identification is based on immunohistochemistry; tumor cells express several PDC markers, but at the same time lack of some of them and/or express de novo other ones; furthermore they lack antigens identifying T-, B- and myeloid cells. Table I reports the most significant markers useful for BPDCN diagnosis. A practical diagnostic approach to diagnose for BPDCN and especially to differentiate It from Acute Myeloid Leukemia includes positivity for at least 3 of 5 among common BPDCN markers (CD4, CD56, CD123, TCL1, BDCA2/CD303) and negativity for CD3, CD20, myeloperoxidase, lysozyme and CD34.

On gene expression profiling BPDCN has a unique signature, distinct from myeloid and lymphoid acute leukemias [5]. Compared with normal PDC BPDCN showed increased expression of genes involved in Notch signaling and NFKB activation, the latter representing a potential therapeutic target [5]. The oncogenic program of BPDCN is under the control of the transcription factor TCF4 (E2-2), which also represents the master regulator of normal PDC differentiation. Most BPDCN have an abnormal karyotype and up to 75% show complex abnormalities (≥ 3), the most frequent including loss of 12p (12p13) (in 30% of cases associated with deletion of *ETV6* gene), 9p (9p21.36q), 6q (6q23-qter) and 13q (13q13-21) [1 6].
Cumulative data based on different analytical approaches revealed a mutation profiling most frequently involving *TET2* (19%-80%), *ASXL1* (29%-44%), *NRAS* (27%) and *ATM* (21%), and more rarely *APC, BRAF, IDH2, KIT, KRAS, MET, MLH1, RB1, RET, TP53, VHL, IKZF3, ZEB2* and *NPM1.* Recurrent rearrangements of the proto-oncogene *MYB* have been reported especially in the pediatric variant, while *8q24*/*MYC* rearrangements has been detected in a subset of cases showing "immunoblastoid" cytology. More recently Sapienza et al. [7] identified that 15/16 BPDCN cases showed mutations of at least one of 25 epigenetic regulator genes (e.g.: *ASXL1, TET2, SUZ12, ARID1A, PHF2, CHD8*), indicating an undermined epigenetic regulatory program, affecting the integrity of the methylation program.
The clinical course of BPDCN is characterized by a rapid progression to systemic disease via hematogenous dissemination [1 4]. Treatment of BPDCN is not standardized. Complete remission may be achieved with local treatment in cases with isolated skin presentation. In patients in first complete remission allogeneic transplantation has been advocated as the best way to obtain long-term survival, even in elderly patients using reduced-intensity conditioning.
Significant results were recently reported using the immunotoxin SL-101 targeting the interleukin-3 receptor alpha (CD123), which is generally overexpressed by PDC blasts [8]. Moreover, data showing effectiveness in some patients by agents targeting the NF-κB pathway aberrantly activated in BPDCN [9], the BCL2 protein strongly expressed by tumor cells [10], or by hypomethilating epigenetic agents Decitabine and Azacitidine [7], pave the way to new therapeutic options in patients affected by BPDCN.

Tab. I. Immunophenotypic profile of BPDCN.compared to normal PDC

POSITIVE IN NORMAL PDC POSITIVE IN BPDCN	CD4, CD123, TCL1, BDCA2/CD303, E2-2/TCF4, CD2AP, BCL11a, SpiB
Positive in normal PDC negative or aberrant in BPDCN	CD68 and CLA (negative or dots) Granzyme B: negative
Negative in normal PDC positive in BPDCN	Consistent CD56 BCL2 Frequent CD7 CD33 Occasional CD2, CD5, CD7, CD10, CD13, CD79a, CD117, MUM1/IRF4, BCL6, S100 TdT (30%) - Ki-67 > 30%

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Mercoledì, 16 ottobre 2019

PATOLOGIA NEFROLOGICA E PATOLOGIA ULTRASTRUTTURALE

Sala Parigi – 12:30-17:00

Innovazioni strumentali e potenzialità applicative in TEM E SEM

Moderatore: G. Cenacchi

LA TERZA DIMENSIONE: UNA NUOVA VITA PER LA MICROSCOPIA ELETTRONICA

C. Moscheni

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Per oltre sei decenni la microscopia elettronica (ME) è stata il metodo elettivo di *imaging* per lo studio ultrastrutturale di campioni biologici. Più recentemente, si è assistito all'emergere di nuove tecniche automatizzate di ME per l'osservazione e l'analisi tridimensionale di cellule e tessuti su scala nanometrica con una risoluzione assiale

senza precedenti. Tali progressi della ME per la ricostruzione 3D di campioni biologici consentono una più rapida ed affidabile acquisizione di immagini seriali, sia riducendo considerevolmente la possibilità di introdurre errori attribuibili ad un'inadeguata capacità tecnica dell'operatore nel tagliare e maneggiare un numero elevato di sezioni, sia evitando l'acquisizione dell'immagine di ogni singola sezione mediante ME a trasmissione. Pertanto queste innovazioni tecniche hanno indubbiamente aperto nuove opportunità di ottenere dati 3D da materiale biologico, ma differendo significativamente nello spessore di volume analizzabile e nella risoluzione assiale, risulta importante valutare preventivamente quale metodologia risponda in modo specifico al singolo progetto di ricerca. Inoltre, l'entusiasmo per il potenziale di queste tecniche ha generato set di dati di dimensioni considerevoli, rendendo l'elaborazione e l'analisi delle immagini il collo di bottiglia per la maggior parte degli studi.

Corso base di Nefropatologia, I parte

Introduzione: G. Mazzucco - S. Pizzolitto

IL RUOLO DEL CLINICO NEL MANAGEMENT DELLA BIOSPIA RENALE

C. Rollino

SCU Nefrologia e Dialisi - Ospedale San Giovanni Bosco - Torino

Il ruolo del nefrologo è quello di porre l'indicazione clinica alla biopsia renale, eseguirla, impostare la terapia sulla base del quadro istologico e monitorare l'evoluzione della nefropatia. La diagnosi precoce, l'epidemiologia e la comprensione delle nefropatie dipendono quindi in gran parte dall'atteggiamento del nefrologo nei confronti della biopsia renale.
Poiché la letteratura internazionale nello scorso quinquennio mostrava una tendenza al declino dell'esecuzione delle biopsie renali e dati italiani mostravano un numero di biopsie renali effettuate inferiore a quello di molti altri paesi pur con proporzione invariata intorno al 20% di nefropatie uremizzanti senza diagnosi, nello scorso quinquennio la Società Italiana di Nefrologia (SIN) ha avviato un percorso per implementare la cultura della biopsia renale tra gli stessi nefrologi e limitare le notevoli disparità di esecuzione e lettura. A questo scopo ha organizzato un corso di aggiornamento itinerante per l'Italia (7 sessioni) e dibattiti congressuali e nel 2015 ha dato l'impulso alla creazione di una commissione congiunta SIN-SIAPEC per redigere i requisiti di esecuzione della biopsia renale e quelli di diagnostica nefropatologica.
La commissione, costituita da 6 anatomo-patologi e da 6 nefrologi clinici, ha prodotto due documenti che sono pubblicati sul sito della SIN e che riguardano ogni aspetto della biopsia renale.
I requisiti clinici includono modalità di ricovero, scelta degli operatori, indagini preparatorie, consenso informato. Viene puntualizzata la necessità di collaborazione interdisciplinare tra nefrologi e nefropatologi. Sono descritte le caratteristiche auspicabili del centro di nefrologia presso cui si effettuano le biopsie, che dovrebbe eseguire almeno 30 biopsie renali all'anno, essere situato in un ospedale provvisto di radiologia interventistica o con convenzione con centri di radiologia interventistica di ospedali vicini, possedere uno staff minimo di 2 nefrologi abilitati all'esecuzione della biopsia. Il nefrologo che interviene nell'esecuzione della biopsia dovrebbe avere un'esperienza ecografica attestata se opera da solo (formazione certificata di almeno 300 biopsie renali) oppure essere coadiuvato da un collaboratore ecografista certificato. Vengono poi puntualizzate gli esami preparatori, la correzione dei tempi di stillicidio, gli aghi da utilizzare, la procedura bioptica, la sorveglianza del paziente dopo la manovra.
Il documento dei requisiti minimi in diagnostica nefropatologica medica sottolinea che un laboratorio di nefropatologia accreditato dovrebbe essere equipaggiato con le metodiche necessarie per l'analisi in microscopia ottica, in epifluorescenza e in microscopia elettronica, il nefropatologo dovrebbe avere una expertise di 120 casi/anno e aver acquisito una specifica formazione certificata presso centri di riferimento nazionali e/o esteri di almeno sei mesi e/o aver esperienza di almeno 1000 biopsie renali, la diagnosi nefropatologica dovrebbe essere affidata a un patologo, il referto effettuato con un sistema di refertazione informatizzato e la sua consegna avvenire nell'80% dei casi entro 5 giorni lavorativi.
Questi importanti documenti, seppure non costituiscano per la loro struttura vere e proprie linee guida, sono tuttavia l'unico riferimento redatto dalla società scientifica e rappresentano quindi la base per lo sviluppo delle linee guida richieste dalla legge Gelli.

L'APPROCCIO METODOLOGICO ISTOLOGICO ED IMMUNOISTOCHIMICO NELLA DIAGNOSI DELLE GLOMERULONEFRITI PRIMITIVE E SECONDARIE

A. Barreca

Anatomia e Istologia Patologica 1U, AOU Città della Salute e della Scienza, Ospedale Molinette, Torino

Glomerulonephritis (GN) are diseases characterized by increased glomerular cellularity due to proliferation of local cells and/or leukocyte infiltration.
Nowadays the reporting of GN in kidney biopsies is not standardized and this constitutes a great bias both for patient care and realization of multicenter clinical trials. Therefore it's crucial to use an etiology/pathogenesis-based classification of GN combined with a standardized pathology kidney reporting of GN as recommended in the Mayo Clinic / Renal pathology Society Consensus meeting in 2015 [1] and confirmed in 2019 [2]. On the basis of etiology/pathogenic type there are main five classes of GN: immune-complex mediated GN, Pauci-immune GN, anti-glomerular basement membrane antibody GN (anti-GBM GN), Monoclonal Ig-mediated GN and C3 glomerulopathy (Tab. I).
The etiologic classification of GN is made on the basis of immunofluorescence (IF) microscopy findings in combination with light microscopy (LM) and electron microscopy (EM) features.
Immune complex-mediated GN is the most heterogeneous group and includes different specific disease entities, such as IgA nephropathy and IgA vasculitis, lupus nephritis, infection-related GN and most rare glomerular entity like fibrillary GN. In this group there are also membranoproliferative GN mediated by immune complex de-

Tab. I. Pathogenic classification of GN [1].

Pathogenic Type	Specific Disease Entity	Pattern of Injury: Focal or Diffuse	Scores or Class
Immune-complex GN[a]	IgA nephropathy, IgA vasculitis, lupus nephritis, infection-related GN, fibrillary GN with polyclonal Ig deposits	Mesangial, endocapillary, exudative, membranoproliferative, necrotizing, crescentic, sclerosing, or multiple[b]	Oxford/MEST scores for IgA nephropathy ISN/RPS class for lupus nephritis
Pauci-immune GN	MPO-ANCA GN, proteinase 3-ANCA GN, ANCA-negative GN	Necrotizing, crescentic, sclerosing, or multiple[b]	Focal, crescentic, mixed, or sclerosing class (Berden/EUVAS class)
Anti-GBM GN	Anti-GBM GN	Necrotizing, crescentic, sclerosing, or mixed[b]	
Monoclonal Ig GN[a]	Monoclonal Ig deposition disease, proliferative GN with monoclonal Ig deposits, immunotactoid glomerulopathy, fibrillary GN with monoclonal Ig deposits	Mesangial, endocapillary, exudative, membranoproliferative, necrotizing, crescentic, sclerosing, or multiple[b]	
C3 glomerulopathy	C3 GN, dense deposit disease	Mesangial, endocapillary, exudative, membranoproliferative, necrotizing, crescentic, sclerosing, or multiple[b]	

MEST, mesangial hypercellularity, endocapillary hypercellularity, segmental sclerosis, interstitial fibrosis/tubular atrophy; ISN/RPS, International Society of Nephrology/Renal Pathology Society; EUVAS, European vasculitis study group.
[a]Some pathologists use the terms immune complex–mediated GN, monoclonal Ig–associated GN, etc. It is up to the discretion of the pathologist to use these terms.
[b]Multiple patterns include two or more patterns of injury. The patterns should be stated (e.g., focal mesangial proliferative, crescentic, and sclerosing or diffuse necrotizing, crescentic, and sclerosing).

position. The common feature for this group is the presence of Ig on IF (mainly granular or smudgy/flocculent in fibrillary GN), often with co-deposits of complement. The LM pattern of glomerular injury is instead quite variable (ranging from minimal lesion to membranoproliferative pattern), linked in part with the specific disease type, and it's possible for some types of GN to have multiple patterns (e.g. lupus nephritis).

Some of these diseases, such as IgA nephropathy and lupus nephritis, have specific classification systems [3-5].

Pauci-immune necrotizing and crescentic GN is typically negative for Ig or complement, although small amounts of Ig and C3 (≤ 1+ granular staining in a segmental distribution) are sometimes observed. 80-90% of patients have serologic positivity for ANCA (ANCA-associated GN) against myeloperoxidase (MPO) or proeteinase 3 (PR3). The glomerular pattern of injury is characterized by extracapillary proliferation with various stage of disease (cellular, fibrocellular and fibrous crescents). In 2010 a pathologic classification for ANCA associated-GN was published [6].

Anti-GBM GN shows on IF strong linear positivity for Ig (mainly IgG) along the GBM, often accompanied by C3 granular staining. The characteristic etiologic finding is the presence of circulating anti-GBM antibodies. The glomerular pattern is typically an aggressive crescentic and necrotizing GN, with large and cellular crescents. Recently an atypical form of anti-GBM GN has been described [7], which shares the same features on IF (linear staining for Ig) but with different LM patterns (endocapillary, mesangial or membranoproliferative gn, rarely with focal crescents) and clinical course.

Monoclonal Ig-mediated GN has monotypic Ig deposits on IF in the glomeruli and/or along tubular basement membranes. The observation of monotypic deposits with light chain or heavy chain restriction on IF is mandatory for the diagnosis. This group includes some diseases with non-organized deposits on EM, such as proliferative glomerulonephritis with monoclonal Ig deposits (PGNMID) and monoclonal Ig deposition disease (MIDD), and others with organized deposits on EM like immunotactoid glomerulopathy, cryoglobulinaemic glomerulonephritis type I and II and fibrillary GN with monoclonal Ig deposits [8]. On LM there are different patterns: membranoproliferative one is the most common for PGNMID and immunotactoid GN, while MIDD is typically characterized by nodular glomerulosclerosis. Many patients with these diseases have an underlying monoclonal gammopathy/paraproteinemia.

C3 glomerulopathy is a new recently introduced pathological entity characterized by the presence of dominant C3 deposits in the glomeruli with absent or minimal Ig [9]. C3 glomerulopathy is correlated with abnormalities in the control of the alternative pathway of complement. This group includes two entities on the basis of EM findings: dense deposits disease and C3 GN, both of them characterized by variable morphologic patterns.

Finally this etiologic classification is based on an integrated approach associated mainly with IF findings and pathogenic features, in some diseases with specific EM aspects.

Apart from these diseases is the complex group of podocytopathies, characterized by podocyte foot process effacement, that includes focal segmental glomerulosclerosis and minimal change disease.

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Mercoledì, 16 ottobre 2019

Sala 500 – 18:00-19:30

SESSIONE PLENARIA

Letture Magistrali

TRACKING COLORECTAL CANCER EVOLUTION

A. Bardelli

When metastatic cancers are challenged with targeted agents almost invariably a subset of cells insensitive to the drug emerges. As a result, in most instances, targeted therapies are only transiently effective in patients. Strategies to prevent or overcome resistance are therefore essential to design the next generation of clinical trials. How can we overcome the near-certainty of disease recurrence following treatment with targeted agents? Addressing this question means considering as a target not "only" individual oncogenes but also the evolving nature of human tumors. We used colorectal cancer (CRC) as a model system to test the hypothesis that by understanding tumor's evolution, the emergence of drug resistance can be controlled. We find that clonal dynamics can be monitored in real time in the blood of patients, and liquid biopsies can be used to intercept the emergence of resistant clones before relapses are clinically manifest. We discovered that a multistep clonal evolution process driven by progressive increases in drug fitness underlies the development of resistance in cells and patient avatars. To have long-term efficacy, the use of targeted therapies must take into account the continuous evolution of cancer cells, that is to say, therapies must adapt to tumor evolution. One possibility is to anticipate the changes the tumors will make. For example, by knowing in advance how CRC cells overcome resistance to EGFR blockade, we devised further rounds of therapy. These findings became the bases of clinical trials aimed at targeting cancer evolution and exploiting liquid biopsies to monitor the emergence of drug resistance and to design further lines of therapies.

Giovedì, 17 ottobre 2019

Sala 500 – 14:00-18:30

PATOLOGIA MAMMARIA

Neoplasia mammaria ed immunogenicità

Moderatori: A. Sapino, S. Bianchi

IMPLICAZIONI CLINICHE DELLA VALUTAZIONE DELL'INFILTRATO LINFOCITARIO NEI CARCINOMI DELLA MAMMELLA

M.V. Dieci

Although breast cancer is not traditionally considered immunogenic, a growing body of evidence suggest that certain breast cancer subtypes, namely triple-negative and HER2-positive, may exhibit a strong infiltration by immune cells.

The TIL (tumor infiltrating lymphocytes) International Working Group has established consensus guidelines for the evaluation of TILs in breast cancer on hematoxylin and eosin-stained slides (Salgado R, Ann Oncol 2014; Dieci MV, Semin Cancer Biol 2018).
Starting from the pivotal study by Denkert et al (J Clin Oncol 2010), showing that increased levels of TILs were associated with increased rate of pCR, a finding that was subsequently confirmed in a larger cohort (Denkert C, Lancet Oncol 2018), several groups have investigated the role of TILs in breast cancer.
Recently, TILs have reached level of evidence 1b as prognostic factor for triple negative breast cancer patients treated with adjuvant anthracycline-based chemotherapy (Loi S, J Clin Oncol 2019). Based on this prognostic value, the St Gallen International Consensus Guidelines 2019 panel endorsed the routine evaluation of TILs in primary triple negative breast cancer, by specifying that, at the moment, the data do not support TILs as a biomarker to guide treatment de-escalation.
With regards to HER2-positive breast cancer, a number of studies in the last couple of years have confirmed a positive prognostic role for TILs. Moreover, the analysis of TILs in the ShortHER trial, comparing the standard 1 year vs 9 weeks of adjuvant trastuzumab combined with chemotherapy, has suggested that patients with high TILs might be good candidate for treatment de-escalation (Dieci MV, Ann Oncol 2019).
With regards to hormone receptor-positive patients, although TILs have been confirmed to predict pathological complete response to neoadjuvant chmeotherapy (Denkert C, Lancet Oncol 2018), the evidence on the association between TILs and prognosis are more controversial, possibly confunded by the potential immunomodulatory effect of endocrine therapy (Dieci MV, Cancer Treat Rev 2016).
Finally, in the era of immunotherapy, there is evidence from some clinical trials that TILs may also predict the efficacy of immune-checkpoint inhibitors (Adams S, Ann Oncol 2019; Loi S, Lancet Oncol 2019). However,

a deeper understanding of TILs predictive role is warranted.

Istotipi particolari

Moderatori: P. Querzoli, A.Rizzo

ADENOMIOEPITELIOMA NELLA WHO 2019

Maria P. Foschini, Angelo G. Corradini.

Anatomia Patologica Ospedale Bellaria, Dipartimento di Scienze Biomediche e Neuromotorie, Università di Bologna.

La prossima edizione del Blue Book WHO sulle neoplasie della mammella non vedrà sostanziali modifiche nella classificazione dell'adenomioepitelioma (AME).
Saranno presenti due capitoli: AME ed AME maligno.
AME. La definizione di AME rimane quella di una neoplasia bifasica composta da strutture ghiandolari rivestite da doppio strato di cellule, epiteliali e mioepiteliali. Lo strato di cellule mioepiteliali deve essere prominente. Nella stesura di tale definizione si è discusso molto su quanto lo strato di mioepitelio dovesse essere "prominente". Infatti, anche in lesioni mammarie benigne, quali adenosi sclerosante, possiamo trovare strutture ghiandolari rivestite da epitelio e mioepitelio. Purtroppo, al momento, non ci sono studi che possano stabilire criteri oggettivi e riproducibili, per porre la diagnosi di AME. Questo può essere un problema, soprattutto nelle agobiopsie pre-operatorie, dove si vede solo una parte della lesione e sono presenti fenomeni artefattuali legati al prelievo.
Terminologia da utilizzare: era stato proposto di utilizzare una terminologia unica per le lesioni della mammella e delle ghiandole salivari, utilizzando per entrambe le sedi, il termini di carcinoma epi-mioepiteliale a basso o ad alto grado di malignità. Tuttavia questa proposta è stata scartata perché si ritiene che AME della mammella, quando si presenta come benigno, possa avere una prognosi ottima. Qualche perplessità viene tuttavia espressa nella sezione prognosi.
AME può insorgere in qualsiasi parte della mammella; quando interessa la zona retro-areolare, può presentarsi con secrezione ematica dal capezzolo.
All'istologia AME può presentare architettura tubulare, a cellule fusate, lobulata e a crescita papillare intraduttale. Le relative diagnosi differenziali sono discusse.
AME può essere suddiviso in due gruppi, a seconda dell'espressione o meno di recettori per l'estrogeno. Tali gruppi differiscono anche per il profilo molecolare [2].
La prognosi di AME senza atipie è sostanzialmente buona, quando asportato chirurgicamente in maniera completa. Tuttavia va tenuto presente che, seppur rari, casi di metastasi sono descritti anche in AME senza atipie [5 6]. AME con crescita polipoide può recidivare, se non escisso con ampio margine.
Un problema ancora non risolto è l'impatto delle mitosi atipiche nella prognosi. Secondo il testo AFIP (2009), il rischio di recidive aumenta se AME presenta un numero di mitosi atipiche superiore a 3 per 10 campi a forte ingrandimento [8]. Tuttavia, al momento, non sono stati pubblicati lavori che confermino o smentiscano questo valore di cut-off.
AME maligno (AME-M). AME viene definito maligno quando compaiono aspetti francamente carcinomatosi a carico della componente epiteliale o mioepiteliale. Quando gli aspetti di malignità coinvolgono entrambe le componenti, si può parlare di carcinoma epi-mioepiteliale.
La componente maligna può avere un aspetto estremamente variabile [1 3 7]. Quando la trasformazione maligna interessa la componente epiteliale possono comparire aspetti di carcinoma duttale infiltrante non altrimenti specificato, di carcinoma lobulare, mucoide ecc. Quando la trasformazione maligna interessa la componente mioepiteliale, essa presenta le caratteristiche del carcinoma mioepieliale. La componente maligna può inoltre avere le caratteristiche di carcinoma metaplastico, in tutte le sue varianti.
I casi riportati di trasformazione maligna di entrambe le componenti, epiteliale e mioepiteliale sono rari [4].
La prognosi è difficile da stabilire, dipende in gran parte dallo stadio alla presentazione e dal tipo di componente carcinomatosa.
In generale recidive e metastasi possono comparire anche a distanza di molti anni. Inoltre le metastasi sono prevalentemente ematogene. I casi con metastasi per via linfatica sono rari.
In generale viene comunque raccomandata estrema prudenza nella gestione delle pazienti con AME-M, data la scarsa conoscenza ancora disponibile.

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Giovedì, 17 ottobre 2019

Sala Londra – 14:00-18:30

QUALITÀ E SICUREZZA

Qualità e Sicurezza nella gestione dei dati in Anatomia Patologica

I Sessione

Moderatori: O. Nappi, G. L. Taddei

IL NUOVO REGOLAMENTO IN MATERIA DI PRIVACY (GDPR): APPLICAZIONE NELL'AMBITO DEI SERVIZI DI ANATOMIA PATOLOGICA

F. Crivelli

Con il regolamento UE 2016/679 meglio conosciuto come General Data Protection Regulation (GDPR) adottato il 26 aprile 2016 e pubblicato sulla Gazzetta Ufficiale della Unione Europea il 4 maggio 2016, l'Unione Europea si propone come obiettivi quello di rafforzare la protezione dei dati personali di cittadini dell'Unione europea e dei residenti nell'Unione europea, sia all'interno che all'esterno dei confini dell'Unione europea (UE), restituendo ai cittadini il controllo dei propri dati personali, semplificando il contesto normativo che riguarda gli affari internazionali, unificando e rendendo omogenea la normativa privacy dentro l'UE.
Il testo affronta anche il tema dell'esportazione di dati personali al di fuori dell'UE e obbliga tutti i titolari del trattamento dei dati (anche con sede legale fuori dall'Unione europea) che trattano dati di residenti nell'Unione europea ad osservare e adempiere agli obblighi previsti.
Dalla sua entrata in vigore, il GDPR ha sostituito i contenuti della direttiva sulla protezione dei dati (Direttiva 95/46/CE) e, in Italia, ha abrogato gli articoli del codice per la protezione dei dati personali (d.lgs. n. 196/2003) con esso incompatibili.
Il "Regolamento Parlamento Europeo 27 aprile 2016 n. 2016/679/UE", non necessita di approvazione da parte dei singoli governi essendo immediatamente operativo ed è entrato in vigore il 25 maggio 2018.
Il Regolamento prevede alcune definizioni:
"dato personale" è: "qualsiasi informazione riguardante una persona fisica identificata o identificabile ("interessato"); si considera identificabile la persona fisica che può essere identificata, direttamente o indirettamente, con particolare riferimento ad un identificativo come il nome, un numero di identificazione, dati relativi all'ubicazione, un identificativo online o a uno o più elementi caratteristici della sua identità fisica, fisiologica, genetica, psichica, economica, culturale o sociale." (art. 4 comma 1).
"trattamento" è: "qualsiasi operazione …. compiuta con o senza ausilio di processi automatizzati … applicata ai dati personali …. come la raccolta, la registrazione, l'organizzazione, la strutturazione, la conservazione, l'adattamento o la modifica, l'estrazione, la consultazione, l'uso, la comunicazione mediante trasmissione, diffusione o qualsiasi altra forma di messa a disposizione …. la cancellazione o la distruzione" (art. 4, comma 2.).
"interessato" è: il titolare del diritto ad un trattamento "corretto e trasparente" dei propri dati ed ha diritto di essere informato (diritto all'informativa) di una serie di dati che colui che raccoglie i dati è tenuto a dargli per poterli trattare. (elenco contenuto negli art. 13 e 14).
"informativa" è: il documento con il quale il "titolare del trattamento" fornisce all' "interessato" le informazioni che la legge prevede gli vengano fornite al momento in cui quest'ultimo fornisce i dati ed è indispensabile per poter poi trattare i dati personali.
Il **"trattamento"** è lecito solo se e nella misura in cui (art. 5.)
L' "Interessato" ha espresso il consenso al trattamento dei propri dati personali per una o più specifiche finalità;
c) d) e) (…omissis…)
f) Il "trattamento" è necessario per il perseguimento del legittimo interesse del titolare del trattamento a condizione che non prevalgano gli interessi o i diritti e le libertà fondamentali dell'interessato che richiedono la protezione dei dati personali, in particolare se l'interessato è un minore.
Occorre in ogni caso tenere presente che "È vietato trattare dati personali che rivelino l'origine razziale o etnica, le opinioni politiche, le convinzioni religiose o filosofiche, o l'appartenenza sindacale, nonché trattare dati genetici, dati biometrici intesi ad identificare in modo univoco una persona fisica, dati relativi alla salute o alla vita sessuale o all'orientamento sessuale della persona" (art. 9 comma 1).
Tali ultimi dati di "categorie particolari", anche "sensibili" possono essere trattati soltanto se: (art. 9):
a) L'interessato ha prestato il proprio consenso esplicito al trattamento di tali dati personali per una o più finalità specifiche;
b) il trattamento è necessario per assolvere gli obblighi ed esercitare i diritti specifici del titolare del trattamento o dell'interessato in materia di diritto del lavoro e della sicurezza sociale e protezione sociale, nella misura in cui sia autorizzato dal diritto dell'Unione o degli Stati membri o da un contratto collettivo ai sensi del diritto degli Stati membri, in presenza di garanzie appropriate per i diritti fondamentali e gli interessi dell'interessato;
c) …omissis…
d) Il trattamento è effettuato, nell'ambito delle sue legittime attività e con adeguate garanzie, da una fondazione, associazione o altro organismo senza scopo di lucro che persegua finalità politiche, filosofiche, religiose o sindacali a condizione che il trattamento riguardi unicamente i membri, gli ex membri o le persone che hanno regolari contatti con la fondazione, l'associazione o l'organismo a motivo delle sue finalità e che i dati non siano comunicati all'esterno senza il consenso dell'interessato.
e) f) h) i) j) …omissis…
Per quanto riguarda le ricadute sulle strutture complesse di Anatomia Patologica, fermo restando quanto già previsto dal D.Lvo 163/2003 il punto fondamentale è il capo IV del Regolamento Generale che prevede gli obblighi riferiti al titolare e al responsabile del trattamento (sezione 1) e la sicurezza dei dati (sezione 2).
Ai fini del regolamento è necessario dichiarare con apposito registro le attività che vengono svolte e quali sono

le possibili necessità all'interno di dette attività (art. 30) e di conseguenza le responsabilità in capo al titolare del trattamento e ai responsabili.
Per quanto riguarda la sicurezza dei dati mota enfasi viene messa nel rispetto della inviolabilità del dato per cui dotarsi di password personali da modificare ogni tre mesi in caso di archivio elettronico per chi accede al dato con programmi che siano in grado di tracciare ogni accesso, dotarsi di armadi con chiusura a chiave o eventualmente di accesso controllato negli spazi di archivio.

APPLICATION OF DATA TRACEABILITY FROM CLINICAL SERVICES TO THE SECTION OF SURGICAL PATHOLOGY

A. Nottegar

The issue of traceability in the service of surgical pathology is becoming one of the most important aspects during the routine daily activity. Traceability is generally defined as a multi-step process that tracks all elements, which can define, modify or specify a product; in this context, it can regard a cytological sample, a biopsy or a surgical specimen, tracked from the clinical/surgical services to the section of surgical pathology. The first passages are usually understimated in their importance, also because they happen out of the pathology units, but are still of critical importance. Indeed, these first steps impact on the origanization and on the activity of professionists that do not work into pathology sections. Thus, they must learn how to use the software of pathology units, from generating the electronical request to obtaining a barcode, which shlould identify the material through all its workflow. Once the material arrives in the pathology section, the traceability becomes more articulated. Indeed, here it starts with the acceptance of material, a phase that is usually coordinated by a technician. This first step is very important and includes a check on what is declared on the clinical request and what is present in the received box. After this first step, the traceability ensures that all the next passages will be strictly monitored and can be followed by technicians and pathologists. The phases of gross sampling (if needed), inclusion (formalin-paraffin passages), cut and slide preparation can be easly reported on the specific software, which nowadays are designed ad-hoc for surgical pathology services. The most recent advances in this line have been represented by the possibility of accompanying each specimen with a matched bidimensional-bar code, which now can be printed also on microscopical slides. The traceability appears today a fundamental topic for all pathology services: it is necessary for decreasing the possibility of mistakes (e.g. material exchange), as well as for legal puroposes, since it represents one of the keys to demonstrate the presence of high standars of quality controls of the pathology sections. The new branch of digital pathology is taking into account the issue of traceability, and represents a promising step forward to improve this complex but still very important process.

TRACKING SYSTEM IN ANATOMIC PATHOLOGY: SIMPLY A TOOL TO PREVENT AND REDUCE ERRORS?

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Tracking solutions have relatively recently made their appearance in Anatomic Pathology laboratories in Italy [1]. Moreover, about a lustrum ago by then, the Ministry of Health recognized the usefulness of such a tool in preventing and reducing pre-analytical and analytical errors in anatomic pathology [2].
Nevertheless, tracking systems are not so widely available in Anatomic Pathology laboratories in Italy nowadays. Such situation might be explained by the type of organization of pathology laboratories in Italy, in general: implementation of tracking solutions in laboratories dealing with a relatively small number of specimens per day might be considered too expensive by Administrators.
Similarly, due to the relatively small number of case dealt generally in Anatomic Pathology services in Italy, new technologies, recently made available by industry, might reveal a negative balance of costs-benefits: again, this would represent a missed opportunity of quality improvement.
We would like to discuss the benefits of tracking system and automation in Anatomic Pathology in terms of laboratory efficiency and, above all, in terms of patient safety and staff accountability: the lab manager should be aware that people, patients as well as employees, must be the target of any decision and choice.

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II Sessione

Moderatori: A. Fabiano, F. Vecchio

COMMUNICATION OF DATA IN ANATOMIC PATHOLOGY

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The collection and sharing of data are a growing trend in healthcare, and Anatomic Pathology is no exception. Among medical specialties, anatomic pathology produces many types of data that are of relevance in protecting the public health.
Every day, public and private health services send a variety of data to the Anatomic Pathology services.
Such data (patient name and demographics, responsible physicians /staff, dates / times, specimen types / procedure, clinical history, location and types of any lesions present, clinical findings, relevant investigations and previous treatment, previous histology / cytology results, presumptive clinical diagnosis, tests ordered, laboratory results, etc…) are shown on the request form that accompanies each biological sample (histological and cytological) and on the container of the biological sample itself.
Thereafter, the Anatomic Pathology service, in addition to the data present on the request form, sends back other data (gross description, microscopic description, final pathologic diagnosis, tumor size, cell type, histological grade, lymph node status, margin status, stage, prognostic markers results, flow cytometry/molecular/genetic results, name and signature of the responsible pathology, name of staff/technicians, billing information/codes, imaging, etc…) to the requesting clinician in the form of a conclusive report.
Anatomic pathology report communicates to different teams involved in patient management information that is required for, or useful in, the proper disease management, prognosis, and patient health. With newer diagnostic modalities becoming available and treatment also affected by several other morphologic, molecular, and genetic features, the pathology report has become a data-rich entity at the core of the multidisciplinary management of the patient. Data contained in pathology report, in addition to be used primarily for patient care, may be shared or exchanged between pathology services and other care providers and institutions for different purposes such as tumor registries, epidemiologic studies, translational research, and quality improvement activities.
Attempts have been made to describe communication competencies in pathology. A summary of the consensus reached by the European Association of Pathology Chairs and Program Directors regarding the "Pathologist as Communicator" indicates that as communicator the pathologist (i) facilitates exchange of information and participative case management between medical experts in the context of the dynamic exchanges which form part of a patient-centered medical encounter, (ii) enables patient-centered diagnostic communication through shared decision-making and effective dynamic interaction with caregivers and other health care professionals, (iii) accurately conveys relevant information and explanations to colleagues and other professionals and fosters the development of common understanding on issues, problems, and plans to develop a shared plan of care, and (iiii) conveys effective oral and written information about a case [1].
Communication is an essential element of good clinical practice in whichever branch of medicine and can be defined as the "*exchange of information between a sender and a receiver*" [2]. According to Scott, communication is about sending, receiving, and understanding information and meaning. He claimed that "receiving" and "understanding" are the most important operations in the communication process, since the response of the receiver defines whether the communication attempt is successful or not [3].
Effective communication of health information requires that at least four important aspects be considered. They are: (i) the content of communicated information, which should satisfy the information needs of health consumers, (ii) the communication tools that are used to communicate the information, (iii) the communication formats that are used to present the information and (iiii) the typefaces of the communicated information, which includes several aspects such as fonts, sizes and styles.
Communication skills are essential for the working of a pathologist; they are necessary for obtaining information from and communicating information to requesting physicians and other health care professionals. Achieving quality in communication in surgical pathology is not as simple as dictating a report and delivering it to the surgeon who generated the specimen.
In addition to diagnostic accuracy, elements that contribute to optimize communication should include (a) managing physicians' expectations for turnaround time, (b) managing critical and unexpected pathology results and (c) managing intraoperative consultation. Moreover, (d) assuring adequate completeness of the report with the use of synoptic checklist with discrete data fields facilitates secondary uses of pathology data. Finally, (e) using of report formatting recommendations as (i) "*headlines*" to emphasize key elements to draw attention to the main diagnosis, (ii) "*maintaining continuity of layout*" to help clinicians identify diagnostic and prognostic information, (iii) "*optimize the density of information*" in family units that is more easily understood and preserved, and (iiii) "*reduce non-essential information*" that competes with essential information for the reader's attention, contribute to a better comprehension of the reports itself [4-6].
Common routes of communication with the requesting clinician are represented by: (i) *printed versions of report* sent out in sealed envelopes to identified addressees are beyond any doubt secure modes of communication; (ii) *communication of report by phone* is certainly a good way if the clinician and the pathologist at the two ends of the telephone line know each other and are directly involved in the case; the verbal transmission of results is a potential patient safety issue, due to the possibility of misinterpretation or transcription errors and the potential that verbal reports are either not read or filed in the relevant patient's record; (iii) *uploading digital report into the patient files*, via protected interfaces, can be considered a secure mode of communication; (iiii) *communication of report by fax* is acceptable only if measures have been taken to assure that only a very restricted, well-known circle, of persons have access to the fax machine that receives pathology reports and ; (iiiii) *electronic means of communication* including "e-mail", "text messaging", and various alert and broadcast systems are all potential media that may be used to deliver surgical pathology results if are done in the context of a closed network used by a group of related professionals and adequately protected by coding algorithms [7].
Because the ultimate product of anatomic pathology

service is "pathology report" and because that communicating data contained in the report is vital to patient management decisions, the security of those data is now an important issue for pathology services. Therefore, understanding and implementing modern methods of data communication security is an important part of a state-of-the-art anatomical pathology service.

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USEFULNESS OF A QUESTIONNAIRE FOR DATA COLLECTION IN THE ANATOMIC PATHOLOGY SERVICES.

Survey of the Gruppo di Studio Siapec-IAP "Gestione, Qualità e Sicurezza per l'Anatomia Patologica"

R. Giardini[1], R. Colombari[2], G. Santeusanio[3]

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With the explosion of pathology data, their security has now become an important issue for pathologists to be properly considered. Our activity generates a wide range of data both digital and on paper support, most of which have pertinence with the privacy and safety of the patient, affecting the quality of our services and our responsibility. Understanding modern security concerns and technologies is critical for implementing state-of-the-art pathology services today (1,2). With the aim to remind to all those are responsible of Structures of Anatomic Pathology services the need to pay attention to the topic, and to obtain a picture of the national situation regarding the protection of sensitive data in Anatomic Pathology services, an e-mail was sent to the directors of all the anatomic pathology centers in Italy in order to describe the aims of the survey. The questionnaire, which was signed up by a Siapec-IAP working group (Gestione, Qualità e Sicurezza per l'Anatomia Patologica) consisted of 20 questions listed below.

Information and training

1) Do you think the topic of data protection is relevant?
2) Is there a specific document in your department that reminds all operators of this issue?
3) In the last year, have meetings been organized on the subject?
4) Do you believe that data protection is under the responsibility of the department management?

Protection of paper data

1) Are paper documents always protected effectively, so that they cannot be consulted by outsiders?
2) Are the paper documents kept and protected from possible photocopies or thefts and damages (for example, fire or flooding)?
3) Are the people who can access the archives defined?
4) Is a procedure for accessing and consulting archives defined?

Data protection

1) Is the computer system in use protected?
2) Do you know the protection system?
3) Are the passwords definitely personal and confidential?
4) Is there a document that clarifies the responsibility of the holder of each password?
5) Is there a procedure to periodically update passwords?
6) Is there a periodic backup system?

Communication and diffusion of data

1) Are there precise indications on how to deliver and diffuse reports?
2) Specifically: to whom are positive outcomes for outpatient patients delivered in a sealed envelope?
3) Do you make photocopy of the cytohistological slides requested by the patient for review?
4) Do you request a deposit for the delivery of cytohistological slides or inclusions?
5) Who to do you communicate a diagnosis by telephone?
6) Do you send the cytohistological slides to a Colleague for a second opinion without requiring the patient's permission?

The collected data, including the response percentages as an index of the perceived interest and utility of such a questionnaire, as well as the evaluation of the predictable differences in data management among different Institutions, will be discussed at the afternoon session "Quality, safety and data management in Anatomic Pathology" next October the 17th 2019. We hope this event will represent the starting point for the production of corporate documents on the subject, which might be applied in a national-wide manner.

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Giovedì, 17 ottobre 2019

Sala Madrid – 14:00-18:30

PATOLOGIA APPARATO DIGERENTE

II Sessione

Young Pathologist Slide Seminar

Moderatori: L. Saragoni, L. Mastracci

SYNCHRONUS FACIAL CUTANEUS METASTASIS FROM INTESTINAL ADENOSQUAMOUS CARCINOMA

P. Parente[1], C. Covelli[1], P. Parrella[2], M. Castelvetere[1], C. Clemente[1], F. Fiordelisi[1], T.P. Latiano[3], E. Maiello[3], P. Graziano[1]

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Amongst colorectal neoplasms, intestinal adenosquamous carcinoma (ASC) is an uncommon histotype, representing approximately 0.05% to 0.20% of all colorectal malignancies [1] The occurrence of cutaneous metastasis from intestinal neoplasm is also very rare [2].
Compared to conventional adenocarcinoma, ASC exhibits an aggressive behavior showing advanced disease with regional and distant metastasis at onset [3]. The liver is the most common site of metastasis from ASC, followed by the peritoneum and the lung 4. In addition, just a few data on its molecular landscape are available [5].
To best of our knowledge, it has not been described colorectal ASC skin metastastis [6].
Here we report a case of synchronous colorectal ASC and cutaneous facial metastasis in a 70-year-old man. The patient was admitted to our Hospital with abdominal pain and a diagnostic colonoscopy revealed the presence of a large mass in the left colon. Biopsies showed histological features of adenosquamous carcinoma and a left hemicolectomy was subsequently performed.
A synchronous nodular skin lesion was also identified on the cheek and the excisional biopsy showed dermal-ipodermal localization of ASC consistent with metastasis from the primary colorectal cancer. Following surgery and chemotherapy, the patient developed one liver and two further cutaneous metastasis.
We illustrate the first case of single synchronous facial skin metastasis from colorectal ASC without other visceral involvement and compared molecular analysis of primary and metatastatic lesions are also described.

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Giovedì, 17 ottobre 2019

Sala Madrid – 15:00-16:20

SESSIONE SPECIALE "PATHOLOGY ASSISTANT"

Patology Assistant: una nuova figura in Anatomia Patologica

Moderatori: M. Barbareschi, M. Rugge

IL PATHOLOGY ASSISTANT NEL PANORAMA NORMATIVO ITALIANO

G. Gardini

Normativa attuale. La normativa sui Tecnici Sanitari di Laboratorio Biomedico (TSLB) è contenuta principalmente nel DM 745/94 e Legge 42/99. Queste due leggi sanciscono il superamento del concetto di professione sanitaria ausiliaria introducendo la logica del profilo professionale che sostituisce la logica del mansionario. La logica del mansionario era basata su una elencazione di compiti e attribuzioni ai quali l'esercizio professionale doveva attenersi e quindi limitarsi; la logica del profilo professionale prevede invece un'ampia autonomia e responsabilità. Il DM 745/94 attribuisce al TSLB il seguente profilo:" svolge attività di laboratorio di analisi e di ricerca relative ad analisi biomediche e biotecnologiche ed in particolare di biochimica, di microbiologia, di citologia e di istopatologia". In sintesi i TSLB svolgono con autonomia professionale le procedure tecniche necessarie all'attività diagnostica su materiale biologico. L'esame istologico consta di una serie di procedure tecniche che portano alla diagnosi pertanto tali procedure possono essere svolte dai tecnici; solo la diagnosi istopatologica rimane un atto medico di cui il Patologo ha la responsabilità.
Esperienze internazionali. Nel Nord America dalla fine degli anni 80 nasce la figura del Pathology Assistant (PA), professionista sanitario, non medico, altamente qualificato, che nei servizi di Anatomia Patologica, in affiancamento al patologo, è responsabile tra l'altro dell'esame macroscopico e del campionamento dei pezzi operatori.
Nuove prospettive. In Italia il Contratto Nazionale di Lavoro (CCNL) 2016/2018 per il Comparto Sanitario per

la prima volta, in analogia a quanto già succede per il Personale Medico, tende al riconoscimento della professionalità introducendo le figure del professionista specialista e del professionista esperto anche per i TSLB. "La posizione di professionista specialista è attribuita al professionista laureato delle citate professioni sanitarie in possesso del master di primo livello di cui all'art. 6 della Legge n. 43/06". "La posizione di professionista esperto è attribuita al professionista che ha acquisito competenze avanzate, tramite percorsi formativi complementari regionali ed attraverso l'esercizio di attività professionali, anche in virtù di protocolli concordati tra le rappresentanze delle professioni interessate, di quelle mediche e dell'area sanitaria più in generale.
Conclusioni. alla luce dell'attuale normativa, delle prospettive aperte dal nuovo, basandosi anche sull'esperienza americana del PA, pensiamo che anche in Italia vi siano i presupposti normativi, scientifici e culturali per avere nelle sale di campionamento TSLB capaci di descrivere e campionare i pezzi chirurgici, ovviamente in collaborazione con il patologo cui spetta sempre la diagnosi istopatologica.

PERCORSI DI FORMAZIONE UNIVERSITARIA

A. Maiorana
Università degli Studi di Modena e Reggio Emilia

Presso l'Università degli Studi di Modena e Reggio Emilia è stato attivato un Master di I livello in "Tecniche Istopatologiche in Anatomia Patologica". Il Master mira a fornire ai tecnici di laboratorio una preparazione specifica nel settore del campionamento, preparazione e conservazione delle biopsie e dei prelievi operatori. Con l'ausilio di lezioni teoriche e del tirocinio pratico, il partecipante viene posto in condizione di sviluppare competenze specifiche per: 1) includere il materiale bioptico (agobiopsie e biopsie endoscopiche) prelevato da organi diversi; 2) orientare e campionare punch e losanghe cutanee; 3) riconoscere e descrivere le lesioni macroscopiche piu' frequenti nei campioni chirurgici; 4) campionare i pezzi operatori più complessi, in collaborazione con il medico anatomo-patologo; 5) gestire le varie attività del laboratorio di istopatologia. Un corso speciale è finalizzato all'apprendimento della tecnologia delle macrosezioni (inclusione, processazione, taglio al microtomo, immunoistochimica). Momenti di apprendimento specifico sono focalizzati sulla preparazione degli esami intraoperatori al congelatore e la inclusione in paraffina di materiale citologico (citoinclusi); vengono anche approfonditi gli ambiti relativi alla preparazione e microdissezione del materiale da sottoporre a valutazioni di biologia molecolare in-situ o estrattiva e alle problematiche connesse con la raccolta di materiale biologico per le biobanche, quali raccolta del consenso informato e criopreservazione.
Le lezioni teoriche vertono su: a) Tracciabilità, raccolta, trasporto, conservazione e archiviazione di tessuti per indagini diagnostiche in Anatomia Patologica; b) Classificazione istopatologica delle neoplasie con grading e staging e inquadramento delle lesioni non neoplastiche; c) Descrizione macroscopica e tecniche di campionamento delle biopsie e dei pezzi chirurgici; d) Anatomia Patologica speciale dei vari organi ed apparati; e) Tecniche di campionamento e processazione degli esami intraoperatori; f) Tecniche per l'esecuzione di macrosezioni, citoinclusi, prelievi per biobanche; g) Tecniche per biologia molecolare, in-situ o estrattiva. La prova finale prevede un esame pratico e la discussione di una tesi su tematiche pertinenti al Master.

IL PA (PATHOLOGY ASSISTANT): SVILUPPO PROFESSIONALE ED ORGANIZZATIVO

M. Cadei

Lo sviluppo professionale delle figure tecnico-sanitarie (nuove competenze), è uno degli argomenti che ha maggiormente caratterizzato, negli ultimi anni, il mondo della sanità.
La costituzione dell'Ordine delle Professioni Sanitarie (Legge 3 del 2018) e la Responsabilità Professionale (Legge 24 del 2017) sono i due maggiori aspetti entro i quali si incardina una professione, quella del Tecnico Sanitario di Laboratorio Biomedico (Tslb), che con l'acquisizione del titolo di studio universitario, ha innalzato la propria preparazione professionale e scientifica.
Nel Laboratorio di Anatomia Patologica, la figura dell'Assistente Patologo (in italiano) o Pathology Assistant (in inglese), è oggetto di discussione da alcuni anni ma, nell'ultimo periodo in particolare, ha subito una spinta decisiva, in conseguenza dell'istituzione dei Master specialistici in alcune università italiane.
Ciò a portato ad attuare, all'interno di alcuni Laboratori di Anatomia Patologica, scelte organizzative con Tecnici di Laboratorio che hanno acquisito questo titolo post-laurea di I°livello.
AITIC, Associazione Italiana dei Tecnici di Istologia e Citologia, che dal 2002 si occupa di formazione scientifica e professionale, da molto tempo discute al proprio interno, dell'acquisizione di nuove competenze, da parte dei Tslb, con il relativo riconoscimento professionale.
Ciò che però ha sempre contraddistinto il pensiero dei componenti il Consiglio Direttivo è la tutela dei professionisti dal punto di vista legale e normativo e lo svolgimento di attività di laboratorio, con adeguata preparazione e formazione.
Questi due aspetti, a nostro avviso molto rilevanti, hanno trovato riscontro anche nei risultati di un "questionario" distribuito ai partecipanti al Corso Nazionale AITIC del Maggio 2019, dove è stato confermato il pensiero della nostra associazione.
Nel rimarcare la nostro disponibilità al dialogo con i Patologi abbiamo, di buon grado, accolto di confrontarci in una sessione "speciale" del Congresso Triennale SIAPEC, dove potranno essere messi in evidenza i punti di forza e le criticità di una "nuova competenza" che deve trovare il giusto riconoscimento (giuridico ed economico) in una quotidianità che presuppone aspetti organizzativi e gestionali nei laboratori di Anatomia Patologica di tutta Italia.

Giovedì, 17 ottobre 2019

Sala Parigi – 15:00-16:20

DERMOPATOLOGIA

Patologia melanocitaria

Moderatori: C. Clemente, A. Cassisa

TC NEL LINFONODO SENTINELLA: CARATTERISTICHE DEL MELANOMA PRIMITIVO E FOLLOW-UP

A.M Cesinaro

Introduzione. Il significato clinico di poche cellule positive ai marcatori melanocitari nel linfonodo sentinella (SLN) del melanoma è stato oggetto di dibattito. I dati di sopravvivenza sembrano confermare l'impostazione in base alla quale il SLN va considerato negativo qualora tali cellule non mostrino le caratteristiche morfologiche del melanoma primitivo; al contrario, in presenza anche di una sola cellula con le caratteristiche di melanoma, il SLN è da considerarsi positivo [1 2].

Materiali e metodi. Abbiamo estratto dal nostro archivio di Anatomia Patologica dell'Azienda Ospedaliera di Modena tutti i casi di pazienti sottoposti ad asportazione di SLN per melanoma, esaminati nel periodo dall'1/1/2016 al 30/6/2019. Il protocollo istopatologico da noi adottato è sovrapponibile a quello proposto recentemente da Cook et al. [3], con la differenza che lo step a 50 micron è applicato a tutti i linfonodi, qualunque sia il loro diametro, mentre il marcatore immunoistochimico preferenziale è il "melanoma cocktail". Su un totale di 173 pazienti trovati, quelli con SLN negativi sono stati 129 (74,5%); di questi, 19 (11% del totale) presentavano nevi capsulari, uno mostrava abbondante deposito di pigmento da tatuaggio, e un altro era sede di linfoma follicolare. I pazienti positivi sono risultati 44 (25,5%). Dopo aver adottato un cut-off di 200 µ per il *tumor burden*, 16 SLN (9,2%) risultavano avere cellule tumorali (TC) singole o in cluster < 200 µ, mentre 28 (16,2%) avevano depositi metastatici > 200 µ (diametro minimo riscontrato: 500 µ). Sono stati considerati in dettaglio le lesioni primitive e i SLN dei 16 pazienti con *tumor burden* < 200 µ.

Risultati. I 16 pazienti (9 maschi e 7 femmine) erano di età compresa fra 19 e 79 anni (media: 52 anni); una paziente aveva meno di 20 anni ed un altro meno di 30 anni. Sei lesioni erano localizzate sul tronco; 5 sull'arto inferiore, di cui 2 sul dorso del piede; 2 sull'arto superiore; 3 in sede acrale (1 sulla pianta del piede, 1 sul tallone e 1 sul palmo mano). Lo spessore di Breslow variava fra 0,9 e 9 mm (media 2,5 mm; mediana 2 mm). L'ulcerazione era presente in 6 casi. La stadiazione patologica è stata la seguente: pT1b: 2 casi; pT2a: 3; pT2b: 2; pT3a: 3; pT3b: 3; pT4b: 1; in due casi il primitivo non era disponibile. La morfologia dei 14 melanomi primitivi disponibili era di tipo epitelioide (10 casi), spitzoide (1 caso), nevoide (1 caso), DPN-like (1 caso), blu epitelioide-like (1 caso). La caratterizzazione biomolecolare (BRAF ed NRAS) è stata eseguita su materiale appartenente a 10 pazienti (9 melanomi primitivi, 1 metastasi pleurica): 5 casi sono risultati wild-type, due casi avevano la mutazione di BRAF p.V600E ed uno di BRAF p.V600K, due casi avevano la mutazione di NRAS p.Q61L. Le caratteristiche delle TC nel SLN sono state le seguenti: una singola cellula in 2 casi, una in sede sub-capsulare e una in sede parenchimale; 8 casi con un cluster in sede sub-capsulare, un caso con due clusters in sede sub-capsulare; 4 casi con un cluster in sede parenchimale, un caso con due clusters in sede parenchimale. La morfologia delle cellule era sovrapponibile a quella del tumore primitivo. In 4 casi coesisteva un nevo capsulare. Tutti i pazienti hanno avuto un ampliamento della sede del melanoma primitivo. Cinque pazienti (31%) sono stati sottoposti a svuotamento linfonodale loco-regionale, risultato negativo. Un paziente ha sviluppato un adenocarcinoma prostatico a distanza di 12 mesi dal melanoma, un altro ha sviluppato un secondo melanoma, mm 0,3 di spessore di Breslow. Due pazienti risultano persi al follow-up. Per i restanti 14 pazienti, il follow-up va dai 4 ai 39 mesi (media 19,5 mesi). Tre pazienti su 14 (21%) sono andati in progressione di malattia, dopo 3, 12 e 16 mesi. Una paziente di 19 anni è deceduta per melanoma metastatico dopo 20 mesi, due pazienti maschi, di 70 e 74 anni, sono vivi con malattia metastatica dopo 16 e 29 mesi rispettivamente; gli altri sono vivi, senza recidive linfonodali o progressione di malattia. Dei 129 pazienti con SLN negativo, il follow-up era disponibile in 113. Di questi, 5 (4%) sono andati in progressione di malattia a distanza di 5, 14, 15, 20 e 23 mesi, e sono tuttora vivi. Dei 28 pazienti con SLN positivo (minimo *tumor burden:* 500 µ), il follow-up era disponibile in 26. Di questi, 5 (19%) sono andati in progressione di malattia a distanza di 3, 7,11, 20 e 32 mesi, e sono tuttora vivi.

Conclusioni. Con i limiti legati ai piccoli numeri della casistica ed al breve follow-up, i nostri dati mostrano come la presenza di TC nel SLN abbia un significato clinico. Considerando un *tumor burden* < 200 µ, 3 casi su 14 pazienti sono progrediti, con anche un decesso. I tre casi, tutti a morfologia epitelioide, e ulcerati, erano i primi due in stadio pT3b e pT4b, con rispettivamente uno e due clusters di TC in sede sotto-capsulare, il terzo (la paziente deceduta) era in stadio pT2b, e aveva un unico piccolo cluster di TC in sede parenchimale. A parità di follow-up, la differenza nelle percentuali di progressione di malattia fra i casi con SLN negativo e quelli con SLN con TC è risultata significativa (4% vs 21%, $p < 0,05$). La percentuale di casi in progressione con SLN positivo è risultata lievemente inferiore rispetto ai SLN con TC, ma il dato potrebbe spiegarsi con il follow-up relativamente breve, che non permette, per giunta, di estrapolare dati di sopravvivenza, da confrontare con quelli riportati in letteratura [4].

Vi sono aspetti ancora controversi relativi all'interpretazione delle TC nel SLN, legati essenzialmente alle caratteristiche del primitivo. Laddove una morfologia epitelioide può non dare adito a dubbi interpretativi, una morfologia nevoide risulta di più ardua valutazione. È ben nota, inoltre, la discussione sul significato biologico della localizzazione metastatica nei primitivi spitzoidi. Anche lesioni con pattern DPN o simil-blu, che abbiano un'attività mitotica ed una atipia citologica tali da non poter rientrare tout court nello spettro

delle lesioni sicuramente benigne [5], possono avere TC nei SLN di altrettanto problematica interpretazione. La relativamente bassa frequenza di questo tipo di lesioni e la non ancora completa conoscenza del loro comportamento biologico, da una parte suggeriscono un atteggiamento prudenziale nella gestione clinica del paziente, dall'altra rendono auspicabile la condivisione, fra patologi, di casistiche con lungo follow-up.

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Patologia infiammatoria e linfoproliferativa

Moderatori: C. Clemente, A. Cassisa

SKIN IMMUNE SYSTEM: ASPETTI DI FISIOPATOLOGIA APPLICATA ALLA DIAGNOSTICA

A. Cassisa

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Skin is the larger human organ and immune homeostasis is one of its main function.

Although histological examination of normal skin does not account for the complexity of the lymphoid component, skin resident lymphocytes represent a very large component of the immune system accounting for about 20 billion of cells, double the number of blood circulating compartment.

Parabiotic animal models and monoclonal antibodies blocking specifically circulating lymphocytes have contributed to better characterize and understand the cutaneous resident compartment.

Lymphocytes interact with several professional and nonprofessional immune cells. Langerhans cells, macrophages, dendritic histiocytic cells among the professional and endothelial cells, keratinocytes, dermal fibroblast in the second group.

Resident cutaneous lymphocytes represent a heterogeneous population. Dynamic phenotypic and functional switching can occur in response to a wide range of molecular triggers. Bacteria and lipid metabolism influence immune homeostasis by a sensing mechanism mediated by glycolipid and lipid-specific presenting molecule. CD1a, a lipid presenting molecule, is typically expressed by Langerhans cells. They are an unique type of histiocyte in that originate from yolk sac and fetal liver in the transition period from hematologic to metabolic organ. Rhythmic expansion of LC dendrites patrols epidermal microenvironment. Antigen encountering makes them maturie from "antigen processing cell" to "antigen presenting cell" skilled to migrate to lymph nodes where activate naïve T cells. Functional polarization it's part of this process so dendritic cells are the first that modulate T cell response. In spongiotic dermatitis Langerhans cell hyperplasia is sustained by recruitment of blood borne histiocytes whose function differs from native Langerhans cells. Langerhans cell dependent T lymphocyte activation is not a so rapid and efficient mechanism of protection. Other cellular players take part in cutaneous immune responses leading relevant implications in the pathogenesis of inflammatory and neoplastic skin diseases. Resident lymphocytes, among others, are involved in both acquired and innate mechanisms. Most of their functions is still unexplored.

A brief overview of the cutaneous resident lymphoid population will follow.

- T resident memory (TRMs) have TCR-specific rearrangement for antigenic epitopes already encountered. Despite their sessile properties due to the expression of CD103 and CD69a, they maintain a certain grade of recirculation. Sharp-edged lesions and recurrence in the same place as see in Psoriasis, mycosis fungoides and fixed drug eruption, correlate with their residency properties. TRMs rapidly activate and expand following antigen re-challenged without the intervention of circulating lymphocytes. Fixed drug eruption represents an interesting model in this respect. CD8+ TRMs play a relevant role in maintain long-term tumor dormancy. Targeting TRM cells in melanoma may be a therapeutic strategy to induce tumor immune equilibrium.
- TRMs expressing CD4 coreceptor are involved in mycosis fungoides pathogenesis although it is not clear if the neoplastic transformation takes part before or after they take residency. Sezary syndrome, although morphological overlapping, originates from circulating central memory lymphocytes.
- NK (natural killers) lack TCR and represent the most stable residential category as their number remains almost unchanged for all life. There are regulated by a lot of inhibitor and activating signals that stop or initiate immune response against cancer and virus infected cells. Recently attention has raised about their potential use in immunotherapy against melanoma.
- LTi (lymphoid tissue inducers) arise from fetal liver and are involved in tertiary lymphoid follicles formation sustained by Th17. It hasn't yet been delineated their role in the formation of reactive organized lymphoid structures in pathologies such as: lymphocytoma cutis, infective conditions, autoimmune diseases.
- ILC (Innate-like lymphoid cells)/ helper-like ILCs express a TCR with limited rearrangement characteristics (invariant) include: iNKT (invariant Natural Killer T) and MAIT (Mucosal associated invariant T) These categories are activated by glycolipids presented respectively by MHC-like molecule CD1d and MR1 .

iNKT are detected in great numbers in lesional skin of psoriatic patients, direct interaction with CD1d bearing keratinocyte induce epidermal proliferation a pathological diagnostic clue. Invariant T seem to have a role in allergic contact dermatitis.

- yδT express a peculiar TCR with an extended extracellular portion that can interact with specific epitopes as well as with cytokines in the microenvironment. Specifically, tumor microenviroment modulates functional balance between tumor promoting and tumor rejecting yδT functional subtypes.

Functional shifting makes it difficult to understad resident T lymphocytes role in every contest. Fortunately, from a practical point of view, different lymphoid subsets are regulated by common transcription factors activators (ie T-Bet, GAta3, RORyt..) that favor the production of a prevalent cytokine. For example, IL17, one of the therapeutic targets of psoriasis, is produced by both TRMs and iNKT, both regulated by RORyt transcription factor. A specific cytokine also correlates with histological pattern (ie: epidermal hyperplasia, acanthosis, microabscesses, etc…).

On the contrary an inflammatory pattern may indicate a specific inflammatory pathway.

In conclusion, an imbalance of skin resident lymphocytes has relevant implications in both inflammatory and neoplastic conditions so it deserves to be intensely investigated.

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L'importanza della correlazione clinico-patologica: short cases

MULTIPLE MUCOSAL EROSIONS MASQUERADING PARANEOPLASTIC PEMPHIGUS IN A 48-YEAR-OLD WOMAN

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Background and objectives. Paraneoplastic pemphigus (PNP) is an autoimmune muco-cutaneous variant of pemphigus associated with an underlying neoplasm, most frequently hematopoietic, and mostly occurring in patients in their fifth to eight decades. The minimal criteria generally accepted for diagnosis include: clinical features of severe and persistent stomatitis with or without polymorphic cutaneous eruptions, histological features of acantholysis and/or interface changes, presence of anti-plakin autoantibodies, and demonstration of an underlying associated neoplasm [1-4]. Immunofluorescence is a useful diagnostic tool for the diagnosis of PNP. In direct immunofluorescence (DIF) of the mucocutaneous lesions, IgG autoantibodies and/or complement deposits are observed in the intercellular spaces and/or along the basement membrane region [4]. Serum autoantibodies can be detected by indirect immunofluorescence (IIF) assays using human skin, monkey or guinea pig esophagus, or other substrates, including rat bladder, myocardium, and lung. In particular, the bladder is rich in plakins such as envoplakin, periplakin, and desmoplakins, but lacks desmogleins. Therefore, despite its relatively low sensitivity (86%), IIF using rat bladder is highly specific (98%) to discriminate PNP from other variants of pemphigus that do not harbor anti-plakin autoantibodies [4 5]. The most widely used treatment for PNP is systemic corticosteroids which can be combined with other immunosuppressive drugs [6 7]. However, the clinical efficacy of combination therapy and prognosis largely depend on the underlying malignancy [8].

Herein we describe a case of paraneoplastic mucosal pemphigus occurring in a 48-year-old woman with a history of colon and breast carcinoma.

Methods. The patient presented with a 5-year-history of painful erosive lesions localized on the lips, buccal mucosa, gingiva, tongue and in the vulvo-vaginal area. She was diagnosed with colon carcinoma and breast carcinoma 3 and 8 years before, respectively.

Results. A diagnosis of Behçet disease was initially rendered in another Institution and anti-TNF-alfa and corticosteroids were administered. ELISA for anti-desmoglein antibodies was negative. A first mucosal biopsy showed a complete mucosal disepithelialization with underlying band-like lymphocytic infiltrate which led to the diagnosis of erosive lichen. Hydroxychloroquine and systemic corticosteroids led to the worsening of the clinical picture. Subsequently two biopsies of the oral mucosa were performed showing a focal area of suprabasal acantholysis with cleft formation and intense lichenoid lympho-histiocytic and plasmacellular infiltrate in the chorion and basal vacuolar interface changes, respectively. At the same time DIF demonstrated intercellular IgG deposits, whereas C3 was found on the lower layers. IIF on rat bladder showed intercellular IgG antibodies, while ELISA was positive for anti-envoplakin autoantibodies. The association of histological, immunopathological and clinical features led to the definitive diagnosis of PNP. Intravenous immunoglobulins and rituximab were administered with a partial clinical remission 26 months after the diagnosis.

Conclusion. An accurate clinical history and clinical-pathological correlation is mandatory when dealing with bullous diseases. Paraneoplastic pemphigus is a rare variant of pemphigus with a peculiar clinical and immunopathological setting. Confirmatory immunofluorescence studies are required to render a correct diagnosis.

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DERMATOSI PUSTOLOSA EROSIVA DEL CUOIO CAPELLUTO: UN CASO

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Riportiamo il caso di una donna di 80 anni giunta all' osservazione per la comparsa da qualche mese di un'ampia area alopecica del cuoio capelluto, con focolai di erosione ed essudazione purulenta.
In anamnesi la donna riferiva l'asportazione di un carcinoma basocellulare, precedentemente alla comparsa della lesione.
Le indagini microbiologiche per la ricerca di batteri e miceti erano risultate ripetutamente negative.
L'esame istologico mostrava un denso infiltrato infiammatorio dermico "a banda", costituito da linfociti e plasmacellule, con granulociti neutrofili ed alcune cellule giganti da corpo estraneo e focali aspetti di scollamento dermo-epidermico. Si associava inoltre una scomparsa pressoché totale dei follicoli piliferi con persistenza dei muscoli erettori del pelo e miniaturizzazione dei follicoli residui.
L'immunofluorescenza diretta aveva dato esito negativo.
Sulla base dei dei dati anamnestici, clinici ed istopatologici, è stata formulata la diagnosi di dermatosi erosiva pustolosa (DEP) del cuoio capelluto, una rara affezione infiammatoria ad eziologia sconosciuta, che colpisce preferenzialmente donne di razza caucasica, di età avanzata, caratterizzata dallo sviluppo di pustole, erosioni superficiali e croste, che esitano in un'alopecia cicatriziale. Sebbene l'eziopatogenesi sia sconosciuta, i fattori che sembrano correlarsi a questa dermatosi sono l'atrofia cutanea e/o un trauma.
La diagnosi differenziale si pone nei confronti di varie patologie, tra cui cheratosi attinica, infezioni batteriche o fungine, pioderma gangrenoso, dermatite factitia, pemfigo foliaceo, pemfigoide cicatriziale, psoriasi pustolosa.
La diagnosi di DEP implica necessariamente la correlazione anatomo-clinica.
Il trattamento si basa sull'utilizzo di corticosteroidi topici ad alta potenza o di tacrolimus.

Giovedì, 17 ottobre 2019

Sala Roma – 14:00-18:30

PATOLOGIA FETOPLACENTARE

L'autopsia fetale e neonatale: anomalie, varianti e malformazioni vascolari

Moderatori: E. Fulcheri, L. Resta

APPROFONDIMENTO DELLA TEMATICA SU 7 SETTORI CORPOREI SPECIFICI QUALI: TESTA/ CRANIO

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Sixty percent of vascular anomalies (including vascular malformations and vascular tumors) in children are found in the head and neck area. These lesions can present throughout antenatal, perinatal and childhood development and can present as simple/isolated, combined and those associated with other anomalies in the context of genetic syndromes.
It is fundamental that the pathologist must be able to recognize these entities both in the foetal/neonatal/pediatric autopsy and in the surgical pathology activity.
In the autoptic practice the vascular malformation could be complicated by the presence of haemorrhage or massive thrombosis, especially in the brain; despite these confounding factors the pathologist must identify the anomalies causing the haemorrhage/thrombosis, finding that is very difficult to recognize radiologically in these cases.
In the head/cranial/brain area the most frequent vascular anomalies are: infantile hemangioma, congenital hemangioma, tufted angioma, arteriovenous malformations, venous malformations, lymphatic malformations. There are a variety of vascular malformations of the central nervous system (CNS). Some of these are aggressive, high flow lesions and have a risk of hemorrhage or other complications; others behave in a more benign manner. Knowledge of the imaging findings of these lesions, along with the findings that might indicate which ones may hemorrhage or have other associated poor outcomes, aids in making treatment decisions.
Major syndromes associated with vascular malformations are A) Associated with low flow malformations: Sturge-Weber syndrome, Klippel-Trenaunay syndrome, Proteus syndrome, Cutis marmorata telangectasica congenita, Adams-Oliver syndrome, Bean syndrome, Maffucci syndrome, Gorham-Stout disease; B) Associated with high flow malformations: Parkes Weber syndrome, Klippel-Trenaunay syndrome, Rendu-Osler-Weber Syndrome, Wyburn-Mason syndrome, Cobb syndrome, Cowden syndrome, Ehlers-Danlos syndrome, CLOVES syndrome, Bannayan-Riley-Ruvalcaba syndrome.
Anatomopathologic diagnosis of these vascular anomalies is also supported today by genetic tests, particularly those studying the mutational status of PIK3CA.

Vascular anomalies can compromise vital organs such as the airway or the liver, lead to cardiovascular compromise, or put the fetus at risk for coagulopathies. The possibility of complications from these lesions emphasizes the importance of their detection in utero. In fact, accurate prenatal diagnosis can improve prenatal care and neonatal outcome.
To make the best correlation the pathologis must dispose of all the radiologic images and reports performed on the patient, including postmortem in utero MR.

TRATTO SOVRAORTICO/COLLO

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Venous vascular malformations of the upper thorax and neck region are rare, while anomalies are more common and possible anatomical variants are even more frequent. Moreover, anatomical variants of the venous circulation are known to be generally more frequent than those of the arterial circulation (interpersonal variants).
The classification of venous vascular malformations of the systemic circulation comprises:

- Persistent left superior vena cava;
- Absence of inferior vena cava;
- Venae cavae entering the left auricle;
- Levoatrial cardinal vein.

The classification of venous vascular malformations of the pulmonary circulation comprises:

- Total anomalous pulmonary venous return;
- Partial anomalous pulmonary venous return.

Malformations involving the superior vena cava and the brachiocephalic and jugular branches are relatively few. By contrast, anomalies of venous return, in the sense of venous branches displaying anomalies of outlet site, pathway or calibre, are more frequent and less exhaustively classified. Some of these constitute situations of compensation for an anomalous arterial circulation.
To understand venous vascular malformations, we need to refer to the structures that form earliest in the chest. This means briefly running through the fundamental stages of this process.
The cardinal veins constitute the main system of venous drainage in the embryo. Symmetrically, on the right and left, the anterior cardinal veins drain the cephalic portion, while the posterior cardinal veins drain the remaining part of the body. Medially, the supracardinal veins are located postero-medially to the cardinal veins, and constitute the system of drainage of the intercostal veins. The anterior and posterior cardinal veins unite to form the common cardinal veins. Subsequently, a long arciform anastomosis forms between the anterior cardinal veins; this is a pre-requisite to the formation of the left brachiocephalic vein, so that most of the venous blood from the left side of the head and the left upper extremity of the body is channelled towards the right.
The superior vena cava is formed from the right common cardinal vein and the proximal portion of the right anterior cardinal vein. The distal portion of the right anterior cardinal vein will subsequently constitute the right brachiocephalic trunk. The subclavian vein and the external and internal jugular veins will flow into the brachiocephalic trunk.
The terminal extremity of the left posterior cardinal vein, which drains into the brachiocephalic trunk, is conserved as a small vessel, which will constitute the left superior intercostal vein. The distal portion of the left anterior cardinal vein and its branches will constitute the left subclavian and cervical venous system.
With the obliteration of some of the posterior cardinal veins, the supracardinal veins become more important. The right intercostal veins from IV to XI will empty into the right supracardinal, which, together with part of the posterior cardinal vein, will form the azygos vein. On the left, the intercostal veins from IV to VII will empty into the left supracardinal vein, which, under the name of hemiazygos vein, will empty into the contralateral azygos vein through an anastomosis.
In the fetus, the main venous system can be deemed to be structurally complete from week XIII onwards, though its components will obviously be more difficult to distinguish in the earlier gestation periods.
In an autopsy examination aimed at detecting venous vascular malformations, it is essential to adopt a correct methodological approach. Indeed, it is common experience that hurried evisceration and routine ablation of the body of the sternum makes it impossible to carry out proper inspection, not so much of the superior vena cava as of the brachiocephalic trunk and branches.
In the last three years, we have implemented this technique in 62 out of a total of 307 autopsies, excluding very early gestational ages (13-15 gw) and subjects with major macerative alterations due to protracted retention in the uterus.
Some of our case records concern malformations already identified echographically in the prenatal period, but which required autopsy confirmation; others concern ex novo cases without prior echographic study.
The complex process by which the venae cavae develop explains the great variability of their smallest branches and the relatively frequent deviation from the normal pattern of development.

ZONA MEDIASTINICA

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All the vascular lesion of the mediastinum can be classified according the Mulliken and Glowacki classification in two categories based on endothelial cells characteristics: hemangiomas and vascular malformations. This classification was updated in 1996 into vascular tumors and malformations, the last group including simple (namely capillary, lymphatic, venous, arteriale) and combined forms.
Vascular malformations are structural abnormalities that may include any combination of capillary, arterial, venous and lymphatic components. Mediastinal vascular malformations are infrequently seen in clinical practice. Most of them are asymptomatic or may present with symptoms secondary to mass effect or compression of the neighbouring organs. We can distinguish abnormalities of the arterial compartment, mostly expression of defect of embryogenesis and frequently associated with congenital heart diseases and venous vascular malformations that are the most common type of vascular mal-

formation seen in clinical practice. The following is a list of anomalies and variants subdivided as: a) aortic arch anomalies (including aberrant right subclavian artery, innominate artery compression syndrome, right arch mirror image, double aortic arch and aortic coarctation); b) anomalies of pulmonary arteries (agenesis, sling, patent ductus arteriosus); c) anomalies of pulmonary veins (partial anomalous venous return, "scimitar" syndrome); d) anomalies of systemic veins (left superior vena cava, azygos continuation of IVC). The aberrant right subclavian artery or "arteria lusoria" is the most common anomaly of the aortic arch, occurring in 0,5 to 2,5% of individuals and it is the first arch anomaly to have been described. It is usually asymptomatic and it is often discovered in course of evaluation of other mediastinal anomalies. In a percentage ranging from 68 to 91% is associated with cardiac anomalies as septal defects, left-sided cardiac lesions and cono-truncal anomalies. In innominate artery compression syndrome the brachiocephalic (innominate) artery is located more to the left and may compresses the trachea anteriorly; the right arch mirror image is a mirror-image variety of the left arch, mostly asymptomatic and associated with congenital heart disease in 98%, mostly tetralogy of Fallot. The double aortic arch forms a complete ring that encircles esophagus and trachea.

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Approfondimento della tematica su 7 settori corporei specifici quali:

TRATTO VENO-PORTALE

V. Toto

Anatomia Patologica, ASST Santi Paolo e Carlo, Milano

The improving of ultrasound technique with Doppler modalities has opened a new era of studying fetal circulation. Increasing number of physiological data add important information about fetal blood distribution in fetal compartments and the role of the shunts, in particular ductus venosus (DV), showing substantial individual variations and important changes with gestational age.
In the fetus there exists a unique situation, in which three venous systems, umbilical, portal and DV, form
a functionally inseparable blood vessel, conveying the highly oxygenated blood from the placenta into the left atrium. The characteristic high-velocity blood flow of the DV prevents mixing with the low-oxygen blood flow from the inferior vena cava (IVC) and the hepatic veins. Each of these three venous components can be absent, hypoplastic or displaced, causing shunting into the systemic veins.
A whole integrated approach to fetal circulation, besides a deep knowledge of congenital anomalies of the portal venous system, is essential not only for proper prenatal diagnosis in a correct surgery planning, but also to understand some unexplained fetal condition leading to intrauterine growth retardation (IUGR) or even to stillbirths. For instance fetus with ductus venousus atrophy could have a vulnerability when facing hypoxemic states and it can be also the primary cause of fetal hypoxia as the obstruction of the placental venous flow return can result in impaired gas exchange and placental edema.
Furthermore, although being a rare group of malformation, they can frequently represent only the tip of the iceberg of a syndrome or a genetic condition, considering that DV blood flow evaluation is today systematically performed in the first trimester screening for aneuploidies and has become part of the daily clinical practice.
In this new contest, the pathologist has gained an essential role: besides discovering, studying and classifying the different malformation of the portal venous system, he has to face clinician's doubts in order to answer crucial question about IUGR or intrauterine fetal death and to guide genetic counseling.

ZONA DEL TRIPODE CELIACO

M. D'Armiento

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For decades, arterial variations of the abdominal aorta have attracted the attention of anatomists and radiologists due to their prominent significance in surgical specialties. The first description of normal and aberrant celiac trunk (CeT) anatomy was published in 1756 by Haller. Lipshutz seems to have been the first who suggested a classification of the celiac trunk into four types. Later, Adachi presented a more detailed classification, while the two most commonly used classifications
were proposed by Morita and Michels.
The celiac trunk (CeT) is the major branch of the abdominal portion branches of the aortic artery and, together with the superior mesenteric and inferior mesenteric arteries, participates in the abdominal viscera vascularization through a series of anastomoses. It usually originates slightly below the diaphragm opposite the intervertebral disk T12/L1. Normally it supplies abdominal portion of the esophagus, the stomach and the upper duodenum, the liver, the gallbladder and the extrahepatic biliary tracts, the spleen and the majority of the pancreas. Anatomical variants of the CeT and its further subdivisions were the object of numerous morphological and dissection studies. Its length varies from 15-30 mm. The most frequent branches are: common hepatic, splenic and left gastric. Through years numerous studies reported significant variations of this vessel, i.e. in the form of double, triple or quadruple branching pattern with some unusual arteries which originate from it [1] and also the absence of CeT, these variation may have implications in surgical approaches and thus they should be taken into consideration when planning surgical interventions on the abdominal part of the esophagus, stomach,duodenum, liver, pancreas, gallbladder and spleen and for diagnostic and interventional radiologists. Despite the short length of the CA, it is affected by a wide range of pathologic conditions, including mesenteric ischemia due to intrinsic occlusion (secondary

to causes such as atherosclerosis or thromboembolic events) and extrinsic compression from masses or the median arcuate ligament,
(MAL) know as Dunbar syndrome (abdominal pain, epigastric bruit, and weight loss) a rare disease resulting from compression of the celiac axis by fibrous attachments of the diaphragmatic
crura, the median arcuate ligament [2].
Half the population having a variation from the classic pattern of the CeT bifurcating into the hepatosplenic trunk and left gastric artery .
In children the most frequent variation in the anatomy of CeT is observed in the branching of the hepatic artery [3].
Understanding CeT and hepatic artery anatomy is important not only in preventing iatrogenic injuries but also in planning surgical procedures in children. Five types of CeT variations were identified according to Song's classification in which 'hepatosplenic trunk + left gastric artery + superior mesenteric artery' was the most prevalent. One hundred-twelve (64.4%) of the 174 patients had normal CHA anatomy; however, 62 (35.6%) had variations. Six types of CHA variations were identified according to Michel's and Hiatt's classification. The most common was "replaced left hepatic artery originating from left gastric artery" [2 3]. Together with many other arterial anomalies, can be found celiac trunk stenosis in rare sindromic form: The Alagille syndrome (autosomal dominant, rare, multiorgan genetic disease caused by genetic mutations arising from the Notch signaling pathway: JAG1 and NOTCH 2) and some forms, very rarely, of atresia of the bile ducts. Liver transplantation is needed in children with Alagille syndrome and some forms of atresia of the bile ducts [4].
Understanding the coeliac trunk (CeT) and hepatic artery anatomy is important not only in preventing iatrogenic injuries but also in planning surgical strategies [1 2.]
The pathologis role is very important to describe the variation of celiac trunk by autoptic study. Indeed numerous studies about variations in the anatomy of the celiac trunk have been carried out also with the purpose of proposing a new classification on this topic [2 3].
The pathologis role is very important to knowledge of the different anatomic permutations ,essential to guide the interventional radiologist for endovascular procedures, such as hemorrhage control, transarterial interventional therapy.
The pathologist role is very important to describe the possible variants of CeT anatomy at autopsy and, also, to diagnose the syndromic forms.

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ARTI SUPERIORI E INFERIORI

V. Nardini

Congenital limb anomalies/malformations associated with vascular anomalies may be related to different conditions: genetic, environmental, drugs, etc. The phenotype of such anomalies are more often of reduction type (terminal transverse defects and terminal longitudinal defects) and may be unilateral or bilateral. These anomalies/malformations may occur in isolated forms or in association with other malformations or may be part of a syndrome. In the last 150 years, more than fifty attempt of classifications were proposed.
In general, limb defects due to embryo-fetal vascular anomalies result in disruptions and in structural anomalies (reduction defects, duplication defects, dysplasia). Vascular disruptions, intervening during embryogenesis (mostly between the IVth and VIIIth week of gestation, when limb bud emerges end outgrowhs from the flank of the embryo) may be the result of fail of the normal vascular limb tree or by the compression by an hematoma or by hypoperfusion (emboli, thrombi, hemorrhagic necrosis of tissues), also related to uterine-placental, placental-fetal unit or fetal unit blood flow.
Several conditions may cause vascular disruptions: genetics (chromosomal aberrations, single gene disorders of genes involved in limb development), teratogenic (drugs, toxics), environmental (maternal illness such as hyperthermia, diabetes, infections or pregnancie-associated conditions like TRAP syndrome).
The rule of a complete and exhaustive fetal autopsy is to describe the specific anatomic vascular limb anomalies (isolated, in association or part of a syndrome) in order to detect the cause of the anomaly for prevention purposes (environmental, teratogenic ethiology) or for risk estimation (genetic counseling) for later siblings.

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Giovedì, 17 ottobre 2019

Sala Atene – 14:00-18:30

PATOLOGIA ULTRASTRUTTURALE E NEFROLOGICA

Corso base di nefropatologia Parte II

Introduzione: G. Monga - G. Mazzucco

METHODOLOGICAL APPROACH IN THE EVALUATION OF THE RENAL BIOPSY: THE BASIC LESIONS OF THE TUBULE-INTERSTITIAL DISEASE

L. Resta[1], M.e Rossini[2], D. Piscitelli[1], M.G. Fiore[1], R. Rossi[1]

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The renal tubule is composed by proximal tubule, loop of Henle, distal convoluted tubule, and collecting duct (embryologically independent from the nephron)
1st step: recognition of functional parts of the tubule. The proximal tubule has cylindrical cells, eosinophilic cytoplasm mitochondrial rich, evident brush border, PAS positive reabsorption droplets. The loop of Henle has thin and thick cell with different amount of cytoplasm and round nuclei. The distal tubule has pale cells smaller then the proximal one, without brush border. In the collecting duct the cells increase in length in the medulla. The normal interstitium is scarcely represented in the cortex and more abundant in the medulla. The vascular component is evident near the tubules.
2nd step: content of the tubule. Normally the tubular lumen is empty. In condition of acute tubular injury the lumen is occupied by granular eosinophilic protein material of cytoplasmic origin and occasional neutrophils. Other compact organized material as hemoglobin or myoglobin, bile pigment are an important key for diagnosis. In chronic situations, the condensation of pre-urine in the tubules forms a homogeneous eosinophilic material similar to the thyroid colloid (thyroidization).
3rd step evaluation of interstitium. Presence of neutrophils is an expression of acute reaction in several conditions: bacterial injury, drug allergy, acute transplant rejection and other. Lymphocytes are present in several immunologic condition (tubulitis). Lymphocytes C4d positive in peritubular capillaries are considered as characteristics of chronic humoral rejection. Giant cell granulomas are present in infections (mycobacteria and other agents), drugs, Crohn disease, sarcoidosis. Altered leukocytes and/or hematopoiesis mark a hemo-lymphoproliferative condition. Mineral deposits as referred in calcinosis and in urate nephropathy.
4th step: immunofluorescent deposits. Immunofluorescence may be useful in detecting intraluminal (light chains in gammopathy), intracellular (high reabsorption in proteinuria; antibodies in autoimmune diseases) deposits, and in studying basal membrane (autoimmune diseases and drugs).
5th step: electron microscopy. With ultrastructural examination we may consider the status of the tubular cell cytoplasm (endoplasmic organelles, microvilli, crystals), the presence of sub epithelial deposits, the aspect of basal membrane (thickness, laminations).
The final diagnosis is the result of the "cultural" evaluation of all these findings in comparison with the clinical parameters.

Interventi preordinati di diagnostica ultrastrutturale e nefropatologica

Moderatore: G. Cenacchi

THE ROLE OF ELECTRON MICROSCOPY IN THE DIAGNOSIS OF PLEURAL NEOPLASTIC DISEASES

F. Fortarezza[1], F. Pezzuto[1], M. Della Barbera[1], C. Giraudo[2], A. Bellini[1], F. Rea[1], F. Calabrese[1]

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Background. The introduction of immunohistochemistry and molecular pathology has progressively restricted the use of transmission electron microscopy (TEM) for diagnostic purposes, especially in the oncologic field. However, TEM should represent a useful adjuvant tool in challenging cases as in cases of malignant pleural mesothelioma (MPM). International guidelines[1] point out the usefulness of TEM in establishing the correct diagnosis when histological and immunohistochemical results are inconclusive. We describe two cases of malignant pleural mesothelioma in which electron microscopy was useful for a more correct diagnosis.

Case I. An unexposed, 69-year-old woman presented a right pleural effusion. She had a history of right breast cancer treated with a quadrantectomy with adjuvant chemo-radiotherapy followed then by right mastectomy for locoregional recurrence. Medical thoracoscopy showed significant pleural effusion and multiple visceral and parietal nodularities. Computed tomography scan confirmed the irregular pleural thickening of the right base. At histology a diffuse epithelioid growth pattern with occasional papillary structures was seen in the fragments. Immunohistochemistry showed weak positivity for calretinin, D2-40 and GATA3; while negativity for TTF1, GCDFP-15, BAP1, HER2 and progesterone receptor, and estrogen receptor positive in only 5% of neoplastic cell. The immunohistochemical findings were inconclusive. TEM was carried out using by formalin-fixed paraffin-embedded blocks. Ultrastructural analyses showed cells with fragmented and elongated microvilli without glycocalyx, giant desmosomes and perinuclear tonofilaments. Based on these findings the final diagnosis was MPM. The patient underwent a pleurectomy/decortication after adjuvant chemotherapy. The histological analysis of the surgical specimen confirmed further the diagnosis of MPM.
Case II. An unexposed, nonsmoker 41-year old man was admitted to the emergency room due to an accidental fall and tibial fracture requiring surgery. Pre-

surgical testing was unremarkable except for the chest X-ray that showed a large posterior, pleural-based, and well-defined opacity in the right hemithorax. The CT confirmed the presence of a large well-defined lesion with a broad base adherent to the posterior pleura at the level of the upper segment of the right lower lobe without any evidence of surrounding atelectasis or pleural effusion. The mass demonstrated a heterogeneous contrast enhancement due to a solid central portion and peripheral cystic and necrotic-hemorrhagic areas. According to the features at imaging and the unremarkable clinical history, the fibrous solitary tumor of the pleura and its malignant variant were initially listed among the differential diagnoses. The patient underwent a complete surgical resection of the tumor. The histological analysis diagnosed a localized malignant mesothelioma (LMM). The tumor showed epithelioid feature strongly positive immunostaining for cytokeratin (MNF116), calretinin, D2-40 and weak nuclear WT1. The LMM is an extremely rare pleural tumor and, up to now, little is known about its biological behavior and prognosis. To confirm this unusual diagnosis an ultrastructural examination was performed. The neoplastic cells showed the typical features of mesothelial origin (elongated microvilli, giant desmosomes, intermediate filaments) and confirmed the diagnosis of LMM.

Conclusion. The two cases presented a diagnostic challenge in defining a precise histotype of the pleural tumors. In both cases, for different reasons (inconclusive immunostaining data – case I – and unusual mesothelial tumor – case II), the ancillary use of this tool allows us to carry out a more precise diagnosis.

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Venerdì, 18 ottobre 2019

Sala 500 – 09:00-13:00

IMMUNOISTOCHIMICA

I Sessione

MICROFOLD CELLS AND M-CELL MARKERS IN NORMAL CONDITION AND DISEASE

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Microfold cells or "membranous" cells (M-cells), are a specialized epithelial component of the Mucosa-Associated Lymphoid Tissue (MALT). Historically, M-cells were identified in the follicle-associated epithelium (FAE) of the Peyer's patches (PPs) and are mostly represented in the distal tract of the ileum, but they have been reported, to a lesser extent, through the small and large bowel. It is presently well-known that M-cells also occur in other MALT outside the gut, such as the bronchus-associated lymphoid tissue and the nasal-associated lymphoid tissue, where they act as key cellular player of the immune microenvironment [1 2]. In particular, M-cells are able to bind many luminal antigens and microbes transferring them by transcytosis to the underlying lymphoid follicle tissue: here, dendritic cells and lymphocytes can initiate the antigen-specific mucosal immune responses and launch IgA production [3]. M-cells exert their function through various membrane receptors for bacteria (Vibrio cholerae, Campylobacter jejunii, Mycobacterium tuberculosis, Shigella spp., Salmonella spp., Escherichia coli, Yersinia spp.), viruses (MMTV virus, polioviruses, reoviruses and HIV) prions and parasites (Cryptosporidium), but they also bind and transport synthetic microparticles 4. Besides these specific receptors, M-cells express some pattern recognition molecules such as TLR-4 and TLR-2 and therefore they are widely interacting with microbiota [5].

It has been estimated that M-cells account for only 5-10% and 2% of the epithelial cells of the FAE in mice and humans, respectively 6. M-cells are not histologically detectable with Hematoxylin-and-Eosin staining: ultrastructural analysis was instrumental to clarify the morphological features [7]. At a variance with adjacent enterocytes, end-differentiated M-cells show scattered, short microvilli and the baso-lateral membrane displays the characteristic infoldings harboring antigen presenting cells and lymphocytes, which confer the "membranous appearance" to these cells [6].

The low amount and the lack of a universal marker hampered so far studies on M-cells across different species and different sites. Earlier, several approaches have been used for identifying molecules specifically expressed in M-cells ranging from classic cell cultures to organoids and in vivo study on mice. Recently, gene expression profiling and transcriptome analyses provided new insights on M-cell expression markers and transcription factors.

Like any other intestinal cell type, M-cells derive from Lgr-5+ epithelial intestinal stem cells, located at the lowest portion of the intestinal crypts [8]. M-cell differentiation, both in humans and animal models, is thought to be the result of the interaction between Lgr-5+ stem cells and RANKL expressing stromal-inducer cells [9]. The transcription factor Spi-B is of great importance for M-cell development and its expression is conserved among different species, although these cells can arise independently from Spi-B [8 10-12]. In a recent study, early activation of TRAF6 has revealed to be essential for M-cell lineage commitment, due to its role in activating both canonical and non-canonical NFkB signaling pathways and inducing the subsequent expression of M-cell markers: Marcksl 1 and Annexin 5 are the earliest expressed molecules and limited to the crypt; CCL-9, Tnfaip2 and GP2 are expressed later.12 The HMG-box family transcription factor SOX8 is strictly correlated to terminal differentiation of M-cells and specifically binds the GP2 promoter region, supporting terminal functional maturation and acquisition of antigen uptake and transcytosis capabilities [13].

Notably, M-cell differentiation could be induced also by extrinsic factors: in vitro and in vivo experiments showed a critical interaction with neighboring B lym-

phocytes. In mice, a CCL-20 chemotactic gradient produced by the epithelial cells of the FAE can recall B220+,CCR6hi,CD11cint,CD19+, IgM+,IgD+ B-cells, specifically residing in subepithelial dome of PPs, and induce M-cell development in a RANKL-independent way [14]. Moreover, human adenocarcinoma cell line Caco-2 have been reported to convert into M-like cells in the presence of Raji cells, a human cell line derived from transformed Burkitt lymphoma cells [15]. Taken together, the current view is that the main physiological pathway of M-cell differentiation is induced by RANK/RANKL axes, while the interaction with B cells seems to support M-cell differentiation or maintain differentiated M-cells [7].
In particular, GP2 expression is considered the marker of terminal M-cell differentiation and is actually one the most common immunohistochemical markers for identification of M-cells in human and mice [16]. GP2 (glycoprotein-2) is specifically expressed by zymogen granules of pancreatic acinar cells, where it was firstly described [17]. This glycoprotein shares also structural homology with the Tamm Horsfall protein, or Uromodulin, expressed in the thick ascending limb of Henlès loop [18]. In kidney, Uromodulin can bind E.coli enabling its clearance with the urine flux; likewise GP2 is a receptor for a subset of commensal and pathogenic fimbriated bacteria such as S. Typhimurium and E. coli [3].
About the possible role of M-cells in different pathologies, data are even more fragmented. Many intestinal pathogens may exploit in some cases properties of M-cells to invade the host and cause infections, as demonstrated in knock-out mouse models [11 19 20]. Moreover, some bacteria and inflammatory molecules can rapidly increase the number of M-cells, thus amplifying the interactions with the microbes [11 16].
A role for M-cells in the pathogenesis of inflammatory bowel diseases was suggested in a study using a mouse model of colitis in which a significant increase of colonic M-cells was observed in comparison with controls [21]. Although the precise function of these cells in pathogenesis or immune regulation is still not clear, another recent study showed a substantial remodeling of colon cellular composition in human ulcerative colitis: in this setting, an increase of M-cells was associated with the recruitment of immune cells to sites of inflammation both expressing several chemokines, like CCL20 and CCL23, and delivering antigens able to rewire Th17/Treg balance [22].
There are small data on the hypothetic neoplastic counterpart of M-cells, the so-called gastrointestinal-associated lymphoid tissue- or "dome"- carcinoma of the gut [23]. These rare carcinomas have a typical submucosal localization and have a significant lymphoid infiltrate with germinal centre formation which confers the dome-like appearance and are not associated with microsatellite instability, sporadic hypermethylation event or EBV infection. Even though the morphologic reminiscence of PPs and the putative origin from M-cells, there are still not convincing proofs on the real presence of these cells in dome carcinoma. In addition, no studies have been published yet about the occurrence of M-cells in reactive as well as neoplastic lymphoproliferations of gut-associated lymphoid tissues.
In conclusion, studies on M-cells are still hampered by their rarity and the lack of a universal marker for their specific identification. The possibility to improve our ability to recognize them by immunohistochemistry necessarily depends on the knowledge of origin and differentiation programs of these enigmatic cells. Thus actually, M-cells are one of the most intriguing and less known cell types which, for their properties, deserve more attention and study in the forthcoming years.

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II Sessione

IMMUNOHISTOCHEMISTRY IN ENDOMETRIAL HYPERPLASIA AND ENDOMETRIAL CARCINOMA: PROGNOSTIC STRATIFICATION

A. Travaglino

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Endometrial carcinoma (EC) is the most common gynecologic malignancy in the Western World. EC often arises from endometrial hyperplasia (EH). In both EH and EC, prognostic stratification is crucial in order to adopt an adequate treatment [1 2].

EH is a heterogeneous entity, which include both hyperplastic conditions reactive to estrogens and precancerous lesions. On this account, the 2014 WHO classification subdivides EH into "EH without atypia" (benign) and "atypical EH" (premalignant). However, differentiating between these two conditions is often difficult based on morphology alone. Several immunohistochemical markers have been studied to improve the differential diagnosis. Among these, PTEN has played a major role, as it is regarded as the key molecule of endometrial carcinogenesis. However, PTEN has repeatedly shown low sensitivity and low specificity in identifying premalignant EH [1]. Other molecules, such as Bcl-2 and β-catenin, appear highly specific but too little sensitive to be useful as stand-alone markers [3 4]. Overall, PAX2 seems to be the most accurate marker for differentiating between benign and premalignant EH [5]. On the other hand, ARID1A is a promising prognostic marker for the risk of occult cancer in atypical EH [6]. Predictive markers of response have also been assessed in EH conservatively treated; while several markers were found to be associated with the response (including estrogen and progesterone receptors), none of them showed sufficient accuracy to be determining in the clinical practice [7 8].

The prognostic stratification of EC is currently based on morphologic parameters such as tumor grade, histotype and myometrial invasion. However, this approach is likely to change in the near future. In fact, The Cancer Genome Atlas (TCGA) has shown that EC clusters into four prognostic molecular subgroups: *POLE* (with good prognosis), microsatellite-instability (MSI, with intermediate prognosis), copy-number-low (with good-to-intermediate prognosis) and copy-number-high (with poor prognosis). Immunohistochemical surrogates of molecular markers have shown acceptable costs and reliability to be used in the clinical practice. Aside from *POLE* assessment, which requires sequencing, the TCGA subgroups can be immunohistochemically identified with loss of mismatch-repair proteins (surrogate of MSI) and p53 overexpression (surrogate of copy-number-high) [2]. In addition, L1CAM overexpression has been identified as a further marker of poor prognosis, while nuclear β-catenin is under investigation as a possible surrogate of *CTNNB1* mutation (a marker of intermediate prognosis within the copy-number-low subgroup) [9]. Ongoing trials are assessing whether such a novel molecular classification can replace the current system for the risk assessment [10].

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Venerdì, 18 ottobre 2019

Sala Madrid – 09:00-13:00

PATOLOGIA MOLECOLARE

Slide Seminar

Innovazione nella predittività - Prima parte

Moderatori: G. Fontanini, R. Franco

TUMOR MUTATION BURDEN IN SOLID TUMORS

U. Malapelle

Department of Public Health, University of Naples Federico II.

To date, an innovative therapeutic approach for cancer patients is represented by immune-checkpoints inhibitors (ICIs) [1]. In fact, monoclonal antibodies targeting Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) and Programmed Death 1 (PD-1)/Programmed Death Ligand-1 (PD-L1) showed an increasing progression free survival and overall survival in different solid tumors respect to chemotherapy [2-4]. As for other target treatments, ICIs demonstrated their efficacy in a limited percentage of patients. For this reason, the identification of positive or negative predictive biomarkers is still necessary. In this setting, careful attention was paid in different cancer types (i.e., melanoma, head and neck malignancies, urothelial and renal carcinoma, metastatic colorectal cancer, and pancreatic cancer) to the immunohistochemical expression of PD-L1. On the overall, despite it represents an imperfect biomarker, PD-L1 showed a positive predictive value for ICIs administration in non small cell lung cancer (NSCLC) patients [5]. In addition to PD-L1 expression, other biomarkers are under investigation. For example careful attention was paid on tumor-infiltrating immune cells, gene expression analysis, mismatch- repair deficiency, and tumor mutational landscape. Another possible biomarker for ICIs is represented by the so called "tumor mutational burden" (TMB). In particular, there were evidences that a high number of non-synonymous mutations, detected by using large gene next generation sequencing (NGS) panels that cover at least one mega-base, may predict response to ICIs in either high or low PD-L1 expression patients [6 7]. Further studies are required for the definition of the role of TMB and other biomarkers for ICIs administration.

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Venerdì, 18 ottobre 2019

Sala Madrid – 09:00-13:00

PATOLOGIA CARDIOVASCOLARE

Casi Complessi in Patologia Cardiovascolare

CASO N°2

A. Marzullo

Anatomia Patologica Universitaria, Università degli Studi di Bari

We report the case of a newborn delivered at 35th week of gestation with diagnosis at birth of transposition of great arteries associated with an abnormality of the origin of circumflex coronary artery from the right coronary artery. After the surgical correction, the patient developed an acute myocardial ischemia; consequently, he was submitted to an aortic-coronaric bypass. Two days later the child died of a multi-organ failure. At autopsy, together with the surgical correction, the heart showed an enlargement of the left ventricle and thinning of the free wall. Histologically, beside the aspects of coagulative necrosis (hypereosinophilic cytoplasm and nuclear changes, contraction band necrosis), we noted the presence of an intracytoplasmic deposition in some cardiomyocytes of a basophilic pigment with the shape of small granules that resulted positive at von Kossa stain. The calcific deposits occur in the more recently damaged myocytes. They are granular, representing foci of mitochondrial calcium uptake and deposition of calcium phosphate. Calcium overload in reperfused myocardium is considered to be deleterious since disturbance of calcium balance activates multiple mechanisms that target cell destruction and significantly interfere with repair processes. Other authors have suggested the possibility that the calcification process is part of the remodelling process, not necessarily affecting long-term cardiac contractility. Successively, we reviewed the histology of ten hearts belonging to patients submitted to cardiac surgery for malformation and died of intervening complications. In about half of the cases, we found similar lesions not

necessarily in combination with the obvious effects of a myocardial ischemia. We suggest that at least in some cases the effects of an ischemia-reperfusion mechanism could lead to a myocardial failure.

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CASE N. 4

C. Ricci [1], C. Bertuzzi[2], C. Agostinelli[2], O. Leone[1]

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A 75-year-old male with no significant medical history visited his doctor with fever, vomiting and abdominal pain. One month before he had mild cough and constipation. After examination and ultrasound, which showed mild splenomegaly, the patient was discharged with antibiotic and NSAIDs therapy. Four days later, the patient went to his local Emergency Department with persistence of the symptoms. An ECG showed sinus tachycardia, incomplete right bundle branch block and a tendency to flattened t waves. A blood test showed marked hyperleukocytosis (121460 WBC/μL), so the patient was urgently transferred to the S.Orsola-Malpighi Hospital Emergency Department. On admission, an abdominal radiography showed right bowel dilation without hydro-air levels and the patient was admitted to the Gastroenterology Department. Four hours later, the patient complained of dyspnea, abdominal pain and diffuse tympanism. A rectal probe was inserted with symptom improvement. The patient was now eupnoic and had normal blood pressure and oxygen saturation; the ECG was similar to the previous one. Five hours later, the patient complained of an attack of abdominal pain and then suddenly died.

The autopsy was jointly performed by pathologists expert in haematopathology and in cardiovascular pathology. The autopsy not only diagnosed acute myeloid myelo-monocytic leukemia, but was the essential factor in identifying the cause of death (fatal right-sided heart failure) and also the mechanism of cardiac death, related to the marked hyperleukocitosis.

Venerdì, 18 ottobre 2019

Sala Istanbul – 08:30-13:00

CITOLOGIA

II Sessione

Moderatori: G. Troncone, A. Crescenzi

MONITORING NSCLC PATIENTS IN THE 'LIQUID BIOPSY' ERA: ROLE OF MORPHOLOGICAL EVALUATION OF CYTOLOGICAL SAMPLES

E. Vigliar

Dipartimento di Sanità Pubblica, Università degli Studi di Napoli "Federico II"

Background. Nearly two thirds of lung cancer patients are diagnosed at a late stage of disease and the only pathologic material guiding diagnosis and therapy is often represented by small biopsy or cytological specimens. Therefore, in the era of personalized medicine with an increasing need for molecular testing, cytological specimens comprise a crucial component in clinical management of patients with lung cancer. Non-small cell lung cancer (NSCLC) harbouring epidermal growth factor receptor (EGFR) mutation, usually progress after an initial response to tyrosine-kinase inhibitors (TKI). Liquid biopsy enables with a simple blood draw the accurate detection of EGFR p.T790M mutation, the most common resistance mechanism, avoiding the more invasive tissue re-biopsy. However, in a subset of cases, resistance mechanisms are more complex featuring both genetic and morphological changes.

Case presentation. The case of a 67 years-old woman, affected by an EGFR mutated lung adenocarcinoma and treated by TKI is reported. At disease progression, the patient developed a morphological transition to squamous cell carcinoma in association to the arising of a PIK3CA p.E542K mutant subclone.

Conclusion. This case illustrates the relevance of accurate methods for tissue collection, triage for molecular testing and processing and emphasizes that the morphological evaluation of cytological findings, related to acquired treatment response, has still a role in the era of "liquid biopsy".

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II Sessione

Moderatori: G. Troncone, A. Crescenzi

A CASE REPORT OF THREE ACQUIRED MECHANISMS OF TKI RESISTANCE DETECTED IN CYTOLOGICAL AND PLASMA SAMPLES OF EGFR-MUTANT LUNG ADENOCARCINOMA

G. Alì[1], R. Bruno[1], A. Proietti[1], A. Chella[2], A. Ribechini[3], G. Fontanini[4]

[1] Unit of Pathological Anatomy, University Hospital of Pisa, Italy; [2] Unit of Pneumology, University Hospital of Pisa, Italy; [3] Endoscopic Section of Pneumology, University Hospital of Pisa, Italy; [4] Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, Italy

The present case report describes the infrequent coexistence of the epidermal growth factor receptor (EGFR) T790M mutation, squamous cell transformation, and MET amplification as resistance mechanisms to treatment with tyrosine kinase inhibitors. The patient was a 38 year old male, diagnosed with an advanced lung adeno¬carcinoma by endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) and cytological on-site rapid evaluation (ROSE). The molecular analysis performed on smear and cell-block showed positivity for the EGFR exon 19 deletion, therefore the patient was treated with erlotinib (150 mg/day, orally) and radiotherapy for bone lesions. After 12 months, the patient developed resistance (lung, brain, and bone lesions). Cytological examination of the pleural effusion confirmed an adenocarcinoma positive for the EGFR exon 19 deletion and the T790M mutation within exon 20. In addi¬tion, the EGFR mutations were concomitantly detected in circulating cell free tumour DNA. Due to the presence of the T790M mutation, the patient underwent osimertinib therapy (80 mg/day, orally), which resulted in a partial tumour regres¬sion at the 2 month follow up, whereas the sternal lesion showed progression. The fine-needle aspiration of sternal lesion revealed a squamous cell carcinoma positive for the EGFR exon 19 deletion and MET amplification. The patient continued osimertinib therapy, whereas the squamous lesions were treated with radiotherapy. However, after 6 months, the patient developed new disease progression and died of acute renal failure. The present case underlines the importance of cytological sample adequacy for morphological diagnosis and molecular analysis, together with monitoring drug resistance by plasma based assessments of cftDNA, to better describe the mechanisms of resistance to TKI inhibitors and to optimize therapeutic regimens.

Venerdì, 18 ottobre 2019

Sala Parigi – 09:00-13:00

PATOLOGIA SPERIMENTALE

Moderatori: M. Ponzoni, C. Tripodo

IL DEFICIT DI IL-1R8 CONDIZIONA LO SVILUPPO DI PATOLOGIE LINFOPROLIFERATIVE-ASSOCIATE AD AUTOIMMUNITÀ

F. Riva

Il legame tra infiammazione cronica e sviluppo di tumori è nota da diverso tempo ed è avvalorato da dati epidemiologici e molecolari. In particolare condizioni patologiche di autoimmunità (systemic lupus erythematosus, rheumatoid arthritis and Sjogren's syndrome) rendono i pazienti più suscettibili allo sviluppo di linfomi a cellule B Non Hodgkin's. Il meccanismo alla base della trasformazione maligna delle cellule B in normale proliferazione è stato soltanto parzialmente svelato. È stato dimostrato infatti che l'infiammazione cronica, una costitutiva stimolazione e signalling del B cell receptor, e mutazioni che esitino nella costitutiva attivazione del fattore trascrizionale NF-kB sono tra le cause più frequenti di malattie linfoproliferative e linfomi. Interleukin-1 Receptor 8 (IL-1R8) è un recettore appartenente alla superfamiglia di recettori per IL-1 e Toll-Like receptor (TLR) con attività regolatoria negativa dell'attivazione di NF-kB dipendente dal signalling di alcuni membri della stessa famiglia, come ad esempio IL-1R1, IL-18R, TLR4, TLR7 e TLR9. Studi precedenti hanno dimostrato il ruolo di IL-1R8 nella patogenesi di malattie linfoproliferative gravi e malattie autoimmuni lupus-like, ma anche nella progressione maligna della leucemia linfocitica cronica, dovuta ad attivazione costitutiva di NF-kB dipendente da MyD88. Abbiamo quindi studiato se la concomitanza di uno stato di autoimmunità con una risposta infiammatoria esagerata dovuta alla mancanza di IL-1R8 potesse anche ricapitolare la linfomagenesi associata ad autoimmunità.
Abbiamo osservato che in topi *lpr*, proni allo sviluppo di una sindrome Lupus-like, anziani (12 mesi), la mancanza di espressione di IL-1R8 causa un aumento nell'espansione di cellule linfoidi con perdita della normale architettura anatomica di milza e linfonodi, che in circa il 50% dei casi evolve nello sviluppo di linfoma diffuso a grandi cellule B (DLBCL). Analisi molecolari e studi di espressione genica hanno dimostrato che il pathway di NF-kB risulta attivato in modo costitutivo negli splenociti Il1r8-/-/lpr. Per dare un valore clinico a tali osservazioni fatte nel modello murino, abbiamo dimostrato che IL-1R8 è espresso a livelli significativamente inferiori nel DLBCL umano rispetto a cellule B normali e rispetto a linfomi considerati meno gravi o precursori del DLBCL, come il linfoma follicolare. Infine abbiamo osservato che una maggior espressione di IL-1R8 correla positivamente con un outcome migliore della malattia in termini di overall survival. I nostri risultati dimostrano che IL-1R8 svolge un'importante funzione di controllo dell'attivazione del-

le cellule B e della loro trasformazione neoplastica indotta dalla stimolazione autoimmune. L'identificazione di geni o pathway molecolari associati all'attività di IL-1R8 (apoptosi o riparazione del DNA) potrebbero contribuire alla definizione di nuovi target e approcci terapeutici per la cura del DLBCL.

MUTAZIONI DI P53 E LORO IMPATTO SUL FENOTIPO TUMORALE.

G. Del Sal

Dipartimento Scienza della Vita-Università degli studi di Trieste, Laboratorio Nazionale CIB-Trieste, Istituto FIRC Oncologia Molecolare, Milano

Il prodotto dell'oncosoppressore TP53 è un fattore trascrizionale che controlla l'attivazione di centinaia di geni, i cui prodotti sono essenziali per regolare processi fondamentali nel mantenimento dell'integrità del genoma e dell'omeostasi cellulare, quali riparazione del DNA, apoptosi, autofagia, senescenza, metabolismo cellulare ed altri. TP53 è uno dei geni mutati più frequentemente nei tumori umani: la maggior parte di tali mutazioni portano alla produzione di proteine p53 (mutp53) che sono incapaci di svolgere le normali funzioni, ma promuovono l'acquisizione di caratteristiche tumorali quali proliferazione, disseminazione e chemoresistenza. Nei tumori le proteine mutp53 sono espresse a elevati livelli, il che rappresenta una condizione necessaria per la loro azione pro-tumorale. Studiando i meccanismi molecolari che inducono la stabilizzazione di mutp53 abbiamo scoperto che la pathway dell'acido mevalonico (responsabile della biosintesi del colesterolo e di altre importanti molecole che controllano vie di segnalazione e rimodellamento del citoscheletro di actina), regolando la funzione della proteina RhoA, è responsabile dell'accumulo di mut-p53 nei tumori solidi. La proteina RhoA è fondamentale nel controllare la dinamica del citoscheletro di actina in risposta a forze di tensione generate da stimoli meccanici, quali la rigidità della matrice extracellullare (ECM). Questi risultati dimostrano quindi che la stabilizzazione di mut-p53 dipende da segnali di natura metabolica e meccanica (Mantovani et al Cell Death and Diff. 2019; Ingallina et al. Nature Cell Biology 2018).

Sabato, 19 ottobre 2019

Sala 500 – 08:00-13:00

PATOLOGIA ENDOCRINA

Stratificare l'aggressività clinica in patologia endocrina

Moderatori: F. Basolo, G. Pelosi

Neoplasie tiroidee differenziate

NONINVASIVE FOLLICULAR THYROID NEOPLASM WITH PAPILLARY-LIKE NUCLEAR FEATURES (NIFTP) AND TUMORS OF UNCERTAIN MALIGNANT POTENTIAL (UMP)

G. Tallini

Bologna, Italy

In the routine practice of pathology a combination of four basic morphologic features is utilized to diagnose tumors of follicular cell derivation: i) papillary growth pattern; ii) follicular growth pattern iii); presence of a tumor capsule and of its invasion (capsular or vascular invasion); iv) presence of alterations of nuclear morphology typical of papillary carcinoma. The relative weight given to these four features has shifted considerably over the years: it has now become apparent that all of them need to be taken into account in a balanced manner. Some encapsulated thyroid nodules pose considerable diagnostic problems because of uncertainties whether the nuclear changes are sufficient to justify a diagnosis of papillary carcinoma, or whether there is bona fide capsular or vascular invasion. The 2017 WHO classification promotes a pragmatic approach for these difficult cases. It has acknowledged the term of "Non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)" for non-invasive neoplasms of thyroid follicular cells with a follicular growth pattern and nuclear features of papillary carcinoma that are well-developed (non-invasive follicular variant papillary carcinoma) or expressed incompletely. This new terminology includes many tumors that in the past have been diagnosed as encapsulated follicular variant papillary carcinoma. When non-invasive, the encapsulated follicular variant papillary carcinoma has an extremely low malignant potential, with a recurrence rate < 1%. It is important to recognize that NIFTP is not a "new" tumor, but simply a new diagnostic term: the absence of the "carcinoma" label makes it easy to avoid aggressive forms of treatment for the patients. The new WHO classification also endorses the concept of "tumors of uncertain malignant potential" to diagnose cases with equivocal signs of invasion (of the tumor capsule and/or of vessels):"Well differentiated tumor of uncertain malignant potential (WDTUMP)" for tumors with well to incompletely expressed nuclear alterations of papillary carcinoma, and "Follicular tumor of uncertain malignant potential (FT-UMP)" if the nuclear

features of papillary carcinoma are absent.
The consistent utilization of the NIFTP and UMP diagnostic terms is essential. Data are accumulating to support their use in the practice of thyroid pathology.

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Slide seminar - Caso 1: Polmone

PITFALLS IN THE IMMUNOHISTOCHEMICAL EVALUATION OF NEUROENDOCRINE MARKERS IN LUNG TUMOURS

F. Pezzuto[1], F. Fortarezza[1], G. Natale[1], G. Pasello[2], F. Rea[1], F. Calabrese[1]

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In the era of precision medicine, immunohistochemistry plays a critical role in the diagnosis and classification of tumours. In the 2015 WHO classification, the category of "neuroendocrine tumours" was recognized. Invasive neuroendocrine tumours comprise three subtypes: SCLC, large cell neuroendocrine carcinoma (LCNEC), and carcinoid tumours (typical/atypical). Focusing on LCNEC, the diagnosis of LCNEC requires not only neuroendocrine morphology but also immunohistochemical expression of at least one of the three neuroendocrine markers, i.e. chromogranin A, synaptophysin, or CD56. The staining for these markers is not specific and other possible differential diagnoses should be considered. We describe the case of a middle-age smoker woman that underwent a CT-guided lung biopsy for cough and haemoptysis. The histological evaluation showed a neoplastic proliferation with the morphological features of a neuroendocrine neoplasia. The immunostaining detected a weak and focal positivity for cytokeratin, TTF1 and synaptophysin. Thus, the final diagnosis was non-small cell lung cancer (NSCLC) with neuroendocrine features, in favour of LCNEC. The patient was treated with three cycles of cisplatin without any response and an increase in tumour size. A surgical approach was programmed. The lower left lobectomy showed a neoplastic mass and micro-metastases in 2 lymph-nodes. The immunohistochemistry was the same as the biopsy with a weak synaptophysin staining in less than 10% of tumour cells. Based on this equivocal pathological findings and clinical information of a very aggressive tumour, we considered new emerging entities with these features. A strong and diffuse NUT nuclear immunostaining was revealed and the final diagnosis was NUT carcinoma. NUT rearrangement by molecular analysis confirmed the presence of the chromosomal translocation. NUT carcinoma is extremely aggressive with a median survival of 7 months. It is currently considered a rare tumour but the real incidence need to be more in-depth investigated when ambiguous pathological findings are detected and clinical information of very aggressive mass is reported. No specific chemotherapeutic regimen has been approved but some trials are ongoing, thus it is important to explore the eventual occurrence of NUT expression.

Slide seminar - Caso 2: Tiroide

STRATIFICARE L'AGGRESSIVITÀ CLINICA IN PATOLOGIA ENDOCRINA: LA TIROIDE.

C. Ugolini

Anatomia Patologica 3 Universitaria, Dipartimento di Medicina di Laboratorio, AOUP Pisa

Background. Il carcinoma tiroideo è considerato raro rappresentando poco più dell'1% di tutte le neoplasie maligne. Nelle ultime due decadi, però, la sua incidenza è più che raddoppiata e la causa di questa crescita è da attribuirsi principalmente ad un aumento delle diagnosi di carcinoma papillare, che rappresenta oltre l'80% di tutte le neoplasie tiroidee. Il carcinoma papillare tiroideo (PTC) viene suddiviso, quasi esclusivamente in base alle caratteristiche morfologiche, in diverse varianti. Attualmente vengono distinte numerose varianti che presentano andamento clinico sostanzialmente diverso tra loro. Il carcinoma papillare variante a cellule alte (TCPTC) per esempio è caratterizzato da un più alto tasso di mortalità, recidive, metastasi linfonodali e a distanza rispetto a gran parte delle altre varianti. Nonostante il continuo incremento nell'incidenza dei PTC, sono ancora pochi i meccanismi genici identificati che possano differenziare le varianti di PTC per patogenesi ed anche per prognosi. La più conosciuta e più studiata è sicuramente la mutazione puntiforme V600E del del proto-oncogene BRAF. Questa mutazione promuove la tumorigenesi attivando il mitogen-activated protein kinase (MAPK) signaling pathway, un pathway cruciale per la proliferazione e sopravvivenza cellulare. Questa mutazione è stata descritta nella variante classica del PTC ma soprattutto nei TCPTC ed è stata associata ad un fenotipo aggressivo. Oltre alla mutazione V600E di BRAF più recentemente altre mutazioni sono state identificate ed associate a progressione tumorale e peggior outcome. La mutazione del promotore della telomerase reverse trascriptase (TERT) per esempio è stata associata ad una peggiore prognosi ed ha un effetto sinergico con la mutazione di BRAF, peggiorando in modo significativo la prognosi quando entrambe le mutazioni sono presenti contemporaneamente. Altre mutazioni importantissime nei PTC sono quelle del gene RAS, nelle sue isoforme H-K-N-RAS. Queste mutazioni sono in grado di attivare in modo duplice sia il MAPK pathway che il phosphatidylinositol 3-kinase/Akt (PI3K/AKT) pathway promuovendo la tumorigenesi nei PTC nella variante follicolare (FVPTC).

Metodi. Revisione della letteratura e delle linee guida della patologia molecolare associate ai carcinomi papillari tiroidei usando PubMed search per "molecular pathology and thyroid cancer progression" (dal 2010) e dati dai più importanti Molecular pathology groups' websites. Revisione di alcuni casi clinici con valutazione molecolare approfondita delle più frequenti mutazioni e del follow-up.

Risultati. Attualmente le analisi molecolari stanno diventando parte fondamentale nelle diagnosi e prognosi di neoplasie di diversi organi. Nei carcinomi tiroidei hanno trovato un importante ruolo diagnostico, prognostico ed anche predittivo sia nella diagnostica citologica preoperatoria che nella diagnostica istologica. Diverse alterazioni geniche come la mutazione *BRAFV600E* soprattutto nei carcinomi papillari e nei carcinomi scarsamente differenziati ed anaplastici che direttamente originano da questi la mutazione del promotore del gene TERT sono diventate anche importanti nella stratificazione del rischio di recidiva, metastasi e mortalità nei PTC ed anche importanti indicatori di terapia. Anche le fusioni di ALK per esempio STRN-ALK, e l'inattivazione di p53 stanno iniziando ad assumere sempre più importanza, essendo comprovate alterazioni driver nello sviluppo di carcinomi scarsamente differenziati, da carcinomi differenziati, in modelli murini.

Sfortunatamente nei carcinomi orfani di terapia come il carcinoma anaplastico, le mutazioni di questi geni svolgono ruoli predittivi marginali, mentre iniziano ad avere importanza gli studi sul background immunitario.

Conclusioni. La morfologia e l'andamento clinico sono sostenute e guidate da alterazioni molecolari, le quali possono essere un valido aiuto diagnostico, prognostico e terapeutico.

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Sabato, 19 ottobre 2019

Sala Londra – 08:30-13:00

NEUROPATOLOGIA

Workshop

La neuro-oncologia molecolare oggi: from bench to bedside.

Moderatori: P. Cassoni, R. Boldorini

MOLECULAR NEURO-ONCOLOGY, NEW TRENDS, AND EMERGING ISSUES

G. Finocchiaro

The tremendous progress in the sequencing of the exomes and transcriptomes of different brain tumors generated a sizable number of data with a potential translational impact. This is particularly true for gliomas. For lower-grade gliomas, the critical relevance of IDH1-2 mutations and of LOH 1p-19q has been firmly established. For glioblastomas (GBM), a more complex subgrouping has been proposed with two robust categories: proneural and mesenchymal GBM. The clinical impact of this sub-grouping, however, is still debated. One reason for this is the understanding that these subgroups may co-exist in the same tumor and that their prevalence might be influenced by environmental (e.g. hypoxia) or therapeutic stimuli (e.g. survival to chemotherapy or radiation therapy). Along this line, it appears that the identification of a group of GBM cancer stem cells (CSC) that play a key role in GBM recurrence or progression has to be considered dynamically: as a consequence, the effort to find a universal marker for CSC has been essentially unsuccessful.

Other emerging issues in neuro-oncology are related to the role that immunotherapy may play to evade the stalemate of radiochemotherapy as a standard treatment. In GBM, unfortunately, trials with checkpoint inhibitors like nivolumab have failed to provide encouraging results. The response to immunotherapy with dendritic cells is possibly more interesting, but still based on small, uncontrolled trials. Here, two issues appear of considerable relevance. The first has to do with the number of mutations in the tumor, the Tumor Mutational Burden (TMB): high TMB seems associated with a better response to immunotherapy: however, the threshold to define high TMB is not obvious. Gliomas have low TMB but we and others found that about one fifth at recurrence after chemotherapy shows a very high TMB. Whether this high TMB will make these tumors more amenable to immunotherapy is an open question that deserves investigation. A second important emerging issue is that of the tumor microenvironment. A number of cytokines, chemokines, and factors contribute to making the microenvironment in gliomas highly immune suppressive. A deeper knowledge of the key players in this scenario is critical to identify effective strategies for pro-immune modulation.

LA NEUROONCOLOGIA MOLECOLARE NELLA GESTIONE CLINICA DEL PAZIENTE PEDIATRICO

M. Massimino

Fondazione IRCCS Istituto Nazionale dei Tumori

Background. The 2016 edition of the World Health Organization Classification of Tumors of the Central Nervous System utilizes integrated diagnosis incorporating both morphologic and molecular features. Despite tremendous successes in understanding their biology in the last decade, brain tumours remain the highest cause of mortality and morbidity rates in childhood and adolescence.

Methods. In the field of pediatric neuro-oncology this has meant a great deal of diagnostic changes especially for medulloblastoma, other embryonal tumors and high-grade glioma patients but the impact on therapeutic decision is a modulation between still important clinical features of patients at different ages and these new acquirements.

Results. Some entities, now more precisely molecularly such as diffuse midline gliomas with histone H3.3 mutations and embryonal tumours with multi-layered rosettes (ETMR), plus molecularly defined subsets of hemispheric high-grade gliomas, medulloblastomas and atypical teratoid/rhabdoid tumours, are still associated with a universally fatal outcome to date. Other entities achieve a better cure rate but with severe late effects of disease and treatments and also require improvements of current treatments. We can only be successful in paediatric neuro-oncology when taking a multi-disciplinary approach – ranging from preclinical teams through to all involved specialized clinical disciplines. These need to address the definition of "preclinical evidence", understanding of the special microenvironment in the brain and its clinical implications, definition of sampling and molecular diagnostics standards (including advanced biomarkers such as liquid biopsies), standards of advanced neuroimaging, neurological/neuropsychological trial endpoints, local delivery trials, radio-sensitizing trials, advanced supportive care, trials in the context of neurodevelopment/neurocognitive late effects and autopsy programs to benefit the next generation of patients, etc.

Conclusions. An improved understanding of the molecular genetics, epigenetics, and cellular biology underpinning childhood brain tumours will potentially enable more effective and less toxic treatment strategies to be developed and implemented. This could spare children from the severely detrimental consequences associated with conventional treatment protocols and improve the outlook for patients with currently incurable disease.

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Sabato, 19 ottobre 2019

Sala Madrid – 10:00-13:00

PATOLOGIA PEDIATRICA

Slide seminar

Mai troppo vicino, mai troppo lontano

Quando i dettagli morfologici o molecolari sviano dalla diagnosi o aiutano ad ottenerla

MYELOID SARCOMA INVOLVING THE KIDNEY AND THE ILIAC BONE

M.E. Errico, V. Donofrio

Pathology Unit, AORN Santobono Pausilipon, Naples, Italy

Background. Myeloid sarcoma (MS) is a rare extramedullary proliferation of myeloid precursors that effaces the tissue architecture at the site of origin; it may arise de novo, as well as preceding or coinciding with acute myeloid leukemia or a myeloproliferative disorder. MS can be located in any part of the body, the skin, soft tissues, orbit, and central nervous system being the most frequent sites, and in rare cases it may present at multiple localizations.
Herein we describe a case of myeloid sarcoma involving the kidney and the iliac bone.
Case. An 11 years old boy presented with a history of right inguinal pain and intermittent fever; XR revealed an osteolytic lesion of the right iliac bone, clinically suspected of osteomyelitis, and subjected to biopsy, that instead showed morphologic features of a small blue round tumor. The following CT confirmed the structural alteration of the ilium and also showed a mass involving the poles of the left kidney; the patient then underwent tru-cut biopsy from the renal lesion. Immunohistochemical findings supported the final diagnosis of myeloid sarcoma in both lesions, without bone marrow involvement. The patient started chemotherapy for AML and five months after the diagnosis he is in remission.
Conclusion. The diagnosis of myeloid sarcoma can be challenging, particularly in patients with no prior history of hematologic disorders or when it involves unusual anatomic sites.The possibility of MS should be considered when dealing with unusual undifferentiated neoplasms that cannot be categorized as any small blue round cell tumor.

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POSITIVE MYOGENIN STAINING: A POTENTIAL DIAGNOSTIC PITFALL IN NEUROMUSCULAR CHORISTOMA.

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Objective. Neuromuscular Choristoma (NMC) is a rare developmental lesion characterized by the endoneurial intercalation of mature muscle fibers among peripheral nerve fibers that tipically involve the lumbo-sacral and brachial plexus and has been reported in association with desmoid-type fibromatosis. CTNNB1 mutation in NMC has been recently described. The possible differential diagnoses include fetal rhabdomyoma and rhabdomyosarcoma. Here we describe a case of NMC with patchy positive myogenin immunostaining as a potential diagnostic pitfall.
Materials and methods. A 3 month infant was referred to our hospital because of a left supraclavicular mass present at birth with functional impairment of the hand, suspected for a malignacy. Two subsequent needle core biopsies were done to achieve the diagnosis. Immunohistochemistry was perfomed on tissue sections obtained from formalin-fixed, paraffin-embedded (FFPE) tissue blocks. For CTNNB1 mutational testing Genomic DNA was extracted from FFPE tissue and amplified by PCR using primers specific for CTNNB1 exon 3. PCR products were sequenced.
Results. The histological examination of the biopsies revealed similar picture consisting of a proliferation of short bundles of skeletal muscle cells with isolated rhabdomyblast-like elements surrounded by spindle cells. Hyaline fibrosis surrounding isolated skeletal muscle elements was observed at the periphery of the lesion. Immuhistochemically muscular fibers were positive for desmin while spindle cells positive for S100 protein and GLUT1 formed a thin network around the skeletal muscle fibers. Myogenin was positive in 15% of the muscular cells with rhabdomyoblastic-like features embedded at the periphery of the lesion. A diagnosis of neuromuscular choristoma was given. A CTNNB1/b catenin mutation was identified (c.121A > G, p.T41A).
Conclusion. Myogenin is a reliable immunohistochemical marker of rhabdomyosarcoma however it may be expressed in other contexts associated with skeletal muscle regeneration or proliferation. It has been reported in rhabdomyomas whereas it is not found or is very weak in rhabdomyomatous hamartoma. The current case demonstrates that myogenin may be expressed in Neuromuscular Choristoma and may rapresent a diagnostic pitfall. The presence of CTNNB1 mutation supports the relationship with desmoid-type fibromatosis.

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Sabato, 19 ottobre 2019

Sala Lisbona – 08:00-13:00

PALEOPATOLOGIA

Moderatori: G. Nesi, L. Ferrari

I Sessione

DERMATOLOGY IN PALEOPATHOLOGY. HISTORY, METHODOLOGIES AND PERSPECTIVES

R. Gaeta

Division of Paleopathology, Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa, Italy

Background. Skin represents the largest organ in the body and, thanks to its characteristics, generally shows an excellent state of mummification. It is a fundamental and extensive source of biological information since it can reveal signs of infections, tumors or metabolic alterations. Moreover, it is an important indicator for socio-cultural conditions because it may report trauma, injuries or cult practices such as the use of tattoos.
Methods. A revision of the English literature about the most significant research in paleodermatology and the various methodologies applied over time has been made. The study of the published papers is instrumental in hypothesising the impact of new technologies on the study of the mummified skin.
Discussion. It is not surprising that the skin was the first mummified tissue studied with 'modern scientific' techniques. The first work date back to the 19th century and was performed with a simple observation at the microscope after rehydration. The studies on Egyptian mummies are numerous because of the large amount of bodies, and because it was the first large basin for paleopathological studies. Gradually, however, the horizons have expanded on all continents. The reported alterations are varied and include: infectious diseases (e.g. smallpox virus, syphilis, pustular dermatosis); neoplasms (e.g. basal cell carcinoma, common wart); metabolic lesions (e.g. comedones); traumatic lesions (e.g. lacerations, perforations); cultural signs (tattoo). The most frequently used technique (as well as the first to be implemented) is the simple microscope observation with haematoxylin-eosin stain after rehydration. Subsequently, immunohistochemical stains were applied to differentiate the components of the tissue and to highlight possible alterations. Recently, in paleodermatology have been applied sophisticated methods like the Fourier Transform Infrared (FTIR) spectroscopy, the Scanning Electron Microscope (SEM) and time-of-flight secondary ion mass spectrometry imaging using cluster primary ion beams (cluster-TOF-SIMS).
Conclusions. The analysed papers demonstrate that the skin shows an extraordinary resistance to the decay and proves to be an inexhaustible source for paleopathological information. In fact, the examination of mummified tissue allows us to understand the prevalence of the dermatological diseases in ancient populations. The resulting picture is also very significant from the epidemiological point of view since the publications cover a period of millennia and all the continents have been involved. Finally, the studies demonstrate that paleopathology can benefit from the use of new techniques and indeed can be one of the most important field of experimentation. The sector is constantly expanding: in the future, DNA studies will be able to unveil new information currently impossible to detect.

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SKIN PALEOPATHOLOGY IN ITALIAN RENAISSANCE MUMMIES (16TH CENTURY)

G. Fornaciari

Division of Paleopathology,Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa

The Basilica of Saint Domenico Maggiore, which dates to the beginning of the 14th century, is one of the largest and most important churches in Naples, Italy. The monumental sacristy of the Basilica contains, in a suspended gateway close to the vault, 40 wooden sarcophagi with the mummies of ten Aragonese kings and other Neapolitan nobles who died in the 15th and 16th centuries (Fornaciari, 2007). The study of these mummies has produced important results for paleopathology, with cases of venereal syphilis, *condyloma acuminatum*, exanthema by Hepatitis B Virus (HBV) and basal cell carcinoma.
Mary of Aragon (1503-68) was a typical example of a

Renaissance noblewoman, whose beauty attracted the admiration of the humanists. She was a member of the literary circle of Ischia, founded by the Renaissance poetess Victoria Colonna, friend of Michelangelo.
Examination of her artificial mummy revealed some white-yellowish cicatricial areas on the right arm and on the left arm a linen bandage intertwined with ivy leaves covering a 1 x 1,5 m ulcer with a brownish-black irregular surface at the base; within the ulcer was an irregular spherical object composed of vegetable filaments immersed in sulphur, a substance commonly used for skin diseases at that time. Radiographs revealed soft-tissue ulceration of the left upper arm but no skeletal abnormalities. For immunohistochemical study we used indirect immunofluorescence. Slides were incubated with human *Treponema pallidum* antibody (Sclavo), washed, and covered with fluorescein-labelled goat anti-human-IgG. Many 10-20 µm filaments fluoresced an intense yellow-green and had the characteristics of treponemes. Fragments from the bottom of the ulcer were rehydrated and fixed and studied by electron-microscopy. The spiral-like structures had electron microscopic appearances typical of spirochetes. Histological, immunochemical, and ultrastructural findings thus demonstrated treponemal infection and the cutaneous ulcer is typical of third stage luetic gumma (Fornaciari et al., 1989). This noblewoman, who lived in southern Italy, was affected by tertiary venereal syphilis, just known as "Neapolitan disease" in the Renaissance.
Further examination revealed, in the right paravulvar region, a large pedunculated branching skin neoformation (about 12 x 3 mm), which we rehydrated and assessed microscopically after Masson's trichromic staining. Light microscopy indicated an exophytic papillary skin lesion with thickened epidermis and less-dense internal tissue with dilatated vessels. These macroscopic and histological features suggested the presence of anogenital warts. To detect the presence of HPV, we amplified DNA extracted from a sample of the lesion with L1 consensus primers GP5+/GP6+, promoting the amplification of a 141 bp sequence from 25 distinct genital HPVs. We used the amplified DNA fragment for direct hybridisation with oligonucleotides HPV 6, 11, 16, 18, 33, revealing the presence of HPV 18, a virus with high oncogenic potential. To confirm the results, the amplified fragments were cloned and sequenced. Automated sequencing of several clones confirmed infection with HPV 18 and also revealed the presence of JC9813 DNA, another putative novel HPV with low oncogenic potential. The diagnosis of *condyloma acuminatum* in palaeopathology is important because HPV 18 plays an important part in the pathogenesis of some epithelial cancers of the female genital tract and also because this discovery of HPV in a mummy could pave the way for further research on the secular evolution of these viruses (Fornaciari et al., 2003).
The mummy of an anonymous 2-year-old boy, radiocarbon (^{14}C) dated to 1569 ± 60 years AD, revealed a widespread vesiculopustular exanthema type eruption. The macroscopic aspects and the regional distribution suggested smallpox. Light microscopy, on samples of skin with vesicles rehydrated according to Sandison confirmed this possibility. Transmission Electron Microscopy (TEM) showed, among the residual bands of collagen fibres, elastic tissue, pyknotic nuclei, and membrane remains with rare desmosomes, many egg-shaped, dense virus-like particles (250 nm x 150 nm), composed of a central dense region surrounded by a zone of lower density. After incubation with human anti-vaccinia-virus antiserum, followed by protein-A/gold complex immunostaining, the particles were covered by protein-A/gold. The controls were uniformly negative. This Neapolitan child, who died some four centuries ago, seemed affected by a severe form of smallpox (Fornaciari and Marchetti, 1986).
However, shotgun sequencing following enrichment for Human Variola Virus (VARV) revealed no evidence of VARV in this mummy. The identification of a consistent number of HBV reads in multiple tissue samples (distal femur, fronto-parietal bone with skin, thigh muscle, temporo-maxillary skin and leg skin) yielded sequence reads mapping closely to viral sequences from the *Hepadnaviridae*, i.e. Hepatitis B Virus (HBV) of genotype D, still predominant in the Mediterranean region today. Interestingly, analyses of modern and ancient HBV samples returned results consistent with the absence of temporal structure. These results have several important implications for the study of HBV evolution. Such a phylogenetic pattern implies that the currently circulating viral genotypes must have been associated with their specific host populations long before the 16th century, and hence supports a long association of HBV with human populations (Patterson Ross et al., 2018).
Given these results, a new interpretation is that the child was not suffering from smallpox at the time of death, but rather Gianotti-Crosti syndrome caused by HBV infection, a rare clinical outcome of HBV that presents as a papular acrodermatitis in children between 2 and 6 years (Snowden and Badri, 2019).
The natural mummy of Ferdinando Orsini, 5th Duke of Gravina (who died in 1549 about 60 years old) revealed a widely destructive lesion of the right orbit and of the root of the nose. X-ray of the skull confirmed the extensive loss of bone with an osteolytic process involving the entire adjacent bone structures. Histology revealed several *lacunae* destroying the normal lamellar bone, with masses of necrotic epithelial-like cells inside the largest *lacunae*. The border between the bone and the underlying neoplastic tissue was sharp, and the brownish, epithelial-like tumour revealed dark margins, like a palisade, separated from the bone by clefting spaces. Histology allowed the diagnosis of a largely destroying basal cell carcinoma, with skin ulceration and osteolysis (hence the Latin name of *ulcus rodens*). This case of cancer is very important because it represents 1 of the only 5 cases of malignant soft-tissue tumour diagnosed in paleopathology (Gaeta et al., 2017).
The present cases well emphasize the relevance of paleo-dermatology studies in paleopathology.

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II Sessione

Moderatori: E. Fulcheri - R. Gaeta

LA PATOLOGIA CUTANEA NELLE ARTI FIGURATIVE

R. Coda

La ricerca di immagini di malattie nelle Arti figurative del passato è stata praticata da molti illustri medici, storici della Medicina e paleopatologi. A differenza delle illustrazioni di trattati medici che sono spesso viziate dalla necessità di aderire al testo, le immagini artistiche scaturiscono da una genuina osservazione della realtà e permettono spesso delle diagnosi agevoli, utilizzando criteri morfologici e classificativi moderni. In questo tipo di studio, la patologia dermatologica occupa un posto privilegiato perché è spesso presente in superfici esposte e quindi può essere compresa nei ritratti. Inoltre, la maggior parte dei grandi flagelli che hanno afflitto l'Umanità in passato, come peste, lebbra, vaiolo, sifilide e tubercolosi presentano rilevanti manifestazioni cutanee. Lo studio iconografico consente di identificare con buona attendibilità una vasta gamma di malattie dermatologiche, in particolare, nevi, epiteliomi, cheratosi e cisti di vario genere. Inoltre, in alcuni casi, permette di riconoscere patologie desuete, di fare luce sul significato sociale di alcune infermità nelle civiltà del passato, di comprendere antichi criteri classificativi e, sul versante umanistico, di chiarire il reale tema o significato dell'opera.

VENUS IN TURIN. SEXUALLY TRANSMITTED DISEASES IN THE COLLECTION OF ROYAL INSTITUTE OF PATHOLOGY.

L. Ferrari

Division of Pathology Cardinal Massaia Hospital Asti; Departments of Oncology Pathology Unit University of Turin

Objectives. The Pathology Collection of the University of Turin houses 300 wet specimens dating back to the end of XIX century and beginning of XX century. Many of the these specimens are in original condition, with original fluids, jars and labels on which the number of autopsy, date and original diagnoses are written. The ancient autopsy reports are also kept in the Institute of Pathology. They describe the most relevant pathological findings and the final cause of death. To investigate the sexually transmitted diseases in the Pathology Collection a selection of these case was performed among the wet specimens. Subsequenly an archive search was carried out to investigate the pathological finding on their autopsy report.
Material and methods. There are 29 cases of venereal diseases, according to original diagnoses reported on original label. In the Pathology Collection there are also older dry specimens too, that show cases of aortic aneurisym generally due to syphilis.The most representative wet specimens of venereal diseases were sampled by conservative approach to re-evaluate the diagnosis. H&E was carried out to study the morphology of the specimens. A compartive study was carried out on the ancient pathology text of Pio Foà, to understand the exact meaning of the diagnoses on the labels.
Results. The morphology of these old wet specimens appears in most cases comparable with the current ones. A case of cutaneous "condylomatosis due to venereal cause" shows the typical coilocytosis of these lesions. p16 expression was negative, confirming a low grade lesion. A case of "vulvar adenocarcinoma" has been re-evaluated as a squamous carcinoma, Cytokeratin MNF116 expression was positive. Others cases of syphilitis aortitis, chronic vaginalitis in syphilitis orchitis and gummas of the lung confirmed morphologically the original diagnosis.
Conclusions. The sexually transmitted diseases were widespread in Turin at the time the wet specimens of the Pathology Collections were collected. The Director of the Institute Professor Pio Foà was very concerned about this problem and therefore wrote some books to educate the youth, demonstrating a very modern mentality. This study shows the accuracy of old diagnosis with only one wet specimens with a different modern diagnosis, as happened in a previous study on dry specimens. These results confirm again the value of the old Pathology Collections as historical and biological archive.

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Sabato, 19 ottobre 2019

Sala Istanbul – 08:00-12:30

AITIC

II Sessione

Moderatori: A. Cimino - C. Fasson

AITIC-ACADEMY: I PROGETTI DELL'ASSOCIAZIONE PER LA FORMAZIONE E L'AGGIORNAMENTO PROFESSIONALE

M. Cadei (Tslb), F. Caruso (Tslb)

AITIC-Academy, gruppo di lavoro nato dall'Associazione AITIC a fine 2016, ha sviluppato alcune progettualità di carattere formativo che hanno l'ambizioso obiettivo di coniugare la formazione degli operatori (Tslb di Anatomia Patologica) e la collaborazione tra Enti (Associazioni tecnico-scientifiche, Università) e Aziende che operano sul mercato della nostra disciplina medica. Tra i diversi progetti che stiamo perseguendo, quello sulla percezione dei fattori di rischio nel Laboratorio

di Anatomia Patologica, è sicuramente il più strutturato perché è scaturito da un questionario che abbiamo somministrato ai colleghi con differenti provenienze sul territorio nazionale, che ci hanno indotto a fare una riflessione approfondita e che ci ha condotto alla conseguente realizzazione dello "Sportello della Sicurezza". Ne parliamo sinteticamente nell'abstract che presentiamo e che troverà approfondimento nella relazione che svolgeremo a "due voci" durante la sessione.

La percezione dei fattori di rischio nel laboratorio di anatomia patologica in applicazione alla normativa del d.Lgs 81/08 e smi

Ciò che affermano e indirizzano le prese di posizione della collettività e del singolo sono i processi legati alla percezione ed alla interpretazione della realtà.

La percezione del rischio è univoca, ognuno di noi decide autonomamente se di tenere conto o evitare la situazione di un rischio. Ogni nostra attività consta sulla percezione che noi abbiamo del rischio ed è il risultato di una sua valutazione a livello conscio o inconscio. Il processo percettivo del rischio è influenzato dalle emozioni generate nel momento in cui veniamo a conoscenza di un nuovo pericolo e quale possibile danno può arrecarci.

La percezione individuale del rischio:

- è influenzata da esperienze vissute, si tendono a sottovalutare i rischi legati alle abitudini di lavoro, come il mancato utilizzo di DPI, i rischi che si presentano quotidianamente nell'allestimento dei preparati, e quelli a bassa probabilità (es. infortunio causa di un tagliente);
- si basa sull'esperienza personale o di altri;
- cambia in rapporto all'accettabilità collettiva del rischio, le variabili da cui dipende sono: il tempo, i luoghi, la cultura, la tipologia del gruppo di lavoro, i valori personali e culturali, l'età ed il sesso.

La percezione del rischio è legata alla conoscenza dei pericoli, alla sensazione di immunità quando si ha familiarità con le situazioni, all'immediatezza del danno, alla libertà di volere riconoscere o meno il danno, ai pericoli presenti e la loro frequenza, all'esposizione soggettiva.

Quando facciamo una valutazione personale su ciò che riteniamo più conveniente fare. Un TSLB che lavora manipolando un reagente chimico o un agente biologico e non utilizza Dispositivi di Protezione: probabilmente percepisce il pericolo derivante dall'operazione molto inferiore rispetto al vantaggio che trae dal velocizzare il lavoro.

La propensione al rischio diminuisce se gli eventi sono ritenuti incontrollabili dal soggetto e dipendenti da forze o avvenimenti esterni, aumenta se gli eventi sono ritenuti gestibili dal singolo. Ad esempio coloro che ritengono di poter controllare i fattori che possono portare ad un disastro, come gli automobilisti che pensano di essere particolarmente abili nel guidare l'auto. Sentirsi troppo sicuri ci rende insicuri.

Il Rischio è percepito negativo quando non è legato ad un obiettivo importante, e riteniamo che non sia conveniente e non ci porti vantaggi soprattutto economici o pratici.

Il Rischio è percepito positivo quando è associato ad una motivazione rilevante, promette vantaggi immediati, gli svantaggi non sono immediatamente evidenti.

Il Decreto Legislativo n. 81/2008 "Testo Unico in materia di sicurezza e salute nei luoghi di lavoro" propone un sistema di gestione (preventivo e permanente) della sicurezza e della salute in ambito lavorativo definendo in modo chiaro le responsabilità e le figure in ambito aziendale per quanto concerne la sicurezza e la salute dei lavoratori, e prevede l'uniformità della tutela dei lavoratori e delle lavoratrici sul territorio nazionale attraverso il rispetto dei livelli essenziali delle prestazioni concernenti i diritti civili e sociali, anche riguardo alle differenze di genere, età ed alla condizione delle lavoratrici e dei lavoratori immigrati. La valutazione dei rischi, "anche nella scelta delle attrezzature di lavoro e delle sostanze o dei preparati chimici impiegati, nonché nella sistemazione dei luoghi di lavoro, deve riguardare tutti i rischi per la sicurezza e la salute dei lavoratori, ivi compresi quelli riguardanti gruppi di lavoratori esposti a rischi particolari, tra cui anche quelli collegati allo stress lavoro-correlato, secondo i contenuti dell'accordo europeo dell'8 ottobre 2004, e quelli riguardanti le lavoratrici in stato di gravidanza, secondo quanto previsto dal decreto legislativo 26 marzo 2001, n. 151, nonché quelli connessi alle differenze di genere, all'età, alla provenienza da altri Paesi e quelli connessi alla specifica tipologia contrattuale attraverso cui viene resa la prestazione di lavoro".

Al "XXV Corso Nazionale di Riccione 15-18 maggio 2018 TSLB: evoluzione o ri(e)voluzione" è iniziato il percorso è stato somministrato un questionario sulla percezione del rischio indicando diverse tipologie di rischio a cui può essere esposto un TSLB nel laboratorio di Anatomia Patologica.

In occasione del Corso Nazionale 2019 è stato aperto "Lo sportello della Sicurezza" la cui finalità è di stabilire un dialogo tra i TSLB per la problematica della Salute e Sicurezza in ottemperanza del D.Lgs 81/0 e smi.

Sabato, 19 ottobre 2019

Sala Parigi – 08:00-12:30

PATOLOGI OLTRE FRONTIERA

I Sessione

Moderatori: P. Giovenali, L. Viberti

PATOLOGI IN AFRICA? STRATEGIE DI COMUNICAZIONE PER I NON ADDETTI AI LAVORI

R. Cerri

Cos'è la comunicazione? Dal latino "cum" (con) e "munire" (legare, costruire) e, sempre dal latino, "communico" (mettere in comune, far partecipe); si intende il processo e le modalità di trasmissione di un'informazione da un individuo a un altro.

La comunicazione fa parte della nostra vita, è impossibile non comunicare: dal nostro primo vagito, alla prima parola, alle nostre facce stanche dopo una giornata di lavoro. Ogni espressione, gesto, sguardo dicono qualcosa di noi alle altre persone, indipendentemente dalla nostra volontà.

La comunicazione è una caratteristica che accomuna gli

esseri viventi a partire dalle cellule fino agli organismi più complessi: senza di essa non c'è vita.
La comunicazione nel mondo del non profit assume un ruolo strategico, in quanto rappresenta l'elemento cerniera tra le organizzazioni e i propri pubblici, attraverso cui attivare i contatti, gestire rapporti, creare e mantenere reciproca fiducia, ascoltare e chiedere ascolto.[1]
Come arrivare ai finanziatori di un progetto di cooperazione sanitaria? Una buona divulgazione ci permette di arrivare a un numero sempre maggiore di persone, aumentando la possibilità di far conoscere la mission, di incrementare il numero dei volontari e di mettere in atto un efficace fundraising.
Il ruolo del comunicatore in questo caso è quello di un mediatore tra l'ambito medico scientifico e i non addetti ai lavori che deve avere la capacità di prendere un argomento complesso e sconosciuto al pubblico e di trasformarlo in qualcosa di fruibile come un'immagine o un'emozione, poiché sono proprio queste che spingono le persone ad interessarsi al nostro lavoro.
L'importanza e l'urgenza di informare i non addetti ai lavori sul ruolo fondamentale dell'anatomia patologica, si scontra con l'idea collettiva che il patologo sia in realtà una figura più simile ad un Coroner o a personaggi televisivi alla Dr. House.
Alla base c'è il problema di far capire al pubblico di cosa ci occupiamo; senza questa presa di coscienza sarà molto difficile riuscire far capire l'importanza dei nostri progetti.
Siamo in un'epoca nella quale sui social media e sul web sono le immagini a suscitare le emozioni più grandi: basta visitare i social o i siti delle più celebri ONG per vedere decine di meravigliose e struggenti immagini di chirurghi, bambini, donne, famiglie, scuole, animali; immagini toccanti che sicuramente sono molto più potenti ed efficaci di un patologo che guarda dentro ad un microscopio.
Anche la gratificazione personale che si ha nel donare è diversa: ci rende molto più fieri e più buoni dare da mangiare a un bambino o acquistare una protesi per un padre di famiglia, piuttosto che comprare un microtomo! Il sentimento e la forza che queste immagini suscitano dentro di noi non sono comparabili.
La sfida dell'awareness sull'anatomia patologica è ancora aperta: APOF e SIAPEC stanno cercando di far conoscere l'impegno del patologo alle persone. I social e il web ci vengono in aiuto, ma crediamo che una buona parte di informazione possa essere fatta dai patologi stessi: lavorando assieme per far riconoscere e per far cambiare la percezione globale del nostro lavoro.
Soprattutto in questo momento storico in cui è presente un clima di marcata ostilità, e dove il termine ONG è diventato quasi un dispregiativo, è necessario correre ai ripari: occorre cambiare metodo di comunicazione che deve andare oltre al solito vecchio racconto del "bene" che facciamo in Africa; bisogna cambiare le modalità di racconto, per restituire alle persone quella fiducia che hanno perso.
Andare oltre significa non cedere alla tentazione di emulare la marea di spot che mostrano bambini malnutriti, sporchi e sofferenti e genitori in lacrime; questo tipo di narrazione pietistica che vede il salvatore occidentale come unica risorsa per i poveri africani è un fenomeno noto come "white saviour complex" ovvero il complesso del salvatore bianco.
Abbiamo invece bisogno di comunicare trasparenza e credibilità, raccontando gli aspetti più veri di APOF: la nostra professionalità, le soddisfazioni e le difficoltà quotidiane che affrontiamo ogni giorno lavorando in paesi culturalmente, geograficamente ed economicamente molto diversi dal nostro.
Il futuro della comunicazione di APOF prevede nuovi sviluppi: oltre alla nostra presenza online (website, social, newsletter), verrà incrementata l'offerta comunicativa mediante una maggior presenza sul territorio: gli incontri con il pubblico rimangono, anche nel mondo virtuale di oggi, uno dei mezzi di comunicazione più efficaci, non limitandosi esclusivamente alla raccolta fondi ma facendo conoscere in maniera più diretta i nostri progetti e il nostro impegno.
Altrettanto importante è il metodo di comunicazione, che va adeguato al tipo di canale che vogliamo utilizzare e al nostro target: per questo motivo sarà importante dare maggior spazio ai nostri volontari e ai beneficiari dei nostri interventi che sono i veri protagonisti dei nostri progetti.

Bibliografia

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II Sessione

Moderatori: S. Guzzetti, G. Nesi

RELAZIONE MEDICO-PERSONA MIGRANTE: DALL' ACCOGLIENZA ALL' INCLUSIONE

R.R. Pepe

Passare da ciò che è giusto o sbagliato (etica) a ciò che si può fare e non si può fare (governance).
Il passo successivo è che tipo di governance? Una governance sottrattiva nella quale il singolo attore (sia medico che persona migrante) non può avere la presunzione di controllare il tutto, pur nel rispetto del principio di "soggettività" di ogni essere umano (Morin E. 1980, pag 78: "Ogni essere vivente, dal batterio all' homo sapiens, per effimero, particolare, marginale che sia, si prende come centro di referenza e preferenza, si dispone nel più naturale dei modi al centro del suo universo e in esso si auto-trascende, cioè si innalza al di sopra del livello di tutti gli altri esseri"). Si tratta qui di sapere o apprendere la difficile disciplina del "non-padroneggiare" (Manghi S., 2004, pag. 14) e del saper inclinare lo specchietto retrovisore della propria automobile al fine di ridurre al minimo l' angolo cieco delle cose. Lo specchio non è un oggetto qualsiasi, né solo una felice metafora epistemologica, e ha avuto nella storia dell' incontro con l' altro un ruolo particolare accanto alle sue cose inutili (perline, pettini, ecc.) grazie alle quali esploratori, missionari, agenti commerciali "scambiarono" beni o risorse preziose trasformando ad uno stesso tempo la coscienza di sé delle popolazioni colonizzate. Il riflesso nel quale l' altro fu invitato a osservarsi, contribuì infatti a forgiare (ad alterare) la sua esperienza. Lo specchio è in definitiva anche un oggetto impregnato di violenza,

di seduzione e di inganno, un commercio di percezioni (da Viveiros de Castro E., 2017, pag. 200), e quando si vogliono comprendere le altrui ontologie, le altrui nozioni di persona, o tout court le altrui epistemologie, la storia – quella coloniale come quella pre-coloniale – non può lasciare da parte nemmeno per un istante la capacità di apprendere dalle pregresse esperienze-errori (Beneduce R. e Roudinesco E., 2005, pag7-27). In questi dodici anni di assistenza sanitaria rivolta alle persone migranti, mi sono posto molte domande: alcune di esse son rimaste senza risposta; altre hanno ricevuto risposte da parte dei miei stessi pazienti. E così proseguendo ho trovato consolazione anche in risposte ottenute dalla letteratura e dall' essermi posto in un rapporto professionale proattivo con colleghi più esperti di me e con amici disposti a stare ad ascoltare i miei racconti.

Sabato, 19 ottobre 2019

Sala Atene – 08:00-12:30

SESSIONI SPECIALI

ACC - SIAPEC - AIOM: i progetti comuni

Moderatori: C. Doglioni - G. Mazzoleni

ALLIANCE AGAINST CANCER

R. De Maria Marchiano

President of Alliance Against Cancer; Scientific Vice-Director of the Fondazione Policlinico Universitario "A. Gemelli", IRCCS

Alliance Against Cancer (ACC) is the largest Italian organization for cancer research. It was established in 2002 by the Italian Ministry of Health as a network of six high standard Institutes for Research, Hospitalization and Health Care (IRCCS). Over the years, ACC broadened its network to the other Italian cancer institutes in order to develop specific advanced projects in clinical and translational research. Indeed, many new full and associate members joined ACC, which currently comprises the National Institute of Health, 26 research-oriented hospitals and 3 scientific/patient organizations, such as the Italian Sarcoma Group, the Italian Association for Cancer Patients (AIMaC) and the National Center of Oncology Adrotherapy Foundation (CNAO). The ACC network project aims to bring diagnostic innovations and the most advanced therapeutic procedures to the patients.

With the aim of establishing clinical trial programs for patients treated in Italian cancer centers and facilitating their access to innovative drugs currently under development, ACC set up ten Working Groups (WGs) on the major cancer types (lung, breast and colorectal cancers, melanomas, glioblastomas, sarcomas, and hematological cancers) as well as clinical research (Genomics, Pathology and Biobanking, and Immunotherapy).

As the spread of genome sequencing is revolutionizing oncology, both in genetic risk analysis (therefore in cancer prevention programs) and in improving cancer treatment, ACC, with its network of IRCCS, has started research programs aimed at generating and testing of low-cost genetic panels.

The ACC Lung Oncochip

Lung cancer is the leading cause of cancer death worldwide and non-small cell lung cancer (NSCLC) accounts for approximately 80%-85% of cases. The majority of patients are diagnosed with advanced or metastatic disease.

Thanks to technological advances, several molecular alterations responsible for the development of oncogene-addicted tumours have been identified over the last ten years. The use of next-generation sequencing (NGS) has recently entered the routine practice, thus allowing the concurrent evaluation of multiple mutations in multiple genes. Most of these mutations are actionable (ie, treatable with specific drugs) or oncogenic drivers, since they can be targeted with selective inhibitors or contribute to driving the oncogenic process.

In the era of precision medicine, chemotherapy can no longer be regarded as the standard treatment for all patients. Rather, treatments will be based on each patient's unique genetic makeup.

ACC recently developed a targeted sequencing panel (the ACC Lung Oncochip) for the detection of genomic alterations in 182 actionable genes, including 139 translocations and 141 germline variants from 86 genes. Thanks to its network of IRCCS, ACC is now prospectively validating the ACC Lung Oncochip panel with the aim of improving the performance of molecular diagnostics to give better and new therapeutic opportunities to NSCLC patients.

The GerSom project

In about 5-10% of all tumors, patients are carriers of specific genetic mutations (called germline) that increase the risk of tumor onset compared to the general population and can be transmitted to children. The identification of inherited (germline) mutations affecting *Cancer Predisposing Genes (CPGs)* in cancer patients and the subsequent identification of the same mutations in their families led to the programming of diagnostic surveillance plans (ie, diagnostic *imaging* in breast or colon cancer) or risk reduction (for example, aspirin in colorectal cancer) in individuals with inherited CPGs mutations. The presence of CPG mutations has important implications for the treatment of tumor, the definition of patients' prognosis (for example, probability of relapse and new primary or secondary tumors), and the treatment of complications not associated with cancer (for example renal dysfunction in patients with mutations of some CPGs). Furthermore, there is a considerable overlap between CPGs and genes mutated in tumors.

At present, the diagnostic pathways for identifying actionable mutations (mutations that are informative for prognosis and treatment) and those for mapping genetic risk (hereditary variants of CPGs) are distinct. The simultaneous analysis of the somatic and germline mutations will allow to identify an increasing number of CPGs and to lower the cost of the genetic profiling analysis.

ACC has generated a low-cost gene panel for simultaneous analysis, at diagnosis, of the somatic mutations (for the definition of the best therapy) and hereditary

variants (for the identification of the genetic risk of cancer). This panel, with more than 450 genes, will be initially validated on patients with ovarian cancer, patients with triple-negative breast cancer, in young patients with breast cancer and in young patients with colorectal cancer.
The aim is to get it into the clinical routine as a tool for molecular screening to bring the benefits of medical genomics to patients.

Data Analysis and Artificial Intelligence
Another key activity of ACC is the collection and elaboration of metadata coming from both research and clinical practice. A huge number of new data are entering the clinical routine. Technologies like genomics, transcriptomics, radiomics, proteomics, and digital pathology, together with data coming from wearable and implantable devices, will be more and more available in the clinical practice. However, the increasing complexity of such omics data requires the development of combinatorial innovations in ICT and big data analysis applied to the healthcare. The goal is to deliver more efficient error-free personalized treatments and individual-specific drug selection in cancer and cancer-related comorbidities while enabling feedback data loops for preventive healthcare strategies. Thus, in collaboration with the other IRCCS networks, ACC is planning to develop a solid infrastructure for data elaboration. We envision that such platform will connect all the biophysical and digital technologies required to handle and exploit the forthcoming data revolution in healthcare.

ALLIANCE AGAINST CANCER: THE WORKING GROUPS

P. De Paoli, G. Cilibero, P. Pelicci, A. Sapino, R. De Maria

ACC Executive Committee

Alliance Against Cancer (ACC) was established in 2002 by the Italian Ministry of Health. At the beginning ACC was mostly involved in networking among different cancer centres including telemedicine, patient centerness and biobanking. In the last few years, ACC has decided to refine its strategy and to reinforce the mission as the national network for translational research in oncology. To realize this ambitious plan, the organization of ACC has been substantially modified by the introduction of two different types of organisms, the working groups and the committees. The WGs are cancer type defined of have a more wide, transversal, goal providing research and innovation in omic sciences. Committees have more strategic duties, such as providing interactions with cancer organizations at the EU level or developing innovative, non profit, clinical trials.

As previously mentioned, WGs are mostly cancer type specific, include both clinicians and translational researchers and are coordinated by a secretary with the aid of one clinician and one preclinical researcher. Presently, the following working groups have beem established a) Genomics b) lung cancer c) breast cancer d) colorectal cancer e) melanoma f) immunotherapy g) glioblastoma h) sarcomas i) oncohematology l) radiomics.
The research of working groups is partially supported by funds from Ricerca Corrente of the Italian Ministry of Health; in addition, working groups are invited to submit research projects to national and international granting agencies.
The ACC Genomics working group functions as a source of translational research to be applied to the tumor based working groups. The genomic research of ACC has been realized through investments in NGS technology in each of the 25 participating centres, the creation of ACC-Bioinformatics and Genomic technologists and common wet and bioinformatic workflows.
The ACC lung WG has been extremely important fo ACC because it launched the first national genome-screening pilot project (June 2018).
The breast WG has developed and observational prospective retrospective, multi-site, single country trial: Genomic test aiming to identify actionable mutations in Hormone Receptor (HR) negative/HER2 positive or triple negative (TN) breast cancer resistant to neoadjuvant therapy.
Research activities of the colon WG were devoted to deciphering gene damage and expression profiles in paired primary colon cancers and liver metastases.
The program of the glioblastoma WG includes two different projects: the first regards the genetic caracterization of more than 100 GB cell lines derived from patients. In particular the WG has identified a cell line bearing the p.V60E mutation of BRAF that expresses a peculiar sensitivity pattern to Dabrafenib. The second project regards the set up of an NGS panel to identify genetic variants, amplifications and deletions. The Melanoma working group has similarly developed two projects: a)Validation of a new next generation sequencing panel for the mutational analysis of histopathological samples of patients with metastatic melanoma treated with BRAF and MEK inhibitors or with anti-PD-1 antibodies b) Predictors of therapeutic response in metastatic melanoma through NGS e technologies organoids (OTSs).
The most relevant results of the sarcoma WG have been the validation of different specific panels for gene fusions characterizing sarcomas and the preparation of patient derived xenografts and 3D cultures derived from osteosarcoma, Ewing sarcoma and rabdomiosarcoma patient samples. This project has been supported by an IMI-2 grant ITCC PEDIATRIC PRECLINICAL POC PLATFORM (ITCC-P4).
The immunotherapy WG established a strong collaboration with the lung WG and tried to develop an integrated approach to predict, by the use of specific biomarkers,the therapeutic response in patients treated with first line checkpoint inhibitors.
As previously mentioned, the ACC organogram includes also Committees, that have more strategic and organizational goals. In particular it is worth mentioning the activities of the ACC OECI Committe and of the ACC Clinical Research committee.
The ACC OECI Committee includes representatives from ACC members undergoing Accreditation or Reaccreditation according to OECI standards. The goals of this committee are:

- To coordinate at the national level the reaccreditation process of italian cancer centres;
- To support OECI in reinforcing cancer mission in Europe;

- To support an active role of OECI working groups, especially by enrolling young scientists throughout Europe.

Forza is the project prepared by the ACC committee for Clinical Research; this project aims to create a network including cancer centers to foster academic clinical research in Italy.
FORZA will set up a group of internal monitors and auditors in order to control the quality of ongoing academic studies. The training plan will be developed in collaboration with AIFA and the Italian Ministry of Health. FORZA will also create a web platform for the comprehensive management of every stage of the trial process, from the regulatory aspects to the data capture. The platform will be available for ACC Promoters.

MERCOLEDÌ 16 OTTOBRE 2019

Miscellanea 1
Sala Atene – 12:30 - 14:30

PATOLOGIA SPERIMENTALE

COMBINED TRANSCRIPTOMIC AND GENOME WIDE CHROMATIN ANALYSIS OF LUNG ADENOCARCINOMA 3D SPHEROIDS LEADS TO THE IDENTIFICATION OF B4GALT1 AS A NEW FACTOR FOR CANCER STEM CELLS

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Background. Lung cancer is the leading cause of cancer related deaths. According to the Cancer Stem Cells (CSC) hypothesis a population of cancer cells with stem cell properties is responsible for tumor propagation, drug resistance and disease recurrence [1-3] we set up culture conditions for cancer cells deriving from MPEs of several patients affected by the most frequent form of lung cancer, namely the subset of non small cell lung cancers (NSCLC. Hence the study of the mechanisms responsible for lung cancer CSCs propagation is expected to provide a better understanding of cancer biology and new opportunities for therapeutic interventions [4-6] the strong need to identify mechanisms of chemoresistance and to develop new combination therapies. We have previously shown that Stearoyl-CoA-desaturase 1 (SCD1.

Methods. The Lung Adenocarcinoma (LUAD) human cell line NCI-H460 cells was grown either as 2D cultures or as 3D spheroids known to be enriched in the expression of cancer stem cell markers. Transcriptomic analysis was carried out on total RNA extracted from 2D vs 3D cells. In parallel genome-wide chromatin accessibility studies of 2D vs 3D cultures were carried out using the Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq) in order to identify major changes in gene regulatory regions. RT-PCR were performed to validate RNA sequencing Results. TCGA datasets were interrogated to establish correlations between selected genes overexpressed in 3D cultures and survival.

Results. RNA sequencing revealed a global transcriptional rewiring of 3D vs 2D cultures in NCI-H460 cells. In particular, 3D spheroids showed a significant enrichment of gene pathways involved in oncogenesis and cancer progression, including Epithelial-Mesenchymal Transition, Hypoxia, Cholesterol homeostasis and Apoptosis. In contrast, ATAC-seq analysis revealed a smaller number of genomic loci differentially accessible between 3D vs 2D cultures. By integrating RNA-seq and ATAC-seq data, we found that a large proportion of promoters associated to expressed genes were consistently accessible to regulatory factors. A close examination of the genomic landscape of genes upregulated in 3D vs 2D cultures, led to the identification of B4GALT1 as the top candidate, with three regulatory sites differentially opened in 3D vs 2D cells which may represent active enhancers. B4GALT1 gene encodes a type II membrane-bound glycoprotein which transfers galactose to N-linked sugar chains of glycoproteins, and which has been previously proposed to promote cancer cell proliferation and drug resistance. Interrogation of available gene expression datasets showed that higher expression of B4GALT1 at diagnosis is linked with poorer survival in LUAD but not in LUSC patients. Finally, B4GALT1 was validated as a stemness factor since its silencing caused strong inhibition of 3D spheroid formation and of the expression of stemness markers.

Conclusions. Combined transcriptomic and chromatin accessibility study of 3D vs 2D LUAD cell cultures allowed to identify differentially expressed gene networks. Our Results led to the identification of B4GALT1 as a new factor involved in the propagation and maintenance of LUAD CSCs.

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RECEPTOR EDITING MACHINERY IS EXPRESSED IN TUMOR INFILTRATING T CELLS

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The importance of T-cell responses in many cancer settings is testified by the correlation between T-cell infiltration and outcome (1). The tumor microenvironment controls T-cells activation via immune checkpoints (IC) such as PD1/PDL-1/-2 and CTLA-4/B7-1/-2 (2). Mechanisms other than IC involved in tumor escape from T-cell recognition and response are poorly characterized.
TCR revision is an effective tolerance process reported to occur in peripheral T cells under specific conditions (3), and as a consequence of revision process triggering, mature T cells reinduce the expression of RAG1 and RAG2 recombinase proteins, which is inhibited in mature T cells (4).
Aim. of our study was that of investigating the hypothesis that TCR revision may occur in the cancer microenvironment as a potential immune-escape mechanism.
We selected two mouse models of Breast Cancer, namely 4T1 (triple negative model, 12 mice) and TS/A (luminal model, 14 mice), and performed Immunohistochemistry (IHC) for T-cell infiltration (CD3) and duplex RNAscope for RAG1/RAG2 mRNA hybridization.
Comparative analysis of IHC and RNAscope was performed on slide scans with Aperio ImageScope.
Seventy-three cases of human Triple Negative Breast Cancer were also selected for in situ analysis of intratumoral T-cells (CD3), TdT expression, and RAG1/RAG2 mRNA hybridization.
We found evidence of RAG1/RAG2 expressing elements with lymphoid morphology in 4T1 and TS/A tumor foci. Some of RAG1/RAG2-expressing elements corresponded to CD3+ T cells (Fig. 1a-b).

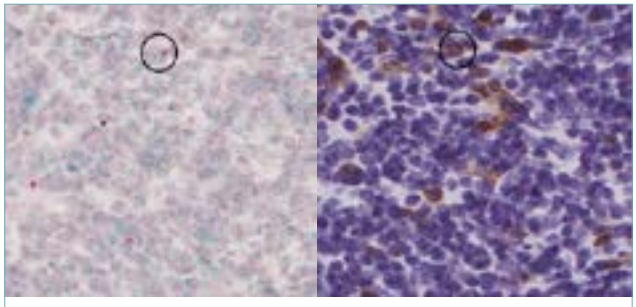

Fig. 1a. 4T1.

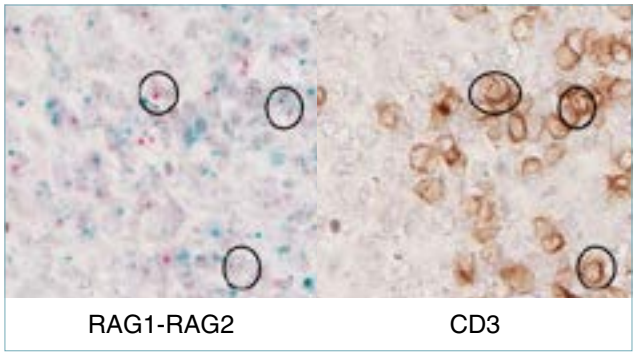

Fig. 1b. TSA.

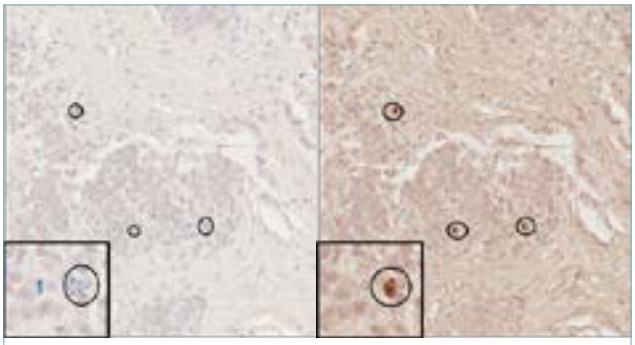

Fig. 1c. TBC.

In human TNBCs we confirmed the presence of RAG1/RAG2 positive elements that also co-expressed TdT, suggesting an undergoing recombination and editing (Fig. 1c).
In conclusion:

- RAG1/RAG2 mRNA expression in peripherals breast-cancer associated T cells may occur;
- RAG1/RAG2 mRNA expression is complemented by TdT protein expression;
- The hypothesis of a revision of tumor antigen-specific TCRs deserves further investigation.

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PATOLOGIA CARDIOVASCOLARE

ANOMALY OF THE CIRCUMFLEX CORONARY ARTERY: WHEN A "SIMPLE" CLASSIFICATION IS NOT SUFFICIENT TO PREDICT THE CLINICAL IMPACT. A CASE REPORT

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Man, about 60 years old, in good nutrition. During a day-hospital for treatment of gonarthrosis, he goes into cardiocirculatory arrest and dies. At autopsy, an anomaly of the circumflex coronary artery is found, which originated at right angles from the initial tract of the right coronary artery, in proximity (<1 cm) of its origin. The artery in question runs posteriorly between the aorta and the right atrium, and then, crossing the left atrium, moves towards the obtuse margin of the heart.

In section, the presence of a subocclusive stenosis (90%) of the origin of the abnormal circumflex was detected. In addition, severe coronary artery sclerosis of the remaining epicardial coronary branches was found, with a maximum stenosis of 50% of the anterior descendant.
The histopathological evaluation concluded by an acute myocardial ischemia with the presence of signs of previous posterolateral infarction of the left ventricle, in a subject with severe stenosis of the circumflex branch with anomalous origin from the right coronary artery.
In medical literature, this anomaly has been included in various classifications based on different criteria. The current clinic-morphological classification, practically, has been proposed in order to predict the possibility to generate heart attack or sudden cardiac death (Clinical relevance). According to these data, the ectopic origin of the circumflexed coronary artery by the right coronary artery is classified as "benign", that is as an anomaly that is not able, by itself, to be able to lead neither to myocardial ischemia, nor to sudden death, but only able to determine various degrees of atherosclerotic disease. At most, this anomaly could be correlated with cardiac surgery problems. We suggest that the angulated origin of this branch could predispose to the formation of an atherosclerotic plaque and the consequent occlusion of the vessel.
In our case, however, we report the death of a patient with a coronary anomaly, which although classified clinically as "benign" led to the death of the patient.

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EMATOPATOLOGIA

TIGIT EXPRESSION IN THE COMPLEX NICHE FOR REED-STERNBERG CELLS IN HODGKIN'S LYMPHOMA

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Introduction. Classic Hodgkin's lymphoma (cHL) is a B-cell lymphoma in which malignant Hodgkin Reed-Sternberg (HRS) cells are sparse and surrounded by an inflammatory Background. However, the lymphoma achieves to escape host immune surveillance and, without therapy, is rapidly lethal. In last period, the use of PD-1/PD-L1 check-point inhibitors resulted highly effective in cHL. 1 In 2016, the US FDA approved Nivolumab for the treatment of cHL that has relapsed or progressed after autologous haematopoietic stem cell transplantation (ASCT) and post-transplantation Brentuximab-Vedotin (BV) 2. Unfortunately, not all cases express PD-L1 and are responsive to its inhibitor, moreover primary refractoriness and acquired resistance after a period of response are major problems with these inhibitors 3. In recent times, the novel co-inhibitory receptor TIGIT (T cell Ig and ITIM domain) a transmembrane glycoprotein of the poliovirus receptor (PVR)/–nectin superfamily, has been shown to be an interesting candidate for novel checkpoint therapies 4 5. Several groups reported that TIGIT expression was elevated on CD8+ Tumor Infiltrating Lymphocytes (TILs) and regulatory T cell (T reg) in a variety of tumors 6 7. Accordingly to these evidences, TIGIT has a crucial role in inhibiting the tumor-directed immune response and, as a consequence, might be attractive as a target for immunotherapy 8.
Aim. of our study was to investigate the expression of TIGIT within the inflammatory Background. in cHL, in order to elucidate the role of the HRS cell niche and to find a potential new target for inhibitor therapy.
Materials and methods. We enrolled 34 consecutive patients with cHL referred to Campus Bio-Medico University Hospital for diagnosis and treatment. Formalin fixed-paraffin embedded lymph node of these patients were characterized by immunohistochemical method with CD20 (Clone L26, Dako), CD3 (Polyclonal Rabbit, Dako), CD30 (Clone Ber-H2, Dako), CD15 (Clone Carb-3, Dako), CD4 (Clone 4B12, Dako), CD45 (Clones 2B11+PD7/26, Dako), and Pax5 (Clone DAK-Pax5, Dako) antibodies. Seriate paraffin sections from archive blocks of these cases were used for adjunctive immunohistochemistry with anti PD-1 (Clone NAT105, Cell Marque), PD-L1 (Clone QR1, UCS Diagnostic) and TIGIT (Clone TG1, Dianova) antibodies. Immunoreactions were achieved with automatized advanced staining system for immunohistochemistry with a controlled onboard environment (Omnis, Dako), and visualized with diaminobenzidine. According to Menter et al., only expression of PD-L1 on RS cells was considered and PD-L1 positivity was defined as at least 5% positively staining tumor cells 9. The score for TIGIT immunohistochemistry was assigned as follows: Score 0: no evidence of lymphocytes with membranous staining within the tumor environment. Score 1: sparse, faintly stained non-tumoral lymphocytes within the tumor environment, near the RS cells. Score 2: presence of a discrete quote of non-tumoral lymphocytes with moderate membrane staining around the RS cells. Score 3: evidence of a circle of non-tumoral lymphocytes with intense membrane staining surrounding the RS cells.

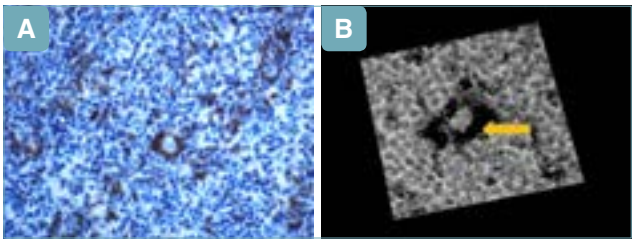

Fig. 1. A) Immunohistochemistry for TIGIT in a case of cHL. Note the peritumoral distribution of TIGIT+ lymphocytes. **B)** Confocal image from immunohistochemistry in a TIGIT+ case. The black area surrounding the HRS cell is the TIGIT positive niche (arrow).

Results and discussion. Immunohistochemistry for TIGIT showed that this immuno check-point is expressed in the niche around the HRS cell in 19/34 patients (56%), moreover all 15 TIGIT negative cases showed PD-L1+ in the HRS cells (100%). Using a 3D reconstruction by confocal microscopy we demonstrated that TIGIT+ lymphocytes are topographically arranged around neoplastic cells suggesting an immune-suppressive functional role for the well-known microenvironmental CD57+/PD-1+/CD4+ T cell rosettes surrounding RS cells 10. Few data are currently available about TIGIT expression in cHL. Based on our Results it seems to be a relevant immunoescape way in this disease. Finally, the evidence that TIGIT+ cases were PD-1+ while TIGIT- cases were PD-L1+ (P=0.0004) suggests that the two checkpoints might be mutually exclusive.

Conclusion. Our preliminary data showed a complex immunocheckpoints interaction in the HRS cell niche and encourage further studies evaluating the role of TIGIT as target for immunotherapies in cHL taking into account that anti-TIGIT antibodies are currently on study on a phase I study in patients with advanced or metastatic tumors (NCT02794571).

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INTERPLAY BETWEEN ONCOGENES AND NONCODING RNAS IN SUBTYPES OF NON-HODGKIN B-CELL LYMPHOMAS

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Oncogenes and epigenetic modifiers shape regulatory loops and circuits involving target genes. MYC controls the transcription of 15% of genes encoded in the human genome including many noncoding RNAs (1). The oncogene TCL1A is expressed at specific stages of the B-cell development and is a coactivator of survival of lymphoma cells (2). TCL1A induced GC B-cell malignancies when dysregulated in pEmu-B29-TCL1 transgenic (TCL1-tg) mice. The epigenetic modifier EZH2, the catalityc member of the polycomb repressive complex 2 (PRC2), that represses the gene transcription by tri-methylation of the lysine 27 of the histone H3, is mutated or deregulated in subsets of non-Hodgkin B-cell lymphomas (NHBCL) (3). We previously identified microRNAs associated to MYC in NHBCLs (4). Here, we investigated the interplay among oncogenes, epigenetic regulation and noncoding RNAs. To this aim, we assessed the immunohistochemical expression of MYC, TCL1A and EZH2 in 75 tissues of five NHBCL subtypes, Burkitt lymphoma (BL), diffuse large B cell lymphoma (DLBCL), primary mediastinal B cell lymphoma (PMBL), mantle cell lymphoma (MCL) and follicular lymphoma (FL), and in 11 reactive lymph nodes (rLN) as reference. Positive cell count was obtained using the cellSense Dimension software (Olympus). The expression analysis of noncoding RNAs was performed by microarrays. $TCL1A^+$ cells were in greater number in BL compared to MCL, FL and DLBCL, respectively, and absent in PMBL. $EZH2^+$ cells were present in decreasing order of frequency in PMBL, BL, DLBCL, MCL and FL. $EZH2^+$ cells increased in parallel to the FL grade. The average number of MYC^+, $TCL1A^+$ and $EZH2^+$ cells was greater in any NHBCL series compared to rLNs. Overall, MYC^+, $TCL1A^+$ and $EZH2^+$ cells were present in decreasing order of frequency in BL, DLBCL, PMBL, MCL and FL, according to aggressiveness of lymphoma subtypes. $EZH2^+$ and $TCL1A^+$ cell counts correlated positively in FL ($P<0.01$). $EZH2^+$ and MYC^+ cell counts correlated positively in MCL ($P<0.0001$), and also through the five NHBCL subtypes ($P<0.001$) (Fig. 1). No correlation among MYC^+, $TCL1A^+$ and $EZH2^+$ cell counts was observed in rLNs, indicating the pathological relevance of lymphoma-specific correlations. Unique patterns of noncoding RNAs, including tRNAs, located at conserved genomic loci discriminated NHBCL subtypes from rLNs. Ten and 36 noncoding RNAs were significantly differentially expressed (Q value < 0.05) between MCL or DLBCL and rLNs, respectively (Figs. 2A and 2B). MYC^+,

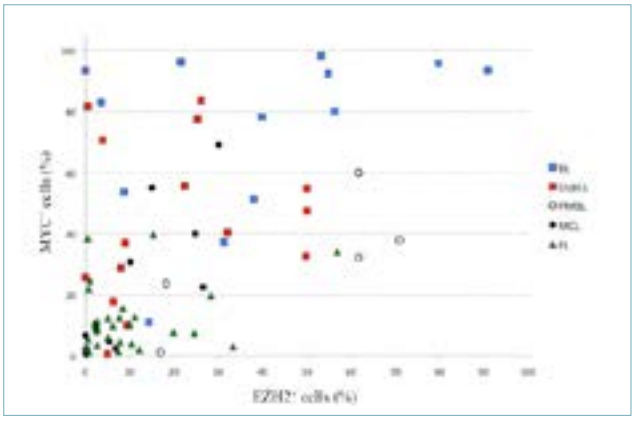

Fig. 1. Distribution of MYC^+ and $EZH2^+$ cell in 5 NHBCLs.

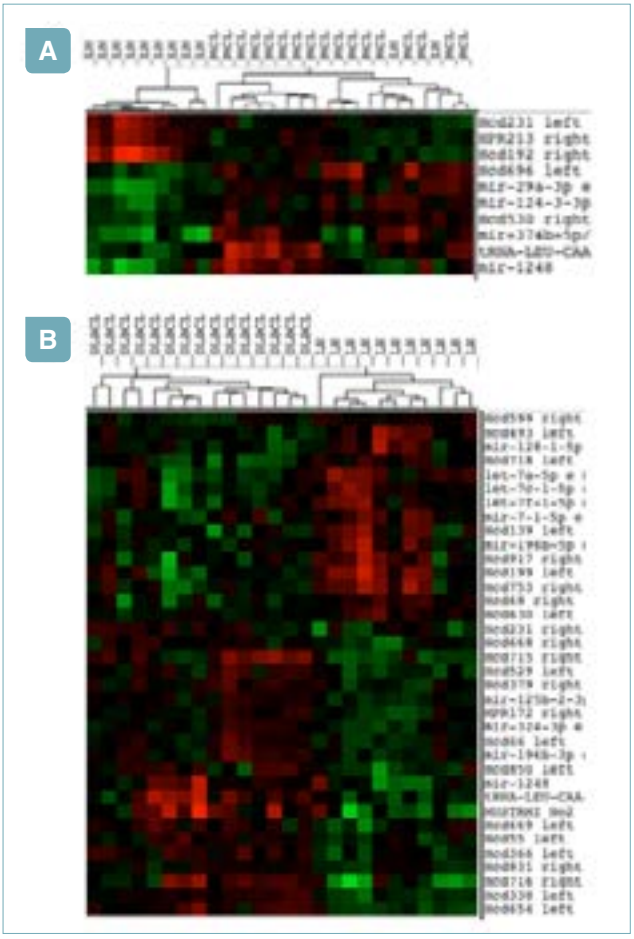

Fig. 2. Noncoding RNAs differentially expressed in MCL (**A**) and DLBCL (**B**) *vs* rLNs.

TCL1A$^+$ and EZH2$^+$ cell counts in NHBCL correlated with the expression level of noncoding RNAs.

MYC, HDAC3, and PRC2 were demonstrated to form a repressive complex tethered to miR-29 and miR-26a promoter elements to epigenetically repress transcription of these microRNAs in MYC-expressing lymphoma cells (5). Our data introduce new factors potentially involved in the MYC-noncoding RNA-EZH2 circuitries that support lymphomagenesis. TCL1A is targeted by microRNAs that are differentially expressed in NHBCLs and regulated by MYC. This evidence makes TCL1A a new potential player in oncogenic circuitries that support lymphoma cells blocked at specific stage of B-cell maturation. The regulatory network that integrates MYC, TCL1A, EZH2 and noncoding RNAs highlights potential pathways to be explored in the context of future clinical approaches.

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CO-EXISTANCE OF HISTIOCYTIC SARCOMA AND ROSAI-DORFMAN DISEASE SHARING THE SAME *KRAS* MUTATION

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Objective. Since its discovery, Rosai-Dorfman disease (RDD), also known as sinus histiocytosis with massive lymphadenopathy, has been considered a non-malignant reactive process [1 2]. However, the recent discovery of oncogenic mutations in some cases of RDD has challenged this concept supporting the hypothesis of a clonal disease [3-8].

Here we report a case of RDD presenting with cervical lymphadenopathy and homolateral mass of the nasal cavity (Fig. 1), histologically displaying features of both

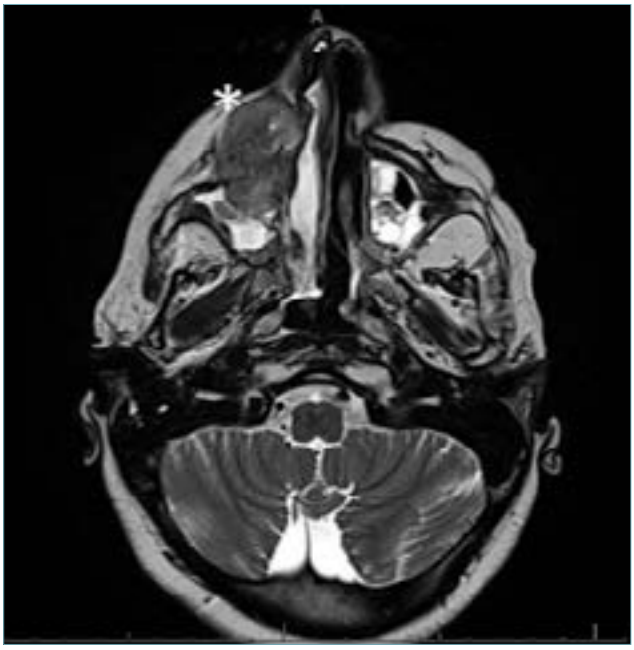

Fig. 1. MRI of the mass of the right nasal cavity.

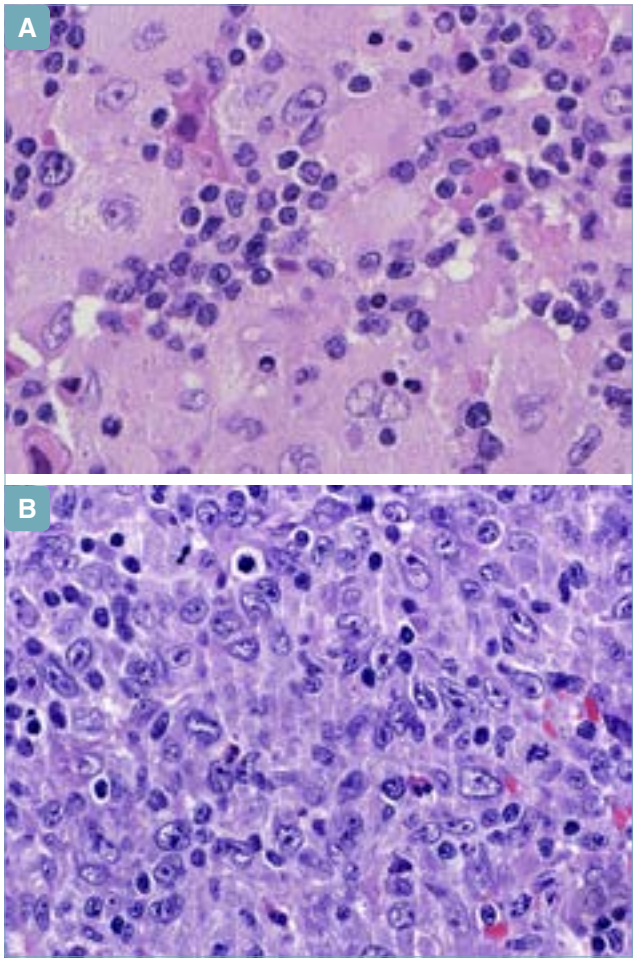

Fig. 2. lymph node: RDD component in figure **A** and atypical histiocytic infiltration in figure **B**.

RDD and histiocytic sarcoma. Both components underwent immunohistochemical and molecular analysis in order to evaluate their mutational status and, eventually, the presence of common alterations.

Materials and methods. Specimens were fixed in 4% neutral buffered formalin and embedded in paraffin. Immunohistochemical analysis was performed applying the following primary antibodies with the Bond-Max (Leica Biosystems, Melbourne, Australia) automatic immunostainer: CD1a (Dako, Glostrup, Denmark); CD68 (PG-M1, Dako); CD163 (10D6, Thermo Scientific, Fremont, CA); Langerin/CD207 (Monosan); Mib/Ki67 (Ventana, Tucson, USA); Lysozyme (Dako); p16 (CINtec, Mannheim, Germany); p53 (Thermo Scientific); PTEN (6H2.1, Dako); S100 (Leica Biosystems).

DNA was extracted from micro-dissected nodal areas showing classical RDD and atypical features and applied to a "Hot spot Panel" (PGM, Ion Torrent), including 50 genes involved in cancer; identified mutations were subsequently confirmed by Sanger sequencing.

Results. Clinical history: a 38-year-old male, with history of radiotherapy for nasopharyngeal carcinoma (13 years earlier), presented with a submucosal endonasal mass and multiple homolateral cervical adenopathies. In the suspect of carcinoma recurrence, a nasal biopsy and a single nodal resection were performed.

Histological findings: the lymph node (*Figg. 2-3*) showed areas morphologically and phenotypically (CD68+, CD163+, Lysozyme+, S100+, CD1a-, CD207-) consistent with classical RDD and areas composed by cohesive sheets of pleomorphic cells, displaying a similar immunophenotype, but with marked atypia and numerous mitoses, including atypical ones. Emperipolesis was obvious in the RDD areas but also recognizable in the atypical histiocytes. By immunochemistry, in both components, PTEN and p16 protein were not expressed by tumor cells, while p53 was positive in about 70% of them. The nasal biopsy showed only the atypical histiocytic infiltration. A diagnosis of combined RDD and RDD-like histiocytic sarcoma was provided.

Molecular findings: in both components, the same *KRAS* p.K117N mutation was identified by NGS panel and confirmed by Sequenom and Sanger sequencing.

Therapy: after diagnosis, induction chemotherapy with doxorubicin and ifosfamide was started and MRI showed a progressive reduction of nasal mass and the adenopathies, with complete response after five cycles. Lack of residual tumor was confirmed on maxillectomy and lymph node dissection specimens. During the post-operative follow up (16 months), no local/regional nor distant recurrences were found. No additional therapy was performed.

Conclusions. Cumulative evidence shows association of RDD to driving clonal mutations, the majority (*17 cases*) involving MAPK/ERK pathway genes (i.e. *KRAS, NRAS, MAP2K1, ARAF*) and only rarely *BRAF (2 cases)* and *SMAD4 (1 case)*. (3-8) Notably, successful treatment of aggressive forms of RDD by MAPK kinase inhibitors have been described confirming the pivotal role of activating mutations in RDD [6].

Transformation or association of RDD and histiocytic sarcoma has been reported in two cases [9 10], one occurring in a patient affected by ALPS [9 10]; notably, no comparative molecular studies were performed in these cases.

Here we report the first case of combined RDD and RDD-like associated histiocytic sarcoma. The presence of similar phenotypical features and of a common molecular profile, with KRAS p.K117N mutation, suggest malignant transformation of RDD, possibly related to previous radiotherapy.

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BONE MARROW REACTIVE FEATURES IN PATIENTS AFFECTED BY AGGRESSIVE LYMPHOMAS

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Objective. The frequency of bone marrow involvement differs according to the lymphoma subtype[1]. To date, the presence of neoplastic infiltration in the bone marrow is the main focus of the studies performed on bone marrow biopsies of patients suffering from lymphoid neoplasms[2]. Less frequently attention has been paid to other non-neoplastic alterations that may be present even without neoplastic infiltration. We believe that this aspect is worthy of further investigation, due to the potential role that these reactive modifications could play. The Aim. of this study is to investigate the reactive features assessed in bone marrow biopsies of patients with aggressive lymphoma.

Materials and methods. We studied 59 bon marrow biopsies of patients with aggressive lymphomas (AL group) and 34 bone marrow biopsies of patients with indolent lymphomas (IL group). All patients, aged between 22 and 80 years, have been selected from the database of the Pathological Anatomy Service of the University Hospital "Policlinico Tor Vergata" of Rome. We observed the following parameters by optical microscope: cellularity, characteristics of haematopoietic lineages, lymphocytic infiltrate, plasmacellular infiltrate, reticulin grade, deposit of substances.

Results. In patients with AL, we observed increased eosinophils in 20/59 cases. Moreover, alterations of megakaryocytic lineage were detected in 50/59 cases. Thirty-three patients belonged to the group without bone marrow infiltration and 17 to the group with bone marrow infiltration. The alterations detected in the group without bone marrow infiltration were hyperplasia of the megakaryocyte series (24/33), presence of loose and/or dense clusters of megakaryocytes (18/33; Fig. 1), and presence of dysplastic megakaryocytes (21/33; Fig. 2). In the group of patients with bone marrow involvement, the alterations detected were hyperplasia of the megakaryocyte series (8/17), presence of loose and/or dense clusters of megakaryocytes (13/17), and presence of dysplastic megakaryocytes (4/17). In IL patients no significative alterations of the three medullary cell lines were found.

Conclusions. Our study has demonstrated for the first time, at best of our knowledge, in patients affected by aggressive lymphomas the presence of reactive eosinophilia and megakaryocytic hyperplasia and dysplasia in bone marrow. These changes present higher frequency in subjects without bone marrow neoplastic infiltration. Until now, these modifications have been described only in Hodgkin lymphoma and in other T-lymphomas and solid tumors[3]. These alterations probably are a non-specific reactive phenomenon, not correlated to histotype and bone marrow infiltration. It can be linked to a hypoxic state of bone marrow due to the release of cytokines, related to the presence of neoplastic cells, which induce oxidative stress[4-5]. Our Results could have important consequences in clinical settings, as the alterations found may contribute to the development of symptoms, associated with those that directly depend on lymphomas. In this context, measures to reduce the levels of oxidative stress could be a benefit for the patient and could facilitate, for example, the management of complications.

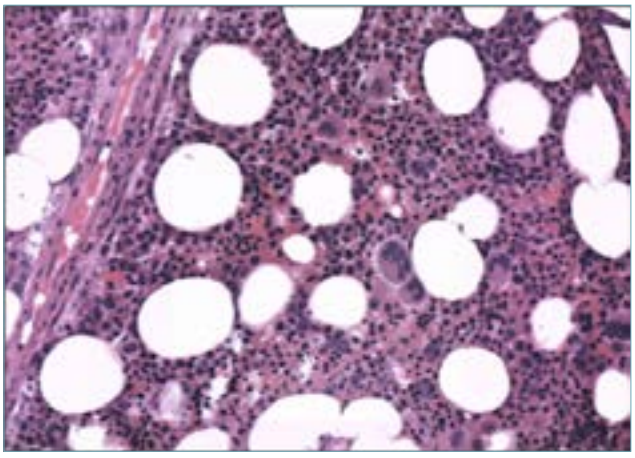

Fig. 1.

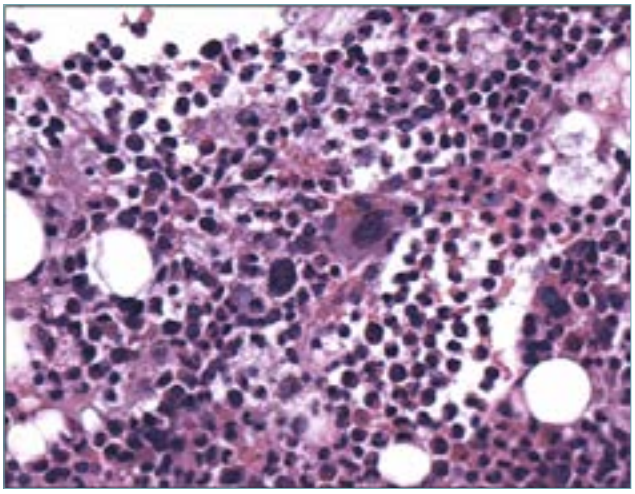

Fig. 2.

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UROPATOLOGIA

COMBINING MOLECULAR IMAGING AND PATHOLOGY FOR DETECTION OF HIGH GRADE CANCER LESIONS EXPRESSING BONE BIOMARKERS

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Cancers of the prostate represent the second cause of cancer-related death in the worldwide in men, with 1.6 million new cases and 366,000 deaths annually [1]. Although screening programs such as prostate-specific antigen (PSA) analysis were associated with a more of 50% reduction in prostate cancer mortality [2], the 5-year survival for patients with bone metastatic prostate cancer lesions is still 30% [3]. Therefore, the discovery of new biomarkers, as well as the development of in vivo analysis capable to identify the prostate lesions with high metastatic potential, could represent an extraordinary possibility to reduce prostate cancer mortality.

We recently reported very preliminary data about the possible use of 18F-choline PET/CT analysis in the detection of prostate lesions with high propensity to develop osteoblastic bone metastatic lesions [4]. In this study, for the first time, we described the presence of prostate cancer cells with morphological and molecular characteristics of osteoblasts (Prostate Osteoblast-Like Cells - POLCs), demonstrating the putative association between their presence and the development of bone metastasis within 5 years from histopathological diagnosis 4. Also, we found that POLCs origin was linked to epithelial to mesenchymal transformation (EMT) and subsequent osteoblastic differentiation stimulated by Bone Morphogenetics Protein (BMP) 2 [5]. Therefore, the main Aim. of this study was to investigate the possible association between ^{18}F-choline uptake and histopathological features of prostate biopsies such as Gleason Geoup and the expression of both epithelial to mesenchymal transition and bone mineralization *in situ* biomarkers.

Methods. We retrospectively enrolled 79 consecutive prostate cancer patients underwent to both ^{18}F-choline PET/CT analysis and prostate bioptic procedure (within 30 days from PET scan). SUV average values were collected from ^{18}F-choline PET/CT analysis. Hematoxylin and eosin sections were use for diagnosis and Gleason Group evaluation; paraffin serial sections were used to investigate the expression of the following molecules: vimentin (epithelial to mesenchymal transition), BMP-2, RUNX2, RANKL, RUNX2, VDR and PTX3 (bone mineralization).

Results. Prostate biopsies were classified in adenocarcinomas according to WHO 2016 [6]. Histological classification showed an heterogenous population including both low/intermediate grade and high-grade prostate cancers. As concern the comparison between Gleason Group and age, no significant differences were observed. A significant increase of ^{18}F-choline uptake in high grade prostate lesions (Gleason Group value ≥8) was found. Also, linear regression analysis showed a significant correlation between ^{18}F-choline uptake and the number of vimentin, RANKL, VDR or PTX3 positive prostate cancer cells. Conversely, we observed no significant association between ^{18}F-choline uptake and the expression of bone biomarkers involved in early phases of osteoblast differentiation (BMP-2, RUNX2). Lastly, we noted an increase in the expression of all investigated *in situ* biomarkers in high grade prostate cancer.

Conclusion. The research of new prognostic and predictive imaging biomarkers for diagnosis and prognosis of prostate cancer represents a very demanding challenge of the scientific community. However, only few molecules investigated in preclinical animal models are successfully translated into the clinical practice. Thus, an important perspective in this field could be the improving of currently knowledge, both technical and biological, on diagnostic analyses already available, such as PET/CT scan. In line with this idea, we used histo-pathological data to verify the possible use of 18F-choline PET/CT in the early identification of prostate lesions with metastatic potential evaluated in term of GG and the presence of POLCs. Although preliminary, data here reported can lay the foundation for the use of 18F-choline PET/CT as diagnostic tool capable to identify undifferentiated prostate cancer lesions expressing bone biomarkers.

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PARVALBUMIN IMMUNOHISTOCHEMICAL EXPRESSION IN THE SPECTRUM OF PERIVASCULAR EPITHELIOID CELL (PEC) LESIONS OF THE KIDNEY

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Aim. Angiomyolipoma is the most common benign renal tumor[1] and it is the prototype of the renal perivascular epithelioid cells (PEC) lesions, which display a variety of histological patterns, all characterized by an immunohistochemical distinctive reactivity for both smooth muscle and melanocytic markers [2-4].
To date, the current World Health Organization (WHO) identifies among angiomyolipomas two main subgroups, classic and epithelioid variant[5], distinguished on the basis of morphology and clinicopathological characteristics, and recognizes in the latter variant a potentially aggressive behaviour[6].
This study Aim.ed to evaluate, in the spectrum of PEC lesions, the immunohistochemical expression of parvalbumin, a low molecular weight calcium-binding protein[7], present in both excitable and non excitable cells[8] and constantly expressed in chromophobe renal cell carcinoma [9].
Matherials and methods. Fifty-three PEC lesions have been retrieved from the files of the University of Verona Hospital Trust between 2000 and 2019 and from the files of Peschiera Hospital between 2011 and 2019. Each case was re-evaluated by two expert pathologists (GM, AC) and categorized according to the morphology. Sections from tissue blocks of each tumor and 120 control cases (50 clear cell renal cell carcinomas, 20 papillary renal cell carcinomas, 20 chromophobe renal cell carcinomas, 20 oncocytomas and 10 clear cell papillary renal cell carcinomas) were immunohistochemically stained with parvalbumin (clone PARV-19, dilution 1:500, Sigma-Aldrich St. Louis, MO, USA). A semiquantitative evaluation of the immunostaining was performed and the positivity of parvalbumin was assessed as the percentage of cell stained by the antibody, ranging from 0% to 100%.
Results. The case cohort was composed of thirty-eight female patients (72%) and fifteen male patients (18%). The patients' ages at diagnosis ranged from 6 to 80 years (mean 54, median 55). The tumors ranged in size from 0.7 to 17 cm (mean 4.8, median 3). Thirty-seven cases (70%) were classified as classic angiomyolipoma, seven (13%) as epithelioid angiomyolipoma/pure epithelioid PEComa, five (9%) as leiomyoma-like angiomyolipoma, one (2%) as sclerosing angiomyolipoma, one (2%) as intraglomerular angiomyolipoma, one (2%) as lipoma-like angiomyolipoma and one (2%) as angiomyolipoma with epithelial cysts (AMLEC).
Overall, parvalbumin immunostain was found in fourty-three PEC lesions (81%) and absent in the remaining ten cases (19%). Classic angiomyolipomas were positive in almost all cases (97%), displaying a percentage of positive cells varying from less than 1% to 100%. None of the 7 epithelioid angiomyolipomas/pure epithelioid PEComas expressed parvalbumin. Leyomyoma-like angiomyolipomas were positive in four of five cases (80%); the only sclerosing angiomyolipoma in the cohort was negative, while cases classified as lipoma-like angiomyolipoma, intraglomerular angiomyolipoma and AMLEC were positive.
Among the control tumors, all oncocytomas and chromophobe renal cell carcinomas were positive for parvalbumin and none of clear cell renal cell carcinomas, papillary renal cell carcinomas and clear cell papillary renal cell carcinomas showed any immunohistochemical expression.
Conclusions. We demonstrated the expression of parvalbumin in almost all perivascular epithelioid cell (PEC) lesions of the kidney but not in the epithelioid angyomyolipoma/pure epithelioid PEComa. This finding is useful for diagnostic purposes, particularly in the differential diagnosis with the renal eosinophilic tumors such as oncocytoma and chromophobe renal cell carcinoma. Interestingly, the latter is known to arise four time more frequently in kidneys bearing angiomyolipomas.

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EXPRESSION ANALYSIS OF THE RING LIGASE PRAJA2 AND EGFR IN CLEAR CELL RENAL CELL CARCINOMA

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Aim. Renal cell carcinomas are the most common tumors in the kidney, and clear cell carcinoma (ccRCC) is the most frequent subtype [1]. Molecular mechanisms of cancerogenesis in ccRCC are only partially known. Our Aim. was to investigate the expression of Praja2 and the Epidermal growth factor Receptor (EGFR) in ccRCC, and to analyse their functional interaction. EGFR is a tyrosine-kinase receptor that activates cell proliferation. Praja2 belongs to a growing family of RING E3 ligases

widely expressed in mammalian tissues. Praja2 controls the stability of protein kinases and scaffold [2 3], and it is involved in the proliferative pathways of human glioblastoma [4]. However, the role of Praja2 in ccRCC cells and its functional interaction with the EFGR are not known.

Methods and materials. Expression of Praja2 and EGFR was evaluated by immunoistochemistry and biochemical analysis. Two tissue microarrays (TMA) were generated containing a selection of 54 cases of ccRCC. The expression levels of Praja2 and EGFR were evaluated on TMA sections using a score from 0 (no expression) to 3+ (high expression). 18 cases of tumoral frozen samples, and 18 normal renal tissues were collected. We also performed a western blot analysis with specific antibodies for Praja2 and EGFR on protein lysates from the selected ccRCC tumor and normal tissues. In addition, we evaluated the levels of Praja2 and EGFR in human kidney cells (HEK293) subjected to genetic silencing of praja2.

Results. Immunohistochemistry and western blot studies revealed that Praja2 was dramatically downregulated in ccRCC, compared to normal kidney tissues. In contrast, expression of EGFR was highly increased in tumor samples, compared to normal counterpart. Immunofluorescence experiments in cultured kidney cells demonstrated that Praja2 partly colocalized with EGFR at cell membrane and its genetic downregulation increased EGFR immunostaining at cell membrane.

Hitherto, the expression of Praja2 in renal cancer were not known. Here, we show that Praja2 is strongly downregulated in ccRCC and its expression showed to be inversely correlated with EGFR levels. Genetic silencing of Praja2 leads to accumulation of EGFR at cell membrane, suggesting a role of the E3 ligase in the regulation of EGFR pathway.

Conclusions. This study described, for the first time, that Praja2 is dramatically downregulated in ccRCC tumor tissues, and its levels inversely correlated with EGFR. This highlights a potential role of Praja2 in the control of EGFR signaling cascade. Further studies are necessary to better define this mechanism and the pathogenic role of Praja2 in ccRCC.

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SEARCHING FOR EXTRA-RENAL EXTENSION IN RENAL CELL CARCINOMA: THE IMPORTANCE OF RENAL SINUS SAMPLING

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Introduction. Renal cell carcinoma is the most frequent malignant tumor of the kidney in the adulthood consisting in the 90% of all renal tumors [1]. Overall survival depends mostly on stage of the disease and then on histology and grade according to the recent WHO [1 2].

Stage of the disease varies according to two main characteristics: size of the tumor (pT1,2) and extension outside the renal parenchyma (pT3+). As specified by the pT suffixes, the extra-renal extension worsens the prognosis, irrespective of the size of disease, putting the case in higher stage, as well as a nodal positive disease (Stage III) [3]. Extra-renal extension encompasses invasion of fat (perirenal and/or renal sinus) or invasion of pelvicalyceal system; or renal vein or its segmental branches. Wider extension of disease includes vena cava, Gerota's fascia and ipsilateral adrenal gland involvement. Obviously, the above pT distinction is not only morphological, but relates to a sharp difference even in the prognosis: 5 ys overall survival of pT1-2 diseases is 92,6%, while, for pT3 it is 66,7% [4]. It becomes very important to correctly recognize and to accurately evaluate the extra-renal extension, already starting at macroscopic handling [5 6], to avoid inadequate staging and therefore inadequate therapy. A recent and interesting study has demonstrated that there is high inter-observer variability in the proper staging of RCC [5]. According to recent ISUP recommendations [6], already during the macroscopic handling it is mandatory to evaluate all the crucial sites of extra-parenchymal extension. Furthermore, ISUP recommendations also indicate how to correctly identify that at microscopic level, while on the other hand, there are no equally well-defined guidelines for assessing intra-parenchymal venous branches invasion.

Aim. In order to reduce inter-operator variability, and to avoid under-staging of diseases, and to more precisely define patients prognosis, we have thought that introducing a standardization of the macroscopic approach along with precise microscopic evaluation of the renal sinus, could be useful.

Materials and methods. Starting on May 2017, on cases doubtful for invasion of renal sinus, we have introduced a more extensive sampling of renal sinus according to ISUP recommendations [6] and an immunohistochemical evaluation by desmin and CD31 on slides suspicious for intra-parenchymal venous branches invasion. At November 2018, we have searched our files collecting all cases diagnosed as RCC in the last 36 months, dividing them into two groups: the first including cases diagnosed in the 18 months before may 2017, the second including cases diagnosed after the introduction of the above standardized macro/micro protocol. Cases have been selected by one of the author (FF) and all slides have been reviewed independently, without any information about the original diagnosis and staging, by two pathologists (GC, MM). In case of divergent opinions, the slides have been submitted to a third experienced pathologist (MGM) in order to get agreement.

From each case have been reported the clinic-pathological characteristics: gender, age (dividing patients in 3 groups: up to 50 ys; 51-70 ys; more than 70 ys), side, size, histotypes, grade, TNM, stage and, for pT3x, site of invasion). Then we have compared the Results of the two groups, by Chi-squared test, Fisher's exact test and t-test confident that the eventual variances were due to the different methodical approaches, instead to a casual distribution of diseases.

Results. In the 2 groups we collected, respectively, 139 and 141 cases of kidney surgical specimens. Out of them, 54 cases in each group were radical nephrectomy and these two groups constitute the object of our study.

Gender: in the 1st group there are 42 males and 12 females; in the 2nd group, 36 males and 18 females which is consistent with the literature data [1] and has no significant difference (p=0.33).

Age: mean age is 61.94 in the 1st group and 63.8 in the 2nd without significant difference (*t-value*= -1.27; p=0.20), neither stratifying for age groups (Chi-square test: 4.2532).

Globally, the distribution of histotypes has the same percentage in the 2 groups and reflects the literature data [1]. Neither stratifying the histotypes distribution by age group or by sex there is difference compared to literature.

About the grade, the 2nd group shows a more homogenous distribution of cases in the 4-tier scale if compared to the 1st where G2 are more abundant. However the statistic tests do not demonstrate any significant difference. As estimated, grade and size are directly proportional [1].

The local extension (pTNM, staging and invasion) between the 2 groups displays a higher percentage in the last one. Considering the single extra-renal structures interested by tumor (pT3+), perinephric and renal sinus fat invasions are about the same in the two groups (p=0,6). The greatest difference is represented by the intra-parenchymal branches of renal vein invasion: 9 cases (16,7%) in the 2nd group vs 1 case (1,8%) in the first one (p=>0,05).

Stratifying these 9 cases by age, sex, histotypes, size and grade, there is a clear and direct correlation with size and grade.

Discussion. RCC is a common tumor whose prognosis depends greatly on the time of diagnosis and on stage. Stage I-II tumors (i.e. tumors limited to parenchyma without extra-renal extension and without nodal involvement) have a better prognosis than stage III tumors which show extra-renal extension or loco-regional lymph node metastases, reflecting better overall survival [4]. In order to get the right microscopic evidence about extra-renal extension of the disease it is deemed a generous macroscopic sampling of critical sites [5 6] and a precise evaluation at microscope, even by immunohistochemistry, if necessary.

We have introduced a more precise and standardized sampling technique Aim.ed at obtaining this piece of information. The result of this study Results support our thesis, mostly about the infiltration of the intra-parenchymal branches of renal vein, which have been previously underestimate. In fact in the two groups of our study, although the distribution of pT3+ cases is quite similar without any statistical significant difference, the main variance is in the site of invasion. Now, as pT3a encompasses cases with fatty invasion (perinephrinc and renal sinus) and intra-parenchymal vein invasion, the percentage of pT3a cases into the 2 groups is analogous. But, if we take into account the single site of involvement, the venous thrombosis, which is the focus of our study, reveals an higher and important significance in the 2nd group.

Unfortunately, due to the limited follow-up time available, we can't appreciate the real clinical benefits of this piece of information, but referring to the literature data, the likelihood that patients could gain a more suitable treatment following a more accurate staging is very high. Furthermore, our data could represent a starting point for future studies.

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PD-L1 EXPRESSION IN PATIENTS WHO RECEIVED ACTIVE SURVEILLANCE FOR METASTATIC RENAL CELL CARCINOMA: VALIDATION COHORT

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Background and Aim. Active surveillance (AS) is one possible option to manage patients with a recent diagnosis of metastatic renal cell carcinoma. Unfortunately, there are no predictive factors to select patients eligible for AS. We previously reported that PD-L1 expression is useful to stratify patients' prognostication; in the actual study we Aim.ed to analyze the potential of PD-L1 expression in improving the selection of patients eligible for AS in an extended cohort of cases (validation).

Patients and Methods. Patients who started AS at our institution from January 2007 to April 2016 with available diagnostic tissue samples were included. Kaplan-Meier method was used to estimate survivals according to the expression of PD-L1 (E1L3N, Cell Signaling Technology, Danvers, MA).

Results. 52 patients (validation cohort) received AS and 43 were analyzed for PD-L1 expression, using two cut-offs (≥1% and ≥5%) to define positivity. The cut-off of 5% was associated with a difference in the median AS: 9.8 months in PD-L1+ group compared to 26.4 months

in PD-L1- group (p=0.025). This difference remained significant even when adjusted for IMDC prognostic class - good vs. intermediate/poor - (HR=2.53, 95%CI 1.18 - 5.44; p=0.018). No differences in OS were found. When the value ≥5% for PD-L1 positivity was considered, among the 6 cases with positive expression on the primary tumor, only 1 (16.6%) had the same degree of expression on the metastatic sites; among the 22 cases with negative expression on the primary tumor, 7 (31.8%) had positive expression on the metastatic sites.
Conclusions. We validated that the expression of PD-L1 is a prognostic factor that can help in the selection of patients for AS. Moreover, we confirmed the heterogeneity of PD-L1 expression between primary and metastatic tumor lesions (32% versus 19% of positive cases); ≥50% of positive cancer cells were observed in respectively 9% and 6% of cases. Harmonization with other PD-L1 commercially available clones need to be verified.
Key Words. mRCC; active surveillance, PD-L1, tumor heterogeneity, survival.

PD-L1 EXPRESSION IN BLADDER PRIMARY IN SITU UROTHELIAL CARCINOMA: EVALUATION IN PATIENTS BCG UNRESPONSIVE AND BCG RESPONDERS

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Objective. Carcinoma in situ (CIS) is believed to be a precursor of MBIC that may arise from these flat high grade, superficial urothelial lesions. CIS accounts for approximately 10% of all bladder tumors. While primary CIS is a preinvasive lesion, secondary CIS means the coexistence of CIS and high grade urothelial carcinoma that might be invasive; moreover secondary CIS is associated with an higher rate of progression of the tumor and a worse prognosis.Therapeutic options for urothelial CIS are limited and in order to inhibit disease progression and recurrence, current guidelines recommend transurethral resection (TURBT) followed by intravesical administration of Bacillus of Calmette-Guerin (BCG). Approximately 30-40% of patients fail the BCG therapy with recurrence and progression and although the precise mechanism of BCG failure is not clear, numerous studies in the literature indicate that immune resistance or immune evasion may be critical factors in the mechanism of BCG failure.
Materials and methods. In the present study, we examined the expression of PD-L1 both in neoplastic epithelial cells and in stromal inflammatory cells in 65 patients with diagnosis of CIS, 28 patients with no evidence of recurrence or tumor progression after BCG therapy and 37 patients not responders to BCG therapy, in order to verify if the PD-L1 expression could identify patients resistant to BCG treatment. Moreover, Aim.ed to compare the reproducibility of the Results with three different MoAbs, we analyzed on the same cases the immunoreactivities of anti PD-L1 MoAbs such as SP263, C23 and SP142.
Results. Our Results have showed that PD-L1 expression in tumor cells and in Immuno Cell compartment is higher in BCG unresponsive group than in BCG responders, but only the PD-L1 22C3 expression in tumor cells seems to be associated with recurrence of disease (p=0,035; OR 0,1204 CI 95% from 0,0147 to 1,023)
Conclusions. Our data seem to demonstrate that the PD-L1 22C3 expression could help to identify CIS that fail the BCG therapy, supporting the hypothesis that enhanced levels of intratumoral PD-L1 22C3 expressed by the tumor cells may cause the antitumoral efficacy of BCG immunotherapy to wane, thereby permitting CIS to recurrence and potentially to progress to more invasive forms of cancer.

NUCLEAR RE-LOCALIZATION OF EMERIN AS A MARKER OF NUCLEAR MEMBRANE INSTABILITY AND BIOMARKER OF PROSTATE CANCER MALIGNANCY

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Background. Prostate cancer is the most common solid cancer affecting men in the EU representing 25% of all male new cancer cases diagnosed [1]. Gleason score, based on morphologic features (such as glandular architecture and variation in nuclear shape and size), alongside changes in chromatin amount and distribution, remains the basic criteria for microscopic diagnosis and the best predictors of prostate cancer outcome. Furthermore, the prognosis of patients is mainly dependent on the presence or absence of distant metastasis [2].
Nuclear aberrations have long been a part of the histopathological features used in diagnosis and stratification of cancer, and their importance in prostatic cancer have been well recognized, and as the roles of the nuclear envelope components are being deciphered in molecular detail there are chances to develop the ability to predict accurately, at the time of diagnosis, which patients are likely to develop metastatic prostate cancer in order to optimize disease management with adjuvant therapy.
One of the mechanisms involved in this transformation is characterized by a reduction and/or mislocalization of the inner nuclear membrane protein, Emerin. Consistent with this, depletion of emerin provokes nuclear shape instability and promotes metastasis. By visualizing Emerin localization, evidence for nuclear shape instability was observed in cultured tumor cells, in experimental models of prostate cancer, in human prostate cancer tissues, and in circulating tumor cells from patients with metastatic disease [3].
Additionally, the identification of patients in need of aggressive follow-up is complicated by the challenging

multifocal nature of the disease which is one of the most confounding and complex factors. In fact, the individual prostate cancer foci may have different aggressiveness and progress independently of one another.
The other important aspect of tumor heterogeneity is the relationship between primary and metastatic tumors, which can impact not only disease progression but could also influence the clinical course of the disease [4]. Thus treatment of patients still remains a difficult decision-making process that requires physicians to balance clinical benefits, life expectancy, comorbidities, and treatment side effects.
Direct detection of nuclear envelope irregularities by labeling nuclear membrane proteins, such as emerin, has resulted in a more Objective. definition of the shape of the nucleus.
Tracking the abundance of molecular components of the nuclear envelope could potentially be used for the prognostic assessment of cancer patients.
Methods. In this study we performed a quantitation of Emerin mislocalization using a bioinformatic workflow. Our principle cohort is comprised of 352 patients, that received a prostatectomy at Vanderbilt University Medical Center between 2000-2012. Immunofluorescent staining was performed on a TMA generated using prostatectomy tissue. The TMA contains normal, tumor and LN tissue along with control. An image analysis and informatics pipeline detected and quantified the appearance of distinct Emerin-positive particles in tumor tissues associated with nuclear membrane instability. The hypothesis that nuclear membrane instability quantified in this manner associated with the presence of metastatic disease was further tested in a cohort of 36 men that presented with metastatic disease at time of biopsy (and did not receive a prostatectomy).
Results. While nuclear membrane instability, as defined by the presence of Emerin-postive particles, was evident in tumor tissue, patients with a Gleason score ≥4 often exhibited a decreased amount of detectable nuclear Emerin positive. The loss of nuclear Emerin suggests that nuclear membrane instability causes Emerin the be shed from the nucleus. This was not true for the nuclear protein Lamin A. The correlation between loss of Emerin and aggressiveness of the disease and its metastatic potential could lead to the finding of a potential role of Emerin as metastasis suppressor.
Conclusions. The Results identify Emerin as a mediator of nuclear shape stability in cancer and show that the use of NMI as a biomarker could have a role in screening, diagnosis, risk stratification and treatment for this disease. Considering the novelty of this observation, this study represents a reference study for prostate cancer researchers which can be used as a starting point to plan new studies.

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TESSUTI MOLLI

UTILITY OF 13Q14 DELETION IN THE DIAGNOSIS OF SOFT TISSUE TUMORS

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Aim.s. Myofibroblastoma, spindle cell lipoma and cellular angiofibroma are three relatively uncommon benign soft tissue tumors with overlapping morphological and immunohistochemical features. They also share the same chromosomal anomaly: the loss of material from the 13q14 region. Based on these observations, it is likely that these tumors represent morphological variations in the spectrum of a single biological entity: the "*13q14 family of tumors*". Although the diagnosis of each single lesion is usually straightforward when all the characteristic features are well represented, diagnostic problems may arise if tumors exhibit unusual morphology. The Aim. of this paper is to emphasize the diagnostic utility of FISH analysis in 4 diagnostically challenging soft tissue tumors belonging to the *13q14 family of tumors.*
Materials and methods. The cases were recovered from the archives and the consulting activity of Anatomic Pathology of University of Catania. The following cases were selected: i) case n.1: a lipomatous tumor with ambiguous morphological features; ii) case n.2: a bland-looking spindle cell tumor with minimal lipomatous component; iii) case n.3: a small round cell tumor with myofibroblastic differentiation arising of the oral cavity; iv) case n.4: a small round cell tumor with myofibroblastic differentiation arising in forearm. Clinical data were obtained from the original pathological reports. For each case, sections stained with Hematoxylin & Eosin (EE), as well as a variable number of paraffin-embedded blocks were available. All slides stained with E.E. have been revised to confirm the original histological diagnosis. F.I.S.H. analysis for the deletion of the *FOXO1* gene located on the 13q14.11 region was performed. A specimen was interpreted as deleted if only 1 fusion signal was detected in more than 22% of the nuclei evaluated. One positive (spindle cell lipoma) and 1 negative control (normal tissue) were included.
Results. Case1.
A 26 year-old woman presented an inguinal mass measuring 4 cm in greatest diameter. This tumor was composed of lobules of mature adipocytes set in a myxoid stroma containing univacuolated lipoblasts, as well as a prominent plexiform network of arborizing capillary-like vessels (plexiform-type vascular pattern; "crow's foot"

vascular pattern). Based on these features the diagnosis of myxoid liposarcoma was originally rendered. Although a variable number of mature adipocytes can be seen in myxoid liposarcoma, however its typical cellular component, i.e. non-lipogenic small-sized round to short spindle-shaped cells, was lacking in our case. In addition, after a meticulous search, it was possible to identify small areas that were closely reminiscent of spindle cell lipoma. These areas consisted of bipolar, short spindle cells set in a myxoid stroma containing rare ropey eosinophilic collagen fibers, as well as rare multinucleated floret-like cells. Immunohistochemically the short spindle cells were stained exclusively with CD34 and focally with CD10 and bcl2-protein.
Case 2.
Tumor consisted of bland-looking spindle cells, with the exception of a single area (less than 1 mm) containing mature adipocytes. The cells were arranged in fascicles with interspersed ropey or keloid-like collagen fibers. Immunohistochemistry revealed a diffuse positivity exclusively for vimentin and CD34.
Cases 3 and 4.
Although tumors were located, respectively, in the oral cavity of a 15 year-old girl and in the subcutis of the forearm of a 62 year-old man, both were composed of bland-looking, small-sized rounded to ovoid cells variably set in a predominant loose myxo-edematous to focally fibrous stroma. These cells were admixed with a minority (3%) of small-sized epithelioid or short spindle cells. The most striking feature was the presence of numerous round- to stellate-shaped thick hyalinized collagen bands, sometimes resembling amianthoid-like fibers. In addition keloid-like collagen fibers were scattered among neoplastic cells. Numerous small- to medium-sized thin-walled blood vessels were also seen. Mitoses ranged from 1 up to 6 per 10 HPF (high power field). Nuclear atypia, atypical mitoses and necrosis were absent. Immunohistochemically the neoplastic cells showed a fibro/myofibroblastic profile with staining for vimentin and focally for desmin.
F.I.S.H. analyses: in all cases, tumors exhibited monoallelic loss of *FOXO1/13q14* loci as indicated by the presence of 1 fusion red/green signal, supporting the hypotesis that these tumors are part of "*13q14 family of tumors*". Based on the morphological and immunohistochemical features the tumors were classified as follows: i) case n.1: *"spindle cell lipoma with atypical features, including lipoblasts and plexiform-type vasculature*"; ii) case n.2: "*low-fat spindle cell lipoma*"; iii) case n.3: "*small round cell myofibroblastoma of the oral cavity*"; iv) case n.4: "*small round cell myofibroblastoma of the forearm*".
Conclusions. The present study emphasizes the diagnostic utility of F.I.S.H. analyses for 13q14 deletion in the recognition of benign soft tissue tumors with unusual morphology, such as spindle cell lipoma and myofibroblastoma, which can be confused with other spindle or round cell malignant tumors.

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UNUSUAL SITES FOR SOLITARY FIBROUS TUMOR: A SERIES OF 7 CASES

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Objectives. Solitary fibrous tumor (SFT) is a relatively uncommon soft tissue fibroblastic tumor first described in the pleura. In the last two decades, there was increasing evidence that this tumor may arise ubiquitously from somatic and deep soft tissues, as well as from parenchymal organs [1 2]. In the majority of cases the histologic diagnosis of SFT is straightforward, but some difficulties may occasionally arise, due to the wide variability of its cyto-architectural patterns, especially when it arises at unusual anatomic sites. Most SFTs are histologically benign, but a minority (5%) may show an aggressive clinical course with local recurrence and/or distant metastases [3]. We herein report a series of 7 cases of SFT, including benign and malignant tumors, arising at unusual sites, focusing on diagnostic difficulties in making the correct diagnosis. The role of STAT6 as a reliable immunomarker is emphasized.
Materials and methods. The cases were recovered from the archives and the consulting activity of Anatomic Pathology of University of Catania. A total of 7 cases of SFT (5 histologically benign and 2 malignant) arising from the breast parenchyma, kidney, oral cavity and retroperitoneum were selected. Clinical data were obtained from the original pathological reports. For each case, sections stained with Hematoxylin & Eosin (EE), as well as a variable number of paraffin-embedded blocks were available. All cases were tested with STAT-6 antibodies to confirm the diagnosis.
Results. *Breast tumors*: a 62-year-old woman underwent radiological screening for breast carcinoma. Mammography and ultrasound revealed a single, well-circumscribed nodule measuring cm 2 in greatest diameter. A 81-year-old man presented a nodule with pushing margins, measured 3 cm in greatest diameter. In both cases a needle core biopsy was performed showing a proliferation of CD34-positive spindly cells set in a fibrous stroma containing medium-sized blood vessels with hyalinization of their walls. Surgical excision was advised. In both cases, histological examination revealed a cellular tumor with the characteristic features of SFT, but lacking the typical branching vascularization. The diagnosis was SFT was rendered based on the diffuse expression of STAT-6, CD34, CD99 and bcl2.
Kidney tumors: a 31-year-old woman presented a mass of her right kidney, which at radiological imaging was consistent with a renal cell carcinoma. Tumor, measur-

ing 8.6 cm in its greatest diameter, completely replaced the cortex and the medulla of the middle region of the kidney, with compression of the renal pelvis. A 34-year-old woman presented with a 9-cm solid mass at the upper pole of her left kidney. The patient underwent left nephrectomy. A 39-year-old woman presented a 5 cm mass of her left kidney, which compressed the pelvis. The patient underwent left nephrectomy. Tumor was located in the peripelvis soft tissues, closely adherent, but without extension, to the kidney. Histological examination, in all three cases, revealed the characteristic features of SFT: alternating hypercellular and hypocelluar areas composed of bland-looking spindle cells exhibiting a haphazard to focally fascicular or storiform growth patterns, with abundant interspersed thick bundles of hyalinized collagen; ectatic or angulated blood vessels with perivascular hyalinization, some of them with a branching haemangiopericytoma-like pattern were frequently seen. Mitotic activity ranged from 1 to 3 mitoses/10HPF. All tumors were diffusely stained with STA6, CD34, CD99 and bcl-2 protein. Notably one renal case (39-year-old woman) showed an additional component, grossly visible as a well circumscribed nodule measuring 3 cm in greatest diameter, that histologically was represented by a high-grade pleomorphic sarcoma N.O.S., abruptly merging from the surrounding typical SFT. Mitotic activity in this area ranged up 6 mitoses/10 HPF. Only rarely were atypical mitoses seen. Interestingly the sarcomatous component maintained both STAT6 and CD34 expression, along with an unexpected S100 protein diffuse staining. The final diagnosis of "*SFT with sarcomatous dedifferentiation*" was rendered.

Oral cavity: a 54-year-old woman presented a swelling of her left cheek. TC showed a 6 cm solid mass measuring 6 cm in maximum diameter, occupying the left cheek with extension to the pterigo-palatine fossa. The mass was radically excised. Histologically, the tumor was composed predominantly of intersecting short fascicles of tightly packed, bland-looking spindle-shaped cells with a moderate amount of eosinophilic cytoplasm. Mitotic activity was low (1 mitosis/50 HPF), and no atypical mitoses were seen. Cellular pleomorphism, necrosis, and infiltration of surrounding tissues were not observed. Tumor had an overall resemblance to leyomioma. However, the focal detection of a hemangiopericytoma-like pattern, perivascular hyalinization, alternating hypercellular and hypocellular areas, and deposition of dense keloid-type collagen, was more consistent with a SFT. Immunohistochemistry, revealing diffuse and strong staining for STAT6, CD34, CD99, bcl-2 protein, along with no expression of myogenic markers, supported the diagnosis of "*SFT with leiomyomatous-like features*".

Retroperitoneum: a 91-year-old man presented a mass in the retroperitoneum, posterior to and displacing the bladder. The tumor, measuring 15 cm in its greatest diameter, was radically excised. Histological features were consistent with a SFT, but focally neoplastic cells exhibited severe nuclear pleomorphism, high mitotic activity (>4 mitoses/10 HPF) and multiple foci of tumor necrosis. Immunohistochemically the neoplastic cells were positive for STAT-6, CD34, bcl2 and CD99. Based on morphological and immunohistochemical features, the diagnosis of "*malignant solitary fibrous tumor*" was rendered.

Conclusion. The anatomic distribution of SFT is very wide and the differential diagnosis with other potential mimickers is challenging, especially if tumor arises at an unexpected anatomic site. In our opinion this is mainly due to the fact that a lesion that would have been easily diagnosed in its expected clinical context, when present in an unexpected location it might be unrecognized ("Man from Istanbul" by Dr. Lauren V. Ackerman). The present paper emphasizes that the diagnosis of benign or malignant SFT can be rendered confidentially even at unusual sites if pathologist is aware of the possibility that this soft tissue tumor can be ubiquitously encountered. Diffuse nuclear staining for STAT-6 is the most specific immunomarker for supporting the diagnosis [3].

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UNUSUAL MESENCHIMAL LESIONS OF THE SKIN: A CLINICO-PATHOLOGICAL STUDY OF 4 CASES

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Aim.s. Apart from dermatofibroma, mesenchymal tumors or tumor-like lesions of the dermis and subcutis are rare entities [1-3]. Potentially, each soft tissue neoplasm occurring in deep sites could also appear superficially, showing morphological, clinical and prognostic differences. Furthermore, some benign mesenchymal dermal lesions can simulate malignant neoplasms and, on the other hand, malignant neoplasms can show a bland-looking morphology simulating a benign tumor [3]. Accordingly the recognition of rare benign and malignant soft tissue lesions arising primarily in the skin is crucial for assuring patient a correct prognostic information and therapeutic strategy [1-2].

The Aim. of the present paper is to emphasize the diagnostic clues of 4 unusual mesenchymal tumors of the dermis, such as proliferative fasciitis, mammary-type myofibroblastoma, sclerosing epithelioid fibrosarcoma and pleomorphic rhabdomyosarcoma.

Materials and methods. The case were retrieved from the archives of Anatomic Pathology of University of Catania. The following unusual cases were selected: i) one case of proliferative fasciitis; ii) one case mammary-type myofibroblastoma; iii) one case of sclerosing epithelioid fibrosarcoma; iv) one case of pleomorphic rhabdomyosarcoma. Clinical data were obtained, and for each case, sections stained with Hematoxylin & Eosin (EE), as well as a variable number of immunohistochemical analysis and paraffin-embebbed blocks were available.

Results. Case no. 1: a 13-year-old boy showed a

dermal-subcutaneous lesion in the right auricular region, measuring 2.5 cm. Histological examination revealed an intradermal hypercellular lesion consisting of large-sized atypical cells with abundant eosinophilic cytoplasm and one or two vesicular nuclei containing prominent nucleoli; the majority of these cells exhibited a ganglion-like morphology; less frequently the cells showed a spindled morphology. The stroma was predominantly fibrous with focal myxoid areas containing a mild to moderate inflammatory infiltrate. Despite the presence of pleomorphic cells, the mitotic count was low (1 mitosis x 10 HPF). Notably, only after a meticulous search it was possible to identify a single atypical mitosis. A focal extension to subcutis was seen. The cells were diffusely positive for vimentin, and focally positive for XIIIth factor. All other tested antibodies were negative. Based on the morphological and immunohistochemical features, the diagnosis of "*intradermal proliferative fasciitis*" was rendered. The patient is well after a follow-up of 10-years.

Case no.2: a 58-year-old man presented with an oval swelling on the forearm. Histological examination showed a dermal proliferation of bland-looking spindle cells, arranged into short fascicles interrupted by thick keloid-like collagen bands. Mitoses were rare (1 mitosis/10 HPF). Focal extension into subcutis was seen. Neoplastic cells were diffusely stained with desmin, and focally with CD34 and alpha-smooth muscle actin. The diagnosis of mammary-type myofibroblastoma was rendered. Patient is well after 9 years of follow-up.

Case no.3: a 52-year-old man showed a nodular, painless nodule measuring 1 cm, located in the right auricle region. Histologically a proliferation of small- to medium-sized epithelioid cells, with eosinophilic cytoplasm and nuclei with mild pleomorphism, was seen. The cells were set in a predominantly fibrous stroma and were arranged into single cells, nests or linear cords, focally resembling invasive lobular carcinoma of the breast. They were stained for vimentin and focally for EMA and MUC-4, whereas cytokeratins, S-100 protein, p63, GFAP, desmin, myogenin, alpha-actin, beta-catenin, CD31, CD34 and STAT-6 were negative; nuclear staining for INI-1 was maintained. The diagnosis of "sclerosing epithelioid fibrosarcoma" was rendered. Three local recurrences were observed during 5 years of follow-up.

Case no.4: a 35-year-old woman had a cutaneous nodular lesion in the right scapular region, with focal ulceration of the epidermis. Histologically a hypercellular tumor was seen in the dermis with extension into subcutis. Tumor was composed of spindle to epithelioid to rhabdoid cells with severe nuclear pleomorphism, high mitotic activity (16 mitoses x 10 HPF), as well as numerous atypical mitoses. The spindle cells were focally arranged into a fascicular pattern. The collagen fibers and the lymphatic vessels of the dermis were diffusely infiltrated. Immunohistochemically neoplastic cells were diffusely stained with pan-cytokeratins, EMA, vimentin, and INI1, while only a focal expression of desmin and myogenin was documented. No reactivity was obtained with other antiboides. The diagnosis of "*pleomorphic rhabdomyosarcoma of the skin*" was rendered. The patient died after 11 months from surgical excision of diffuse metastatic disease.

Conclusions. In our opinion, the diagnosis of mesenchymal dermo-hypodermic tumors represents a challenge for pathologists because of the numerous overlapping morphological and immunohistochemical features[1-3]. We think that it is the unusual/unexpected site than makes difficult the diagnosis. Pathologist should be aware that proliferative fasciitis can be encountered in the dermis and that rare (no more than one) atypical mitoses are not per se signs of malignancy. In addition there is the possibility that mammary-type myofibroblatoma, sclerosing epithelioid fibrosarcoma and pleomorphic rhabdomyosarcoma may occur superficially in the dermis/subcutis. With regard to pleomorphic rhabdomyosarcoma the possibility of a diffuse expression of epithelial markers should be kept in mind to ovoid a misdiagnosis of metastatic pleomorphic carcinoma. The identification, at least focal, of myogenic markers, is crucial for the correct diagnosis.

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MERCOLEDÌ 16 OTTOBRE 2019

Miscellanea 2

Sala Atene – 15:00 - 17:00

DIGITAL PATHOLOGY

CLINICAL APPLICATION OF A REAL-TIME TELEPATHOLOGY SYSTEM FOR FROZEN SECTION DIAGNOSIS IN COMPARISON WITH OPTICAL MICROSCOPE

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Background. The imbalance between the increasing demand of highly specialized service and the reduction of specialists able to release this service, is a global challenge for Pathology. This situation applies also to the setting of intra-operatory diagnostic: here the broad presence of Surgical divisions contrasts with the contraction of Pathology departments, progressively concentrated in few hospitals. The use of e-pathology device, such as remote-control microscopes, offers a possible solution to this imbalance.
Aim. To prove the non-inferiority of function of a remote-control, real-time microscope named Nano-Eye Device (NED) with the optical microscope (OM) for intra-operatory histological diagnosis.
Methods. The study was designed into two phases: discovery and validation. During the discovery phase features influencing the process of adaptation to NED were investigated in detail, focusing on the turnaround time (TAT). Validation phase investigated the diagnostic concordance between NED and OM; as well as sensitivity, specificity and accuracy of NED in intra-operatory histological diagnosis.
Results. During the discovery phase 250 cases were examined. TAT of NED was longer than that of OM (112 ± 89.8 versus 36 ± 37.9 seconds) and influenced by the difficulty of the specimen, age of pathologist and the type of the specimen. In the validation phase (185 cases) TAT of NED reduced significantly to 92 ± 86.3 sec (p: 0.01). NED showed a concordance rate of 98% with OM; the sensitivity 95.65%), specificity (100%) and diagnostic accuracy (98.87%) of NED were equal to that of OM. NED failed to work in 6% during the discovery phase and 4% in the validation.
Conclusions. Taken as a whole, the functionality of NED is comparable to OM. It can be the alternative choice for hospital lacking on-site pathology services and one of the tool of e-pathology.

IMPROVEMENT OF EXTERNAL INTRAOPERATIVE PATHOLOGY CONSULTATION USING UNMANNED AERIAL VEHICLES (DRONES)

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Intraoperative pathology consultation with frozen section analysis is a key procedure to characterize a lesion, to confirm adequacy of surgical margins and to guide operative decision-making for optimal patient care. Small hospitals that do not reach an adequate workload for an on-site pathology service usually require pathology consultants or, alternatively, specimen transportation by medical cars. Both these solutions, however, incur at great expense in terms of delayed diagnosis and involved personnel. Telepathology represents a valid alternative, but it still requires equipment and technicians in each hospital. Moreover, telepathology does not allow gross examination of the specimen by the pathologist in charge for the microscopic diagnosis.
Recently, drones have emerged as innovative mode of operations to deliver health services such as transport of blood samples for laboratory testing. Our Aim. was to test the feasibility and reliability of drone transportation

Fig. 1. Customized drone during the flight.

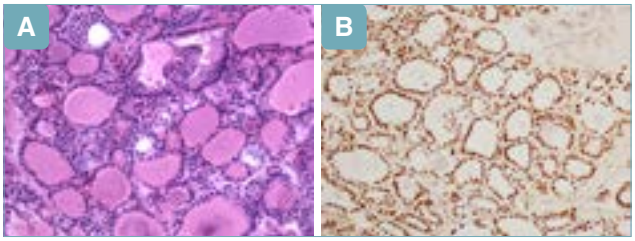

Fig. 2. Thyroid Specimen. Both morphology (a) and immunohistochemical reactivity for TTF1 (b) resulted well preserved in samples transported by drone.

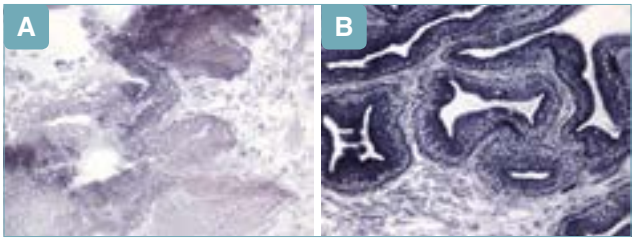

Fig.3. Galbladder specimen. Loss of NADH activity in the mucosal cells is evident in the transported specimens (a) before temperature adjustment in the box. NADH activity is well preserved in a second transported specimen (b) after a more accurate temperature control during the flight.

of surgical samples submitted for intraoperative consultation, and to assess the factors affecting the Results. To carry out the experiment, a commercial drone has been customized to transport biological samples according to current requirements for packaging of samples with biological risk. The monitored parameters were: accelerations, internal and external temperatures, ground and vertical speeds.
We analyzed tissues from different organs by evaluating morphological features as well as immunohistochemical and enzymatic properties of frozen samples after the flight, compared with corresponding samples stored at similar conditions but without transportation. Our Results demonstrated similar preservation of morphological features and immunohistochemical reactivity in flown and stationary sample pairs, while a reduction in enzymatic activity was evidenced in two out of seven transported specimens. Adjustment of temperature stability inside the box during drone transportation significantly improved the enzyme activity preservation.
Our study is, to our knowledge, the first oriented to develop novel technological solution able to exploit the use of autonomous vehicles in the field of frozen section diagnosis. Based on these observations, customized drones with adequate transportation properties may represent a valid alternative in overcoming problems related with people's mobility and, at the same time, dramatically reduce operational and environmental costs.

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A DIGITAL PATHOLOGY SYSTEM IT'S NOT JUST TO POSSESS A SCANNER
THE ANATOMIC PATHOLOGY OF BIELLA EXPERIENCE WITH WHOLE SLIDE IMAGING FOR PRIMARY HISTOPATHOLOGICAL DIAGNOSIS

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Objectives. The adoption of a digital pathology system (DPS) for primary diagnosis is being tested at the Anatomic Pathology of the Biella hospital. Digital pathology is a process by which a microscope slide is scanned and virtualized into a digital image file that pathologists can then examine, even remotely, on a computer screen for routine reporting. In this context, it is important to define the terms, a DPS it is not to just a scanner, it is the complete integration between the laboratory information system, a scanning software, the whole slide image (WSI) repository and the web platform that allows virtual slides to be shared through the internet. The Aim. of this project is twofold. Firstly, to assess whether this technology is suitable to safely and accurately perform routine diagnostic activities of a medium-size surgical pathology department, as already tested by others [1]. Secondly, with respect to the histology laboratory, how this technology fits into the workflow, to what degree it can determine a work overload and how it can contribute to quality control in the pre-analytical and analytical phase [2]. The long term target is to verify whether digital pathology can change the pathologists frame of mind to improve the attitude toward a real time, constant interaction through a network of colleagues, ready to expand their knowledge base in order to optimize speed, accuracy and quality. This latter aspect is relevant because it is well known that in Italy 81% of pathologists will leave work in the next seven years and right now there is a severe shortage of pathologists, especially in certain subspecialty branches.

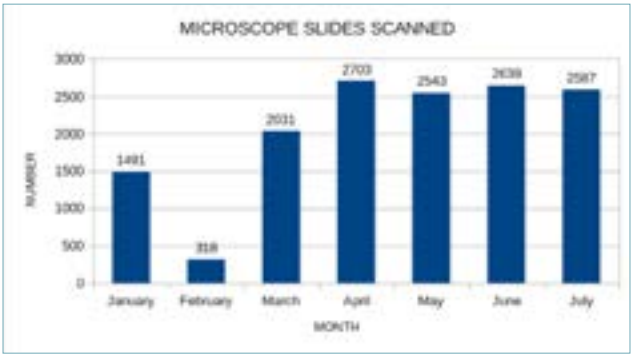

Fig.1. Number of microscope slides scanned.

Materials and methods. The ASL-BI Anatomic Pathology department handles about 10.000 specimens annually in a a hospital with 482 beds. Our histopathology laboratory is now equipped with a Nanozoomer-XR high slide capacity scanner (Hamamatsu Photonics K.K., Japan) able to rapid automatic processing of up to 320 slides, dynamic focus and image quality judgment functions. This instrument was interfaced with the Winsap laboratory information system (Engineering, Torino, Italy) by a barcoded string module that allows the download of slide data to the scanner software. Scanned slides were saved as ndpi files in a network attached storage (NAS) with a 20 TB capacity (Dell EMC2). A web platform (Cloud Pathology Group, Milan, Italy) for remote access to virtual slide trays was permitted by a specifically designed server that allowed case searching, selection and whole slide imaging (WSI) with a viewer that enabled histological representation of up to a 40X resolution. Access to the web served virtual slides was independent from the geographical location of the workstation, it was platform independent from the point of view of the operating system (Windows, Apple, Linux etc.) and the kind of browser used (Google Chrome, Mozilla Firefox, Internet Explorer, Microsoft Edge, Opera were tested). Each operator used dual monitor workstations one of which was a 27" Barco display (Kortrijk, Belgium) with a 3280 x 2048 resolution. The scanner and its workstation was placed at the end of the histol-

Fig.2. Storage space used.

ogy laboratory workflow to optimize slide loading on the racks and focusing options settings by each operator. Image processing for nuclei counting on IHC slides and for measurements were performed with the options available in the viewer. External public domain software such as Imagej with plugins such as Immunoratio (http://wsiserver.jilab.fi/old-jvsmicroscope-software/) was also used.

Results. When it was decided to implement this technology, the first problem we faced was the added workload on lab technologists. A series of new details for a correct digital scanning of microscope slides emerged as critical factors: the uneven thickness of a paraffin section, bubbles, incorrect coverslip position, defective printing of the Datamatrix code on the labels and selection of the correct focus hot spots on tissue sections. In our hands, when more than 200 slides a day were to be scanned, the workload on the single operator could be up to 5 hours. If this first step has been correctly accomplished the percentage of rescans can be lower then 1% for slide batch. Most of the slides were scanned at a 20X resolution with few exceptions, like special stains for Helicobacter Pylori. That resolution was considered more than sufficient for over 90% of specimens. In a few cases, when a second opinion was required, a 40X resolution was deemed necessary for a conclusive diagnosis. In our hands, a constant interaction between lab technicians and pathologists is indispensable for determining the appropriate levels of quality in whole slide scanning. The second problem that emerged in the systematic use of our DPS was the response time, when virtual slide fields were accessed randomly or when magnification was changed. That was much influenced by the load of LAN and WAN networks. The average final rendering time on screen that we experienced was between 2 and 5 seconds. When testing phase was over and our DPS was fully operational, systematic scanning of all microscope slides performed with a throughput of around 2500 virtual slides generated. That means a reserved memory space of 1400 GB/month that, when extrapolated out to a full year, amounts to about 16.8 TB of network storage.

Conclusions. None of the problems we faced became a real obstacle for the primary diagnosis of routine histology and all pathologists became more and more confident with remote WSI diagnosis even from home. The availability of a DPS gave to our workflow incomparable advantages that significantly improved the quality of daily routine. One, and overall the most important, was to be able to histologically study the history of a single patient by accessing in a few seconds to the histology of all past biopsies and surgical specimens of that case. This was perceived by all pathologist of our department as a sort of Copernican revolution, a paradigm shift in histopathology. Second opinion were possible almost in real-time with the same effort of a phone call. Digital image analysis of prognostic and predictive markers was not a high technology whim, but a tool to achieve more solid and reproducible Results. It is quite evident that there still are challenges, such as higher bandwidth requirement, reliability of the operating systems, constant assistance from the IT departments. These, however, are not an obstacle that cannot be resolved or that could hinder investments in a DPS now and in the near future.

Acknowledgments. This work was supported by the "Fondazione Edo ed Elvo Tempia" and the "Fondazione Cassa di Risparmio di Biella"

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NEW DIRECTIONS FOR THE ASSESSMENT OF THE PROLIFERATIVE COMPARTMENT OF ORAL SQUAMOUS CELL CARCINOMA BY IMAGE ANALYSIS ON HEMATOXYLIN-EOSIN STAINED SECTIONS.

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Introduction. The assessment of the proliferative compartment is widely used to stratify the risk of malignant solid tumors. To this Aim., one among the most used tools in surgical pathology, is the IHC labelling index for the cell proliferation marker Ki67/MIB1[1].The prognostic value of this marker, however, is often hampered by the extreme heterogeneity of the cut-off values used to stratify risk categories, this making the proliferation data based on Ki67/MIB1 immunostaining often weakly reproducible[2]. To date, the prognostic role of Ki67 in Oral Squamous Cell Carcinoma (OSCC) is still a matter of debate by scientific community[3]. We developed a new algorithm to evaluate the proliferative tumor cells fraction, based on the application of QuPath[4], an open-source software, on OSCC Hematoxylin and Eosin stained (H&E) digitalized histological sections.

Materials and methods. For each OSCC FFPE sample, we used two consecutive sections, respectively stained with H&E and immuno-stained for Ki67/MIB1. We performed the automatic positive and negative cells recognition on Regions of Interest (ROIs) in Ki67/MIB1 whole slide images, using QuPath integrated algorithms, extracting a total of 33 features. Among them, Hematoxylin Optical Density (HOD) resulted as the best feature to distinguish Ki67/MIB1 positive from negative cells. As

a second step, we automatically detected tumor cells in H&E slides within the same ROIs, generating a "false colors map", based on HOD through QuPath measurements map tool, calculating then the predicted percentage of positive cells.
Results and conclusion. "False colors map" nearly coincided with the actual immunohistochemical slide Results, allowing the prediction of Ki67/MIB1 positive cells in selected ROIs, giving the pathologist a fast method to identify the proliferating compartment of the tumor simply through the quantitative assessment of H&E slides. Although this technique needs to be finely-tuned and tested on larger series of tumors, the digital analysis approach appears as a promising tool to quickly forecast the tumor's proliferation fraction on simple H&E stained digital section.

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PATOLOGIA MOLECOLARE

MOLECULAR EPIDEMIOLOGY OF EGFR, KRAS, BRAF, ALK, AND CMET GENETIC ALTERATIONS IN SARDINIAN PATIENTS WITH LUNG ADENOCARCINOMA

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Background. Lung cancer is the most incident neoplastic disease, and a leading cause of death for cancer worldwide. Knowledge of the incidence of genetic alterations, their correlation with clinical and pathological features of the disease, is nowadays crucial for selecting the best clinical management of patients with lung adenocarcinoma. Our study Aim.s to describe the molecular epidemiology of genetic alterations in five driver genes and their correlations with the demographic and clinical characteristics of Sardinian patients with lung adenocarcinoma.
Methods. 1,440 consecutive Sardinian patients with a histologically proven diagnosis of lung adenocarcinoma made from January 2011 through July 2016 were included in the study. *EGFR* mutation analysis was performed with Sanger sequencing and Pyrosequencing for all of them, while *KRAS* and *BRAF* mutations were searched in 1,047 cases; *ALK* alterations were determined with fluorescence in situ hybridization in 899 cases, and cMET amplification in 788 cases. Testing was dictated by the gradual introduction of the single analyses in clinical practice, and the availability of sample tissues for testing.
Results. Nine-hundred-sixty-three (67%) of the 1440 patients enrolled were males, and the mean age was 66 (range 30-88). *KRAS* mutations were the most common genetic alterations involving 22.1% of the cases and being mutually exclusive with the *EGFR* mutations, which were found in 12,6% of them and were significantly more frequent in females and never smokers. *BRAF* mutations, *ALK* rearrangements, and *cMET* amplifications were detected in 3.2%, 5.3%, and 2.1% of the cases, respectively. Concomitant mutations were detected only in a few cases. **Conclusions.** Almost all the genetic alterations studied showed a slightly lower incidence in comparison with other Caucasian populations. Concomitant mutations were rare, and they probably have a scarce impact on the clinical management of Sardinians with lung adenocarcinoma. Most patients did not present any genetic alterations in the genes evaluated, therefore further studies with newer approaches are necessary to establish novel therapeutic targets.

VALIDATION OF A FULLY-AUTOMATED DEVICE FOR THE ASSESSMENT OF *BRAF* P.V600E AND *NRAS* ALTERATIONS IN THYROID FINE-NEEDLE ASPIRATES: TOWARD A SAME-DAY CYTOLOGICAL AND MOLECULAR DIAGNOSIS FOR A RAPID AND TAILORED TREATMENT

C. De Luca[1], S. Campione[2], R. Sgariglia[1], G. Gragnano[1], M. Nacchio[1], F. Conticelli[1], M.L. Maresca[1], P. Galasso[1], F. Pizzo[1], P. Pisapia[1], G. De Dominicis[2], C. Bellevicine[1], U. Malapelle[1], E. Vigliar[1], G. Troncone[1].

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Aim. Fine-needle aspiration (FNA) represents a relevant diagnostic tool for patients affected by thyroid nodules. This diagnostic method is safe, well tolerated, economic and rapid. In fact, thanks to rapid-on site evaluation (ROSE) of the aspirated material, when a dedicated cytopathologist is available, a same-day FNA diagnosis can be rendered, relieving the patient anxiety and achieving high diagnostic accuracy [1]. Unfortunately, up to 30% of thyroid FNA are classified as indeterminate by microscopy alone, hampering the possibility for the patient to rapidly obtain a definitive actionable diagnosis. [2]. Although several molecular assays are available to better stratify the risk of a patient with an indeterminate FNA diagnosis [3], these tests are designed to improve the diagnostic certainty of thyroid cytology. This review summarizes the early published experience with the commercially available versions of these tests: the Afirma Gene Expression Classifier, ThyGenX (formerly miR-Inform these solutions take usually long time, require dedicated operators and are not always available at the clinics were the FNA are taken. The introduction of a

fully automated molecular device (Idylla™) [4], may allow a same-day rapid on-site molecular evaluation (ROME) by assessing the most frequent oncogenic alterations occurring in thyroid neoplasms (i.e. *BRAF* p.V600E and *NRAS*) along with ROSE.
This study Aim.s to verify the technical feasibility of a ROME by using FNA leftovers.
Materials and methods. The study is carried out in two steps to validate the ROME: during the first step, we analyzed, using Idylla™ *NRAS-BRAF* cartridge, n=25 FNA simulations on thyroid surgical specimens and their corresponding formalin-fixed paraffin-embedded (FFPE) histological sections, to compare the mutational status.
In the second step we analyzed n=25 extracted DNA from thyroid FNA, collected in a vial filled with a preservative nucleic acid solution after the preparation of the smear(s) for the microscopy. In this set of FNA, the DNA extracted from the vials were previously tested by a validated molecular technique and further processed the Idylla™ cartridge in order to verify the mutational status of each FNA.
Results. The Results obtained in the first step showed an high sensibility and specificity with a fully agreement between the simulated FNA and matching FFPE sections. In particular, 12/25 cases were mutated (10/25 *BRAF* p.V600E and 2/25 *NRAS* p.Q61R/K). In the second step the test performed on real thyroid FNA showed a perfect reproducibility between the standard molecular assay and Idylla™ system.
Conclusions. In conclusion, the fully automated molecular device Idylla™ can perform a rapid on-site molecular evaluation in thyroid FNA and may be used for the same-day molecular test of intederminate cases also in centers without expertise in molecular pathology, relieving the patient anxiety and allowing a rapid and tailored treatment.

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FEASIBILITY OF THE IDYLLA *EGFR* MUTATION ASSAY ON ARCHIVAL STAINED CYTOLOGICAL SMEARS: A PILOT STUDY

G. Gragnano[1], C. De Luca[1], F. Conticelli[1], A. Leone[2], M. Salatiello[1], P. Galasso[1], P. Pisapia[1], G. Di Maiolo[1], M.L. Maresca[1], L. Rosalba Grillo[2], A. Iaccarino[1], C. Bellevicine[1], U. Malapelle[1], E. Vigliar[1], G. Troncone[1]

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Aim. The Idylla epidermal growth factor receptor (*EGFR*) Mutation Assay has been designed and validated to process formalin-fixed paraffin-embedded sections without the necessity of a preliminary step involving DNA extraction [1 2]. This study focused on the possibility to scrape directly into the cartridge the cellular material from archival smears from patients with non-small cell lung cancer (NSCLC) [3].
Materials and methods. The study was carried out into two parts. Firstly, we performed the Idylla *EGFR* Mutation Assay on previously genotyped archival stained smears from n = 39 NSCLC patients. In this series, n = 14 cases harbored an *EGFR* mutation (n = 11 *EGFR* exon 19 deletions and n = 3 *EGFR* exon 21 point mutation), detected by fragment length or TaqMan assays [4]. Subsequently, we analyzed whether de-staining of the smears could reduce Background. fluorescence.
Results. In n = 11 cases (78.6%) the Idylla *EGFR* Mutation Assay correctly detected the presence of *EGFR* mutations. On the overall, the concordance among different methodologies was higher for *EGFR* exon 19 deletions (10/11) than for *EGFR* exon 21 p.L858R. This latter was underestimated due to a high Background. fluorescence in channel 2, where the *EGFR* exon 21 p.L858R mutation was identified. This limitation was in part reduced by de-staining the smears.
Conclusions. This study showed the feasibility of the Idylla *EGFR* Mutation Assay on archival stained smears without the necessity of a preliminary DNA extraction [3]. Further studies are required on de-stained material in order to reduce problems related to the Background. fluorescence.

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DESIGN OF A QUANTITATIVE 4-MULTIGENE EXPRESSION ASSAY FOR THYROID NEOPLASM ON CYTOLOGICAL SAMPLES

M. Nacchio, R. Sgariglia, I. Migliatico, F. Pepe, P. Pisapia, E. Clery, E. Vigliar, C. Bellevicine, U. Malapelle, G. Troncone

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Aim. Thyroid nodules cytological diagnosed as indeterminate need further molecular assessment to better define the risk of malignancy (ROM) and the necessity to adopt lobectomy instead of total thyroidectomy.[1, 2] In our experience, we performed molecular analysis on indeterminate nodules (Tir3A and Tir3B, following the Italian

consensus for the classification and reporting of thyroid cytology [3]) by using DNA and RNA extracted from an aliquot suspended, after the fine needle aspiration (FNA) procedure, into a vial of nuclease-free water (Invitrogen Ambion; Thermo Fisher, Waltham, Massachusetts). The vial is then stored at -20°C until the final cytological diagnosis is available. The nucleic acid obtained from the vial is analyzed with a real-time polymerase chain reaction (RT-PCR)-based procedure with the EntroGen Thyroid Cancer Mutation Analysis Panel Kit (EntroGen, Inc, Woodland Hills, California), which covers *BRAF* p.V600E; *KRAS* codon 12 and 13, *NRAS* codon 61, and *HRAS* codon 12, 13, and 61 point mutations; and *RET*/*PTC1* (fusion between *RET* and *CCDC6* genes), *RET*/*PTC3* (fusion between *RET* and *NCOA4* genes), and *PAX8*/*PPARg* fusions.[4] In order to avoid the possibility that the analysis were carried out in absence of epithelial cells, a relevant role was played by the analysis of expression of different genes normally expressed in thyrocytes. The Aim. of the study was to validate a TaqMan assay for the expression of different genes normally expressed in thyrocytes on RNA extracted from cytological material suspended into the vials.

Materials and methods. By using Primer Express Software (Thermo Fisher Scientific, Waltham, MA) 8 primers pairs and relative (n = 4) TaqMan Probes to analyze the mRNA expression level in parathyroid hormone (PTH), tireoglobulin (Tg), paired box 8 (PAX8), calcitonin (CALCA) were designed. In addition to normalize the obtained Results a commercially available G6PDH assay (cod. Hs00188728_m1, Thermofisher) was carried out. Each probe was florescent labeled with FAM fluorophore by using Minor Grow Binding compost (MGB) as a quencher. To verify the specificity of mRNA level detection, the RNA extracted from 5 cytological samples, positive for each gene considered and one negative control, previously evaluated by using immunohistochemistry (IHC) were analyzed.

Results. The preliminary Results showed that in each positive sample, assessed by IHC, a specific amplification curve was obtained, while in negative control, the absence of amplification was reported. In Figure 1 an example of positive sample (CALCA) and negative one was reported.

Conclusions. Basing on preliminary Results, our quantitative 4-multigene expression assay for thyroid neoplasm on cytological samples can be prospectively evaluated in routine diagnostic setting to complete the mutational status assessment in order to avoid false negative Results.

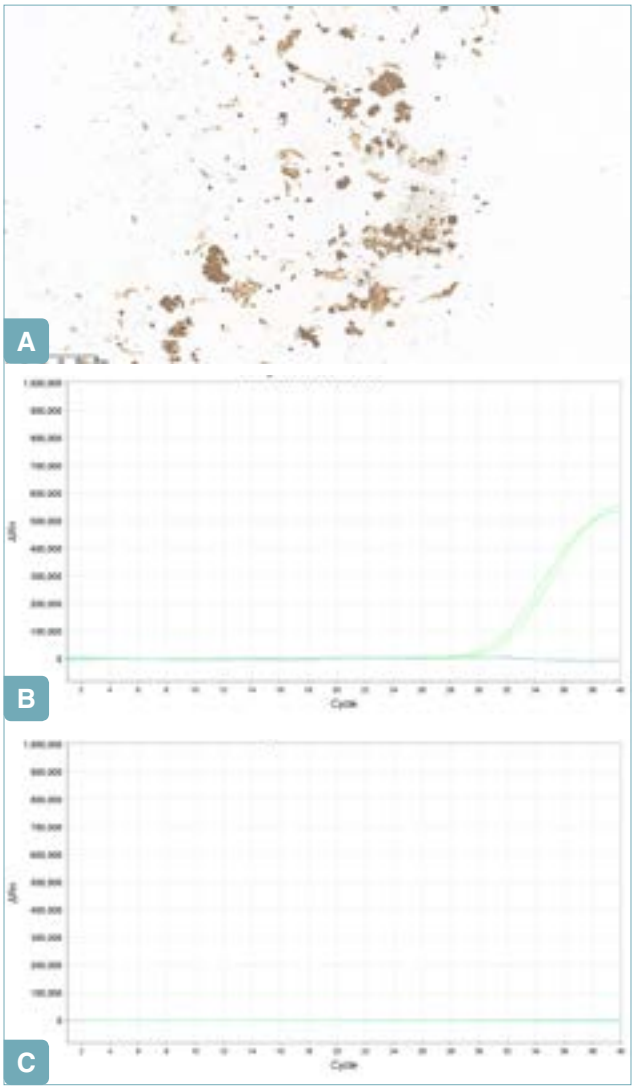

Fig. 1. In the figure was reported an example of positive IHC (A) and RT - PCR (B) sample for CALCA and RT - PCR amplification plot for negative control (C).

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EVALUATION OF A NOVEL LIQUID BIOPSY-BASED COLOSCAPE ASSAY FOR MUTATIONAL ANALYSIS OF COLORECTAL NEOPLASIA AND TRIAGE OF FIT+ PATIENTS: A PILOT STUDY

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Aim. Circulating cell free tumor DNA showed an increasing clinically value and was useful for cancer detection and monitoring after target treatments [1]. To

date, in Europe the primary screening test for colorectal cancer (CRC) identification is represented by the Fetal Immunochemical Test (FIT) [2] When FIT featured a positive result (FIT+), patients undergo to immediate colonoscopy [3]. The positive predictive value (PPV) is usually 25%. In this study, we report our experience using the ColoScape assay panel in FIT+ patients in order to identify mutations in the *APC*, *KRAS*, *BRAF* and *CTNNB1* genes [4].

Materials and methods. We performed this assay on n = 52 prospectively collected whole-blood samples obtained from FIT+ patients. These patients were enrolled in the CRC screening programme carried out in ASL NAPOLI 3 SUD. In all cases colonoscopy was available.

Results. On the overall, the ColoScape assay panel showed a sensitivity for advanced adenomas of 53.8% and a specificity of 92.3%. The PPV was 70.0% and negative predicitive value (NPV) was 85.7%. Interestingly, a less than suboptimal DNA input led to n = 4 of the six positive cases missed by ColoScape. Of note, an increase in sensitivity (53.8% to 69%) can be obtained when considering these cases as inadequate.

Conclusions. In conclusion this study showed that ColoScape is a fascinating and promising tool for CRC screening, and further studies are warranted in order to validate its use for the triage of FIT+ patients [4].

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A SECOND WORLDWIDE RING TRIAL ON IMPROVED QUANTITATIVE CYTOLOGICAL MOLECULAR REFERENCE SPECIMENS: FOCUS ON NEXT GENERATION SEQUENCING

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Aim. Artificial cytological molecular reference specimens were generated in a cytocentrifuge/cytospin format with well-annotated genomic data. In a previous study, these samples showed their feasibility on next generation sequencing (NGS) platforms [1]. To better reflect the challenges of cytological material, in this "second round" of the study, slides were optimized to contain a lower number of cells (2×10^5) and to be stained by the different laboratories before DNA extraction [2 3].

Materials and methods. Seventeen worldwide laboratories received and analyzed, following their standard workflow, n = 4 cytological molecular reference slides (slides A-D) harboring mutations at different allelic frequency (AF; 10%, 5%, 1% and wild-type). Each slide was engineered to harbor the following mutations: epidermal growth factor receptor (*EGFR*) c.2235_2249del15 p.E746_A750delELREA, *EGFR* c.2369C>T p.T790M, Kirsten rat sarcoma viral oncogene homolog (*KRAS*) c.38G>A p.G13D, and B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) c.1798_1799GT>AA p.V600K.

Results. Slides A and B (10% and 5% AFs) showed a high reproducibility on *EGFR* and *KRAS* mutation detection among laboratories. Conversely, a lower concordance was reported on slide C (1% AF). In fact, either *EGFR* or *KRAS* mutations were undetected by 10 of the 17 laboratories (58.82%). Subsequently, Results were revised by laboratories after visual inspection of the generated reads. Ten mutations, that had been previously missed in the first-look analysis, were correctly detected. On the other hand, *BRAF* c.1798_1799GT>AA p.V600K showed a lower concordance rate, in particular in the first-look analysis, for any AF due to the presence of an endogenous high frequency *BRAF* c.1799T>A p.V600E [3].

Conclusions. Cytological smears are unique specimen preparations that cannot be reproduced or replaced once they have been sacrificed for molecular testing [4]. Our data, on artificial genomic reference standards showed a high degree of concordance in the detection of mutations at 10% and 5% AFs, whereas the detection of low-abundance mutations is still challenging and may require a visual inspection of sequencing reads.

However, artificial cytological molecular reference specimens may replace cytological samples as a valid tool to evaluate consistency and reproducibility of NGS Results on cytological material.

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A MULTICENTER STUDY EVALUATING THE PERFORMANCE OF SIRE® PANEL IN CLINICAL SETTING

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Aim. Predictive molecular pathology plays a key role in the management of cancer patients for the administration of target therapies.[1, 2] In this setting, next generation sequencing (NGS) represents a fascinating tool for that allow the analysis of different targets for several patients, simultaneously.[3, 4] A relevant issue is represented by the implementations of this novel technology in the different laboratory workflows. The Aim. of this study was the evaluation of the analytical and clinical performance of a laboratory developed panel (SiRe®) among different laboratories on routine colon and lung cancer specimens.

Materials and methods. Four Italian laboratories were enrolled in our study. All of them received the SiRe® NGS kits. These latter consisted in the primer pool covering n = 42 amplicons (568 actionable mutations in 6 genes) and a dedicated protocol for library preparation and sequencing run on Ion Torrent (Thermo Fisher Scientific, Waltham, MA) platforms.[5] A total n = 60 Formalin Fixed Paraffin Embedded specimens from the routine were analyzed by the different laboratories. Among these samples, n = 20 from each laboratories were selected and distributed to the centers. These latter were adopted to assess the inter-laboratory reproducibility rate.

Results. On the overall, a high analytical performance in terms of sequencing run parameters of the SiRe® panel was evaluated on the total n = 240 colon and lung cancer samples among all laboratories joining the study. In addition, a high inter-laboratory agreement (100%) was obtained among the set of shared samples. In particular, we assessed that the concordance rate relative to the allelic frequencies distribution, as measured by ICC, was 0.989.

Conclusions. SiRe® NGS panel represents a robust and valid diagnostic tool in colon and lung cancer routine setting.

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DESIGN OF A NEXT-GENERATION SEQUENCING CUSTOM GENE PANEL FOR THE RISK STRATIFICATION OF INDETERMINATE THYROID FINE NEEDLE ASPIRATES

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Aim. Fine-needle aspiration (FNA) is an accurate and cost-effective tool in the evaluation of thyroid nodules.[1, 2] However, molecular techniques may help cytopathologists and clinicians in the risk stratification of indeterminate cases.[3, 4] Although next-generation sequencing (NGS) is a promising technique for the molecular testing of thyroid FNA specimens, thyroid-specific cancer gene panels are not commercially available.[5, 6] In this proof-

of-concept study, we developed a specific NGS panel to use in ultra-deep sequencing to identify the DNA based clinical relevant alternations to better stratify the risk of thyroid FNA showing indeterminate microscopic features.

Materials and methods. Our panel ("NexThyro") covers the hot - spots regions in 7 genes (KRAS, NRAS, BRAF, HRAS, RET, GNAS and TERT) involved in thyroid cancer of follicular and parafollicular origin. The Ion AmpliSeq Designer suite v5.3.1 with hg19 was used as reference genome to develop a customized panel. A single primer pool leading to the selection of 70 amplicons (ranging from 100 to 150 bp) enabled us to cover all COSMIC annotated mutations (246) in the selected exons of the target genes. The amplicon design covering 1.44 kb of genomic DNA was optimized for the simultaneous analysis of 40 samples with the 520 chip (Thermofisher, Foster City, CA, USA) on an S5 GS platform (Thermofisher). We preliminarily evaluated the panel performance in term of library construction on DNA derived from artificial control with a pool of DNA derived cell lines (OncoSpan - Horizon, Cambridge, UK) by using 4 dilution (10%, 5%, 2%, 1%) point with a wild - type DNA extracted from SKBR3 cell line, replicated 10 times, comparing the shape of Libraries constructed analyzed on TapeStation 4200 platform by using DNA High Sensitivity Kit (Agilent Technologies, Santa Clara, CA) with the relative data obtained by software assisted designed process.

Results. NexThyro had a high performance in term of quantity and quality of the libraries constructed. In particular the shape of constructed libraries closely related with the expected bioinformatic Results obtained by software assisted designed process (Fig. 1).

Conclusions. The preliminary Results show that NexThyro NGS panel could be suitable for the use in the cytopathology practice, although further analytical and clinical validation on real cytological samples are required.

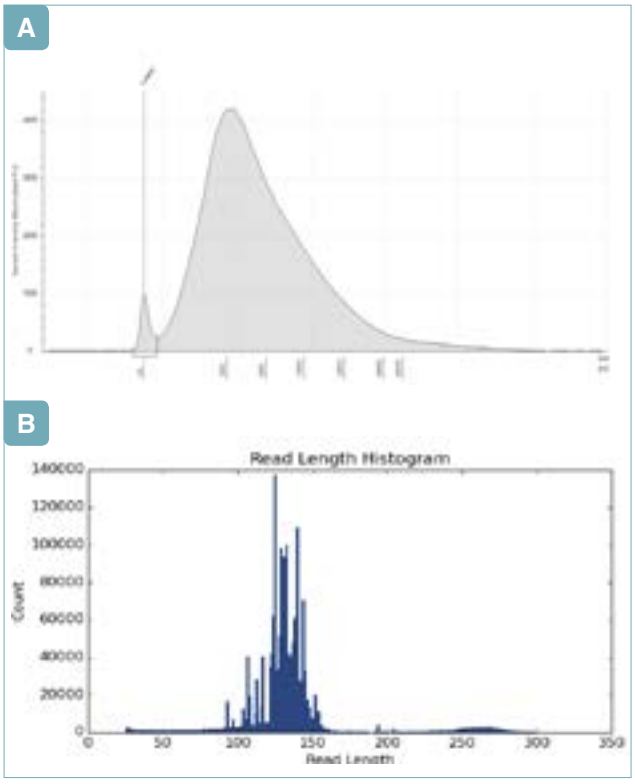

Fig. 1. An example of one NGS library prepared by using our "NexThyro" panel. **A)** Electropherogram obtained by using DNA sensitivity kit on Tape Station Platform (Agilent); **B)** Expected bp range and coverage distribution evaluated by using Torrent Suite (Thermofisher).

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A MULTICENTER TRIAL FOCUSING ON THE IMPLEMENTATION OF *EGFR* PREDICTIVE MUTATION ANALYSIS IN CLINICAL PRACTICE ON CELL FREE CIRCULATING TUMOR DNA

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Aim. A relevant percentage (up to 30%) of advanced non small cell lung cancer (NSCLC) patients do not have any tissue specimen available for the molecular assessment of epidermal growth factor receptor (*EGFR*), in order to administrate tyrosine kinase inhibitors (TKIs).[1] In this setting, cell free circulating tumor DNA (ctDNA) represents a valid specimen to avoid this issue, in particular for patients in "basal setting" (before any treatment) when tissue is inadequate or not available or in "progression setting" for the detection of *EGFR* exon 20 p.T790M resistance mutation.[2-4] However, technical protocols for molecular analysis of *EGFR* performed on ctDNA extracted by peripheral blood are very complex and is very difficult to validate internal workflow in clinical practice for each single laboratory. This trial Aim.s to generate a "network" of n = 12 Italian laboratories, who evaluated a real - time PCR (RT-PCR) approach (*EGFR* Therascreen kit) for *EGFR* exon 20 p.T790M resistance mutation. Results were compared whit those obtained by next generation sequencing (NGS) platforms performed in the Predictive Molecular Pathology laboratory of the University "Federico II".
(Gene Reader - and,
Materials and methods. N = 12 Italian laboratories joined the study. A total of n = 24 NSCLC patients were enrolled after the development of resistance to a treatment with an *EGFR* TKIs of first or second generation. For each patients plasma and serum samples were available for the molecular analysis.
Results. On the overall, all (n = 48; 100%) ctDNA samples were correctly analyzed by the RT-PCR approach by the different laboratories and a total of n = 5 samples (10,41%) harbored an *EGFR* exon 20 p.T790M resistance mutations. We then analyzed ctDNA extracted by serum and plasma from the same patients with two different techniques: Gene Reader (Qiagen, Hilden, Germany) and Personal Genome Machine (PGM - Thermofisher, Waltham, USA), and they have detected respectively n = 5 (10,41%) and n = 12 (25%) of p.T790M point mutation.
Conclusions. The study showed the feasibility of ctDNA analysis for the identification of *EGFR* exon 20 p.T790M. Further analysis are still ongoing to better define the concordance rate among the different technologies.

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MONOALLELIC GERMLINE MUTATIONS IN THE *PMS2* GENE RELATED TO LYNCH SYNDROME

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Introduction. Lynch Syndrome (LS) represents the most common form of hereditary colorectal and endometrial cancer, and is caused by pathogenic variants in the DNA mismatch repair (MMR) genes. While standard mutation-detection methods apply well to the *MLH1*, *MSH2* and *MSH6* genes, *PMS2* mutation testing has been problematic because of the presence of pseudogenes and frequent gene conversion events. Over the years, it has become clear that monoallelic germline mutations in the *PMS2* gene play a far more critical role than initially thought; however, the *PMS2*-related from of LS remains poorly defined. The present work Aim.ed to review the contribution of *PMS2* mutations to the ULSS1 Dolomiti (Veneto) cohort of patients with LS.
Materials and methods. Tumours were subjected to immunohistochemical (ICH) staining for MMR gene products (Roche/ Ventana, Rotkreuz, Switzerland), microsatellite instability (MSI) (HNPCC kit; Experteam, Venice, Italy) and *BRAF* gene mutation analysis (Diatech Pharmacogenetics, Jesi, Italy), according to the manufacturers' instructions. *MLH1* promoter methylation analysis was as described previously [1]. Direct Sanger sequencing was used to analyse MMR genes. The multiplex ligation-dependent probe amplification (MLPA) technique was used to evaluate large genome rearrangements (LGRs) (MRC-Holland probes).
Results. Since 2012, a multi-specialist group at the ULSS1 Dolomiti (Veneto) has been established for the diagnosis and clinical management of patients affected by LS. Over the years and in a gradual manner, a universal screening strategy based on MMR protein IHC was introduced and corroborated, in appropriate cases, by MSI evaluation, *BRAF* mutation analysis and an *MLH1* promoter hypermethylation test. To date, about 250 cases, including colon and endometrial cancer, have been subjected to first-level investigations. This medium led us to select 77 candidate patients for second-level molecular analysis of the appropriate MMR gene, based on IHC Results. Despite the technical difficulties that hamper *PMS2* molecular testing, i.e. the existence of pseudogenes with a high grade of similarity to the functional counterpart, our laboratory offers a complete *PMS2* second-level analysis for the identification of both small sequence variants and LGRs. At our centre, *PMS2* molecular testing is performed in all cases harbouring PMS2- IHC solitary loss and, according to the international guideline [2], in cases characterized by combined MLH1/PMS2-IHC loss in which a previous *MLH1* sequence analysis was not informative. To date, a total of 32 *PMS2* full-gene tests have been performed, six of which were conducted on colorectal cancer with PMS2-IHC solitary loss. All six of these cases were positive for pathogenic *PMS2* mutations. Twenty-six of the 32 instances concerned a tumour harbouring combined MLH1/PMS2 IHC loss with a non-informative *MLH1* test, one of which was positive for a *PMS2* pathogenic muta-

tion. Overall, the offered molecular characterization has led to the identification of seven cases affected by PMS2 pathogenetic mutations. In particular, two of these seven cases were affected by a c.137G>T nucleotide substitution, which represents a founder mutation that has been reported in the international scientific literature. Three of the seven cases harboured the following micro-deletion/duplication: c. 1281delT, c.1150_1151delTT and c.576dupT. These mutations, which are not listed in the LOVD- InSiGHT and ClinVar International databases, are frameshift mutations that introduce a premature STOP codon with the consequent generation of a truncated protein. In the latest update of the LOVD-InSiGHT classification criteria (Version 2.4 June 2018), they were classified as pathogenetic variants. One of the seven cases harboured a c.383C>A single-nucleotide substitution. This variant represents a nonsense mutation that creates a premature STOP codon. It is not listed in the LOVD-InSiGHT database, but is present in ClinVar as a single submission, and is classified as being pathogenetic. Another of the seven cases was characterized by an LGR consisting of an exon 12-13-14-15 heterozygous deletion. This gene rearrangement is not currently catalogued in the international LOVD-InSiGTH database, even though other deletion events concerning the same genetic region (between exons 11 and 15) are reported and classified as being pathogenetic. **Discussion.** The documented low penetrance of *PMS2* mutations relative to the other MMR genes, such as *MLH1* or *MSH2*, implies that *PMS2* mutations will be underdiagnosed in the clinical setting, where mutation analyses are applied only to individuals displaying the high-risk features of keen family history or early- onset LS-related cancer. By contrast, the universal screening strategy that was gradually introduced at our centre has proved to be very useful in identifying potential cases with *PMS2* gene mutations, as shown in the present work, in which 9% (7 out of 77) of the patients analysed were affected by a *PMS2* pathogenic mutation. As reported in the literature, our data confirmed that lone loss of PMS2 IHC expression is suggestive of LS with a primary defect in the *PMS2* gene. In our cohort of patients, six of the six cases (100%) cases with only PMS2 IHC staining loss showed a pathogenic mutation, even if cases with only PMS2 loss and an *MLH1* germline pathogenic mutation are reported in the literature. In the latter case, MLH1 staining retention can be explained by admitting the existence of a genetic defect that preferentially affects protein functionality, rather than protein antigenicity. The finding of one case that was characterised by combined MLH1/PMS2 staining loss, *MLH1* germline non-informative test and a *PMS2* germline c.137C>A founder mutation was particularly impressive. These scenarios are quite rare, and the failure of expression of MLH1 can be explained by supposing the onset of a double somatic MLH1 mutation, rather than a germline one, which is responsible for the loss of its expression. The technical difficulties in the molecular analysis of *PMS2*, which are caused by the existence of many pseudogenes, have led to a less-detailed characterization of its variants compared with the other MMR components. The finding that four out of the seven mutations identified in our study were not listed in international databases confirms that the *PMS2* gene mutation panorama has been only partially explored. Three of these four new mutations were nonsense or small insertion/deletion mutations, as are the most frequent genomic variation types in MMR genes. By contrast, one of these four cases was an LGR involving the 3′ end of *PMS2*, a genomic region that is especially challenging to analyse because of the presence of the *PMS2CL* pseudogene, which has more than 98% sequence identity with the functional gene. This finding is fascinating considering that the international literature states that LGR accounts for 5-20% of all mutations within MMR genes [3], with a prevalence of *MSH2* deletions [4] and a much lower frequency of exonic *MSH2* and *MLH1* duplications. The incidence of LGR may vary according to the population studied. While the southern Italian and Sardinian community exhibited a frequency of LGR that was quite low and mostly represented by *MSH2* sizeable genomic deletions, the ULSS1 Dolomiti Veneto cohort of patients was characterized by a high rate of exonic duplications involving the *MSH2* and *MLH1* genes, as described previously [5]. To our knowledge, the *PMS2* exon 12-13-14-15 heterozygous deletion detected here in a patient of Sardinian origin is the second report of a *PMS2* LGR in Italy, the first one having been reported by Lo Monte et al., who reported a single case of a *PMS2* exon 3-4 duplication in a cohort of southern Italian patients with LS [6]. These Results underscore the need for further study of the spectrum of *PMS2* gene mutations, for its entire delineation, and reinforces the importance of performing accurate *PMS2* germline testing to obtain a much more detailed estimate of the prevalence of *PMS2*-related LS forms and develop more accurate clinical management and personalized surveillance programs.

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PERIHILAR AND DISTAL EXTRAHEPATIC CHOLANGIOCARCINOMAS SHOW DIFFERENT MOLECULAR ASSETS AND SHARE MYC COPY GAINS AS AN INDEPENDENT POOR PROGNOSTIC MARKER

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Purpose. Extrahepatic cholangiocarcinoma (ECC) constitutes 80% of bile duct cancer cases, yet it is less defined than intrahepatic cholangiocarcinoma from the molecular point of view. ECC is classified into two subtypes with different site of origin and clinical features:

distal extrahepatic (DECC) and perihilar colangiocarcinoma (PHCC). However, recent high throughput molecular studies analysed ECC as a single entity, with few exception relying on a limited number of cases. A better knowledge of the molecular differences between PHCC and DECC may inform therapeutic decisions and improve patients outcome.
Experimental Design. Transcriptomic and genomic alterations were investigated by next-generation sequencing in a discovery set of 7 DECCs and 7 PHCCs, and validated on 45 DECCs and 40 PHCCs using custom gene panels, FISH and immunohistochemistry.
Results. DECC and PHCC shared genomic alterations in several cancer-related genes, including *KRAS*, *TP53*, *SMAD4*, *ARID1A*, *CDKN2A* and *PIK3CA. KRAS* and *TP53* were the most frequently mutated genes overall (42% and 43% of cases respectively). However, DECC had significantly more frequent *TP53* mutations, while *KRAS* mutations were more frequent in PHCC (p=0.0047). Copy gain of *MDM2* (p=0.006) and *CCND1* (p=0.02) were also enriched in DECC. Trascriptomic analysis identified a 40-gene signature able to distinguish two histotypes.
Conclusions. This is the first study reporting different molecular profiles of perihilar and distal extrahepatic cholangiocarcinomas. *KRAS* mutations are enriched in PHCC while the p53 pathway (*TP53* mutation and copy gain of its inhibitor *MDM2*) is heavily affected in DECC with consequent impact on prognosis. A second novel finding, common to both ECC subtypes, is MYC copy gain association with poor prognosis. Trascriptomic analysis also supports differences about pathogenic development of these two subtypes. Several potentially actionable alterations have been reported in both subtypes and need further evaluation.

COLD FORMALIN FIXATION PRESERVES DNA INTEGRITY IN FORMALIN FIXED PARAFFIN EMBEDDED SAMPLES: PREMISES FOR A BETTER QUALITY OF DIAGNOSTIC AND EXPERIMENTAL PATHOLOGY

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Aim.s. Formalin fixation represents the standard method to preserve tissue specimens for diagnostic pathology and archival formalin fixed paraffin embedded (FFPE) samples represent a source of tissues for both diagnostic and experimental pathology [1]. It is well known that formalin fixation induces severe fragmentation of nucleic acids, thus affecting the feasibility of high-throughput molecular downstream analyses from FFPE specimens [2]. We Aim.ed at evaluating whether formalin fixation at 4°C [3] could better preserve DNA quality from FFPE specimens.
Materials and methods. Paired samples from 38 cancer specimens were formalin fixed at room temperature (*stdFFPE*) and at 4°C with pre-cooled formalin (*coldFFPE*), respectively. Two independent cohorts were prospectively collected, cohort A (collected 6 years prior to the study, n=21) and cohort B (collected at time of the study, n=17). DNA was extracted from paired tissues. In a subgroup of 14 cases from cohort B, DNA was recovered twice, i.e. at time of collection and six months after collection. The DNA integrity was evaluated by using a qPCR-based kit calculating a quality control (QC) score (=dimensionless number tending to 1 for highly conserved DNA).
Results. Cold formalin fixation led to less DNA fragmentation compared to DNA extracted from *stdFFPE* samples (mean QC values: 0.36 *versus* 0.69, $p < 0.0001$). Comparable QC scores were obtained between *coldFFPE* tissues of cohorts A and B, conversely DNA integrity was significantly lower in *stdFFPE* of cohort A compared to cohort B ($p < 0.0001$). For the 14 cases for which DNA was recovered at time of collection and 6 months following collection comparable QC scores were obtained in *coldFFPE* samples ($p=0.131$), whereas a statistically significant reduction in QC scores (35% mean percentage loss) was observed in the *stdFFPE* samples (p =0.0001).
Conclusions. A monitored formalin fixation at 4°C Results in high values of QC score, which are less influenced by storage over time, thus ensuring a less degree of DNA fragmentation compared to that obtained FFPE tissues fixed at room temperature. This cold formalin fixation protocol may represent a valuable alternative to standard fixation to ensure high-quality DNA for downstream high throughput molecular analyses.

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MOLECULAR HETEROGENEITY IN "BIPHASIC" COMBINED MUCINOUS NON-MUCINOUS COLORECTAL ADENOCARCINOMA, REVEALED BY NEXT GENERATION SEQUENCING

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Objective. The coexistence of distinct histological variants in the same colorectal tumor is unfrequently

observed. “Biphasic” mucinous/non-mucinous adenocarcinoma represents a possible combination. The identification of specific molecular profiles for each histopathologic variant in the same mass could improve the knowledge of tumor biology and be significant for post-surgical treatment.

Materials and methods. Four colorectal adenocarcinomas (from 2 male and 2 female patients; age: 70 years) with combined “biphasic” mucinous/non-mucinous adenocarcinoma were assessed for mutational status. Analysis was conducted using two different Targeted Next Generation Sequencing technologies. Myriapod NGS-IL 56G Onco panel (Diatech Pharmacogenetics) on MiSeq platform (Illumina) allows the simultaneous identification of hotspot mutations of 56 clinically relevant genes: ABL1, AKT1,ALK, APC, ATM, BRAF,CDH1,CDKN2A, CSF1R, CTNNB1, DDR2, DNMT3A, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, FOXL2, GNA11,GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MA2K1, MET, MLH1, MLP, MSH6, NOTCH, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, ShKT11, SMAD4, SMARCB1, SMO, SRC, TP53, TSC1, VHL. Molecular Results was confirmed by Oncomine Solid Tumour DNA Kit applied on Ion-S5 platform (Thermofisher Scientific, both), that identifies somatic variants in selected regions of 22 cancer-related genes: EGFR, ALK, ERBB2, ERBB4, FGFR1, FGFR2, FGFR3, MET, DDR2, KRAS, PIK3CA, BRAF, AKT1, PTEN, NRAS, MAP2K1, STK11, NOTCH1, CTNNB1, SMAD4, FBXW7, TP53. The molecular profile of each histological component was assessed and correlated with histomorphological features.

Results. Tumors were located in the right colon (1 cases) and in the left colon (3 cases) and occurred as ulcered masses with averaged diameter of 4 cm. 2 tumors were composed of mucinous adenocarcinoma and non-mucinous poorly differentiated adenocarcinoma, 1 tumor was composed of mucinous adenocarcinoma and non-mucinous low grade adenocarcinoma (G2) and 1 tumor was composed of mucinous adenocarcinoma and serrated adenocarcinoma. 2 tumors had stage 3 (pT3pN1apM0) and 2 had stage 2 (pT3pN0pM0). The mutational profile of the tumors can be summarized as follow: 2 tumors showed partially different molecular profiles in their components [BRAF(V600E)/PTEN(p.N323fs and p.K267fs) mutations in mucinous component and BRAF(V600E)/PIK3CA(p.E542K)/SMARCB1(p.G33R) mutations in non-mucinous poorly differentiated adenocarcinoma in one case and BRAF(V600E)/PIK3CA(p.I112N) mutations in mucinous component and BRAF(p.V600E)/AKT1(p.R23W)/SMAD4(p.V126D)/RET(p.C634Y)-CDH1(p.N371D)/CDKN2A(p.W110)/FGFR3(p.G382D) mutations in non-mucinous poorly differentiated adenocarcinoma, in the other case]. One tumor exhibited totally different mutations in the two components [KRAS(p.G12S)/PIK3CA(E547Q)/APC(R1460) mutations in the mucinous component and NRAS(p.G12D)/SMAD4(p.W524)/p53(p.G245S) mutations in the non-mucinous poorly differentiated adenocarcinoma]. In the last case the two components (mucinous and non-mucinous serrated components) showed identical mutations, represented by KRAS(p.G12A)/KIT(p.V731I) mutations. Mutations occurred in genes included in both panels were confirmed by both NGS technologies, suggesting their analytical reliability. Overall, BRAF-, RAS- and PI3KCA- mutations were the most frequent mutated genes. BRAF- and RAS- mutations were equally distributed in the two part of the tumor masses. PI3KCA- and PTEN mutations were most frequently detected in mucinous component, while SMAD4 or p53 mutations were more frequent in the non-mucinous poorly differentiated component.

Conclusions. Although with the limitation of the number of the samples, the coexistence of different histological components with distinctive biomolecular alterations in the same tumor strengths the concept of tumor biomolecular heterogeneity. Complex multiple molecular profiles seem to be most frequently associated with the occurrence of unfavourable histopathological features. Thus, the “biphasic” histological variability must be considered in the assessment of the biological behaviour of the tumors and their targeting to oncological treatments.

LIQUID BIOPSY IN REAL WORD CLINICAL PRACTICE OF NON-SMALL-CELL-LUNG CANCER (NSCLC): A MULTI-INSTITUTIONAL EXPERIENCE

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Background. Circulating tumor DNA (ctDNA) from liquid biopsy is a recognized valid option for EGFR testing as a source of tumor genetic material in NSCLC when resistance to I-II generations TKI-therapies occurs or upfront, or if tumor is not easily amenable to sample.
Multidisciplinary approach and patient context are crucial for accurately interpreting molecular findings and guarantee an optimal management of non-small cell lung cancer (NSCLC) patients.
A multi-regional survey for patients treated with EGFR TKI from January to December 2018 was conducted regarding use of liquid biopsy for NSCLC in clinical practice.

Methods. Aim. of the study was describing the use of EGFR testing by liquid biopsy in clinical practice of seven major lung-cancer centers in North Eastern Italy, with regard about workflow in clinical setting.
A multi-regional survey about clinical practice of EGFR ctDNA testing in the period January-December 2018 was conducted.

Results. Overall 316 patients (360 samples) have been screened for ctDNA EGFR, with a medium number of 1.1 samples/patient.
Seven out of seven (100%) centers used commercially available real time CE-IVD tests on two different diagnostic platforms: 2/6 (33%) Cobas® EGFR Mutation Test v2 (Roche) and 4/6 (67%) Easy® EGFR (Diatech pharmacogenetics). NGS or droplet digital PCR were used by one center each, as second level confirmation methods.
All institutions reported EGFR as the main gene tested for clinical purposes in plasma samples of NSCLC, other genes (i.e. KRAS/BRAF) were occasionally tested upon oncologist request.
Blood drawing was uniformly managed between all centers with sampling performed in the same hospital of EGFR testing by qualified nurse staff, in 6/7 (86%) centers in the Medical Oncology Unit in predefined sessions dedicated to liquid biopsy analysis and in 1/7 (14%) center in blood sampling center. All institutions asserted to collect blood in EDTA tubes, rapidly delivered to molecular lab and to separate plasma within 2 hours. Only one center declared the occasional use of Cell-Free DNA BCT® (Streck) tubes.
Plasma separation was done within 30 minutes (43%), 1 hour (43%) and 2 hours (14%) by the same laboratory who did EGFR testing.
Molecular analysis was performed in Surgical Pathology Department in 6/7 (86%) institutions and in 1/7 (14%) in Oncologic Molecular Pathology Laboratory.
Reports were drawn up within 24 hours in 3/7 (43%) centers and within 3-5 working days in 4/7 (57%) centers by biologist 6/7 (86%) or oncologist 1/7 (14%).
Among all, 108 (34%) patients were tested at the time of diagnosis with an EGFR mutation rate of 15%.
At progression to TKI treatment, 208 (66%) patients were tested. T790M positive rate was 55/122 (45%). Inconclusive cases (negative for both T790M and known actionable mutation) were 86 (41%). All centers stated that histo/cytological re-biopsy was suggested in these cases.
Conclusions. Real-world experiences about EGFR testing in liquid biopsies revealed substantially homogeneous habits among interviewed centers, with a technical approach coherent with national guidelines and literatures data.
We noticed a consistent number of liquid biopsies at the time of diagnosis mainly related to some reference centers that collect specimens from several institutions.
However, overall data collected from the network of interviewed centers suggested a revision of workflows from sampling during bronchoscopy Aim.ed to obtaining better specimens suitable both for cito-histological diagnosis, predictive biomarkers assessment and multitargeted wide molecular profiling.

PDL-1 EXPRESSION IN PROSTATE CARCINOMA: A PROGNOSTIC MARKER OVEREXPRESSED IN SPOP MUTATED CARCINOMAS

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Introduction. In the last few years, molecular profiling allowed us to identify seven specific genetic mutations or fusions that characterize about 74% of prostate carcinomas, thus defining seven specific molecular subtypes that may show distinct biological behavior, with potential prognostic and therapeutic implications. Interestingly, a specific prostate carcinoma sub-type was characterized by the inactivating mutation of the SPOP gene. This subtype has been shown to have a prevalence spanning from 6 to 15% of all prostate carcinomas.
SPOP is a cullin-based E3 ubiquitin ligase that has a central role in the degradation of several proteins. Its inactivating mutations represent a key point in prostate carcinogenesis affecting various signaling cascades, particularly the steroid receptor one, the hedgehog and the JNK cascades. Recently, Zhang and colleagues demonstrated that SPOP mutations can reduce PDL-1 degradation with a significant upregulation of its expression in neoplastic cells.
In this study our Aim. was to investigate the SPOP mutation in prostate carcinoma, correlating our Results with PDL-1 expression, with clinical and pathological features.
Materials and methods. We selected 82 prostate fine needle agobiopsy specimens, formalin fixed and paraffin embedded. Patients had an average age of 73 years. The biopsies were divided into two groups: the first group was constituted by 36 low grade specimens (gleason grade group of 2-3), and the second group was constituted by 46 high grade specimens (gleason grade group of 4-5).
We evaluated PDL-1 expression with immunohistochemistry with an anti-PDL-1 antibody (Dako PD-L1 22C3 pharmDx, Agilent Pathology Solutions). We used the Tumor Positive Score (TPS) and the Combined Positive Score (CPS) to evaluate the PDL-1expression. We defined samples to be positive when TPS and CPS PDL-1 expression was ≥ 1% of the neoplasm. Afterwards we searched for SPOP mutations using RT-PCR and exone-sequencing (exons 5-7).
Results. We found SPOP missense mutations in 7 out of 82 samples (8,54%): 6 samples had a exone 6 mutation (4 samples had the F133V mutation, 1 had the F133L mutation, 1 had the Y123D mutation); 1 had the exone 5 F102V mutation. These mutations determine a functional alteration of the SPOP protein. All SPOP mutated samples showed to have a higher expression of the PDL-1 protein, with TPS values in a range of 20-25%, and CPS values in a range of 30-35. Moreover, we found a positive expression of PDL-1 in 29 SPOP wild-type samples, with a TPS expression within a range of 1-35%, while CPS expression was in a range of 3-45. SPOP mutations were significantly associated to PDL-1 overexpression (p=0.0022; Fisher's exact test). Notwithstanding, this association, 29 out 82 (35,36%) SPOP wild type samples showed an upregulation of PDL-1. Overall PDL-1 expression was found to be positively associated with gleason grading; as a matter of fact the low grade specimens were found to be positive in 11 out of 36 samples (30,55%), while the high grade specimens were found to be positive in 25 out of 46 specimens (54,3%; p=0.0437; Fisher's exact test).

Discussion. SPOP was found to be mutated in 8,54% of our samples, confirming the literature data that showed a strong association with high expression of PDL-1, independently from specific mutations. Interestingly, we found that SPOP mutated samples had also the highest expression profiles of our samples. This data seems to confirm the possible role of SPOP and the cullin-based E3 ubiquitin ligase in the regulation of PDL-1 expression, becoming a possible molecular marker in the cancer immunotherapy. Moreover, we found PDL-1 to be associated with higher Gleason grades, such a result is probably linked to the higher tumor mutational burden of this prostate cancer subgroup. Nothwithstanding, we found that 35,4% of SPOP wild-type prostate cancer have an overexpression of PDL-1, suggesting that other molecular pathways could be implicated in the upregulation of PDL-1 in prostate cancer. However, our SPOP mutated specimens were not numerous enough in order to make definitive statements and further research is needed in order to get more conclusive Results.

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BRCA AND OVARIAN CANCER: IMPLEMENTING ROUTINE GENETIC TESTING. THE GENOA EXPERIENCE

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Objectives. Ovarian cancer (OC) is the fifth cause of cancer death among women in western countries. The advanced stage at diagnosis (FIGO III-IV) is the main cause of its high mortality rate. The evaluation of OC BRCA status has important therapeutic and prognostic implications, therefore genetic testing is recommended at diagnosis of OC. Our Institute (Policlinico San Martino, Genoa) investigated the reliability of the detection of germline and somatic BRCAm from formalin-fixed-paraffin-embedded (FFPE) specimens in a routine lab set.

Materials and methods. We included patients who underwent germline BRCA testing for the period 2017-2019 and with histological diagnosis of High Grade Serous Ovarian Cancer (HGSOC) (n=19). All the cases were reviewed by a pathologist expert in gynecological pathology. The paraffin block for each patient was chosen according to: optimal fixation and storage, high representativeness of the entire neoplasia, high tumor cellularity (>20%), low percentage of stroma cell, fibrosis and necrosis. From each selected sample, macro sections (six sections of 10 µm thickness) were obtained for molecular analyses. DNA was isolated using the QS GeneRead DNA FFPE treatment kit and its quality was assessed by the Qubit 3.0 Fluorometer. Somatic BRCA analysis was performed with Oncomine BRCA panel on the Ion GeneStudio S5 System. Parameters for analysis excluded SNVs with: variant allele frequency (VAF) <5%, coverage <500X, quality score (PHRED) < 30. Pathogenetic variants (PVs) were validated by Sanger sequencing. Samples were analyzed blindly to their germline status.

Results. PVs were detected in 10 of 19 cases (52,63%): of these, 8 (80%) were germline. All known germline variants were detected by the somatic analysis.

Conclusions. The implementation of BRCA testing in the routine diagnostic workflow of OC benefits from the strict collaboration of skilled clinician, geneticist and pathologist. An optimal preanalytical phase is required to obtain satisfactory genetic Results from FFPE specimens.

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CITOLOGIA

DIAGNOSTIC ACCURACY AT DETECTING BREAST CANCER METASTASES IN AXILLARY LYMPH NODES OF CYFRA 21-1 MEASUREMENT OF FINE NEEDLE ASPIRATION FLUID WASHOUTS

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Objectives. Axillary nodal status remains an important determinant of prognosis and therapeutic strategy in patients with a newly diagnosed breast cancer. In malignant epithelial cells expressing CK19 a 18.4 KDa fragment (CYFRA 21-1) of this cytoskeletal filament is released into circulation during apoptosis by caspase-3, a cleaving enzyme. This CK19 fragment is soluble in serum and therefore readily detectable. The Aim. of our work was to assess the diagnostic accuracy of CYFRA 21-1 level measurement in needle washout fluid of ultrasound-guided fine-needle aspiration biopsy (US-FNAB) of axillary nodes in breast cancer patients. The method presented in this study, in extension of what we have already published (2), may prove to be a reliable assay for rapid detection of axillary tumor deposits, with an approach less prone to human error.

Materials and methods. Between 2012 and 2019, in all patients with a newly diagnosed breast cancer, suspicious axillary lymph nodes were sampled with a US-FNAB. Of a total of 258 biopsies were performed 252 were included in the study, six cases were removed from the dataset because proved to be CK19 negative by immunohistochemistry (IHC). Needle contents were expelled onto a labeled slide for smears and processed for cell block histology. Needles were then washed with 1 ml of saline solution and in the wash-outs CYFRA 21-1 was tested. CYFRA 21-1 levels were measured with an enzyme-linked immunosorbent assay. The cutoff value for positive samples that expressed CK19 by immunohistochemistry (IHC), was determined by a receiver operating characteristic (ROC) curve. The optimal cutoff point was set by the Youden's index, where true positive rate is high and the false positive rate is low. Based on this value, sensitivity, specificity, positive and negative predictive values were then calculated, using cytology Results as a reference standard. Statistical analyses were done using Scikit-learn (1) a Python language based, open source tool, for data data analysis built on NumPy, SciPy, and Matplotlib libraries. Non parametric statistics (Mann-Whitney U-test) was used to compare tumour marker values of cytologically positive and negative lymph nodes.

Results. The area under the ROC curve (AUC) close to 1.0 allowed a reliable estimate of cutoff value that was set to 1,98 ng/ml for CYFRA 21-1 concentration in needle wash out fluids (Fig. 1). Of the 252 axillary lymph nodes sampled, 178 were positive on cytology and were

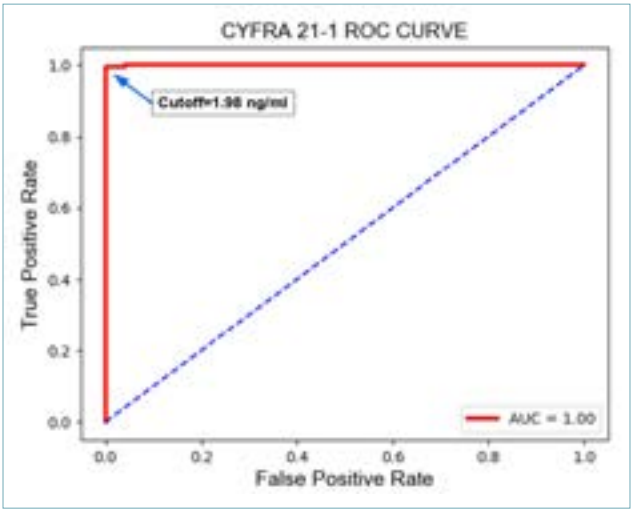

Fig. 1. ROC curve with cutoff value.

N1 as confirmed by surgery while 74 were negative both on smears and by tumor marker testing. Median CYFRA 21-1 concentration of positive samples was 108.65 ng/ml, whereas in the negative nodes its median concentration was 0.84 ng/ml (P <0.0001) (Fig, 2). Using the CYFRA 21-1 cutoff value we were able to clearly separate negative from positive samples (P value < 0.00001 by Chi square) and the following contingency table could then be generated:

	CYFRA +	CYFRA -	
Positive cytology	177	1	178
Negative cytology	0	74	74
totals	177	75	252

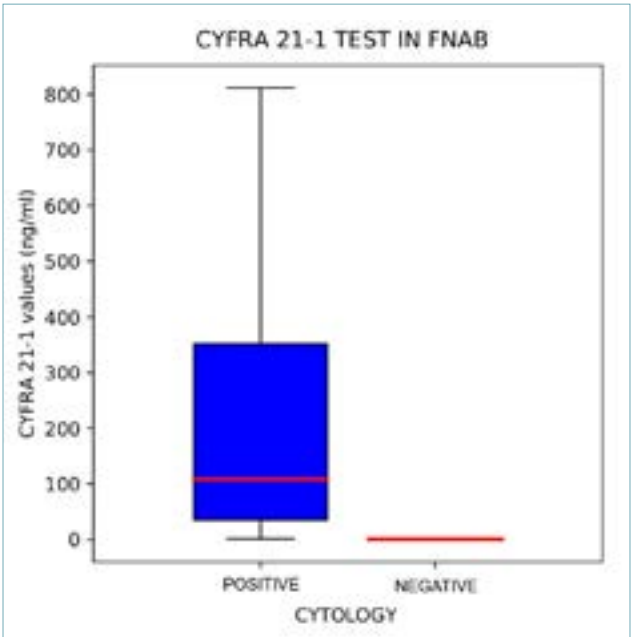

Fig. 2. CYFRA 21-1 concentration in positive and negative cytology.

The test a sensitivity was 0.98 and its specificity 1.0. Positive and negative predictive values were 1.0 and 0.95 respectively. No false positives were detected, one false negative was identified in a biopsy with inadequate sampling. Interestingly of the six cases that were were positive on cytology and CK19 negative by IHC, two had CYFRA 21-1 values above cut off (75,6 and 2,88 ng/ml).
Conclusion. The purpose of our study was to verify the diagnostic accuracy of CYFRA 21-1 levels in axillary lymph node US-FNAB washouts, testing whether detection of this tumor marker above a certain cutoff level could be diagnostic of breast cancer metastases. Our intent was to demonstrate the feasibility of a method reliable enough to be a faithful replacement of traditional cytology, solely based on personal experience of pathologists. Rendering this diagnostic process independent from human factors in all aspects (slide smearing, fixing, staining, cell block preparation etc.) will certainly increase its accuracy and reproducibility. The CYFRA 21-1 ROC curve AUC with a performance measurement of 1.0 shows that this test is a very effective binary classifier, able to reliably separate positive from negative samples. These Results indicate the overall accuracy of our assay. It is important to stress the fact that CYFRA 21-1 immunoassay do not only replaces cytology but also CK19 IHC on cell blocks, a tedious and time consuming procedure. In fact the turn around time of a CYFRA 21-1 test takes no more than 25 minutes, the time needed by the immunosorbent assay to complete its tasks. The tumor marker measurement demonstrated also to be very sensitive since it was able to identify two positive lymph nodes that by IHC apparently did not express CK19.

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OUTCOME AND DIAGNOSTIC REPRODUCIBILITY OF THYROID CYTOLOGY INDETERMINATE SIAPEC/SIE 2014 TIR3 CATEGORIES IN A CONSECUTIVE SERIES OF 302 CASES

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Background. Although proposed more than five years ago, the clinical impact of the SIAPEC/SIE 2014 diagnostic classification for thyroid cytology has been addressed in few studies. All of them were Aim.ed at evaluating the malignancy rate and at assessing the relative prevalence of each individual category (1-4). So far, no study determined the intra-observer and inter-observer reproducibility of the diagnostic scheme, with special reference to the sub-classification of indeterminate diagnoses into TIR3A and TIR3B categories, which was the most relevant novelty of this classification.
Aim. To test the prevalence, malignancy risk and reproducibility of TIR3A and TIR3B cytological diagnoses defined according to SIAPEC/SIE 2014 classification (adopted at our Institution from February 2015).
Materials and methods. A total of 302 consecutive cytological samples with TIR3 diagnoses were retrospectively collected from February 1, 2015 to December 31, 2018 from the Pathology files of the San Luigi Hospital, Orbassano, Turin. All samples included from 2 to 6 smears and the corresponding cell block preparations. The prevalence of these cases (including both TIR3A and TIR3B) over the total number of thyroid cytological diagnoses in the same period was compared with that of a comparable time-period before the application of the SIAPEC/SIE 2014 (years 2011-2014).
Histological diagnoses of surgically resected cases were retrieved from the Pathology files of four major Hospitals in the Turin area dedicated to thyroid surgery, and the malignancy rate for both TIR3A and TIR3B was assessed. Reproducibility was assessed as either concordance of diagnoses in repetitive cytological samples from the same thyroid nodule or as diagnostic agreement between different observers. For this latter analysis, among the 302 cases, 141 consecutive cases from years 2015 and 2016 were independently reviewed by an experienced thyroid pathologist (MV) who was also responsible for the original diagnoses (to assess intra-observer agreement) and by a senior resident in pathology (FM) (to assess inter-observer agreement). All slides were reviewed blind of the original cytological diagnoses and of the histological outcomes, whenever available. Finally, the characteristics of TIR3A samples that at surgery proved malignant or borderline (including non-invasive follicular tumors with papillary-like nuclear features - NIFTP or well-differentiated tumors of uncertain malignant potential - WDT-UMP) were compared to those benign at histology. Evaluated parameters included clinical (sex, age, multinodularity, location, and size), ultrasound (echogenicity, halo, microcalcifications, vascolarization, "taller-than-wide" size) and pathological (cellularity, presence of degenerative changes and atypia of unknown significance) features.
Results. The overall prevalence of "indeterminate" diagnoses from 2015 to 2018 was 302/1603 (19%), including 207 TIR3A (13%) and 95 TIR3B (6%). Predominant oncocytic features were observed in 32/207 (15%) TIR3A and 13/95 (14%) TIR3B cases. These figures were similar to those of TIR3 diagnosed in years 2011-2014 (261/1680, 16%). Surgery was performed in 48 of 175 TIR3A cases with available follow up (27%) and in 86 of 89 TIR3B cases with available follow up (97%). Malignancy rate for TIR3B was 39%, whereas the malignancy rate for TIR3A was 17%. When NIFTP or WDT-UMP were included into the benign group, the malignancy rate was 28% for TIR3B and 6% for TIR3A. A second cytological sample was obtained in 26 TIR3A and in 1 TIR3B cases, with a concordant diagnosis in 13/27 cases (48%), only. Intra-observer overall agreement was 122/141 (86%), with a concordance of 85/98 (86%) for TIR3A and 37/43 (86%) for TIR3B. Interestingly, the intra-observer agreement raised from 53/66 (80%) to 69/75 (92%) comparing diagnoses of year 2015 and year 2016, respectively. Inter-observer agree-

ment between expert and in-training pathologist was lower, with concordance rates of 95/141 (67%) overall, 69/98 (70%) for TIR3A and 26/43 (60%) for TIR3B. Discordant diagnoses mostly included TIR3A versus TIR3B and *vice versa*, whereas a minority of cases were re-classified into other categories (including TIR1, TIR2 and TIR4). Clinical and cyto-pathological characteristics were not significantly different in histologically malignant as compared to benign TIR3A cases, except for a larger nodule size (38 vs 26 mm, respectively) (p=0.005).

Conclusions. In a real life experience from a large consecutive series of thyroid cytological diagnoses, the sub-classification of TIR3A and TIR3B introduced by the SIAPEC/SIE 2014 system slightly increased the overall prevalence of "indeterminate" diagnoses. Malignancy rate was strongly influenced by the proposed WHO 2017 re-classification of encapsulated follicular-patterned lesions, especially in the TIR3A category. Re-sampling of a TIR3 lesion resulted in a concordant diagnosis in less than half of the cases. Agreement among observers of TIR3 sub-classification into TIR3A and TIR3B highly depends on pathologist's training. Finally, no criteria except for size are predictive of malignancy in the TIR3A category.

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RATE OF MALIGNANCY AND FINAL OUTCOME IN INDETERMINATE THYROID LESIONS CLASSIFIED AS BETHESDA CATEGORY III (AUS/FLUS): A RETROSPECTIVE STUDY

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Introduction. Ultrasound guided FNAC is a standard procedure for thyroid nodules management and selecting patients for surgical treatment. The implementation of The Bethesda System for Reporting Thyroid Cytology (TBSRTC) has improved the quality of FNAC reporting and has reduced the overall rate of unnecessary surgery. However, Bethesda category III (atypia of undetermined significance [AUS]/follicular lesion of undetermined significance [FLUS]) still remains a controversial subject in the classification of FNAC specimens as a result of variability in cytopathologic interpretation, its heterogeneity and difficulty to determine the true rate of malignancy (ROM). Based on 2008 TBSRTC the AUS/FLUS diagnostic rate and ROM are <7% and 5-15% respectively. There have been various outcome studies on AUS/FLUS demonstrating that this category may represent up to 20% of thyroid diagnosis[1] with a reported ROM variable and significantly higher than that proposed by 2008 TBSRTC[2]. Possible reasons for this wide variation include differences in patient demographics and/or characteristics, technical issues including the adequacy of sampled material, the optimization of cellular preservation and slide preparation, the level of comfort/expertise of the reporting pathologist, and publication bias[3]. The 2017 TBSRTC recalculated the ROM for the different categories, particularly for AUS/FLUS (which becomes 10-30% instead of 5-15% of the previous edition). Despite AUS/FLUS has been extensively studied since the advent of TBSRTC, calculating the ROM associated with this interpretation remains challenging because only a minority of cases undergo excision.

Materials and methods. The laboratory information system of our institution was searched to obtain diagnostic reports recorded from January 2016 and December 2018 related to thyroid FNACs classified as AUS/FLUS. During the study period an overall of 6498 thyroid FNACs were performed (5138 patients), including 535 AUS/FLUS (453 patients). The thyroid FNAC service in our institution is provided by endocrinologists. FNACs were done under ultrasound guidance. FNACs preparation consists of a direct smear, air-drying and staining with May-Grunwald Giemsa; the needle was then rinsed into Cytolyt (Hologic) and collected for ThinPrep™ liquid-based cytology. The data of follow-up modalities including clinical follow-up with or without thyroid ultrasound, molecular testing, repeat FNA and surgical intervention were collected, allowing for a minimum follow-up period of 6 month. The final outcomes were classified as benign, malignant or undetermined.

Results. A total of 6498 FNACs were performed during the period of study; 453 (7%) patients (535 FNACs) were diagnosed as AUS/FLUS. 255 (56,3%) of AUS/FLUS patients had repeat FNAC, 104 (23%) went directly to surgery, 94 (20,7%) were followed up clinically by ultrasound scan (USS). 405 (89,4%) patients had molecular testing done on either initial AUS/FLUS specimen or on follow-up. Of the 104 patients who had direct surgery after the first AUS/FLUS diagnosis, 56 (53,9%) cases were malignant and 48 (46,1%) were benign (Tab. I). The Results of the repeat FNA are: 121 (47,4%) benign, 81 (31,8%) AUS/FLUS, 8 (3,2%) FN/SFN, 1 (0,4%) malignant and 44 (17,2%) non diagnostic (Tab. I).

The Results of the final outcomes as determined by all modalities are represented in Table II; there was an overall ROM of 17,6%.

50/54 patients with an histological diagnosis of papillary carcinoma/papillary microcarcinoma had molecular testing for BRAF V600E mutations: 62% were BRAF+.

Conclusions. At our institution the AUS/FLUS diagnostic rate was 7% and ROM was 17,6%. The frequency of AUS/FLUS diagnostic category is at the upper limit of the frequency suggested by TBSRTC. The ROM observed is slightly higher than the 5-15% proposed by 2008 TBSRTC but this falls within the 10-30% recommended by the 2017 Bethesda Conference.

Tab. I. Histological correlation.

Follow-up histology	Direct surgery cohort	Repeat FNA cohort						Tot
		Non diagnostic	Benign	AUS/ FLUS	FN/SFN	SM	M	
Nodular hyperplasia	24	1		9				34
Hashimoto thyroiditis	1							1
Follicular adenoma	19	1		11	1			32
Hürthle cell adenoma	3				2			5
NIFTP	1							1
Papillary microcarcinoma	7			1				8
Papillary carcinoma	35			1			1	37
Follicular variant of papillary carcinoma	9							9
Follicular carcinoma	2							2
Medullary carcinoma	2							2
Metastatic adenocarcinoma	1							1
Total	104	2	0	22	3	0	1	132

FN/SFN=follicular neoplasm, suspicious for follicular neoplasm; SM=suspicious for malignancy; M=malignant; NIFTP= noninvasive follicular thyroid neoplasm with papillary-like nuclear features.

Tab. II. Outcome analysis.

		Final outcome*		
		Benign	Malignant	Inconclusive
Repet FNAC cohort	255	204	3	48
Direct surgery cohort	104	48	56	0
Clinical follow-up and molecular testing	94	24	0	70

*Determined by histology, repeat FNAC, molecular testing and clinical ultrasound scan follow-up.

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PRIMARY EFFUSION LYMPHOMA (PEL) IN HIV-NEGATIVE PATIENTS A RARE UNDERDIAGNOSED ENTITY: REPORT OF NINE CASES FROM A SINGLE INSTITUTION

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Objectives. Primary effusion lymphoma (PEL) defines a very rare B-cell non-Hodgkin lymphoma that grows in liquid-phase within body cavities (pleural, peritoneal, pericardial). Its underlying infection cause is HHV-8. PEL is included as separate entity (ICD-O code 9678/3) in the 2017 WHO classification of tumors of hematopoietic and lymphoid tissues. No epidemiological data are available from cancer registries. Cytology is essential for the diagnosis. We Aim to share our experience about PEL outside HIV-infection by describing clinical features, cytology and molecular characteristics, in order to raise awareness of this entity among pathologists in Italy, an HHV-8 endemic country.

Materials and methods. For the current study, we selected 9 cases previously diagnosed as PEL in HIV-negative patients from a pool of 12,500 effusions (1995-June 2019) at the Pathology Department, Sapienza University Hospital. Each case was reviewed for clinical and laboratory data. Cytological characteristics pooled into Background. inflammatory cells and lymphomatous cells were evaluated and quantified. The immunostaining panel included HHV-8/LANA; EBV/LMP; CD138, CD38, CD30, CD45, EMA, CD20, CD3, Ki-67, kappa and lambda light chains. HHV-8 detection and clonality analysis were performed by Real-Time PCR and PCR, respectively.

Results. The patients were 8 men and 1 woman with a median age of 81 (range 40-93 years), with birthplace in central (n=6) and southern Italy (n=3). Six patients had pleural cavity PEL and 3 had peritoneal cavity PEL. All 9 patients had sings/symptoms of massive effusions, no lymphadenopathies >2cm, and elevated C-reactive Protein (CRP); 2 patients had B-symptoms, 4 had elevated serum LDH levels, 5 had anemia, 2 had CD4 lymphocytopenia and thrombocytopenia. Survival varied widely: <6 months (n=4 subjects); 23 months (n=1); 55 months (n=1); alive/free of PEL in follow-up after 14 and 2 months (n=2); lost at follow-up (n=1).

Effusions showed a predominant medium-to-large cell pattern with a few anaplastic cells (mitoses x10H-PF: 5-12; apoptosis/100 cells: <1%-36%) in a Background. of varying number of inflammatory cells (eosinophils>lymphocytes). All cases were strongly positive for HHV-8/LANA, CD45 and EMA but negative for EBV/LMP, and showed loss of B/T-cell differentia-

tion. CD138 and CD38 were variably expressed; Ig light chains showed restriction in 3 cases out of 5 tested. Clonal bands indicating Ig gene rearrangements were seen both in heavy and light chains. The detection of HHV-8 revealed a very high number of HHV-8 viral copies (tested in 7 effusions). The number of EBV viral copies (tested in 6 effusions) was as well elevated, except in one case (negative).

Conclusions. Considering the limited literature of HIV-negative PEL, our study is so far the largest case series based on clinical information, morphology, immunophenotype, and molecular characteristics. In our institution, HIV-negative PEL has emerged as more common than HIV-positive PEL during the last two decades. It is conceivable that a number of cases remain underdiagnosed for the gap of awareness that many pathologists and clinicians have about PEL outside HIV-infection. It is our hope that this study will contribute to set up large collaborative studies in Italy.

This study is dedicated to the memory of Francesco Lo Coco, Professor of Hematology (1955-2019)

A COMBINED APPROACH FOR THYROID FINE NEEDLE ASPIRATION: LIQUID BASED CYTOLOGY, CELL BLOCK AND IMMUNOHISTOCHEMICAL METHODS WITH THE IMPLEMENTATION OF THE CELLIENT ™ AUTOMATED CELL BLOCK SYSTEM

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Background. Ultrasound Fine Needle Aspiration Cytology (FNAC) is the gold standard diagnostic test for the diagnosis of thyroid nodules. It is a cost-effective procedure which rapidly provides specific diagnosis with minimal complications. It plays an important role in the determination of patients who could undergo surgical treatment reducing the rate of unnecessary surgeries. However, studies have shown that up to 30% of FNAC were diagnosed previously as “atypical,” “indeterminate” and “suspicious” for malignancy [1]. Traditionally FNAC produce cytological material into a smear, which frequently does not allow as many immunohistochemical markers as we need. For some years the liquid base cytology technique has been applied to FNAC allowing also to store cytological samples, enabling to carry out immunocytochemistry, molecular test or cell block (CB). However, due the intrinsic low cellularity of the cytological sample, the traditional techniques of CB fail particularly when caught by a fine needle (23-25 G). Lately introduced automatically CB system is able to standardize CB, also in a low cellularity setting. Cellient™ Cell Block System (Hologic Corporation, Marlborough, MA, USA) can produce standardized CBs with a higher cellularity than traditional CB methods and also achieve excellent Results performing immunohistochemistry (IHC) and molecular techniques [2, 3].

Objective. Our Aim. is both to validate and standardize a diagnostic algorithm, which could allow both a better diagnostic classification of the indeterminate lesions and patients’ risk stratification.

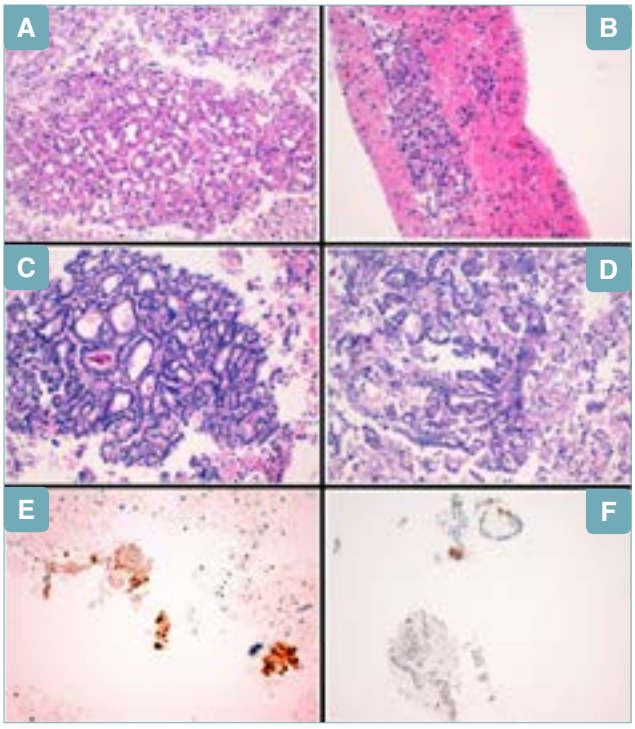

Fig. 1. cCB slides with a pure Hurthle cell morphology(A); micro follicular pattern into Hurthle cell lesion (B); benign pattern (C); papillary pattern (D); Ciclin D1 overexpression (E); Ciclin D1 low expression (F).

Materials and methods. During the period from March 2018 to August 2019, 1808 US-FNA performed with a 23-25G needle by capillary action into thyroid nodule, were fixed in Cytolyt solution and routinely processed with ThinPrep (Hologic). After examination of the slides, a Cellient™ CB (cCB) was requested when it resulted/was suggestive of atypia/follicular lesion of unknown significance. In a minority of cases, it was performed also on benign or evidently malignant samples. Pathologists evaluated the material obtained, recordering cellularity, cellular morphology and diagnostic category according to the Italian Reporting System for Thyroid Cytology[4] and immunohistochemical Results for HBME-1, BRAF and Galectin 3. Expression of cyclin D1[5] in thyroid nodules was determined by immunohistochemistry and a semiquantitative valuation was performed using H-score method [6].

Results.1808 FNAC with an average age of 57 years (range 13-90 years), final diagnoses were not diagnostic [TIR1] (291; 16%), benign [TIR2] (1142; 63%), low risk indeterminate lesions [TIR 3A] (193; 11%), high risk indeterminate lesions [TIR 3B] (89; 5%), suspect for carcinoma [TIR 4] (20; 1%), diagnostic for carcinoma [TIR 5] (73; 4%). 134 (7%) cases with near to adequate cellularity were selected for cCB with a provisional diagnosis of: TIR1 (4; 3%), TIR2 (6; 4%), di TIR3A (84; 60%), TIR3B (19; 16%), TIR4 (11; 8%) e TIR5 (10; 7%). Afterwards, cCB slice were confronted with provisional diagnosis, the final diagnosis was concordant in 66 (49%) cases. Into discordance group all TIR1 cases became adequate (TIR2); TIR3A cases were reclassified into TIR2

(20; 21%), TIR3B (19; 23%) and TIR 4/5 (4; 5%); TIR3B cases were reclassified into TIR3A (4; 21%) and TIR4/5 (5; 26%); all TIR 4 cases became TIR5. Among the indeterminate lesions, 28 (21%) showed a pure Hurtle cell morphology and 54 (40%) showed a prevalent follicular architecture. In a selected number of atypical lesions, the immunolabeling for cyclin D1 was also performed in order to highlight the lesions with the greatest proliferative potential. The average H-Score was 75 for TIR3A, 80 for TIR3B, 200 for TIR4, 270 for TIR5; thus, we observed the higher the Ciclin D1 the higher the probability of neoplastic lesion was.

Conclusions. In the present study, the combination for LBC and Cellient™ CB, has increased the cellularity evaluated with great architectural detail information and more defined cytological details, mostly for Hurtle cell lesions, in particular into TIR3A and TIR3B category (Fig. 1). Finally, a better availability of slices to IHC was obtained. In centers with a high volume of thyroid FNAC, LBC allow to perform rapid and cheap diagnosis but into indeterminate lesions cCB could be useful to better stratify the diagnostic category. Among these our preliminary Results confirm the possible role of Cyclin D1 as a marker of proliferation to select cases to send at surgery or to perform molecular tests.

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ROLE OF CYTOLOGY IN THE DIAGNOSES OF SUBCENTIMETRIC INDETERMINATE LESIONS

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Objectives. Thyroid nodules are a common medical detection found in 4.7% of patients at physical examination by palpation alone and 10-67% of patients by ultrasound (US). Although the overall malignant rate is at around 5-10%, it is between 9 and 15% at fine needle aspiration cytology (FNAC). Guidelines have been clearly defined for large thyroid nodules, but controversy emerged about nodules smaller than 1 cm. The 2015 American Thyroid association (ATA) defined specific guidelines for assessment of thyroid nodules including those for small nodules. Experts suggest that suspicious US features

BSRTC Category	Total	Percent	Percent ROM
Unsat/ND	3	1.3	33.3
Neg/Benign	24	10.7	0
AUS/FLUS	9	4	22.2
FN/SFN	9	4	11.1
FNHCT/SFNHCT	2	0.9	0
Susp Malign	69	30.7	97.1
Pos Malign	109	48.4	100
Totals	225	100	

	Total number	ROM
Indeterminate lesions (III+IV)	20	15%
Malignant categories (V+VI)	178	97.1%

Fig. 1.

seem to merit referral for US-FNAC. The management of a diagnosis of indeterminate categories in a subcentimetric nodule is a relevant issue for pathologists and clinicians. A further issue in their diagnosis and management has been the recent introduction of NIFTP that kept its size above 1 cm. The purpose of this study is to evaluate our series of subcentimetric thyroid nodules to determine their rate of malignancy (ROM), the indeterminate diagnoses and the diagnosis of NIFTP.

Materials and methods. The thyroid archival databases were searched at a tertiary medical centre (Fondazione Policlinico Universitario "Agostino Gemelli"-IRCCS) for a 40 month timeframe (January 2015-May 2018.). A total of 225 thyroid FNA cases of lesions smaller or equal to 1 cm with available histological follow-up were identified. Cases were classified according to The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC). IRB approval was obtained and the majority of nodules were evaluated and biopsied under ultrasound (US) guidance by clinicians and radiologists. All FNAC specimens were processed with liquid-based cytological (LBC) method using Thin Prep 5000™ processing (Hologic Co., Marlborough, MA, USA) and Papanicolaou staining. In addition second LBCs for each case were performed if the initial evaluation showed low cellularity. All the cytological and histological sections were evaluated by cytopathologists and pathologists with experience in diagnosing thyroid pathology and those cases whose interpretation was equivocal were submitted to the diagnostic judgment of the other pathologists until a final agreement was achieved. The entire series included 158 female and 67 male patients with age ranging from 9 to 82 years. The table below and the

result section describe the distribution of the cases for the different cytological categories including the risk of malignancy (ROM).

Results. The series included 225 lesions ≤1cm out of 891 cyto-histological thyroid lesions. According to TBSRTC we found: 3 cases in category I (1.3%), 24 in category II-Benign Lesion (BL, 10.7%), 9 in III- Atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS, 4%), 11 in IV-Follicular Neoplasm/Suspicious for follicular neoplasm (FN/SFN, 4.9%), 69 in V-Suspicious for malignancy (SM, 30.7%) and 109 in VI-Positive for malignancy (PM, 48.4%). Our result found that the majority of cases in the category VI and V were subcentimetric (82% and 80% respectively). None of our BL had a malignant outcome regardless of the size. The rate of ND was slightly high due to the difficulties in obtaining adequate material from small nodules. Furthermore a 33.3% ROM was reported in ND underlying the need to repeat FNAC and combined the yield with the clinical and radiological findings. The proportion of III/IV was globally 8.9%. The malignant rate for indeterminate lesions (III+IV) was 15%; with 22.2% for category III and 11.1% for category IV. The malignant rate for the malignant categories (V plus VI) was 97.1%, with only two follicular adenomas diagnosed in the category V. None of these cases was diagnosed as NIFTP and it did not affect the ROM for the indeterminate categories.

Conclusions. This study shows that a large proportion (80%) of subcentimetric thyroid nodules, sampled by FNA, is malignant. Since these nodules are small, they can be difficult to biopsy and often result in an unsatisfactory diagnosis. However, the unsatisfactory diagnosis should not be ignored since the ROM for this category is much higher than that for the usual thyroid nodule (ROM 5-10%, TBSRTC). The low ROM for the FN/SFN and FNHCT/SFNHCT diagnoses reflects the lower incidence of follicular-patterned malignancies among subcentimetric nodules. Conversely, the high incidence of malignant cytology diagnoses (48.4%) indicates that a greater proportion of subcentimetric thyroid carcinomas show overt features of papillary carcinoma.

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ROSETTE AND PSEUDO-ROSETTE PATTERNS: MORPHOLOGICAL EVIDENCE IN THE DIFFERENT THYROID LESIONS. ARE THEY SPECIFICALLY LINKED TO PTC VARIANTS?

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Background. The detection of rosette-like clusters of tumor cells in thyroid carcinoma has been reported only in the columnar cell variant of papillary thyroid carcinoma (CCV-PTC). Despite the fact that a cytological diagnosis of PTC variant is not encouraged by the Bethesda system for reporting thyroid cytopathology (TBSRTC), the identification of specific features, linked with the PTC variants, might be helpful in reducing possible misinterpretation of thyroid fine needle aspiration cytology (FNAC). For thyroid gland, even though some authors documented rosettes in thyroid cancers diagnosed as MTC, Sen et al assessed the presence of a rosette-like pattern in a case of well-differentiated thyroid follicular carcinoma diagnosed as columnar cell variant of papillary thyroid carcinoma (CCV-PTC). To the best of our knowledge, for the first time in literature, Sen et al reported the occurrence of rosette-like structures also on fine needle aspiration cytology (FNAC). The possible association of rosette pattern with CCV-PTC might be a new additional diagnostic criterion useful for identifying different PTC variants on thyroid cytology. We investigated the correlation of architectural patterns with the different PTC variants.

Method. We analyzed all FNAC diagnosed as suspicious for malignant (SFM) and positive for malignancy (PM) with histological follow-up and collected from January 2018 to December 2018. Cytological cases were processed with liquid based cytology (LBC). The cytology cases were classified and diagnosed according to the New Italian Working Group SIAPEC-IAP classification. All of cases were re-evaluated and then re-classified according to The Bethesda System for Reporting Thyroid Cytology II (TBSRTC, 2017). For this study, analyses were conducted using TBSRTC terminology.

Results. The series included 115 lesions with FNAC ranged in size from 5 to 65 mm (average size 14,93mm and median size 12mm). Cytological diagnoses included: 50 suspicious for malignancy favoring PTC (SM-V) (%) and 65 malignant (M-VI) (11%). All cases had histological follow-up resulting into 100% malignancy. The histological diagnoses included: 30 classic PTC and 85 PTC variants (46 tall cell variants-TCV, 13 hobnail variant, 3 solid variant, 6 Warthin-like, 8 follicular variant, 5 columnar variant and 4 mixed tall and columnar variants). The morphological evaluation was conducted analyzing the different architectural patterns. Specifically papillary structures, with prevalence of microfollicular and follicular architecture and with a rosette or pseudorosette like morphology were searched in each case. Our data confirmed that solid clusters were present in 100% of cases, and isolated cells in 111 out of 115 (96.5%). However, papillary and pseudopapillary pattern was found in 111 cases (96.5%) and microfollicular pattern in 87 cases (75.6%). Specifically rosette-like architecture was found in 33 cases (28.7%) including 12 TIR4 and 21 TIR5 cases. Analyzing the distribution of the rosette-like pattern, it was mostly associated with both TCV (14 cases-42.5%) and hobnail variant of PTC (21.2%). The amount of rosette-like pattern was equally distributed regardless of the percentage of tall cell component (30% as cut-off level of tall cells).

Conclusions. Some architectural patterns may be recog-

nized in specific PTC variants and they may be helpful in reducing the risk of misinterpretation and/or suggesting the correlation with specific PTC variants. In our series, the presence of rosette-like pattern has been observed mostly in TCV and Hobnail variant of PTC representing 63.6% of the cases with rosette-like pattern

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PD-L1 EXPRESSION IN THYROID CYTOLOGY CORRELATES TO MALIGNANCY AND IT REPRESENTS A POSSIBLE PROGNOSTIC MARKER

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Background. Programmed death-ligand 1(PD-L1) expression is emerging as an important predictive biomarker in anti-PD-L1 cancer immunotherapy. Its role has been clearly defined in a variety of human cancers and linked to poor prognosis and resistance to anticancer therapies. In fact, the analysis of PD-L1/PD-1 has been evaluated on several cancers including advanced stage lung cancer, mostly non-small cell lung cancer (NSCLC), head and neck carcinoma, melanoma and urogenital cancers showing a prevalence ranging from 50% to 20%. PD-L1 expression in these cancers using immunohistochemistry (IHC) has been validated only on formalin fixed, paraffin embedded (FFPE) histological samples. None of the FDA-approved PD-L1 antibodies have been clinically validated on cytological specimens not fixed in formalin. The majority of studies to date have instead dealt with the use of FFPE cytology cell-blocks (CB). Its role in thyroid cancers has not been well defined as well as its application on fine needle aspiration cytology (FNAC). For thyroid pathology, the expression of PD-L1 has been reported in a few series. Recently, Chowdhury et al documented that PD-L1 expression of tumor cells correlated with poor prognosis in thyroid cancer, with a greater risk of recurrence, and shortened disease free survival. Other published studies proposed the administration of anti-PD-L1 immunotheraphy in patients that are unresponsive to radioiodine or other chemotheraphy measures. None of these articles addressed the evaluation of the diagnostic and prognostic role of PD-L1 in thyroid cytological samples, particularly liquid based cytology (LBC). Studies validating the use of different cytological specimens are needed to optimize tissue utilization in diagnostic testing also for thyroid lesions. We demonstrated the performance of PD-L1 on liquid based cytology (LBC) and whether PD-L1 could be a biomarker of malignancy or aggressive disease.

Methods. From January 2018 to March 2019, 206 thyroid lesions, diagnosed by FNAC as indeterminate lesions (IL), suspicious for malignancy (SFM) and malignant (M), were enrolled. We performed PD-L1 staining on both LBC and the corresponding histology.

Results. The FNAC cohort included 20 benign negative controls, 42 AUS/FLUS, 33 FN/SFN, 53 SFM and 58 M. AUS/FLUS included 3 goiters, 32 follicular adenomas (FA), one NIFTP, 5 invasive variant of papillary thyroid carcinoma (I-FVPTC) and 1 follicular carcinoma (FC) whereas FN/SFN included 24 FAs and 9 malignancies (4 I-FVPC, 1 NIFTP, 3 PTC, 1 oncocytic follicular carcinoma (OFC). The 53 SMs were diagnosed on histopathology as 2 FA, 5 NIFTP, 15 I-FVPC and 31 PTC whilst the 58 Ms included 5 NIFTP, 5 I-FVPCs, and 48 PTC. Increased plasma membrane and cytoplasmic PD-L1 expression was found in 79 cases (38.5%) including 61 PTC (conventional and variants). Negative PD-L1 expression was found in NIFTP and FAs. The majority of our positive PD-L1 cases had more than 30% staining, but less than 50% positive tumor cells (61 cases) and showed moderate cytoplasmic and membranous staining. Indeterminate expression was found in the tall cell variant of PTC (TCV), with only 10% positive cells. Weak PD-L1 expression was found in 19 oxyphilic neoplasms. $BRAF^{V600E}$ was mutated in 15% of PD-L1 positive malignancies. $BRAF^{V600E}$ was mutated in 15% of PD-L1 positive malignancies.

Conclusions. In conclusion, this study shows that the quantification of PD-L1 immunostaining in LBC slides is feasible and correlates well with paired histological tissue interpretations. Our finding suggest that PD-L1 expression in thyroid LBC cases may serve as a marker of malignancy. Specifically, our data reveals that PD-L1 expression in thyroid LBC specimens correlates with PTC, the aggressiveness of these tumors, and in a subset of patients with $BRAF^{V600E}$.

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IMMUNOCYTOCHEMICAL APPROACH TO INDETERMINATE THYROID NODULES

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Objective. Fine needle aspiration cytology (FNAC) has been widely used in the diagnosis of thyroid carcinoma and can provide reliable preoperative diagnostic Results, however follicular patterned lesions (FP) are a challenging category for which the exclusive morphology evaluation is not able to define the nature so that some ancillary techniques have been developed to make a correct diagnosis achievable.

In these last years, immunocytochemistry using HBME-1

and Galectin-3, has been referred as helpful in differentiating low and high risk follicular lesions whereas BRAF-1 activating mutations have been identified in 29-69% of papillary thyroid cancers (PTC). The accurate evaluation of $BRAF^{V600E}$ mutation in preoperative FNAC specimens is important for making management decisions in thyroid nodules. The introduction of the alternative Liquid based cytology (LBC) might simplify their application.
Our Aim. is to evaluate the role of LBC in the application of an algorithm combining immunocytochemistry and BRAF mutation in predicting the outcome and management of FP.
Materials and methods. From April 2019 through June 2019, we enrolled 24 LBC cytological cases classified as indeterminate lesions with low risk (TIR3A) and 4 cases of indeterminate lesions with high risk (TIR3B). An immunocytochemistry panel including HBME-1 and Galectin-3 was developed and applied to 16 TIR3A cases. BRAF gene mutation was evaluated using $BRAF^{V600E}$ specific antibody in 14 TIR3A cases. All TIR3B, TIR4 and TIR 5 were simultaneously studied with ICC panel and $BRAF^{V600E}$ antibody.
Results. ICC panel (HBME-1+Galectin-3), performed on 20 indeterminate cases, resulted positive in only one TIR3A case (6,25%), with 100% of the 18 TIR3 cases studied showing a BRAF wild type. In the 8 malignant TIR4, ICC resulted positive in 6 cases (75%) and 25% had BRAF positivity. All the 3 malignant TIR5 lesions expressed a positive panel showing a BRAF mutation in one case. Two malignant cases with simultaneous ICC panel and BRAF positivity underwent surgery, histologically resulting as papillary thyroid carcinoma.
Conclusions. Immunocytochemistry and BRAF gene mutation can be successfully carried out on LBC processed material. Our cytological algorithm combining morphology and ancillary techniques may be able to better define the nature of follicular lesions and may contribute to a correct clinical management of patients.

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FINE NEEDLE ASPIRATION CYTOLOGY FOR THE PRE-OPERATIVE DIAGNOSIS OF SOFT TISSUE TUMORS: TECHNIQUES, OPPORTUNITIES AND PERSPECTIVES THROUGH A SINGLE INSTITUTION EXPERIENCE

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Introduction. Soft tissue tumors comprise a great variety of common and rare neoplasms with overlapping features [1]. Their diagnosis is based on the evaluation of histological parameters, such as architectural pattern, vasculature and stromal component, the presence and extent of necrosis and mitoses, which are difficult to assess on small incisional biopsies in a pre-operative setting [2]. It is known that some of these tumors are driven by recurrent molecular alterations whose discovery has led to the implementation of highly specific diagnostic tests. They include: molecular techniques detecting pathogenetically relevant, distinctive molecular alterations and immunohistochemical surrogates for pathogenetically relevant, distinctive molecular alterations. Furthermore, the development of new immunohistochemical stains indicating tumor line of differentiation with higher specificity than conventional stains facilitates the diagnosis of those tumors lacking known pathognomonic alterations. Fine needle aspiration cytology (FNAC) is gaining acceptance for the pre-operative assessment of soft tissue tumors. FNAC represents a versatile, poorly expensive and well tolerated diagnostic technique which can reach and sample both superficial and deep-seated lesions with minimal risks for the patient. Moreover, the introduction of the previously discussed tests with high diagnostic specificity, which are easily applied to cytological samples, greatly enhances the diagnostic yield of cytology, allowing a definite diagnosis in most cases.
Objectives. We present a series of exemplificative cases of different soft tissue tumors assessed by FNAC in the pre-operative setting at our Institution (University *"Luigi Vanvitelli"* of Naples, Pathology Unit). In all cases, the cytological diagnosis was supported by the use of special immunostains or molecular techniques. The applied techniques and feasibility of the procedures are commented. In addition, the cytological diagnosis is compared with the final histological diagnosis.
Material and methods. The series include: 1) soft tissue tumors with pathogenetically relevant molecular alterations detected by molecular techniques: a synovial sarcoma of the lung and a desmoplastic small round cell tumor of the abdominal cavity; 2) soft tissue tumors with immunohistochemical surrogates for pathogenetically relevant molecular alterations: a solitary fibrous tumor of the pancreas; 3) soft tissue tumors with highly specific immunohistochemical markers indicating line of differentiation: a recurrent chordoma and a gastrointestinal stromal tumor of the stomach; a high grade chondroblastic osteosarcoma of the chest wall.
Results. In the vast majority of cases, cytology could reach a definite diagnosis which was further confirmed by histological post-operative examination.
Conclusions. In expert hands, FNAC can be confidently accurate in subtyping and grading mesenchymal neoplasms and thus guide the clinical management of the patient [3,4]. Moreover, it can also Aim. at a definite diagnosis in some cases with the aid of immunoistochemical and molecular tests with high diagnostic specificity [5]. Importantly, rapid on site evaluation performed by a cytopathologist provides direct information about the adequacy of the cytological specimen and helps reducing to a minimum the number of non-diagnostic procedures. The cytopathologist plays a determinant role in the whole process, from sampling of the mass to the final report.

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CYTO-HISTOLOGICAL CORRELATION OF TIR3A AND TIR3B THYROID NODULES AFTER THE IMPLEMENTATION OF ITALIAN REPORTING SYSTEM FOR THYROID CYTOLOGY

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Objectives. To evaluate histological outcomes and malignancy rates of a well characterized series of thyroid nodules diagnosed as TIR3A or TIR3B after the implementation in 2014 of the new Italian Reporting System for Thyroid Cytology (IRSTC).[1] To date, there are few studies addressing this issue.[2,3]

Materials and methods. We report a retrospective analysis of thyroid FNA from 4454 nodules diagnosed in our institution from January 2015 through December 2017, according to IRSTC. The overall distribution of cytological diagnoses was the following: TIR1, 17.3% (n=766); TIR1c, 1.9% (n=86); TIR2, 60.2% (n=2666); TIR3A, 12.5% (n=555); TIR3B, 4.6% (n=205); TIR4, 2.4% (n=105); TIR5, 1.6% (n=71). We considered histological outcome and calculated malignancy rates of all thyroid nodules classified as TIR3A or TIR3B and submitted to surgery in house.

Results. A total of 130 patients underwent surgical resection for indeterminate cytology (17.1%), with rates of surgery of 9.9% for TIR3A (n=55) and 36.5% for TIR3B (n=75), respectively. Out of 55 TIR3A nodules, 9 (16.5%) were malignant at histology, namely 7 papillary thyroid carcinomas (PTC) and 2 follicular thyroid carcinomas (FTC); the remaining were 32 (58.2%) hyperplastic nodules, 13 (23.6%) follicular adenomas, and 1 NIFTP. Regarding TIR3B, out of 75 TIR3B nodules, 28 (37.3%) were malignant, namely 25 PTC and 3 FTC; the remaining were 18 (24%) follicular adenomas, 2 NIFTP, 1 NET (G1), and 26 (34.6%) hyperplastic nodules.

Conclusions. In our experience, TIR3A and TIRB malignancy risks (16.5% vs. 37.3%) were slightly higher than those expected according to ISRTC (about 10% for TIR3A and 15%-30% for TIR3B). This is somewhat in line with several studies addressing TIR3A/TIR3B malignancy risks.[4] Our Results support the validity of TIR3A/TIR3B sub-categorization in sorting-out nodules with dissimilar risks of malignancy; conversely, TIR3A/TIR3B sub-categorization seems ineffective to discriminate adenomas (23.6% in TIR3A vs. 24% in TIR3B).

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THE ROLE OF THYROID FNAC WITH MOLECULAR ANALYSIS IN CLINICAL PRACTICE: TRIESTE EXPERIENCE

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Objectives. To evaluate the impact of genetic molecular analyses in the diagnosis and treatment of thyroid indeterminate and suspicious lesions (Tir3a, Tir3b and Tir4 categories of the Italian classification).

Materials and methods. We collected the data of fine needle aspiration cytology (FNAC) exams performed on thyroid nodules at the University Hospital of Trieste in 2008 and 2018. In order to compare the cytological diagnoses with histology, we collected the data about thyroid surgery in 2008 and 2009 for the first case series and in 2018 and 2019 for the latter. Data about genetic molecular analyses - where available - were also included. All these data were analysed by mean of a Microsoft Excel® worksheet. We included in the analysis all cases with an indeterminate or suspicious cytological diagnosis (Tir3a, Tir3b and Tir4).

Results. 630 and 647 thyroid FNAC exams were performed in 2008 and in 2018 respectively. An indeterminate (Tir3) diagnosis was made in 95 cases (15.05%) in 2008 and in 91 cases (14.06%) in 2018; 19 (3.06%) and 19 (2.94%) lesions were assessed as suspicious for malignancy (Tir4) in 2008 and in 2018 respectively. Genetic molecular analyses were performed preoperatively in only 4.50% of all indeterminate and suspicious diagnoses in 2008, compared with 81.13% in 2018. Overall, the rate of surgical treatments was 9.05% in 2008 and 8.04% in 2018, with a prevalence of hemithyroidectomies performed in 2008 (29.82% of all surgical treatments) compared to 2018 (17.31%). Among patients with Tir3 nodules, 30.43% underwent surgery in 2008 versus 23.86% in 2018.

Considering only the cases in which a cyto-histological comparison was possible - and excluding the so-called “occult papillary micro-carcinomas” - the risk of malignancy (ROM) of Tir3 was 20,69% in 2008 and 19,95%

in 2018. According with literature, it was much higher (66.67%) in cases with indeterminate cytology which were found to carry a mutation in the genes BRAF, NRAS, KRAS or PIK3CA. Surprisingly, separating Tir3a and Tir3b (which are referred in the literature as "low-risk" and "high-risk" indeterminate lesions, respectively) in the case series from 2018, in which this division was always made in the routine diagnostic, the ROM was slightly higher (20.00%) in the Tir3a category than in the Tir3b (18.75%).

The ROM was 92.31% in 2008 and 87.50% in 2018 among Tir4 diagnoses. Two Tir4 cases were diagnosed as benign (both Hürtle cell adenomas) on histology. In both cases, preoperative genetic analyses did not show any recognized mutation. All the suspicious cases with BRAF mutations were confirmed as malignant (papillary carcinomas) in the surgical specimen.

Conclusions. The actual risk of malignancy is difficult to evaluate in thyroid pathology, since most malignant tumours have low progression rates and, even when misdiagnosed as benign and not adequately treated, take a long time before becoming clinically significant. In our analysis we had to consider only those lesions which underwent surgery, with a relatively short follow-up period, and this affects the comparability of our data with the literature. However, the data from the two periods taken into consideration (2008 and 2018) have been compared to each other and show a slight reduction in the ROM of Tir3 and Tir4 lesions overall. The distinction of Tir3a and Tir3b diagnoses in the case series of 2018 did not show the expected increase in the ROM in Tir3b lesions. Nevertheless, genetic molecular analyses showed to be very useful in identifying those lesions which had the most probability of being malignant on the surgical specimen.

Routine use of genetic molecular analyses in thyroid cytology is useful in the assessment of indeterminate and suspicious nodules, optimizing the Results of FNAC in term of risk of malignancy, and reducing the need of diagnostic hemithyroidectomy.

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VENERDÌ 18 OTTOBRE 2019

Miscellanea 3
Sala Roma – 08:30 - 13:00

PATOLOGIA APPARATO DIGERENTE

PD-L1 EXPRESSION IN GASTROESOPHAGEAL DYSPLASTIC LESIONS: BIOLOGICAL, DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS

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** Equally contributed*

Background. PD-1/PD-L1 checkpoint immunotherapy has been recently approved for gastric (GC) and gastroesophageal junction adenocarcinomas (GEC) and PD-L1 immunohistochemical evaluation represents a promising predictive biomarker in this subgroup of neoplasms. However, no systematic data are available on PD-L1 expression in gastroesophageal preinvasive lesions.
Materials and methods. A series of 125 dysplastic lesions (52 low-grade and 73 high grade) and 30 early stage intestinal-type GC and Barrett's related GEC were investigated for immunohistochemical expression of PD-L1. Cases were considered as PD-L1 positive in the presence of a Combined Positive Score (CPS) ≥1. In all cases DNA mismatch repair proteins status was also investigated.
Results. PD-L1 was positive in 48 (31.0%) samples. A higher prevalence of PD-L1 positive cases was observed among esophageal specimens compared to gastric ones (p=0.0003), in high-grade and adenocarcinoma samples in comparison to low-grade dysplasia (p<0.0001), and in lesions with DNA mismatch repair deficiency (p=0.028). Among 30 cases with matched dysplastic-adenocarcinomatous samples, discordant PD-L1 status was observed in 12/30 (40%) cases.
Conclusions. This is the first study that systematically assessed PD-L1 expression among gastric and Barrett's related dysplastic lesions. In such cases, PD-L1 expression status should be investigated on routine endoscopy biopsies by gastrointestinal pathologists.

PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF APPENDICEAL MUCINOUS NEOPLASMS

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Background. Appendiceal mucinous neoplasms are a heterogeneous group of diseases, which are associated to the occurrence of pseudomyxoma peritonei (PMP) or of mucinous adenocarcinoma. The molecular landscape of appendiceal mucinous tumors was investigated profiling *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *RNF43*, and *SMAD4* gene mutational status and assessing the expression of the proteins of the DNA mismatch repair (MMR) complex and of p53. The molecular profile of these neoplasms was also compared with that of similar lesions occurring within colic mucosa (i.e. the serrated carcinogenetic pathway) or in the pancreas (i.e. intraductal papillary mucinous neoplasm; IPMN).
Materials and methods. Slides obtained from paraffin-embedded tissue samples (H&E) of 30 Caucasian patients were reassessed by 2 expert gastrointestinal pathologists. Standard PCR amplification and Sanger sequencing were used to study *RNF43* and *SMAD4* mutational status. Sequenom MassARRAY System and the Myriapod Colon Status kit were used to study hotspot regions of the *KRAS*, *NRAS*, *BRAF* and *PIK3CA* genes. Immunohistochemical staining was performed to assess MMR proteins status and p53 expression.
Results. A total of 33 lesions were analyzed: 1 hyperplastic polyp (HP), 13 serrated adenomas (SA; 3 with dysplasia), 2 tubular adenomas with high-grade dysplasia (THG), 1 tubulovillous adenoma with low-grade dysplasia (TVLG), 13 low-grade appendiceal mucinous neoplasm (LAMN), 2 high-grade appendiceal mucinous neoplasm (HAMN), and a mucinous adenocarcinoma (K). In 3 patients 2 lesions coexist: HP with TVLG; LAMN with HAMN; THG with K. Mismatch repair proteins resulted proficient in all cases but one; *SMAD4* was wild type in all the tested cases. *KRAS* was mutated in 50% of cases. Altered p53 IHC expression was observed in five lesions (3 LAMN, 2 HAMN). p.V600E *BRAF* mutation was found in 4 cases (1 THG, 3 SA). *GNAS* appeared mutated in 3 cases (1 HAMN, 1 LAMN, 1 SA), like *RNF43* (1 HAMN, 2 LAMN).
Conclusions. The high prevalence of *KRAS* mutation suggests a critical role in appendiceal mucinous neoplasm. *GNAS* mutations always presented with *KRAS* ones, suggesting a role as additive step in the cancerogenic pathway. In 10% of cases, *RNF43* was found mutated. The analogies in phenotypic aspect between appendicular mucinous lesions and serrated colon adenomas/polyps do not reflect in similarities of genetic landscape; instead, the relatively high frequency of mutations observed in the *GNAS*, *RNF43* and *KRAS* genes support a common genetic Background. with pancreatic IPMNs.

IMMUNOPHENOTIPIC INTRATUMOR HETEROGENEITY IN METASTATIC SPORADIC NEUROENDOCRINE NEOPLASMS OF THE GASTROENTERIC TRACT

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Key words: GE-NENs; intratumor heterogeneity; mTOR pathway; p70S6K; 4EBP1; SSTR5

Background. Neuroendocrine neoplasms (NENs) are an emerging issue both in research and in clinical practice. The interest for these diseases stems from the fact that they can develop in almost all anatomical sites, their worldwide increasing incidence and their unpredictable biological and clinical behaviour on the basis of currently available biomarkers, especially regarding Gastro-Entero-Pancreatic NENs (GEP-NENs). Novel targeted drugs have been recently introduced into GEP-NET clinical practice. However, neither reliable predictive biomarker is still available, nor the diagnostic, prognostic and predictive value of intra-tumor phenotypic and molecular heterogeneity has been studied in these neoplasms. The molecular landscape of GEP-NENs is essentially an unexplored continent.

Aim of the study. The main Aim. of the present work is to explore the potential implications of intra-tumoral heterogeneity in GE-NENs. In particular, in order to optimize patient's management, it is important to evaluate if the same treatment could be effective both against primitive tumor and metastases and if a single biopsy could be useful to direct therapeutic choices despite the intra-tumoral heterogeneity.

Maherials and methods. A case-study of 31 consecutive matched primitive and metastatic (both nodal and hepatic) GE-NENs was collected and intra-tumoral heterogeneity was evaluated by means of phenotypical immunohistochemical features. This is the largest casuistry of such type ever collected in field of GE-NENs. The differential expression of Ki67 and 6 druggable targets throughout the progression of natural history of the disease (PTEN, ph-mTOR, ph-p70S6K, ph-4EBP1, SSTR2 and SSTR5) was assessed. Differences between matched samples were tested by applying the Wilcoxon matched pairs signed rank test.

Results. Immunohistochemical characterization of the matched samples resulted in the following findings: grading progression occurs in 16% of cases; the loss of the PTEN activity does not differ significantly between primary tumor, loco-regional nodal locations (LNLs) and metastases (MTs); the activation of the mTOR complex characterizes the vast majority of GEP-NENs, consistently between primitive tumors, LNLs and MTs; the activity of the final effectors of the mTOR pathway (p70S6K and 4EBP1) is significantly stronger in MTs rather than in primary tumors; concerning the evaluation of somatostatin receptor 2A (SSTR-2A) expression-level, there are no significant differences between primary neoplasms, LNLs and MTs, whereas expression of the somatostatin receptor 5 (SSTR-5) prevails in MTs in statistically significant way. Comparing with their well-differentiated counterparts, both neuroendocrine carcinomas included in the casuistry revealed a unique molecular landscape, thus confirming their nosographic autonomy and entailing the need for a dedicated diagnostic-therapeutic approach.

Conclusions. The mentioned Results suggest that the characterization of the molecular profile of GEP-NENs should be performed on a tissue sample taken from the metastases rather than from primary tumor. Intra-tumor phenotypic and molecular heterogeneity in GEP-NENs may impact the tailoring of treatments.

CLINICOPATHOLOGICAL FACTORS ASSOCIATED WITH BRAF-V600E MUTATION IN COLORECTAL SERRATED ADENOMAS.

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Aim. Colorectal serrated polyps are a group of epithelial neoplasm characterised by a saw-tooth glandular profile.[1-3] In 2010, the World Health Organization (WHO) provided a standardised classification of colorectal serrated polyps, identifying three different lesions: hyperplastic polyp, sessile serrated adenoma/polyp (SSA) and traditional serrated adenoma (TSA)[1]. While hyperplastic polyps constitute the earliest step in the serrated pathway, SSA and TSA have a more immediate risk of progression to cancer[4,5]. However, such classification does not reflect the molecular landscape of these lesions. In fact, both SSA and TSA may or may not bear BRAF-V600E mutation, which is considered a marker of poor prognosis in colorectal carcinoma The Objective. of this study was to identify clinical or pathological factors associated with BRAF-V600E mutation in SSA and TSA.

Materials and methods. A systematic review and meta-analysis was performed by searching electronic databases from January 2011 to January 2019 for studies assessing the association of BRAF-V600E mutation with clinical or pathological features of serrated adenomas. Odds ratio (OR) was calculated for each factor; a P-value <0.05 was considered significant.

Results. Forty studies assessing 3511 serrated adenomas (2375 SSAs and 1136 TSAs) were included. BRAF-V600E mutation was significantly associated with proximal localisation (OR = 2.71; P < 0.00001) and cytosine-phosphatase-guanosine (CpG) Island methylator phenotype-high (CIMP-H status) (OR = 4.81; P < 0.0001) in both SSA and TSA, with polyp size <10 mm (OR = 0.41; P = 0.02) in TSA, and with endoscopic pit pattern II-O (OR = 13.11; P < 0.00001) and expression of mucins MUC5A5 (OR = 4.43; P = 0.003) and MUC6

(OR = 2.28; P < 0.05) in SSA. Conversely, BRAF mutation was not associated with age <70 years (OR = 1.63; P = 0.34), age <60 years (OR = 0.86; P = 0.79), female sex (OR = 0.77; P = 0.12), flat morphology (OR = 1.52; P = 0.16), presence of any dysplasia (OR = 1.01; P = 0.59), serrated dysplasia (OR = 1.23; P = 0.72) and invasive cancer (OR = 0.67; P = 0.32), nuclear β-catenin expression (OR = 0.73; P = 0.21) and p53 overexpression (OR = 1.24; P = 0.82).

Conclusions. BRAF-V600E mutation is associated with proximal localisation and CIMP-H status in both SSA and TSA, with size <10 mm only in TSA, and with expression of MUC5A5 and MUC6 and endoscopic pit pattern II-O at least in SSA. In serrated adenomas, BRAF-V600E mutation does not seem to be associated with age and sex, with the prevalence of dysplasia and cancer and with the morphology of the dysplastic component.

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MMUNOHISTOCHEMICAL EXPRESSION OF P27^{KIP1} IS A PREDICTIVE MARKER OF RESPONSE TO CDK4/6 INHIBITORS IN COLORECTAL CARCINOMA

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Objectives. About the 45% of Colorectal Cancer (CRC) harbors RAS mutation (RAS^{MUT}), determining an intrinsic resistance to anti-EGFR targeted therapy (1). In preclinical models, small inhibitors like Palbociclib (PD), a selective inhibitor of Cyclin-Dependent Kinase 4/6 (CDK4/6), have been proposed to impinge on downstream effectors and overcome the effect of RAS^{MUT} (2). To date, there are no biomarkers able to select patients who may mostly benefit from these therapies (3). A recent study pointed to the critical role of p27^{kip1} in the resistance to small inhibitors in RAS^{MUT} lung tumors (4). p27^{kip1} (hereafter p27) is a member of CIP/KIP family of CDK inhibitors, an oncosuppressor that maintains intricate relationship with RAS, in both physiologic and tumoral context, as we previously described (5, 6). Here, we Aim. to verify the possible role of p27 in conferring resistance to PD in RAS^{MUT} CRC and establish if it could act as a predictive biomarker of response, to guide clinicians in tailoring the therapeutic choice.

Materials and methods. Immunofluorescence (Leica TSP8 confocal microscope) and immunohistochemical staining (Mouse monocl. p27 Ab, Transd. Lab), wet lab and cellular techniques were in accordance to standard protocols of our lab (6). *In vivo* experiments were in accordance with European legislation and ethical guidelines of our Animal Wellbeing Organism. After tumor onset, 100mg/Kg of Palbociclib (MedChemExpress) was administrated daily and luciferase activity was weekly tracked (Ivis Caliper System).

Results. We analyzed p27 protein expression by Western Blot analysis (WB) in a panel of 15 CRC cell lines, observing high variability. We selected RAS^{MUT} CRC cells with high- (SW480) or low- (SW620) expression of p27, and we tested their sensitivity to PD in dose-response curves. Results showed that SW480 (p27 high) were more resistant to PD compared with SW620 (p27 low). Accordingly, lentiviral silencing of p27 in SW480 increased their sensitivity to PD and, conversely, p27 overexpression in SW620 cells increased their resistance, corroborating the correlation between p27 expression and CDK-inhibitor response (Fig. 1). To evaluate the effects of PD treatment on p27 localization, we performed immunofluorescence assay showing a time-dependent accumulation of both nuclear and cytoplasmic p27 in PD-treated cells (Fig. 2). This result prompted us to generate PD-resistant CRC cells, thus after a continuous exposure to PD (1 μM of PD for 1 month), we selected SW480 and SW620 resistant pools. WB and IF analyses showed not only an upregulation, but also a nuclear accumulation of p27 in PD-resistant CRC cells, compared to parental cells in which p27 was preferentially localized into the cytoplasm (Fig. 3). To evaluate whether these changes could have an impact in tumor response in vivo, we set up an ortothopic xenograft model of CRC. We injected HCT116 cells stably expressing a luciferase reporter and transduced with lentiviral particles encoding for a scramble sequence or a short-hairpin RNA vectors against p27 (respectively shNT and shp27) in the cecum of immunocompromised NOD/SCID mice (male, 5-7 weeks old) and tracked the luciferase signal to evaluate the tumor onset. Once the tumors were engrafted, we randomly assigned mice in two groups, respectively treated with vehicle or PD. As expected, silencing of p27 *per se* strongly increased tumor growth. However, under PD treatment, CRC tumors derived from shp27 displayed a significantly increased response respect to shNT tumors (Fig. 4).

In order to verify if p27 expression could be actually exploited as histological biomarker, we carried out immunohistochemical staining of a cohort of 157 CRC specimens (66% RAS mutated, 44% wild type). Although we did not observe any correlation between RAS-mutational status and p27 expression/localization, we reported a strong correlation between low/absent p27 expression and the mucinous histotype (p= 0.00055).

Conclusions. Our Results strongly support that p27 expression levels impact on PD-sensitivity in CRC. The

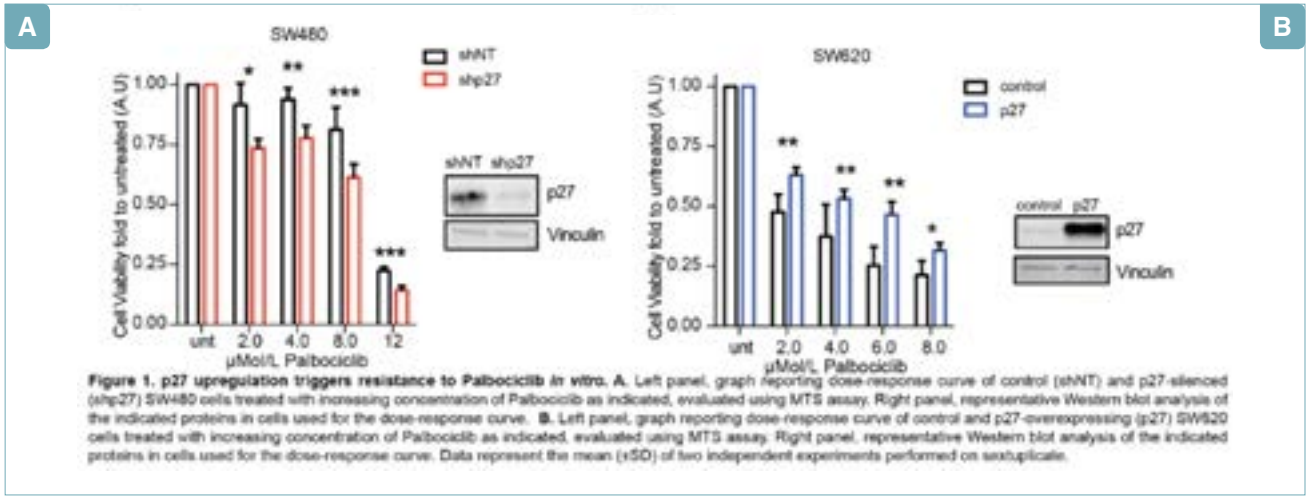

Figure 1. p27 upregulation triggers resistance to Palbociclib *in vitro*. A. Left panel, graph reporting dose-response curve of control (shNT) and p27-silenced (shp27) SW480 cells treated with increasing concentration of Palbociclib as indicated, evaluated using MTS assay. Right panel, representative Western blot analysis of the indicated proteins in cells used for the dose-response curve. **B.** Left panel, graph reporting dose-response curve of control and p27-overexpressing (p27) SW620 cells treated with increasing concentration of Palbociclib as indicated, evaluated using MTS assay. Right panel, representative Western blot analysis of the indicated proteins in cells used for the dose-response curve. Data represent the mean (±SD) of two independent experiments performed on sextuplicate.

Fig. 1.

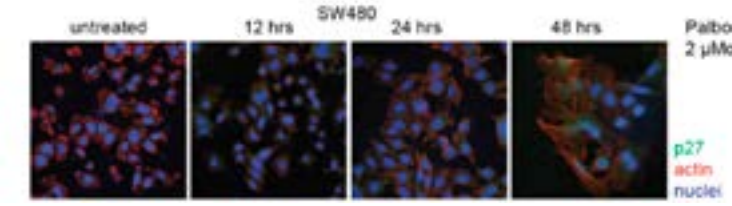

Figure 2. PD pressure induces a strong upregulation of nuclear p27 *in vitro*. A. Representative confocal images of time-course immunofluorescence assay performed in SW480 cells treated for the indicated time with 2 μMol/L Palbociclib.

Fig. 2.

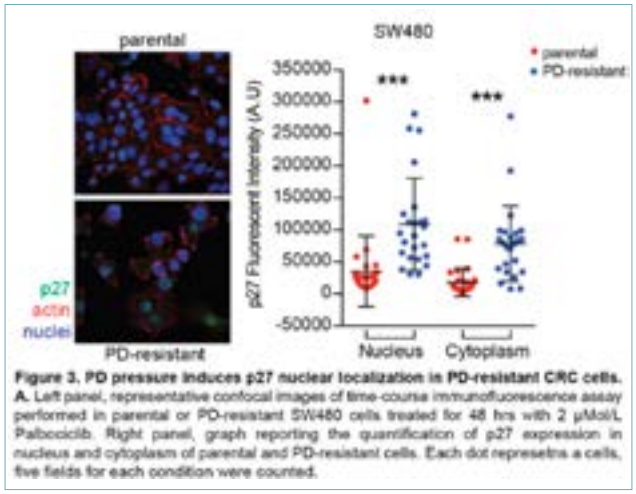

Figure 3. PD pressure induces p27 nuclear localization in PD-resistant CRC cells. A. Left panel, representative confocal images of time-course immunofluorescence assay performed in parental or PD-resistant SW480 cells treated for 48 hrs with 2 μMol/L Palbociclib. Right panel, graph reporting the quantification of p27 expression in nucleus and cytoplasm of parental and PD-resistant cells. Each dot represetns a cells, five fields for each condition were counted.

Fig. 3.

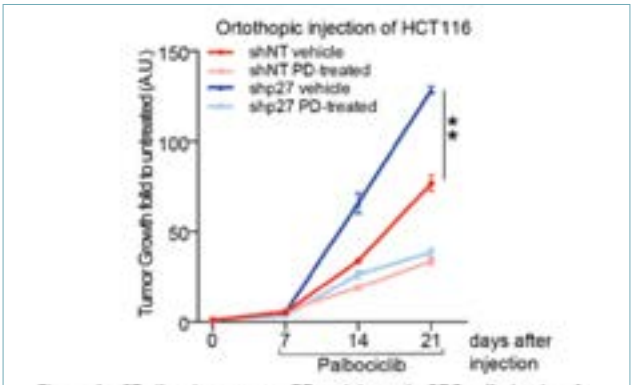

Figure 4. p27 silencing rescues PD resistance in CRC cells *in vivo*. A. Graph reporting the Luciferase activity folded to untreated in mice orthotopically injected with control (shNT, n=4) or p27-silenced (shp27, n=4) HCT116 cells. After tumor appearance mice were daily treated with vehicle (2 mice/group) or 100mg/kg of Palbociclib (2 mice/group).

Fig. 4.

mechanism through which p27 induces resistance will be the Objective. of our future studies. It is well known that p27 is rarely mutated or lost in human cancer (5), therefore immunohistochemical analysis could represent a feasible and valuable tool to investigate p27 expression, to establish at the time of diagnosis which patients may benefit the most from PD administration. Finally, our study reveals that p27 expression is significantly down modulated in high grade CRC with a mucinous component, suggesting that this cluster of patients could be more sensitive to the treatment with CDK inhibitors.

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CLAUDIN-18 EXPRESSION IN ESOPHAGOGASTRIC ADENOCARCINOMAS: A TISSUE MICROARRAY STUDY OF 523 MOLECULARLY-PROFILED CASES

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Key words: Gastric cancer; Claudin-18; predictive biomarkers; immunohistochemistry

Background. Gastric (GCs) and gastro-esophageal (GECs) carcinomas are the third leading cause of cancer-related death world-wide with a combined incidence of 1.4 million cases annually. Claudins (CDLNs) are a family of at least 27 transmembrane proteins and are the major component of the tight junctions (TJ). CDLNs are mainly localized in the apical regions of the cellular membrane and play a critical role in cell-cell adhesion, maintenance of cell polarity and in selective paracellular permeability. Different CLDNs are expressed in various tissues and can be altered during carcinogenesis. Claudin-18 (CLDN18) is a highly specific TJ protein of the gastric mucosa. An isoform of CLDN18, the Claudin 18.2 (CDLN18.2), has recently emerged as an innovative drug target for metastatic gastric cancer due to its trans-membranous localization. Claudiximab (IMAB362) is a monoclonal recombinant chimeric antibody (IgG1) specific for the CLDN18.2. The antibody can bind CLDN18.2 on the cellular surface inducing the activation of antibody and complement-dependent cytotoxicity. IMAB362 is currently tested in several clinical trials for treatment of advanced gastric carcinomas alone or in combination with standard chemotherapy, showing a favorable safety profile and promising preliminary Results in terms of clinical efficacy.
Aim. To investigate CLDN18 expression in a large mono-Institutional series of GCs and GECs by IHC focusing on its association with the clinico-pathological and molecular parameters.
Materials and methods. We investigated the immunohistochemical profile of CLDN18 (using a semi-quantitative pathology H-score of the tumor cells membranous staining), p53 (considered aberrant if a complete loss or diffuse and strong nuclear immunostaining was present in neoplastic cells), p16 (using a four-tier classification), E-Cadherin (considered altered if complete loss or markedly reduced membranous staining: >30% was present), MSH2, MSH6, MLH1, PSM2 (defining samples MMRd when one or both proteins resulted negative), HER2 (using the four-tier modified Herceptest score for biopsies), and PDL-1 (scoring only tumor cells expression, and using 1% cut-off) in a large series of 523 primary gastric carcinomas (GCs; n=408) and gastroesophageal carcinomas (EGCs; n=115) and 135 matched and synchronous nodal metastases. The status of HER2 and EBER by means of chromogenic *in situ* hybridization (CISH) were also evaluated. In order to assess the intratumoral markers expression variability two neoplastic areas from two separate formalin-fixed, paraffin-embedded (FFPE) blocks were selected and tissue cores (1 mm diameter) were punched out of these areas; whereas, from the 135 synchronous nodal metastases, 2 tissue cores were obtained.
Results. High membranous CLDN18 expression was present in 150/510 (29.4%) primary cases and in 45/132 (34.1%) metastases. A 38.8% of the cases showed significant CLDN18 intratumoral variability among the different tissue microarray cores obtained from the same tumor. Positive membrane CLDN18 expression was statistically associated with: tumor sites with higher prevalence among non-antral GCs ($p = 0.016$); Lauren Classification with higher prevalence in diffuse pattern ($p = 0.019$); and EBV-associated cancers ($p<0.001$). No association emerged between CLDN18 expression and age/sex, grading, staging, Ming Classification, p53, p16, E-cadherin, HER2 or MMRd in both primary tumors and in metastatic nodes.
Conclusions. CLDN18 is frequently expressed in gastric and gastroesophageal cancers; this was significantly associated with gastric corpus location, diffuse-type GC and with the presence of EBV infection. Further studies should investigate the prognostic significance of CLDN18 heterogeneity in order to implement its test into clinical practice.

CIRCULATING MICRORNA EXPRESSION PROFILING IN THE DETECTION OF EARLY NEOPLASTIC TRANSFORMATION DURING BARRETT'S CARCINOGENESIS

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Key words: miRNA; Barrett's esophagus; metaplasia; expression signature; liquid biopsy

Background. The main intent of secondary prevention strategies for Barrett's esophagus (BE) patients relies in the prompt identification of patients with dysplasia (i.e. Intra epithelial neoplasia; IEN) and early-stage Barrett's adenocarcinoma (BAc). Despite the adequate characterization of the molecular landscape characterizing Barrett's carcinogenesis, no tissue and/or circulating biomarker has been approved for clinical use.
Materials and methods. A series of 25 serum samples (12 BE, 5 High Grade-IEN and 8 BAc) were analyzed for comprehensive miRNA (i.e. miR-92a-3p, miR-151a-5p, miR-362-3p, miR-345-3p, miR-619-3p, miR-1260b, miR-1276, miR-381-3p, miR-502-3p, and miR-3615) profiling. In order to validate the Results Droplet Digital PCR was used. In order to understand the cellular source of circulating miR-92a-3p, its expression was analyzed in endoscopy biopsy samples by both qRT-PCR and ISH analyses.
Results. Ten miRNAs were found to be significantly dysregulated: seven were upregulated (i.e. miR-92a-3p, miR-151a-5p, miR-362-3p, miR-345-3p, miR-619-3p, miR-1260b, and miR-1276) and three downregulated (i.e. miR-381-3p, miR-502-3p, and miR-3615) in High Grade-IEN/BAc samples in comparison to non-dysplastic BE. All the identified miRNAs showed significant ROC curves in discriminating among groups with AUC values range of 0.75-0.83. Results were validated by droplet digital PCR in two out of three tested miRNAs. The analysis of endoscopy biopsy samples by both qRT-PCR and ISH showed that, as observed in serum samples, miR-92a-3p was over-expressed in HG-IEN/BAc samples in comparison to naïve esophageal squamous mucosa and BE and was mainly localized within the epithelial cells, supporting neoplastic cells as the main source of the circulating miRNA.
Conclusions. Our data further demonstrated that circulating miRNAs are a promising mini-invasive diagnostic tool in the secondary follow-up and management of BE patients. Larger multi-Institutional studies should validate and investigate the most adequate miRNAs profile in discriminating BE patients in specific risk classes.

HISTOPATHOLOGY OF CELIAC DISEASE: POSITION STATEMENT OF THE ITALIAN GROUP OF GASTROINTESTINAL PATHOLOGIST (GIPAD-SIAPEC)

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Celiac Disease (CeD) is an immune mediated inflammatory disorder of the small intestine, affecting genetically susceptible individuals when exposed to gluten. Small intestinal biopsy interpretation has been the "gold standard" for the diagnosis of celiac disease (CeD) for over 50 years; although sensitive and specific serological tests are today available for the diagnosis, the histopathological features from the mucosal biopsies continue to play a key role in the diagnostic approach when CeD is suspected. Such diagnostic approach requires a multidisciplinary team in which both the pathologists and the gastroenterologists work together, in order to optimize tissue sampling and diagnostic interpretation [1-3]. Growing evidences about problems and pitfalls in the histopathological diagnosis of CeD, make a literature review necessary. A selected group of pathologists from the Italian Group of Gastrointestinal Pathology (GIPAD-SIAPEC), together with a member (TR) of the Italian Society of Technicians (AITIC) and a CeD gastroenterologist expert (CC), have defined nine statements to update the Italian position on the correct methodological approach[4], morphological characteristic of CeD[4], it's complications and the recent condition of Non Celiac Gluten Sensitivity (NGCS) [6]. In such diagnostic setting the architectural anomalies in the duodenal mucosa, namely glandular hyperplasia and villous atrophy, and the intraepithelial T-lymphocytes count are well highlighted in the Marsh classification[7] and its subsequent modification fromOberhuber[8], as well as the Corazza-Villanacci classification[9,10]. Ancillary tests such as anti-CD 3 stain is useful for a precise count of the intraepithelial T lymphocytes when CeD or Non-Celiac Gluten Sensitivity (NCGS) are suspected[11-12]. Moreover the use of anti-CD 4 and anti CD 8 stains are recommended in patients not responding to the gluten-free diet, in order to confirm a diagnosis of Refractory Celiac Disease (RCD), in a proper clinical setting[5].

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ENDOSCOPIC RESECTION WITH THERAPEUTIC INTENT ON A SERIE OF "*EARLY COLORECTAL CANCERS*". A HISTOPATHOLOGICAL AND MOLECULAR STUDY.

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Background and aim. The implementation of screening programs and the widespread use of gastrointestinal endoscopy have increased the detection of malignant colorectal polyps and the diagnosis of early (pT1) colorectal cancer (eCRC), which can be eligible to endoscopic resection. Nevertheless, the histopathological evaluation of such lesions is often influenced by subjectivity and experience of the pathologist; this leads to interobserver variability in the histological diagnosis causing questionable patient management. Therefore, more Objective. parameters indicating a higher risk of incomplete resection and of lymph node involvement and the need for additional intestinal resection are required. We have performed a retrospective study on a series of 43 eCRCs to evaluate the role of global DNA hypomethylation as a complementary biomarker in the management of eCRC.

Materials and methods. Reports from the electronic archives of the Pathology Unit of the "Maggiore della Carità Hospital (Novara) were searched for endoscopically-resected eCRCs diagnosed between January 2016 and August 2018. Of the 55 cases identified, 12 were excluded due to sample fragmentation, which hindered proper histopathological assessment or because formalin-fixed paraffin-embedded tissue blocks were out of the archive. Selected cases were reviewed by two experienced pathologists in order to evaluate the following histopathological data: associated adenomatous component, grading, width and depth of submucosal invasion, lymphovascular invasion, tumor budding presence and grade, resection margin status; for patients who had required additional intestinal resection based on initial histopathological examination of the resected polyp, lymph node involvement was also considered.
For each lesion, a representative tumoral area was selected and dissected (by either manual macrodissection or laser microdissection) to extract DNA for long interspersed element-1 (LINE-1) hypomethylation analysis, which was performed by PCR-pyrosequencing.
Associations between histopathological data and LINE-1 methylation status were tested by Fisher's exact test; for continuous variables, the Wilcoxon rank-sum test was used. The methylation status difference between adenomatous and adenocarcinoma component of each lesion was evaluated by Wilcoxon signed-rank test.

Results. Our final cohort study consisted of 43 lesions resected by polipectomy, either endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD).
Twenty-five of 43 patients (58%) underwent intestinal resection in addition to endoscopic resection because of high risk histopathological parameters on the endoscopic specimen. In 4 of these, an upstaging of T status was found in the surgical specimen (one lesion was upstaged to pT2 and three lesions were upstaged to pT3); lymph node metastases were identified in 4/43 patients (9%), of which two had also hepatic metastases.
An adenomatous component was observed in 34/43 lesions.
LINE-1 methylation levels <60% were observed in 10/41 lesions. No association was found between LINE-1 hypomethylation and the histopathological parameters considered. When LINE-1 methylation status was compared between the adenomatous component and the adenocarcinoma within a single lesion, the adenocarcinoma showed statistically significant lower methylation levels (p=0.018).

Conclusions. The management of patients with eCRC diagnosis requires a close cooperation between endoscopist and pathologist in order to decide the most appropriate treatment. Nevertheless, this is burdened by interobserver variability and a lack of uniformity in the cutoffs of histopathological parameters. The methylation status of LINE-1 in eCRC can be easily evaluated on archive formalin-fixed, paraffin-embedded specimens and can be used as an additional tool in order to assess the best management for such lesions.

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ROLE OF CELLULAR SENESCENCE IN THE NATURAL HISTORY OF PRIMARY SCLEROSING CHOLANGITIS

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Background. Cellular senescence (CS) is a physiological mechanism of irreversible cell cycle arrest in response to damage that acts to prevent the uncontrolled replication of injured cells. Recent data supports the role of CS in the pathogenesis of human chronic liver diseases, particularly in cholestatic disorders. Primary sclerosing cholangitis (PSC) is a rare cholangiopathy of unknown etiopathogenesis. A few studies suggested the involvement of CS in PSC, but its role in the natural history of the disease has not been clarified so far.
Aim. To evaluate the tissue expression of CS markers in PSC patients, and its correlation with clinical-pathological features and disease progression.
Materials and methods. Twenty-eight PSC patients of the Gastroenterology Unit of the University Hospital of Padua, with at least one available liver biopsy and a median follow-up of 145 months, were retrospectively enrolled. Clinical and laboratory data, including the Mayo Risk Score and the Amsterdam/Oxford Model, were collected in all cases. Thirty-five biopsies were included in the study, and re-evaluated by two experienced pathologists. Grading and staging of the disease were assessed according to Nakanuma. Immunohistochemical stains for CS markers p16 and p21 were performed and semi-quantitatively scored by a three-tier scale based on the positivity extent. p16 stain was evaluated both in the native bile duct (NBD) and in the ductular reaction (DR), while p21 expression was assessed in the native bile duct and in periportal hepatocytes (PH).
Results. A significant relationship was found between the hepatitis activity of the disease and p21 expression (p=0.002 and p=0.009 in NBD and PH, respectively). A strong association between the presence of cholestasis-related histological lesions and both p21 (p<0.0001) and p16 (p=0.005) expression was also observed. Overexpression of p16 (both in NBD and DR) and p21 (in NBD) was associated with a higher amount of liver fibrosis (p=0.03, p<0.0001, and p=0.004, respectively). An increased expression of p16 in DR and p21 in NBD was also related to a more advanced disease stage (p=0.0004 and p=0.002, respectively). p16 expression was strongly correlated to the prognostic clinical scores: the Mayo Risk Score (p=0.007 and p=0.01 in NBD and DR, respectively) and the Amsterdam/Oxford Model (p=0.04 and p<0.0001 in NBD and DR, respectively).
Conclusions. The overexpression of senescence markers in PSC is associated to advanced stages of the disease and correlate with prognostic clinical scores. This suggests that cellular senescence may play a role in the pathogenesis and progression of the disease.

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NECROPTOSIS IS ASSOCIATED WITH A BETTER SURVIVAL IN INTRAHEPATIC CHOLANGIOCARCINOMA

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Background. Intrahepatic cholangiocarcinoma (ICC) has a poor prognosis and few therapeutic options. Necroptosis is a form of programmed cell death whose pathway includes Receptor-Interacting Protein Kinase 3 (RIPK3) that subsequently activates the necroptosis executioner Mixed Lineage Kinase domain-Like (MLKL). In turn, RIPK3 may be activated by RIPK1 and other proteins involved in innate immunity. Active caspase-8 inhibits necroptosis by cleaving RIPK3 and RIPK1 and triggers the apoptotic pathway. Thus, the necroptotic cascade is active only when apoptosis signaling is blocked and caspase-8 inhibited. The spilling of necroptotic cancer cells into the tumor microenvironment induces CD8 T-cells priming and provides anti-tumor immune response. Necroptosis has been studied in different liver diseases, but little is known regarding its role in ICC.
Aim. To assess the immunohistochemical tissue expression of the necroptosis-related proteins RIPK3, phosphorylated-MLKL (p-MLKL), and RIPK1 and the apoptosis-related protein caspase-8 in a cohort of ICCs, and its relationship with tumor infiltrating CD8-positive T-cells and patients' survival.
Materials and methods. Sixty-one consecutive patients who underwent curative hepatic resection for ICC were enrolled. None received any therapy prior to surgery. Immunostainings for RIPK3, p-MLKL, RIPK1, and caspase-8 were performed and semi-quantitatively scored by a four-tier scale based on the percentage of positive cancer cells as follows: 0=<5%, 1+=5-30%, 2+=31-60%, and 3+=>60%. CD3 and CD8-positive lymphocytes were counted by using a computer-assisted method.
Results. A 3+ expression of RIPK3, p-MLKL and RIPK1 was associated with a significant decrease of perineural invasion (p<0.001 for all of them) and nodal metastasis (only with RIPK3; p<0.001). A 3+ expression of RIPK3 and RIPK1 was related to an improvement of the overall survival (p=0.01 and p=0.02, respectively). A reduced expression of caspase-8 was associated with a higher p-MLKL expression (p=0.001). Moreover, a strong RIPK3 expression correlated with a high grade CD8-positive T-cells tumor infiltration (p<0.001), which, in turn, seemed to be related to a better prognosis (p=0.05).
Conclusions. The overexpression of the necroptosis-related proteins RIPK3 and RIPK1 is associated with a higher CD8-positive T-cells tumor infiltration and a better prognosis in ICC. This may lead to new therapeutic options.

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A HISTOLOGICAL ANALYSIS ON SPATIAL DISTRIBUTION AND TISSUE CHANGES IN TRANSARTERIAL CHEMOEMBOLIZATION WITH RADIOPAQUE DRUG ELUTING BEADS IN HEPATOCELLULAR CARCINOMA

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Introduction. Transcatheter arterial chemoembolization (TACE) is a standard therapy for intermediate–hepatocellular carcinoma (HCC). Drug-eluting beads (DEBs) have been imposed as novel drug-delivering agents for TACE, which involves the infusion of higher concentrations of drugs within the target tumor and determine a selective obstruction of tumor-feeding artery, leading to ischemic effects. The most commonly used microspheres are loaded with doxorubicin, and are able to release drug in a controlled and long-standing manner. As the beads were not radio-dense, there had been no way of precisely locating them in the target tissue, until radiopaque microspheres have been developed. New types of radiopaque microsphere (R-DEBs) have been recently introduced and they allow a real-time visualization of bead localization to enable intra-procedural and post-procedural feedback with imaging technique.[1] A morphological analysis of liver tissue in patients treated with R-DEBs has not been performed, so far[1,2].
The Aim.s of this study were to map R-DEBs microspheres in target and non-target liver tissue and to determine if specific beads-related histological lesions may occur in non-target tissue.
Materials and methods. This retrospective case-control single-center study was performed at a tertiary referral center. All patients, who underwent liver transplantation for HCC between March 2016 and December 2018 at the University Hospital of Padova, and received locoregional DEB-TACE before transplantation, were initially included. Among these, 20 patients with follow-up imaging studies (contrast-enhanced multi-phase computed tomography and/or magnetic resonance), were retrieved: 10 consecutive explanted livers from HCC cirrhotic patients previously treated with 70-150 μm non-radiopaque DEB-TACE (control group) and 10 consecutive explanted livers from HCC cirrhotic patients previously treated with 70-150 μm R-DEB TACE (study group). All the interventional radiology imaging studies were reviewed by a radiologist (CA) to identify all types of locoregional treatments received by patients (including TACE and radiofrequency ablation) and the localization of the specific target-nodule treated by DEB-TACE. Assessment of response to DEB-TACE was histologically performed by two pathologists (MG and DS), by a retrospective review of the slides. The percentage of tumor necrosis (defined as volume of necrotic areas divided by total tumor volume) was also evaluated.

Tab. I. Presence of biliary damage in peritumoral (PT) and distant tissue (DT, and presence of gallbladder damage) in R-DEB TACE and DEB-TACE.

	Presence of biliary damage in:		Presence of gallbladder damage
	PT	DT	
LUMI	1/10 (10%)	0/10 (0,0%)	0/10 (0,0%)
Non-LUMI	4/10 (40%)	6/10 (60%)	5/9* (55,6%)
P-value	0,303	0,011	0,011
*One non-LUMI patient underwent cholecystectomy before TACE.			

During the retrospective review, the number of single beads and beads clusters (defined by more than 4 adjacent beads) were assessed in the target nodule, in the peritumoral tissue (PT) (which is the liver tissue within 3 cm from the target nodule), in distant tissue (DT) (more than 3 cm from the target nodule), in the gallbladder and by the hepatic hilum. Moreover, the presence of biliary damage in the PT and DT, as well as gallbladder damage, was reported. The presence of neovascular tissue in the target nodule was investigated, as well. Statistical analyses were performed by SPSS statistical software (version 20.0, IBM, Armonk, New York, United States). T test and Fisher Exact test were used when appropriated. Test Results were deemed statistically significant when p value was <0.05.
Results. In patients treated by R-DEB, the histological percentage of tumor necrosis and the number of clusters and single beads in DT were significantly lower than those treated by non-radiopaque TACE (p=0,029, p=0,006 and p<0,001, respectively). The number of clusters in PT seems to be decreased in the study-group (p=0,06). The number of single beads in the gallbladder was significantly lower in the R-DEB group (p=0,041) as well as the number of clusters, even if it was not significantly evident (p=0,081). In the study group, we found a lower risk of biliary damage in DT (p=0,011), but not in PT (p=0,303). Besides, we found a lower risk of gallbladder damage in patients treated by R-DEB TACE (p=0,011). As expected, there was no evident difference about the number of clusters and single beads in the target-nodules in the two groups. Only in the control-group, one patient had developed clinically symptomatic acute cholecystitis.
Conclusion. This preliminary study is the first to provide a histopathological assessment of morphological changes due to treatment with R-DEB, compared to non-radiopaque beads. It demonstrates that TACE with R-DEB is associated with a more precise infusion of the microspheres and, consequently, a decreased risk of biliary and gallbladder damage.

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MIRNA PROFILE DISTINGUISCES GIST SUBGROUPS WITH DIFFERENT PROGNOSTIC IMPACT: A FEATURE WITH POTENTIAL CLINICAL IMPLICATION?

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Introduction. Gastrointestinal stromal tumor (GIST) prevalence in the general population differs greatly between clinically overt cases (~0.0014%) and so-called microGISTs (<1cm) (10-35% in systematic studies from autopsy/surgical specimens). Additionally, microGISTs often show sclerosis/calcification, thereby supporting the occurrence of spontaneous remission. Intriguingly, it is not possible to identify which microGIST will eventually evolve into overt/malignant GIST. Consequently, despite follow-up is admissible in a subset of small, pathologically diagnosed GISTs, presently it is impossible to definitively assess the lack of aggressiveness of unresected GISTs. Existing data support the role of microRNA (miRNA) expression profile in GIST tumorigenesis and progression. Given the increasing efficiency of endoscopic techniques in biopsy yield, allowing a reliable histotypic/molecular characterization of small subepithelial tumors, indolent microGISTs could be identified provided solid distinctive features were defined, thereby avoiding unnecessary surgery.
Method. Expression of 384 miRNAs was assessed through the TapMan Advanced miRNA Human A Card (Applied Biosystem) in 5 micro GISTs, 5 very low risk/ low risk GISTs (VLR/LR GISTs) and 5 metastatic GISTs. All cases harbored a KIT-exon-11 mutation.
Results. 23 miRNAs were found significantly differentially expressed between microGISTs and metastatic GISTs, 63 miRNAs between microGISTs and VLR/LR GISTs, 22 miRNAs between VLR/LR GISTs and metastatic GISTs (Limma R/bioconductor software package. Ritchie et al. Nucl Acid Res 2015;43:e47). Accordingly, Hierarchical clustering based on different miRNA expressions showed well-defined clusters related to the type of lesion (microGISTs, VLR/LR GISTs or metastatic GISTs). Functional enrichment of differentially expressed miRNAs showed several target genes involved in SCF-KIT/PDGF pathways.
Conclusion. Our Results are consistent with a robust difference in miRNA profile between KIT-exon-11 mutant microGISTs, VLR/LR GISTs and metastatic GISTs, allowing a reliable separation based on an epigenetic standpoint. miRNA profiling has the potential for identifying indolent microGISTs. Further studies are ongoing Aim.ing to clarify these issues.

IBD AND NON-IBD COLITIS DIAGNOSIS PROJECT: THE ITALIAN PATHOLOGISTS IG-IBD-GIPAD GROUP EXPERIENCE

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Objective. Today the histopathological diagnosis of inflammatory bowel diseases (IBD) and non-IBD colitis is still a field full of difficulties for pathologists [1-3]. Although in most cases the correlation between clinical data and accurate histological examination is decisive, sometime further diagnostic investigations and a second opinion by expert pathologists dedicated to gastrointestinal pathology is request [4]. The digitization of histological slides and the increasing use of telepathology, with the possibility of case-sharing, has improved diagnostic accuracy, supporting the growing competence of generic pathologists and gastrointestinal pathologists. On January 2019, the group of Pathologists of the Italian Association for the Study of Chronic Intestinal Inflammatory Diseases (P-IG-IBD) and Italian group of pathologists (GIPAD-SIAPEC), with particular experience in IBD diagnosis, devised a project based on case-sharing network of IBD and NON-IBD colitis in adult and in childhood. Through a constantly updated and easily accessible "forum", users could comment all cases uploaded to the server and insert their own cases to share opinions and experiences. The Results in 6 months of the project are presented in this study.
Materials and methods. One case per week with clinical data was uploaded; after 6 months of activity, an anonymous multiple-choice test with 20 questions, divided into three sessions has been sent to all participants. It was composed by three parts: A - attendance to the forum activities; B - accessibility to the scanned slides; C - personal comments and suggestions.
Results. 40 pathologists attended the project; 55% of these were confident with digital slides. Approximately 53% were invited by the P-IG-IBD group while the remaining 47% were invited by a member already attending the forum; the others registered indepentely to IG-IBD site; 40% of the participants constantly accessed the site; 26% commented more than 75% of cases. The remaining 25% had no free access from their office or were not able to download the slides. 14% of pathologists showed high confidence with digital slides while 86% showed moderate to poor confidence because of the incorrect display of the slide fields, the lack of experience or the complexity of some cases. Overall, 60% of the participants found the forum relevant for practical training while 40% defined the experience as relevant but not educational.

Conclusions. Our experience has shown that the sharing of scanned slides using a dedicated forum is an useful and innovative diagnostic tool that improves the histological knowledge of IBD and non-IBD. Although our Results demonstrated a quite relevant pathologist's participation to the forum, further steps are required in order to motivate this promising practice activity.

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C-MYC AS A KEY-MARKER IN THE COLORECTAL CANCER RESISTANCE TO EGFR INHIBITORS

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Objectives. Despite the outstanding success of EGFR inhibitors in the treatment of metastatic colorectal cancer (mCC), targeted therapy (TT) leads inevitably to the acquired resistance stemming from various molecular mechanisms such as activation of compensatory kinases. Alterations in the transcriptional factor c-MYC could be involved in the TT resistance. We here analyzed the role of c-MYC pathway in a TT treated cohort of KRAS, NRAS and BRAF wild-type mCC patients, and in a subgroup of liver metastases subjected to conversion chemotherapy that led to surgical treatment. Results were correlated with the patients' clinical characteristics and biological tumor features. Moreover, we identified specific altered genes and miRNAs, linked to c-MYC pathway, as possible new molecular targets to overcome the anti-EGFR resistance.

Materials and methods. The expression of c-MYC was assessed in 121 RAS and BRAF wild-type mCC samples before treatment with anti-EGFR+Folfiri therapy and in 33 subsequent metastases collected during TT or in TT resistance phase. In this cohort, and in two cancer cell lines, we also analyzed the expression of miRNA143 and miRNA145, and we performed a c-MYC Targets PCR Array to identify possible downstream pathways involved in the c-Myc upregularion.

Results. Patients with low c-MYC expression (LME) showed a significant higher PFS and OS respect to those with high c-MYC expression (HME) ($p<0.0001$ and $p=0.0016$). The involvement of c-MYC in the anti-EGFR resistance mechanism was also highlighted by the significant increase of HME cases in liver metastases resected after or during TT treatment in comparison to the primary tumor ($p=0.0012$). This data was functionally reinforced by the significant association between the anti-EGFR resistance molecular alterations, arisen in the liver TT treated metastasis, and the HME ($p=0.034$). We demonstrated that patients with HME had a significant reduced miRNA-143 and miRNA-145 expression ($p=0.0002$ and $p=0.0005$), involved in the c-MYC downregulation and EGFR activation in the HME mCC, in primary CC and metastatic samples. Restoring the miRs expression in two KRAS mutated cancer cell-lines, we observed a c-MYC downregulation with a lowering of the cell proliferation and migration after cetuximab exposure. Using two mutated KRAS CC cell lines, we showed that the upregulation of these two miRs was able to downregulate the expression of c-MYC.

With the Objective. of understanding the molecular mechanisms underlying the c-MYC-induced resistance, we performed a microarray analysis that individuates some significant downstream genes that could be a specific druggable target. The analysis demonstrated that the anti-EGFR resistance particularly involved some genes belonging to the cell cycle, to the apoptosis and to signal transduction pathways (about 30%, 20% and 20% respectively). On the contrary, other pathways, such as the apoptotic, signal transduction and cell growth and proliferation pathways and the DNA repair, RNA processing and binding factors pathways showed a relatively reduced alteration of gene expression. Finally, several scientific publications have reported the association between c-MYC gene function and its nuclear localization. In this sense, the immunohistochemical expression, which has a significant correlation with the nuclear expression of the gene (nuclear fraction), as we highlighted with the western-blot analysis in CC cell lines, represents an excellent method for functionally evaluating c-MYC.

Conclusions. Our data demonstrated that c-Myc could play a pivotal role in the cetuximab resistance of mCC and in the kinome reprogramming resistance that develops through alternate routes of kinase pathway activation. In this scenario, the c-MYC blockade, acting on upstream regulators or downstream to activated genes, in association with other specific anti-kinase inhibitors could circumvent the acquired resistance to anti-EGFR preventing the kinase reprogramming.

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HISTOPATHOLOGICAL EXAMINATION OF IBD BIOPSIES: PRACTICAL POSITION STATEMENTS OF THE ITALIAN GROUP FOR THE STUDY OF INFLAMMATORY BOWEL DISEASE (IG-IBD) AND ITALIAN GROUP OF GASTROINTESTINAL PATHOLOGISTS (GIPAD-SIAPEC)

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Objectives. Inflammatory Bowel Disease (IBD) are chronic bowel disorders that encompass two specific diseases: Ulcerative Colitis (UC) and Crohn's Disease (CD). The distinction between these two entities each other as well as the differential diagnosis from non-IBD colitis, is based on a variety of clinical settings and histological features. Diagnosis, activity status, mucosal healing and relapse of the diseases required careful histopathological examination of biopsy specimens [1-3]. Thus, for the increasing evidence of pitfalls in the histopathological diagnosis of IBD, the pathologists of the Italian Group for the Study of Inflammatory Bowel Disease (IG-IBD) and those of the Italian Group of the Gastrointestinal Pathologists (GIPAD-SIAPEC) defined a series of statements in order to clarify the histomorphology of IBD and the correct methodological approach for their diagnosis.

Materials and methods. Statements were defined after a critical review of the current literature among the IBD issue. The work of the pathologist's team received the support of members of the Italian Association of Laboratory Technicians (AITIC) and approved by the National IBD Patients' Association (AMICI-ONLUS).

Results. The statements are summarized as follows: **Statement 1**: *methodological approach for the management of the biopsies.* A minimum of two endoscopic samples from at least five sites along the colon, rectum and ileum are required; in the suspicious of CD, endoscopy and biopsies of the upper gastrointestinal tract are suggested. Anatomical orientation of the samples is made easier by placing biopsies on already-cut cellulose acetate filters. **Statement 2:** *the histological features of UC*. Continuous transmucosal inflammation characterizes the disease. Non-active inflammation, active inflammation parameters and structural changes define the active or, non-active status of the disease, healing or relapsing. Basal cell plasmacytosis, eosinophils and mucosal distortion are detected. Histopathology of pediatric UC differs from adult UC. **Statement 3***: degrees of inflammation.* Mild, moderate and severe inflammation is quantified by the presence and the quote of the inflammatory cells. Activities and relapse are defined by the presence of granulocytes. **Statement 4:** *histological mucosal healing.* Colonic mucosa free from active inflammation, erosions or crypt abscesses. **Statement 5:** *Histological features of pouchitis and cuffities*. In these conditions it is important to pay particular attention to alterations of villi, number of T intraepithelial lymphocytes, lamina propria inflammation and superimposed infections. **Statement 6:** *The optimal requirements for histopathological report in UC*. It based on a clear description of the morphological elements mandatory for a diagnosis: grade of inflammation, basal plasmocytosis and crypt architecture. **Statement 7:** *The histological features of CD***.** It includes sectorial transmucosal non-active inflammation parameters (non-active inflammation, basal plasmacytosis, eosinophils), active inflammation parameters (neutrophil-mediated epithelial glandular injury), typical granulomas and sectorial architectural distortion of glands. **Statement 8:** *Extra ileocolic disease and pediatric CD***.** Antrum, duodenum oesophagus as well as anal and oral disease are included in the extra ileocolic localization. Absence of non-necrotizing granuloma should characterize these setting of disease. **Statement 9:** *The optimal requirements for histopathological report in CD*. A clear description of the morphological elements mandatory for a diagnosis: sectorial distribution of inflammation, basal plasmacytosis and granulomas. **Statements 10***: Diagnosis of dysplasia*. Mucosal cytological and architectural alterations are classified as low- and high-grade; indeterminate of dysplasia should be reserved only for selected cases. The risk of cancer development from dysplasia is greater in colonic and rectal mucosa when compared with ileal mucosa. Serrated lesions can also be observed. **Statement 11:** The optimal requirements for histopathological report of dysplasia in CD and UC are based on a clear description of the morphological elements mandatory for a diagnosis: grade of dysplasia-single or multiple foci of dysplasia-grade of infiltration in submucosa.

Conclusions. Histological diagnosis of IBD requires specific pathological competence. The acknowledgment of peculiar morphological features is fundamental for precise definition of UC, CD and their clinical variants. The statements purposed in this paper Aim. to underline some points useful for the correct methodological approach for the IBD diagnosis and its histopathological report, by the pathologists.

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CONCORDANCE IN HISTOLOGIC DIAGNOSIS OF NEOPLASTIC LESIONS IN INFLAMMATORY BOWEL DISEASE: RESULTS FROM A NATIONAL SURVEY IN ITALY

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Background. Inflammatory bowel diseases (IBD) are chronic inflammatory conditions of gastrointestinal tract, involving significant fractions of western world populations. Due to the long lasting mucosal damage, the disease increases the risk of developing preneoplastic and neoplastic conditions. In a metanalysis [1] the probability of CRC in patients with UC increases with the duration of the disease, and another recent study confirmed the trend [2]. On the contrary, in a Danish study the overall RR for CRC decreased from 1.34 (1979 -1988) to 0.57 in 1999 -2008, while in patients with CD the overall RR for CRC was 0.85 and did not change over time [3]. On the other hand, in another analysis the incidence of CRC in patients with IBD was 60% higher than in the general population and essentially stable over time [4]. Therefore, the detection of preneoplastic (dysplastic) lesion and a diagnostic approach with low interobserver variability between pathologists is essential in the management of IBD patients, in order to decrease the risk of progression towards invasive, neoplastic lesions. A concordance study on 4 pathologists and 38 cases of dysplasia demonstrated a fair agreement (k=0,4) [5] A similar study demonstrated that the lowest level of agreement in the dysplasia group was for the category indefinite for dysplasia ($\varkappa$ = 0.251). Negative and high grade dysplasia diagnosis reached the highest level of agreement with $\varkappa$ values of 0.822 [6].

Objective. The study design consisted in a multicentre survey about diagnostic agreement in a large series of preneoplastic lesions of IBD-affected patients based on digital images, given the scant literature on this topic. The effective occurrence of the disease and the disease stage was considered in the study.

Tab. I.

Case	Blocks (site of samplings with uniform morphology)							% of blocks with concordant answers about occurrence of dysplasia
	First endoscopy			Second endoscopy			Third endoscopy	
Block	1A	1B	1C	2A	2B	2C	3A	
Case n. 01	CONC	CONC	CONC	CONC	CONC	CONC		100,00%
Case n. 02	DISC			DISC			CONC	33,33%
Case n. 03	CONC	CONC	DISC	CONC	CONC	DISC	CONC (*)	71,43%
Case n. 04	CONC			CONC	DISC	CONC	CONC	80,00%
Case n. 05	DISC			CONC	DISC			33,33%
Case n. 06	CONC	DISC						50,00%
Case n. 07	CONC	DISC		CONC	CONC			75,00%
Case n. 08	CONC	DISC						50,00%
Case n. 09	DISC							0,00%
Case n. 10	DISC			DISC				0,00%
Case n. 11	CONC	CONC	DISC					66,67%
Case n. 12	DISC							100,00%
Case n. 13	CONC	DISC				DISC		50,00%
Case n. 14	CONC	CONC						100,00%
Case n. 15	CONC							100,00%
Case n. 16	DISC	DISC						0,00%
Case n. 17	DISC							0,00%
Case n. 18	CONC	DISC	CONC					66,67%
Case n. 19	CONC	CONC						100,00%
Case n. 20	DISC	DISC						0,00%
							mean	61,11%
(*) surgical specimen (see Materials and methods)								

Tab. II.

Subject	% concordant answers (*)
evidence of inflammatory bowel disease	85
differential diagnosis RCU/Crohn's disease	65
evidence of dysplasia	50
differential diagnosis Sporadic Adenoma/DALM/ALM	50

Materials and methods.
Collection criteria. The study enclosed biopsy specimens from 30 colonoscopies and 1 surgical specimens, related to 20 patients with a clinical pattern of IBD, and collected from 4 reference centres in Italy.
Quiz profiles. Digital slides were obtained from the histologic material and the files were uploaded in an open source learning platform. For each endoscopy of the selected cases, sampling sites with similar morphologic pattern were aggregated in 54 "blocks", and a series of close-ended questions about (A) the occurrence of IBD (active, in remission, absent, not evaluable) and (B) the evidence of dysplasia (low grade, high grade, not obvious, undefined) have been submitted for every block. For each case, a final comprehensive evaluation about (1) the occurrence of IBD (present, absent, unsuitable for assessment), (2) the disease classification (Ulcerative Colitis, Crohn's disease, material not eligible for the differential diagnosis, differential diagnosis not possible for lack of clinical data), (3) the occurrence of IBD related dysplasia (IBD with dysplasia, dysplasia not IBD-related, dysplasia with untestable coexistence of IBD, absent) and (4) the classification of dysplasia (sporadic adenoma, ALM, DALM, sampling unfit for classification) were provided.
Participants. 20 gastrointestinal pathologists from as many centres in Italy were included in the study and were invited to answer the above-mentioned questions; every case was structured as an independent investigation and the participants were asked to answer the quizzes concerning all the endoscopies of any single case to complete successfully the test.
Results. 325 (81,2% - 3217 answers) of the 400 tests (20 cases x 20 participants) were successfully concluded. In order to define the agreement about the single questions, we chose to consider the answers as concordant when more than 75% of the participants selected the same response. Based on these criteria, agreement about occurrence of dysplasia was evaluated for every single block (sample sites of an endoscopic mapping with similar morphologic characteristics - see Materials and methods) (Tab. II) the percentage of cases with concordant answers about the conclusive questions of the 20 cases are resumed in Table I.
Conclusions. In the present survey, a preliminary data analysis demonstrated a good agreement about the occurrence of IBD, and a lower agreement about the evaluation of the occurrence of dysplasia and the classification of dysplasia in IBD. Analysis of the correlation between agreement and clinical-histologic parameters could get a picture of the diagnostic process in clinical practise in our country, and could provide interesting spotlights on diagnostic algorithms in this field.

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INTEROBSERVER CONSISTENCY ON THE HISTOLOGICAL ASSESSMENT OF SPASMOLYTIC POLYPEPTIDE-EXPRESSING METAPLASIA

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Introduction. Autoimmune gastritis (AiG), an organ-specific disease restricted to oxyntic mucosa, is mediated by a complex interaction between autoantibodies (against parietal cell proton pump and/or intrinsic factor), and sensitized T-cells. The long-standing, non-self-limiting inflammation Results into progressive destruction of the oxyntic glands, which are replaced by atrophic, non-metaplastic and metaplastic, mucosa (*i.e.* intestinal metaplasia [IM] and spasmolytic polypeptide-expressing metaplasia [SPEM]). Assessing histologically oxyntic atrophy (an essential step in gastritis staging) involves the distinction between the different atrophic phenotypes, which includes non-metaplastic (disappearance of glandular units replaced by a fibrotic lamina propria), and metaplastic (IM and/or SPEM) atrophy. SPEM is defined as antral-like (pseudo-pyloric metaplasia in hematoxylin-eosin [H&E]) mucosa, expressing immunohistochemical (IHC) markers natively associated to antral glands (trefoil factor 2 [TFF2]). Aim.s of this study were: i) to test the inter-observer consistency in the assessment of atrophy (*i.e*. non-metaplastic atrophy, IM and SPEM) in routine H&E histology; ii) to verify if/how TFF2-IHC modifies the histological assessment of SPEM, as obtained by H&E; iii) to explore if the SPEM-score, as obtained by applying TFF2-IHC, may result in any modification of the gastritis OLGA-stage as assessed by H&E.
Materials and methods. The study considered a series of 434 biopsy sets obtained from histologically-proved, serologically-confirmed AiG. Inclusion criteria were as follows: i) availability of a standard set of gastric biopsy samples (*i.e.* Sydney System compliant sets); ii) availability of immunohistochemical profiling, including TFF2 (Proteintech Group, Inc. IL, USA, 1:1500 dilutions; rabbit IgG). The study involved two pathologists working at the same Department (one GI expert [MR] and one in training [SAD]), where they shared the same diagnostic criteria and daily practice. To test the interobserver consistency (IOC) in the H&E assessment of the mucosa atrophy spectrum (non-metaplastic atrophy, IM atrophy, SPEM), 78/434 randomly selected biopsy sets (H&E stain) were considered by pathologists, who indepen-

Tab. I. H&E interobserver consistency (78 biopsy sets).

	k coefficient
corpus MI	0,7656
antral MI	0,620
SPEM score	0,8295
non-metaplastic atrophy	0,0314
"global" corpus atrophy score (including all the spectrum of atrophic lesions)	1
antral atrophy score (including all the spectrum of atrophic lesions)	0,926
OLGA stage	0,9536

Tab. II. H&E versus TFF2-IHC consistency (434 biopsy sets).

	k coefficient
SPEM score	0,8365
corpus atrophy score	0,8997
OLGA stage	0,889

dently reported the atrophy scores. For each of the considered variables, the obtained score values were tested by *k* statistics.
All the original 434 histological sets (H&E and TFF2-IHC) were re-considered (SAD), by histologically scoring the whole spectrum of atrophy subtypes (OLGA staging tutorial[1]). In the biopsy samples obtained from the oxyntic mucosa, SPEM-atrophy was assessed by both H&E and TFF2-IHC; the consistency between the 2 score-values (H&E versus TFF2-IHC) was tested by *k* statistics.
Finally, to test if TFF2-IHC SPEM-scores could result in modifying: i) the "global" oxyntic score value, ii) and/or the conclusive OLGA-staging, this study compared the allocation of cases by stage when TFF2-IHC was applied additionally. The strength of association between OLGA stage and the pathological was obtained by applying Student's t-test and the Wilcoxon non-parametric test for matched pairs, as appropriate. The statistical analysis was performed using the STATA 8.0 software (Stata Corporation, College Station, TX, USA).
Results. Among the 78/434 biopsy sets reviewed by two pathologists, the IOC was tested by means of *k* statistics. IOC was ranked as 'good' in corpus IM (no change=84,6%; *k coefficient* = 0,7656), as well as, in antral IM (no change=93,6%; k coefficient= 0,620), and as 'excellent' in SPEM (no change=88,5%; *k coefficient*=0,8295). The IOC was ranked as 'weak' in non-metaplastic atrophy (no change=28,2%; higher score=71,8%; *k coefficient*=0,0314; the discrepancy range: 5% to 15%). 'Excellent' inter-observer consistency was documented in the assessment in the score of both "global" oxyntic (no change =100%; *k coefficient* =1), and "global" antral atrophy score (no changes=98,7%; *k coefficient* =0,926) and, ultimately, in OLGA stage assessment (no change=98,7%; *k coefficient* = 0,9536) (Tab. I). When all the 434 cases were considered, no differences emerged by comparing the SPEM-atrophy scores as obtained from H&E *versus* TFF2-IHC assessments, ('excellent' *k* statistics; no change=85,7%; higher score at TFF2-IHC=7,4%, and lower score at TFF2-IHC=6,9%; *k coefficient*= 0,8365). Furthermore, no differences emerged in corpus atrophy scores (no change=94,0%; higher score at TFF2-IHC=3,9%, and lower score at TFF2-IHC=2,1%; *k coefficient*= 0,8997). Finally, "excellent" consistency emerged in the allocation of cases by OLGA-stage: no change in 95,2%; higher stage at TFF2-IHC in 2,9%, and lower stage at TFF2-IHC in1,8%; *k coefficient*= 0,889 (Tab. II).
Conclusions. In AiG, the present Results demonstrate that the TFF2 stain does not significantly improve the histological assessment of SPEM. TFF2-IHC, however, may help pathologists who are not familiar with gastritis atrophy-assessment. In such a diagnostic setting, OLGA-staging of AiG is not significantly modified by the additional information achievable by including TFF2-IHC to the routine histological stain.

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NATURAL HISTORY OF AUTOIMMUNE GASTRITIS: PROSPECTIVE LONG-TERM FOLLOW-UP CLINICO-PATHOLOGICAL STUDY

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Introduction. Autoimmune Gastritis (AiG) is a chronic inflammatory disease mediated by autoantibodies against parietal cell (proton pump and/or intrinsic factor), and sensitized T-cells. In AiG, the inflammatory/atrophic lesions exclusively involve the oxyntic mucosal compartment. The reduced acid-production due to the oxyntic atrophy Results in loss of intrinsic factor, vitamin-B12 deficiency and, eventually, into pernicious anemia[1]age 54 (18-79. Gastrin hypersecretion due to hypo-chlorhydria promotes the proliferation of oxyntic enterochromaffin-like (ECL) cells, thus creating the biological Background. where neuroendocrine tumors (NET) may develop. Based on the well-established correlation between gastric atrophy and gastric cancer (GC), the OLGA staging system consistently ranks the GC risk associated with the different score/topography of gastric atrophy. After the initial AiG diagnosis, the America Society for Gastrointestinal Endoscopy recommends a single endoscopic evaluation for neoplastic lesions [2]. Among those AiG-patients showing linear and/or micronodular hyperplasia, a 3- to 5-years endoscopy surveillance has been suggested. Of note, however, the available guidelines do not specifically consider AiG patients being, more in general, applicable to any atrophic gastritis with active or eradicated *H. pylori* infection. Despite improvements on the current knowledge about AiG, no reliable markers are available for identifying patients at higher risk of gastric NET, and the precancerous meaning of AiG is largely debated.
Patients e method. A consecutive series of 217 AiG patients with histologically-proved, serologically-confirmed AiG, and with no (previous or active) histologically proved *H. pylori* infection, was prospectively collected.

At their enrollment, all patients underwent an initial endoscopy (Padua University Hospital); a follow-up endoscopy with biopsy sampling was always available (interval time ranging from 11 to 204 months; mean/median follow-up time= 84/72 months, respectively). Inclusion criteria were as follows: i) no epithelial neoplastic lesions; ii) availability of a gastric biopsy compliant with Sydney guidelines; iii) at least 2 biopsy sets collected per patient (initial-T0, and last-T1); iv) availability of immunohistochemical profiling, including chromogranin A (clone DAK-A3; DAKO). In total, 434 biopsy sets (as obtained from 217 patients) were considered. In all the available tissue samples, *H. pylori* status was further tested by genomic DNA extraction and RealTime-PCR for *Hp* detection; at the (post-recruitment) molecular testing 4/217 (1,8%) were weakly positive. All the original biopsy sets (Hematoxylin-Eosin and Giemsa stains) were histologically reconsidered ([SAD] blinded to patients' endoscopic/clinical information) and the atrophy score was assessed (OLGA staging tutorial[3]an international group of pathologists (Operative Link for Gastritis Assessment). ECL-cell hyperplasia (as supported by the appropriate immunostaining) was assessed according to established criteria, distinguishing linear *versus* micro-nodular hyperplasia. Among micro-nodular ECL-hyperplasia cases, those showing more than 5 ECL aggregates per biopsy sample were defined as "adenomatous". The strength of association between OLGA stage and the pathological and patients' demographic features was obtained by applying Student's t-test and the Wilcoxon nonparametric test for matched pairs, as appropriate. Significance was inferred at a P-value of less than 0.05. The statistical analysis was performed using the STATA 9.4 software (Stata Corporation, College Station, TX, USA).

Results. In all, 434 biopsy sets obtained from 217 patients were considered. At the T0 endoscopy, the M:F ratio was 53:164 (mean age= 55,89 years; range= 15-91,5). Males mean age was significantly higher (M=59,21 years; range=28-80; F= 54,82 years; range=15-83; p=0,04). *H. pylori*-DNA was identified in 4/217 (1,8%) of cases. The gastritis OLGA stage significantly increased from T0 to T1 (no change= 172 cases [79,2%]; higher stage at T1= 30 [13,8%], lower stage at T1= 15 [6,9%]; p=0,04). The oxyntic atrophy showed non-significant progression from T0 to T1 (no change= 179 cases [82,5%]; higher stage at T1= 24 [11,1%]; lower stage at T1=14 [6,4%]; p= 0,07) (Table). Among the 4 *H. pylori*-positive cases, one progressed from stage II to stage III. At both T0 and T1, epithelial neoplastic lesions were never observed. In 18/217 patients (8,3%) an incidental NET was histologically documented (tumor grade: G1= 17; G2= 1). The mean age of NET-patients (M:F=5:13) was similar to that of non-NET patients (NET patients: mean age 58,1 years; range=31-76 years; non-NET patients: mean age 56,4; range=15-91,5; p=0,47). All NET-patients were *H. pylori*-negative and coexisted with ECL linear/micronodular hyperplasia and with oxyntic atrophy, involving more than 60% of the oxyntic biopsy specimens (score 3). Adenomatous ECL-hyperplasia was observed in 47/217 cases (21,6%); in particular, adenomatous ECL-hyperplasia was documented in 15/18 (83,3%) NET and 32/199 (14,7%) non-NET patients. Among these adenomatous patients, 45/47 (95,7%) showed score 3 oxyntic atrophy. The presence of adenomatous hyperplasia was an independent risk factor for gastric NET (risk ratio 22,3; 95%CI = 7,9-62,8).

Tab. I. 217 AiG patients: OLGA stages al the initial and latest endoscopy follow-up.

		T1: OLGA Stage					
T0: OLGA Stage		0	I	II	III	IV	Total
	0	4	5	0	0	0	9
	I	2	15	8	1	0	26
	II	1	3	146	15	1	166
	III	0	1	8	7	0	16
	IV	0	0	0	0	0	0
	Total	7	24	162	23	1	217

Conclusions. In this large cohort of AiG-patients, the long-term endoscopy and histology follow-up did not documente any gastric epithelial precancerous/cancer lesion. In Western countries, the prevalence/incidence of both AiG and Gastric NETs are consistently increasing 4; moreover, fragmentary (even contradictory) information is available on the NET (AiG-associated) natural history. While AiG-related NETs account about 70-80% of all gastric NET, no reliable markers are available in order to identify patients at higher risk of gastric neuroendocrine tumors. This long-term follow-up study provides evidence that adenomatous ECL-hyperplasia is an independent risk factor for developing NET. Moreover, the prevalence of adenomatous ECL-hyperplasia was significantly higher in advanced corpus atrophy involving more than 60% of the biopsy oxyntic specimens.

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LINE-1 TRASCRIPTS EXPRESSION AND METABOLIC SWITCH AS MARKERS OF EARLY TRANFORMATION IN ADENOMAS SUBJECTED TO HIGHLY OXIDATIVE DNA DAMAGED

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Objectives. Accumulation of G>T transversion at specific gene loci and DNA global demethylation are proper of *MUTYH* associated polyposis (MAP), a model of he-

reditary syndrome characterized by unrepaired oxidative DNA damage[1]. Long Interspersed Nuclear Element-1 (L1), which are repeated sequences activated in cancer through DNA demethylation, can be partially regulated by Sirtuin 6 (Sirt6), an histone deacetylase and ADP-ribosyl-transferase, that counteracts the expression of key glycolytic genes[2], such as lactate dehydrogenase (LDH-A), a marker of metabolic switch and oxidative damage. A key branching point in the glycolytic pathway for this metabolism is the production of pyruvate, which in anaerobic conditions is converted to lactate. During this metabolic switch, LDH-A increases and pyruvate dehydrogenase (PDH) complex (PDC) is inhibited by pyruvate dehydrogenase kinase (PDK)[3].

We hypothesized a crosstalk between L1 activation and genetic and/or metabolic alterations in the MAP model. To this Aim., we compared the L1-coding gene (*ORF1/2*) expression with the *SIRT6*, *LDH-A*, *PDK1* and *PDK3* metabolic-associated gene transcript level and/or the presence of MAPK gene mutations in MAP adenomas.

Materials and methods. the analysis compared formalin fixed paraffin embedded (FFPE) samples from 13 MAP patients with 29 samples from sporadic adenomas and, as controls, 17 samples of colorectal normal mucosa. Specimens were collected at the Candiolo Cancer Institute and at the Anatomic Pathology Unit of University of Insubria between 2008-2015. The expression analysis of L1-coding gene (*ORF1*/*ORF2*), *SIRT6*, *LDH-A*, *PDK1* and *PDK3* genes was evaluated by RT-PCR and tested for associations with the presence of the most common colorectal cancer mutations (*KRAS*/*NRAS*/*BRAF,* and *PIK3CA*), analysed by mass-spectrometry (MALDI-TOF). Associations with different adenoma features (histotype, dysplasia, polyp dimension etc.) were also tested.

Results. MAP adenomas displayed higher L1 gene expression levels compared to normal mucosa (p-value ≤ 0.0001) and were prevalently detected in MAP and sporadic adenomas with villous architecture.

MAP polyps were characterized by higher *LDH-A* mRNA expression but lower *SIRT6* transcript level compared with normal mucosa (p-value ≤0.0001). Accordingly, *PDK3*, the most common PDK isoform in the colonocytes, showed the higher levels in MAP adenomas compared with normal mucosa (p-value = 0.008).

As expected, MAP adenomas showed also a high frequency (53%) of mutations, prevalently *KRAS*/*NRAS* codon 12 mutation (37%), reflecting the well-known increased susceptibility to oxidative damage at this nucleotide position as can be seen in patients whose adenomas were concomitantly mutated for two/three genes. However, the higher expression of L1 genes and the consequent "metabolic switch" was found mainly in MAP wild-type adenomas, whereas MAP mutated polyps displayed only an increased *SIRT6* expression.

Conclusions. The early stages of oxidative damaged colorectal tumorigenesis can be driven by the L1 product expression, controlling a precocious metabolic switching. Notably, this process seems to be independent of the presence of activating mutations. The L1 transcripts expression and the "metabolic switch" could be exploited as early colorectal cancer transformation markers.

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ESOPHAGEAL CANCER IN THE VENETO REGION: WHEN PATHOLOGY-BASED CANCER REGISTRY DOES NO FIT TO THE EXPECTANCIES

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Introduction. In the last decades, consistently all over the industrialized world, a decreased incidence of esophageal cancer (EC) has been reported (1,2,3). However, data about: i) the prevalence of the most involved esophageal sub-sites; ii) the histological cancer sub-types; iii) the cancer staging; iv) the neoadjuvant therapies and their clinico-pathological effectiveness are fragmentary reported, or completely lacking.

This study is Aim.ed to assess the status of EC registration in the (pathology-based) Cancer Registry of the Veneto Region (Registro Tumori del Veneto [RTV]). The study particularly focused on the current adequacy of the collected clinico-pathological variables as registered by the RTV between years 1987-2015.

Methods. The ECs incidence rates were computed using the RTV data, as collected between the years 1987 and 2015 (regional population covered by the RTV in the considered time-interval: 2.475.000). Incidence rates were standardized according to the European standard population 2013.

The study further considered the clinico-biological variables, as collected from a subgroup of subjects (313.668; years: 2000-2015) living in a well-defined Regional area (Verona). The pathology reports of incident ECs were considered. In such a population of EC-patients, the following clinico-pathological variables were checked, as potential indicators of the cancer status at the diagnosis, or of the cancer clinical outcome: i) anatomic site (upper, medium, lower esophagus); ii) surgical intervention (performed *versus* not performed); iii) cancer stage at the initial diagnosis (pT, pN); iv) Mandard Index of tumor regression.

Results. In the considered regional population (2.475.000 inhabitants; years: 1987 to 2015), the EC incidence rates steadily decreased, with an annual percent change of -3.3% in men and -1.6% in women. The incidence rate in 1987 was 26.2/100,000 in men, and 4.2/100,000 in women, and it dropped to 8.9 and 2.2/100,000 in 2015, respectively. In a subgroup of subjects (69 incident ECs as assessed in a population of 313.668 subjects; years: 2000-2015; Table), the study explored the clinico-pathological cancer information, as

Tab. I. Main clinico-pathological features of 69 Esophageal Cancers as reported by the clinical and pathology records (reference population).

		Number	%
Gender	male female	54 15	78,3 21,7
Age	<60 61-69 70-79 >80	3 23 27 16	4,4 33,3 39,1 23,2
Period	2000-2003 2004-2007 2008-2010 2013-2015	15 14 20 20	21,7 20,3 29,0 29,0
Histology	squamous carcinoma adenocarcinoma carcinoma NOS	50 15 4	72,5 21,7 5,8
Surgery	performed not performed	5 64	7,2 92,8
Mandard index	available not available	5 64	7,2 92,8
Stage T	available not available	13 56	18,8 81,2
Stage N	available not available	12 57	17,4 82,6

available from the collected pathology records. Among this test-population (about 80% were males, and more than 95% of them were older than 60 years old), the cancer sub-site was reported in only 2 cases (2,9%). According to pathology records, only 5 cases (7.2%) underwent surgical intervention. According to clinical and pathological data, T-Stage was available in less than 19% of the cases and the Nodal status in less than 18%. The Mandard Index was available for 5 cases (7,2%), overall.

Conclusions. Till December 31st 2015, the Veneto Cancer Registry covered a population of 2.475.000 regional inhabitants (currently, the covered population accounts for 4.900.000 [100% of the Veneto region population]). This study assessed the status of EC registration in the Regional Cancer Registry between the years 1987-2015. In the considered interval time, the incidence rate of ECs (all the histotypes) significantly decreased from 1987 to 2015. In a small (not representative) sub-group of the covered population, very scanty clinico-pathological information was additionally achievable through consulting the pathology reports of 69 incident ECs (population 313.668 subjects; years: 2000-2015). These unsatisfactory Results unequivocally support the priority of consistently expanding the collection/registration of clinical-biological data concerning esophageal cancer patients. The current disappointing situation prompts implementing several procedural improvements: i) to standardize data collection and consistently coding of the considered variables; ii) to chronologically update the cancer data collection/registration; iii) to reconsider critically the clinico-pathological variables to be included in patient-record; iv) to accelerate the nation-wide registration of the oncological disease. These combined implementations will allow an efficient remodulations of the strategic interventions in both cancer (primary and secondary) prevention, and therapies.

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PATOLOGIA MAMMARIA

MICROCALCIFICATIONS DRIVE BREAST CANCER OCCURRENCE AND DEVELOPMENT BY MACROPHAGES-MEDIATED EPITHELIAL TO MESENCHYMAL TRANSITION.

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Introduction. In breast tissues, microcalcifications play a crucial role in early cancer diagnosis [1,2]. Indeed, approximately 50% of non-palpable breast cancers are detected by mammography exclusively through microcalcification patterns [3], revealing up to 90% of ductal carcinoma in situ [4]. They can be classified according to their appearance on a mammogram based on the Breast Imaging Reporting and Data system [5], or by their physical and chemical properties [6]. In a recent paper, we demonstrated, for the first time, the presence of magnesium-substituted hydroxyapatite (Mg-HAp), which was frequently noted in breast cancer but never found in benign lesions [7]. In our experience, CO calcification is often associated with benign lesion, whereas hydroxyapatite (HA) is related both to benign and malignant lesions [7]. Recent evidences suggest that the morphological appearance of mammographic microcalcifications is associated with patient prognosis. In fact, patients harboring small breast tumors with casting type calcifications in the mammograms have a poor survival rate for this tumor size category [8-9].

Methods. This study Aim.s to investigate: a) the putative association between the presence of microcalcifications and the expression of both epithelial-to-mesenchymal transition and bone biomarkers, b) the role of microcalcifications in the breast osteoblast-like cells formation, and c) the association between microcalcifications composition and breast cancer progression. For *ex vivo* studies, we collected 174 biopsies on which we performed immunohistochemical and ultrastructural analysis. *In vitro* experiments were performed to demonstrate the relationship among microcalcification, BOLCs development and breast cancer occurrence. After experimental phases breast cell lines were used for morphological, immunocytochemical and western blot analysis.

Results. Histological investigations demonstrated the significant increase of breast osteoblast-like cells in breast lesions with microcalcifications respect to those without microcalcifications. *In vitro* data displayed that in presence of calcium oxalate and activated monocytes breast cancer cells undergo epithelial to mesenchymal transition. Also, in this condition cells acquired an osteoblast phenotype thus producing hydroxyapatite. To further confirm *in vitro* data, we studied 15 benign lesions with microcalcification from patients that developed a malignant condition in the same breast quadrant. Immunohistochemical analysis showed macrophages' polarization in benign lesions with microcalcifications. Noteworthy, in all patients with benign lesions associated with calcium oxalate microcalcifications, the subsequent malignant lesion was characterized by the presence of hydroxyapatite.
Conclusion. Our data shed new light about the role of microcalcifications in breast cancer occurrence and progression. In particular, we proposed a model for breast cancer carcinogenesis based on the capability of calcium oxalate calcifications to induce macrophages-epithelial-to-mesenchymal transition. In conclusion, this study can contribute to the re-evaluation of the role of microcalcifications in the management of breast cancer patients laying the foundation for the development of clinical analysis capable to identify the risk of breast cancer occurrence and progression based on the in vivo detection of microcalcifications.

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BREAST SEROMA: A CHALLENGING CYTOLOGICAL DIAGNOSIS.

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Aim. Peri-implant breast seroma is a late clinical presentation of augmentation mammoplasty or reconstructive surgery with breast implants[1]. Cytological evaluation of breast seromas is a common clinical approach, showing mainly an inflammatory reaction or, more rarely, a breast implant associated anaplastic large cell lymphoma (BIA-ALCL)[2,3]. We present a case of a woman presenting with peri-implant breast seroma.
Case presentation. A 55-year-old woman with history of breast cosmetic augmentation with textured surface implants presented with left breast swelling. The patient was admitted at our breast clinic and the ultrasound (US) examination of the left breast revealed a scant peri-implant fluid collection. An US-guided fine needle aspiration biopsy (FNA) was then performed: 6 mL of cloud, yellowish fluid were collected. The sample was concentrated by centrifugation; cytospins were prepared and Papanicolaou stained. A cell block (CB) was prepared from residual material.
Results. Papanicolaou stained cytospin preparations and hematoxylin-eosin stained CB slides showed a high cellular sample composed by medium to large-sized discohesive atypical cells with irregularly-shaped, hyperchromatic nuclei; prominent nucleoli were oc-

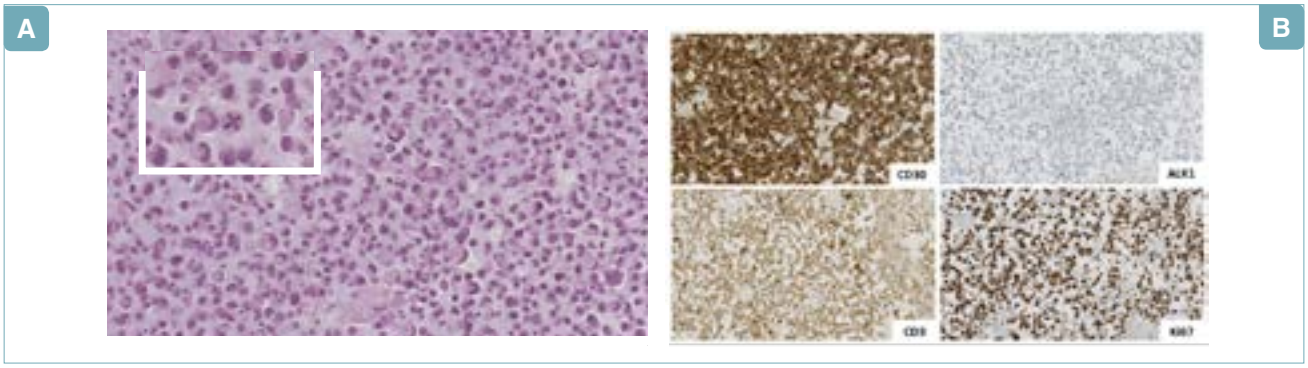

Fig. 1. (A) Cytophatological features of breast implant associated anaplastic large cell lymphoma in seroma aspirates. (B) Immunohistochemical studies on cell block material from the breast seroma.

casionally evident. Larger cells showed peripherally-located, "horseshoe" shape nuclei and abundant clear cytoplasm; cytoplasmic vacuoles were occasionally observed. Apoptotic cells and atypical mitoses were also observed. A diagnosis BIA-ALCL was suspected. Immunohistochemical (IHC) evaluation showed strong and diffuse CD30 positivity, diffuse CD3 positivity and ALK1 negativity in atypical cells; Ki-67 labeling index was > 80%.
IHC evaluation confirmed the BIA-ALCL diagnosis. Next generation sequencing (NGS) analysis by a multiple-gene panel showed FGFR3 and TP53 single-nucleotide polymorphism (SNP); pathogenic hotspot mutations in analyzed genes were not found.
Pre-operative simultaneous ^{18}F-FDG PET/MRI was performed confirming the presence of a fluid collection around the left breast implant that showed mild tracer uptake. No areas of abnormal enhancement were detected within the breast tissue and no pathological axillary lymph-nodes were found. Patient underwent implant removal and subsequent histological control confirmed the FNC diagnosis.
Conclusion. FNA confirmed to be a valuable approach for BIA-ALCL diagnosis4. The 2019 National Comprehensive Cancer Network (NCCN) guidelines on diagnosis and treatment of BIA-ALCL suggest to collect as much fluid as possible (minimum 50 mL)5; however, the present case demonstrates that an appropriate management of cytological samples allows a comprehensive morphological, IHC and molecular evaluation, even when scant (<10mL) peri-implant fluid is collected.

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PET/TC-DETECTED MYOFIBROBLASTOMA OF THE BREAST WITH ATYPICAL/BIZARRE CELLS: A CASE REPORT

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Background. Myofibroblastoma (MFB) is a rare benign mesenchymal tumor which usually occurs in the breast parenchyma of both females and males. In its classic type, MFB is usually composed of bland-looking spindle cells which exhibit immunohistochemical and ultra-structural features of both fibroblasts and myofibroblasts, and arranged in short, haphazardly intersecting fascicles interrupted by thick keloid-like collagen fibers/mats (1). Over the last two decades, the morphological spectrum of mammary MFB has been broadened by the recognition of several variants, including infiltrating, cellular, fibrous/collagenized, epithelioid/deciduoid, lipomatous, myxoid, and palisaded/Schwannian variants (1). The recognition of lipomatous, myxoid and epithelioid/deciduoid-like variants is crucial to prevent an overdiagnosis of malignancy, especially when evaluating small incisional biopsies. The presence of mono- or multinucleated atypical/bizarre cells has been occasionally reported as a focal finding, especially in the myxoid variant (2,3).
In the present study, we report a unique case of PET/TC-detected mammary MFB which was predominantly (80% of cells) composed of mono- and bi-nucleated atypical/bizarre cells. Given the alarming morphology tumor may be confused with a high sarcoma N.O.S. Only after a careful search, the identification of a focal residual area consistent with a conventional mammary-type MFB was helpful for achieving a correct diagnosis.
Materials and methods. Surgical specimen was submitted for histological examina-tion in neutral-buffered 10% formalin, dehydrated using standardtechniques, embedded in paraffin, 5mm sections cut and stained with hematoxylin and eosin (H&E). Immunohistochemical studies were per-formed with the labeled streptavidin-biotin peroxidase detection system using the Ventana automated immunostainer (Ven-tana Medical Systems, Tucson, AZ). The antibodies tested were vimentin; alpha-smooth muscle actin; desmin; myogenin; h-caldesmon, S-100 protein; CD99; CD34; bcl-2, CD10, cytokeratins (AE1/AE3 clone); epithelial membrane antigen (EMA); p63; estrogen receptor (ER); progesterone receptor (PR); all from Dako, Glostrup, Denmark. Appropriate positive and negative controls were included.
Case presentation: A 80-year old man with a history of thoracic and aortic aneurysms presented to our hospital for a solitary pulmonary 1,4 cm nodular lesion in the left lower lobe detected during follow-up CT scan evaluation. The patient underwent diagnostic PET-CT scan for staging purposes, which evidenced moderately increased uptake in the lung nodule with a maximum standardized uptake value (SUV) of 24. In addition, a nodular solid lesion with faint metabolic uptake (SUV 3) in the upper outer quadrant of the left breast was detected. Ultrasonography of the breast mass revealed a circumscribed nodular hypoechoic lesion with internal vascularity, without calcifications, measuring 2,5 cm in its greatest diameter. To further characterize the breast lesion, and its possible relation with the lung nodule (metastasis?), an ultrasound-guided core needle biopsy was performed. A proliferation of polygonal, epithelioid/deciduoid-like cells with severe nuclear atypia was observed. Neither mitoses nor necrosis was seen. Immunohistochemistry revealed diffuse staining for vimentin and CD34 and only focal immunoreactivity for desmin. The provisional diagnosis of "*atypical mesenchymal lesion of uncertain ma-*

lignancy" was rendered with the recommendation of histological evaluation in surgically-resected sample. The patient underwent surgical excision of the breast nodule with a rim of normal breast parenchyma. Gross examination revealed a well-circumscribed, unencapsulated tumor mass of 2.5 cm in its greatest diameter. The cut surface showed a fibro-myxoid mass, whitish in color. Histological examination revealed an unencapsulated tumor with pushing, lobulated margins, predominantly (80%) composed of atypical mononucleated or multinucleated cells with epithelioid, deciduoid and spindle cell morphology, These neoplastic cells exhibited abundant eosinophilic cytoplasm and nuclei with severe pleomorphism, smudged chromatin, evident nucleoli and pseudoinclusions. Mitotic activity was very low (≤ 1/50 HPF). Atypical mitoses and necrosis were absent. Neoplastic cells, mostly arranged haphazardly or in solid nests, were set in a fibro-myxoid stroma containing numerous thick, keloid-like fibers. Focally, lobules of mature adipocytes were seen within the tumor, but mammary ducts and lobules were lacking. Only after a meticulous search of the whole tumor mass, focal hypercellular areas composed of spindle-shaped cells, arranged in short fascicles interrupted by thick collagen fibers, were observed. These areas were consistent with classic-type mammary MFB. Immunohistochemically, neoplastic cells were positive for vimentin, CD34, desmin, estrogen/progesterone receptors, CD99, CD10, and bcl-2 protein. None of the other markers tested was positive. Ki67 expression was very low (3%). On the basis of the morphological and immunohistochemical features, a diagnosis of "*atypical/bizarre cell myofibroblastoma of breast" was rendered*"

Conclusions. the presence of mono- or multi-nucleated atypical/bizarre cells has only rarely been reported as a focal finding in the context of mammary MFB (2,3). The present case contributes to widen the morphological spectrum of stromal tumors of the breast, emphasizing the existence of a new diagnostically challenging variant of mammary myofibroblastoma, consisting of atypical/bizarre cells as the main cytotype. This tumor should be included in the list of the benign soft tissue lesions, especially leiomyomas and schwannoma/neurofibroma, that may exhibit a degenerative-looking atypia in the setting of low mitotic activity and absence of atypical mitoses and necrosis. The main differential diagnosis included pleomorphic sarcoma and carcinoma. Although the unusual morphology of the present case, a correct diagnosis can be rendered confidentially on the following features: (i) identification of focal residual areas with the morphological and immunohistochemical features of the classic-type mammary MFB; (ii) presence of thick, keloid-like collagen fibers interspersed in the tumor stroma; (iii) demonstration of fibroblastic/myofibroblastic differentiation (vimentin+, CD34+, desmin+).

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ANOMALOUS NUCLEAR DELOCALIZATION OF CYTOCHROME P450 1A1 (CYP1A1) IN BREAST CANCER PATIENTS FROM GEOGRAPHICAL AREAS CHRONICALLY EXPOSED TO ENVIRONMENTAL POLLUTANTS

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Background. In 1997 the World Health Organization defined the Campania Region as a geographical area with a high risk of incidence for tumors linked to environmental factors. Breast carcinoma (BC) has a highest incidence in the female and is one of best represented in the areas with a high environmental impact in Campania (1). The dioxins are the main pollutants in these areas, produced by continuous combustion of urban/industrial wastes. Chronic exposure to dioxins Results in hyperactivation of the detoxification pathway mediated by Cytochrome P450 1A1 (CYP1A1), a phase-I enzyme that metabolizes endogenous and xenobiotic substrates. CYP1A1 expression, suppressed under physiological conditions, is induced in the presence of substrates *via* transcriptional regulation of the aryl hydrocarbon receptor (AhR) (2). The majority of BCs constitutively express CYP1A1, in which it can be also involved in tumor progression (3).

Aim. In this study a series of BC patients were selected from areas with different environmental impact to evaluate CYP1A1 expression and its cell sub-localization.

Methods. CYP1A1 expression was evaluated by ISH and IF on all tissue samples, and by RT-PCR and WB on selected specimens.

Results. Preliminary analysis showed an abnormal nuclear delocalization of the enzyme only in BC patients from areas chronically exposed to pollutants (Fig. 1). Nuclear import is mainly mediated by a complex with importin-α, importin-β1 and a cargo, through the recognition by importin-α of nuclear localization signals (NLSs) within the cargos (4). cNLS Mapper allowed the identification of a NLS in CYP1A1, potentially involved in nuclear translocation. The importin-mediated transport mechanism was confirmed by a double immunofluorescence (importin-α/CYP1A1) on selected BC samples, showing a clear co-localization of both proteins in the nuclei of tumor cells (Fig. 2).

Furthermore, CYP1A1 expression was evaluated by RT-PCR and WB analyses in BC and healthy control tissues. Typical Results of WB performed on total protein extracts showed a different pattern of immunoreactive bands, possibly due to the presence of spliced variants of CYP1A1 in cancer samples, as preliminarily confirmed by mass spectrometry and proteomic tools. On the transcriptional side, initial analyses indicated the presence of variants with a different distribution between healthy and cancer tissues.

Conclusions. To clarify their potential role as toxicity markers, we are characterizing CYP1A1 variants involved in the nuclear delocalization and performing

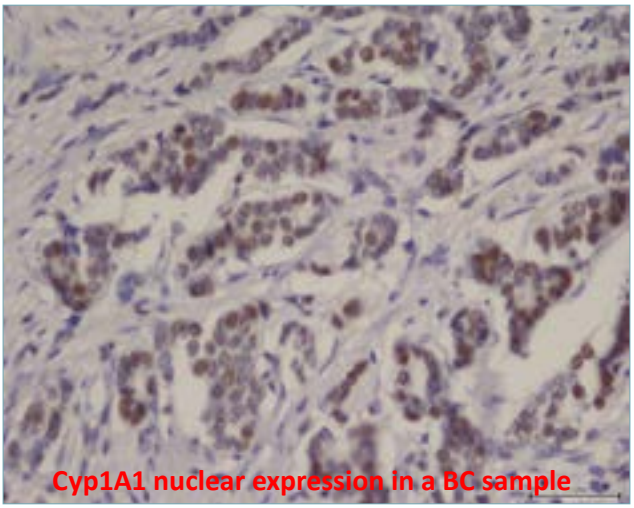

Fig. 1.

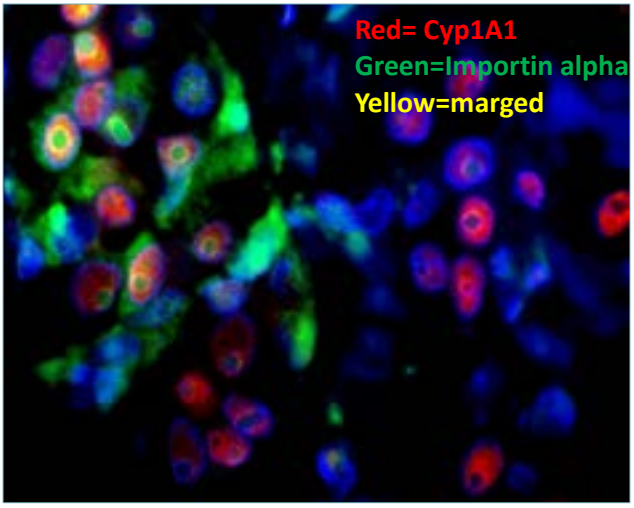

Fig. 2.

functional in vitro studies to better understand associated molecular mechanisms.

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COMPARISON BETWEEN ROUTINE METHODS AND RT-QPCR ASSESSMENT (MAMMATYPER®) OF BREAST CANCER BIOMARKERS: NEW APPROACH FOR IMPROVING MOLECULAR CLASSIFICATION

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Aims. The standards of immunohistochemistry (IHC), the main method used for biomarkers testing in breast cancer[1-3], have been efficiently ameliorate by decades of quality control efforts. However, computational pathology and reverse transcription quantitative PCR (RT-qPCR) may also hold promise for additional substantial improvements. In this regard, the present study compared routine methods and RT-qPCR evaluation of breast cancer biomarkers. The original Results (ORI) of the routinely used IHC for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2), proliferation marker Ki67 and fluorescence in situ hybridization (FISH) with the findings of manual (REV) and semi-automated digital image analysis (DIA) re-evaluation of the original IHC slides; moreover, both the IHC (ORI, REV and DIA) and the FISH Results were related to the RNA expression data of *ERBB2*, *ESR1*, *PGR* and *MKI67* from MammaTyper®(MT) gene expression assay.

Materials and methods. The available material of 96 women who underwent surgery for invasive breast carcinoma in the period from 2010 to 2012 (formalin-fixed and paraffin-embedded - FFPE - blocks, Hematoxylin and Eosin - H&E - slides and IHC slides) was retrieved from the archive. The previously reported Results for ER, PR, Ki67, HER2 and FISH were registered as ORI. An experienced pathologist (EC) performed both REV and DIA blind re-evaluation, which involved both tumor classifications and grading on the original H&E slides, according to international recommendations[1-5]. Furthermore, a re-evaluation of ER, PR, Ki67 and HER2 on the existing IHC slides were performed[1-3]. The HER2 equivocal cases, after re-evaluation, were subjected to FISH analysis (dual-probe, Leica HER2 FISH system), the Results of which were recorded according to the ASCO/CAP 2013 updated guidelines.

The MT test was performed in all 96 cases, using 10 µm sections from FFPE blocks, in order to extract total RNA, following the step described by the manufacturers' instructions[6]. The determination of the expression levels of *ERBB2*, *ESR1*, *PGR* and *MKI67* by RT-qPCR was obtained using the CE-marked MammaTyper® IVD kit (BioNTech Diagnostics) on the CFX96™ (BIO-RAD®) platform.

Statistical analysis both to compare ORI, REV, DIA data and to correlate Results from ORI, REV, DIA, FISH with RNA expression data was performed.

Results. Correlation for ER and PR was excellent between ORI IHC and Results from REV, DIA and RT-qPCR. As regards HER2, 10 out of 96 discrepant cases were detected in ORI versus REV comparison. Among these, only 1 case was finally classified as equivocal after comparison of ORI, REV, DIA and RT-qPCR. For Ki67, 22 (23%) cases were categorized differently by

either REV alone, DIA alone or both. Most of the discrepant Ki67 cases changed from low to high between ORI and REV/DIA. Thirty-two (33%) cases resulted discrepant between RT-qPCR and any IHC assessment of Ki67, 29 cases of these showed high *MKI67* expression.

Conclusions. Assessment of the breast cancer biomarkers ER, PR, HER2 and Ki67 at the RNA level shows high degree of correlation with IHC and compares well with correlations between original with subsequent independent manual or semi-automated IHC re-evaluations. Intrinsic marker properties, such as the type of protein and RNA frequency distributions or spatial heterogeneity in whole sections may interact with interpretation bias to shape the extent of inter-observer or inter-method variability. The use of methods with wider dynamic range and higher reproducibility such as RT-qPCR may offer more precise information about endocrine responsiveness, improve Ki67 standardization and help resolve HER2 cases that remain equivocal or ambiguous by IHC/FISH. Moreover, the use of RT-qPCR for the breast cancer biomarkers determination could both improve the molecular classification and allow a more appropriate patients'treatment.

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RANKL, BMP2 AND PTX3 AS BONE METASTASIS MARKERS IN INFILTRATING LOBULAR BREAST CARCINOMA: PRELIMINARY RESULTS

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Introduction and objectives. About 10% of breast cancer patients without evidence of bone metastases at the time of diagnosis will have a first disease relapse in bone within 5 years from primary diagnosis [1]. Bone metastasis (BM) are more frequent in Infiltrating Lobular Carcinoma (ILC), bone representing 92% of metastatic site, if compared with Infiltrating Ductal Carcinoma (IDC) [2]. Recently, cancer cells with osteoblastic differentiation - Breast Osteoblastic-like Cells (BOLCs) - have been described as a potential reliable biomarker predictive of BM [3]. Indeed high expression of Receptor Activator of Nuclear Factor kB Ligand (RANKL), Bone Morphogenetic Protein-2 (BMP-2) and PTX3, antigens involved in the mineralization and osteoblastic differentiation, has been reported in breast cancer [3]. The Aim. of this study was to investigate the potential role of RANKL, BMP-2 and PTX3 as biomarkers of BM risk in ILC.

Materials and methods. Sixteen ILC breast samples from the Breast Unit of San Giovanni-Addolorata Hospital (Rome) database de-identified were included in the study. All experimental procedures were carried out according to the Declaration of Helsinki.

Patients were stratified according to the presence of bone metastasis. Group 1 included 6 cases with bone metastasis (40 months median follow-up). Group 2 included 10 cases of ILC free of disease at the same follow-up time. The clinic-pathologic record included: age at diagnosis, pT and pN at surgery, histological grade (G), multifocality, Lymphovascular Invasion (LVI), Hormone Receptors (ER and PR), Ki67, c-erbB2(neu)

Tab. I. List of antibodies.

Antibody	Clone	Source
Estrogen Receptor	SP1	Ventana-Roche
Progesteron Receptor	1E2	Ventana-Roche
Ki67	Mib-1	Ventana-Roche
c-erbB2(neu)	4B5	Ventana-Roche
BMP-2	Rabbit clone N/A	Novus Biologicals
PTX3	MNB1	AbCam
RANKL	12A668	AbCam

Tab. II. Clinical and molecular markers results.

	Group 1	Group 2	P
Age	61	66	n.s.
pT1	2/6 (33%)	5/10 (50%)	n.s.
pN+	4/6 (66%)	4/10 (40%)	n.s.
multifocality	2/6 (33%)	6/10 (60%)	n.s.
LVI	1/6 (16%)	1/10 (10%)	n.s.
G3	1/6 (16%)	4/10 (40%)	n.s.
ER+	6/6 (100%)	10/10 (100%)	n.s.
PR+	5/6 (84%)	6/10 (60%)	n.s.
Ki67	18%	14%	n.s.
c-erbB2(neu)	Negative (0)	Negative (0)	n.s.
BMP-2	627/mm2	171/mm2	0.03
RANKL	270/mm2	0	0.05
PTX3	214/mm2	105/mm2	n.s.

status. For each sample, paraffin serial sections were used for immunohistochemical analysis of BMP-2, PTX3 and RANKL. Table I shows the antibodies.
The slides were digitalized by iScan Coreo (Ventana, Tucson, AZ, USA). The number of neoplastic cells positive for each antibody was assessed in an area of at least 1 cm^2 and was expressed as positive cells per mm^2. Results were analyzed by Student T-Test and binomial Pearson χ^2-test.
Results. The clinical data and the Results of the present study are reported in Table II.
Age, tumor stage, multifocality, LVI, Ki67, Hormone Receptor and c-erbB2 status did not show any statistically significant difference. The number of BMP-2 and RANKL positive cells was higher in Group 1 than in Group 2 with a significant difference (BMP-2 p 0.03; RANKL p=0.05). Even if in Group 1 the amount of PTX3 positive cancer cells doubled as compared Group 2, no significant differences were observed.
Discussion and conclusions. There are experimental and clinical evidences of strong osteotropism of breast cancer cells [4], especially for what concern the IDC [5,6]. A better understanding of the molecular and cellular mechanisms underlying the formation of bone metastases could improve therapies and patients quality of life. Bone is one of the main sites of metastases in ILC, but to date no work in literature defines a relationship between expression of molecules able to induce osteoblastic differentiation of breast cancer cells and to predict bone metastases risk in ILC.
In this study, we compared the expression of BMP-2, PTX3 and RANKL in a subset of ILC with or without bone metastasis at the same follow-up time. Our Results, although based on a limited number of cases, show a significant rise of BMP-2 and RANKL expression in patients that will develop bone metastasis (Group 1 vs Group2). BMPs, cytokines belonging to the transforming growth factor-β superfamily, and RANKL represent the most powerful osteoblast differentiation factors. Our hypothesis is that BMP-2 could play a role determining mesenchymal-like cancer cells to transform in BOLCs, able to drive bone metastatization. The increase of the series is in progress.

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LOW-GRADE ADENOSQUAMOUS BREAST CARCINOMA ARISING IN A EXTENSIVE COMPLEX SCLEROSING LESION - A CASE REPORT

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Objective. Low-grade adenosquamous carcinoma (LGASC) is a rare variant of metaplastic breast cancer. Unlike other variants of metaplastic carcinoma and despite having a triple negative immunophenotype it is usually an indolent lesion with favorable prognosis. Due to its morphology and low-grade nuclear atypia it may mimic benign lesions and therefore its diagnosis can be challenging in core biopsy samples.
Materials and methods. We describe the case of a 43-year-old woman who came to our attention for investigation of a palpable lesion in her right breast.
Results. A 43-year-old woman, with no familiar history of breast cancer, presented with a painful nodule in the right breast. Palpation revealed a 2 cm hard nodule in the transition of the inner quadrants (Q1-Q3). There were no skin alterations, no nipple discharge or axillary lymphadenopathies. The left breast was normal. Ultrasound imaging showed a 13x15mm nodule with spiculated contours and hypoechoic heterogenous structure. The core biopsy performed in this area revealed fragments of a complex sclerosing lesion (CSL) encompassing foci of an atypical intraductal epithelial proliferation displaying weak and heterogeneous estrogen receptor (ER) expression and lacking basal cytokeratin (CK14) expression. The core biopsy was therefore classified as a B3 lesion. A second core biopsy was carried out that showed similar alterations and received the same classification.
The patient was subjected to a magnetic resonance (MR) imaging, which showed a wide (64x18 mm) and heterogenous area of enhancement ("non-mass" type) associated with minor areas of enhancement with a linear distribution and with "clustered ring" features.
An excisional biopsy of the lesion was performed. In the context of a breast parenchyma with diffuse sclerosing adenosis we identified a central sclero-elastotic area with a neoplastic infiltrative proliferation composed of small tubular structures with round to comma-like shape. The epithelial cells showed low to intermediate-grade nuclear pleomorphism, were of triple negative phenotype and expressed myoepithelial cell markers, such as p63, which featured a circumferential staining in some of the tubular structures. The tubular structures populated a fibro-elastotic stroma with lymphocytic infiltration. A diagnosis of low-grade adenosquamous carcinoma (LGASC) of the breast was rendered.
In addition, ductal and lobular structures around the LGASC displayed a neoplastic intraepithelial proliferation featuring a cribriform and solid architecture. The neoplastic cells showed round nuclei with sometimes evident nucleoli, they lacked CK14 expression and displayed a heterogeneous pattern of ER expression (weak

nuclear staining in a fraction of neoplastic cells; remaining cells lacking ER expression). Some of the DCIS foci were 1,5 mm from the superficial margin.
In consideration of the MR features and of the proximity of the DCIS to the margin the multidisciplinary group favored a skin-sparing mastectomy with sentinel node excision.
At final histological examination two foci of DCIS (11 mm and 5 mm) in a breast parenchyma with diffuse sclerosing adenosis and signs of the previous surgical intervention (fibrosis and foreign body multinuclear cell reaction) were diagnosed. There were no signs of invasive carcinoma and the sentinel nodes (n=3) were free of metastasis.
Conclusion. Herein we report a case a low-grade adenosquamous carcinoma associated with DCIS arising in an extensive complex sclerosing lesion, that was proven challenging for several reasons.
First, due to the absence in the core biopsies of squamous differentiation and to the fact that glandular structures of LGASC can express myoepithelial cell markers, we were not able to render a diagnosis of LGASC, which may have helped in the proper planning of the surgical intervention.
Second, the accompanying DCIS had unique features because, even though morphologically it showed only intermediate grade nuclear atypia, immunophenotically it expressed weak and heterogeneous ER.
Finally, the MR imaging features were suspicious for a larger but unspecific involvement and the histopathological analysis that revealed in situ carcinoma in the context of CSL but also in the surrounding parenchyma characterized by diffuse sclerosing adenosis made the therapeutic decision about re-excision versus radiotherapy more challenging. The therapeutic intervention of a radical mastectomy was eventually proven correct.
The description of this case highlights the need of multidisciplinary discussion for proper breast cancer patient diagnosis and management, in particular in the context of discrepancy between radiological features and diagnosis on core biopsy, as well as in the context of rare histologic types of breast cancer.

HISTOLOGIC SUBTYPING AFFECTING OUTCOME OF TRIPLE NEGATIVE BREAST CANCER: A LARGE SARDINIAN POPULATION-BASED ANALYSIS

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Introduction. Triple Negative breast cancers (TNBCs) account for 10-20% of all invasive breast cancer. TNBC is most prevalent in young women, <50 years of age, shows aggressive clinical behavior, high histological grade and poor prognosis, and is responsible for about 25% of deaths for breast cancer. TNBC encompasses a heterogeneous group of tumors with different clinicopathological features and genetic-molecular alterations [1], and is prevalently histologically categorized as high-grade invasive ductal carcinomas of no special type (IDC-NST). Other histologic "special types" (HST) as metaplastic, medullary and adenoid cystic carcinomas are still included among TNBC. These special phenotypes substantially differ in terms of biologic behavior and clinical course [2].
Recent studies evaluated the outcome of special types of TNBC, showing that distinct prognostic implications may derive from the specific histotype of TNBC, and highlighting that the identification of these special types has a significant clinical utility and should be considered in therapeutic algorithms [3-6].
The Aim. of this study was to analyze a uniquely large cohort of TNBC patients enrolled in Sardinia in order to evaluate morphologic heterogeneity and to determine the prognostic significance of special types compared with non-specific TNBC variants.
Methods. This study was based on data obtained from "*TNBC Database*" including pathological characteristics and clinical records of 1009 TNBCs diagnosed between 1994 and 2015 in four major oncologic centers from Sardinia. Kaplan-Meier analysis, log-rank test and multivariate Cox proportional-hazards regression were utilized for overall survival (OS) and disease free survival (DFS), according to TNBC histologic types.
Results. The overall distribution of TNBC according to histologic type was as follows: tumors from 745 patients (78%) were classified as IDC-NST, 62 (6.5%) as lobular, 43 (5.5%) as apocrine, 42 (4.4%) as metaplastic, 39 (4.1%) as medullary; other histological types, such as mucinous (0.2%), mixed (0.7%), papillary (0.7%), adenoid cystic (0.6%) and micropapillary (0.2 %) were less common.
At five-years, OS was 92.1%, 100%, and 94.5% for patients with apocrine, adenoid cystic and medullary carcinoma, respectively; patients with lobular and metaplastic carcinoma showed the worst OS, with 79.7% and 84.3% respectively. At ten-years, a favorable prognosis was reported for patients with adenoid cystic (100%) and medullary (94.5%) carcinoma, whereas patients with lobular carcinoma showed the worst prognosis (73.8%).
Conclusion. Taking IDC-NST as a reference, our data showed that patients with lobular and metaplastic carcinoma had poor survival outcomes at 5- and 10-years follow-up; patients with adenoid cystic and medullary carcinoma had excellent prognosis at 5- and 10-years follow-up, and patients with apocrine carcinoma had good outcome until 5-years, with similar values to IDC-NST at 10-years. Patients with medullary carcinoma had the best DFS, representing an independent prognostic factor.
Current clinical data indicate that patients with triple negative lobular carcinoma or metaplastic carcinoma are less responsive to chemotherapeutic agents, contrary to patients affected by other histologic types of TNBC, increasing the need to identify unambiguous

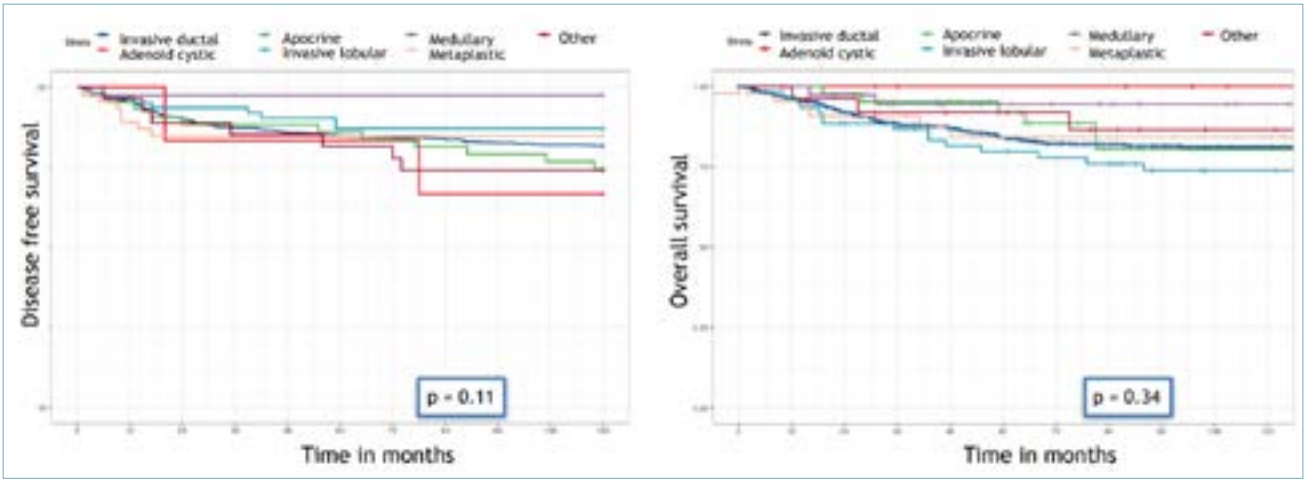

Fig. 1. Kaplan-Meier curves of Disease Free Survival (A) and Overall Survival (B) of patients affected by breast cancer Triple Negative according to histologic types.

molecular target against which to develop specific targeted therapy. On the other hand, patients with TNBC histologic variants as medullary, apocrine and adenoid cystic carcinomas, which are known to share a better prognosis, could be treated with less aggressive or even without chemotherapeutic regimens, according to the stage of disease.
Nowadays, TNBC histologic features have been poorly pondered in the clinical management of patients. Our study strengthens previous clinical and experimental data in favoring greater integration between histologic and molecular characterization of these variants, which should be considered together in the choice of therapeutic regimens.

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SERINE PROTEASE-INDUCED COLLECTIVE DETACHMENT OF BREAST CANCER CELL AND CORRELATION WITH LYMPHOVASCULAR INVASION IN BREAST CANCER

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Background. High levels of the serine protease neutrophil elastase (NE) correlate with poor outcome and endocrine resistance in breast cancer patients. It is not clear if released NE is related to tumor progression or is simply a sign of reactive responses to progressing tumors. In both scenarios, high levels of NE might create a favorable environment for breast cancer invasion and metastasis.
Estrogen receptor positive (ER+) MCF7 breast cancer cells in NE-addicted medium grow in suspension as 3D-spheroids, resembling the micropapillae of a human breast cancer characterized by high propensity to metastasize.
We hypothesized that NE may produce disarrangement of tumor cell adhesion to the substrate fostering neoplastic lymphovascular invasion (LVI) and metastasis.
Objectives. The Aim. of this study was to compare *in vitro* the effect of NE with that of other proteases on the growth-pattern of breast cancer cell lines with different estrogen receptor (ER), HER2 and E-cadherin (E-CAD) status and Epithelial Membrane Antigen (EMA) expression. Finally, we compared the experimental Results with those derived from human breast carcinomas with neoplastic embolization evaluating a possible correlation with the presence of NE+ polymorphous neutrophilic granulocytes (PMNs).
Materials and methods. ER+/E-Cadherin (E-CAD)+/HER2- (MCF7, T-47D, ZR-75-1), ER+/E-CAD+/HER2+ (BT-474), ER-/E-CAD-/HER2+ (SK-BR-3) and ER-/E-CAD-/HER2- (MDA-MB-231) cells were grown with serine proteases (NE, cathepsin-G), hyaluronidase and collagenase. To reproduce *in vitro* the environment of *in vivo* ER+ human breast cancers, co-cultures of MCF7 cells and α-smooth-muscle actin (SMA) positive human mammary fibroblasts (CAFs) isolated from an ER+ human breast carcinoma were set up. EMA expression was assessed by immunocytochemistry. ELISA BrdU assay was used to assess proliferation of MCF7 NE induced 3D-spheroids following tamoxifen treatment.
A cohort of consecutive invasive carcinomas showing either focal, moderate or extensive LVI was collected.

Representative tissue blocks of the neoplastic embolization were selected to perform immunohistochemistry. As a control group we collected invasive breast carcinomas with no evidence of peritumoral LVI and without lymph-node metastasis (pN0). Lobular carcinomas were excluded.

Results. NE and cathepsin-G led to 3D-spheroid formation of ER+/E-CAD+ cells only. During NE-induced MCF7 3D-spheroid formation the luminal Epithelial Membrane Antigen (EMA) remained along the external border of cell clusters, which faced cancer associated fibroblasts in co-cultures experiments. MCF7 3D-spheroids were tamoxifen resistant.

In a cohort of human breast carcinomas with LVI, the MCF7-3D-spheroid-alike pattern was the most prevalent in tumor emboli. Immunohistochemical NE expression was mainly detected in polymorphous neutrophilic granulocytes (PMNs) within vessels and in the stroma. PMNs were significantly higher in breast carcinomas with LVI. In fully developed metastases within lymph-nodes, which reverted the EMA expression as in primary tumor, no NE+ PMNs were observed.

Conclusions. In conclusion, our Results provide indirect evidence on how high levels of NE may negatively act on patient prognosis by creating a favorable environment for potential breast cancer invasion and metastasis.

BENIGN MYOFIBROBLASTIC-EPITHELIAL LESIONS OF THE BREAST WITH INFILTRATIVE MARGINS: CLINICO-PATHOLOGIC STUDY OF 5 CASES

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Aims. Fibroepithelial lesions of the breast are biphasic lesions that usually comprise fibroadenomas and phyllodes tumors. The Aim. of this study was to investigate a series of benign biphasic lesions of the breast lacking the characteristic cyto-architectural features of both fibroadenomas and phyllodes tumors, most of them previously labeled with the term of "hamartomas or myoid/muscular hamartomas or benign fibro-adenomatous lesions of the breast." We focused on a series of five lesions which shared, as an unexpected feature, a prominent myofibroblastic stromal component with, at least focally, finger-like infiltrative margins. The provisional descriptive term "Benign Myofibroblastic/Epithelial Lesions of the Breast" is proposed.

Materials and methods. All cases labeled as "hamartomas or myoid/muscular hamartoma or benign fibroadenomatous lesions of the breast" were retrieved from the archives of Anatomic Pathology of the University of Catania. Among a series of 42 cases, we selected five cases which shared as the most striking feature a prominent proliferation of stromal cells with the morphological and immunohistochemical features of myofibroblasts which, at least focally, exhibited infiltrative margins. Three patients underwent needle core biopsy that showed a proliferation of vimentin/alpha-smooth muscle actin-positive, bland-looking spindle cells variably admixed with benign epithelial structures (lobules, ducts, cysts, typical ductal hyperplasia). All lesions were surgically removed. Patients are well without any evidence of local recurrence.

Results. All patients were women with an age ranging from 31 to 52 years. The lesions were incidentally detected by mammography performed as screening examination. Radiologically these lesions appeared as dense nodules, ranging in size from 1 cm to 2,5 cm, with circumscribed margins, only focally irregular in two lesions. Histological examination of the surgically excised lesions showed a common morphological theme, consisting of biphasic lesions composed of an epithelial and stromal component. The former consisted of cysts, florid adenosis and simple to more complex typical ductal hyperplasia, while the latter was represented by a variable fibro-myxoid stroma rich in bland-looking spindle cells with eosinophilic cytoplasm. These cells, exclusively stained with vimentin and a-smooth muscle actin (no staining for desmin, h-caldesmon, S100, HMB45, pan-cytokertins, EMA, p63, beta-catenin) were interpreted as myofibroblastic in nature. Rare mitoses were found, but neither atypical mitoses nor necrosis were seen. Two lesions, exhibiting an exclusive myxoid stroma with interspersed myofibroblastic cells focally arranged in short fascicles, were reminiscent of nodular fasciitis. In other two cases, the myofibroblastic cells, set in a prominent fibrous stroma, were closely packed and arranged into more well defined fascicles. The stromal component looked like desmoid-type fibromatosis. In one case the stroma was variably fibro-myxoid and the myofibroblastic cells were haphazardly arranged. In all the lesions the pericanalicular and intracanalicular growth patterns, typically seen in fibroadenoma, were lacking. Similarly, the morphological hallmarks of phyllodes tumors, including the broad leaflike papillae, increased stromal cellularity and periductal stromal condensation, were absent. The most striking and unexpected feature was the presence of, at least focally, infiltrative margins, namely the myofibroblastic cells showed a finger-like infiltration into the fibro-fatty breast tissue.

Conclusions. The present study contributes to widen the morphological spectrum of the benign biphasic epithelial and stromal lesions of the breast. We focused on a previously unrecognized group of biphasic lesions characterized by a prominent myofibroblastic stromal component with variable fibro-myxoid stroma and infiltrative margins, for which the descriptive term "Benign Myofibroblastic/Epithelial Lesions of the Breast" is proposed. Awareness of these lesions is crucial to avoid confusion with other bland-looking spindle cell lesions of the breast such as nodular fasciitis, desmoid-type fibromatosis, or low-grade fibromatosis-like spindle cell carcinoma, especially when evaluating on small biopsies.

PROGNOSTIC FACTORS IN PHYLLODES TUMORS OF THE BREAST: RETROSPECTIVE OBSERVATIONAL MONOCENTRIC STUDY

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Background. Phyllode tumors (PT) of the breast are rare fibro epithelial tumors accounting for less than 1% of all primary breast tumors. PT exhibit a heterogeneous clinical outcome, with both local and distant recurrence. The main purpose of our research is to investigate the morphological, histological and clinical characteristics of all types of PT and to correlate them with the rate of PT-related local and distant relapses. We also explored correlation between the clinico-pathological characteristics and the cumulative incidence of PT-related relapse and death in order to identify possible prognostic factors.
Methods. We performed a retrospective observational mono-institutional analysis of 166 patients with PT of the breast diagnosed between July 2001 and April 2018 at the Pathology Unit of University of Padua. For all patients we have evaluated the pathological parameters - histological grade, dimensions of the primitive tumor, stromal cellularity, number of mitosis per 10 HPF, stromal atypia, stromal overgrowth, necrosis and intertumoral heterogeneity. The stromal cellularity was evaluated in the most cellular areas. Mild stromal cellularity was assigned when was observed twice cellularity of normal perilobular stroma with evenly spaced nuclei without overlapping nuclei. Marked cellularity was considered when present overlapping nuclei and closely spaced cells. Moderate cellularity showed intermediate characteristics between mild and marked stromal cellularity. The mitotic activity was also evaluated in the most cellular areas, counting the number of mitotic figures per 10 HPF at 40X magnification: < 5 mitosis/10HPF; 5-9 mitosis /10HPF; or > 9 mitosis/10HPF. The PT were divided into 3 categories based on their stromal atypia: mild, moderate and marked. As mild stromal atypia we considered the presence of cells with small, uniform nuclei with absence or inconspicuous nucleoli. Marked atypia was assigned in the presence of cells with variable size and shape, irregular nuclear membrane and prominent nucleoli. Moderate degree was assigned to these cases that showed intermediate characteristic between mild and marked atypia. We considered the presence of stromal overgrowth when we were able to identify stromal proliferation without accompanying epithelial elements in at least one low powered field (4X). The PT were divided into three categories: benign, borderline and malignant following the established criteria of WHO (1). The cumulative incidence of PT-related recurrence and PT-related death were calculated. We considered as PT-related relapses the first ipsilateral breast relapse, axillary lymph node relapse or distant relapse not taking into consideration contralateral PT. For all the statistical analysis, we have used software SPSS version 25.0. We have considered statistically significant result if $p < 0.05$
Results. Most of our patients were diagnosed with benign PT (115 patients, 69.3%), borderline PT (30 patients, 18.2%) and malignant PT (21 patients, 12.7%). The median age at diagnosis is 41 years (16-85) with statistically significant distribution of the age based on the histological grade. The age at diagnosis was higher in case of higher tumor grade ($p<0.001$). The median dimension of the primitive tumor is 31 mm (4-250) and is positively correlated with the histological grade of the tumor ($p<0.001$). We found a statistically significant positive correlation between the presence of necrosis and the histological grade. Intertumoral heterogeneity was detected in 12 cases (7%): 2 cases (1%) benign PT; 1 case (0.6%) borderline PT and 9 cases (5.4%) malignant PT.
One hundred and forty-nine patients had adequate follow up. At a median follow-up of 97.7 months (95% CI 82.5-113.0), PT-related recurrences were observed in 8 (8.1%), 2 (6.7%) and 4 (20.0%) patients with benign, borderline and malignant PT, respectively (p 0.212). In 5 cases (35.7%) we have noticed an upgrade of the tumor grade on the relapse, 2 patients were diagnosed with borderline relapse and primitive benign PT, 2 patients were diagnosed with benign PT and relapse as malignant PT and 1 patient was diagnosed with borderline PT and relapsed as malignant PT. Cumulative incidence of PT-related recurrence at 4 years was 6.9% for benign, 6.8% for borderline, 21.3% for malignant histology (p 0.273). Stromal atypia and intertumoral heterogeneity significantly correlated with the risk of PT-relapse ($p=0.031$, p 0.005 respectively). We observe a tendency for a higher risk of relapse when tumor dimension is over 5 cm or the patient is between 35 and 49 years. PT-related deaths occurred in 4 patients (3 with malignant and 1 with borderline tumor), all experiencing distant metastasis. The median time for relapse is 22.9 months (2.4-72.7 months).
Conclusions. PTs are rare fibro epithelial tumors with unclear behavior and clinical management. We reported a large retrospective series of patients with PT and we found that cellular atypia and heterologous differentiation could be considered as predictive factors for recurrence. Further prospective studies are required to validate a prognostic stratification of patients to improve the therapeutic approach.

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EPITHELIOID STROMAL CELL HYPERPLASIA OF THE BREAST: A POTENTIAL PITFALL OF MALIGNANCY

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Objectives. Epithelioid stromal cell changes in benign neoplastic diseases of the breast is a rare event that can

be encountered in diabetic mastopathy, autoimmune disorders and in benign tumors, especially myofibroblastoma (1,2). We herein report three rare cases of a benign lesion, labeled with the descriptive term "*epithelioid stromal cell hyperplasia of the breast*", which apparently occurred in patients without any history of diabetes or other immune disorders. Radiologic features were not specific, but did not show features suggestive of malignancy. Notably in two cases, a misdiagnosis of malignancy was made on core biopsy.

Materials e methods. All cases herein presented had been seen in consultation. For each case, core biopsy and surgical specimen were available. All available slides, including H&E and immuhistochemical stainings, were reviewed. For all cases, additional unstained paraffin sections, clinical data and follow-up information were obtained from the referring pathologists. There were 2 females and 1 male patient. Age at diagnosis ranged from 44 to 79 years. No clinical history of long-term oral estrogen or sex-steroid therapy, as well as of insulin-dependent diabetes mellitus or autoimmune disorders, was present. In all cases, radiologic features were not specific. Two cases were incidentally discovered on routine screening mammography for breast cancer (cases no.1 and no.2). In case no.1, a hypoechogenic nodule measured 20 mm in greatest diameter and an ultrasound-guided needle core biopsy was performed. The diagnosis of "*invasive lobular carcinoma, with expression of ER and PR but negative for HER2 and e-cadherin*, was rendered. Patient underwent quadrantectomy and sentinel node biopsy was also performed. In case no.2 the radiological diagnosis was "*focal asymmetric density*" and a core biopsy was performed, accordingly. The diagnosis of "*proliferation of CD34-positive epithelioid stromal cells, likely benign*" (C3), was made. A lumpectomy was performed. In case no.3 (a 79-year-old man) a sub-areolar nodularity, consistent with gynecomastia, was detected by ultrasound. A core biopsy was performed with the provisional diagnosis of "*highly suspicion for invasive malignant tumor, likely sarcoma with epithelioid morphology"* (C4). A lumpectomy was performed. All patients are well with no recurrence after a follow-up period ranging from 24 to 58 months.

Results. Histological examination on surgical specimens revealed the following basic common theme: stromal proliferation of medium- to large-sized cells which displayed an epithelioid morphology. Only a minority of spindly stromal cells was seen. The cells showed well-defined borders and a relatively abundant pale to slightly eosinophilic cytoplasm with round, centrally- or eccentrically-placed nuclei containing small nucleoli. In two cases (case no.1 and case no.3) binucleated cells were focally present. The epithelioid cells were variously arranged in single cells or as cohesive cells forming small clusters. Only focally they exhibited a single-file growth pattern. Stroma was extensively collagenized, with a variable amount of thin to thick eosinophilic collagen bundles interspersed among cells or cell clusters. Cytological atypia, consisting of mild nuclear pleomorphism, was observed in two cases (case no.1 and case no.3), but mitoses and necrosis were absent. Immunohistochemically, the epithelioid stromal cells were stained with vimentin, CD34, ER and PR. Only focally, a-smooth muscle actin was detected in case no.3. Based on the clinical, radiologic, morphological and immunohistochemical features, the descriptive term "*epithelioid stromal cell hyperplasia*" was proposed. Fibro-cystic changes, extensive stromal fibrosis and florid gynecomastia were additional features associated, respectively, in cases no.1, no.2, and no.3.

Conclusions. The present paper contributes to widen the morphological spectrum of the benign mammary stromal changes. We describe a lesion labeled "*epithelioid stromal cell hyperplasia*" which is not associated with diabetic mastopathy or autoimmune disorders, emphasizing the potential diagnostic problems encountered on core biopsy. This lesion should be kept in mind by the pathologists when facing epithelioid cells, as the sole or predominant cytotype, on core biopsy to avoid a misdiagnosis of malignancy, especially invasive lobular carcinoma. Radiologic information is crucial for a correct interpretation in that a nodular mass with circumscribed borders is suggestive of a benign tumor, especially myofibroblastoma, whereas a lesion with infiltrative margins favors the diagnosis of invasive lobular carcinoma. If imaging is negative or ambiguous, pathologist should perform a wide immunohistochemical panel, including cytokeratins. A fibroblastic profile with expression of ER/PR, along with a relatively bland-looking appearance of the epithelioid cells, is strongly suggestive of epithelioid stromal cell hyperplasia.

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WHEN CORE BIOPSY CAN HINDER SOME FEATURES OF BREAST CARCINOMA: CASE REPORT

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Introduction. Breast cancer with osteoclast-like stromal giant cells is considered a rare morphological variant of no special type breast carcinoma according to WHO classification (1), while other authors consider it as a special type (2). Searching the literature, there are a few cases described, mostly as case report.

Clinically, these tumors are similar to other breast carcinomas, although their morphological characteristics, both macroscopic and microscopic, are unusual. In fact, the macroscopic feature is so unique that this rare histological type can be suspected already at eyesight. It is curiously brown or red brown, and bulges above the surrounding parenchyma when divided on fresh tissue. On formalin fixed specimens, the color tends to be darker, suggesting a melanoma metastatic.

At microscope, the main feature is the presence of giant cells sometimes in the stroma, sometimes in the glandular lumens, which however are not neoplastic (1,2). The giant cells are large, rich of cytoplasm containing centrally located numerous nuclei, some of them with nucleoli. The carcinoma cells may be arranged in tubules, glands, papillae, or may show mucinous or lobular pattern. Numerous inflammatory cells, lymphocytes,

monocytes, histiocytes occupy the stroma, along with many extra-vascular red blood cells and hemosiderin which definitively gives the color to the tumor .

Case description. A 45 years old woman, during her screening ultrasonography programmed, was found with a 8 mm solid mass in upper outer quadrant of right breast, with irregular margins, and inhomogeneous internal echoes, suspicious for carcinoma. An ultrasound-guided core needle biopsy of the lesion was performed and a diagnosis of gland-forming breast carcinoma with many erythrocytes, hemosiderin and unusual giant cells osteoclast-like was rendered. The neoplastic cells showed a middle grade of atypia. No further assays were performed.

Two weeks later, the patient underwent a quadrantectomy with sentinel lymph node as recommended by the interdisciplinary tumor board.

The node was negative when evaluated by One Step Nucleic Acid Amplification.

The breast specimen was sent for intraoperative evaluation of margins and on the cut surface, the lesion showed a red brown color congruous with a reaction to the previous core biopsy, without showing any bulging as expected. Furthermore, 24 hours later, after fixation, the lesion did not show a peculiar characteristic and color as expected. These features questioned the core biopsy diagnosis. All the lesion was then obviously sampled and embedded in paraffin for routine histological processing.

Microscopic examination showed the fibrous reaction of previous core biopsy, foci of ductal carcinoma in situ and a little (3 mm) residual carcinoma glands and tubules forming. Surprisingly there were no remaining giant cells osteoclast-like.

Immunohistochemistry for prognostic purposes in order to address the best therapeutic choices was performed on the surgical specimens. The tumor was highly responsive for estrogen receptors (>95%) while negative for progesterone receptors with a positive internal control. Ki67 was 8% and there was no over-expression of her2 (score 0 according to ASCO/CAP 2018 guidelines). CD68 was negative confirming the absence of giant cells.

At this point, the slides of previous case were retrieved for a second examination on one hand, and for performing immunostains even on the core biopsy on the other hand.

The second morphological evaluation confirmed the presence of osteoclast-like giant cells. The immunohistochemistry confirmed the high positivity of estrogen receptors and the negativity of her2. But, in contrast, showed highly rate of immunostaining for progesterone receptors (>95%) and higher Ki67 labeled index, which was 18%.

The CD68 immunostain decorated intensely all the giant cells seen at morphology confirming their monocyte lineage.

Conclusions. The distinctive feature of this case is that the diagnoses rendered on core biopsy and on surgical specimen are different and both of them are quite right.

Many times we have experienced circumstances where previous diagnostic procedures have completely removed the lesions.

This case is, to the best of our knowledge, the first case described, where the previous diagnostic procedure has removed the peculiar characteristic of the tumor, so much so that to lead to a different diagnosis of histological type.

Furthermore, there is also an important clinical impact: unusually in this case, the core biopsy, in addiction to osteoclast-like giant cells, has removed also all the neoplastic cells immunoreactive for progesterone receptors inducing oncologists to consider the case as a less hormone-responsive tumor and suggesting a potential more aggressive treatment (3).

In our case, according to ASCO/CAP guidelines recommendations (4), we performed again the immunohistochemistry for routine biomarkers of the tumor because of the negativity of progesterone, and also CD68 because of the previous diagnosis.

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BREAST CANCER LUMINAL A AND LUMINAL B WITH PAIRED METASTASES: ESTABLISHED IMMUHISTOCHEMICAL MARKERS, MICRORNA EXPRESSION PROFILE AND PROGNOSIS

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Background. About 70% of all breast cancers (BC) express estrogen receptor (ER) alpha and belong to the molecular subtypes luminal A or luminal B.[1]993 cases of invasive mammary carcinomas were assessed immunohistochemically for estrogen receptor (ER; 1D5 Intrinsic BC subtypes are properly defined using molecular technologies, however surrogate definitions are typically based on the immunohistochemical (IHC) staining for ER, progesterone receptor (PgR), human epidermal growth receptor factor 2 (HER2), and Ki-67 antigen. The IHC-based classification is more widely applicable at lesser cost.[2] The natural history of luminal A and luminal B tumor is usually less aggressive, in fact 50% of relapses occur more than 5 years after diagnosis and moreover relapses can occur even after 10 or even 20 years

of follow-up.[3] The most effective strategy to treat these hormone-sensitive tumors is the inhibition of estrogen receptor action using hormonal therapy. Nevertheless, some patients do not respond to hormonal therapy (primary or de novo resistance), and patients who do respond could eventually relapse (acquired resistance). In the past decade, the role of microRNAs in the progression of endocrine-resistant breast cancer has become of keen interest in developing biomarkers and therapies to counter metastatic disease.[4] MicroRNAs are small RNA molecules of 18 to 22 base pairs that regulate the expression of target mRNA by inhibiting the translation or degrading the transcripts. MicroRNAs deregulation was described in human BC comparing miRNA microarrays from cancerous and normal mammary tissues.[5]

Aim.s. The primary Aim. of the present study was to investigate the correlation of ER, PgR, HER2 status and Ki67 expression between luminal A or luminal B, and their paired metastases. The second Aim. was to assess the role of microRNAs as diagnostic biomarkers of BC.

Materials and methods. Patients who had histological diagnosis of metastatic breast cancer from January 2007 through December 2015 were identified by the Institutional database. Clinicopathological data were reviewed retrospectively. ER, PgR, HER2 and Ki-67 IHC expression (Ventana Benchmark automatic stainer) were evaluated on every specimen. HER2 concordance status was defined as negative (score 0/1+) positive (score 3+), and equivocal (score 2+); the equivocal group was subsequently tested for gene amplification by Silver in situ hybridization Dual-probe to fall finally into negative or positive set. Primary BC was classified in luminal A (ER+, PgR+, Ki67 ≤ 20%, HER2-), luminal B1 (ER+, PgR+, Ki67>20%, HER2-) and luminal B2 (ER+, PgR+, Ki67>20%, HER2+). Six microRNAs, namely miR-26a, miR-181b, miR-125b, miR-221, miR-200c, miR-101-3p were selected because previous studies demonstrated their involvement in mechanism of endocrine resistance. The droplet digital polymerase chain reaction technique has been used to yield an absolute quantification of nucleic acid concentrations. The microRNAs expression was analysed on the formalin-fixed paraffin embedded samples both in primary BC and metastasis and normal breast tissue.

Results. Seventy-seven patients were included. Twenty-five (32%) patients were luminal A, 39 (51) luminal B1 and 13 (17%) luminal B2. The sites of metastases were liver (43%), skin (26%), lung (13%), ovary (10%), and brain (8%). The rate of liver metastases by luminal types A, B1, and B2 were 32%, 49%, and 46%, respectively. Differentiation grade 2 was significantly associated with the occurrence of distant metastases (p = 0.005). Only one case turned out not to express ER in the liver metastasis. Ki67 expression was more than 20% in metastatic lesion compared to paired luminal A (p = 0.025). Accurate follow-up and regimens of endocrine therapy, chemotherapy, radiotherapy, and immunotherapy (either primary BC or metastatic lesions) was available only in 48 patients. Luminal A had the longest disease-free survival (88.14 ± 11.56 months) whereas shorter disease-free survival was observed in luminal B1 and luminal B2 subtypes (65.59 ± 7.96 months and 66.80 ± 12.00 months, respectively). Luminal A overall survival (214.87 ± 24.91 months) was greater than luminal B1 (152.53 ± 12.25 months) and luminal B2 (116.48 ± 14.87 months). The expression levels of all six miRNAs (miR-26a, miR-221, miR-101-3p, miR-125b, miR-181b, miR-200c) between normal breast tissue and breast cancer were significantly different. Therefore, the biology of primary tumour includes a dysregulation of miRNAs. In addition, the metastases presented different expression levels of all miRNAs compared to normal breast tissue. Of note, only miR-26a expression levels were significantly different between metastasis and primary BC.

Conclusion. BC metastases can change their genotype and/or phenotype. However, these mechanisms are not fully understood. The evaluation of microRNA profiles, the established IHC markers (ER, PgR, HER2 and Ki-67), and other pathological features of tumor specimen (histological type and grade, tumour size, lymph node status, vascular invasion) are essential to assess prognosis.

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PATOLOGIA TESTA COLLO

INTERACTION BETWEEN PDL1-MEDIATED IMMUNOCONTEXT AND PHLOGISTIC MARKER COX-2 IN TUMOR MICROENVIRONMENT OF ORAL SQUAMOUS CELL CARCINOMA: A TISSUE MICROARRAY STUDY

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Background. Oral squamous cell carcinoma (OSCC) is a devastating disease causing substantial morbidity and mortality with an incidence of approximately 540,000 new cases annually worldwide. Despite promising advancements in the conventional therapeutic approaches currently available for OSCC patients, many problems are still unsolved. In recent years, monoclonal antibodies targeting programmed cell death-ligand 1 (PD-L1) constitute a promising cancer immunotherapy (1). How-

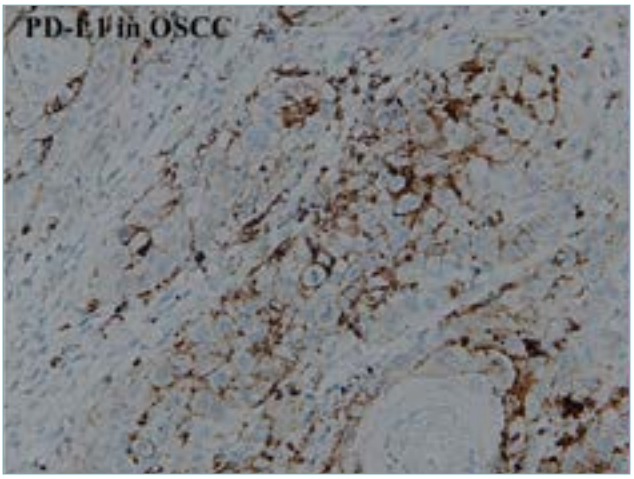

Fig. 1.

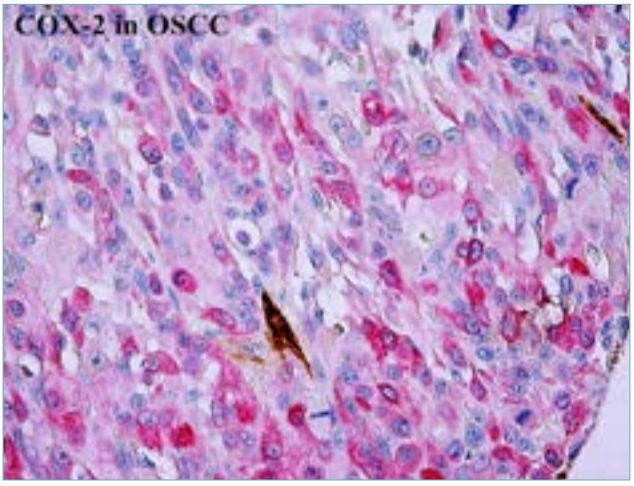

Fig. 2.

ever, the role of PD-L1 in OSCC remains controversial. Cyclooxygenase-2 (COX-2) has a main role in the promotion of cancer cell growth (2,3) and it is able to create an Immunosuppressive Microenvironment involved in the progression of different human cancers (4).

Aim. The Aim. of the present study was to investigate the expression level of PD-L1 in OSCC and examine its relationship with COX-2 expression and different clinic-pathological features.

Methods. In this study, we built a prognostic TMA with 119 OSCC samples, representative of superficial part and deep invasive front of the tumor, to investigate, COX-2 proteins expression and PDL1, correlating them with clinic-pathological parameters, outcomes and therapeutic data. Statistical tests used in this study were Chi-square test and Pearson's correlation analysis that has been performed using SPSS software.

Results. PDL1 expression in lymphocytes has been significantly related to COX-2 expressing plasma-cells (P=0,001). PDL1 expression in lymphocytes has been significantly correlated to COX-2 positive Tumor infiltrating lymphocytes (TIL) (P=0,048). PDL1 distribution is related to COX-2 intensity of expression (P=0.02).

Conclusions. Our Results revealed a co-expression of the PDL1 and COX-2, suggesting that they play complementary roles during oral carcinogenesis. This relationship has already been described in melanoma and breast cancer (5, 6), emphasizing their clinical relevance in the era of precision medicine. In near future researches on the importance of COX-2 expression related to immunotherapy should be evaluated to improve therapy response.

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PROGNOSTIC ROLE OF MTOR EXPRESSION AND MASPIN PATTERN IN LARYNGEAL SQUAMOUS CELL CARCINOMA

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Background. The long-term survival rates for patients with laryngeal squamous cell carcinoma (LSCC), especially in advanced and/or relapsed cases, have not improved in recent decades. There is an undeniable need for novel, more effective diagnostic and therapeutic strategies to improve the chances of overall survival for patients with advanced LSCC. Maspin (mammary serine protease inhibitor), a member of the serine protease inhibitor superfamily, is a tumor suppressor gene. Its subcellular localization in the nucleus of cancer cells is essential to its tumor suppressor activity. As a tumor suppressor, maspin interacts with the cellular signaling pathways of different oncogenes. The mammalian target of rapamycin (mTOR) is a serine/threonine kinase located downstream from the phosphatidylinositol 3-kinase (PI3K)-Akt pathway, which is often activated in many neoplastic diseases: an upregulated mTOR signaling can promote tumor growth and progression in cancer

cells through various mechanisms. The main Aim. of this study was to conduct a preliminary investigation into the possible relationship between mTOR and the nuclear tumor suppressor Maspin in LSCC.

Materials and methods. mTOR (monoclonal rabbit antibody, clone Y391, diluted 1:500, Novus Biologicals, Littleton, CO, USA) and maspin (monoclonal mouse antibody, clone EAW24, diluted 1:100, Leica) immunohistochemical expression, with specific attention to maspin subcellular pattern, were evaluated in 79 consecutive LSCCs. Three areas were chosen by the pathologist, irrespective of their position in the carcinoma tissue, and the subcellular maspin expression pattern was assessed in 500 carcinoma cells in each area. mTOR assessment was performed in the same areas using an image analysis (IA) workstation comprising a conventional Zeiss Axioskop light microscope (Zeiss, Jena, Germany) with a color digital, Peltier-cooled videocamera (MicroPublisher 5.0 RTV) connected to a personal computer with IMAGE-PRO PLUS software version 7 for Windows (Media Cybernetics, Bethesda, MD, USA). In all cases, 1378 x 954 µm areas of tumor tissue were examined with a 495-point sampling grid superimposed by the program on the image acquired with a x50 field of view. After counting the points that intercepted the positive and negative areas, the positive area fraction was calculated and recorded as a percentage (%).

Results. Considering the whole series, univariate statistical analysis showed that mTOR expression was significantly higher in patients whose disease recurred (p=0.009). The DFS rate was also significantly shorter in cases of LSCC with an mTOR expression ≥11.55% (p=0.049). Multivariate analysis showed that N-status (p=0.002) and mTOR expression (p=0.037) retained their prognostic significance in relation to cancer recurrence. In a subgroup of LSCCs with a non-nuclear maspin pattern, mTOR expression was significantly higher in patients whose disease recurred. Multivariate analysis disclosed that N-stage (p=0.012) retained its independent prognostic significance for disease recurrence in this setting. mTOR expression showed a trend towards independent significance in terms of carcinoma recurrence (p=0.083).

Conclusions. In conclusion, the present study, which is the first to have investigated the possible combined prognostic role of maspin pattern and mTOR expression in LSCC, supports the idea that mTOR is related to an aggressive phenotype in LSCC: a higher mTOR expression was significantly associated with a higher carcinoma recurrence rate. Interestingly, when maspin was expressed in the nucleus, mTOR was unassociated with LSCC prognosis, due possibly to an interaction of this tumor suppressor with mTOR's proliferative pathway. Further investigations are needed to establish the potential of incorporating modern mTOR inhibitors in multimodality or multitarget strategies against advanced LSCCs, also considering the role and expression of other tumor suppressor genes.

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TUMOR BUDDING AS AN INDEPENDENT MARKER OF LYMPH NODAL METASTASIS AND OUTCOME IN STAGE I-IV LARYNGEAL SQUAMOUS CELL CARCINOMA SURGICAL AND BIOPTIC SAMPLES

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Background. Laryngeal squamous cell carcinoma (LSCC) is one of the most relevant cancer of the head and neck region and it is considered as a heterogeneous disease in term of prognosis and outcome. To date, tumor budding (TB) is one of the novel factors that seems to be able to stratify LSCC progression risk, but the assessment method has not yet been standardized. The Aim. of this study was to assess TB in LSCC surgical and paired bioptic specimens and to evaluate its possible association with prognostic features and outcome.

Methods. 72 formalin-fixed and paraffin embedded surgical specimens of LSCC and the related previous biopsies have been evaluated for TB according to the international recommendations developed for colorectal cancer (ITBCC) 2016: tumor budding was scored by 2 pathologists on hematoxylin and eosin-stained slides and scored as BD1 (low grade), BD2 (intermediate grade), and BD3 (high grade); cytokeratins were used only in questionable cases. Results were correlated with clinic-pathological findings and outcome.

Results. In surgically resected LSCC, TB was significantly associated with pT (p = 0.01), the pattern of invasion (p = 0.003) and with the presence of lymph-node metastasis (p = 0.01); at the multivariate analysis, TB resulted the only factor significantly associated with lymph-node status. Moreover, TB in paired biopsy samples resulted significantly associated with TB in surgical specimens (p = 0.0001) and with lymph-node metastasis (p = 0.03). Considering the TB impact on the patients' outcome, Kaplan-Meier curves showed that patients with high grade TB more frequently had recurrent disease at the end of the follow-up (p = 0.04).

Conclusion. TB in LSCC, evaluated according to the ITBCC 2016 score-system, is associated with the presence of lymph node metastasis and a recurrent disease at the end of follow-up. For the first time, we correlated the TB in surgical and biopsy samples and we found a significantly correlation with the two data. So, TB could

become an important tool, also at the presurgical level, to prevent LSCC poor outcome and to find a targeted surgery and follow-up for the patients.

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IMAGING ACCURACY IN PREOPERATIVE STAGING OF T2-T3 GLOTTIC CARCINOMA

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Objectives. Surgery is the best therapeutic options for patients with laryngeal squamous cell carcinoma. In locally advanced stages (T3) the choice between conservative (open partial horizontal laryngectomy OPHL) and radical laryngectomy depends on clinical staging, which is mostly based on the information provided by Computed Tomography (CT) and on endoscopic evaluation. Glottic T3 classification is a very broad category and may include lesions with a considerably different prognosis depending on their location, specifically the anterior commissure and the posterior paraglottic space (Fig. 1). These sites are susceptible to tumor invasion and represent favorite "escape routes" for extralaryngeal tumor extension. Recent studies have proposed to consider the anatomical involvement of these structures, in particular the posterior paraglottic space, for a more accurate staging of laryngeal cancer. Despite its universal application, it is not clear to what extent CT scan can accurately recognize tumor invasion at crucial sites, which are relevant for tumor staging and prognostic correlation. To shed light on this aspect, we undertake a retrospective analysis of a series of patients who underwent OPHL for cT3-T4 glottic carcinoma, comparing imaging data with the Results of histopathological examination of the specimen obtained at surgical resection, which we considered as the gold standard for the diagnosis of tumor extension.

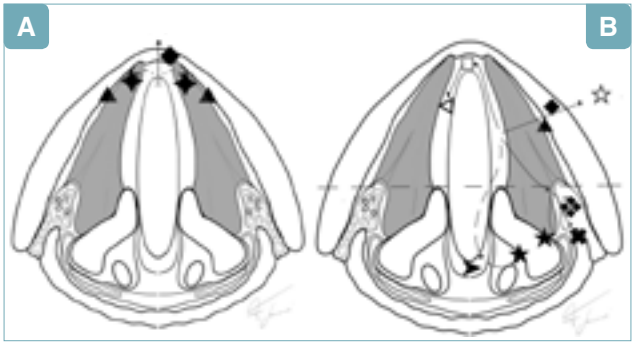

Fig. 1. Fig. 1. A, left): anterior commissure involvement and pattern of spread (◆ = thyroid cartilage, ✦ = vocal cords, ▲ = anterior paraglottic space); B, right): anterior glottis and posterior paraglottic space involvement: anatomical barriers and different pattern of spread (◆ = thyroid cartilage, ▲ = anterior paraglottic space, ☆ = extra-laryngeal spread, ❖ = posterior paraglottic space, ★ = cricoarytenoid joint, ✖ = piriform sinus, ➤ = posterior commissure).

Methods: We selected for this retrospective study study all patients submitted to OPHL in our Center between 2005 and 2018 with available archival CT imaging and histopathological reports and samples. All CT imaging exams were re-evaluated with the help of an experienced H&N radiologist, providing the cT staging and an estimation of the disease extension at critical sites as represented in Figure 1. Histopathological reports and specimens were similarly re-evaluated.
In both cases we registered the extension of tumor invasion at the anterior commissure, the vocal cords and the posterior paraglottic space, distinguishing the following levels: for the anterior commissure, invasion limited to the mucosal layer (a), full thickness extension reaching the inner cortex of the cartilage (b), extra-laryngeal spread in the pre-epiglottic space (c); for the glottis: invasion of the mucosal layer or thyroarytenoid muscle (a), invasion of the anterior paraglottic space up to the cartilage (b), extra-laryngeal spread (c); for the posterior paraglottic space: invasion limited to the mucosal layer (a), involvement of the crico-arytenoid unit or full thickness extension reaching the inner cortex of the cartilage (b), extra-laryngeal spread (c). Finally, the diagnostic accuracy of CT scan assessment of tumor extension in the three considered areas was determined respective to the histopathological evaluation considered as the gold standard.
Results. The study included 37 patients, 30 males (81,1%) and 7 females (18,9%), with a median age of 62 years (37-76). The rates of concordance in the assessment of tumor extension at each level between imaging and histopathology and overall accuracy of CT versus the gold standard are summarized in Table I.
Conclusions. The performance of CT in the staging of laryngeal tumor invasion at critical anatomic sites was suboptimal. In particular, sensitivity was low for full thickness invasion at all sites, and for extralaryngeal extension in the anterior glottis. On the other hand, high rates of false positives are probably related to the presence of perilesional edema, which can not be reliably distinguished from neoplastic tissue. While new imaging methods, in particular magnetic resonance, are proving more accurate in the definition of tumor extensions, their widespread application in patient diagnostic evaluation is delayed by higher costs and limited expertise. For this reason, intraoperative evaluation of surgical margins and anatomical extension is recommended in patients undergoing OPHL to avoid unexpected pT upstaging and the consequent necessity of salvage surgery or adjuvant radiotherapy.

Tab. I.

	True Pos	True Neg	False Pos	False Neg	Sensitivity	Specificity	Accuracy
Anterior commissure							
Tumor presence	17	11	2	7	70,83%	84.62%	75.68%
Superficial extension	8	11	1	6	57.14%	91.67%	73.08%
Full thickness invasion	1	26	3	1	50.00%	89.66%	87.10%
Extralaryngeal extension	3	31	1	2	60.00%	96.88%	91.89%
Anterior glottis							
Tumor presence	35	1	0	1	97.22%	100%	97.30%
Full thickness invasion	14	3	13	4	77.78%	18.75 %	50%
Extralaryngeal extension	2	34	0	1	66.67%	100%	97.30%
Posterior paraglottic space							
Tumor presence	9	18	5	5	64.29%	78.26 %	72.97%
Superficial extension	0	17	0	3	0	100%	85.71 %
Full thickness invasion	3	21	4	2	60%	84%	80%
Extralaryngeal extension	2	30	5	0	100%	85.71 %	86.49%

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UNUSUAL SOFT TISSUE TUMORS OF THE HEAD & NECK

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Objectives. Soft tissue tumors of the head and neck region in both children and adults are relatively rare. They encompass numerous entities with different biological behavior, ranging from benign to highly aggressive tumors (1-3). Due to the rarity of these lesions in this anatomic site, serious differential diagnostic problems may arise. The following diagnostically challenging cases will be presented: i) spindle cell lipoma with unusual morphology of the tongue; ii) solitary fibrous tumor of the oral cavity; iii) low-grade fibromyxoid sarcoma of the parapharyngeal space; iv) cystic multilocular ancient schwannoma of the soft tissues of submandibular gland; v) desmoid-type fibromatosis of the nasal cavity; vi) epithelioid haemangioma of the nasal cavity. Differential diagnosis will be discussed with emphasis on the main diagnostic clues.

Materials and methods. A series of 55 cases soft tissue tumors of the head and neck was retrieved from the files of the pathology archives of Anatomic Pathology of University of Catania. Only six cases were unusual enough, in terms of localization and morphology, to deserve a separate presentation. Patients had an age raging from 4 to 63 years. Surgical specimens were submitted for histological examination and immunohistochemical studies were performed using a wide panel of antibodies.

Results. Case n.1.

A 4-year-old boy presented a 1.4 cm mass located in the gingivo-buccal sulcus of the lower dental arch. Histological examination revealed, apart from a predominant small round cell component admixed with a minority of short spindle cells, interspersed islands of mature adipose tissue, as well as foci of myxoid stroma with ropey-like collagen fibers. The cells were stained exclusively with vimentin and CD34. This tumor was labeled as "*spindle cell lipoma of the tongue*".

Case n.2.

A 54-year-old woman presented a swelling of her left cheek. MR imaging showed a solid mass measuring 6 cm in greatest diameter, occupying the left cheek with extension to the pterigo-palatine fossa. Tumor was radically excised and histological examination revealed the typical morphological features of solitary fibrous tumor as seen in the pleura or soft tissues. Immunohistochemical expression of CD34 and STAT-6 confirmed the diagnosis of "*solitary fibrous tumor of the oral cavity"*.

Case n.3

A 57-year-old woman presented with painless, slowly-expanding mass in the right side of the neck. CT showed a large, well-circumscribed, isodense mass that measured approximately 8 cm X 3 cm X 4 cm, involving and entirely filled the right parapharyngeal space. Histological examination showed fibrous areas alternating with myxoid areas, composed of bland-looking spindle cells arranged into short intersecting fascicles or whorls. Numerous capillary-like blood vessels with branching or curvilinear configuration were seen. A striking feature was the presence of variably-sized pseudorosettes composed of hyalinized collagen surrounded by a palisade of neoplastic cells. Immunohistochemistry revealed diffuse expression of vimentin and MUC4, and focally of EMA. The diagnosis of "*low-grade fibro-myxoid tumor of the parapharyngeal space*" was rendered.

Case n.4.

A 70-year-old woman presented an indolent swelling in the right submandibular region. MR confirmed a well-defined nodular lesion of the right submandibular gland with a solid and cystic component. Histologically examination revealed, in the soft tissues of sub-mandibular gland, a multilocular cystic mass with the typical features of "*ancient schwannoma*". The cystic degeneration was so prominent and extensive that the tumor appeared radiologically as a solid-cystic mass. Positivity for S-100 protein confirmed the diagnosis of "*cystic multilocular ancient schwannoma*".

Case n.5
A 62-year old-women showed a mass in nasal cavity. MR confirmed a solid mass with focal destruction of the underlying bones. Histological examination revealed a proliferation of palely eosinophilic fibroblasts and myofibroblasts arranged parallel in sweeping and long fascicles with infiltrative margins. Immunohistochemically, spindle cells were positive for alpha-smooth muscle actin and β-catenin. A diagnosis of "*desmoid-type* fibromatosis of the nasal cavity" was rendered.
Case n.6
A 49-year-old man presented with a nasal mass. The lesion was surgically removed and histological examination showed a well circumscribed vascular proliferation composed of numerous small to medium-sized, thin-walled blood vessels lined by plump, epithelioid endothelial cells with abundant eosinophilic cytoplasm and vesicular nuclei. Endothelial cells protruded into the vascular lumina in a "hobnail" or "tombstone" pattern. No mitoses nor pleomorphism was found. Immunohistochemical staining for CD31 and ERG confirmed the vascular nature of the lesion and a diagnosis of "*epithelioid haemangioma of the nasal cavity*" was rendered.
Conclusion. The spectrum of the soft tissue tumors of the head and neck region is wide and the differential diagnosis may be very challenging, especially if the pathologist is not familiar with such tumors. We think that it is the unusual/unexpected site that makes difficult the diagnosis. The application of strict morphological criteria, along with the interpretation of the immunohistochemical Results, is crucial for a correct diagnosis.

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TWO UNUSUAL CASES OF ONCOCYTIC LESIONS OF THE PAROTID GLAND

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Background. Oncocytes are epithelial cells presenting an aberrant accumulation of mitochondria that determinates the typical eosinophilic granular cytoplasm[1]. They may occur in many different sites, such as salivary glands, thyroid, kidney, esophagus, hypophysis.
Oncocytic changes represent a confounding morphological finding in differential diagnosis of several pathological entities [2].
Cases presentation. We report two peculiar cases of parotid gland neoplasms with oncocytic features.
The first one occurred in a 73-year-old male, presenting in October 2018 with a persistent unilateral, painless hard-elastic swelling at the left angle of the mandible.
FNC resulted suspicious for malignancy (category V sec. Milan system). A superficial parotidectomy was performed.
At histopathological examination, the lesion was composed of a peripheral cystic area and a central, multinodular solid component, representing respectively 10% and 90% of the entire lesion, clearly separated from each other. The cystic area was composed of bilayered oncocytic cells arranged on a lymphoid stroma with prominent germinal centers, configuring a Warthin tumor. The solid component was characterized by a multinodular proliferation of oncocytes morphologically compatible with oncocytoma. In both the components, oncocytic cells were positive for keratins; p63 was expressed exclusively by cells with a basal derivation, located in the cystic part of the tumor. The Ki 67/MIB-1 L.I. was </= 5% (in oncocytic cells).
A diagnosis of "hybrid tumor", Warthin Tumor (WT) with oncocytoma, was performed.
The second case was from a 50-year-old male, with multiple nodules within the left parotid gland, detected by clinical examination in June 2019. Histopathological analysis revealed multiple, unencapsulated, solid nodules of closely packed oncocytes, without mitotic figures, and a larger, central, oncocytic nodule, clearly surrounded by a thick capsule. These morphological aspects were interpreted as Multifocal Nodular Oncocytic Hyperplasia (MNOH) with a central oncocytoma.
Discussion. Oncocytic modifications are frequent in salivary glands lesions and represent an important problem for the differential diagnosis. It is thought they represent a metaplastic reaction to adverse events and senescence, probably associated with specific molecular alterations[4]. It has been hypothesized that senescence causes a functional reduction of mitochondrial enzymes with a consequent increase of cytoplasmatic mitochondria. Oncocytic changes, as the result of the salivary tissue ageing, may occur either in benign or in malignant contests[1].
According to the fourth edition of WHO Classification of Head and Neck Tumors, benign conditions with oncocytic differentiation are nodular oncocytic hyperplasia, oncocytoma and WT, as opposed to oncocytic carcinoma characterized by evident histological atypia, absent capsule, nuclear pleomorphism, mitotic figures with atypical mitoses, and infiltration of surrounding parenchyma[5].
Several salivary gland tumors, such as pleomorphic adenoma, myoepithelioma, duct carcinoma, acinic cell carcinoma, Mammary Analogue Secretory Carcinoma (MASC) and mucoepidermoid carcinoma, may present areas of oncocytic metaplasia, that make the differential diagnosis frequently challenging[6].
Conclusions. To the best of our knowledge, few cases of hybrid tumors characterized by WT with Oncocytoma are reported in the literature[7], and the coexistence of Oncocytoma and MNOH is an extremely rare eventuality. These two cases support the hypothesis that the contemporary presence of multiple oncocytic patterns may occur as the result of a transition between different oncocytic entities.
The oncocytic neoplasms' development is likely based on a "progression model", starting with oncocytic metaplasia, that needs a particularly accurate investigation[8].

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PD-L1 (CLONE E1L3N) EXPRESSION IN LOCALIZED AND ADVANCED SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

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Background and aim. Currently, FDA-approved immunotherapies for head and neck squamous cancer (HNSCC) patients include pembrolizumab with platinum and fluorouracil for all patients and as a single agent for patients whose tumors express PD-L1 CPS (combined prositive score) ≥1. Nivolumab and pembrolizumab are also approved for treatment in the platinum-failure setting for refractory or metastatic HNSCC patients.
Increased understanding of histology-specific considerations, potential biomarkers, and further characterization of HPV- and EBV related cancers will also greatly assist in future therapeutic development, administration and management.
In our study we Aim.ed to address the presence of PD-L1 immunoexpression in morphological different settings of squamous cell carcinoma of the head and neck
Methods. Squamous cell carcinomas of the head and neck (HNSCC) with available formalin-fixed, paraffin-embedded tissue blocks were recruited from the files of the University of Verona Hospital Trust, from 2007 to 2013. PD-L1immunohistochemical expression has been assessed using the platform-independent test (E1L3N Cell Signaling Technology, Danvers, MA).
CPS was assessed. Positivity cut-offs of ≥1, 5, 25 and 50% were stratified on positive cases. p16 immunostaining and HPV-test was performed.
Results. 51 squamous cell carcinomas were studied. 23 cases (45%) staged without advanced or metastatic profile, the remaining were locally advanced/or metastatic to lymph-nodes. Positive (≥1, 5, 25 and 50%) PD-L1 expression was respectively observed in 34%, 19%, 15% and 9% of cases.
Only 11 patients (12%) were positive for high-risk human-papillomavirus (HPV) at RNA in situ hybridization (ISH). Immunohistochemical expression of p16 was observed in 13 (25%) carcinomas. There was no correlation between p16, HPV-ISH positivity ($p = 0.79$) and PD-L1 expression.
Conclusion. The presence of PD-L1 expression is significant in different cohorts of squamous cell carcinoma of the head and neck region. No correlation between p16 expression, HPV-ISH positivity and PD-L1 expression has been observed. Though, harmonization with other PD-L1 clones is required.

VENERDÌ 18 OTTOBRE 2019

Miscellanea 4
Sala Atene – 08:30 - 13:00

PATOLOGIA FETOPLACENTARE

NEW PARAMETERS TO EXTIMATE THE TIME OF DEATH IN STILLBORN

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Aim. Stillbirth, defined as delivery of a fetus who has died in utero after 20 weeks' gestation[1]. Fetal death is an important obstetric problem, accounting for approximately half of perinatal death[2]. For many stillbirths, the etiology and time of fetal death are unknown. Fetal deaths result from diverse causes: maternal diseases, such as diabetes and preeclampsia; placental disorders, such as placental underperfusion and abruption placentae; fetal congenital anomalies; complications of multiple gestation[1]. Our goal was to extimate the time of death in stillborn considering new parameters compared to those taken in the studies present in the litterature. In the literature there are few studies that deal with the topic and the parameters considered are: the extent of external maceration which comprises skin colors, cord color, eylid color, cranium, desquamation, mummification[2]; characteristic histologic alterations (autolysis) in hematoxilin and eosin-stained tissue, these changes consist of gradual loss of nuclear basophilic staining, progressive cytoplasmic eosinophilia, and eventual loss of most cellular detail[1]; placental histologic examination[3].
Materials and methods. Our is a retrospective study performed analyzing the autopsy examination of 150 dead fetuses of woman who were treated at the Gynecological clinic of the Federico II Polyclinic of Naples over the period 2013-2019. One hundred stillborns were identified for which the timing of fetal death was accurately determined by clinical studies and for autopsy color photographs of good quality were available for review. For each case, we have considered: birth-to-autopsy interval, gestational age at the time of death, clinical pathologic evidence of acute or chronic fetal stress, presence of fetal hydrops, presence of amniotic or fetal infection. We have considered the extent of external maceration in a stillborn fetus (color, desquamation, mummification), characteristic histologic alterations, placental histologic examination and length of the femur establisched radiographycally.
Conclusions. Based on our study and the data in literature we can conclude that external esamination, histologic changes identifiable in hematoxylin and eosin-stained fetal tissue and placental histologic examination seem to be useful for determining the approximate time of death in many stillborn fetuses. However, these parameters can be modified by variables such as a fetus closed in the amniotic sac, infections, etc. Therefore, the evaluation criteria of tissue maturation (despite the maceration) and the evaluation of the skeletal segments (radiographic length of the femur or presence of ossification nuclei) are a more reliable parameters, as the maceration of the bone tissue happens in longer time and they serve to evaluate both growth and of maturation.

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THE COMPUTERIZED MICRO TOMOGRAPHY: A NEW DIAGNOSTIC TOOL FOR FETAL CARDIAC PATHOLOGIES

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Background. The diagnostic process in cases of stillbirth, sudden infant death syndrome (SIDS), and sudden unexpected death in infant (SUID) is a challenge. In such cases, the heart should always be carefully analyzed: the exclusion or confirmation of diseases affecting this organ is, in fact, a fundamental step in a correct diagnostic process (1,2). The need to develop new diagnostic methods appears increasingly evident, if we hope to achieve a more accurate identification of cardiac pathological processes. Recently the use of the computerized tomography (micro-CT) for the detailed analysis of fetal hearts has been proposed in the literature (3).

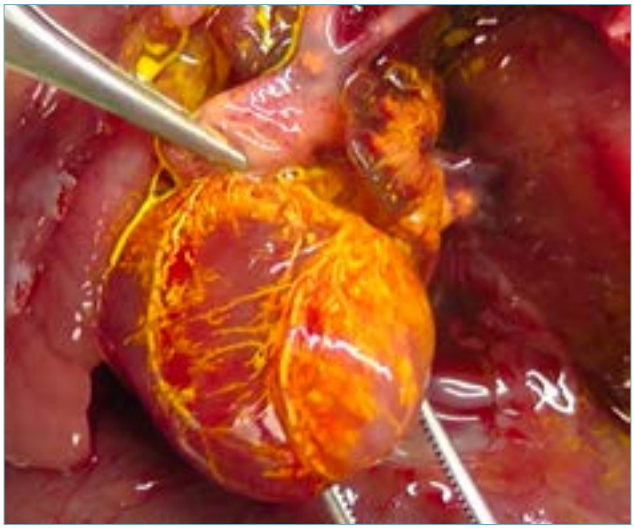

Fig. 1. Coronary perfusion after clamping aorta.

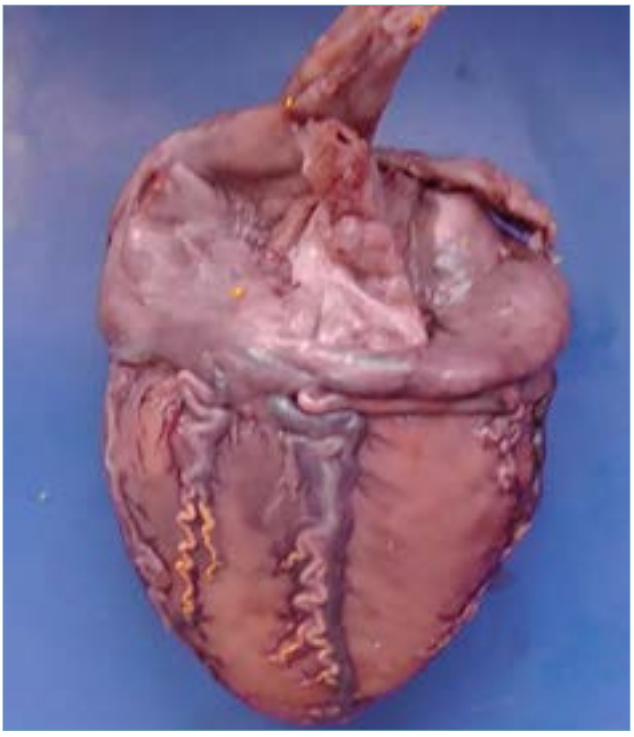

Fig. 2. Anomalous coronary path.

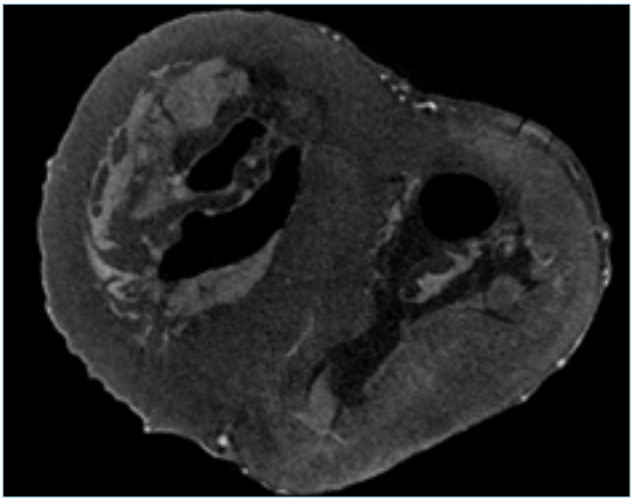

Fig. 3. Ventricle cavities of a 10 week fetal heart (2D).

Objectives. The study Aim.s to develop a reliable and reproducible method for the study of fetal hearts by micro-CT

Methods: 12 fetal hearts were selected. 5 out of 12 were treated with 5% formalin and subsequently with lugol. 3 were treated by injection of a polymerizing radiopaque substance (Microfil) into the coronary circulation via the aortic route after 5% formalin fixation. 4 fresh specimens were treated with EDTA injection and polymerizing radiopaque substance (Microfil); subsequently they were fixed in formalin. Following these operations, all the hearts were scanned by micro-TC. The data obtained were analyzed using the CTvox® and DataViewer® softwere for reconstruction in two and three sample sizes.

Results. The use of lugol as a radiopaque medium allowed an impregnation of heart chambers and large vessels. The use of Microfil on hearts already fixed determined the partial possibility of highlighting the coronary tree of the hearts examined. The use of EDTA and pre-fixation polymerising substance allowed the possibility of highlighting the cardiac vascular tree.

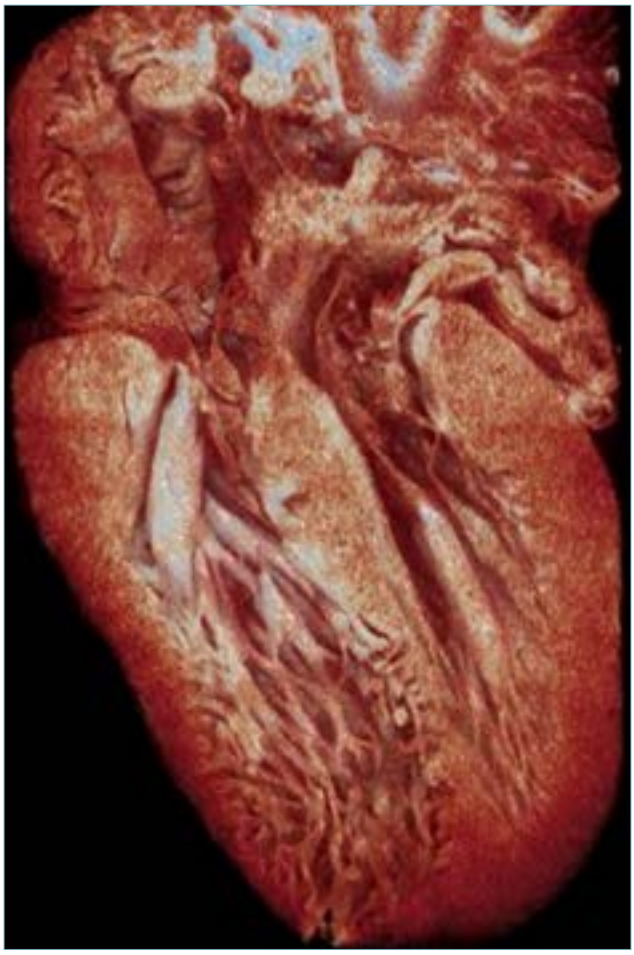

Fig. 4. 3D reconstruction of a 17 week fetal heart.

Conclusions. The study shows that the use of micro-CT in the examination of fetal hearts is certainly possible. In particular, the use of lugol allows a clear highlighting of the cardiac chambers and the vascular peduncle; the use of EDTA and polymerizing substance allowed an accurate analysis of the cardiac coronary tree. Ultimately it is possible to conclude that micro-CT is a reliable and reproducible tool in the study of fetal hearts. In addition, the computerized analysis of the data makes it possible to hypothesize that this method will also permit the printing of 3D models of the hearts being studied, usable for diagnostic and didactic purposes.

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PATOLOGIA PEDIATRICA

HISTOLOGICAL FEATURES OF THE LUNG IN FATAL CASES OF PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN: A FIVE-YEAR RETROSPECTIVE ANALYSIS IN TWO MAJOR CENTERS IN THE NORTHWEST OF ITALY.

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Persistent Pulmonary Hypertension of the Newborn (PPHN) occurs in about 1.8-2 per 1000 of both term and preterm infants, often complicating their clinical course and requiring their immediate transfer to a neonatal intensive care unit (1-2). Moreover, it is associated with an increased risk of an adverse outcome.

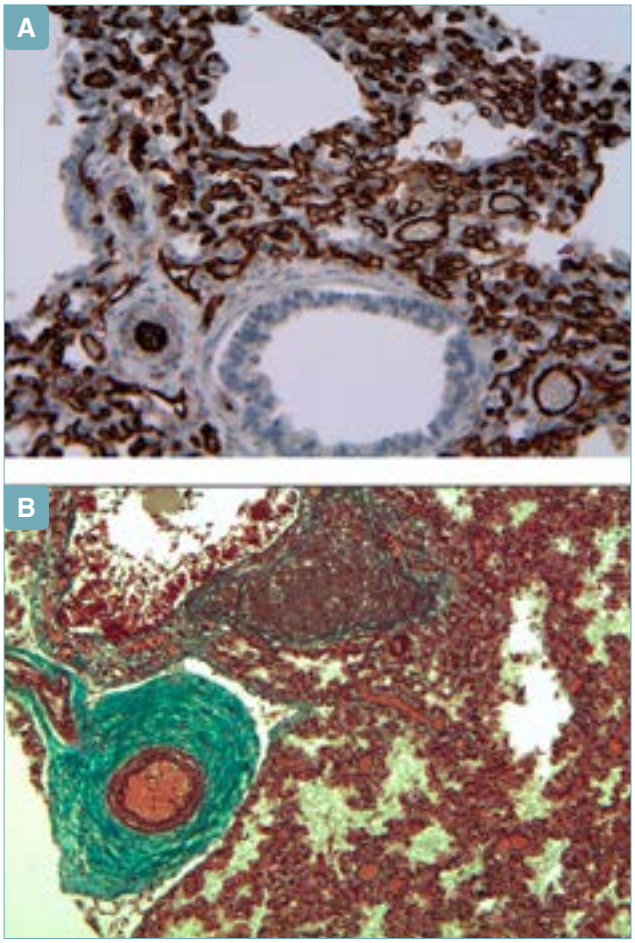

Fig. 1. A) Case SA242/2015. PPHN associated to a transposition of great vessel and occlusive ductus arterious. Newborn died few hours after birth. Both lungs show diffuse thickening of alveolar septa associated to abnormal and chaotic capillaries proliferations. ICC CD31, 250X; B) Case Sa184/2016. Congenital diaphragmatic ernia. Reduction of alveolar spaces associated to atelectasia. The walls of small peripheral pulmonary arteries are thickened due to hypertrophic smooth muscle cells in the media. The vessel wall is thickened, and the lumen narrowed because of increased wall thickness, due to thickening of adventitia. Tricromic 250X.

Definition. PPHN is defined as a failure to achieve or sustain the physiological decrease in pulmonary vascular resistance at birth associated with the persistence of the typical intrauterine right-to-left shunt. PPHN may eventually lead to life-threatening circulation failure; it is one of the main causes of unexpected death of neonates, with a mortality rate of about 10-20% of the affected patients (5).

Clinical aspects. Clinically, PPHN is characterized by tachypnoea, severe cyanosis, acidosis, as well as rapidly increasing hypoxaemia.

Nevertheless, PPHN that occurs suddenly and shortly after birth is usually fatal, despite evidence-based treatments. PPHN remains a challenge in therapeutic management, because the complex etiopathogenesis is yet to be fully explained. Moreover, prenatal assessment of PPHN is very difficult and not always possible.

The Aim of this study was to identify specific histological markers characterizing pathologies responsible for severe and fatal cases of PPHN and to appreciate the suitability and the Results of therapies adopted by clinicians, potentially helping to clarify cases of medical malpractice litigation.

Materials and methods. The present study comprised a five-year retrospective analysis of data from neonatal autopsies collected in two hospitals in the northwest of Italy, “Città della Salute e della Scienza di Torino, Presidio Ospedaliero OIRM Sant Anna” (Torino) and “Ospedale Pediatrico G. Gaslini” (Genova).The research showed that from January 2014 to December 2018 the hospitals in Torino and in Genova performed respectively 1312 and 456 autopsies, of which 86 and 111 were on neonates. After reviewing the medical history of all 197 cases, 39 autopsies involving neonates affected by PPHN (18 in Torino and 21 in Genova) were selected.

Paraffin-embedded tissue sections from the lungs of all 39 selected cases were cut in 4-µm slices and stained with different histological and immunohistochemical stains. Specific histological parameters (degree of lung development, morphological features of bronchioles, alveolar veins and capillaries, interstitial septa structure, presence of thickening in arteriolar layers, hyaline membranes, intralveolar histocytes, interstitial or endoalveolar inflammation, and therapy-induced pulmonary alteration) were reevaluated with light microscopy.

Result. Although PPHN is a distinctive and unique clinical manifestation, our research showed at least 5 different histological patterns.

1) A group of extremely preterm or preterm neonates (27/39 cases), where PPHN is determined histologically by compromised intrauterine development and maturation of alveolar and vascular pulmonary structures. Recent publications suggested this altered growth in the lung may be related to a maternal-placental malperfusion.

2) PPHN associated with impaired lung development due to an extrinsic compression (diaphragmatic hernia, 6/39 cases). In this group the main histological feature is characterized by pulmonary hypoplasia and vascular constrictive lesions involving pre- and intra-acinar pulmonary arteries. The vessel wall is thickened, and the lumen narrowed because of increased medial thickness, due to both hypertrophy and hyperplasia of smooth muscle cells, or due to thickening of intima and adventitia, even though the exact mechanism of this vascular

abnormality is not well known.
3) PPHN associated with pulmonary overflow during fetal development, usually determined by a specific cardiac malformation (6/39). In this group the main histological feature is pulmonary microvasculopathy characterized by focal/diffuse thickening of alveolar septa associated with abnormal and chaotic capillary proliferations. Usually this alteration is abruptly fatal and unresponsive to any attempted therapeutic assistance.
4) PPHN associated with altered and congested pulmonary microcirculation caused by platelet micro-aggregates (in case of sepsis) or vascular abnormal contraction (in case of meconium inhalation) (4 case out of 39).
5) PPHN associated with genetic mutations (4 out of 39 cases). With our current state of knowledge, we are able to identify only a few rare forms of genetic syndromes. In our research, we report 3 cases of alveolar capillary dysplasia with misalignment of the pulmonary veins (ACD/MPV). ACD/MPV is a rare and lethal disorder principally affecting the vascular development of the lungs. Genetic studies have identified associations with genomic alterations in the locus of the transcription factor FOXF1. This condition is rare, but its identification is very important due to the risk of recurrence in future pregnancies.
In some cases, an overlap of the main distinctive features aforementioned has been observed.
Conclusion. Our study showed that PPHN represents 20% of the causes of neonatal death. It also identifies a very heterogeneous histopathogenesis, associated with multiple structural or functional lung malformations.
Our proposal for a new form of classification offers to paediatricians and pathologists insight into the importance of an in-depth knowledge of histological criteria in PPHN, which is often responsible for sudden death of neonates during after birth hospitalization.

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NEUROPATOLOGIA

ULTRA-MUTATED *IDH* WILD-TYPE GLIOBLASTOMAS IN PATIENTS YOUNGER THAN 55 YEARS SHOW DEFECTIVE MISMATCH REPAIR, MICROSATELLITE INSTABILITY AND GIANT CELL ENRICHMENT

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Objectives. Glioblastoma (GBM) is the most frequent malignant primary tumor of the central nervous system. It carries dismal prognosis, with most GBM patients dying within 18 months since diagnosis in spite of treatment.
According to the World Health Organization Classification, GBM is subdivided into IDH-mutant and IDH wild-type (wt), on the basis of the mutational status of IDH1 and IDH2 genes. IDH-wt GBM carries worse prognosis than IDH-mutant one. It is considered primary, i.e. arisen de novo, and mainly affects older subjects (>55 years, median: 62 years). From a molecular standpoint, IDH-wt GBM is defined by the absence of IDH mutations and it is commonly characterized by EGFR amplification, PTEN mutations/deletions and CDKN2A deletions. Since IDH-wt GBM has the lowest incidence between 18 and 55 years, its molecular asset has not been specifically investigated in this age subgroup. Therefore, this study Aim.ed to analyze the mutational spectrum of IDH-wt GBMs from adult patients younger than 55 years.
Materials and methods. Sixteen (14.8%) IDH-wt GBMs were found among 108 GBMs from patients aged 18-50 years diagnosed at Messina Polyclinic, Italy, and were explored for mutations, copy number variations, tumor mutational load (TML) and mutational spectrum by a 409 genes panel.
Results. Eight cases (50%) had TML > 9 mutations/Megabase (Mb) and were considered to be hypermutated. Among those, two cases (12.5%) had TML > 100 mutations/Mb and were classified as ultra-mutated. One ultra-mutated GBM had MSI and two somatic mutations in MSH2. The other ultra-mutated GBM had microsatellite instability (MSI), a somatic MSH6 mutation and a germline POLE mutation, which is classified in ClinVar database as of uncertain significance and annotated by PolyPhen software as probably damaging. The mutational spectrum of this ultra-mutated case showed predominance of C>T over T>C transitions, and also a significant proportion of C>A transversions (Fig. 1) with four peaks in the trinucleotide contexts CCT, TCT, GCT, and ACT. This mutational pattern corresponds to signature SBS14 in COSMIC database, which has been associated with the concurrent impairment of POLE and MMR functions.
Both ultra-mutated GBMs featured at least 25% giant cells.
The overall survival of 8 patients with hypermutated GBMs was significantly longer than that of patients with non-hypermutated GBMs (P=0.04).
Conclusions. Although IDH-wt GBMs are commonly characterized by worse prognosis, we identified a hypermutated subgroup in adults <55 years that had improved prognosis.
Two cases were ultramutated and they were characterized by the presence of at least 25% giant cells, MMR mutations and MSI. Ultra-mutated GBMs may represent a subgroup of giant cell GBM. Since anecdotal reports showed that pediatric gliomas with high TML respond to immune checkpoint inhibitors, ultra-mutated IDH-wt GBM might be potential candidates to immunotherapy.

DEREGULATED EXPRESSION OF THE IMPRINTED *DLK1-DIO3* REGION IN GLIOBLASTOMA STEM-LIKE CELLS: TUMOR SUPPRESSOR ROLE OF LNCRNA *MEG3*

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Background. Glioblastoma (GBM) stem-like cells (GSCs) are thought to be responsible for the maintenance and aggressiveness of GBM, the most common primary brain tumor in adults. This study Aim.s at elucidating the involvement of deregulations within the imprinted *DLK-DIO3* region on chromosome 14q32 in GBM pathogenesis.
Methods. RT-PCR analyses were performed on GSCs and GBM tissues. Methylation analyses, gene expression and Reverse-Phase protein Array profiles were used to investigate the tumor suppressor function of *MEG3*.
Results. Loss of expression of genes and non-coding RNA within the *DLK1-DIO3* region was observed in GSCs and GBM tissues compared to normal brain. This down-regulation is mainly mediated by epigenetic silencing. Kaplan-Meier analysis indicated that lower expression of *MEG3* and *MEG8* lncRNAs significantly correlated with short survival in GBM patients. *MEG3* restoration impairs tumorigenic abilities of GSCs *in vitro* by inhibiting cell growth, migration and colony formation and decreases *in vivo* tumor growth reducing infiltrative growth. These effects were associated with modulation of genes involved in cell adhesion and EMT.
Conclusions. In GBM, *MEG3* acts as a tumor-suppressor mainly regulating cell adhesion, EMT and cell proliferation, thus providing a potential candidate for novel GBM therapies.

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1P/19Q FISH ANALYSIS FOR THE DIFFERENTIAL DIAGNOSIS OF PITUITARY POSTERIOR TUMORS: PRELIMINARY FINDINGS

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Objectives. Pituitary posterior tumors are rare which according to the fourth edition of The World Health Organization Classification of endocrine tumors, include a distinct group of low-grade neoplasms of the sellar region and are characterized by the immunohistochemical expression of thyroid transcription factor-1 (TTF-1). The entities are spindle cell oncocytoma (SCO), pituicytoma, granular cell tumor of the sellare region and sellar ependymoma and all these tumours are classified as grade I, because initial reports showed their indolent clinical behavior. However, several recurrent SCOs were reported after its initial description, suggesting that this tumor may have a potential for recurrence.
SCO and pituicytoma have close morphological resemblance and although differential diagnosis of these two entities is essential in view of different recurrent potential, it may be challenging at times.
SCO is histologically characterized by the presence of spindle oncocytic neoplastic cells and it is usually immuno-positive for EMA and negative for GFAP. Pituicytoma also shows spindle cells and is generally negative for EMA and positive for GFAP; conversely, pituicytomas negative for GFAP and SCO negative for EMA have been also reported. The genetic alterations of pituicytomas and SCO have been only barely investigated.
In a preliminary study exploring the copy number variation in two SCOs, we found that both cases showed focal deletions of chromosomes 1 and 19.
In this study we Aim.ed to analyze 1p/19q codeletion, assessed by Fluorescent in Situ Hybridization (FISH), to point the chromosomal alterations as potential molecular hallmark of SCO and serve in the differential diagnosis toward pituicytoma.
Materials and methods. Ten cases have been tested including 6 SCOs, 2 pituicytomas and 2 control tumours were considered.
All cases has been formalin fixed and paraffin embedded and submitted to immunohistochemistry by using TTF-1, chromogranin, synaptophysin, cytokeratins, EMA and GFAP. In two cases, ultra-structural findings were also performed.
We performed FISH analysis to assess 1p/19q codeletion on all cases. Serial section of each case were processed with commercially available (LSI 1p36/19q13 Dual-Color Probe Sets (Vysis/Abbott, Molecular Europe, Wiesbaden, Germany) assays, following manufacturer-recommended protocols. Slides were examined by Olympus BX61 fluorescence microscope equipped with a 100x oil immersion Objective. and a triple band pass filter for simultaneous detection of Spectrum Orange, Spectrum Green, and DAPI signals. Two hundred non-overlapping nuclei, containing a minimum of two reference probe signals, were counted. The ratio of 1p/1q and 19q/19p was calculated by dividing the number of orange and green signals. Correction for artefactual nuclear truncarion was assessed.
Results. At immunohistochemistry, all cases were positive for TTF-1 and negative for chromogranin and synaptophysin. All SCOs had at least focal positivity for EMA (around 10%), while all pituicytomas were negative for EMA. In 2 SCOs, ultra-structural microscopy showed neoplastic cells with cytoplasm packed with swollen mitochondria.
4/6 (67%) of SCOs showed 1p/19q codeletion (nuclei with single fluorescent signals ranging from 45 to 67%, mean 55%), while 2/6 (33%) displayed chromosomes 1 and 19 gains (>30% of neoplastic nuclei with ≥3 fluorescent signals for both chronosomes). No 1p/19q alterations were found in pituicytomas (range single signals ranging from 2% to 34%). Nuclear truncation was estimated to cause up to 30% of artefactual false single signals. Two controls revealed absence of 1p/19q chromosome codeletion.
Conclusions. All SCOs had chromosomes 1 and 19 alterations, which were not observed in any pituicytomas. Although these findings need to be validated in larger

cohort of tumurs, they suggest that chromosomes 1/19 deletions or gains may be a distinct character of SCO and may be used in the differential diagnosis with pituicytoma in challenging cases.

PATOLOGIA ENDOCRINA

SECOND OPINION IN THYROID PATHOLOGY: CHANGES AFTER THE NEW WHO 2017 TERMINOLOGY FOR ENCAPSULATED NON INVASIVE FOLLICULAR-PATTERNED LESIONS.

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Background. Diagnostic criteria of the follicular variant of papillary thyroid carcinoma have been a matter of debate for a long time. Nuclear features may be equivocal and incomplete for a definite diagnosis in the absence of invasion, and diagnostic reproducibility is very low, even among experts (1). As a consequence, some years ago the term well-differentiated tumor of uncertain malignant potential (WDT-UMP) was coined for those cases of follicular lesions with no invasion and with nuclear features equivocal for a diagnosis of papillary carcinoma (2). In recent years, several studies clAim.ed that the pure follicular variant of papillary thyroid carcinoma, when encapsulated and with no vascular or capsular invasion, has an excellent prognosis, thus reducing in these lesions the diagnostic impact of nuclear features for the definition of "clinical malignancy" (3). In April 2016 a new proposal for encapsulated purely follicular thyroid neoplasms was released online, stating that those that lack any evidence of invasion, even if showing papillary type nuclear features (either unequivocal or incomplete) are clinically benign, thus avoiding the term carcinoma (non-invasive follicular thyroid neoplasm with papillary like nuclear features, NIFTP) (4). In this new concept, for follicular lesions, invasion rather than nuclear features is the key issue to define malignancy. Both WDT-UMP and NIFTP have been incorporated in the new WHO 2017 classification of thyroid tumors.

Aim. To verify the impact of the new WHO 2017 terminology for encapsulated non invasive follicular-patterned lesions of the thyroid in the prevalence, pitfalls and diagnostic issues of thyroid pathology consultation cases sent to our Institution for second opinion.

Materials and methods. All cases of thyroid pathology sent for consultation at the Pathology Unit of San Luigi Hospital, Orbassano, Turin, from 2008 to July 2019, have been reviewed and divided into those before and after June 2016, based on the date of publication of the NIFTP terminology. The prevalence of thyroid consultation cases was assessed in the total consultation cases received for endocrine pathology diseases. All cases have been classified according to: sender (pathologist or clinician), confirmation after consultation of the initial diagnosis (if available), major diagnostic issue and final second opinion diagnosis.

Results. Total endocrine pathology consultation cases were 423 before and 244 after June 2016, and among them the total thyroid pathology cases were 176 (42%) and 60 (24%), respectively. Despite the number of endocrine pathology consultation cases/year remained nearly stable from 2015 to 2019, there was a progressive decrease of thyroid pathology cases, from 44% to 19%, respectively. Among thyroid pathology cases, all but one were sent from pathologists. There was no difference in the percentage of diagnoses confirmed at second opinion before or after June 2016 (55% in each group). The major diagnostic issues greatly varied in cases before and after June 2016. In particular, thyroid cases sent for uncertain nuclear features of papillary carcinoma decreased from 53% to 15%, respectively. By contrast, thyroid cases worrisome for the definition of the presence of capsular or vascular invasion raised from 15% to 37%, respectively. Moreover, no case was sent before June 2016 for the definition of infiltrative vs non-infiltrative growth, whereas those cases represented 10% of cases after June 2016. Finally, cases sent for the definition of high grade features (i.e. for the diagnosis of poorly differentiated carcinoma), which are unrelated to the new terminology for encapsulated non invasive follicular-patterned lesions, remained unaltered (10% in both groups). Diagnoses after second opinion reflected what described above. In fact, diagnoses of follicular variant of papillary carcinoma decreased before to after June 2016 from 37% to 17%, respectively, and diagnoses of follicular carcinoma raised from 10% to 18%. Diagnoses of well-differentiated tumor of uncertain malignant potential (WDT-UMP) decreased from 4% to 0% before and after June 2016, respectively, whereas the diagnoses of follicular tumor of uncertain malignant potential (FT-UMP), which is based on equivocal signs of vascular or capsular invasion, raised from 2% to 10%. Diagnoses of papillary carcinoma variants (follicular variant excluded) and of benign lesions remained largely stable, as well as those of poorly differentiated carcinoma (this latter 11% before and 7% after June 2016). As expected, no diagnosis of non-invasive follicular thyroid neoplasm with papillary like nuclear features (NIFTP) was made before June 2016 whereas it represented 12% of second opinion after.

Conclusions. The release of the new terminology for encapsulated non invasive follicular-patterned lesions of the thyroid significantly reduced the number of thyroid cases sent for second opinion, based on the understatement of the diagnostic role of nuclear features to define malignancy in these lesions, as compared to the recognition of the presence of invasion.

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IMMUNE RELATED GENE EXPRESSION PROFILES OF ONCOCYTIC AND NON- ONCOCYTIC POORLY DIFFERENTIATED THYROID CARCINOMAS; CORRELATION WITH CLINICO-PATHOLOGICAL CHARACTERISTICS

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Background. Poorly differentiated thyroid carcinoma (PDTC) is a rare malignant tumor with intermediate features between well differentiated and anaplastic carcinomas. Oncocytic changes, resulting from cytoplasmic accumulation of altered mitochondria, are characterized by markedly eosinophilic and granular cytoplasm, and have been associated to an adverse prognosis in well differentiated thyroid carcinomas. The clinical impact of oncocytic transformation in PDTC is poorly understood.
Objectives. Aim of this study was to (i) analyze the immune-related gene expression profile of PDTC comparing oncocytic cases to classical types by NanoString technology; (ii) investigate PD-L1 immunoreactivity and correlate the observed levels with the gene expression profile and clinicopathological data.
Materials and methods. We selected a series of PDTC operated at Città della Salute Hospital of Torino from January 2000 to December 2017. Upon revision according to the Turin proposal criteria, 48 PDTC were selected, including 23 with oncocytic changes. We performed comprehensive molecular analyses employing NanoString technology using nCounter PanCancer Immune Profiling panel and investigated PD-L1 immunoexpression (utilizing clone 22C3). The gene expression Results and immunohistochemical assessment were correlated with clinicopathological characteristics.
Results. In the group of PDTC with oncocytic changes an up-regulation of several immunity-related genes was observed, including IRAK1, CD274, DEFB1, PSMD7, ITGAL, LAIR2, LY96 ($p<0.01$), and downregulation of NOD1 ($p<0.01$) compared to non-oncocytic cases, that had the reverse pattern. Moreover, oncocytic PDTCs were significantly correlated with the presence of necrosis ($p=0.028$) and tumor infiltrating lymphocytes ($p=0.03$). PD-L1 immunoreactivity was observed in 14/23 (60.9%) oncocytic and 4/25 (16%) classical PDTCs ($p<0.001$). In addition, PD-L1 positive cases were associated to an up-regulation of CD274, CD79B, ITGAL, LY96 and LAIR2 ($p<0.01$), compared to PD-L1 negative tumors. PD-L1 positivity was correlated to a shorter DFS ($p=0.033$) in PDTC, while the oncocytic subgroup only had also a shorter overall survival ($p=0.04$).
Conclusions. The oncocytic variant of PDTC has different gene expression pattern, immunohistochemical profile and prognosis. The up-regulated genes reported in this tumor subset (some of them coinciding with those described in PD-L1 positive lesions), namely, *IRAK1, CD274, DEFB1,* PSMD7, ITGAL, LAIR2, LY96 are associated with adverse prognosis in epithelial tumors, including papillary thyroid carcinoma. Our findings suggest that, having a diverse intrinsic profile, oncocytic PDTC may benefit from novel therapeutic strategies linked to their molecular profile.

MOLECULAR TESTING FOR CYTOLOGICALLY INDETERMINATE THYROID NODULES: A SINGLE INSTITUTION EXPERIENCE

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Objectives. Fine-needle aspiration (FNA) cytology represents the gold-standard procedure in the diagnostic management of the thyroid nodules. However 15-30% of thyroid FNA are cytologically indeterminate. Molecular tests have been recently introduced to improve the diagnostic accuracy of these cases. Aim. of the study was the evaluation of cyto-histological and molecular patterns in a single-institution cohort of patients.
Methods. We reviewed 161 cases with FNA cytology classified according to Bethesda System, followed up in Udine University Hospital in the period 2017-2018 and molecularly classified for a 4-gene panel *BRAF*/*NRAS*/*HRAS*/*KRAS*.
Genotyping was performed on DNA extracted by smears samples, with real time PCR method on a RotorGene system with commercially available reagents (*Kit Easy® Thyroid CE-IVD - diatech pharmacogenetics*).
Results. Among all 161 molecularly characterized cases, cytologically indeterminate cases were 139: 118 (73%) classified as Tyr3a and 21 (13%) as Tyr3b, according to *Italian consensus for the classification and reporting of thyroid.*
Molecular profile of Tyr3a cases was: *BRAF*mut 16 cases (14%), *KRAS*mut 3 (2%), *NRAS*mut 9 (8%), *HRAS*mut 6 (5%), all wild type 84 (71%).
Cytologic Tyr3b cases were molecularly subdivided in: *BRAF*mut 7 cases (33%), *KRAS*mut 1 (5%), *NRAS*mut 2 (10%), *HRAS*mut 0 (0%), all wild type 11 (52%).
Mutations detected were: *BRAF p.Val600Glu*, *KRAS p.Gly12Asp*, *p.Gly12Val* and *p.GlnQ61Arg*, *NRAS p.Gly13Arg*, *p.Gln61Lys* and *p.Gln61Arg*, *HRAS p.Gln61x*. Mutations were all mutually exclusive.
Surgical sample of thyroidectomy was available for 52/139 (37%) citologically reported as indeterminate (Tyr3a/Tyr3b).
Among surgically Tyr3a resected cases 23 out of 39 (60%) had a positive histology for thyroid cancer. Mutation pattern in these Tyr3a positive histology cases was: *BRAF*mut 12 cases (52%), *KRAS*mut 2 (9%), *NRAS*mut 3 (13%), *HRAS*mut 1 (4%), all wild type 5 (22%).
Among surgically Tyr3b resected cases 7 out of 13 (54%) had a histologic diagnosis of carcinoma. Mutation pattern in these Tyr3b positive histology cases was: *BRAF*mut 5 cases (72%), *NRAS*mut 1 (14%) and all wild type 1 (14%).
Any *KRAS*/*NRAS*/*HRAS* (RAS) mutation was detected in 9/16 (56%) of Tyr3a-histologic negative resected patients and 2/6 (33%) of Tyr3b-histologic negative resect-

Tyr3a/Tyr3b	*PPV*	*NPV*	*S*	*Sp*
BRAFmut	**94%**		57%	**95%**
KRASmut	33%		7%	82%
NRASmut	40%		13%	73%
HRASmut	50%		3%	**95%**
RASmut	39%		20%	50%
BRAF-NRAS-KRAS-HRASmut	67%		**80%**	45%
RAS-WT		38%	43%	50%
all-WT		63%	20%	55%

Fig. 1.

ed cases. Only one case reported as surgically negative histology had *BRAF* mutation with Tyr3a cytology.
We interestingly observed a case of a young 42-years-old woman with a cytologic Tyr3a right lobe nodule, radiologically highly suspected (ecographic class 3 based on *AACE/ACE/*AME *2016 guidelines*), with a *KRAS p.Gln61Arg* mutation on smear cytologic sample that underwent total thyroidectomy. Histologic examination showed a microfollicular adenoma and a classic papillary carcinoma with a *BRAF p.Val600Glu* mutation.
Positive predictive value (PPV), negative predictive value (NPV), sensitivity (S) and Specificity (Sp) are summarized in the table.
Conclusions. Our data confirmed the high specificity and positive predictive value of BRAF V600E mutation for malignant nodules detection, supporting its *rule-in* role in indeterminate FNA cytology samples.
The 4-gene panel allowed a higher sensitivity in the detection of malignancy, despite the low sensitivity of mutation in any single gene. It confirms and suggests a possible role of molecular panel analysis in this setting. However, the low positive predictive value both of the 4-gene panel and of each single gene (except of BRAF) confirmed the necessity of managing molecular data in a complete clinical context evaluation, as reported in all guidelines about management of thyroid nodules.

FOLLICULAR THYROID CARCINOMA: SYSTEMATIC ANALYSIS OF MORPHOLOGICAL PARAMETERS TO IMPROVE THE DIAGNOSIS AND ASSESS THE RISK OF TUMOR PROGRESSION

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Introduction: Follicular thyroid carcinoma (FTC) is a differentiated thyroid malignant neoplasm with a worse prognosis when compared with papillary thyroid carcinoma (PTC) especially in cases with vascular invasion. Its diagnosis may be difficult on routine histology, as evidenced by high intra- and inter-variability amongst pathologists.
Objective of the study. To identify morphological parameters with specific utility in diagnosis and determination of the risk of disease progression in a series of FTCs diagnosed in the Pathological Anatomy service of the Umberto I University Hospital, Rome, from 2007-2018. This retrospective study was limited to cases with available clinical follow-up.
Materials and methods. Four anatomic pathologists reviewed blindly and individually 21 FTCs in order to confirm the initial diagnosis. Both cases of FTC with oncocytic cell changes (oncocytic variant of FTC or Hürthle cell carcinoma) and without oncocytic cell features were enrolled. The confirmed FTCs (diagnostic agreement of at least 2 out 4 pathologists), were then further evaluated with regard to a series of morphological parameters including capsular and blood vessel infiltration, pattern of growth, type of neoplastic cells, nuclear atypia, non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) score, tumor necrosis, mitotic activity, and evaluation of Ki67 proliferation index and p53 expression.
Results. In 16 cases (76%) the diagnosis of FTC was confirmed, of which 8 (50%) were minimally invasive, 7 (43.75%) were encapsulated angioinvasive, and 1 (6.3%) was widely invasive. Diagnostic concordance among pathologists was excellent in all 16 FTCs for evaluation of capsular infiltration, vascular invasion foci, evaluation of the NIFTP score, and tumor necrosis.
In 8/16 FTCs, foci of questionable capsular infiltration were also detected. 6/8 FTCs were encapsulated with angioinvasion and had <4 vessels involved, and 5/16 showed foci of questionable vascular invasion.
The group of oncocytic cell carcinomas is characterized by significantly higher values of solid/trabecular growth patterns ($p = 0.001$), Ki 67 ($p = 0.0254$), and p53 ($p = 0.0197$).
The metastatic FTCs had significantly higher values of capsular infiltration foci ($p = 0.0157$) (the cut-off of capsular infiltration foci was > 5.5 with a sensitivity 67% and specificity 92%), vascular invasion ($p = 0.008$), and NIFTP score 1 ($p = 0.002$). The discriminating diagnostic power between the FTC groups with and without metastases was of moderate degree (AUC: 0.7308, 95% CI: 0.3395-1.000).

Conclusions. The current study confirms high inter-observer variability in the diagnosis of FTC. It reiterates the increased aggressiveness of this cancer and suggests a cut-off of > 5.5 capsular infiltration foci as an index of greater aggressiveness, to be confirmed in a larger study. NIFTP score was shown to have potential value in excluding the follicular variant of papillary carcinoma and identifying the group of more aggressive FTCs. Finally, evaluation of Ki67 proliferation index and p53 expression have potential as prognostic factors.
To date, FTC remains a difficult diagnosis and more complex cases should be reviewed by several pathologists whenever possible. Optimally, these cases should be reviewed via a multidisciplinary comparison within a unit dedicated for this purpose.

HOW PERIPHERAL ARE PAPILLARY MICROCARCINOMAS? A MULTICENTER PATHOLOGIC AND CLINICAL STUDY FOR RISK STRATIFICATION

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Background & objectives. Papillary microcarcinoma (mPTC) is defined by the World Health Organization as a papillary carcinoma that measures ≤1.0 cm in size. It usually has an indolent behavior, and it is often found incidentally. mPTCs represent a heterogeneous group of neoplasms and include a subset capable of aggressive behavior with propensity to lymph node metastases, dissemination and recurrence. Many mPTCs are believed to arise at the periphery of the thyroid, but it is currently unclear whether the site of the origin of the mPTC within the gland has an influence on clinico-pathological parameters.
The Aim. of the study is to assess whether the site of the origin of the mPTC within the thyroid impacts on clinico-pathological tumor parameters relevant to risk stratification.
Materials and methods. A multicenter cohort of 298 mPTCs from six Italian medical institutions has been analyzed. The distance of the tumor center from the so called "thyroid capsule" (the interface of the thyroid parenchyma with the surrounding extrathyroidal tissues) was measured micrometrically. Pathological and clinical features of the tumors were analyzed including tumor subtype, microscopic appearance, extent and type of tumor growth, BRAF mutational status.
Results. mPTC arise peripherally, at a median distance of 3.5 mm below the thyroid capsule. Four mPTC groups based on size (> or = 5 mm or < 5 mm) and distance of the edge of the tumor from the thyroid capsule (= 0 mm or > 0 mm) were identified: Group A: Tumors with size > or = 5 mm and distance of the edge of the tumor from the thyroid capsule = 0 mm (Large subcapsular mPTC); Group B: Tumors with size > or = 5 mm and distance of the edge of the tumor from the thyroid capsule > 0 mm (Large nonsubcapsular mPTC); Group C: Tumors with size < 5 mm and distance of the edge of the tumor from the thyroid capsule = 0 mm (Small subcapsular mPTC); Group D: Tumors with size < 5 mm and distance of the edge of the tumor from the thyroid capsule > 0 mm (Small nonsubcapsular mPTC). Univariate analysis demonstrated significant differences between the four groups, with Group A showing the most aggressive features and Group D the most indolent ones. Multivariate analysis correlated Group A tumors with (i) specific microscopic features: presence of psammoma bodies within the tumor, tall cell features, tumor fibrosis, with an inverse correlation to follicular growth pattern; (ii) specific characteristics of tumor growth: infiltrative border, unicentric tumor with intraglandular tumor spread, vascular invasion, psammoma bodies in the parenchyma surrounding the tumor; (iii) specific clinicopathologic features: tall cell or classic papillary carcinoma diagnoses, BRAF V600E mutation, lymph node metastases, American Thyroid Association (ATA) risk group, with an inverse correlation to nodular hyperplasia or to the presence of other thyroid neoplasms, an inverse correlation to a diagnosis of papillary microtumor (PMiT) or that of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP).
Conclusion. Subcapsular mPTC that are > or = 5 mm (Group A tumors) have unfavorable clinico-pathological features and likely represent a subset of mPTC with the potential to progress to larger, clinically relevant tumors. Nonubcapsular mPTC that are < 5 mm (Group D tumors) show bland clinico-pathological features and are likely endowed with limited malignant potential.

CLINICOPATHOLOGICAL CHARACTERISTICS OF AGGRESSIVE WELL-DIFFERENTIATED FOLLICULAR CELL-DERIVED THYROID CANCERS

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Background. Papillary, follicular and Hürthle cell thyroid carcinomas (PTC, FTC, HCC) belong to a group of well differentiated follicular cell-derived thyroid neoplasms. Despite their relatively good prognosis, approximately 10% of these cases show an aggressive behavior, namely, metastasis at diagnosis, locoregional recurrence and/or distant metastasis.

Objectives. The Aim. of this study was to identify histomorphological characteristics that may be predictive of an aggressive behavior in a series of non-anaplastic well differentiated thyroid cancers.
Methods. We revised the pathological file of Città della Salute Hospital of Torino, searching for patients that underwent total thyroidectomy due to well-differentiated thyroid carcinoma, with available follow-up data. We selected 73 cases with an aggressive behavior defined as the presence of metastasis at diagnosis, biochemical or local recurrence, or subsequent development of distant metastases and matched them with 78 controls who resulted free of disease at clinical follow up. Case-control groups were matched according to sex and TNM stage (T and N parameters). All cases were anonymized and re-evaluated by an experienced pathologist and all clinico-morphological data were inserted in a dedicated database.
Results. Clinico-morphological characteristics that significantly correlated with the aggressive cases were younger age at diagnosis ($p = 0.02$), aggressive PTC histological variants such as solid, high cell, hobnail, diffuse sclerosing, extensive angioinvasion in FTC ($p = 0.001$), presence of necrosis ($p = 0.011$), absence of tumoral capsule ($p = 0.009$), presence of angioinvasion ($p<0.001$), tumor capsular invasion ($p = 0.005$), bilateral thyroid presentation ($p = 0.038$), extra-thyroidal extension ($p = 0.002$), diameter of lymph node metastases (1.45 ± 1.1 vs. 0.68 ± 0.88; $p = 0.01$), extra-nodal extension ($p < 0.001$), presence of a intratumoral immune infiltrate ($p = 0.03$), positive surgical margins ($p = 0.019$), and presence of extensive intratumoral sclerosis ($p = 0.004$).
Conclusions. The present study confirmed the presence of certain clinico-histopathological parameters that if correctly identified at the time of diagnosis may possibly predict neoplasms with aggressive potential and identify patients with higher risk of recurrence. Moreover, extensive intratumoral sclerosis, whose impact on prognosis had not previously been described in the literature, is associated with a more aggressive tumor behavior. It may therefore be a useful novel parameter to be included in the pathological reporting checklists, for a more complete prognostic stratification.

THE RATE OF TALL CELL VARIANT IS A PREDICTOR OF THE CLINICAL COURSE OF PAPILLARY THYROID CARCINOMA

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Introduction. Papillary thyroid carcinoma (PTC) is the most common type of malignant neoplasm of the gland. Despite the fact that it pursues a good prognosis, nearly 5 % of thyroid carcinomas may exhibit an aggressive biological behavior and a less favorable prognosis. According to the American Thyroid Association (ATA) guidelines, the presence of an aggressive histologic variant of PTC may be associated with a worse prognosis. In fact, several studies have documented that these variants are associated with the possibility of an incomplete response to radioactive iodine treatment (RAI) and a higher risk of recurrence. Among them, the tall cell variant (TCV) of PTC is the most frequent. The WHO Classification of Endocrine Neoplasms (4th edition, 2017) recommends that the minimal rate of tall cells that should be present in order to diagnose a PTC-TCV is 30% of the neoplastic cells. The Aim. of this study is to evaluate the correlation of different tall cell component rates in PTC-TCV with the most important clinical and histological parameters.
Methods and materials. We analysed 1,644 cases of thyroid cancer taken from the records of the Div. of Pathology of the Foundation "Agostino Gemelli" University Hospital IRCCS of Rome from 1st January 2016 until 30th September 2018. We selected 49 cases of PTC-TCV where we identified 3 groups: 30% of tall cells, TC rate between 31 and 50%, TC rate higher than 50%. All patients underwent surgery, with or without nodal dissection, and subsequent radioiodine treatment. They were classified according to the ATA risk guidelines in low, intermediate and high risk. Clinical responses after treatment were classified as complete response or incomplete response, the latter defined as either incomplete biochemical response (IBR) or incomplete structural response (ISR).
Results. In all of our cases, we evaluated different histological parameters: T according to TNM/AJCC (8th edition 2017), rate of tall cells, lymph node metastasis, extranodal extension (ENE), maximum dimension of lymph node metastasis and presence of vascular invasion.
Thirty-seven out of 49 had a complete response to iodine administration (75.5%), while 12 had an incomplete response (24.5%).
Diagnoses were distributed in 18 T1a (36.7%), 18 T1b (36.7%), 5 T2 (10.2%), 5 T3a (10.2%), 2 T4a (4.08%) and 1 T4b (2.04%). No case was diagnosed as T3b.
35 cases (71.42%) had lymph node metastasis, 7 of these (14.29%) showed ENE.
Lymph node metastasis had dimensions ranging between 0,1 to 3,5 cm, while the average size of lymph node metastasis was found to be 1.2 cm, the median value being 1 cm.
Lymphovascular space invasion (LVI) was present in 12 (24.3%) cases.
Our data showed a statically significant negative correlation between the T according to TNM and the completeness of the response to treatment (p value=0.028, Fisher's exact test), confirming the literature data. Additionally, our case series documented a positive correlation between the percentage of tall cells and T stage (Fisher's exact test, p value=0.007). This correlation might be due to the detection of small TCV with the performance of pre-operative fine needle aspiration cytology. In fact 36 out of 49 (73.5%) cases were diagnosed as T1 category. Interestingly we also found the presence of an association between lower tall cell percentages and complete responses to treatment (Fisher's exact test, p value=0.024).
Conclusions. Even though the series is still limited, our data show a statistically significant difference between the different rates of tall cells and the response to treatment. These preliminary Results underline the capital importance of an exact evaluation of the tall cell rate in thyroid papillary carcinoma, as it may be a predictor of prognostic outcome

and it may affect the clinical management of these patients.

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DERMOPATOLOGIA

POTENTIAL PROGNOSTIC ROLE OF HMOX-1 IN DISCOID LUPUS ERYTHEMATOSUS

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Objectives. Discoid lupus erythematosus (DLE) is a chronic autoimmune disease of the skin and mucous membranes, with minimal visceral involvement. Eziopathogenesis is not still defined but are involved genetic and environmental factors like gene mutations of HLA-B7, -B8, -Cw7, -DR2, -DR3 and -DQw1 or traumatism, stress, sunburn, infections, exposure to cold, pregnancy [1].

The LED diagnosis is based on clinical features, but only histology can confirm it with a skin biopsy. Heme oxygenase or Heat shock protein 32 is a microsomal enzyme that contributes in heme catabolism. Its function is to catalyze the opening of the heme ring with the formation of biliverdin, CO and iron, in the presence of molecular oxygen and reducing equivalents provided by NADPH-cytochrome p450 reductase. In particular, it hydroxylates the methylene bridges and oxidizes Fe2 + to Fe3 +. The heme-oxygenase (HO) system consists of three isoforms:

HO-1 is inducible by oxidative stress; HO-2 is constitutive and is most stimulated by corticosteroids; HO-3 is also constitutive and has recently been isolated in the rat brain.

The Aim.s of this study is to evaluate the expression of EME Oxygenase 1 (HMOX-1) in patients with DLE and to evaluate the relationship between the expression of HMOX-1 and the extent of the inflammatory infiltrate.

Materials and methods. 36 patients with DLE, 18 men and 18 women, with an age range between 30 and 50 years were selected. Naive patients were chosen and biopsies were performed after a six-month wash-out period. The tissue samples were obtained by incisional skin biopsies, then fixed in 10% buffered formalin for 12 hours, included in paraffine, cut to the microtome and stained with hematoxylin and eosin. The morphological diagnosis of DLE was made on the basis of established criteria. In cutaneous biopsy samples we assessed the extent of the inflammatory infiltrate by staining hematoxylin and eosin, quantifying it according to a score (0; 1+; 2+; 3+). The sections were incubated with HMOX1 monoclonal antibody (MA1-112 Thermofisher Scientific), diluted 1:500 in PBS (Sigma, Milan, Italy). Immunohistochemistry positive staining was defined as the presence of brown chromogene. Immunostained slides were separately evaluated by two pathologists (RC and EP), who were blinded to patient identity, clinical status and group identification, using a light microscope. The HMOX-1-staining status was identified as either negative or positive. Immunohistochemical positive staining was defined as the presence of brown chromogen detection within the cytoplasm. The percentage of HMOX-1 positive cells (Extent Score (ES) was independently evaluated by two investigators and scored as a percentage of the final number of 100 cells in four categories: <5% (0); 5-33%(+); 33-66% (++); and >66% (+++). Counting was performed at 200× magnification.

Results. In the hematoxylin and eosin-stained sections from specimens of patients affected by DLE, the extent of the inflammatory infiltrate, according to a score (0; 1+; 2+; 3+), was evaluated.

Then, we evaluated the expression of HMOX-1 in three compartments: epidermis, cutaneous appendages and inflammatory infiltrate assigning a score (0; 1+; 2+; 3+), obtaining the following Results. In the samples in which the score was high (2+; 3+), the expression of HMOX-1 in the cutaneous appendages and in the epidermis was found to be inversely proportional to the extent of the inflammatory score and consequently low (1+). In the samples in which the score was low (1+),·the expression of HMOX-1 in the epidermis and in the cutaneous appendages was found to be inversely proportional to the inflammatory score and consequently high (2+; 3+). Regarding the inflammatory infiltrate, whether poorly or widely represented, HMOX-1 was diffused and intense (2+; 3+), in almost all the cases examined (26/36).

Following biopsy, hydroxychloroquine was administered to patients with a dose of 200 mg per day. Patient follow-up at six months and one year showed symptomatic remission in all cases.

Conclusions. The scientific community has been very attentive to the role of HMOX-1 in the metabolic and inflammatory processes that have occurred in our body since 1989, when HMOX-1 was identified as a 32 KDa protein commonly induced in damaged cells.

Heme oxygenase 1 has anti-inflammatory, antioxidant, antiapoptotic effects and promotes the maintenance of cellular and tissue homeostasis.

The motivation that led us to undertake this study was the interest in deepening the potential in the clinical and therapeutic field of this protein, focusing in particular on the role that HMOX-1 plays in inflammatory skin diseases and, first of all, in DLE.

Despite the presence in the literature of studies on the correlation between over-expression of HMOX-1 and dermatological diseases, such as psoriasis and atopic dermatitis [2-4], and between over-expression of HMOX-1 and systemic diseases, such as systemic lupus erythematosus, a study that connects the HMOX-1 and the DLE does not still exist. From the quantification of the expression of HOMX-1 in the epidermis, in the cutaneous appendages and in the inflammatory infiltrate of 36 patients affected by DLE, an inverse proportional-

ity between the inflammatory score and the HMOX1 expression in the skin and in the appendages was observed.
In samples in which the score was high (2+; 3+) the expression of HMOX-1 in the appendages and in the epidermis was found to be low (1+). In fact, out of sixteen patients with an inflammatory score (3+), thirteen patients had HMOX-1 expression of (0/1 +), or more than 80%. In samples in which the score was low (1+) the expression of HMOX-1 in the epidermis and in the appendages was found to be high (2+; 3+). Out of eight patients with low inflammatory scores (1+), the resultant HMOX-1 expression was high (2+; 3+) on seven out of eight patients in skin (> 85%) and six out of eight patients in skin appendages (75%).
Finally, we deduce a possible use of the expression of HMOX-1 as a prognostic index in the evaluation of the DLE, since its high value corresponds to a low inflammatory score.

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POTENTIAL PROGNOSTIC ROLE OF MIF IN DISCOID LUPUS ERYTHEMATOSUS

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Objectives. Discoid lupus erithematosus (DLE) is a chronic cutaneous disease characterized by inflammatory plaques that may lead to disfiguring scarring and skin atrophy. The LED diagnosis is based on clinical features, but only histology can confirm it with a skin biopsy. Macrophage migration inhibitory factor (MIF) is a cytokine with both pro- and anti-inflammatory actions, that has been linked to the etiopathogenesis of autoimmune diseases [1], such as Multiple Sclerosis and Type 1 Diabetes, but also, in cancer immune evasion [2].
The Aim.s of this study is to evaluate the expression of MIF in patients with DLE and to evaluate the relationship between the expression of MIF and the extent of the inflammatory infiltrate.
Materials and methods. 36 patients with DLE, 18 men and 18 women, with an age range between 30 and 50 years were selected. Naive patients were chosen and biopsies were performed after a six-month wash-out period. 5 surgical specimens from patients with non pathologic skin were also selected. The tissue samples were obtained by incisional skin biopsies, then fixed in 10% buffered formalin for 12 hours, included in paraffine, cut to the microtome and stained with hematoxylin and eosin. The morphological diagnosis of DLE was made on the basis of established criteria. In cutaneous biopsy samples we assessed the extent of the inflammatory infiltrate by staining hematoxylin and eosin, quantifying it according to a score (0; 1+; 2+; 3+). The slides were dewaxed in xylene, hydrated using graded ethanols and were incubated for 30 min in 0.3% H2O2/methanol to quench endogenous peroxidase activity then rinsed for 20 min with phosphate-buffered saline (PBS; Bio-Optica, Milan, Italy). The sections were heated (5 min × 3) in capped polypropylene slideholders with citrate buffer (10 mM citric acid, 0.05% Tween 20, pH 6.0; Bio-Optica, Milan, Italy), using a microwave oven (750W) to unmask antigenic sites. The blocking step was performed before application of the primary antibody with 5% bovine serum albumin (BSA; Sigma, Milan,Italy) in PBS for 1 h in a humid chamber. BSA was used as a blocking agent to prevent non-specific binding of the antibody. Then, the sections were incubated with MIF polyclonal antibody (PA5-27343 Thermofisher Scientific), diluted 1:500 in PBS (Sigma, Milan, Italy). The immunoreaction was visualized by incubating the sections for 4 min in a 0.1% 3,3-diaminobenzidine (DAB) and 0.02% hydrogen peroxide solution (DAB substrate kit, Vector Laboratories, CA, USA). The sections were lightly counterstained with Gill's hematoxylin (Histolab Products AB, Göteborg, Sweden) and mounted in GVA mountant (Zymed Laboratories, San Francisco, CA, USA).
Immunohistochemistry positive staining was defined as the presence of brown chromogen detection in the cell membrane and cytoplasm; renal tubules were identified as positive control to test the specific reaction of primary antibodies used in this study.
Immunostained slides were separately evaluated by two pathologists (RC and GB), who were blinded to patient identity, clinical status and group identification, using a light microscope.
The MIF-staining status was identified as either negative or positive.
Immunohistochemistry positive staining was defined as the presence of brown chromogen detection within the cytoplasm or in the membrane. The percentage of MIF immunopositive cells (Extent Score (ES) was independently evaluated by two investigators and scored as a percentage of the final number of 100 cells in four categories: <5% (0); 5-33%(+); 33-66% (++); and >66% (+++).
Counting was performed at 200× magnification.
Results. We evaluated the expression of MIF in the three compartments: epidermis, skin appendages and inflammatory infiltrate by assigning a score (0; 1+; 2+; 3+), and subsequently obtained the following Results. In cases of DLE with an high inflammatory score (2+; 3+), MIF's low expression (0; 1+) was observed in epidermis, appendages and inflammatory infiltrate. In cases of DLE with a low inflammatory score (0; 1+), MIF's high expression (2+; 3+) was observed in epidermis, appendages and inflammatory infiltrate. In normal skin biopsies, high levels of MIF (2+; 3+) in epidermis and cutaneous appendages were observed.
Conclusions. In the present study, we have performed an immunohistochemical analysis for MIF on DLE lesions and normal skin, as well. We found high levels of MIF at the basal layer of the epidermis and in the cutaneous appendages (eccrine glands and sebocytes) of normal skin. Unexpectedly, in DLE lesions, we observed

a significant negative correlation between the expression of MIF and the severity of inflammation. These data were further corroborated by an analysis of MIF expression levels in publicly available microarray datasets. Overall, these data support a role for MIF in the regulation of homeostasis and inflammation in the skin, and open up novel avenues for the treatment of DLE.

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SECOND OPINION BY EXPERIENCED DERMATOPATHOLOGISTS IMPROVES CLINICAL MANAGEMENT OF PATIENTS WITH DIAGNOSIS OF MALIGNANT MELANOMA IN A REFERRAL REGIONAL MELANOMA UNIT

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Objectives. Malignant melanoma (MM) is one of the most lethal diseases in human oncology. Clinical examination and dermoscopy play a fundamental role in identifying which atypical melanocytic lesions must be excised, but the final diagnosis of MM is based on pathological examination. The diagnosis of melanocytic lesions can be challenging for the general pathologist, and, therefore, a second diagnostic opinion by a pathologist with high expertise in dermatopathology could improve the diagnostic accuracy. We performed a retrospective study to evaluate the effects of a second diagnostic opinion in a Series of melanocytic neoplasms.
Materials and methods. Fifty-four cases of melanocytic lesions were re-evaluated at the Department of Pathology of University "Vanvitelli" (Naples, Italy), between November 2018 and July 2019. All the cases had been originally diagnosed by general pathologists in peripheral Hospitals or private diagnostic centers; re-evaluation was performed by two experienced dermatopathologists. The first diagnoses were compared with the second ones, paying particular attention to divergencies in the assessment of pathological features influencing prognosis, therapy and clinical management of the patients. We defined three kinds of diagnostic discrepancies (Type I, Type II and Type III), to facilitate comparison of the Results. Results. Type I discrepancies (discordant final diagnoses) were observed in 13 out of 54 (24%) cases. Type II discrepancies (concordant final diagnoses with differences in the evaluation of parameters affecting the therapy and the clinical management of the patient) were found in 7 out of 54 (13%) cases. Eleven out of 54 (20.4%) cases presented Type III discrepancies (concordant final diagnoses with differences in the evaluation of parameters with prognostic significance but not affecting therapy or clinical management of the patient). In particular, 8 out of 54 (14.8%) cases showed a different Breslow thickness, 1 out of 54 (1.8%) cases presented differences in both Breslow thickness and presence of ulceration. In 2 out of 54 (3.7%) cases, only other minor prognostic factors (number of mitoses, presence of regression, presence and type of TILs) differed. The stage of the neoplasm changed in 14 (26%) cases, and the indication to perform the sentinel node excision changed in 6 (11%) cases.
Conclusions. The role of the second diagnostic opinion in melanocytic pathology is poorly defined and only few studies, to date, have evaluated its effects on the clinical management of the patients. In our Series, the second diagnostic opinion changed the final diagnosis in 24% (13 out of 54) of the cases, including 3 cases of MM previously considered as benign nevi, and 3 cases of benign nevi previously considered as MM. Impressively, in the 37% (20 out of 54) of all the cases, the second diagnostic opinion changed the clinical management of the patients with consequences on therapy and performed medical procedures. In conclusion, the pathological evaluation of melanocytic neoplasms is one of the greatest challenges in pathology. Consequently, a second diagnostic opinion obtained by a pathologist with a specific training in dermatopathology may improve the clinical management of the patients.

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PRIMARY CUTANEOUS γ-δ T-CELL LYMPHOMA IN A MAN PRESENTING WITH A PUSTULAR RASH

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Primary cutaneous gamma-delta T-cell lymphoma (PCGD-TCL) is a clonal proliferation of mature, activated $\gamma\delta$ T-cell with a cytotoxic phenotype [1,2]. PCGD-TCL is a rare subtype of primary cutaneous T-cell lymphoma (accounting for approximately 1% of all cutaneous T-cell lymphomas), which affect predominantly the adults, with a predilection for the extremities [3,4]. Clinically, it is characterised by patches or plaques, with or without epidermal necrosis [3,5]. Lymph nodes, spleen and bone marrow involvement is rare. The prognosis is generally poor, with a median survival of 12 months [5], and patients with subcutaneous involvement have a worst prognosis compare to those with disease limited to the epidermis or dermis [3].
We present a case of a 73-year old man who presented with pneumonia and a pustular rash on the left leg, clinically considered to be herpes zoster or Sweet

syndrome. An initial biopsy was interpreted as a neutrophilic dermatosis in keeping with Sweet syndrome. The patient was treated with Prednisolone 40mg and Dermovate, without success. Dapsone was added to the treatment with initial good response. However, when the dose of Prednisolone was reduced, the skin lesions reappeared.
Further different lesions developed consisting of reddish, variably ulcerated nodules. Skin biopsies were performed. Histology showed focal epidermal necrosis and a mild to moderate, mononuclear cell infiltrate involving the dermis and focally extending to the subcutis. The lymphoid cells were pleomorphic, with medium to large sized nuclei. By immunohistochemistry the atypical lymphoid cells were positive for CD3, granzyme and TCR-gamma, whereas CD8, CD56, Beta-F1 and EBER-1 were negative. A small proportion of reactive cells showed positivity for CD4, CD5 and CD30. The proliferation index, estimated with Ki67, was high (80%). In addition, a monoclonal rearrangement of the TCR genes was demonstrated.
These features were consistent with the histological diagnosis of cutaneous T-cell lymphoma, $\gamma\delta$- type.
PCGD-TCL is a rare entity and the diagnosis could be challenging. The differential diagnoses is wide and include neutrophilic panniculitis, subcutaneous panniculitis-like T-cell lymphoma (SPTCL), mycosis fungoides and lymphomatoid papulosis with gamma-delta TCR expression (which have an indolent clinical course) [2].

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REFLECTANCE CONFOCAL MICROSCOPY FEATURES OF PATCH-STAGE MYCOSIS FUNGOIDES AND BOWEN'S DISEASE OVERLAP WITH HORIZONTAL HISTOPATHOLOGY: A CASE SERIES WITH POTENTIAL CLINICAL APPLICATIONS

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Objectives. *In vivo* reflectance confocal microscopy (RCM) is a non-invasive technique for real-time, high resolution (horizontal ~1.25 µm, vertical ~5 µm) imaging of the epidermis and upper dermis. It is currently approved as a useful tool for the diagnosis of melanocytic and non-melanocytic skin tumors and some inflammatory and infectious diseases. RCM displays horizontal, *en face* tissue sections of the skin with maximum imaging depth of ~250 µm [1]. Mycosis fungoides (MF) and Bowen's disease (BD) have been investigated with RCM resulting in the identification of some diagnostic features [2,3]. The interpretation of such Results however has been always based on the comparison with vertical conventional histopathological sections that, as known, do not reflect the same plane of RCM images. On the other hand, the use of horizontal histopathological sections (HHS) is common in the evaluation of various types of alopecias, allowing a prompt follicular counts, density and ratio [4].
The Aim. of this paper study was to correlate RCM images of patch-stage MF and BD with HHS from skin biopsies.
Materials and methods. Eight patients (M:5, F:3; median age: 51.4 years) with suspected MF with patch lesions and seven patients (M:4, F:3; median age 56.6 years) with a clinical suspicious of BD were enrolled. For each subject, a representative cutaneous lesion was identified and evaluated by the handheld RCM device. In the selected lesion, 1 set of 30 images (1×1 mm), from the stratum corneum down to the upper dermis was taken from 3 adjacent fields. A total of 900 images were obtained and analyzed. In the same area, two 5-mm punch biopsies were performed in order to obtain histopathological vertical and horizontal sections. Biopsies for vertical sections were processed for diagnosis confirmation only. Samples for horizontal sections were added with extra paraffin to avoid wearing out the stratum corneum with initial slices. Consecutive 5 µm thick sections started from the outer layer of the stratum corneum down to the superficial dermis. Both vertical and horizontal sections were stained with standard haematoxylin and eosin and digitalized using ScanScope Digital Slide Scanner. RCM and HHS images were compared at different depths: stratum corneum, stratum granulosum/spinosum and dermoepidermal junction.
Results. A strong correlation between RCM features and HHS was observed in both MF and BD.
RCM images of patches of MF showed in the epidermis loss of the regular honeycomb pattern (epidermal disarray) and presence of small bright cells (epidermal lymphocytes) scattered throughout the layers. In some fields, darker areas compared to the surrounding epidermis were observed (spongiosis). Small bright cells were detected also at the dermal-epidermal junction, both within and surrounding roundish hyporefractive areas (dermal papillae). Moreover, the presence of lymphocytic epidermotropism with Pautrier microabscesses at RCM perfectly matched with HHS, a finding that was further confirmed with immunohistochemical staining for CD4.
RCM examination of selected cases of BD revealed a thickened stratum corneum and the presence of polygonal, refractile structures in this layer (parakeratosis). At the level of the spinous layer, a loss of the regular honeycomb pattern with marked variability in cellular size and nuclear morphology (atypical keratinocytes) was found. At the dermo-epidermal junction RCM showed rounded dark areas with central structures containing bright elements, interspersed among epidermal keratinocytes, corresponding to the dermal papillae containing

tortuous capillary vessels, arranged in a psoriasiform pattern.

Conclusion. As known in literature, RCM is a useful tool to enhance the clinical diagnosis of MF and BD by showing the peculiar features that we observed in our case series. Undoubtedly, vertical histopathology still remains the gold standard for the diagnosis of both these diseases; however, HHS represents an unequivocal control to match and enforce RCM findings. In our experience [5-7], the correlation between RCM findings and HHS further confirms the diagnostic reliability of RCM. Based on these findings, the use of RCM to select the appropriate site for biopsy sampling in suspected MF and BD lesions may represent an interesting area for future investigation, in order to avoid multiple biopsies often required for a certain diagnosis.

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WT1 IS A HELPFUL IMMUNOMARKER FOR THE DIAGNOSIS OF DERMATOFIBROSARCOMA PROTUBERANS

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Aims There is increasing evidence showing the presence of WT1 protein within the cytoplasm in several tumors, suggesting its complex regulator activity in transcriptional/translational processes (1-2). Interestingly, diffuse and strong WT1 cytoplasmic staining has been observed in both benign and malignant vascular tumors, infantile-type fibromatosis and fibrosarcoma, rhabdomyosarcoma, some neuroblastic tumors, benign and malignant peripheral nerve sheath tumors, gastrointestinal stromal tumors (GISTs) and leiomyosarcomas. The most common malignant soft tissue tumor of the dermis/subcutis is dermatofibrosarcoma protuberans. Although its diagnosis is relatively straightforward in presence of diffuse infiltration of subcutaneous adipose tissue, differential diagnostic problems may arise with cellular dermatofibroma when tumor is dermal-centered or only focally infiltrative into subcutis; similarly deep-seated benign fibrous histiocytoma may be confused with dermatofibrosarcoma protuberans due to its storiform growth pattern and frequent CD34 expression. Accordingly, apart from CD34, there is the need of additional immunomarkers for dermatofibrosarcoma protuberans. The Aim. of the present study was to investigate immunohistochemically the expression and distribution of WT1 (clone 6F-H2) in a series of 57 cases of primary and recurrent dermatofibrosarcoma protuberans and in its main morphological mimickers, such as cellular dermatofibroma and deep-seated fibrous histiocytoma.

Materials and methods. The cases were retrieved from the pathology files of Anatomic Pathology at the University of Catania and Anatomic Pathology of Santa Chiara Hospital of Trento. Clinical data were obtained from the original pathology reports. The following tumors were collected: i) 57 cases of dermatofibrosarcoma protuberans; 11 of these cases were recurrent lesions; 2 primary cases exhibited an additional fibrosarcomatous overgrowth, while 2 primary and one recurrent tumor contained a minority of giant cell fibroblastoma component); ii) 15 cases of dermatofibroma (classic type and cellular variants); iii) 8 cases of dermal scars; iv) 5 cases of deep-seated fibrous histiocytoma. Immunohistochemical analyses were performed using the standard avidin-biotin- peroxidase method (Dako autostainer link 48, Glostrup, Denmark). The antibody against the N- terminal portion of WT1(clone 6F-H2, from Dako) was used. With regard to WT1 immunostaining (both nuclear and cytoplasmic staining), the percentage of positively stained cells was assessed by semi-quantitative optical analysis according to a four-tiered system (<1% of positive cell = negative; ; 1-20% positive cells = focal staining; 21-50% positive cells = heterogeneous staining; >50% positive cells = diffuse staining). Staining intensity was graded into weak, moderate, or strong intensity.

Results. The majority of dermatofibrosarcomas protuberans, namely 95% of cases (54 out of 57), exhibited cytoplasmic staining for WT1. WT1-negative cases were represented by 3 primary, classic-type dermatofibrosarcomas protuberans. The immunohistochemical expression was diffuse, heterogeneous or focal, respectively, in 75%, 15% and 6% of cases. With the exception of 4 cases (classic-type primary dermatofibrosarcoma protuberans) showing a weak to moderate staining in different areas of the same tumor, the staining intensity was strong. All recurrent tumors showed diffuse and strong WT1 cytoplasmic immunoreactivity restricted to neoplastic cells, while the fibroblasts of the associated scar tissue were negative. Notably the neoplastic cells of both the fibrosarcomatous and giant cell fibroblastoma components, found in 2 and 3 cases, respectively, were strongly and diffusely stained with WT1. Nuclear WT1 staining was lacking in all cases.

Neither nuclear nor cytoplasmic staining was obtained in all cases of dermatofibroma (classic type and cellular variants), dermal scars, and deep-seated fibrous histiocytoma tested. WT1 was detected in the cytoplasm of

endothelial cells of intra- and extra-tumoral blood vessels, and this staining served as internal control.

Conclusions. The present study first shows WT1 is a highly sensitive immunomarker for dermatofibrosarcoma protuberans. The potential use of WT1 in routinely practice is supported by the evidence that its potential morphological mimickers, especially cellular dermatofibroma and deep-seated fibrous histiocytoma, are negative. The retained expression by the neoplastic cells, along with negative staining in scar tissue-associated fibroblasts, is helpful in evaluating residual tumor in locally recurrent tumors. Based on these findings, we emphasize that cytoplasmic expression of WT1 is of complementary diagnostic value to the CD34 in confirming the diagnosis of dermatofibrosarcoma protuberans.

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VULVAR PIGMENTED EPITHELIOID MELANOCYTOMA WITH A NOVEL *HTT-PKN1* FUSION

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Pigmented epithelioid melanocytoma (PEM) is a highly pigmented, predominantly dermal melanocytic neoplasm composed by epithelioid and spindled melanocytes. This term was introduced to encompass histologically similar or indistinguishable melanocytic lesions formerly named as "animal-type" or pigment- synthesizing melanoma and epithelioid blue nevus occurring in the setting of Carney complex (CC) or sporadically.

PEM represents a molecular heterogeneous group of low-grade melanocytic tumors characterized by a limited number of specific genomic alteration principally involving protein kinase A regulatory subunit alpha (PRKAR1A) and fusion of protein kinase C alpha isoform (PRKCA). However, in some of these neoplasms no genetic aberrations have been detected.

We performed Next Generation Sequencing (NGS) analysis of a nodular heavily pigmented intradermal proliferation composed of monomorphic epithelioid melanocytes with moderate cytologic atypia occurring on the vulva of a 24-year-old woman.

The FusionPlex Solid Tumor kit (ArcherDX) containing 288 targets in 53 genes was used, and all steps were performed following the Archer's FusionPlex protocol for Illumina (ArcherDX, Inc). The targeted genes included *AKT3, ALK, ARHGAP26, AXL, BRAF, BRD3, BRD4, EGFR, ERG, ESR1, ETV1, ETV4, ETV5, ETV6, EWSR1, FGFR1, FGFR2, FGFR3, FGR, INSR, MAML2, MAST1, MAST2, MET, MSMB, MUSK, MYB, NOTCH1, NOTCH2, NRG1, NTRK1, NTRK2, NTRK3, NUMBL, NUTM1, PDGFRA, PDGFRB, PIK3CA, PKN1, PPARG, PRKCA, PRKCB, RAF1, RELA, RET, ROS1, RSPO2, RSPO3,TERT, TFE3, TFEB, THADA*, and *TMPRSS2*.

Variant Plex Solid Tumor (ArcherDx) for the detection of single nucleotide variants (SNVs), copy number variations (CNVs), insertions and deletions in 67 genes associated with solid tumors was used.

RNA sequencing analysis identified a single fusion composed by the intact catalytic domain of the serine/threonine *PKN1* fused in frame with the N-terminal of *HTT*.

DNA sequencing analysis detected *ATM* c.1229T>C, p.(Val410Ala) missense mutation, COSM21825. No further mutations including *TERT promoter* hot spot mutation were detected.

This is the first case of PEM harboring a novel *HTT-PKN1* fusion. Our case expands the spectrum of molecular events underlying PEM, supporting the diagnosis of these rare melanocytic neoplasms.

Reports of new drivers detected by NGS analysis in challenging melanocytic lesions are of special interest, as they pave the way to larger studies. Further studies on larger series will allow to determine the frequency of *PKN1* fusion in PEM group.

PATOLOGIA GINECOLOGICA

THE ROLE OF ULTRASOUND-GUIDED FNA CYTOLOGY OF GROIN LYMPH NODES IN THE MANAGEMENT OF SQUAMOUS CELL CARCINOMA OF THE VULVA

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Objectives. Lymph node metastases are the most important negative prognostic predictor in vulvar carcinoma: an accurate preoperative assessment of suspicious lymph nodes would be therefore essential for a personalized therapy. The Aim. of this study was to assess the reliability of ultrasound-guided fine-needle aspiration cytology (FNAC) in the preoperative assessment of nodal metastatic disease in 144 patients with vulvar cancer.

Materials and methods. For the current study, we selected 144 patients who had vulvar squamous cell carcinoma with clinically and radiologically suspicious lymph nodes. The case cohort comprised 256 FNAC cases with corresponding nodal resection samples collected from 2016 to 2018 at the Catholic University Hospital (Rome, Italy). Suspicious lymph nodes were evaluated and biopsied under ultrasound guidance by clinicians and radiologists. Criteria for clinical and sonographic evaluation included lymph node size, shape (long:short axis diameter ratio), preservation of an echogenic hilum,

general attenuation and vascularity on Doppler imaging, and lymph nodes that were suspicious for metastatic disease and showed irregular margins with a loss of central echogenicity. Cytologic-histologic correlation was performed by matching the FNAC-sampled nodule with the corresponding histological diagnosis. After the FNAC procedure, all patients underwent wide local excision of the vulvar mass followed by bilateral inguinofemoral lymphadenectomy and radiotherapy if the tumor involved a lymph node or the vulvar margins.

Results. In our study, 87 patients had negative inguinal lymph nodes at histology and 57 had positive lymph nodes. A total of 256 groins were analyzed: at histology 171 were negative and 85 showed at least one metastatic LN. Cytological examination agreed with the subsequent histology in 220 of 256 cases (86%). Our data confirmed that 60 of 85 FNACs had a histologically confirmed metastatic diagnosis, with 25 false-negatives and no false-positive Results. The number of false-negative Results, as also mentioned in other series, might be attributed to an erroneous sampling in which the aspirate failed to extract neoplastic cells. In fact, in 18 cases, the extent of metastatic involvement consisted of a solitary neoplastic focus of 1 mm; 4 cases showed a single metastatic focus of 4 mm; and, in the remaining 3 cases, the extent of metastatic involvement was 6 mm. The false-negative rate, with a sensitivity of 76.92%, represents important preoperative data, requiring a possible repetition of FNAC or, alternatively, sentinel lymph node sampling in this subset of women. Furthermore, even the preparation of a second slide from the liquid-based, stored material did not add any further diagnostic information. On the basis of our Results, we also investigated whether the preoperative FNAC examination of a single suspicious lymph node may predict the nodal status of subsequent lymphadenectomy chain specimens. The statistical analysis of our cases found 100% positive predictive value and 62% negative predictive value. In fact, in our series, all patients who had positive cytology had metastatic disease identified in other surgically resected lymph nodes of inguinofemoral lymphadenectomy specimens. By contrast, 65 of 171 (38%) negative FNAC samples showed metastatic disease in subsequent lymphadenectomy specimens. In these latter patients, the extent of metastatic foci ranged from 0.1 to 0.6 cm. Therefore, we conclude that a positive FNAC result in suspicious lymph nodes is highly reliable in predicting further metastatic nodes. Conversely, a negative FNAC result does not exclude the presence of metastatic disease in both the same and other inguinofemoral lymph nodes. However, according to the surgical status of resected lymph nodes, we observed that negative FNAC Results were always associated with a subcentimetric metastatic deposit. As a result, we may speculate that, in the presence of a negative cytology result, there is a low probability of extensive metastatic disease in other lymph nodes and, mostly, we would expect a subcentimetric metastasis.

Conclusions. To date, the main diagnostic techniques used to preoperatively detect metastatic lymph node disease in vulvar cancer are represented by computed tomography and magnetic resonance imaging; however, both have demonstrated low specificity and sensitivity. Therefore, the common practice to improve patient outcome is represented by inguinofemoral lymphadenectomy. Actually, sentinel lymph node biopsy has become increasingly accepted as an alternative method to lymphadenectomy, especially in the management of patients with early-stage vulvar cancer. The intraoperative evaluation of the sentinel lymph node, representing the first regional station that drains from the primary tumor, offers a high chance to define the negative or positive involvement of the entire regional lymph nodes. In this regard, intraoperative diagnosis would avoid unnecessary lymphadenectomies in patients with early-stage vulvar cancer. Nonetheless, another useful diagnostic tool is represented by ultrasound combined with FNAC. On the basis of these observations, FNAC of suspicious lymph nodes might represent a useful tool in the management of patients with vulvar cancer. In fact, given the 100% specificity of this technique, as also demonstrated in previous studies, a positive result enables the surgeon to immediately perform a bilateral inguinofemoral lymphadenectomy, thus avoiding an unnecessary sentinel lymph node sampling. Moreover, as we demonstrated, FNAC examination is also useful in predicting the presence and extent of metastatic disease in other surgically resected inguinofemoral lymph nodes. Furthermore, the role of FNAC in the management of patients with vulvar cancer is suggested by the fact that FNAC is generally considered easy to perform, reproducible, not expensive, well tolerated by patients, and has encouraging levels of accuracy also in groin lymph nodes.

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MUTATIONAL AND IMMUNOPHENOTYPIC PROFILING OF A SERIES OF 8 TUBO-OVARIAN CARCINOSARCOMAS REVEALED A MONOCLONAL ORIGIN OF THE DISEASE

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Background. Carcinosarcomas manifest a combination of carcinomatous and sarcomatous elements and as such, they constitute fertile ground for the study of intratumour heterogeneity.

Materials and methods. In this study we characterize a series of 8 tubo-ovarian carcinosarcomas for the immunhistochemical expression of epithelial (MNF116, EMA), mesenchymal (Vimentin), neural (S100), neuroendocrine (chromogranin, synaptophysin), muscular (Desmin, Myogenin) and p53 markers, and for the mutational profiling of *KRAS*, *BRAF*, *PIK3CA*, *NRAS*, *TP53* and *DICER1* genes.
Results. Heterologous differentiation was present in six of eight tumors. The carcinomatous components resulted positive for epithelial markers in all of the cases, while two demonstrated additional positivity for neuroendocrine markers. The sarcomatous components were all diffusely positive for vimentin, while only 87.5% and 50.0% of them expressed MNF116 and EMA respectively; rhabdomyosarcomas were positive for desmin and MYF-4, while chondrosarcomas were positive for S100. Regarding p53, all but one tumor showed similar immunoreactivity in both the carcinomatous and sarcomatous components while one case showed a peculiar cytoplasmatic expression. Three of eight cases (37.5%) showed *TP53* mutations, and in two cases the *TP53* mutation was shared by both epithelial and mesenchymal components. *DICER1* mutation was found in all components of one case. Mutations in *KRAS, NRAS, BRAF and PIK3CA* genes were not found in the study cohort.
Conclusions. Our Results highlight the heterogeneity of ovarian carcinosarcomas at phenotypic level. A common mutational signature was observed in both components in 3 out of 4 informative tumors. More studies are required to dissect different levels of ovarian carcinosarcomas heterogeneity in order to define the best therapeutic approaches to these neoplasms.
Key words: Ovarian tumors; carcinosarcoma; mutational analysis; immunohistochemistry

ADENOID CYSTIC CARCINOMA (ACC) OF THE BARTHOLIN'S GLAND: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF A RARE CASE

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Introduction. The primary carcinomas of the Bartholin's gland (BGs), is a rare tumor that accounts for less than 1% of all malignant tumors of the female genital tract. A 66-year-old woman for about two years, in correspondence with the posterior third of the left labium minus, presents a slow growing solid neoformation with lobulated margins of 17 mm x 20 mm, covered with intact mucosa, considered in a first time as a cyst. Given the persistence of the lesion and its tendency to increase, it is subjected to excision.
Materials and methods. The surgical sample is represented by a nodular formation of cm 3 x 3, of pink color, with a granular and shiny surface, of sustained consistency. The material is fixed in formalin and in paraffin embedded. The sections stained with hematoxylin and eosin, PAS and Blu Alcian. The tissue is subjected to a panel of immunohistochemical antibodies: CKAE1/AE3, CK5/6, CK7, CK19, CK20, EMA, p63, SMACT, S-100, CD10, CD34, CD117, ER, PgR, CEA, p53 and Ki67. The morphological aspect of the lesion is clearly neoplastic. For the majority is represented by acinar structures of cribriform appearance, in which the holes are delimited by small flattened cells and are occupied by a dense, weakly basophilic material, PAS and Blue-Alcian positive. Scattered in this context are solid, nodular-looking cell agglomerates, consisting of small roundish, mononuclear cells with poor cytoplasm and a hyperchromatic nucleus. Some of these agglomerates have an entirely solid appearance, while others show a mixed aspect in which the cribriform component is also represented to varying degrees. The latter starts from the periphery of the nodule progressing in a centripetal direction until it is completely occupied. Neoplastic proliferation also occurs in the form of tubular structures delimited by a monolayer cylinder-cubic epithelium above a basal layer consisting of small-medium roundish mononuclear elements. This component, however, is completely minor, accounting for no more than 10% of the proliferation. Also found, is a diffuse perineural permeation and infiltration of periglandular structures. The Results of immunohistochemical research are in cribriform pattern (CKAE1/AE3 +, CK5/6 +, CK7 +, CK19 -/+, CK20 -, EMA +, CEA -, p63 +, SMACT +, S-100 + in neural structures, CD10 -, CD34 -, CD117 -, ER -, PgR -, p53 -/+ and Ki67 >5%), in solid pattern (CKAE1/AE3 +, CK5/6 -, CK7 -, CK19 -, CK20 -, EMA -/+, CEA -, p63 +, SMACT +, S-100 -, CD10 -, CD34 -, CD117 -, ER -, PgR -, p53 -/+ and Ki67 >5%), and in tubular pattern (CKAE1/AE3 +, CK5/6 + luminal, CK7 +, CK19 + luminal, CK20 + luminal, EMA -, CEA +, p63 +, SMACT +, S-100 -, CD10 -, CD34 -, CD117 -, ER -, PgR -, p53 -/+ and Ki67 >5%). The diagnosis of Adenoid Cystic Carcinoma of the Bartholin's Gland was performed.
Discussion and conclusions. In a study of 2011 are retrieved from the literature 79 cases of ACC with an incidence of about 15% of all tumors of BGs gland, in a subsequent paper of 2017, based on the study of 275 cases of primary tumors of BGs obtained from the literature, 77 were ACC, with an incidence of about 30%. In this study, the various tumors are classified by histotype: squamous cell carcinoma 80 (30.7%), adenoid cystic carcinoma 77 (29.6%), adenocarcinoma 65 (25%), transitional cell carcinoma 7 (2.6%), sarcoma 7 (2.6%), neuroendocrine carcinoma 3 (1.1%), adenosquamous 3 (1.1%), epithelioid-myoepithelial carcinoma 2 (0.7%), rare others 16 (6.1%). The adenoid cystic carcinoma of the BGs, accounting for only 0.30% of all female genital tract cancers, is considered a rare malignancy and, therefore, worthy of study and signaling. Like all neoplasms of the BGs, adenoid cystic carcinoma (ACC) has no particular clinical characteristics and is confused with the most frequent abscesses or cysts of the gland. In the various reports, the most constant sign at presentation was vulvar mass in 147 (53.5%). Nevertheless, despite its indolent course, it has a high rate of local recurrence and hematogenous metastatization, especially to the lungs. Characteristic of this neoplasm is the perineural penetration which explains the high recurrence rate even after complete tumor excision. Before starting the discussion of our case it is opportune to refer to the structure and immunohistochemical characteristics of Bartholin's gland. The Bartholin's gland contain three types of

epithelium. The glandular acini are lined by mucinous columnar epithelium. This merges in the ducts with a transitional epithelium and becomes squamous epithelium at the ostia which open on the vestibule vaginalis. The various histological types of tumors present in BGs would be linked to the cell line from which it would take origin. Squamous carcinomas would originate from squamous orificial cells, transitional carcinomas from transitional cells of the excretory duct, adenocarcinoma from acinar cells. More complex, instead, the histogenesis of the adenoidocystic carcinoma similar to that of morphologically similar tumors arising, more frequently, in other locations such as the salivary glands, in the first line, upper respiratory tract, nasopharynx, breast, uterine cervix and brain. It is hypothesised by some Authors that such neoplasms may originate from the reserve cells present in the intercalated small ducts of Bartholin's gland that may have the potential to differentiate into two cell types, myoepithelial and luminal cells. As already noted by various Authors, also in our case, this histotype presents, variously intermingled three morphological patterns: one acinar cribriform; one solid, consisting of small mononuclear cells with a poor cytoplasmic halo and another tubular, consisting of luminal cubic cylinder elements below which a layer of basal cells of small volume is present. In our case, the three patterns are irregularly mixed, with a clear prevalence of that acinar/cribriform. An article specifically devoted to the tumors of Bartholin's gland, for the ACC reports the following immunohistochemical profile: CKAE1/AE3 +, CK8/18 +, SMA +, SMM +, p63 +, S-100 + and EMA +. Overall, the immunophenotypic profile of our case is in line with the data reported in the literature. The expression of various antigens in different morphological patterns allows us some histogenetic considerations hitherto never advanced. The "*primum movens*" would be identified in the solid nodules generated by the proliferation of elements with a morphologic and immunophenotypic myoepithelial profile. From the periphery of these nodules begins the morphological and immunochemical epithelial differentiation that leads to the formation of the cribriform acinar structures and to the tubular ones. Also in our case, there are evidences of the nerve structures permeation phenomena of the surrounding tissues that made the prognosis of these neoplasms very severe as reported several times in the literature.

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LOSS OF PTEN EXPRESSION AS DIAGNOSTIC MARKER OF ENDOMETRIAL PRECANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS.

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Introduction. Endometrial hyperplasias (EH) includes both benign proliferations, caused by unopposed estrogens action, and premalignant lesions. These two conditions are differentiated by two possible histologic classifications: the World Health Organization (WHO) classification, based on cytologic atypia, disregarding glandular complexity, and the endometrial intraepithelial neoplasia (EIN) classification, based on three morphologic parameters (glandular crowding, lesion diameter >1mm, cytology different from adjacent endometrium) and a careful exclusion of benign mimics and cancer[1]. The 2017 European Society of Gynaecological Oncology guidelines recommend the use of immunohistochemistry for tumor suppressor protein phosphatase and tensin homolog (PTEN) to improve the differential diagnosis[1,2], because the mutation of PTEN is the most common molecular alteration found in endometrial carcinogenesis[3,4] and occurs in an early phase[2,4]. We Aim.ed to assess the diagnostic accuracy of immunohistochemistry for PTEN in the differential diagnosis between benign and premalignant endometrial hyperplasia.

Materials and methods. Electronic databases were searched from their inception to May 2018. Studies assessing PTEN immunohistochemistry in endometrial hyperplasia specimens were included. PTEN status ("loss" or "presence") was the index test; histological diagnosis ("precancer" or "benign") was the reference standard. Sensitivity, specificity, positive and negative likelihood ratios (LR+, LR-), diagnostic odds ratio (DOR), and area under the curve (AUC) on summary receiver operating characteristic curves were calculated (95% CI), with a subgroup analysis based on the histologic classification adopted (WHO vs EIN).

Results. Twenty-seven observational studies with 1736 cases of endometrial hyperplasia were included. Pooled estimates showed low diagnostic accuracy: sensitivity 54% (95% CI 50%-59%), specificity 66% (63%-69%), LR+ 1.55 (1.29-1.87), LR- 0.72 (0.62-0.83), DOR 3.56 (2.02-6.28), AUC 0.657. When the WHO subgroup was compared with the EIN subgroup, higher accuracy (AUC 0.694 vs. 0.621), and higher heterogeneity in all analyses, were observed.

Conclusions. Immunohistochemistry for PTEN showed a low diagnostic usefulness in the differential diagnosis between benign and premalignant EH, independently from the histologic classification used (WHO or EIN), although a loss of PTEN expression was associated with endometrial precancer. In the absence of further evidence, the recommendation about the use of PTEN for this purpose should be reconsidered.

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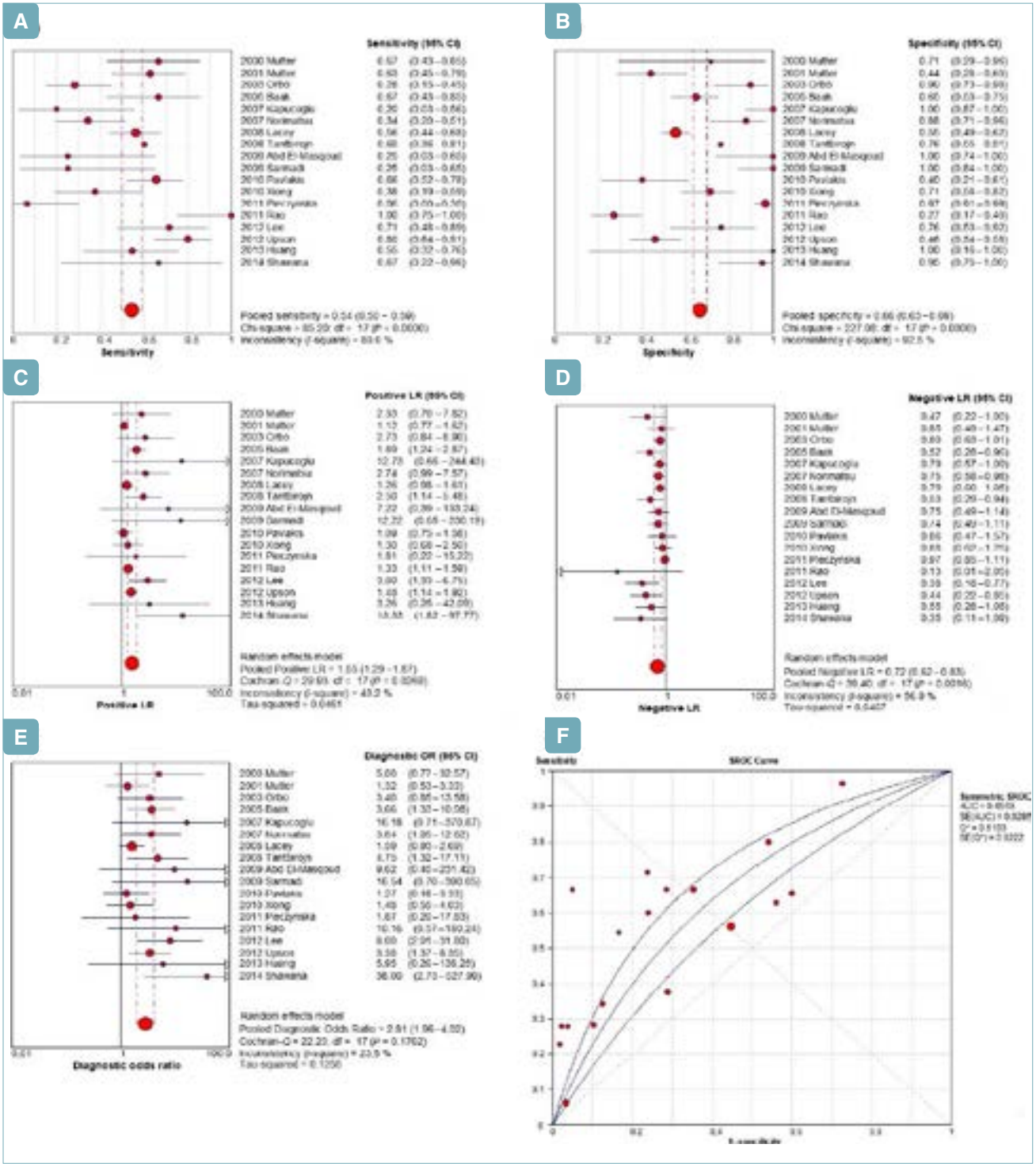

Fig. 1. Forest plots of individual studies and pooled sensitivity (A), specificity (B), positive likelihood ratio (C), negative likelihood ratio (D), and diagnostic odds ratio (E) of PTEN immunohistochemical assessment in differential diagnosis between benign and premalignant endometrial hyperplasia, with summary receiver operating characteristic curves (F).

P63 IMMUNOCYTOCHEMISTRY IN THE CYTOLOGIC DIFFERENTIAL DIAGNOSIS OF SQUAMOUS AND GLANDULAR LESIONS OF THE CERVIX UTERI

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Objectives. The distinction between primary glandular lesions of the cervix uteri and squamous lesion with glandular involvement may be challenging in gyneco-logical cytology[1].

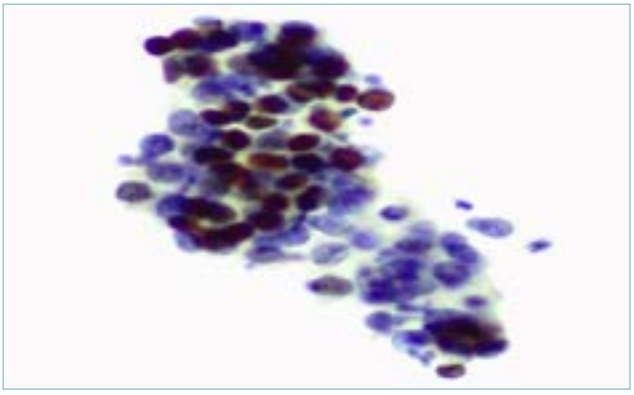

Fig. 1. Abnormal cells positive nuclear staining for p63 confirms their squamous origin (20x).

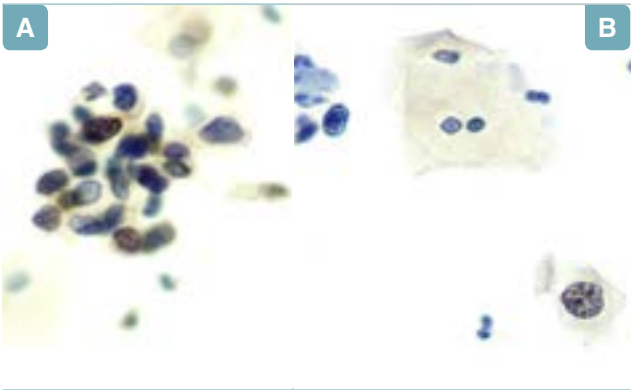

Fig. 2. A) abnormal metaplastic cells positive nuclear staining for p63 confirms their squamous origin (20x); B) abnormal intermediate cells and dysplastic nuclei, positive nuclear staining for p63 confirms their squamous origin (40x).

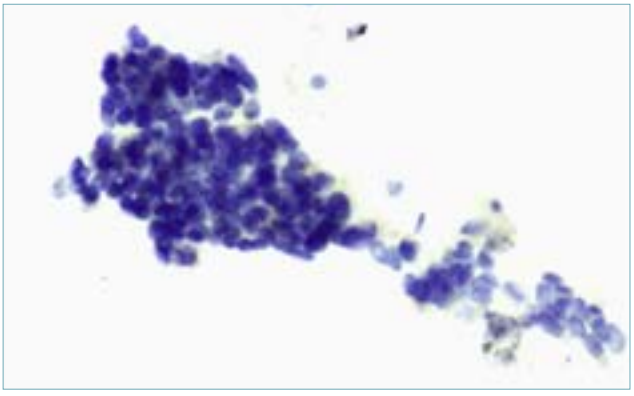

Fig. 3. Abnormal glandular cells negative nuclear staining for p63 confirms their ghiandolar origin (20x).

The Aim. of this study is to evaluate the use of p63 immunocytochemistry in the differential diagnosis between glandular and squamous lesions in cervicovaginal specimens.

Materials and methods. 23 liquid-based cervicovaginal specimens (13 HSIL with features consistent with endocervical involvement; 4 HSIL without features of endocervical involvement; 4 AIS ; 2 AGC) were collected over a 1-year period. AIS and HSIL cases have been confirmed by cervical biopsies while AGC cases had at least cytologic follow-up. Cytologic specimens were obtained using a Pap Test Perfect® and CytoBrush Plus GT ® (Hologic Medscand® Sample Collection Kit) and immediately immersed in fixative solution (PreservCyt® Hologic).Vials were placed into the ThinPrep Processor 5000 AutoLoader (Hologic) [2-3]. Cytologic routine diagnosis was based upon cytopathologic criteria alone[4]. For study purposes, all cases were stained with the p63 monoclonal antibody (7:JUL Leica) using the automated BOND III system (Leica).

Results. In all (100%) thirteen HSIL cases with features of endocervical involvement, p63 was at least focally expressed in hyperchromatic cell groups, although also several p63-negative dysplastic nuclei were evident in all cases (Fig. 1). Four (100%) HSILs without features of endocervical involvement displayed positivity for p63 with variable intensity (Fig 2a-2b). Of the four AIS cases, three (75%) were p63 negative and one (25%) was p63 positive (Fig.3) .

In the two AGC cases one showed only focally and weakly positive p63 staining; the remaining specimen was p63 negative. Both cases had a negative follow-up.

Conclusions. Immunocytochemistry with p63 may increase the cytomorphologic accuracy in cases with hyperchromatic crowded cell groups, particularly when the differential diagnosis between primary glandular lesions of the cervix and squamous lesion with glandular involvement is challenging. Since p63-negative HSIL cells may occur, the interpretation of the p63 immunocytochemistry should however always take in account the morphological features of the dysplastic cells.

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TIME TRENDS IN VULVAR SQUAMOUS CELL CARCINOMA INCIDENCE RATES IN ITALY (1990-2015)

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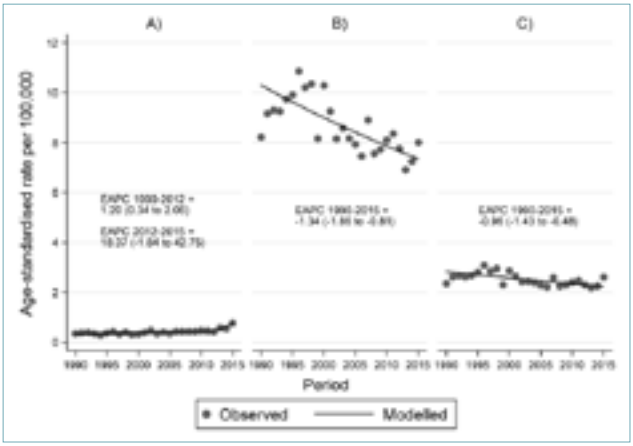

Fig. 1. Trends of incidence rate by age group (A = <60 years; B = ≥60 years; C = whole population).

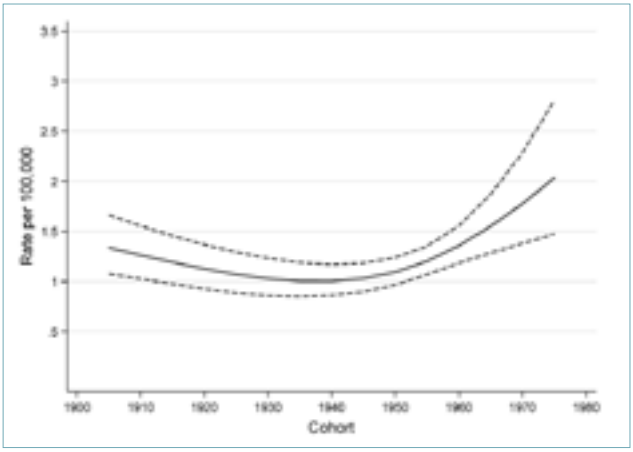

Fig. 2. Curve of incidence rates (1990-2015) by birth cohort, adjusted for the age and period effect.

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Objectives. The incidence of vulvar squamous cell carcinoma (VSCC) has increased for decades in most Western countries (1). This trend has been more pronounced among, and even restricted to, women aged < 50-60 years. The incidence trends in southern Europe have not been studied in a formal fashion. We report a study of time trends in incidence rates of VSCC in Italy.
Methods. Data were obtained from the database of the Italian Association of Cancer Registries (AIRTUM), which currently covers about 70% of total national population. 35 registries participated in the study, covering about 50% of the Italian female population (15,000,000 women). Each registry covered a time period from 3 to 26 years (median 20 years). Vulvar invasive squamous carcinomas (ICD-O 3 classification III revision codes C51, M8050-8084) incident from 1990 to 2015 were included in the study (n. 6,294). Geographic areas (northern, central, and southern Italy) were defined according to the AIRTUM. Age-standardized (Europe) incidence rates were calculated. Data were analyzed based on joinpoint regression models, with calculation of the annual percent change (APC), and age-period-cohort models.
Results. Total incidence showed a regular and significant decreasing trend (APC, -0.96; 95% confidence interval (CI), -1.43 to -0.48). This was entirely due to a significant decrease among women aged 60 years and over (APC, -1.34; 95% CI, -1.86 to -0.81). For younger women, APC was 1.20 (95% CI, 0.34 to 2.06) with a non-significant acceleration thereafter (Fig. 1). This pattern did not vary across geographic areas, nor after exclusion of those registries that contributed data for < 50% of the 26-year study period. The age-period-cohort analysis revealed a risk decrease in cohorts born between 1905 and 1940 and a new increase in cohorts born since 1945 (Fig. 2). This pattern of incidence was substantially confirmed by sensitivity analysis: for women aged ≥60 years, the magnitude of the decreasing trend was marginally modified when any of the three geographic areas was removed from the models and when the analysis was restricted to long-duration registries. The magnitude and the downward direction of the trend observed between 1990-2012 among women aged <60 years were also roughly confirmed.
Conclusions. The decreasing incidence trend of VSCC observed among older women, and the resulting decrease in total rates, are at variance with reports from the greater part of Western countries (2). The upward trend observed among younger women, compatible with an increasing prevalence of human papillomavirus infection, is in line with expectations. In this type of analysis, the cohort effects usually result from changes in lifestyle habits leading to differences in the prevalence of risk factors between different generations. Long term trends of HPV, obesity and smoking prevalence (decreasing in the earliest generations, increasing in younger ones) appear suggestive hypotheses to explain the observed Results (3). Risk factor prevalence requires careful prospective surveillance, given its potential to reverse the current favourable situation.

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A CLINICALLY APPLICABLE INTEGRATED MOLECULAR, IMMUNOHISTOCHEMICAL AND HISTOLOGICAL APPROACH FOR ENDOMETRIAL CARCINOMA

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Background and objectives. Endometrial carcinoma (EC) is a heterogeneous disease and it is becoming increasingly clear that this heterogeneity may be a function of the diversity of the underlying molecular alterations. The integrated genomic analysis by The Cancer Genome Atlas (TCGA) has revealed that EC can be divided into at least four distinct molecular subtypes. These include the ultramutated subtype, defined by mutation in the exonuclease domain of DNA *Polymerase epsilon (POLE)*; the hypermutated, microsatellite-unstable subclass, with deficiency of one or more mismatch repair proteins (MMRd); the copy number-high subtype, which is characterized by mutations in *p53*; and the copy number-low subtype that does not have a surrogate marker.
These subtypes carry significant prognostic as well as predictive information; embracing and incorporating them into clinical practice is thus essential.
The Aim. of our study was to compare and integrate the current histological WHO classification and the surrogate molecular (SM) subtypes, correlating them with important clinico-pathological features.
Materials and methods. A cohort of 91 EC patients who underwent surgical resection with curative intent was enrolled. All cases were reviewed and classified according to the current WHO classification. ECs were also classified into 4 SM subgroups: p53 abnormal, based on mutant-like immunostaining (p53abn); MMR deficient, based on loss of mismatch repair protein expression (MMRd); presence of POLE exonuclease domain hotspot mutation (POLE); no specific molecular profile (NSMP), in which none of these aberrations were present. The clinical and pathological features included: age, BMI, familiarity, FIGO grade, lymphovascular invasion, lymph nodes metastases, myometrial invasion, microcystic elongated and fragmented (MELF) pattern of myometrial invasion, tumor budding, presence of necrosis, stromal and intraepithelial tumor-infiltrating lymphocytes (sTILs and iTILs). Sanger sequencing and Next Generation Sequencing were used to evaluate mutations in the POLE.
Protein expression for mismatch repair (MMR) proteins was evaluated by immunohistochemistry (IHC). MMR alterations were confirmed by microsatellite instability (MSI) analysis using ten mononucleotide repeat markers and methylation status of the MLH1 promoter was measured by a fluorescent bisulfite polymerase chain reaction (PCR).
Results. Complete clinico-pathological data were evaluable from 91 women. The median age of the patients was 63 years, and the median BMI was 26. Lymph nodes metastases were detected in 20 out of 77 cases (26%). Histotype classification included 59 (64.8%) endometrioid carcinomas, 14 (15.4%) undifferentiated/dedifferentiated carcinomas, 13 (14.3%) serous carcinomas and 5 (5.5%) carcinosarcomas. Grade distribution included 25 (27.5%) G1, 26 (28.6%) G2, and 40 (43.9%) G3 tumors. Molecular classification yielded 26 (28.6%) MMR-D, 7 (7.7%) POLE, 27 (29.7%) p53abn, and 31 (34%) NSMP. MMR-D tumors include 17 endometrioid carcinoma and 9 undifferentiated/dedifferentiated carcinomas. POLE mutated tumors include 6 endometroid carcinoma and 1 undifferentiated/dedifferentiated carcinoma. p53-mutant tumors include 8 endometrioid carcinomas, 1 undifferentiated/dedifferentiated carcinoma, 13 serous carcinomas and 5 carcinosarcomas. NSMP tumors include 28 endometrioid carcinomas and 3 undifferentiated/dedifferentiated carcinomas. Histological classification showed significant statistical association with familiarity (p=0.009), FIGO grade (p<0.0001), lymphovascular invasion (p=0.0003), lymph nodes metastases (p=0.04), presence of necrosis (p=0.04), but not with age, BMI, myometrial invasion, MELF pattern of invasion, tumor budding, sTILs and iTILs. Conversely, the surrogate molecular subtyping statistically correlated with FIGO grade (p<0.0001), presence of high sTILs and iTILs (p=0.01 and p=0.0009), MELF pattern of invasion (p=0.01), tumor budding (p=0.002), but not with age, familiarity, BMI, myometrial invasion, lymphovascular invasion, lymph nodes metastases and presence of necrosis.
Conclusion. The surrogate molecular subtyping of endometrial carcinoma is a cost-effective method that allows testing on formalin-fixed paraffin-embedded tissue for genomic-based molecular classification of EC in routine practice. Both histological and molecular characterizations are correlated with different, but important clinico-pathological features. An integrated approach may better define risk assessment, representing a step towards precision medicine and individualized therapies.

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PATHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES OF OVARIAN NEUROENDOCRINE TUMOR: A SINGLE CENTER EXPERIENCE

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Background and objectives. The current pathologic classifications of neuroendocrine neoplasms (NENs) across different organ systems use a range of site-specific terminologies and criteria, creating significant confusion among pathologists and treating clinicians. Primary neuroendocrine tumors of the ovary are exceptionally rare entities accounting for approximately 0.1%

of all ovarian neoplasms. Most of them are clinically benign carcinoid tumors arising in dermoid cysts or rarely identified in other ovarian neoplasms such as germ cell tumor, yolk sac tumor, Brenner tumor and Sertoli-Leydig cell tumor. In the 2014 WHO classification of ovarian tumors, there is no separate category of NENs. This is a shortcoming of the 2014 classification. In the ovary, the NEN types included in WHO 2014 are: (1) carcinoid tumor (subtypes of insular, stromal, trabecular and mucinous carcinoid), which is included in the category of monodermal teratoma and somatic-type tumors arising from a dermoid cyst; (2) small cell carcinoma, pulmonary type; the latter is essentially a SCNEC which is included in the category of miscellaneous tumors and must be distinguished from ovarian small cell carcinoma of hypercalcaemic type. The current classification is only based on morphology and differ from the uniform standard classification terminology for gastroenteropancreatic (GEP) NENs.

The Aim. of the study was to analyze the clinico-pathological and immunohistochemical features of a case series of ovarian neuroendocrine neoplasms.

Materials and methods. Pathology files of "Policlinico di S. Orsola", Bologna, Italy, (2009-2019) were searched, and clinical data were collected from medical records. All cases were reviewed according to the WHO 2014 classification. Ki-67 proliferation index was assessed by digital imaging analysis in order to reclassify the cases according to the GEP NEN classification. Immunohistochemistry for Synaptophysin, Chromogranin, Serotonin, SSTR2a, SSTR5, CDX2, TTF1 and PAX8 was performed.

Results. A total of 27 cases were found, including 12 primary and 15 metastatic neoplasms (appendix, ileum, pancreas, lung, stomach and colon). The median age of the patients was 43 years.

According to the WHO 2014 classification, 6 cases were diagnosed as insular carcinoids, 2 as trabecular, 3 as struma-carcinoids, 1 as ovarian small cell carcinoma of pulmonary type. The neuroendocrine neoplasms were associated with mature cystic teratoma (6 cases), malignant struma ovarii/papillary carcinoma (1 case), immature teratoma (1 case), mucinous cistoadenocarcinoma (1 case), sero-mucinous cistadenoma (1 case), Sertoli cell tumor (1 case). One was a pure ovarian small cell carcinoma of pulmonary type. All carcinoids tumors were staged as pT1a, while the ovarian small cell carcinoma of pulmonary type as pT3c. According to the GEP NEN classification, including Ki-67 proliferation index, 7 cases were classified as NET G1, 3 as NET G2, 1 as NET G3 and 1 as NEC.

According to the immunohistochemical profile, all insular carcinoids were positive for Synaptophysin, Chromogranin, Serotonin and CDX2, with strong expression of SSTR2a, and were negative for SSTR5, TTF1 and PAX8. All struma-carcinoids were positive for Synaptophysin, Chromogranin, with variable expression of SSTR2a, and were negative for Serotonin, CDX2, SSTR5, TTF1 and PAX8. Trabecular carcinoid was positive for Synaptophysin, Chromogranin and SSTR5 and negative for the other markers. Small cell carcinoma of pulmonary type was positive for Synaptophysin, Chromogranin, and TTF1 and was negative for the other markers.

Conclusion. The current morphological classification showed a consistent distinct immunoprofile for each subtype. Nevertheless, the application of GEP NEN classification, comprehensive of Ki-67 proliferation index, is feasible and better discriminates tumor grade. This approach substantiates the importance of a common classification for neuroendocrine neoplasms reducing inconsistencies and contradictions among the various systems currently in use, allowing a more uniform diagnosis and clinical management.

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EFFECT OF ANTI-PARP-THERAPY IN PATIENTS WITH OVARIAN SEROUS HIGH-GRADE CARCINOMA: THE PREDEICTIVE VALUE OF BRCA1 E PARP1 IMMUNOHISTOCHEMISTRY

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Objectives. In the last years, PARP inhibitors were applied for maintenance therapy of high-grade ovarian serious carcinoma (HGOSC) following first-line chemotherapy, as usually suggested in triple negative breast carcinoma cases. Results in HGOSC are more encouraging than in breast carcinoma cases; therefore, anti-PARP therapy is often approved as first-line treatment. Our Aim. is to provide a classification of HGOCS patients basing on the immunohistochemical expression of BRCA1 and PARP1-associated proteins. Thereafter, in this paper we examine the effect of anti-PARP therapy on vital signs. Anti-PARP delivery may depend on the therapeutic response to the tumour, basing on its biological characteristics.

Materials and methods. We studied 19 HGOSC patients receiving surgery and standard chemotherapy (platinum ± taxolo) as well as anti-PARP maintenance treatment. Following common therapeutic indications, Olaparib was administered to 11 patients presenting BRCA1/2 genome mutation as well as Niraparib was applied to 8 wild type cases.

As group on control, we took into account 22 HGOSC patients treated with standard chemotherapy (platinum ± taxolo) between 2010-13 without any maintenance treatment.

This cohort of patients have several common elements. The average age of the focus group is 55.9 as well as 53.9 is the median age of the group on control. The first group is composed by patients with III-IV stage cancer and just 1 patient with I stage cancer (in comparison to 4 cases with I stage cancer of the group on control).

On one hand, we applied Clone MS-110 (Abcam) in order to identify BRCA1-associated protein. On the

other hand, we used the polyclonal antibody by Thermoscientific to identify PARP1-associated protein. We evaluated positive reactions considering the nuclear positive reaction.
Results. The BRCA1 expression was positive 15/19, within a range between 10% and 60%; the PARP expression goes from 15% to 70% without any negative cases. In the group on control, positivity to BRCA1 expression varies within 10% and 80% as well as within 10% and 70% with just one negative case.
The average survival rate is 13 months in group 1 and 82.9 months in the group on control.
Focus group: Comparing DFS and BRCA1 expression, the xy chart shows that the highest the immunohistochemical positivity, the lowest the overall surviving time of patients. Correlation coefficient is r=0.41665. The relation between DFS and PARP1 expression is represented by the value r=0.09, showing a non-correlation between both parameters.
Group on control: Comparing DFS and BRCA1 expression, the xy chart shows that the highest the immunohistochemical positivity, the lowest the overall surviving time of patients. Correlation coefficient is r=0.678. The relation between DFS and PARP1 expression is represented by the value r=0.21, showing a non-correlation between both parameters.
Conclusions. Previous studies on immunohistochemical evaluation of BRCA protein associated to ovarian carcinoma are insufficient and incomplete. Garg et al (2013) identified the "normal" expression in cases having evident expression and the "abnormal" expression in cases showing equivoque or negative expression. According to those authors, the "normal" expression seems to be related to a germline or somatic mutation or to a methylation status. Therefore, low immunohistochemical expression of BRCA1 may represent a group of women with a BRCA mutation or a BRACA-ness condition, both having a far better prognosis, whether anti-PARP administration or not.

PRIMITIVE UTERINE RHABDOMYOSARCOMA OF THE UTERUS IN ADULT PATIENTS: A SYSTEMATIC REVIEW OF A RARE ENTITY

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Objectives. Primitive Uterine Rhabdomyosarcoma (PUR) of the uterus in adult patients is an exceedingly rare entity. Scientific literature is limited to case reports and small series. We reported one case and reviewed the literature.
Materials and methods. PubMed research for "Rhabdomyosarcoma + Uterus" disclosed a total of 197 items. The research has been restricted to English written full papers on adult (19+) patients for the period 2000 - 2018. A total of 44 papers have been reviewed. 18 papers have been excluded and 46 patients (from 26 papers) and a case of our observation have been considered for the purpose of the study.
Results. Mean age resulted 51.15 years (19-79). Uterine corpus appeared the primitive site of the neoplasm in 30 cases while the remaining 16 cases originated from the cervix. In both groups bloody vaginal discharge and abdominal swelling were the main onset symptoms.
20 cases have been diagnosed as Embryonal/Botryoid, 17 cases as Pleomorphic, 7 cases as Alveolar, 1 case as Mixed and 1 case as Spindle. All the cases but 3 underwent to radical hysterectomy variably completed by peritoneal sampling, lymph nodal dissection and omentectomy; in the remaining 3 cases surgery was limited in 1 case to cone biopsy, in 1 case to polypectomy and in 1 case to incisional biopsy. 31 patients were treated also with chemotherapy with variable regimens, 1 case with radiotherapy. The remaining 12 reported cases died of the disease before accessing to cytotoxic therapies.
Globally 18 patients died of the disease (DOD); 5 were alive with disease (AWD) and 17 were negative for residual disease (NED). Mean follow up time was 20.55 months with a marked variability (3 days - 125 months). Differences exist between the histological subtypes. Pleomorphic rhabdomyosarcoma seems to originate exclusively from the corpus of elderly women (mean age 65.53 years) and is aggravated by a worse prognosis (11 DOD, 3 AWD, 2 NED) while Embryonal/Botryoid rhabdomyosarcoma seems to emulate its pediatric counterpart arising more frequently on the cervix of younger women (mean age 51.15 years) and carrying a better prognosis (4 DOD; 1 AWD; 11 NED) and even a successful fertility sparing treatment is reported.
Conclusions. Uterine primitive rhabdomyosarcoma appears globally as a rare entity with relevant differences among its histologic subtypes. A thorough histologic examination is required to exclude a heterologous differentiation in a mixed neoplasm and to correctly identify the specific subtype. Due to its rarity the most appropriate treatment protocol remains to be elucidated.

PRIMARY ENDOMETRIOID ADENOCARCINOMA OF THE VULVA: THE FIRST REPORT.

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Objective. Primary adenocarcinomas of the vulva are uncommon epithelial malignancies accounting for about 2% of all vulvar neoplasms. Although rare, these tumours encompass a wide spectrum of glandular neoplasms including extramammary Paget's disease, sweat gland carcinomas, adenocarcinoma of the mammary gland type, apocrine adenocarcinomas, Bartholin's gland adenocarcinoma, cloacogenic tumors and neuroendocrine carcinomas.
To the best of our knowledge, the endometrioid variant arising in the vulva has never been reported in literature and only a few authors described its occurrence as a

metastasis from an endometrial primary tumor or associated with foci of endometriosis.
Herein we describe a unique case of primary endometrioid adenocarcinoma of the vulva, without clinical and pathological evidence of endometriosis.
Case Report. A 60-year-old Caucasian woman was referred at our Institution for the evaluation of a slow growing, painful, enlarging mass located in the right major labium of the vulva. She denied any gynecological history of dysmenorrhea, endometriosis, vulvar disease, infectious disease or trauma as well as the use of any hormone replacement therapy. On clinical examination, the lesion was an eczematous nodule, measuring 2 cm in maximum size, fixed to the underlying soft tissue. No other patological alterations were found in the controlateral vulvar region, perineal and perianal region, vaginal vestibule, clitoris and periurethral regions. Trans-vaginal ultrasoud examination did not show any pathological alteration.
The patient underwent an excisional biopsy of the vulvar lesion.
The histological examination revealed a malignant neoplastic epithelial lesion. The carcinoma showed a glandular cribriform pattern composed of columnar and cuboidal cells with eosinophilic cytoplasm: some neoplastic areas were purely glandular while others showed a more solid growth pattern. Intermixed with the glandular structures, there were diffuse areas of squamous differentiation. The squamous epithelium near the neoplasia did not show any pathological alterations nor evidence of dysplasia. Immunohistochemistry showed neoplastic cells to be strongly and diffusely posititve for PAX-8, cytokeratin 7, vimentin and for the oestrogen and the progesterone receptors, while it showed a patchy staining for p16 in neoplastic cells. Moreover, these cells were found to be negative for p63. These morphological and immunohistochemical data suggested the diagnosis of a moderately differentiated endometrioid adenocarcinoma of the vulva. In order to exclude a primitive endometrioid adenocarcinoma of the endometrium, the patient underwent histeroscopy: the endometrium did not show any macroscopic alterations and multiple biopsies were randomly taken. Endometrial biopsies showed only a histopathological pattern of glandulocystic atrophy.
After one month, the patient underwent a wider excision of the right labium of the vulva in order to ensure tumor excision radicality because of the proximity of the neoplasm to surgical margins.
The histolopathological examination did not show any residual tumour nor endometriosis.
After 1 year of follow-up, the patient is healthy without evidence of local recurrences or distant metastases.
Discussion. To the best of our knowledge, this is the first work to report primary endometrioid adenocarcinoma of the vulva. The occurrence of endometrioid adenocarcinoma in the vulvar region has only been reported as a secondary localization of an endometrial endometrioid adenocarcinoma. Moreover, the occurrence of endometrioid carcinomas outside the endometrium is usually reported in literature in association with endometriosis. In this case there were no clinical or pathological evidences for endometriosis in the vulvar area, nor in other anatomic sites.
The present case could widen the morphological spectrum of vulvar glandular neoplasms. Pathologists should also consider the rare possibility of a primary endometrioid adenocarcinoma in order to facilitate a correct clinical management.

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HIGH GRADE SEROUS CARCINOMA: A COMPREHENSIVE GENETIC AND CLINICO-PATHOLOGIC STUDY OF A HETEROGENEOUS NEOPLASM.

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Background. Tubo-Ovarian High Grade Serous Carcinoma (HGSC) is a heterogeneous neoplasm that may exhibit two main morphologic patterns: classic and SET (solid, pseudoendometrioid and transitional cell-like carcinoma) variants. The hypothesis of a potential dualistic model of HGSC, with distinct clinico-pathological and molecular features, in particular regarding *BRCA* tumor mutational status, has been proposed but not yet fully understood.
Objective. . To investigate the correlation of HGSC tumor morphology, BRCA mutational status, and clinicopathological features (i.e. age, serous tubal intraepithelial carcinoma - STIC, tumor-infiltrating lymphocytes (TILs) and peritoneal invasion patterns).
Materials and methods. All resection specimens with a diagnosis of HGSC between 2016 and 2019 were collected. All cases have been reviewed considering all the histopathological characteristics blinded to the original pathology report and the molecular features. Complete immunohistochemical analysis was performed in all cases to characterize the tumor and to evaluate TILs. BRCA1 and BRCA2 analysis was performed on DNA extracted from formalin-fixed paraffin-embedded (FFPE) ovarian cancer tissue through Next Generation Sequencing technology using ION Torrent™ Oncomine™ BRCA Research Assay (Life Technologies). Any variants were then validated in tumor DNA and checked in DNA extracted from peripheral blood lymphocytes using targeted Sanger sequencing. In addition, BRCA germline deletions/duplication were investigated via MLPA analysis on constitutional DNA.
Results. 75 HGSC have been reviewed for this study.

BRCA1/2 mutations were found in 40% of cases and was strongly correlated with SET morphology (p=0.0005) and pushing peritoneal invasion pattern (p=0.007). Morphologically, SET features were identified in 47% of the cases compared to 53% of classic HGSC. Compared with classic HGSC, SET tumors showed a statistically significant association with pushing pattern of invasion (p=0.0001), high density of stromal and intraepithelial TILs (p=0.0001) with significant higher count of CD8+ lymphocytes (>100/10 HPF). Serous tubal intraepithelial carcinoma (STIC) was identified in 62.7% of cases, but was not statistically correlated with *BRCA* mutational status and tumor morphology.

Conclusions. SET morphology was observed in almost half of the cases in our series and was statistically associated with *BRCA* mutational status, high TILs and pushing pattern of peritoneal invasion, but not with the presence of STIC. These pathologic differences among the classic HGSC and the SET variant together with their different relations to BRCA mutations allow to identify two possible separable entities, thus supporting the hypothesis of a dualistic model of HGSC, but further studies are needed to investigate their clinical and prognostic implications.

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MELF PATTERN OF MYOINVASION IN ENDOMETRIOID ENDOMETRIAL ADENOCARCINOMA: MORPHOLOGICAL AND MOLECULAR FINDINGS

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Objective. Recent studies have shown that endometrial cancers, even at the same stage and with same histology, can have a different molecular/genomic profile. In particular, interesting data were elaborated by the Cancer Genome Atlas Research Network (TCGA) that has classified endometrial neoplasms in 4 molecular subgroups: 1)*POLE* (ultramutated) tumours 2)microsatellite-instable (MSI [hypermutated]) group of endometrioid tumours 3) copy-number low (endometrioid) tumours, 4) copy-number high (serous-like) tumours. Moreover, recently, there is an increasing interest about the association between Lynch syndrome, MSI and endometrial neoplasms. Mutations in MMR system, easily identifiable even with immuhistochemical methods, account for up to 30% cases of endometrial cancer and are mainly due to the hypermetilation of MLH1 promoter.

In this preliminary study our Aim.s is to examine the possible relationships between the microcystic, elongated, fragmented (MELF) pattern of invasion in endometrioid endometrial carcinoma (EEC) and the classical histopathological parameter, the status of MMR proteins.

Materials and methods. We randomly selected 129 cases of EEC, being 28 with MELF pattern of myoinvasion (MELF population) and 101 without MELF pattern of myoinvasion (NON MELF population) taken from the records of the Div. of Pathology of the Foundation "Agostino Gemelli" University Hospital IRCCS of Rome from 1st January 2017 until 30th December 2018.

In these population, we analyzed different parameters: tumor dimension, stage, FIGO grade, the presence of moderate or diffuse LVI (classified as absent, moderate or diffuse) and MMR proteins (MLH1, PMS2, MSH2 and MSH6)

Results. ***Histopathological Findings.***

26 MELF cases were G2 FIGO (92.86%), 1 case G1 (3.57%) and 1 G3 (3.57%). Among NON MELF population 13 cases were G1 (12.87%), 55 G2 (54.46%) and 33 G3 (32.67%). 14 MELF (50 %) and 48 NON MELF cases (47.52 %) invaded > 50% of the myometrium while cervical infiltration was present in 25 MELF (89.29%) and in 82 NON MELF carcinomas (81.19%).

Among MELF population, 13 cases were Stage IA (46.43%), 12 stage IB (46.43%), 2 stage II (42.86%), 1 stage IIIA (3.57%) and among NON MELF population, 46 cases were Stage IA (45.54 %), 31 stage IB (30.69%), 17 stage II (16.83%), 4 stage IIIA (3.96%), 2 stage IIIB (1.98%) and 1 case stage IVB (0.99%).

8 MELF cases showed no LVI (28.57%), 6 cases had a moderate LVI (21.43%) and 14 cases showed diffuse LVI (50%); 65 NON MELF tumors showed no LVI (64.36%), 12 had a focal LVI (11.88%) and 24 had a diffuse LVI (23.76%).

In MELF pattern, 21 cases (75%) had no nodes metastases, 2 cases (7.14%) showed macrometastases, 2 cases (7.14%) had only micrometastases and 3 cases (14.28%) had isolated tumor cells (ITCs). No case of the NON MELF population had micrometastasis or ITCs, 93 (92.01%) had negative lymph-nodes, 8 cases (7.9%) had macrometastatic lymph-nodes.

The multi-parametric logistic regression analysis revealed that in NON MELF population the risk of lymph-node metastasis is higher with the increase of tumoral dimension and the entity of LVI, while in MELF population this synergic association in determining node metastasis has not been observed.

Molecular/Immunohistochemical Findings.

Loss of MLH1 expression has been observed in 6 MELF (21,43%) and in 33 NON MELF cases (32,67%).

PMS2 was absent in 9 MELF (32,14%) and in 37 NON MELF tumors (36.63%).
MLH1 and PMS2 were both negative in 6 MELF (21,34%) and in 30 NON MELF tumors (29,7%)
MSH6 was lost in 2 MELF (7,14%) and in 7 NON MELF tumors (6,93%)
MSH2 was lost in 2 MELF (7.14%) and in 4 NON MELF tumors (3.96 %)
MSH2 and MSH6 were both negative in 2 MELF (7,14%) and in 3 NON MELF tumors (2,97%).

Discussion. MELF pattern is a particular pattern of myoinvasion by EEC, characterized by the presence of micro-cystic and elongated glands, composed of flat cells with eosinophilic cytoplasm, invading the myometrium with associated fibromyxoid stromal reaction and more frequently associated to low-intermediate grade EEC. According to the recent literature, this particular pattern of invasion seems to be associated with lympho-vascular invasion and lymph node metastasis, despite the low grade of this tumors. However the prognostic role of MELF pattern of invasion remains unclear since the presence of discording data in the literature.
Our data confirmed the low-moderate grade of MELF tumors (94.94% MELF case were G1 or G2 FIGO) compared to NON MELF population.
Moreover, in our data MELF pattern resulted associated with a higher incidence of low-volume nodal metastases compared to NON MELF tumors (p=0.001). The presence of higher incidence of low-volume nodal metastases is also associated with absence of LVI (p=0.037)
Finally, the multi-parametric logistic regression analysis revealed that in NON MELF population the risk of lymph-node metastasis is higher with the increase of tumoral maximum diameter and the entity of LVI; in MELF population, this synergic association in determining node metastasis has not been observed.
This data may support the fact that MELF pattern is an independent prognostic factor in the risk to develop lymph-node metastasis, since the particular stromal reaction that can predispose to epithelial-mesenchymal transition (EMT).
Concerning molecular features, in our case-series there was a higher frequency of mutation of MLH1 in NON MELF endometrial pattern of invasion (32.67% of NON MELF cases *vs* 21.43% of MELF cases) but a higher prevalence of MSH2 loss in MELF pattern (7.14% in MELF population *vs* 3.96% of NON MELF population).
This preliminary data could support the hypothesis of a specific pattern of mutation among MSI in MELF pattern and probably in these group of EEC with worse prognosis an interesting role could be assumed by MSI mutation.
Further studies and larger population are requested to confirm these data.

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THE WIDE MORPHOLOGICAL SPECTRUM OF PRIMARY AND RECURRENT AGGRESSIVE ANGIOMYXOMA: A SERIES OF 36 CASES

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Background. Aggressive angiomyxoma (AAM) is a rare locally infiltrative, non-metastasizing mesenchymal neoplasm arising primarily in the soft tissues of the vulvo-vaginal region, perineum and pelvis of adult women (1). AAM is most commonly found in women of the reproductive age, however sporadic cases have been reported in males, occurring almost exclusively in the genital area (scrotum, spermatic cord, inguinal region, perianal region and pelvic soft tissues) (2). The reported age at presentation is 6-77 years, with the peak incidence occurring during the reproductive years. The female-to-male ratio is 6.6:1. The tumor usually presents as a slow-growing large multilobular or polypoid mass with finger-like projections infiltrating the surrounding soft tissues. On gross examination, it is rubbery and white or soft and gelatinous (1). Histologically, it is a hypocellular tumor composed of neoplastic spindle-shaped cells set in a loose myxoid stroma containing small to medium-sized thick-walled vessels. Despite the bland-looking cytology, AAM is a locally aggressive tumor, with infiltrative growth and high rate of local recurrence (1,2). Wide local excision is the therapy of choice. However, this is difficult at times as the tumour is non-encapsulated and has the same consistency as that of surrounding connective tissue.
In the present study, we present the clinico-pathologic features of a series of 36 cases of AAM, with emphasis on unusual morphological features in both primary and recurrent tumors.

Materials and methods. A series of 36 cases of surgically resected, vulvo-vaginal AAMs was retrospectively collected from the files of the University of Catania, University of Pilsen and Catholic University of Sacred Hearth (Rome). All patients were females, ranging in age from 43 to 65 years. Immunohistochemical studies were performed with the labeled streptavidin-biotin peroxidase detection system using the Ventana automated immunostainer (Ventana Medical Systems, Tucson, AZ). The following antibodies were applied: vimentin, CD34, alpha-smooth muscle actin, desmin, estrogen, progesteron and KI-67.

Results. Our series included 31 primitive tumors and 5 local recurrences of surgically resected AAMs. On gross examination, the majority of tumors presented as unencapsulated, poorly or vaguely circumscribed masses with a gelatinous, myxoid or fibrous cut surface. Tumor size was highly variable, ranging from 1.5 cm to 20 cm in greatest dimension. Histologically, all the selected tumors exhibited the following common basic theme: proliferation of bland-looking small-sized cells set in a myxoid, fibro-myxoid or collagenous stroma,

containing numerous small- to medium-sized blood vessels. The neoplastic cells exhibited spindled, round to ovoid or stellated morphology, with palely eosinophilic cytoplasm. Nuclei were round-to-ovoid in shape, with dispersed chromatin. Mitotic figures and nuclear pleomorphism were rare or absent. All cases exhibited infiltrative borders, often with entrapment of adipose tissue, nerve and skeletal muscle at the periphery. Cellularity was low to moderate, with focal areas displaying a mild hypercellularity, especially around large vessels at the periphery of the tumor. An interesting finding, observed in 11 cases, was the presence of small bundles of spindle-shaped smooth muscle cells with eosinophilic cytoplasm, scattered within the stroma, often around blood vessels. Immunohistochemical findings confirmed the considerable overlap of AAM with that of many other mesenchymal tumors of the lower female genital tract. All cases showed diffuse immunoreactivity for vimentin and desmin, while alpha-smooth muscle actin and CD34 were variably expressed. The majority of cases (70%) exhibited strong nuclear staining for estrogen and progesterone receptors in most of the tumor cells. Labeling for Ki-67 demonstrated a low proliferative index (<1% of tumor cells).

The following unusual morphological features were seen: i) 5 cases (2 primary and 3 recurrent tumors) were extensively hypercellular with a moderate amount of fibro-myxoid stroma; ii) 3 primary and 2 recurrent tumors showed areas of neoplastic spindle-shaped cells with a concentric layered, perivascular "onion skin-like" arrangement; usually the blood vessels had hyalinized walls; iii) 4 primary tumors showed focal areas with microvascular proliferation, as typically seen in glioblastoma; iv) 3 primary tumors showed focal hypercellular areas due to a perivascular condensation of neoplastic cells (most of them with round morphology), closely packed and set in a fibrous stroma; v) two cases showed areas with a leyomioma-like morphology, consisting of spindle cells with moderate eosinophilic cytoplasm and elongated nuclei with small evident nucleoli; vi) two recurrent cases were composed of a hypocellular proliferation of bland-looking spindle cells with wavy nuclei set in abundant fibro-sclerotic stroma, closely reminiscent of neurofibroma; vii) two recurrent cases with extensive stromal hyalinization showed small- to medium-sized blood vessel replaced by fibrous tissue, with complete obliteration of their lumen; these vascular changes resulted into confluent fibrotic nodular structures of variable sizes, closely resembling ovarian corpora albicantia.

Conclusions. AAM is currently included in the category of the "*specific stromal tumors of the lower female genital tract*", together with angiomyofibroblastoma, cellular angiofibroma, and myofibroblastoma (3). Although some overlapping features between these entities do exist, an accurate evaluation of their clinico-pathologic features allows their distinction in most cases. Among these tumors, it is crucial to separate AAM from others for its high rate of local recurrences. Although diagnosis of AAM is usually straightforward if typical morphology is encountered, diagnostic problems may arise when pathologist is dealing with unusual morphological features, especially in recurrent tumors exhibiting hypercellularity or extensive sclerosis and/or neurofibroma-like appearance.

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ATYPICAL ENDOMETRIAL HYPERPLASIA, LOW-GRADE: "MUCH ADO ABOUT NOTHING"

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Background. Atypical endometrial hyperplasia (AEH) is considered a precursor of endometrioid carcinoma. The 2014 World Health Organization (WHO) classification divides endometrial hyperplasia into two categories: hyperplasia without atypia and atypical hyperplasia/endometrioid intraepithelial neoplasia (EIN). However, this classification disregards the degree of nuclear atypia. The Aim. of this study is to show the importance of grading nuclear atypia and estimate the risk of developing endometrial carcinoma following a diagnosis of low-grade or high-grade AEH. In addition, we investigated the potential role of genes known to be involved in endometrial carcinogenesis such as *ARID1A, PIK3CA, PTEN, KRAS, CTNNB1* and mismatch repair genes.

Methods. We reviewed 91 biopsies of AEH from 91 patients who subsequently underwent hysterectomy within 1-year interval. The association between the grade of nuclear atypia at biopsy and the findings at hysterectomy was assessed via a Fisher's exact test. Targeted sequencing was performed in 26 cases of AEH and 4 samples of simple hyperplasia.

Results. The degree of nuclear atypia at biopsy was highly predictive of the findings at hysterectomy ($P=5.0x10^{-25}$). None of the patients with low-grade AEH had a diagnosis of high-grade AEH or carcinoma at hysterectomy; whereas 9 (29%) patients with high-grade AEH in the biopsy also had high-grade AEH in the uterus and 22 (71%) patients had FIGO grade-1 carcinoma. None of the genes tested showed a mutational load significantly associated with the degree of nuclear atypia.

Conclusions. We conclude that in AEH is crucial to assess the degree of nuclear atypia. Our data strongly support that low-grade AEH is inconsequential and question the need of hysterectomy for such patients.

Key words: atypical hyperplasia, low-grade nuclear atypia, high-grade nuclear atypia, endometrial metaplasia, well differentiated (G1) endometrial carcinoma, risk of progression to carcinoma.

IMMUNUNOHISTOCHEMICAL PROGNOSTIC FACTORS IN HIGH-GRADE ENDOMETRIAL NON-ENDOMETRIOID CARCINOMAS (HG-NECS). A PRELIMINARY REPORT

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Introduction. Endometrial carcinoma (EC) is the most prevalent gynecological cancer, with an increasing incidence of aggressive histotypes and mortality in recent years. Beside dichotomic classification in type I and II, recently ECs may be re-classified in molecular prognostic groups. In the present study EC potential prognostic factors were investigated by immunohistochemistry, especially in high grade endometrial non-endometrioid carcinomas (HG-NECs).
Materials and methods. In our study, we considered all high-grade endometrial non-endometrioid carcinomas surgically managed in IRCCS Ospedale Policlinico San Martino from 2013 to 2018, with available follow-up. Surgical specimens have been routinely processed and stained with immunohistochemical technique for Estrogen Receptor (ER), Progesterone Receptor (PR), Ki67, p53, E-cadherin, β-Catenin, Bcl2 and Cyclin D1 for each case. We evaluated the percentage of positive tumor cells (%) for all antibodies, distinguishing it in low and high rate according to the distribution in the study population. Follow-up was reported for disease-free survival (DFS) and overall survival (OS).
Results. 33 cases were eligible. Regarding histotype, 13 ECs were mixed, 9 serous, 6 MMMT, 3 undifferentiated and 2 clear cell carcinoma. About International Federation of Gynecology and Obstetrics (FIGO) stage: 19 ECs were at stage I-II and 14 at stage III-IV. 12 patients suffered from relapsing disease (RLP; mean follow-up 24.6 months); 8 patients died for the disease (DOD; mean follow-up 26,6 months). RLP demonstrated a significantly higher Bcl2 expression (38.2% vs. 8.1%; p=0.003), as well as DOD patients, for whom p reaches a borderline significance value though (34.4% vs. 13.1%; p=0.08). Moreover in DOD patients, neoplasms showed a higher mitotic index evaluated with Ki67 (75% vs. 67%; p=0.02). Patients affected by tumors with high expression of Bcl2 (Bcl2>10%) demonstrated a significant worse disease free survival (DFS) (HR=9.11 95%CI: 2.6-32.4; p=0.0006) and overall survival (OS) (HR=7.63 95%CI:1.7-34; p=0.0084). Also patients with low PR-expressing neoplasia (PR≤10%) had a significant worse DFS (HR=3.74 95%CI:1.2-11.9; p=0.02). OS in these patients had no clear significance value (HR=3.15 95%CI:0.73-13.53; p=0.12).
Conclusions. HG-NECs represent a heterogeneous group of endometrial aggressive neoplasms with a worrisome prognosis and often an advanced stage at presentation. Bcl2 and PR may represent promising immunohistochemical markers to identify a sub-group of patients having an even worse prognosis requiring a careful, close follow-up and eventually an improving post-surgical management.

PATOLOGIA PLEUROPOLMONARE

GRP78 AND PD-L1 EXPRESSION IN MESOTHELIOMA: A POSSIBLE LINK BETWEEN ER STRESS, UPR AND IMMUNECHECKPOINT INHIBITORS.

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Objective. Mesothelioma is a rare pleural and peritoneal malignancy related to asbestos exposure and with poor prognosis. First-line treatment consists of pemetrexed and platinum-based chemotherapy. Despite huge efforts made, therapies for mesothelioma continue to be challenging and the outcome for patients remains disappointedly poor.There is, therefore, an unmet need to identify more reliable diagnostic and/or prognostic markers and to highlight novel molecular targets clAim. ing efficient and tailored therapies.
GRP78, also referred to as BiP, is a molecular chaperone of the endoplasmic reticulum (ER) that aids proper folding of nascent polypeptides. When unfolded proteins accumulate, GRP78 triggers unfolded protein response (UPR), restoring cell homeostasis. Increased expression of GRP78 and mild UPR is constitutive activated in cancer cells, hindering apoptosis, and promoting cell survival, favoring the insurgence of chemoresistance and worsen patient outcome. We have already found that mesothelioma cells display mild constitutive UPR and overexpression of GRP78.
PD-L1 expression is actuallyused as a predictive biomarker forimmune checkpoint inhibitors, being incorporated into multi-parametric predictive therapeuticapproach in numerous tumors. PD-L1 is expressed in a substantial proportion of mesothelioma cases, as measured by FDA-approved companion assays for widely used immunotherapeutic drugs.
Interestingly, a recent study opens up the possibility that PD-L1 protein expression at the surface of cancer cellsmight be correlatedwith the protein levels of UPR proteins. This correlation prospect that PD-L1 expression on the surface of tumor cells might be linked to the UPR and cell proteostasis in general. Thisinteractioncould be targeted by some clinically approved drugs, which were recently reported to target PD-L1 for protein turnover by ER-associated degradation.
Materials. 15cases of mesothelioma have been selected (13 epiteliomorphic and 2 biphasic types). GRP78 (EPR4040 AbcammAb) has been tested through immunohistochemical method on formalin-fixed paraffin embedded bioptic samples. PD-L1 monoclonal antibody Ventana SP263 has been detected on Ventana Benchmark Ultra.
Results. All the cases considered were intensely positive for GRP78, with a valued score 2+ (5 cases, high granular cytoplasmatic positivity, Fig. 1) and 3+ (10 cas-

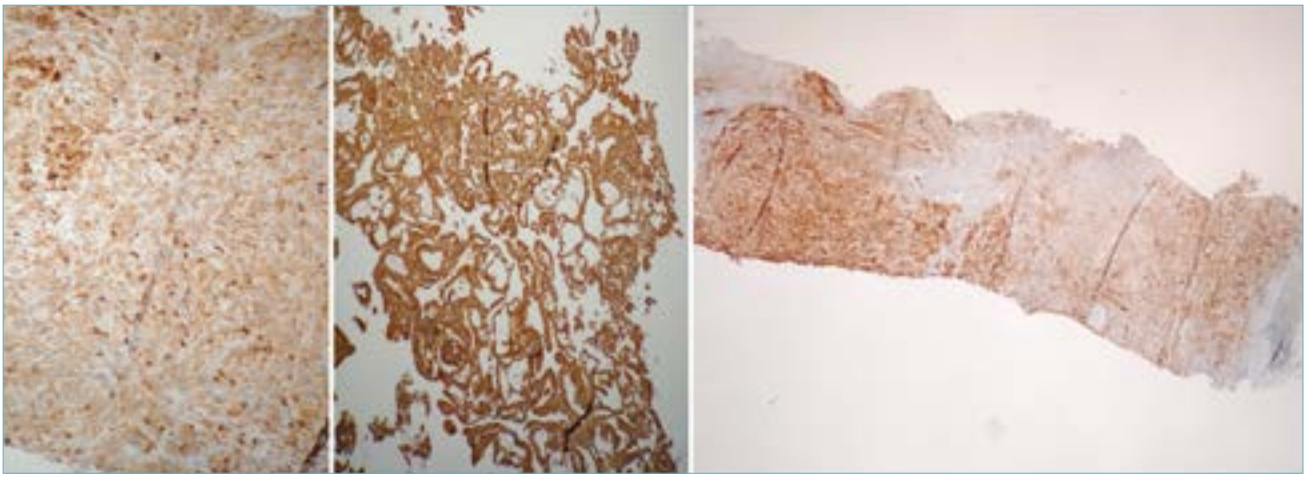

Fig. 1. GRP78 score 2+(20X)Fig 2: GRP78 score 3+ (10X) Fig. 3: PD-L1 high expression (4X).

es, high membrane and cytoplasmatic positivity, Fig. 2). As regards PD-L1 expression, 8 cases resulted positive (expression in more than 1% of neoplastic cells, Fig. 3), independently from morphology and GRP-78 score of positivity.

As regards tumormorphology, GRP-78 score intensity is the same (2+) in the two cases of biphasic mesothelioma, while PD-L1 expression showsnegative in a case and highly positive (than more 50% of neoplastic cells) in the other one.

Conclusion. The treatment of malignant mesothelioma is complex and the survival outcomes rather than the overall survival data are, to date, disappointedly daunting. Moreover, there are also several unresolved issues, such as the lack of predictive biomarkers and adequate tools for response evaluation.

Taken together, our preliminary data indicate that ER stress/GRP78 overexpression play a functional role in mesothelioma by regulating key tumor biology processes. Abnormal ER stress is a critical regulator of immune function in tumor microenvironment, being important for immunotherapy success. In this view, GRP78/UPR might influence PD-L1 expression, affecting the sensitivity of the host's immune system to neoplastic elements. As immunohistochemical detection has become routine diagnostic instrument in the management of tumoral tissues,prognostic stratification of the neoplasia testing GRP78 immunopositivity can be readily implemented to detect biological tumoralcharacteristics, useful inselecting treatment options.

Therefore, the relationship between the ER status/GRP78 overexpression and the clinical and detecting pathological features of the tumor may represent an interesting avenue to better characterize the carcinogenic pathway, influencing the prognostic tool in mesothelioma.

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HETEROGENEITY OF PD-L1 EXPRESSION IN LUNG MIXED ADENOCARCINOMAS AND ADENOSQUAMOUS CARCINOMAS.

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Aims. Immune-checkpoint inhibitors against Programmed cell death protein 1(PD-1) /Programmed death-ligand 1 (PD-L1) have proven to be remarkably effective in Non Small Cell Lung Cancer (NSCLC). PD1/PD-L1 pathway inhibitors represent a recent paradigm shift in the first-line therapy of NSCLCs lacking EGFR, ALK, ROS1 gene alterations, including either squamous and non-squamous histology. PD-L1 expression is currently a prerequisite parameter to select lung cancer patients eligible for treatment with PD-1/PD-L1 inhibitors, particularly in first line of treatment. In unselected lung cancer patients, the response rate was approximately 20%, increasing up to 50% in patients with PD-L1 expression. PD-L1 represents a predictive biomarker in lung cancer, although its heterogeneous expression represents an emerging challenge for accurate biomarker-based patient selection. Lung adenocarcinomas show a high rate of intratumor morphological heterogeneity that may reflect a heterogeneous molecular and im-

munophenotypic profile. Previous data have reported a variable PDL1 expression at different levels, including spatial and temporal heterogeneities.
To date, few data have been provided that account for intratumoral heterogeneity of PD-L1 expression in adenocarcinomas and adenosquamous carcinomas, in relation to the different histological subtypes.
The Aim. of our study was to analyze the intratumoral heterogeneity of PD-L1 expression in different intratumor subtypes and/or growth patterns in a series of mixed adenocarcinomas (mADCs) and adenosquamous lung carcinomas (AdSqLCs).
Methods and materials. A series of lung mixed adenocarcinomas and adenosquamous carcinomas from patients undergoing surgical resection was reviewed and comprehensive histologic subtyping was performed. Lung mixed adenocarcinomas were reviewed according to the new IASLC/ATS/ERS classification, which allows for five comprehensive histologic subtypes of lepidic, acinar, papillary, solid and micropapillary patterns, by a percentage of 5% increments to determine a predominant pattern.Inclusion criteria consisted of surgically-resected adenocarcinoma with 2 or more growth patterns and adenosquamous carcinomas. As many as 73 mADCs and 6 AdSqLCs were selected.
PD-L1 expression was assessed by immunohistochemistry assay using different primary antibodies, particularly anti-PD-L1 antibody SP263 (Ventana Medical Systems Inc., Tucson, AZ, USA) on a Ventana Benchmark Ultra with OptiView Universal DAB Detection Kit (Ventana Medical Systems), and with anti-PD-L1 monoclonal primary antibody Dako 28-8 pharmDx (Agilent Technologies/ Dako, Carpinteria, CA, USA) on an Agilent Link AS-48 automated immunostainer.
Immunostaining was interpreted on the basis of two categories, low positivity (between 1 and 49% of neoplastic cells) and high positivity (more than 50% of neoplastic cells), according to the positivity used for predictive purposes in lung cancer diagnosis.
Results. Overall, PD-L1 expression was observed in 37 out of 79 cases (39.2%) (31 mADC and all AdSqLCs). All PD-L1 positive cases were EGFR wild type, 2 cases harbored concomitantly PD-L1 expression and ALK-rearrangement. PD-L1 expression was heterogeneous in 22 out of 37 PD-L1-positive cases (23.2% mADC and 83% AdSqLCs). PD-L1 expression in 3 out of 5 AdSqLCs patients was exclusively found in the squamous component, while in 2 cases immunostaining was restricted to ADC. PD-L1 expression was observed more frequently in ADC with solid pattern.
Our Results highlighted significant intratumor variability of IHC PD-L1 expression, while a complete agreement between the two different anti-PD-L1 primary antibodies, namely SP263 and 28-8 clones, was observed. PD-L1 positive cases were significantly related to heterogeneous expression (p=0.000). Heterogeneity of PD-L1 expression was significantly related to the presence of micropapillary (p=.028) and solid (p=.017) patterns.
Conclusions. Our data suggest that PD-L1 expression is quite heterogeneous in mADCs and AdSqLCs, partly contributing to explain discrepant Results between biopsy and surgical resections as well as discordant clinical effectiveness in regards with PD-L1 positive or negative ADC diagnosed on cytology/small biopsy. The heterogeneous PD-L1 expression could represent one of the most important limitations for immunotherapy, since the majority of NSCLCs are diagnosed and investigated on cytology and/or small biopsy. Therefore, when analysing ADCs and AdSqLCs, PD-L1 expression should be tested in areas containing different patterns. Pathologists and oncologists should be aware that the examination of PD-L1 expression in small samples of ADCs might not be really representative of the entire neoplasm.

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MOLECULAR ANALYSIS OF SECOND PRIMARY LUNG ADENOCARCINOMAS IN PATIENTS WITH A PREVIOUS DIAGNOSIS OF BREAST CANCER

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Aims. Patients treated for a breast cancer have an increased risk to develop a second primary malignancy at different sites, including the lung [1]. Several studies have suggested radiotherapy for the breast cancer and smoking habit to be directly correlated with the onset of a second primary lung carcinoma [2,3]. Nevertheless, no reports on the molecular profiling of lung adenocarcinomas following a previous breast cancer are on record.
In our study we Aim.ed to compare the mutational frequencies in the most commonly mutated genes in lung cancer between patients with a previous diagnosis of breast cancer and patients without a history of a previous malignancy.
Materials and methods. From the files of the Pathology Units of Candiolo Cancer Institute and Città della Salute e della Scienza Hospital we retrieved 63 lung adeno-

carcinomas diagnosed in patients previously treated for a breast cancer, either *in situ* or invasive (test group), and 82 cases lung adenocarcinomas diagnosed as first malignancy (control group). Histological slides were reviewed by a pathologist to confirm the primary origin of the second lung tumour and to exclude the possibility of a metastatic deposit. When available, clinical data were obtained from clinical databases and reports. DNA was extracted from representative formalin fixed paraffin embedded blocks and subjected to sequencing analysis by Mass Array Sequenom using the Myriapod Lung status kit, which allows to detect hotspot mutations in the 10 most commonly mutated genes in lung cancer (*EGFR, KRAS, BRAF, NRAS, PIK3CA, ALK, ERBB2, DDR2, RET, MAP2K1*).

Results. Due to data fragmentation in the clinical history of several clinical reports information about smoking habit, radiotherapy and histology of breast carcinomas were not available for all the patients. On the other hand, mutational analysis was successfully carried out for all the samples. In the control group 34% of patients were wild type (WT) and 66% harboured a mutation in at least one of the analyzed genes. Among the mutated samples we detected *EGFR*, *KRAS*, *HER2*, and *PIK3CA* mutations in 62%, 27%, 4%, 7% of samples, respectively. In the test group, 29% were WT and 71% mutated (*EGFR*, *KRAS*, *HER2*, *BRAF* and *PIK3CA* mutations were found in 32%, 47%, 11%, 6%, 4% of samples, respectively). There were no differences in the number of mutations within the two groups (p=0.66), however *EGFR* was more frequently mutated in the control group (p=0.0036) while *KRAS* mutations were more frequent in the test group (p=0.041). A different distribution of HER2 mutations was also observed in the two group (11% and 4% in the test group and control group respectively) although not statistically significant (p=0.23).

KRAS mutations in control and test group were significantly correlated with smoking habit (p=0.00026 and p=0.029, respectively), nevertheless the smoking habits were comparable between the two groups (p=0.33). No correlation was found between distinct mutations and other clinical data.

Conclusions. In this study we observed an increased frequency of *KRAS* mutation in lung adenocarcinoma samples from patients with a previously documented history of breast cancer, whereas *EGFR* mutations seem to be less frequently encountered in this population. Although not statistically significant our preliminary data suggest a higher frequency of *HER2* mutations in these tumors. Based on the possible clinical implications, these data need to be confirmed in larger studies exploiting genomic analyses with high throughput assays.

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PRIMARY INTRATHORACIC SYNOVIAL SARCOMA: CLINICO-PATHOLOGIC AND IMMUNOMOLECULAR STUDY OF A MULTICENTRIC CASE SERIES

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Background and aim. Primary intrathoracic synovial sarcoma (SS) is a rare and aggressive neoplasm arising from the lungs, pleura, mediastinum and hearth. Due to its rarity, SS are frequently misdiagnosed as thymoma, mesothelioma or non-small-cell-lung-cancer. Biological and clinical prognostic factors are still understudied. Thus, our Aim. was to provide data on clinico-pathologic, molecular and prognostic features of SS.

Methods. We analysed data from a multicentric series of 52 cases of primary intrathoracic SS. Demographic, clinical, imaging, histologic and immunohistochemistry features were reviewed, as well as data on treatment and clinical outcomes. We also recorded molecular data on EGFR (exons 18-21), c-KIT (exons 9,11,13,17), BRAF (exon 15) and PDGFRs gene mutations. Patient survival was estimated contrasting subjects according to demographic features, clinical variables, histology (monophasic versus biphasic/pleomorphic) and treatment (surgical versus non-surgical).

Results. The case series comprised 32 males and 20 females, with a mean age of 53.9 years at diagnosis. SS mostly originated from the lung (63.4%), followed by the pleura (11.5%) and mediastinum (9.6%), while 16 cases displayed multifocality. Mean tumor diameter was 72 mm (range 16-150). Thirty-eight cases (73%) showed monophasic histology. Evidence of t(X;18) involving SYT-SSX fusion gene was found in all cases. Thirty-six patients (69%) received surgical treatment: alone (26/52=50%), plus chemotherapy (8/52=15.3%) or radiotherapy (3/52=5.6%).

Relapse of the disease was recorded in 32 cases (61.5%) with a median follow up of 21 months. Age ≤ 65 at the time of the diagnosis was associated with a better prognosis (p=0.02). Surgical treatment was associated with a trend for greater survival (median survival time: 22 vs. 16 months, p=0.059) and significantly lower relapse rates (p=0.04).

Conclusions. Primary intrathoracic SS occurring in the pleuro-pulmonary district are rare tumors characterized by a poor prognosis. In our case series, however, surgical treatment was associated with a lower rate of relapse. Whereas, the role of adjuvant therapies still remains controversial.

HISTOLOGICAL TRANSFORMATION IN EGFR MUTATED TKI-TREATED LUNG ADENOCARCINOMA: A SINGLE CENTER REPORT OF FOUR CASES.

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Background. EGFR-mutated (EGFRm) lung adenocarcinoma (ADC) occurs in about 10% of western patients, with exon 19 deletions and exon 21 L858R point mutation being the most common genetic events[1]. First and second generation (1G, 2G) tyrosine kinase inhibitors (TKI) such as Gefitinib, Erlotinib or Afatinib have been representing effective first line treatments for such patients, with relapses occurring generally after 12 months[2]; more recently third generation (3G) TKI Osimertinib became the new gold standard first-line approach. The main two mechanisms of acquired resistance to 1G and 2G TKIs are the development of EGFR exon 20 T790M secondary mutations (about 50% of all cases) and amplification of the MET receptor (15 to 20% of cases) [2]. In addition, the histological transformation of EGFRm ADC into small cell lung cancer (SCLC) and squamous cell carcinoma (SCC) has been described as an important, less common, resistance mechanism to TKI therapy. SCLC transformation has been observed in about 5 to 14% of cases[2] and occurs after both 1G and 3G EGFR TKIs. The transformation to SCC is far less common and its molecular basis unknown: to date, only 13 of such cases have been described[3].

Purpose. In this single center study we Aim. to describe our experience in approaching and evaluating the pathological and molecular features of EGFRm ADC undergoing transformation into both SCLC and SCC in response to first and second line therapy with EGFR TKIs.

Materials and methods. From 2017 to 2019, four cases of advanced ADC harboring EGFR mutations and showing histological transformation under TKI treatment were collected at San Luigi Hospital, Orbassano (Turin), Italy. All cases underwent re-biopsy after first and second disease relapses. Histological and immunohistochemical analyses were performed. Molecular profile with next generation sequencing using the Kit Oncomine™ CE-IVD with Ion Torrent PGM platform was performed, allowing the sequencing of hot-spot regions in *EGFR, ALK, ERBB2, ERBB4, FGFR1, FGFR2, FGFR3, MET, DDR2, KRAS, PI3CA, BRAF, AKT1, PTEN, NRAS, MAP2K1, STK11, NOTCH1, CTNNB1, SMAD4* and *FBXW7* genes. *ALK* and *ROS1* rearrangements were assessed by fluorescent in situ hybridization.

Results. Three patients were female and 1 male, with a mean age of 69 year; 1 was current while the others were never smokers. All four patients had advanced ADC, initially diagnosed by pleural effusion (2 patients) or bronchial biopsy (2 patients) cyto-histological examination. All tumors resulted positive for the EGFR exon 19 deletion, thus undergoing first line treatment with 1G TKI Gefitinib. After a mean period of 11 months all patients experienced a first disease progression and, as recommended by guidelines, a liquid biopsy was performed. In one patient the T790M mutation was detected in circulating tumor DNA (ctDNA), and treatment with 3G TKI Osimertinib was started. Conversely, in the other 3 patients the acquired EGFR T790M resistance mutation was absent at ctDNA analysis and a tissue re-biopsy was performed. At the molecular analysis of the re-sampled tumor tissue, two out of 3 tumors resulted EGFR mutated in both exon 19 (deletion) and exon 20 (T790M point mutation) and underwent treatment with Osimertinib. The last tumor sample (collected from an adrenal metastasis developed in the current smoker), demonstrated SCLC morphology (i.e. small round uniform cells with finely granular chromatin pattern and inconspicuous nucleoli) and immunohistochemistry (i.e. TTF1 positivity, high proliferative activity and evidence of neuroendocrine differentiation, as confirmed by synaptophysin and focal chromogranin A positivity). The NGS analysis showed persistence of the initial exon 19 deletion and did not demonstrate any further mutations in the EGFR gene. ALK and ROS1 were both negative for translocation. This patient finally underwent second line treatment with chemotherapy (carboplatinum-etoposide).

After a median time to progression of 4 months, all three patients under Osimertinib treatment showed disease progression. A new tissue re-biopsy was performed: two out of three tumor samples demonstrated a SCLC, while the third patient demonstrated SCC histological transformation, which was morphologically and immunohistochemically confirmed (i.e. p40 positivity and TTF1 and synaptophysin negativity). The NGS analysis showed that all tumors maintained the original EGFR driver mutation but lacked the previously found T790M resistance mutation. No other relevant differences with previous NGS studies were identified in the mutational profile of the two SCLC-transformed tumors. No ALK and ROS1 fusions were detected in all samples. Interestingly, in the SCC transformation a shift in the mutational status of the CTNNB1 (beta-catenin) gene was observed: in the first tumor biopsy (after Gefitinib treatment) a newly acquired S37F point mutation (COSM5662) was found (with an allele frequency of 16,31%), while the subsequent biopsy (after Osimertinib treatment) harbored the D32V point mutation (COSM5691) with an allele frequency of 37%.

Conclusions. Our observations support the existence of a specific mechanism of resistance to TKIs, involving the selective growth of a resistant clone with non-ADC histo-morphological and immunophenotypical features. The mutational status of the TKI-resistant SCLC clones, which maintained the original exon 19 sensitizing mutation but no longer expressed the T790M, is consistent with recent findings stating that histologically-changed (or transformed) clones directly evolve from EGFR mutant clones early in the course of the disease, prior to the eventual acquisition of other molecular mechanisms of TKI resistance (i.e. T790M mutation)[4]. Resistant clones are, thus, viewed as dynamic populations, changing in prevalence according to the selective pressure of different sequential therapies. Furthermore, we describe the case of an EGFRm ADC undergoing SCC transformation after second line therapy with 3G EGFR TKIs. The resistant clone exhibited morphological and immunohistochemical features consistent with SCC, with NGS analysis still showing persistence of the original exon 19 mutation and absence of the previously found T790M mutation. To date, only few and heterogeneous similar cases have been described[3].

Further studies are necessary to understand the molecular basis of this transformation and to detect a potential

early predictive factor, even detectable on liquid biopsy, to establish a personalized therapeutic approach for this subgroup of EGFR-addicted tumors.

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CITOLOGIA

A CASE OF METASTATIC MALIGNANT MELANOMA OF UNKNOWN PRIMARY SITE: THE CRUCIAL CONTRIBUTION OF CYTOLOGY IN PATIENT'S MANAGEMENT

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Aims. To show a case in whom the Fine Needle Aspiration (FNA) procedure permitted not only the diagnosis of lymph node metastatic malignant melanoma of unknown primary site, but also to re-interpret a previous histological diagnosis in a different way and to address subsequent therapies.

Clinical history. A middle-aged woman referred to our Breast Unit because of a 2cm lump in her right breast. She previously received a FNA with a cytological diagnosis of C3 and a histologic diagnosis of solid papillary lesion with lymphoid stroma on core needle biopsy. She also has a familial history of breast cancer. A triple negative carcinoma was signed out after surgery. In view of adjuvant therapy, she was submitted to instrumental staging. This revealed the presence of two inguinal lymph nodes. FNA was performed on the greatest: two slides were alcohol fixed and stained with Hematoxylin and Eosin (H&E) and Papanicolaou (PAP), three more slides were air dried and stained with May-Grünwald Giemsa (MGG). The remaining material was put in Cytolyt® solution (Hologic) to prepare a slide following ThinPrep® method (Hologic) and a cell block suitable for immunostaining and molecular biology.

Results. The morphological analysis of the slides showed the presence of poorly differentiated epithelioid or fusiform cells with prominent nucleoli and some intranuclear pseudoinclusion, mainly placed in syncytial aggregates; focal, scanty, pigmentation was also found.

As this kind of picture is not typical of breast cancer metastasis, we went back to the breast lump histology to see the similarity. ICC was then performed both on cell block and on histological slides. S100, HMB-45 and Melan A antibodies strongly decorated the tumor cells, whereas the cytokeratin staining was negative.

The cytological diagnosis was: malignant tumor cells referring to lymph node metastasis from malignant melanoma of unknown primary site.

The histologic diagnosis was changed as: Intra-mammary metastasis from malignant melanoma of unknown primary site.

Subsequently, the presence of mutation of the BRAF gene, exon 15p.V600 E/D was found on both the speci-

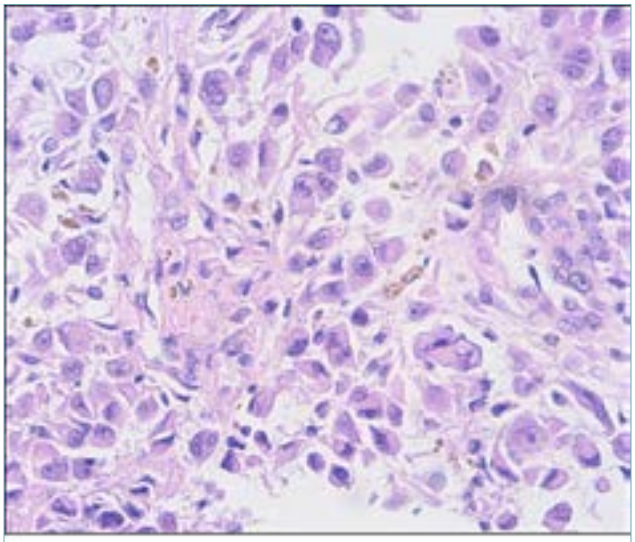

Figure 2. Cell block. Dispersed population of epithelioid and fusiform cells with nuclear pleomorfism, prominent nucleoli and occasional pseudoinclusions. Focal deposits of pigment. (Original magnification x40, H&E).

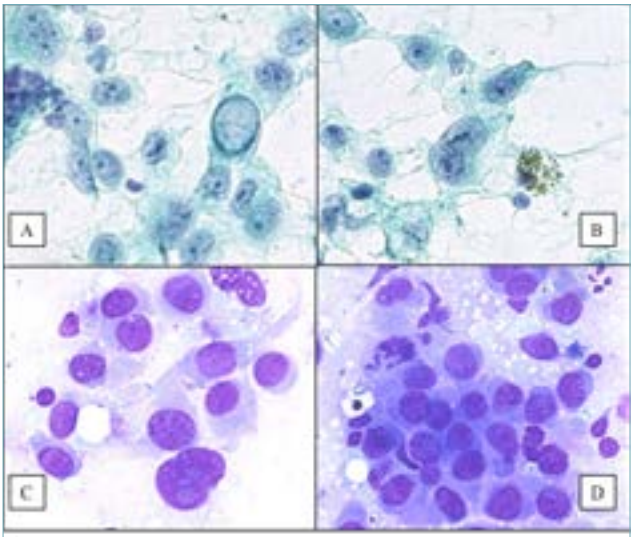

Figure 1. Cytomorphology of metastatic melanoma to the inguinal lymph node. A: intranuclear pseudoinclusion; B: prominent nucleoli and pigment deposits; C: epithelioid and fusiform cells; D: syncytial aggregate. (Original magnification x40, PAP [A, B], MGG [C, D]).

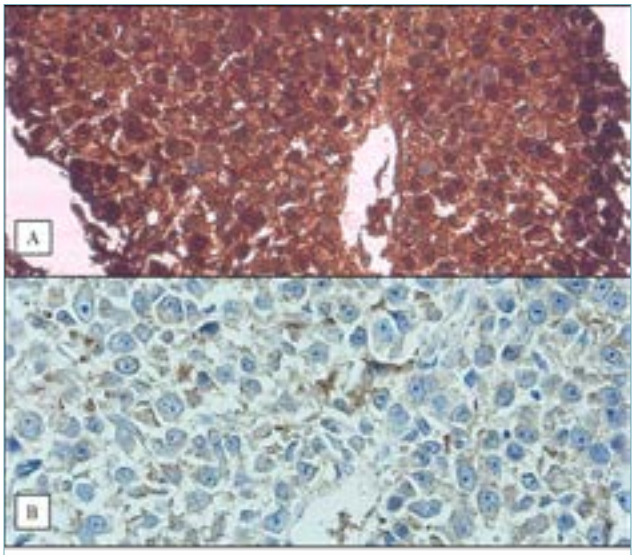

Figure 3. Immunoistochemistry of metastatic melanoma to the inguinal lymph node. A: strong positivity for S-100 protein; B: positivity for BRAF. (Original magnifications x20 [A], x40 [B]).

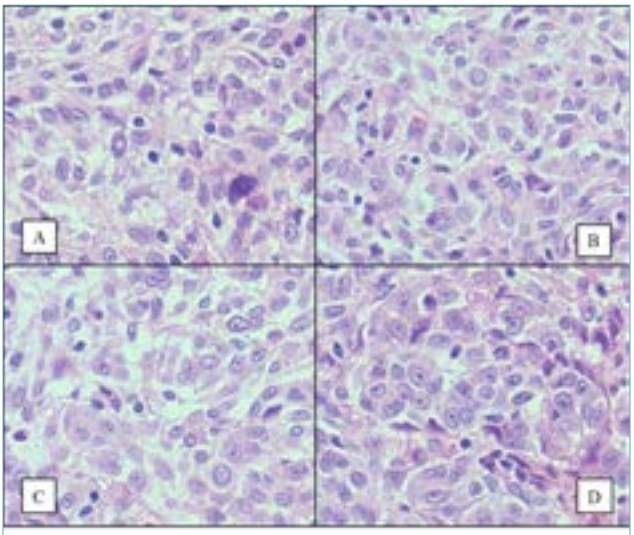

Figure 4. Cytomorphology of metastatic melanoma to breast. A: intranuclear pseudoinclusion; B: prominent nucleoli and deposits of hemosiderin, absence of melanic pigment; C: epithelioid and fusiform cells; D: syncytial aggregate. (Original magnification x40, H&E [A, B, C, D]).

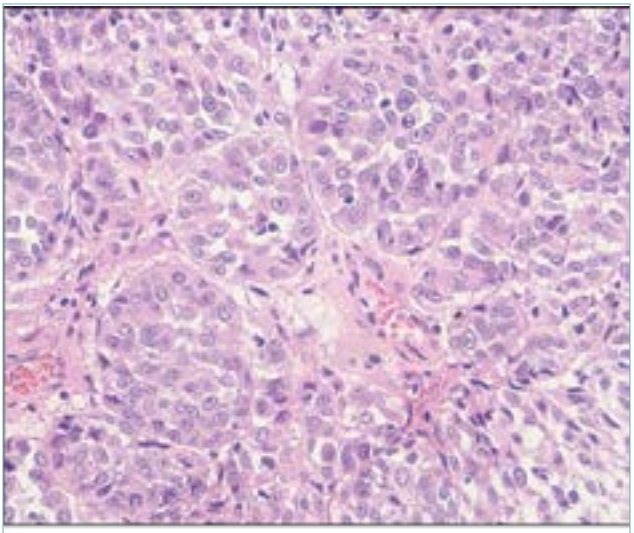

Figure 5. Cytomorphology of metastatic melanoma to breast. Many syncytial aggregates suggesting lobular cancerization. (Original magnification x40, H&E).

mens by means of "Idylla BRAF Mutation test" on Biocartis Idylla System.

Conclusions. Melanoma represents approximately 3% of malignant neoplasm[3]. Metastasis from malignant melanoma can present everywhere, with a large spectrum of appearances, but its common metastatic sites are lymph nodes, liver, lung and brain. Metastatic melanoma to the breast is a very rare event, it can mimic high grade carcinoma and you can avoid the mistake if you only are aware of a history of primary malignant melanoma[2]. In our Institute we detected only 19 cases from 1999 to 2019, 4 of them in the absence of history of primary malignant melanoma. The case we present is one of the 4. FNA is a well-established, minimally invasive technique for the diagnosis of metastatic melanoma (*sensitivity: 97%, specificity: 99%)*[1]. In our case this procedure has been crucial to give the correct diagnosis and address the Clinician with an important tool for therapy as is the presence of BRAF mutation.

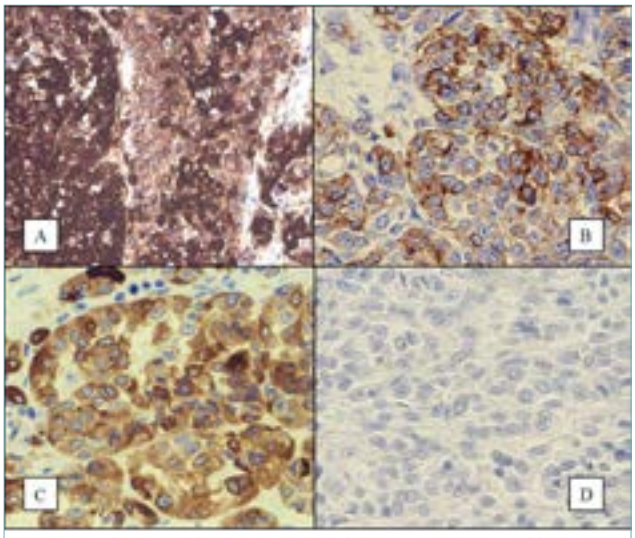

Figure 6. Immunohistochemistry of metastatic melanoma to the breast. Strong positivity for S-100 protein [A], HMB-45 [B] and Melan-A [C], negativity for cytokeratins AE1/AE3. (Original magnifications x20 [A], x40 [B, C, D]).

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FNC *VERSUS* FNB OF BILIO-PANCREATIC LESIONS: ARE THEY REALLY OPPOSING TECHNIQUES OR CAN THEY BE COMPLEMENTARY? A SINGLE INSTITUTION EXPERIENCE

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Objective. Diagnostic assessment of bilio-pancreatic lesions represents a crucial step in medical clinical practice considering that malignant bilio-pancreatic adenocarcinomas remain the most lethal neoplasm of the gastro-bilio-intestinal tract. Recent reports describe an

increase in the incidence of asymptomatic pancreatic nodules however, this earlier detection has not so far translated into better survival rates. In this background the acquisition of biological material by Endoscopic UltraSound (EUS) evaluation has proved to be the most effective technique. In this setting two modalities have come to attention: Fine Needle Cytology (FNC) and Fine Needle Biopsy (FNB). FNC represents a very useful, safe and highly accurate diagnostic tool for assessing and characterizing bilio-pancreatic lesions, as confirmed in several studies. The great strength of FNC is its excellent specificity while there are still conflicting results regarding its sensitivity and sample adequacy. However, many factors influence the diagnostic yield of cytology of bilio-pancreatic lesions, including the size of the needle, the use of tissue blocks versus slides, the use of rapid on-site evaluation (ROSE) for sample collection and the number of passes into the neoplastic lesion. All these factors still remain unstandardized and basically are operator-dependent. This lack of homogeneity of FNC results together with the availability of needles for tissue core biopsy paved the way to histological evaluation of bilio-pancreatic masses by FNB under EUS guidance. According to some Authors, this procedure is both safe and accurate enabling Pathologists to perform difficult diagnoses with the aid of immunohistochemistry. Moreover the preservation of histological material may allow in the near future the possibility to test it for molecular markers and targeted therapies.

Materials and methods. We reviewed our series of 382 diagnostic EUS evaluations of bilio-pancreatic lesions performed at our hospital between 2015 and 2018. The procedure was performed with variable gauge needles (ranging from 19G to 25G with a mean diameter of 23G). In 304 patients a single EUS was sufficient to achieve diagnosis while 33 of them underwent more than one procedure. Cell block could be obtained in 264 cases. Simultaneous FNB was carried out in 74 patients with a 19G needle. Each FNC specimen was fixed in 95% ethanol overnight and stained with Papanicolaou (PAP) method or air dried prior to staining with May-Grunwald Giemsa (MGG). A cell block was also prepared using an agar method and each section was stained with H&E or PAP. FNB was directly fixed in 10% buffered formaldehyde and then routinely processed. Sections were stained with H&E. Immunocytochemical and immunohistochemical analyses were performed according to standard immunoperoxidase methods. The panel of principal antisera included cytokeratin AE1/AE3, cytokeratin 7, cytokeratin 19, p53, Ki67, synaptophisin, chromogranin A, NSE, CD56, CD20, CD3, LCA/CD45, TTF1, vimentin (Ventana).

Results

Clinical features

Our series was composed by 337 patients (173 males and 164 females) with a median age of 68.9 (range from 29 to 82). A large proportion of patients (136/337, 40,35%) were affected by lesions located in the head of the pancreas whereas 34,12% of cases (115/337) were characterized by an anatomic location in the pancreatic body and in the tail; 21 patients (21/337; 6,23%) presented an alteration in the uncinated process. 56 patients displayed lesions in the pancreato-biliary ducts. The dimensional range of the lesions was wide and varied from 3 mm to 150 mm with an average diameter of 27,7 mm. 38 patients (38/337; 11,27%) displayed pancreatic cystic lesions.

Cyto-histological diagnoses

The overall rate of sample adequacy was of 98,69% (377/382 samples) with only 5 inadequate FNC. Among the overall adequate specimens a diagnosis of malignant neoplasm was achieved in 41,11% of cases (155/377) whereas the clinical suspect of neoplasia was not confirmed in 142 cases (142/377; 37,66%). In 37 cases (37/377; 9,81%) a definite diagnosis of malignancy could not be made but the cytological sample was suspicious for malignancy. As for neoplastic histotypes, adenocarcinoma of ductal origin was the most common detected histotype (139/155; 89,67%). Neuroendocrine neoplasms were diagnosed in 12 cases (12/155; 7,74%). Concerning concomitant FNB, our series displayed the following Results. in 58 cases (58/74, 78,37%) cytological and histological diagnoses were concordant with FNB allowing a more deepen immunoistochemical characterization of the neoplasms. As for the remaining 16 discordant cases (16/74, 21,63%) we noticed that in 11 cases FNB was negative while the concurrent FNC displayed a cytological picture of malignancy. In the last 5 cases (5/74; 6,75%) FNB proved to be diagnostic for malignancy in the event of a negative FNC.

Conclusion. As previously stated, diagnostic assessment of bilio-pancreatic lesions plays a crucial role in everyday clinical practice since bilio-pancreatic adenocarcinomas are still one of the most lethal neoplasm of the gastro-bilio-intestinal tract. In this setting FNC and FNB under EUS guidance have become more and more efficient to provide biological diagnostic material. Among malignant neoplasia, as expected, the most common encountered histotype in our series was pancreato-biliary ductal adenocarcinoma, which accounted for nearly 90% of positive diagnoses, followed by neuroendocrine tumors. As for the latter, immunocytochemical analyses carried out on cell block whenever possible allowed us to classify them according to current guidelines. Several studies confirmed the excellent specificity of FNC but controversial data are still reported about its sensitivity mainly due to lack of homogeneity of an operator-dependent procedure. Our series demonstrated an overall rate of adequacy greater than 98% and a high percentage of cases (69,1%) with biological material sufficient to prepare a cell block. The availability of needles for tissue core biopsy paved the way to histological evaluation of bilio-pancreatic masses by FNB under EUS guidance. In our experience the vast majority of FNB (78,37%) confirmed cytological results and allowed a more thorough characterization of the neoplasm by immunohistochemistry. Moreover, in the 6,75% of cases FNB provided enough material to reach diagnoses while FNC was non diagnostic/negative, thus proving to be a complementary procedure to FNC. To answer the title question, we consider that FNC and FNB are operator-dependent techniques and therefore the experience of both endoscopist and pathologist are of paramount importance. Moreover a strict multidisciplinary methodology is needed to achieve the correct clinical-pathological diagnosis. Finally, we would like to underline that FNB enables the storage of formalin-fixed-paraffin-embedded biological material that may be useful for further research of new molecular pathways and/or innovative therapeutic strategies.

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ROLE OF PAX8 IN THE SCREENING IMMUNOCYTOCHEMISTRY PANEL FOR CARCINOMATOUS EFFUSIONS IN WOMEN

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Introduction and objectives. Many of malignant effusion cases with unknown primary have cytomorphological overlap. Due to this diagnostic difficulty, immunocytochemistry (ICC) on cell block can be used to get insight into the primary site. It was aimed to evaluate addition of PAX8 in the screening ICC panel for carcinomatous effusions in women.

Methods & materials. Total 892 effusion samples were evaluated, out of which 75(8%) (50 women & 25 men) comprised of carcinomatous effusion. Study included 44/50 (88%) (38 peritoneal & 6 pleural) samples with adequate cell blocks. Primary panel of ICC markers comprised of calretinin, CK7, CK20, CDX2, WT1, mammoglobin, CK 5/6 and PAX8.

Results. On follow up, the commonest site of primary malignancy (35/44) was ovary with sensitivity, specificity, positive predictive value and negative predictive value of CK 7- 97.14%,11.11%, 80.95 & 50%, of WT-1-51.4%,100%, 100% & 34.6%, of PAX8 71.43%,100%, 100% & 47.37% and of combined WT-1 & PAX8 74.29%, 100 %, 100% & 50% respectively.

Conclusion. Most common cause of carcinomatous effusion among women was was ovary followed by Breast, gall bladder and GIT. WT-1 is a highly sensitive and specific marker for ovarian malignancies, however its sensitivity is lesser than PAX-8. WT-1 is also expressed in mesothelium and is unable to detect mucinous and clear cell adenocarcinoma of ovary, however PAX-8 can identify all Müllerian derived benign or malignant epithelial neoplasms. Since ovarian carcinoma is the commonest cause of malignant effusion among women, it is worth to include Pax-8 with other markers in the screening ICC panel.

Key words: PAX-8, WT-1, effusion, immunocytochemistry

MISSING OR MISLEADING CLINICAL DOSSIER IMPACT ON THE PROPER CYTOLOGICAL DIAGNOSIS: A CASE REPORT OF CERVICAL LYMPH NODE FINE NEEDLE ASPIRATION (FNA).

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Introduction. Cervical lymph nodes can be affected by malignant lymphomas or be the site of metastatic diseases[1]. Most frequently, the latter originate from head and neck primary cancer, but can also come from distant sites[3,4,5], like breast[2], lung, stomach, ovary and uterine cervix[6].

Aims. We want to focus on the importance of receiving cytological specimens together with a complete clinical dossier to assist in the diagnostic path. For this we present a case we recently faced.

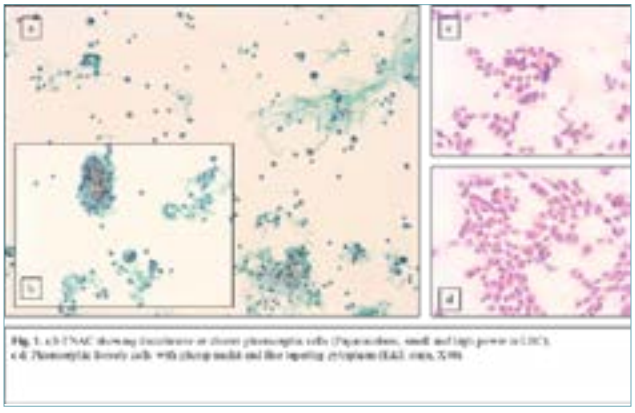
Clinical history. A 65 years old lady came to our Division of Radiology, asking for a FNA on a palpable latero-cervical nodule. The only history she relates is that we are the sole Institution in town where she could find an appointment to do it, being August. The Radiologist didn't ask any more questions, but while performing the ultra sound (US), he found many more nodules than the single palpable one. He performed the FNA[7] and sent the specimen to the Cytology Lab; the slides where stained according to standard procedures (Papanicolaou, May-Grünwald Giemsa). The material put in Cytolyt® solution (Hologic) was used to prepare a slide with ThinPrep® method (Hologic) and a cell block suitable for immunostaining and molecular biology.

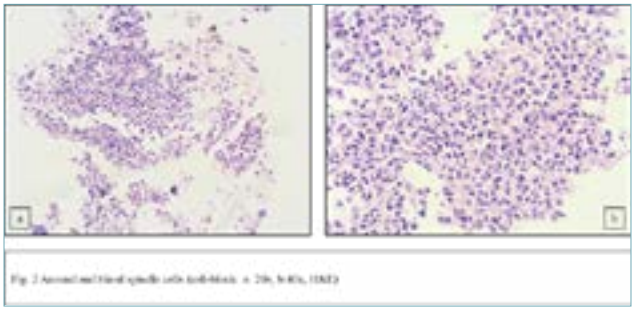
Results. The morphological analysis of the slides showed atypical round or fusiform, medium-sized cells, with high nucleo/cytoplasmic ratio, mainly isolated and dispersed in the background intermingled with small lymphocytes.

First of all, we had to decide if the tumor cells were epithelial or not. Immunocytochemistry (ICC) for LCA, S100 and cytokeratins was performed resulting positive for cytokeratins only.

By this time, the Radiologist came to show us a picture he had taken just after FNA, of a skin lesion over the scalp of the lady. We then asked for p40 and synaptophysin immune reactions: the first one was negative, while the second one turned out positive.

The third step was to explore the path of a neuroendo-





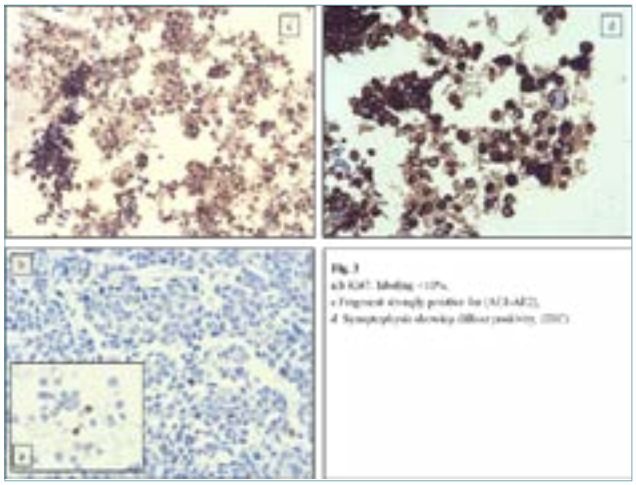


TABLE 1
Immunohistochemical markers associated with the origin of head and neck primary tumor or metastasis.

Day	Markers Panel	Tumor origin	Result
1	LCA,	Lymph-node,	Negative
	CK AE1-AE3,	epithelial,	Positive
	CDX2		Negative
	TTF1		Negative
	S100	Non epithelial	Negative
2	Synaptophysin	Neuroendocrin tumor	Positive
	P40 (Squamous cells carcinoma)	Cervix, Esophagous	Negative
3	Neurofilament protein	CNS	Negative
	MCPyV	Merkel tumor	Negative
	CK20	Merkel tumor	Negative
	Ki-67		5%

Abbreviations: LCA: Human Leukocyte Common Antigen, CNS: Central Nervous System, MCPyV: Merkel Cell Polyomavirus, Ki-67: Proliferation Marker Protein, TTF-1: Thyroid Transcription Factor-1, CDX-2: Caudal Type Homeobox-2

crine tumor of the skin. We asked for cytokeratin 20, neurofilament proteins ICC and Merkel cell-Polyomavirus in situ hybridization, and all these assays were negative

The last step was to try to identify a different primary site and give information about cell proliferation index. ICC for TTF1 and CDX2 was also negative, and Ki67 was 5%.

After seven days and many ICC reactions, a final diagnosis was rendered of neuroendocrine tumor metastatic to lymph node, without any suggestions about the likely primary site.

Conclusions. FNA procedure is an efficient, safe and cost-effective way to reach a quick cytological diagnosis. As cytopathologists we need the same amount of clinical news[8] as any other specialist. Their absence is cell-consuming, time-consuming and contributes to increase the costs for the Lab and the Health System[9].

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MOLECULAR AND CYTOLOGICAL FEATURES IN THYROID CARCINOMAS: FOCUS ON PAPILLARY THYROID MICROCARCINOMA

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Aim. The improvement and the widespread use of the ultrasound (US) screening or thyroid gland, were related to the increasing number of papillary thyroid microcarcinoma (mPTC) diagnosis.[1, 2] In addition to the US approach, fine needle aspiration (FNA) is necessary for the microscopic confirmation of mPTC. [3] However, due to the limited mortality and the indolent behaviour of mPTCs, a more conservative approach is preferred for these patients; thus a correct pre-operative diagnosis is requested.[1, 4] The aim of our study was to verify if cytological or molecular features were able to distinguish mPTC from PTC >1 cm pre-operatively.

Material and methods. We retrospectively collected from our archives a total of n = 179 cases with histological diagnosis of malignancy (n = 96 PTCs and n = 83 mPTCs) with available cytological diagnosis and molecular assessment. Subsequently, we focused on the difference between PTCs and mPTCs in terms of cytological categories sec. Italian consensus for the classification and reporting of thyroid cytology (in particular TIR3A, TIR3B, TIR4 and TIR5 classes) and molecular assessment (Inadequate, Wild-Type, *BRAF*-, *N-H-KRAS*-, *RET*/*PTC*- and *PAX8*/*PPARG*-mutated cases).

Results. On the overall, a high percentage (20.5% vs 11.5%) of histologically confirmed mPTCs were cytological diagnosed as TIR3A, whereas PTCs were more frequently diagnosed as TIR5 (51.0% vs 44.6%). From a molecular standpoint, we identified both a higher number of *BRAF*-mutated (65.6% vs 60.2%) and *RAS*-mutated cases (10.4% vs 6.1%) in PTCs >1 cm in comparison to mPTCs. Conversely, *RET*/*PTC*-mutated cases were higher in mPTCs than in PTCs (4.8% vs 2.1%). However, these results did not reach statistically significance (p = 0.3867 and p = 0.6185, respectively). Conversely, in the FNA classified as TIR3A, we identified a statistically

significant higher frequency of *BRAF*-mutations in PTC >1 cm in comparison to mPTC (p = 0.0293).

Conclusions. In this study we observed that no specific cytological or molecular features could discriminate between mPTCs and PTCs >1 cm. However, we confirmed the potential diagnostic and prognostic role of *BRAF* mutations in indeterminate cytological categories.[5]

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DERMOPATOLOGIA

A 47-YEAR-OLD PATIENT WITH MULTIPLE DESQUAMATIVE PATCHES AND SUBSEQUENT ONSET OF PAPULAR LESIONS: REPORT OF AN UNCOMMON CASE

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Objectives. The association in the same patient of mycosis fungoides (MF), a peripheral T-cell lymphoma, with B-cell malignancies is an unusual event; most of the cases described in the literature are case reports, and case series are strikingly sparse.

We herein present a rare case of a 47-year-old man with a coexistence of MF and primary cutaneous follicle center lymphoma (PCFCL); to the best of our knowledge, only another similar case has been described in the literature [1]: in that case, MF showed a CD4/CD8 double-negative phenotype, and PCFCL was the first malignancy to develop.

Materials and methods. A 47-year-old male patient presented with scaly, erythematous, slightly itchy, patches located at the trunk and in the cervical region. Lesions were noticed by patient four months earlier. Concurrently, small cutaneous papules, measuring 2 cm in their greatest diameter, were found in the right axillary region. He had neither previous history of skin cancer nor familiarity for neoplastic diseases. Two skin biopsies of the trunk and axillary lesion were performed two months apart from each other. Surgical specimens were submitted for histological examination in neutral-buffered 10% formalin, dehydrated using standard techniques, embedded in paraffin, cut to 5 µm, and stained with hematoxylin and eosin. Immunohistochemical studies were performed and the following antibodies were tested: CD3, CD5, CD45RO, CD20, CD4, CD8, BCl-6, CD10, BCL-2 and MIB-1.

Results. Histological examination showed a parakeratotic and mildly spongiotic epidermis. The upper dermis was occupied by small-sized lymphocytic infiltrate with focal band-like distribution. Lymphocytes displayed mild atypia with "halo" features and cerebriform nuclei. Focal epidermotropism, consisting of isolated intraepidermal lymphocytes, was found. Pautrier microabscesses were absent. Immunohistochemically, the atypical lymphocytes were positive for CD3, CD5, and CD45RO and negative for CD20. Moreover, they showed a T-helper phenotype (CD4+ and CD82). Based on both morphological and immunohistochemical features, a diagnosis of early *"mycosis fungoides"* was rendered. The patient underwent local corticosteroid therapy, which led to the complete disappearance of the cutaneous patches. No recurrence of the disease has been noted after 1 year of follow-up. After 2 months, the patient underwent surgical excision of the papules occurring in the axillary region. Histologically, lymphatic follicles were scattered in the upper and medium dermis. They were irregularly spaced, with atypical architecture, including thinning of the mantle zones, reduction of the normal polarization, and absence of intrafollicular histiocytes. The overlying epidermis was spared. Immunohistochemically, follicle center cells were stained with CD20 and BCl-6. CD10, BCL-2, and CD3 were negative. Among follicles, a population of non-neoplastic CD3+ T-cells was seen. MIB-1 proliferation index was low in neoplastic follicles. The patient showed no systemic B symptoms, and the full-body computed tomography scan was negative for superficial and deep lymphadenopathies or organomegalies.

On the basis of the clinical, radiological, and histopathological findings, a diagnosis of *"primary cutaneous follicle center lymphoma"* was rendered. In accordance with the indolent nature of PCFCL, the patient underwent follow-up, consisting of a dermatologic evaluation every 3 months. After 1 year of follow-up, papules reappeared in the same region, and after the excisional biopsy, recurrence of PCFCL was histologically confirmed.

Conclusion. In the few cases reported in literature of coexistence of MF and a B-cell malignancy, MF is more frequently the first to develop and B-cell malignancy, the second. The most common association is between MF and B-cell non-Hodgkin lymphoma, particularly chronic lymphocytic leukemia/small lymphocytic lymphoma, whereas coexistence in the same patient of MF and Hodgkin lymphoma is a more uncommon evenience [2]. Moreover, the coexistence of 2 primary cutaneous lymphomas, respectively, of T-cell and B-cell origin, with no systemic involvement, has to be considered an even rarer coincidence; few reports of association between MF and primary cutaneous marginal zone B-cell lymphoma are present and just another case similar to ours is reported to date [1].

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BALLOON CELL MELANOMA: AN UNCOMMON ENTITY REPRESENTING A DIAGNOSTIC PITFALL IN DERMATOPATHOLOGY.

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Objectives. Balloon cell melanoma (BCM) is an uncommon and diagnostically challenging neoplasm; clinically, it may arise as a soft, firm or rubbery nodular lesion with a polypoid or ulcerated surface, generally located in the lower body [1,2]. and be misinterpreted as dermatofibroma, dermal nevus, Spitz nevus or basal cell carcinoma. In this direction, dermoscopy represents a useful tool for to the clinician. Polarized dermoscopic examination confirms the algorithmic method used for pigmented skin lesions "Chaos and Clues": a central structureless white area related to the balloon cell component, with a rim of structureless very focal pigmented reticular lines (with blue-gray inner pigment and outer brown one), corresponding to the asymmetrical and peripheral distribution of melanin detected in the non-balloon cell component [3]. Other clues to malignancy include polymorphous vessels, visible at the perimeter of structureless white area, both at the superior and lower extremity, as a negative pigment network of linear, dotted or serpentine vessels.

Materials and methods. A 56-year-old female patient presented to a privately working dermatologist with an ulcerated, partially raised and irregularly pigmented skin lesion located at the back. At dermoscopy, irregular, non pigmented, white areas, with a rim of structure-less pigmented reticular lines, alternating with regular and normally pigmented zones were visible; some serpentine vessels were also found near the non pigmented component. An excisional biopsy was made.

Surgical specimen was submitted for histological examination in neutral-buffered 10% formalin, dehydrated using standard techniques and embedded in paraffin. Immunohistochemical studies were performed with the labeled streptavidin-biotin peroxidase detection system using the Dako automated immunostainer (Dako autostainer link 48, Glostrup, Denmark). The following antibodies were tested: Melan-A, HMB-45, S-100 protein and MIB-1.

Results. After the excisional biopsy, histological examination showed an asymmetrical and ulcerated melanocytic tumor, with two distinct cellular components: a congenital compound nevus, in proximity of which dermal aggregates of large, round or polygonal shaped cells with abundant, clear and vacuolated cytoplasm, suggestive for balloon cell changes, with poor stroma interposed, were found. Nuclear pleomorphism, nucleoli with irregularly disposed chromatin and intranuclear cytoplasmic pseudoinclusions were diffusely present in balloon cell component. Few mitoses were also found. Immunohistochemically, both the balloon cells and the melanocytes of the preexisting compound nevus were positively stained with S-100, HMB45 and Melan-A; MIB-1 index was high in balloon cell component. Based on both morphological and immunohistochemical features, a diagnosis of "balloon cell melanoma" was rendered. Lesion infiltrated the reticular dermis (IV Clark level). Breslow thickness measured 2.8 mm. Patient underwent local wide-deep re-excision and sentinel lymph node biopsy and no residual or metastatic disease was found. She is currently healthy and no recurrence of disease has been noted after 6 months of follow-up.

Conclusion. Histologically, BCM is composed of clear, foamy cells, accounting for more than 50% of total neoplasm; they are large, polyhedrical or round shaped and show a vacuolated cytoplasm without pigment. This invasive amelanotic component may be mixed to a second distinct invasive component made of atypical non-mature epithelioid or spindle cells. It was initially believed that balloon cells arised from the transformation of melanocytes to sebocytes as a consequence of glycogen accumulation or lipoid degeneration. This theory has actually been dismissed and, although etiology is still unknown, ultrastructural studies have indicated demyelinization and merging of melanosomes at the origin of ballooning cells and their vacuolated cytoplasm.

The lack of pigment in balloon cells makes BCM difficult to diagnose without special stains that correctly identify melanocytic origin: S-100 protein, Melan-A and HMB-45. BCM positively stains for these markers, whereas PanCK, EMA, fat stains and PAS staining are usually negative. Histopathological differential diagnosis of BCM includes both benign and malignant lesions, such as balloon cell nevus, halo nevus, basal cell carcinoma, metastatic clear cell renal carcinoma, clear cell sarcoma of soft tissues, perivascular epithelioid tumor, histiocytic lesions and liposarcoma [4]. The distinction between BCM and balloon cell nevus, may be particularly challenging [4]: unlike its benign counterpart, BCM shows atypia, increased mitotic activity, high MIB-1 index, nuclear pleomorphism, poor stroma interposed and lack of melanocytic maturation and melanin. Finally, BCM is a challenging diagnosis for both the clinicians and the pathologists and a multidisciplinary approach is recommended to recognize and promptly treat this rare entity.

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UTILITY OF A GEL STAND-OFF PAD IN THE DETECTION OF DOPPLER SIGNAL ON FOCAL NODULAR LESIONS OF THE SKIN.

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Aim. Sonography (US) of the skin is rapidly gaining importance as a non-invasive, accurate, and reproduc-

ible method to detect focal lesions and describe their morphologic appearance. However, despite many accurate descriptions of skin tumors with US, the vast majority of benign and malignant lesions seem to share the same well-defined hypoechoic pattern. Thus, although being highly sensitive in tumor detection, US can be considered poorly specific in tumor characterization[1-4]. Color Doppler US, being able to produce an accurate map of tumoral vessels, is now considered as one of the most sensitive methods to detect signs of malignant neovascularity[5]. However, despite the use of high-frequency transducers and low-flow scanner settings (high transmission frequency, low pulse repetition frequency, minimum wall filter, color gain increased up to the artifacts threshold), it is sometimes difficult or impossible to detect flow signals of the superficial lesions at color- and power-Doppler (CD and PD) US[6]. Gel pad is an aqueous, flexible, easy available, disposable spacer used for the US scan of superficial and difficult-to-visualize areas; improving the transmission of acoustic energy by shifting the focus more superficially; it is a useful tool to achieve a better B-mode US definition of skin lesions with superior probe stability and contact[7], particularly to scan uneven surfaces[8]. In our study, a commercial gel pad was used as a coupling agent for dermatologic US. The purpose was to outline the technical aspects of gel pad application and to evaluate the role of gel stand-off pad in the detection of the otherwise-missed peri- or intra-lesional flow signals on Doppler imaging. This is the first study about this topic.

Materials and methods. A total of 100 superficial lesions have been undergone to an US evaluation: 32 were histologically benign and 68 malignant. Specifcally, the lesions examined were 33 melanomas (33%), 12 basocellular carcinomas (12%), 10 spinocellular carcinomas (10%), 8 cutaneous lymphomas (8%), 5 merkel cell carcinomas, 15 complicated cysts (15%), 8 granulomas (8%), 5 abscesses/cellulitis (5%) and 4 melanocytic nevi (4%). We have used a 7.5–12-MHz linear probe were evaluated prospectively with and without interposition of a gel stand-off pad to detect the presence or absence of vascularization and to classify the vascular pattern. The diagnostic confirmation was obtained by pathologic examination of specimens obtained from a surgical resection (n=67) and US-guided percutaneous fine-needle aspiration cytology/biopsy (n=33). A pathologist with expertise in skin pathology examined the specimens according to standard criteria based on the World Health Organization (WHO) classifcation system.

Results. Peri- or intra-lesional flow was demonstrated in 56% of cases without and in 84% of cases with interposition of a gel stand-off pad; moreover, a statistically significant difference (p value < 0.001) was observed at Chi-square test in the identification of the flow pattern between the use and no use of the pad.

Conclusions. The use of a gel stand-off pad allows the detection of otherwise-missed peri- or intra-lesional flow signals on Doppler imaging, increasing the diagnostic role of this technique in differential diagnosis of skin lesions, but the availability of well-known gold standards, such as physical examination and biopsy with histopathological investigation, will probably limit its diagnostic role to some well-defined fields.

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BALLOON CELL NEVUS: CLINICAL AND HISTOPATHOLOGIC FEATURES

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Introduction

Balloon cell nevus is a rare variant that is characterized histologically by a predominance or complete occurrence of large, vesicular, clear cells, known as balloon cells.

Balloon cells are altered melanocytes with clear vacuolated cytoplasm caused by a defect in the process of melanogenesis. Although rare, balloon cell change has been observed in a variety of melanocytic proliferations, particularly intradermal melanocytic nevi and melanoma. When present, such features may lead to difficulties in diagnosis, particularly with other clear cell neoplasms.

The most common site appears to be the head and neck area, followed by the trunk and extremities, and most commonly under age of 30.

We report an unusual case of the development of balloon cell change in a common melanocytic nevus. The importance of recognizing this change in common nevus to avoid misinterpreting the lesion as malignant is discussed.

Clinical case

Woman, 51 years old, who had melanocytic lesion for about 2 years. On the recommendation of the trusted dermatologist, the lesion (trunk) was removed, and sent to the Department of Pathological Anatomy. Clinically this lesion was referred to as "atypical" and the clinical suspicion was therefore of an "atypical melanocytic nevus"or of a "lentigo maligna". No specific characteristic was dermoscopically recognized.

After sampling, inclusion in paraffin, preparation of sections and staining with hematoxylin-eosin, in the initial sections ordinary melanocytes were present, but, in the next sections, appeared "ballon cells" in the intradermal component. These cells had ample cytoplasm which is finely vacuolated and had small hypercromatic wrinkled

nuclei with scalloped contour. Sometimes these balloon cell melanocytes had scarce melanin pigment and there were, occasionally, multinucleated melanocytes. Mitosis were scarse.
The main differential diagnosis of balloon cell nevus was with balloon cell melanoma.
Some authors (1) consider some crucial points for a correct differential diagnosis between nevus and melanoma "ballon cell":

1. Nuclear pleomorphism (irregular distributed chromatin) and prominent nucleoli throughout the neoplasm (Ballon cell melanoma);
2. The melanocytes appear rather small and monomorphous in balloon cell nevus;
3. Mitotic Fig. s are virtually absent in balloon cell nevus, thus any mitotic Fig. should alert the possibility of melanoma.
4. Clinically, balloon cell nevus is usually seen in younger patients, whereas melanomas are more common in older patients.

Other authors (2) report that the true differential diagnosis between nevus "balloon cell" and melanoma is the possible dysplasia of the melanocytes of the dermoepidermal junction, reserving the last word to the immunohistochemical study.
Immunoistochemical profile was: Melan-A (MART-1) positive in the melanocytes of the junctional and intradermal component. HMB-45 (Melanoma-Ag) negative in the intradermal melanocytes.
BCL-2 and Ciclina D1 were normoespressi.
The diagnosis was of congenital melanocytic cutaneous nevus, with "Ballon Cells" changes.

Comment

The correct differential diagnosis between nevus balloon cells or melanoma ballon cells can be very difficult. Morphological criteria can help, but integration with immunohistochemistry techniques is necessary in order to provide a diagnosis of certainty. Recently, new approaches to molecular biology and gene profiling seem to make this diagnosis more accessible and safe.

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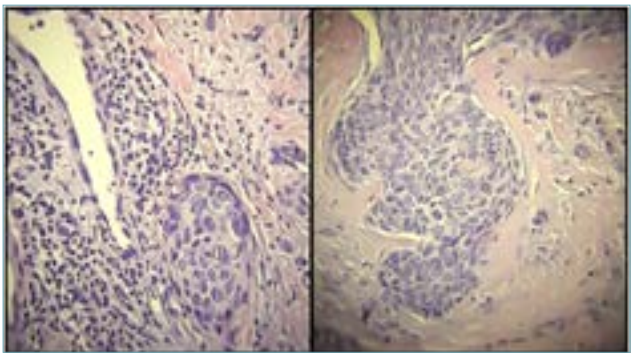

Fig. 1. A Small solid nest (left) and a strand (right) of atypical keratinocytes penetrating the underlying fibrotic dermis.

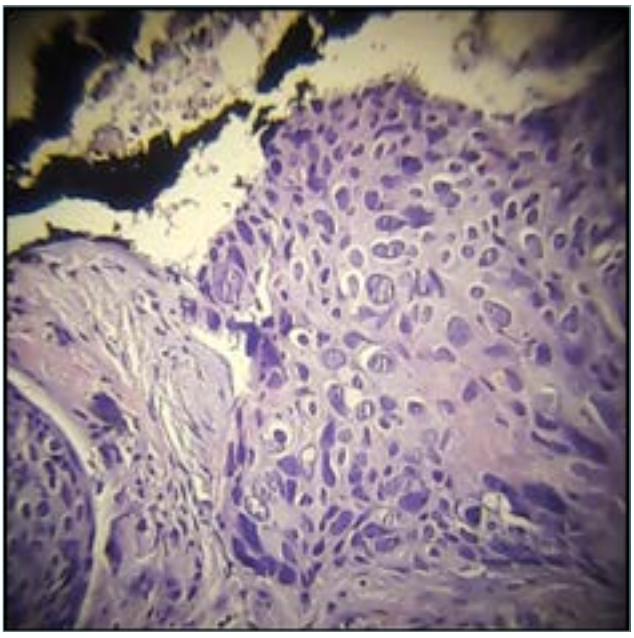

Fig. 2. Pleomorphism of atypical keratinocytes

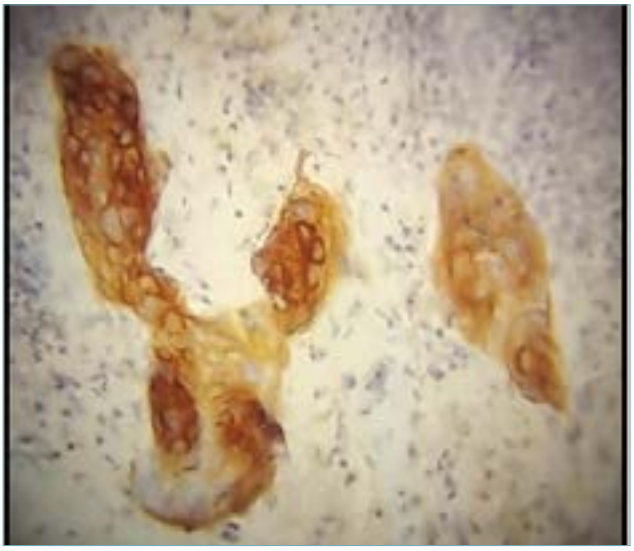

Fig. 3. Positivity for Cytokeratin cocktail AE1/AE3.

A RARE CASE OF ONYCHOLEMMAL CARCINOMA WITH UNDEFINIED CLINICAL DIAGNOSIS

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Background. The onycholemmal carcinoma (OC) is a rare malignant tumor developing from the epithelial cells of the nail bed and only 13 cases are reported in literature. It is easy to confuse this slow-growing and dark-colored lesion with other benign lesions (verruca

vulgaris, onychomicosis), malignant lesions (melanoma and squamous cell carcinoma) and post-traumatic dystrophy. The OC is a distinct entity with its own prognosis but, to date, there is no standard treatment or specific follow up for this pathology. Here, we present the clinical and histopathological features of a case of onycholemmal carcinoma.

Materials and methods. We report the 14th documented case of onycholemmal carcinoma occurring in a 65-year-old male with a clinical suspicion for nail bed melanoma. After total onychectomy and laminectomy, histological examination and immunohistochemistry investigation was performed.

Results. Histologically the lesion was characterized by small solid nests or strand of moderately pleomorphic cells. The neoplastic proliferation was connected to the nail bed epithelium and infiltrated the underlying dermis in a multinodular pattern. The cells showed negativity for Melan-A and positivity for CK AE1/AE3.

The mitotic index was high and occasional atypical mitoses were observed.

Conclusions. The clinical aspects and the slow evolution of this rare lesion leads to erroneous diagnoses. The OC is a distinct entity with peculiar histological features and biological behaviour different from other lesions involving the nail. We believe it is essential to include the OC in the differential diagnosis of these lesions as misdiagnosis can lead to an under-treatment. At the same time the absence of international treatment guidelines can lead to an over-treatment of this rare entity.

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MORPHOLOGICAL AND MORPHOMETRICAL ANALYSIS OF CUTANEOUS SQUAMOUS CELL CARCINOMA IN PATIENTS WITH RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA: A RETROSPECTIVE STUDY

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Background. Recessive dystrophic epidermolysis bullosa (RDEB) is a highly disabling genodermatosis characterized by skin and mucosal fragility and blistering. Cutaneous squamous cell carcinoma (cSCC) is one of the most devastating complications with a high morbidity and mortality rate. Patients with RDEB were reported to have up to a 70-fold higher risk of developing cSCC than unaffected individuals. Immune cells play a role in cancer evolution.

Objective: The aim of our study is to evaluate immunohistological difference between cSCC in RDEB patients versus cSCC in non RDEB subjects.

Methods. A retrospective study of 20 consecutive cases was performed. 5 were biopsies of cSCC taken by 5 RDEB patients; as controls we analysed 10 cSCC in non RDEB subjects (5 primitive, 3 post-burns and 2 post-radiotherapy) and 5 cutaneous pseudoepitheliomatous hyperplasia in RDEB patients. From the paraffin-embedded blocks, 5 µm thick sections were de-paraffinized, hydrated and subjected to immunohistochemical analyses using the antibodies against CD3, CD4, CD8, CD20, CD68. For each patient, mean and standard deviation values for the 10 fields for each antibody were recorded.

Results. We found a significant reduction in immune infiltration in RDEB patients compared to controls. In particular, we have found a reduction in CD3+, CD4+, CD8+, CD20+, CD68+. The presence of CD3 lymphocytes was lower in RDEB patients, both in the cSCC group and cutaneous pseudoepitheliomatous hyperplasia one. This indicates a deficient cell-type immune response in the local peripheral response. cSCC RDEB patients there is a lower expression of CD4 T-helper lymphocytes as compared to the two control groups. This has not been previously reported in literature of cSCC patients affected by RDEB, whereas the increased infiltrate of peritumoral CD4 is known in the other two groups. The deficit of helper lymphocytes has an intrinsic value in quantifying the immune response, featuring a lower antitumoral response capacity as well as a reduced capacity to recruit B lymphocytes, NK lymphocytes and macrophages.

No significant difference was found in size, histopathology, grading, number of mitoses and EGFR expression between the different groups.

Conclusions. Our data show a reduction in immune cell peritumoral infiltration. It can be hypothesized that a condition of immune tolerance toward the tumor develops in epidermolysis bullosa patients, as a consequence of these deficits, that favour the tumor growth. Considering the well-known evolution of cSCC in RDEB as well as the youngest age at diagnosis, we could assume that immune dysfunction can lead the cSCC aggressivity in these patients.

BLASTIC DENDRITIC CELL NEOPLASM: REPORT OF A CASE

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A 72-year-old male, who reports long-term pancytopenia (lengthening of aPTT related to LAC positivity), presented to the dermatology department because of the presence of asymptomatic erythematous purplish patches infiltrated at the level of the scalp, forehead and trunk; those lesions arose two months before. During the dermatological examination the lesions were clinically judged as a pityriasis rosea and a punch biopsy of two of them was performed.

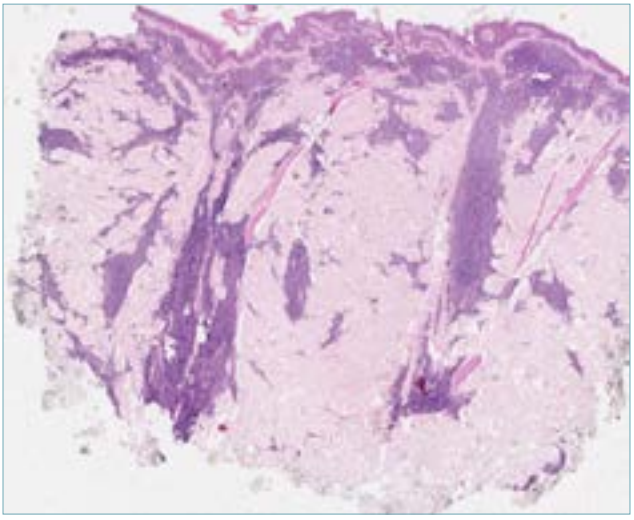

Fig. 1.

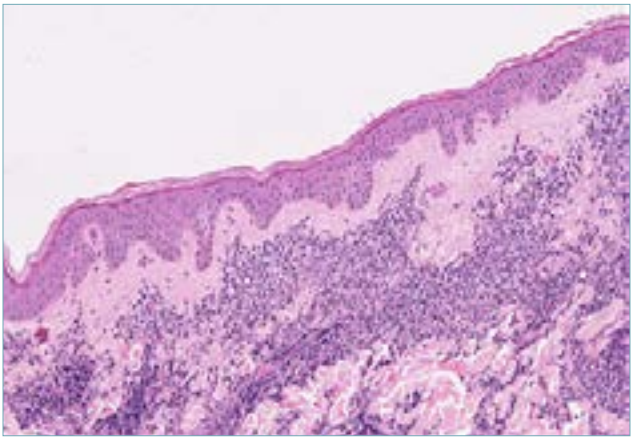

Fig. 2.

The histopathological examination of both lesions showed a dense inflammatory infiltrate composed of medium-sized monomorphic lymphatic cells admixed with large cells with irregular nuclei, arranged in a subepithelial and periadnexal location, without obvious epidermotropism (Figg. 1, 2). No hypodermis in the biopsies were identified.
Immunohistochemical evaluations have been performed, and the inflammatory infiltrate described resulted focally positive for CD3, diffusely positive for CD4, negative for CD8, CD20 and CD30. The Ki-67 labeling index was 70%. The lesion was first thought to be a peripheral T lymphoma, NAS. However, the molecular biology analysis later performed in order to better characterize the lesion, failed to demonstrate a monoclonal population of T lymphocytes. Considering the morphological features, the immunohistochemical and molecular results, the diagnosis of a blastic plasmacytoid dendritic cell neoplasm was favored.
The histological material was also sent for a second opinion to an experienced hematopathologist (Prof. Facchetti, Brescia); the results of the further immunohistochemical studies performed (CD4+CD56+TCL1+CD123+BCL2+), supported the diagnosis.
The patient underwent many other diagnostic exams, and the same neoplastic cells were found in the CSF; because of that a polychemotherapy with HyperCVAD scheme was started. One year later the patient reached a complete remission, he is now in good health and an autologous bone marrow transplant has been programmed.
Blastic plasmacytoid dendritic cell neoplasm is an aggressive tumour derived from the precursor of plasmacytoid dendritic cells, characterized by high skin tropism and a tendency for bone marrow and leukaemic dissemination [1,2]. The median of survival is 10.0-19.8 months, irrespective of the initial pattern of disease. Most cases (80-90%) show an initial response to multiagent chemotherapy, but relapses with subsequent resistance to drugs are regularly observed [3,4,5,6,7].

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ROLE OF BRIT1, CHROMATIN ASSEMBLY FACTOR 1/ P60 AND POLY (ADP-RIBOSE) POLYMERASES 1 IN CUTANEOUS MELANOMA

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Cutaneous melanoma (CM) is the most lethal skin malignancy, with a continuous and dramatic increase in incidence worldwide[1]. To date, there are no reliable prognostic biomarkers in CM. Among the suitable prognostic markers, PARP1 (poly-adenosine diphosphate-ribose polymerase 1) plays a critical role in the regulation of developmental processes, including apoptosis, DNA-repair, epigenetic marking of chromatin, assembly of higher-order chromatin structures and transcriptional activation. Furthermore, recent studies have shown that

CAF1 (chromatin assembly factor 1), an important regulator of the chromatin structure during cell replication and DNA repair, is overexpressed in CM with aggressive behavior[2,3]. In the last few years, there has been a growing interest in the role played by BRIT1 protein (repeat inhibitor of TERT expression 1), also known as MCPH1 (microcephalin 1), in the pathogenesis of many cancers[4]. BRIT1 plays a crucial role in the mitotic and meiotic homologous recombination and is involved in the regulation of genomic stability[4]. BRIT1 represents a key regulator of DNA damage response (DDR) and links chromatin remodeling with DNA damage response in the control of DNA repair[5]. Based on these postulates, we aimed to evaluate the role of BRIT1 as markers of outcome and progression-free survival and to verify whether exist a relationship between BRIT1, PARP1 and CAF1 expression in CM patients. Immunohistochemistry for these three molecules was performed on a series of primary CM retrieved from the archives of the department of Advanced Biomedical Sciences, Section of Pathology, University of Naples "Federico II", Naples, Italy. The results were compared with histopathological and follow-up data of patients. We found that the CM with the worst prognosis and most of CM metastases evaluated showed absent expression or cytoplasmic overexpression of BRIT1 in association with high level of reactivity for CAF1/p60 and PARP1. These data show that this small panel of antibodies, available in any hospital, may provide additional information for a better-defined CM risk stratification and be of benefit in the clinical management and the identification of more personalized therapy for these patients.

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SUBCUTANEOUS PANNICULITIS-LIKE T-CELL LYMPHOMA: A CASE REPORT

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Introduction. Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a relatively rare subtype of cutaneous non-Hodgkin lymphoma where neoplastic T-cells are located exclusively within the subcutaneous fat and display an α/β cytotoxic phenotype[1,2]. Clinical manifestations of this rare disease are often aspecific and its morphological features can resemble those of other cutaneous disorders. This characteristic usually causes a delay in the correct diagnosis.

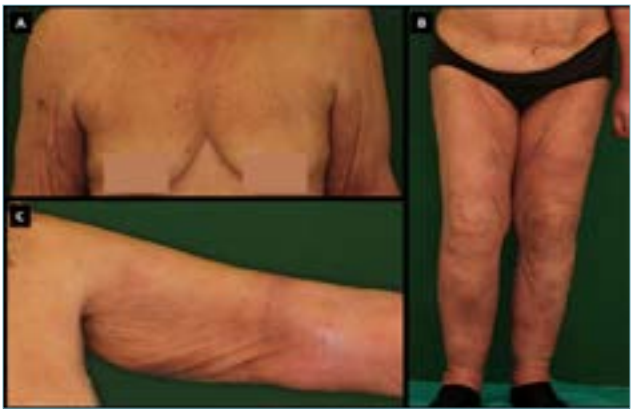

Fig. 1. A, **B**, **C**, hyperpigmented and hardened plaques occupying most of the skin and merging into cuffs on the distal segments of the limbs; the widespread hardened skin forms areas of cutis laxa specially visible on folds

Case report. We present the case of a 70 years old female with a long history of clinical "eczema" who was admitted for hyperpigmented and hardened plaques occupying most of her skin and merging into cuffs on the distal segments of the limbs. On the right upper arm, physical examination revealed subcutaneous nodules overlying the plaques. The widespread hardened skin formed areas of cutis laxa especially visible on folds (Fig. 1). The patient lamented moderate itch and burning sensation. Laboratory tests were normal. No other useful clinical data. These features of slack skin appearance oriented towards the clinical diagnosis of "Granulomatous slack skin T-cell lymphoma", an indolent clinical variant of mycosis fungoides. Thus, a skin biopsy from the right thigh was made.

Results. Histological examination showed mild epidermic hyperplasia, acanthosis, hyperparakeratosis and exoserosis in the stratum corneum. Deep dermis and subcutaneous tissue harbored a dense infiltrate consisting of T-cells (CD3+) mixed with sparse B-cells (CD20+) and a large number of eosinophils (Fig. 2). There were no signs of lymphocytic aggression towards epidermis, skin annexes or blood vessels. Rimming of adipocytes by medium-sized T cells (CD3+) was only focally present. Such histologic picture could conFig. both SPTCL and lupus panniculitis[3.] Differential diagnosis between the two entity is often challenging, as they tend to overlap in later stages; therefore we performed molecular analysis of the TCR genes, showing monoclonal TCR rearrangement. The evidence of monoclonal rearrangement of TCR genes led to the final diagnosis of SPTCL[2].

Conclusions. The peculiarity of our case precisely resides within its atypical history, clinical presentation and morphological features. In our case molecular analysis were pivotal in the final diagnosis. Both Granulomatous slack skin T-cell lymphoma and SPTCL are indolent. SPTCL has an overall good prognosis with a 5-year survival over 80%. Nevertheless in SPTCL hemophagocytic syndrome and angiotropism can occur, worsening prognosis[2]. Correct differential diagnosis between these

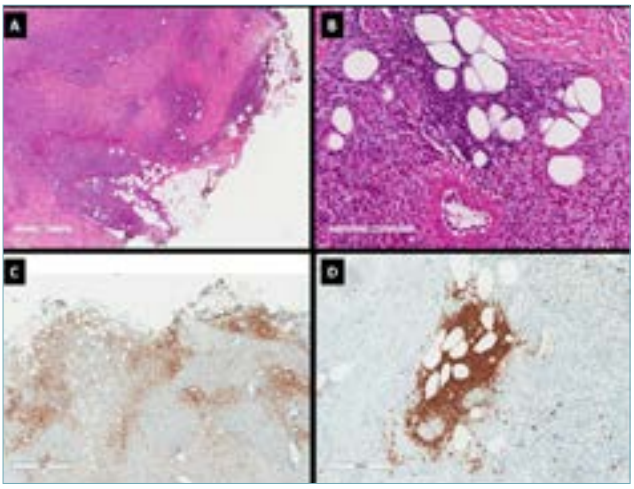

Fig. 2. **A**, Epidermic hyperplasia, acanthosis, hyperparakeratosis and exoserosis in the stratum corneum, deep dermis and subcutaneous tissue harbored a dense infiltrate (H & E, 20×) **B**, focal rimming of adipocytes by atypical small to medium-sized T lymphocytes (H & E, 100×). **C**, Atypical lymphocytes are positive for CD3 (20×) **D**, CD20 (200×).

entities is useful for follow-up and a proper diagnosis can't do without appropriate molecular tests.

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EMATOPATOLOGIA

ANNEXIN A1-POSITIVE SPLENIC DIFFUSE RED PULP SMALL B-CELL LYMPHOMA: BLURRING THE BOUNDARIES OF PRIMARY SPLENIC RED-PULP LYMPHOMAS

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Goals. To describe a rare case of Annexin-A1-positive splenic diffuse red pulp small B cell lymphoma (SDRPL) and to discuss the differential diagnosis of splenic lymphomas, with special regard to the category of splenic B-cell lymphoma/leukemia, unclassifiable (SBCL-U).
Materials and methods. A 70-years-old man presented to our hematology department in 2013 with a 5-year history of mild hepatosplenomegaly and marked lymphocytosis (13x10^3/ul) without cytopenia. No lymph-node enlargements were detected at CT-scan. For diagnostic purposes, a bone marrow biopsy was performed, resulting in a diagnosis of low-grade CD5-negative small B cell lymphoma NOS. The patient undergoes an observational follow up only. Six years later, due to a trauma, a splenectomy was performed.
The histological slides of the spleen specimen were obtained from formalin-fixed, paraffin-embedded (FFPE) tissue blocks, cutting each slide 3 µm thick. The slides were then stained with hematoxylin and eosin (HE) and with antibodies against DBA44/CD76, IgM, IgD, Bcl-2 CD5, CD10, CD20, CD23, CD25, CD79a, Bcl-1, IgG, p53 (Agilent/Dako, Santa Clara, California USA), Annexin-A1, c-Myc (Cell Marque/Millipore Sigma, Rocklin, California, USA), CD123 T-bet (BD Bioscience, Qume Drive San Jose, California, USA). Gomori's reticulin stain was prepared in our laborarory. In order to determine BRAF mutational status, genomic DNA was extracted from formalin-fixed and paraffin-embedded tissue. PCRs for exon 15 of the BRAF gene were performed. PCR products were purified and sequenced with the use of BigDye Terminator Version 1.1 Cycle Sequencing Kit and an automatic sequencer Applied Biosystems® 3130 Genetic Analyzer.
Results. Histological examination of the spleen showed an abundant lymphocyte proliferation, involving the red pulp, made of small to medium-sized cells with scant cytoplasm with diffuse intrasinusoidal growth pattern (Fig. 1 A-C). The lymphoid population displayed a positivity for Annexin-A1, DBA44/CD76, IgM, IgD, Bcl-2 and panB markers (Fig. 1 D-G). CD123 and T-Bet were only partially expressed, whereas CD5, CD10, CD23, CD25, Bcl-1, IgG, c-Myc and p53 tested all negative. Proliferative index marked less than 5% of the cellularity. BRAF gene sequencing was negative for pathogenetic mutations. Despite the positivity for Annexin-A1, the overall clinical, morphological and immunophenotypical features were more suggestive for a SDRPL. Hairy-cell leukemia (HCL) and hairy-cell leukemia variant (HCL-v) were ruled out due to the absence of cytopenia, the presence of lymphocytosis and the different cytology of the cells, as well as to the absence of BRAF mutations.
Conclusion. Primary splenic lymphomas represent less than 5% of all lymphoid neoplasms. SBCL-U is a category of rare lymphoproliferative disorders that involve the red pulp, including SDRPL and HCL-v. These two lymphomas should be differentiated from the more common and better characterized HCL. All the three entities involve the spleen, bone marrow and peripheral

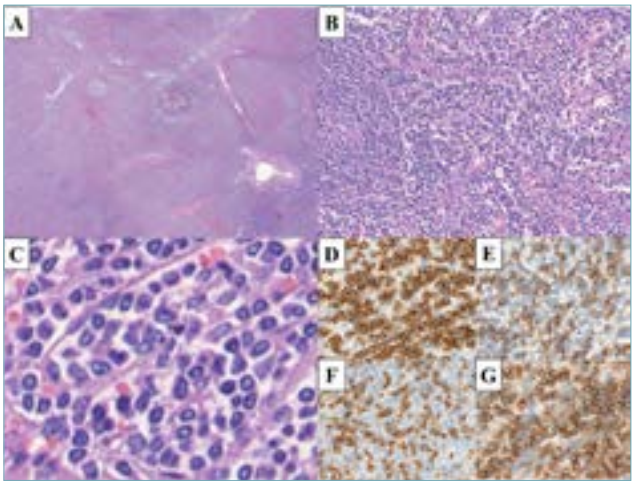

Fig. 1. Histological and immunophenotypical features of our case. A: HE, 4x; B: HE,20x; C: HE, 60x; D; CD79a; E: DBA44; F: Annexin-A1; G: IgM. See the main text for further description.

blood, but the degree of leukocytosis, is typically higher than in HCL. The differential diagnosis between the three entities is based on cytological, architectural and immunohistochemical features. Specifically regarding the immunophenotype, Annexin-A1 is regarded as the most specific marker for HCL, whereas it is generally negative in the other two entities. Annexin-A1 function as an endogenous immunomodulator and has been implicated in cancer cell proliferation, apoptosis, chemosensitivity, metastasis, and invasion. Before our case, only one further Annexin-A1+ SDRPL was described. Considering the relatively recent identification of Annexin A1 as a specific marker of HCL and the highly pleiotropic function of the protein in cancer biology, the positivity for Annexin-A1 on non-HCL lymphomas could be related to a possible heterogeneity of the category of SBCL-U as well as to a biological significance that still needs to be discovered in lymphomas. Future studies should therefore re-confirm the specificity of Annexin-A1 in lymphoproliferative disorders. Our case highlights the difficulties that may be encountered in the diagnosis and classification of cases that do not meet the criteria for any of the well-recognized entities or display overlapping features between them.

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DOUBLE EXPRESSOR AND DOUBLE/TRIPLE HIT STATUS IN A SERIES OF 30 PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMAS: A COMPARISON BETWEEN "LEG TYPE" AND "NOS" ENTITIES

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Goals. Primary cutaneous diffuse large B cell lymphomas (pcDLBCL) are rare lymphoproliferative disorders of the skin. The 2018 WHO classification of skin tumors only recognizes pcDLBCL-leg-type (LT) as a specific entity, whereas the term pcDLBCL "Not Otherwise Specified (NOS)" is restricted to those cases that do not meet the diagnostic criteria for neither pcDLBCL-LT nor primary cutaneous Follicle Center Lymphoma (pcFCL).

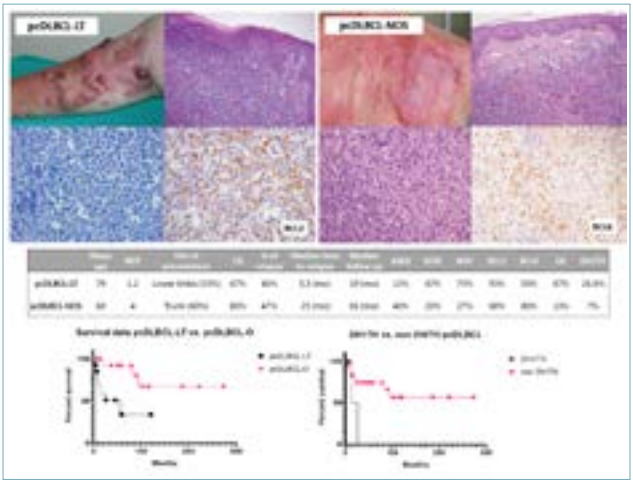

Fig. 1.

pcDLBCL-LT generally displays an abysmal prognosis, whereas pcDLBCL-NOS show a better outcome. The revised 2017 WHO of hematolymphoid tumors placed systemic DLBCL harboring rearrangements of MYC, BCL2 and/or BCL6 (so called "double hits" and "triple hits") in the new category of "High-grade lymphomas" as the presence of such molecular alterations confers a prognostic disadvantage to these patients. We compared on a molecular and immunohistochemical base a cohort of patients diagnosed with pcDLBCL-LT and a series of cases with clinical-pathological features consistent with pcDLBCL-NOS. Our purposes were to assess: 1. the prognostic significance of "double-expressor" (BCL2 and MYC co-expression) immunophenotype; 2. the relevance and prognostic impact of double or triple hit molecular status (MYC rearrangement coupled with BCL2 and/or BCL6 rearrangement) and 3. the relevance and prognostic impact of MYC rearrangements alone.
Material and methods. We retrospectively analyzed a multicentric cohort of 15 patients diagnosed with pcDLBCL-LT and 15 more cases coherent with the category of pcDLBCL-NOS. Therefore, we characterized them at histopathological and molecular level, focusing on the expression and/or rearrangements of BCL2, BCL6 and C-MYC proteins. Molecular alterations were analyzed by mean of FISH for MYC, BCL2 and BCL6 rearrangements.
Results. As expected, pcDLBCL-NOS showed a better prognosis than pcDLBCL-LT (p=0,01), which supports the clinical and pathological distinction of these entities. In the present work we describe four double hit cases (3 pcDLBCL-LT and 1 pcDLBCL-NOS), all characterized by MYC and BCL6 translocations, as well as one case of triple hit pcDLBCL-LT. The DH pcDLBCL-LT cases presented in elderly patients (meanage 79 years), with tumor lesions characterized by a diffuse pattern, a high proliferation rate, a "double expressor" phenotype and a rapid progression. The triple hit case was also a double expressor and showed a rapid progression of disease and an abysmal outcome, with an overall survival of just 13 months after diagnosis. On the contrary, the patient with DH pcDLBCL-NOS did not show a "double expressor" phenotype and was prognostically aligned with other pcDLBCL-NOS cases. Although MYC rearrangements were associated with a tendency towards a worse

outcome (p=0,07), the impact of double expressor status on survival among both all pcDLBCL and pcDLBCL-LT vs pcDLBCL-O cathegories was not statistically significant (p=0,29 and p=0,46 respectively).

Conclusion. Albeit rare, double and triple hit status among pcDLBCL-LT identifies a particular subset of patients with unfavourable outcome. Therefore, this suggests the importance of a routinely screening of pcDLBCL cases for MYC, BCL2 and BCL6 rearrangements. Of importance, a double expressor status alone should not be considered neither of prognostic importance nor a surrogate of the molecular investigation.

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ANGIOIMMUNOBLASTIC T CELL LYMPHOMA, CUTANEOUS PRESENTATION, DESCRIPTION OF TWO CASES

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Introduction. Tfh phenotype is defined by constitutive expression of T and follicle center antigens: CD3, CD4, BCL6, CD10, PD1, CXCL13.

Non neoplastic Tfh lymphocytes have a relevant role in organizing a follicular structure and stimulating germinal center development and B lymphocyte maturation and replication.

Cutaneous lymphoproliferative disorders with a Tfh phenotype encompass a wide spectrum of disease with different clinicopathological features and prognosis. We describe two clinicopathological cases of angioimmunoblastic T cell lymphoma.

First Case. A 63 years old woman presented with a infiltrated plaque in the scapular region,suddenly followed by widespread lesions, of variable dimension involving the entire cutaneous area. Most of the larger plaques developed central ulcer. The complained no symptoms, apart modest itch. No specific ecific dermoscopic pattern was detected.

An incisional biopsy performed on a large, tender nodule on the forearm showed a dense, diffuse dermal proliferation of lymphoid elements, with extension to the the subcutaneous tissue. No significant epidermotropism was detected.

Lymphoid elements were of medium / large size.

Immunohistochemical profile. A relevant portion of lymphocyte coexpressed CD4, CD3 and BCL-6. CD8, CD56, IRF-4, CD20, CD5 and CD30 were negative. Proliferation fraction (Ki67) was 40-70%. CD68+ dispersed histiocytes were present. CD23 highlighted hyperplastic and irregular dendritic-follicular-type cells. Large lymphoid elements CD30 +/IRF4 +, were also represented.

Differential diagnosis comprises: Primary cutaneous gamma / delta T-cell lymphoma; Primitive cutaneous CD8 + T-cell lymphoma, aggressive, epidermotropic; Primary acral cutaneous CD8 + T-cell lymphoma; Cutaneous primordial T-cell lymphoproliferative disorder of small / medium size T CD4 +; Localization of angioimmunoblastic T-cell lymphoma; primary or secondary cutaneous follicular T-cell lymphoma.

The diagnosis with this phenotype Tfh (presence of component B and dispersed follicular dendritic cells) was of cutaneous presentation of angioimmunoblastic T cell lymphoma.

Clinical workout demonstrated massive lymph-node involvement so we can consider the case as cutaneous manifestation of angioimmunoblastic T cell lymphoma with tumorous cutaneous involvement.

Second Case. A 75 years old man presented with maculo-papular lesions on the limbs and trunk.

An incisional biopsy showed a non-epidermotropic perivascular dermal infiltrate (vascular reaction pattern). Perivascular lymphoid cuff were quite dense and lymphoid elements looked activated. The first suggestion was lymphomatoid hypersensitivity reaction.

Immunohistochemical profile revealed coexpression of CD4, CD3, BCL-6 protein, PD-1.

Isolated EBER-positive blasts were detected. A light meshwork of CD21 positive dendritic cells were present. Also, in this case, workup demonstrated a more extensive disease with widespread adenomegaly and the final diagnosis was of cutaneous presentation of angioimmunoblastic T cell lymphoma.

Comment. The two cases presented demonstrate two different patterns of cutaneous involvement of angioimmunoblastic lymphoma, phenotype T. The differential diagnosis with some entities can be done with the help of clinical-anamnestic indicators. In the case of the differential diagnosis with a localization of follicular T-cell. reference is made to the hyperplasia of dendritic follicular cells (CD23 and CD21 +), an aspect that depicts more for angioimmunoblastic T-cell lymphoma.

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MYCOSIS FUNGOIDES WITH DERMAL MUCIN DEPOSITION: AN UNUSUAL VARIANT OF THE MOST CHALLENGING PRIMARY CUTANEOUS LYMPHOMA

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Introduction. Mycosis Fungoides (MF) is defined as an epidermotropic, primary cutaneous T-cell lymphoma (CTCL) composed of small to medium-sized T lymphocytes with cerebriform nuclei and with a T- helper phenotype. MF is the most common type of cutaneous lymphoma, representing almost 50% of all primary cutaneous lymphomas. The classical presentation of the disease is an evolution of patches, plaques, and tumors. Patches of MF are erythematous lesions usually on sun-protected areas. Plaques of MF are characterized by infiltrated, irregular, variably scaling, erythematous, or reddish-brown lesions. Tumors are always the last step of disease evolution with nodules concurrent with other different stage lesions. Histologically, the presence of many intraepidermal lymphocytes in areas with only scant spongiosis represent useful clues for diagnosis. The most common immunophenotypic profile for mycosis fungoides is CD3, CD4, CD45RO positivity and CD20, CD8 and CD30 negativity. Several clinicopathological variants of MF have been described. At the present level of knowledge, mucin deposition is only a concurrent epiphenomenon of the disease, truly justifying the annoveration of mucinous MF between the clinicopathological variants of MF.

Material and methods. We herein report a case of patch stage MF with abundant papillary dermal deposition in a clinical setting of an erythematous patch of the lower abdomen and thigh. We extensively describe the clinicopathological characteristic of our 49 years old patient speculating about a possible interaction between stromal background and neoplastic cell in the mucin deposition pathway.

Result. The histological examination of the biopsy specimen revealed an atrophic epidermis with a moderate lymphocytic infiltrate in a perivascular pattern. The lymphocytes were mostly medium-sized with an irregular nuclear contour, and a cerebriform appearance. The lymphocytes showed evident epidermotropism without spongiosis and merged with an abundant mucinous material in a band-like pattern deposition in the entire papillary dermis. The mucinous material resulted positive for Alcian at 2.5 pH. The lymphoid infiltrate was composed of lymphocytes with a T helper phenotype. Immunohistochemical analysis showed positivity of the lymphoid elements for CD3 and CD4 with a variable expression of T cell associated antigen CD2, CD5 and CD7. The lymphocytes did not express CD8, and other cytotoxic markers such as granzyme, perforin and TIA-1. The interstitial between collagen bundles of the dermis showed a slight increase in the number of Triptase positive mast cell and occasional, isolated, plasmocytoid dendritic cells positive for anti-CD123 antibody.

Discussion. MF is the most common type of cutaneous lymphoma, representing almost 50% of all lymphomas arising primarily in the skin. It is defined as an epidermotropic, primary cutaneous T-cell lymphoma (CTCL) composed of small to medium-sized T lymphocytes with cerebriform nuclei and with a T- helper phenotype (1). Early lesions of MF reveal, in the vast majority of cases, a patchy lichenoid infiltrate in a fibrotic papillary dermis. Epidermotropism of solitary lymphocytes is usually found, but Darier's microabscesses are uncommon. The most common immunophenotypic profile for mycosis fungoides is CD3, CD4, CD45RO positivity and CD8, CD30 negativity. The cells most commonly show an anomalous loss of CD7 expression, followed in frequency by loss of CD2, CD5, or CD3 expression (2). Besides conventional presentations, several variants of MF have been described. Three variants were included separately in the WHO European Organization of Research and Treatment of Cancer (WHO-EORTC) classification of cutaneous lymphomas and are also mentioned in the 2017 revision of the WHO classification of tumors of hematopoietic and lymphoid tissues (3). An abundant dermal deposition of mucin is only rarely reported in patients with MF. Le Boit first described an unusual variant of MF with dermal acid mucin deposition similar to our case (4). More recently Fairbee S et al reported a case of mycosis fungoides associated with extensive dermal fibrosis and mucin deposition (5). The histology and immunophenotype of the dermal spindle-cell population (factor XIIIa–positive) supports a dermal dendritic cell origin. They hypothesized that this increase should be regarded as a consequence of tumour immune escape response mechanisms. On the same line Pileri et al found that in their patients with stage IIB MF there was a statistically significant increase in CD303/BDCA-2+ peripheral dendritic in comparison to stage IA/B (6). They hypothesized that this increase should be regarded as a consequence of tumour immune escape response mechanisms: tolerogenic cytokine expression. by lymphocytes present in MF and immature dendritic cells could lead to accumulation of immature pDCs recruited from the blood, determining an increase in immunosuppression and eventually favouring tumour spread. In addition, factor XIIIa and CD34 positive dermal dendrocytes are known to produce acid mucopolysaccharides (hyaluronic acid) in dermal mucinoses, and in neoplastic conditions. If this ipotesys is true, the mucinous variant of MF could have a more aggressive course. Unfortunately the follow-up of our patient is too short for speculate any possible clinical behaviour. At the present, we are convinced that mucinous MF should be recorded among morphological variant of MF. More cases ad longer follow up are needed to demonstrate clinical and prognostic implication.

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A CASE OF PRIMARY EFFUSION LYMPHOMA IN AN ELDERLY HIV-NEGATIVE PATIENT

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Introduction. Primary effusion lymphoma (PEL) is a rare large B-cell neoplasm of body cavities, associated with HHV-8 infection and manifesting with effusion, without tumor masses or lymphadenopathy (1). It is described typically in HIV-positive patient, seldom in immunosuppressed and elderly (2,3). Neoplastic cells of the effusion show a peculiar immunohistochemical phenotype. PEL is generally associated with a poor prognosis and the therapeutic management is based on the HIV status and the performance status (4).

Case report. We present an unusual case of PEL of an elderly HIV-negative patient with respiratory failure and massive right-side pleural and pericardial effusion. Diagnosis was made on cytospin smears and with the aid of immunohistochemistry performed on cellblock slides. *Results.* Cytological evaluation of pericardial fluid showed rare atypical dyscohesive cells, too few to perform immunohistochemistry. Conversely, pleural fluid, which was subsequently examined, showed a population of large atypical cells with convoluted nuclei, high nucleus/cytoplasm ratio and mitosis. They stained negatively for high and low molecular weight cytokeratins,EpCAM, Chromogranin-A, Synaptophysin, TTF1, CD56, CD15, CD38. They showed very faint positive stain for CD45 and were negative for PAX5, CD20, CD3, Cd79a. Furthermore, strong and diffuse positivity for CD30 and high proliferation rate (Ki67>90%) was evidenced. Finally, HHV-8 (LANA-1) was found to be positive in most nuclei. The diagnosis of "Primary effusion lymphoma" (PEL) "null" lymphocyte phenotype was proposed.

Conclusions. This case is interesting because the patient didn't have any underlying immunodeficiency conditions, except for advanced age. Moreover, the contemporary involvement of more than one body cavities has been rarely reported (5,6,7). As Mediterranean region is considered HHV8 endemic area (8), our case could expand data about epidemiology and clinical features of this disease and highlights the usefulness of immunohistochemistry (mainly with CD30 and anti HHV8 antibodies) in the cytological assessment of atypical cells of body cavities fluids in elderly patients living in HHV8 endemic regions.

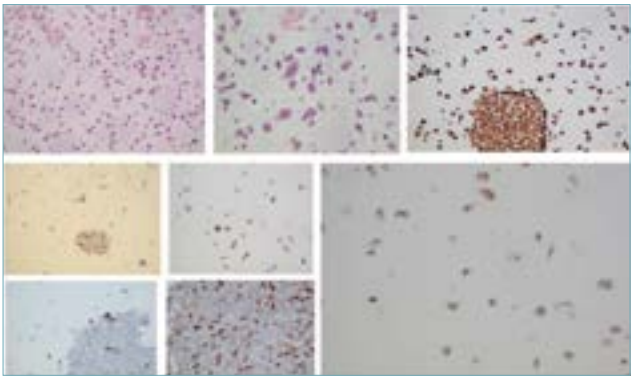

Fig. 1. A,B) large, dyscohesive cells with convoluted nuclei, high nuclear/cytoplasmic ratio and mitotic Fig. s (H&E : A 20X, B 40X); C) Ki67 positivity in more than 90% of nuclei ; D) Weak positive immunostaining for CD45; E) CD30 positive stain; F-G) CD20 (F) and CD3 (G) negative stain. Only few non neoplastic lymphocites are stained ; H) HHV-8 positive stain in most of the neoplastic nuclei.

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ROLE OF CHROMATIN ASSEMBLY FACTOR 1/P60 AND POLY (ADP-RIBOSE) POLYMERASES 1 IN MYCOSIS FUNGOIDES

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Mycosis fungoides (MF) is the most common type of cutaneous lymphoma, accounting for almost 50% of cases[1]. In advanced phases of MF, such as tumor-stage, neoplastic T-cells acquire the ability to resist apoptotic stimuli[2], leading to failures in therapeutic treatments and in long-term remission of disease. Therefore, identifying prognostic markers in MF at early phases appear crucial. Recently, Poly [ADP-Ribose] polymerases 1 (PARP-1) has been identified as a prognostic biomarker for progression to aggressive disease in patients with early-stage MF[3]. Poly ADP-ribosylation is one of the most important epigenetic modifications involving many eukaryotic cell proteins[4]. Several lines of evidence have shown that the poly ADP-ribosylation of histones plays a pivotal role also in the structure, replication, and assembly of the chromatin, in addition to the well-known repair activity of DNA strand breaks[5]. A critical role in chromosome assembly and replication was also shown for the Chromatin Assembly Factor 1 (CAF1) complex, which overexpresses its p60 subunit whenever a hyperproliferative condition occurs in eukaryotic cells[6-7].

This study aimed to evaluate the role of PARP1 and CAF1 in patients with MF when moving from an indo-

lent to a more aggressive phase of the disease. To this end, we examined the immunohistochemical expression of PARP-1 and CAF-1/p60 in a selected series of MF at different stages of disease. Our results showed that patients with tumor-stage MF expressed a higher level of PARP-1 and CAF-1/p60 than patients with early-stage MF. Furthermore, the observation that CAF-1/p60 and PARP-1 were co-expressed in some of advanced-stage MF studied is extremely intriguing and need to be further evaluated. In conclusion, these data are in agreement with the previous reports of CAF-1/p60 and PARP-1 as markers of adverse biological behavior in different solid tumors[7], and they allow us to hypothesize a role for CAF1/p60 as a prognostic marker for MF and to suggest PARP-1 as a potential new target in the treatment of MF.

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IMMUNOISTOCHIMICA

STAPHYLOCOCCUS AUREUS CARRYING PANTON VALENTINE LEUKOCYDIN: IMMUNOHISTOCHEMICAL STUDY OF THE CELL POPULATIONS INVOLVED IN SOFT TISSUE LESIONS IN A SERIES OF SAMPLE FROM PATIENTS ACCESSING THE LUIGI SACCO HOSPITAL – MILAN

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Staphylococcus aureus is a widespread commensal bacterium and pathogen. Approximately 50% to 60% of individuals are intermittently or permanently colonized with S.aureus and, thus, there is relatively high potential for infections [1]. Staphylococcus aureus ranked second among bacterial isolates recovered from bacteremias in Europe in 2008, and the prevalence of S.aureus bacteraemia increased from 2002 to 2008 [2]. In addition to its high prevalence, S.aureus is well known for its ability to acquire resistance to antibiotics [3]. Methicillin-resistant S.aureus (MRSA) was reported in the early 1960s and then ultimately spread worldwide over the next several decades. MRSA is now endemic in health care facilities in virtually all industrialized countries [4]. Community-associated MRSA (CA-MRSA) appeared inexplicably in the 1990s and is currently a major problem in many countries worldwide [5].

Unlike health care-associated MRSA (HA-MRSA) infections, which occur in individuals with predisposing risk factors, CA-MRSA typically causes disease in otherwise healthy individuals. Epidemic events have been described among homosexuals, soldiers, athletes, addicts, children and people over 65 years old [6]. CA-MRSA are more prevalent in skin and soft tissue infection, but life threatening infection as fasciitis and necrotic pneumonia have been described [7].

In contrast to HA-MRSA, CA-MRSA strains are commonly susceptible to the majority of other non-β-lactam antistaphylococcal antibiotics, but their virulence seems to be greater thanks to the synthesis of various virulence factors –i.e. Panton-Valentine leukocidin (PVL), a protein with lyses abilities [8].

Panton-Valentine leukocidin (PVL) represents a key determinant of community-acquired methicillin-resistant *S.aureus* (CA-MRSA) virulence, although not all CA-MRSA produce PVL and its presence is recently found also in hospital–acquired MRSA (HA-MRSA). HA and CA infections due to PVL-positive methicillin-susceptible *S.aureus* (MSSA) have also been described with clinical characteristics similar to that produce by PVL-positive MRSA.

Although many in vivo (mouse and rabbit) models actually are used to study S.aureus pathogenesis, few data are available regarding morphological and biochemical alterations in human samples respect to the molecular features of the pathogen sustaining the infection.

This descriptive and retrospective study was based on the observation and analysis of cell populations involved in response to infection sustained by *S. aureus*, comparing PVL+ and PVL- subjects with the aim of evaluate the eventually differences in tissue lesions.

Matherials and methods. Ten paraffin-embedded samples of cutaneous and subcutaneous tissues with inflammatory/necrotizing lesions were included in this retrospective study (years 2016-2018). The criteria for the patient inclusion in the study were: *S. aureus* positive microbiological test and subsequent molecular PVL characterization. The histology was revised by a pathologist with experience on infectious disease. To characterize the inflammatory infiltrate of the lesions, immunohistochemical analysis were performed on the most representative paraffin block of each case by using the following antibodies: anti-CD45, anti-CD20, anti-CD3, anti-CD68, anti-CD4, anti-CD31, anti-CD138, Ki67 proliferation index. Moreover, Tunel assay for the evaluation of apoptosis was performed. A semiquantitative scale (0-3+) was applied to each immunohistochemistry, excepting Ki67 that was expressed in percentage (%).

Results. Molecular PVL characterization identified 5

PVL positive cases (defined as “PVL+”) and 5 PVL negative cases (defined as “controls”).
At histological examination, no difference was found between the two groups. The samples showed inflammatory infiltrate (from moderate to severe at quantification), with lymphocytes, macrophages and granulocytes. Two cases showed also rare multinucleated giant cells. In three cases, small necrotic areas were present.
Semiquantitative evaluation of inflammatory infiltrate showed the same proportion of CD45 positive cells in both groups. PVL+ had a higher amount of lymphocytes, both CD3+ T lymphocytes and CD20+ B lymphocytes, than controls; on the contrary, the population of CD68+ cells was a few lower in PVL+ than in the other group of cases. No difference was found regarding plasmacells (CD138+ cells) and CD4+ T lymphocytes.
The proliferation index was higher in PVL+ and was also associated to a higher amount of inflammatory cells positive to Tunel assay in this group in respect to what seen in controls.
Conclusions. In the context of skin and soft tissue infections sustained by *S. aureus* harbouring genes responsible for the PVL synthesis, the results of this preliminary study could suggest an influence of Panton-Valentine leukocidin non only in the virulence of Staphylococcus aureus, as already known, but also in the tissue response to infection. PVL+ have an inflammatory population made mainly by lymphocytes and have also an increased turnover of the inflammatory cells, with a higher but balanced rate between Ki67+ and Tunel+ cells.
Of course this study has some limitations, as the number of the samples that will be soon expanded, as the markers for the further characterization of the cell response to the pathogen stress. The frontiers of IHC in the field of bacterial infections are expanding, and we will further work on this exciting research field.

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NEUROPATOLOGIA

PRIMARY WHIPPLE’S DISEASE OF THE CNS PRESENTING WITH INTRACEREBRAL MASS LESION: A CASE REPORT

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Objectives. Whipple’s disease (WD) is a rare, chronic and systemic illness characterized by intestinal involvement but including also a variety of other organs, especially the lymphatic system and the heart. CNS is a major site of involvement in extraintestinal WD and carries a poor prognosis with a mortality rate of approximately 25% within 4 years of diagnosis. Only a small number of cases of isolated CNS form of WD without intestinal manifestation are reported. Men are affected much more often than women and the mean age of onset approaches 50 although the age range extends from childhood to senility. This rare, chronic, multisystemic infectious disorder is caused by the bacterium Tropheryma Whipplei (TW) wich is related to the family of Actinomyces. It is a weakly gram-positive rod shaped bacillus which is not acid fast-positive. It is 1-2 millimicrons in length and has a thick wall, the inner layer of which stains with PAS reagents and thus accounts for the PAS staining pattern of bacilli and their remnants within macrophages. The mechanism by which this agent traverses the blood brain barrier and escapes host defense remains unknown. Successful antibiotic treatment had been reported: patients with WD who are treated with antibiotics get better with the disappearance of bacilli. We report a rare case, with only a small number of cases previously described, of WD confined to the CNS illustrating the diagnostic difficulties in this context.
Material and methods. A of 27-year-old male was admitted in our hospital in May 2018 with recent history of increasingly severe headaches, vomiting, drowsiness. He had no rash, fever, lymphadenopathy or other abnormality on general examination. Routine investigation were all normal. No immunological abnormalities were evident in our patient. An MRI scan showed a high signal mass lesion in the right temporo-insulo-frontal lobe with infiltrative portions that displaced the midline structures and partially enhanced following intravenous gadolinium administration.
Glioma was suspected on the imaging results. Excision of right cerebral lesion was performed by open craniotomy. The tissue was fixed and processed with standard methods for light microscopy.
Results. The histological diagnosis of low-grade astrocytoma was suggested according to the diagnosis of glioma on MRI. During the follow-up, in November 2018, MRI showed a right cerebellar lesion that misured 1 cm, with mass effect on the vermian and paravermian structures. The lesion displaced the IV ventricle to the left. Following the appearance of the new cerebellar lesion, the patient asked for a second opinion at a center of high specialization. On that occasion the diagnosis of WD was suspected. PAS staining highlighted and confirmed the

presence of granular, foamy PAS-positive macrophages. Microscopically, a gliovascular tissue was observed with a remarkable number of large astrocytes and perivascular cuffs of mononuclear cells, as well as smaller collections of foamy macrophages and small nodules or granulomas scattered in grey matter of the cerebral cortex. Those granulomas have been shown to contain strongly positive PAS staining macrophages surrounded by large astrocytes. The number of astrocytes was increased but there was no evidence of moderate/severe atypia, mitosis, necrosis, and the Ki-67 index was 2%. The foamy cells were positive in the immunohistochemical investigations for CD68 (PGM1), negative for GFAP and S100. The liquor examination was normal, including the culture test and Borrelia's research. Negative the search for TW with PCR on brain tissue included in paraffin wax, liquor, feces and urine. The patient started antibiotic therapy. At the next check MRI did not confirm the previous cerebellar lesion.

Conclusions. We report a rare case of WD of SNC presented with symptoms of rapidly evolving raised intracranial pressure in the absence of other systemic symptoms with mass lesion on CT and MRI. It was diagnosed as glioma on the neuroimaging results and initially confirmed by histological examination. The appearance, after a short time, of a new lesion in the cerebellar site, an unusual event in a low-grade astrocytoma, led to a revision of the case and of the brain biopsy at a referential histopathological laboratory center, where WD diagnosis of the CNS was done. Brain imaging techniques are not diagnostic. On clinical ground it can be appreciated that the differential diagnosis of WD of the CNS encompasses a large slice of neurology. The brain biopsy is essential for the diagnosis of CNS WD.

It is particularly difficult when the patient does not show symptoms outside the CNS and the neuroradiological images show expansive-infiltrative brain lesions as in our case, suggestive of neoplasia. Reactive astrocytosis can be mistaken for low-grade glial neoplastic proliferation. Granulomatous diseases, including sarcoid granulomas and tubercolosis can cause diagnostic confusion. The presence of foamy PAS-positive macrophages, scattered between astrocytic proliferation or aggregates in small granulomas, must make the pathologist think of WD of the CNS.

The diagnosis should be confirmed with a PCR assay against Tropheryma Whipplei performed on brain tissue or CSF. It is now the diagnostic method of choice, although its limitations have not yet been defined. In conclusion, although the WD confined to the CNS is very rare, it is necessary for the clinician and for the pathologist to include it among the differential diagnoses of neoplastic diseases and not.

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PATOLOGIA APPARATO DIGERENTE

STEM CELLS IN ADENOCARCINOMA OF THE COLON: THE ROLE OF CD44

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Introduction. Recently, several studies on tumor cells have provided evidence of self-renewing cells, similar to stem cells. They are called cancer stem cells (CSC) and in colorectal carcinoma are reactive for CD44, CD133, EpCAm, CD24 and CD29 [1][2][3][4][5]. CD44 is a multifunctional transmembrane glycoprotein involved in many cellular processes including cell division, survival, migration and adhesion [6]. It is expressed ubiquitously throughout the body. CD44 promotes the progression of colorectal cancer through the activation of low molecular weight hyaluronic acid, which, in turn, activates signaling pathways that promote migration and cellular invasion, or acting as a co-receptor of oncogenes (c-Met and ErbB receptors). CD44 can also inhibit tumor progression by binding to high molecular weight hyaluronic acid, promote interaction with hypophosphorylated merlin, inhibit RAS activation, inhibit CD44-ERM interactions and suppress EGFR activation [7][8]).

Objectives. Our aim was to evaluate CD44 expression in colorectal adenocarcinoma in both cases CDX2 positive and negative.

Materials and methods. We examined 70 consecutive cases of colorectal adenocarcinoma diagnosed between January 2018 and April 2019, ranging in age from 46 up to 89 years, 45 males and 25 females. Tissue samples were routinely processed for histology and stained with hematoxylin-eosin (H.E). In all cases, we evaluated CDX2 and CD44 (HCAM) immunoreactivity using commercial antibodies.

For CD44 interpretation, the following grading score system, based on HER2/neu scheme, was used (Tab. I).

For CDX2 evaluation, the following scoring system was utilized (Tab. II).

Results. By our score systems for CD44 and CDX2, we obtained 16 groups of patients. Data regarding immunoreactivity for CDX2 and CD44 are summarized in Table III. In short, according with the different degree of reactivity for CDX2 and/or CD44, the cases of colon cancer analyzed were differentiated into 16 groups. At the extremes

Tab. I. CD44 grading score.

CD44 expression	Score
Negative or weak membrane staining in less than 10% of tumor cells	0
Weak membrane staining in at least 10% of tumor cells or moderate membrane staining in less than 10% of tumor cells	1+
Moderate membrane staining in at least 10% of tumor cells or intense membrane staining in less than 10% of tumor cells	2+
Intense membrane staining in at least 10% of tumor cells	3+

Tab. II. CDX2 grading score.

CDX2 expression	Score
Negative or nuclear staining less than 5% of tumor cells	0
Nuclear staining in 6%-33% of tumor cells	1+
Nuclear staining in 34%-66% of tumor cells	2+
Nuclear staining in more than 66% of tumor cells	3+

Tab. III. Number of cases divided according to immunohistochemistry.

	CDX2 0	CDX2 1+	CDX2 2+	CDX2 3+
CD44 0	5	1	0	16
CD44 1+	0	0	0	12
CD44 2+	1	0	1	13
CD44 3+	1	0	0	20

of the spectrum we found 2 cases CDX2- and CD44+ and 17 cases CDX2+ CD44-. All the other cases showed a more complex co-expression of the two markers.

Conclusions. Given the complexity of the data obtained, the meaning of the expression of CD44 has to be clarified, especially with regard to the co-expression with the CDX-2.

Our work can make an important contribution to assessing the role of CD44 with regard to the ability to metastasize, local infiltration and response to chemotherapy.

Consequently, the expression of CD44 in CDX-2 negative tumors could indicate a possible target-therapy targeted to CD44.

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COLONIC AMYLOIDOSIS PRESENTING AS LOWER GASTROINTESTINAL BLEEDING: CASE REPORT

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Objectives. Amyloidosis is a heterogeneous group of disorders characterized by deposits of extracellular fibrillar protein, either systemic or organ localized. Gastrointestinal (GI) tract involvement is uncommon, yet in systemic amyloidosis the stomach and duodenum are more frequently involved than the large bowel. GI amyloidosis rarely presents with abdominal pain or acute GI hemorrhage, especially in the absence of clinically evident disease elsewhere. Early diagnosis is important to limit significant long-term effects. We report a case of primary colonic amyloidosis presenting as a lower intestinal hemorrhage.

Case report. A 68-year-old Caucasian man underwent urgent colonoscopy due to acute, massive rectal bleeding along with normocytic anemia. Endoscopic examination revealed congestion, widespread petechiae and fibrin ulcers of mucosa arranged “in mold” from the cecum to the descending colon; from the descending colon to the distal sigma the endoscopic aspect became more accentuated and included remarkable congestion as well as ulcerated and multiple hematic suffusions. Overall, the clinical impression was consistent with mild to moderate pancolitis. The patient had past medical history of parossistic atrial fibrillation, chest pain and hypertension and was on low dose of rivaroxaban and beta-blocker. After colonoscopy, a provisional diagnosis of ulcerative colitis was made: management was conservative and included mesalazine administration. The oral anticoagulant therapy was discontinued. No further bleeding was seen and hemoglobin levels did not decrease. Stool microscopy was reported negative.

Methods. Tissue biopsy specimens obtained during colonoscopy were fixed in formalin, routinely processed and stained with hematoxylin and eosin and the Congo red histochemical method. Congo red-stained sections were evaluated by means of standard and polarized light microscopy.

Results. Microscopic examination of ileal and colonic mucosa showed deposits of homogeneous and amorphous eosinophilic substance in the lamina propria. Such eosinophilic material strongly reacted with Congo red stain and showed apple-green birefringence when examined under polarized light. A diagnosis of colonic amyloidosis was rendered. Further investigations, which included daily urine collection for Bence Jones protein, serum and urine immunofixation, demonstrated a monoclonal protein. Examination of bone marrow biopsy revealed plasma cell myeloma. Ultrasound scan imaging of the heart was consistent with myocardial amyloidosis.

Conclusions. Amyloidosis of the GI tract is uncommon, and clinical manifestations depend on the segment of involved GI tract. GI bleeding due to amyloidosis is rare and has been reported in 10%-40% of patients with GI amyloidosis. Furthermore, the clinical and endoscopic features are different and may mimic other diseases such as inflammatory bowel diseases, malignancies and ischemic colitis. In classic cases, endoscopic features of colonic amyloidosis include ulcerations, diffusely distributed petechiae, nodules, luminal narrowing, loss of haustrations and thickened mucosal folds: such clues were seen in our patient. In fact, in our patient, endoscopic examination initially suggested ulcerative colitis. Although primary presentation with GI hemorrhage is rare, endoscopic examination may raise the index of suspicion, prompting thorough biopsy sampling of intes-

tinal mucosa. Congo red stain remains the diagnostic gold standard as long as it allows demonstration of the distinct green birefringence of amyloid deposits when examined under polarized light. Timely diagnosis is critical to appropriately treat the ongoing cause of amyloidosis and potentially favor regression of the amyloid substance deposits.

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TWO LIVER MASSES: HETEROGENEITY AT ITS BEST

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Introduction. Primary hepatic carcinosarcoma is a rare malignant tumor with poor prognosis composed of a mixture of carcinomatous and sarcomatous elements.
The epithelial component can either be a hepatocellular carcinoma, usually moderately to poorly differentiated, and/or an adenocarcinoma. The sarcomatous component shows morphological evidence of mesenchymal differentiation and may acquire aspects of leiomyosarcoma, rhabdomyosarcoma, chondrosarcoma, fibrosarcoma, or osteosarcoma.
Case reports. We describe two patients with hepatic nodules: (patient 1) a 72-year-old man was admitted to our hospital in October 2018 with a medical history of hepatitis B and C virus infection and liver cirrhosis; (patient 2) a 67-year-old woman presented to medical attention in June 2019 with pre-existing partial gastrectomy for a duodenal ulcer followed by several blood transfusions and evidence of HCV-related hepatitis.
In each patient, a hepatic lesion was discovered by ultrasonography. Subsequently, planar computed tomography (CT) scan revealed two well-defined and markedly heterogeneous masses with different components in the enhanced CT scan, early contrast-enhancement, partial washout in the late phase and steadily hypodense areas with no biliary ducts dilatation. Patient 1 showed a 14 cm large mass, occupying the right hepatic lobe while patient 2 presented with a subcapcsular 3.8 cm nodule, located in medial segment (S4).
Both lesions were radiologically consistent with a primary hepatic neoplasm, more suggestive of atypical cholangiocarcinoma. Associating Liver Partititon and Portal vein ligation for Staged hepatectomy (ALPPS) was performed in the patient 1 while hepatic segmentectomy was performed in patient 2.
At pathological gross examination, the cut surface of both masses showed a white, well-demarcated, solid nodular lesion, partially cystic and exophytic in patient 2. Microscopically, both tumors exhibited overlapping features, mainly consisting of atypical spindle, polygonal and occasionally pleomorphic cells, with a high mitotic index (27 mitoses /10 HPF in patient 1; >30 mitoses/10 HPF in patient 2) and necrosis. In the mass, in patient 1, spindle cells were arranged in sheets and intersecting fascicles, creating a diffuse herringbone appearance. The nodule in patient 2 showed spindle-shaped cells with focal whirling, surrounded by myxoid stroma, areas of ossification, focally producing mature bone.
Epithelial moderate to poorly differentiated neoplastic elements, were seen to co-exist in both neoplasms with a solid growth pattern. More rarely these elements, intermingled with atypical spindle-cells, formed small glandular structures or formation of abortive tubules, producing mucus only focally. Furthermore moderately differentiated atypical hepatocellular elements, with a solid/trabecular pattern, were observed.
All components were intertwined with no sharp demarcation nor transformation zones.
Immunohistochemical analysis revealed epithelial cells with diffuse positivity for Cytokeratins and Hepar-1 while spindle-shaped cells expressed mesenchymal markers such as Vimentin and CD10, but no epithelial and hepatic markers.
Discussion. As already mentioned, our cases share many clinical and pathological similarities. A common medical history of viral infection-related hepatitis has to be noted first.
Both hepatic tumors were characterized by a combination of various types of carcinomatous and sarcomatous elements, such as hepatocellular carcinoma, cholangiocarcinoma and mesenchymal neoplasia. Immunohistochemistry may help to identify the diverse lineage of neoplastic elements with a blended expression of Hepar1 stain in hepatoid and carcinomatous components together with evidence of amphicrine mucous secretion.
In both tumors a diagnosis of hepatic carcinosarcoma was been made.
To guarantee a complete and combined histopathological diagnosis, we draw the attention to the need of extensive sampling documenting the heterogeneity of the neoplasms, especially in large or multiple masses.

MECKEL'S DIVERTICULUM A RARE LOCALIZATION OF PRIMARY NEUROENDOCRINE TUMOR: AN ADDITIONAL CASE.

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Objective. Meckel's diverticulum (MD) is the most common congenital anomaly of the gastrointestinal tract. Complications of this anomaly comprehend a wide variety of manifestations, extending from benign and indolent findings to acute life-threatening conditions and neoplasms. They can occur in adults and they may give rise to bleeding (11.8%), intestinal obstruction (36.5%), inflammation (12.7%), intussusceptions (13.7%). MD may contain also neoplasms (eg adenocarcinomas or neuroendocrine tumours, NETs). NETs are the most common neoplasms found incidentally in MD and rep-

resent approximately a third of MD associated tumours. The purpose of this report is to emphasize that besides its rarity, MD complications related to a coexisting neuroendocrine tumor can occur in adult patients.

Materials and methods. A 57-year-old male patient was admitted to our hospital due to abdominal pain with cramps, distension and vomiting. A clinical diagnosis of acute ileitis was performed and the patient underwent urgent surgery. During abdominal exploration adhesions were found in approximately 15 cm section of the terminal ileum and adhesiolysis was carried out. Moreover a MD without any sign of inflammation was detected, excised and sent to the pathological Unit. Macroscopic examination of the resected MD revealed, in the *cul the sac*, the presence of a well-circumscribed lesion of 0.5 cm, within the thickness of the wall. Histological examination showed a neoplastic population of round, cuboidal cells arranged in nests and fascicles with central nuclei and finely granulated chromatin. Immunohistochemical studies were performed and the neoplasm showed positivity to synaptophysin, NSE and Chromogranine. The neoplastic cell showed a proliferative index Ki67 about 1% and a mitotic index of 3 mitosis/10 HPF. No vascular or lymphatic invasion was found and no metastatic lymph nodes were detected. A diagnosis of well differentiated neuroendocrine tumor of Meckel's diverticulum (NET G2; WHO 2019) was performed, based on the pathological finding. The post-operative period was uneventful with bowel recanalization and the patient was discharged with no complaints and has been followed-up by oncologists since the pathological diagnosis.

Results. MD is the commonest congenital anomaly of the gastrointestinal tract and results from the incomplete obliteration of the omphalomesenteric or vitelline duct during the 5th week of gestation. The usual location is at the anti-mesenteric border of the ileum, 60 cm proximal to the ileocecal valve. It is usually about 2-3 cm in length and usually present in children. The referred locations of MD are related to its complications. Symptomatic diverticula are more common in males than in females, despite the fact that there is no known gender predisposition of asymptomatic MD, and the incidence of complications decreases with age, with the majority occurring in the pediatric population.

Tab. I

Tumor components	Antibody	Patient 1	Patient 2
Sarcomatoid component	Vimentin	++	+
	CD10	+/–	+/–
	Smooth muscle Actin	–	–
	CD34	–	–
	S100	–	–
	Ck 7 / Ck 19	–	–
	Hepar1	–	–
Cholangiocarcinoma component	Vimentin	–	–
	Ck CAM 5.2	+	+
	Ck 7 / Ck 19	+	+
	Hepar1	+	–
HCC component	Vimentin	–	–
	CD10	–	–
	Ck CAM 5.2	+	+
	Ck 7 / Ck 19	–	+/–
	Hepar1	+/–	+

In adult patients, intestinal obstruction is the most common complication, also due to a coexisting neoplasm. In this setting, NETs represent the most common primary tumors of the small intestine and they account for the 33% of cases in this site. Nonetheless they occur rarely in MD and to date only 200 cases have been reported in the current literature. The association between NETs and MD seems to be supported by a common embryological origin due to aberrant interactions between the neural crest and the endoderm. The mean age of patients with an association between MD and NET is 55 years and the incidence is 2.5 times higher in men. The correct therapeutic approach in these situations is controversial. Some authors proposed incidental diverticulectomy as the best treatment, resulting in early detection of NETs and preventing their progression. Moreover, based on the number of asymptomatic MD not resected, it seems likely that incidental NETs are more prevalent than previously thought. The extreme rarity of this lesion makes the clinical behavior and outcome somewhat difficult to predict.

Conclusions. Even though the strategy for adult patients of an incidental finding of MD during surgery performed for other reasons divides the experts. A prophylactic excision is recommended in order to avoid any further risk to develop a neoplasm in MD. Finally, as for any rare case, it must be kept in mind that these particular cases can exist, presenting with many clinical scenarios and underlying that a complicated MD could hide unexpected neoplasm. For this reason the MD surgical specimen must be careful analyzed to avoid diagnostic mistakes from incorrect and inadequate macroscopic examination and sampling.

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VASCULOGENIC MIMICRY IN PANCREATIC ADENOCARCINOMA: IMPORTANCE AS PREDICTIVE MARKER IN CLINICAL AND PATHOLOGICAL STAGING AND PROGNOSTIC IMPLICATION

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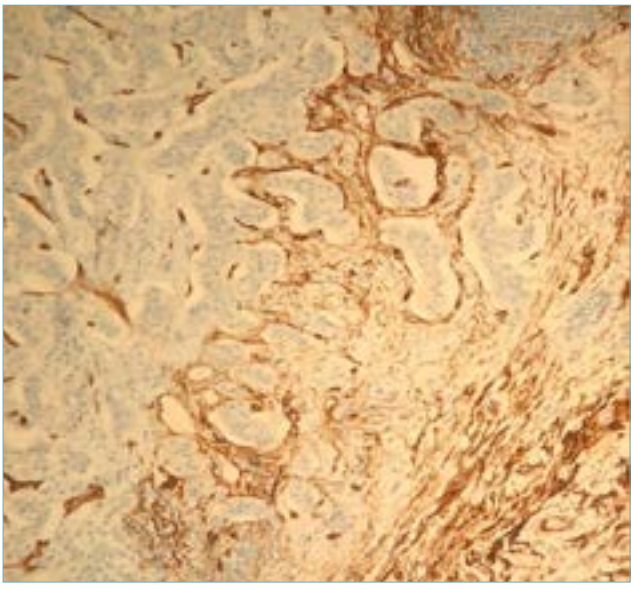
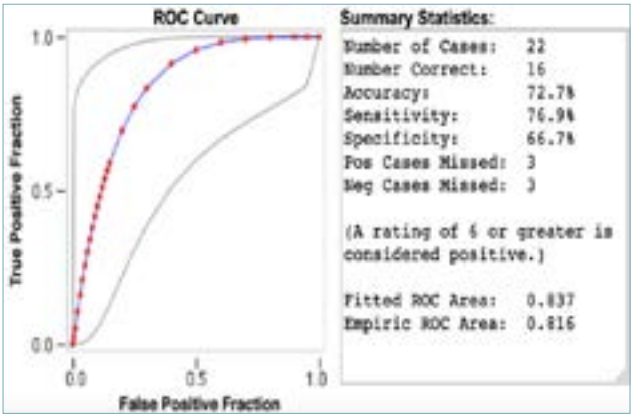
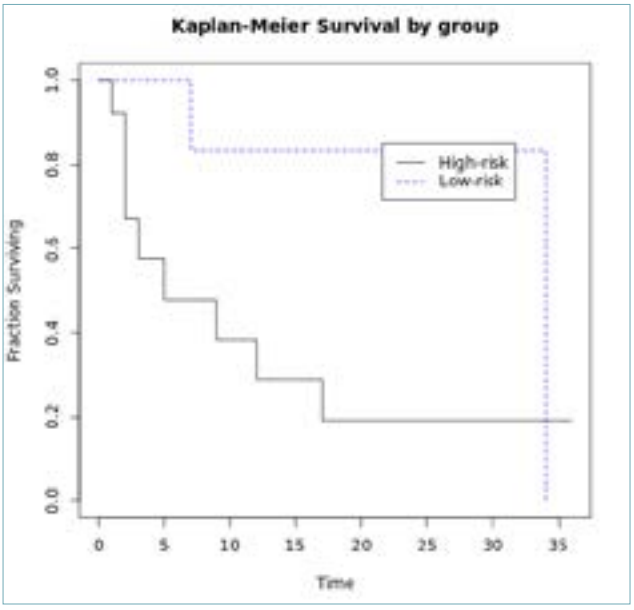
Objectives. The present study is focused on the determination of the phenomena of vasculogenic mimicry (VM) on histologic samples from pancreatic ductal adenocarcinoma in patients undergoing tumor gross-total resection without neoadjuvant chemotherapy. A statistical analysis of the obtained data has been made, in relation to relapse prognosis and its confrontation with







other clinical and pathological factors involved in this outcome.

Methods. 22 cases of pancreatic ductal adenocarcinoma have been considered. The patients underwent to surgery in a period from January 2016 to January 2019, with a known and monitored clinical course, eligible for the vascular mimicry detection. The phenomenon has been carried out through immunohistochemical method using CD34 mAb (Ventana QBend/10) and ERG (Ventana, EPR 3864) on selected sections of neoplasia. Then, the comparison of the two immunohistochemical results at high power field has made possible to recognize vascular neoangiogenetic structures from host primitive vessels. For every case, it has been determined the number of newly formed vessels on three optical fields to evaluate VM quantitatively. VM and TNG classification factors have been taken into account with time of disease-recurrence to draw a Cox Hazard model, to establish how much each factor influences the outcome. From B coefficients of Cox regression model, a score value has been derived to estimate the risk of relapse. Statistical significance of this score (relapse score; RS) prediction and a relative risk-model have been assessed with an univariate logistic regression. ROC (receiver operating characteristic) curve defines the sensitivity and specificity of this score-based prediction system. Kaplan-Meier survival curve determines the probability of recurrence-free survival within 2 groups of low and high risk of relapse based on RM score.

Results. The newly formed vessels in the context of the tumor are the expression of the vascular mimicry phenomenon. It has been identified on morphology: vessels with absence of a basal membrane, presence of pleomorphic nuclei, positivity for CD34 and negativity for ERG (nuclear factor of endothelial cells and EPC) represent typical of neoplastic cells. They marked the difference between tumoral newly formed vessels and host endothelial ones.

In the picture above a selected microscopic field shows the presence of neoplastic vessel with emboli (CD34+, ERG-).

The number of newly formed vessels (VM) on three optical fields of every selected slide has been collected to define quantitatively the VM factor. The strength of association of VM and TNG factors with disease relapse outcome, evaluated with COX regression, showed Hazard Ratios (HR) =3,73(p=0,125) for T; HR=3,11 for N(p=0,179); HR=0,28 for G(p=0,130); HR=1,29 for VM(p=0,043). The dimension of the sample has been the major limit on the statistical significance (with 95% C.I.) of this preliminary study so that for at least 3 variables, casual association with the outcome cannot be excluded. In spite of this problem, in relation to other study on metastasis prognostic factors, B coefficients of regression model for T, N and VM variables has been combined to obtain an RM score on an interval of 10 units from 0 (lowest relapse risk) to 10 (highest relapse risk). It has been assigned 0-1 point for N-/N+ status, 0,1 and 2 points for T1-T2-T3 status and 0 to a maximum of 6/7 point from 0 to 12+ VM vessels. A risk model for the probability of disease relapse in function of RM score has been calculated out of the univariate logistic regression (on 95% C.I.), which gave an O.R. increment equal to 1.88. P-value=0,02 result has confirmed the statistical significance of the model.

A ROC curve has determined the prognostic ability of the RM score (a good sensitivity, specificity and ac-

curacy) highlighting a strong risk model (C-index >0.8): The RM score has been identified 2 groups of patients, the first with low risk and the other one with high risk of relapse with a 0-5 S.I. and 7-10 S.I. (Score Interval). These 2 groups found a different recurrence-free survival prediction based on Kaplan-Meier analysis, the was lower on the high-risk group, as expected (C.I. 95% p=0,041).

Conclusion. Vascular mimicry represents an acquired clinical important phenomenon detectable in different types of malignant tumor. It can be considered as an ubiquitously factor and it might have an important role in resistance against anti-angiogenic therapies. In vitro studies defined also a major role of this phenomenon in cancer invasion and acquisition of metastatic phenotype by neoplastic cells and so metastatic tumor capability. From this last standpoint, the study is finalized to preliminarily examine the clinical significance of VM in tumoral disease-recurrence. The VM phenomenon has to be considered important in determining this outcome next to other clinical and pathologic factors already known, influencing the sensibility of response to targeted therapies.

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GASTROBLASTOMA IN THE ELDERLY

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Background. Gastroblastoma is a rare, unique gastric biphasic tumour affecting both sexes, with metastatic potential. In literature 11 cases have been published, supported by morphology, immunophenotype and, in 4 of them, by the identification of a characteristic *MALAT-GLI1* fusion gene. There is also a twelfth "gastroblastoma", questionable due to the presence of cellular atypia significantly deviating from previously classical examples. A gastroblastoma-like duodenal neoplasm has also been described. Finally, a series of 6 extragastric tumours harboring *GLI1* rearrangements has been published, showing focal cytokeratin positivity and scattered tubular structures in one case (bearing an *ACTB-GLI1* fusion), proposing an entity defined as "malignant epithelioid neoplasm with GLI1 fusions". All reported canonical gastroblastomas occurred within the third decade of life, except a patient aged 56 years. Grossly, these neoplasms form multinodular/lobulated, often partly cystic/haemorragic masses, involving the gastric wall. Histologically, these tumours are biphasic, featuring spindle-cell and epithelioid components, immature but nonpleomorphic, with low mitotic activity. Concerning immunohistochemistry, gastroblastomas variously express vimentin, CD10 and cytokeratins, the former two prevailing in the spindle cells, the latter in the epithelioid ones. The recently reported consistent finding of a *MALAT1-GLI1* fusion gene in gastroblastoma allows a solid differential diagnosis with other biphasic neoplasms such as synovial sarcoma, carcinosarcoma and teratoma. Interestingly, the same genetic defect has been detected in some plexiform fibromyxomas, which are gastric mesenchymal neoplasms definitely benign and lacking biphasic morphology most likely unrelated to gastroblastoma.

Case presentation. We herein report a gastric antral tumour with morphologic, immunophenotypic and genotypic features consistent with gastroblastoma, in a 79-year-old male. The patient presented with dysphagia and weight loss. Contrast-enhanced CT scan showed a 4 cm antral transmural thickening and endoscopic ultrasound detected a 3 cm submucosal, hypoecogenic mass. The patient underwent partial gastrectomy. A 9 cm gastric wall tumour with haemorragic pseudocystic degeneration, diagnosed as paraganglioma, had been excised 51 months earlier in another hospital.

At pathology, the herein reported tumour consisted of three masses, sized 30 mm, 11 mm and 5 mm, 1-to-2 cm apart. Histologically, the intermediate mass was composed in turn of numerous micronodules, with marked propensity to vessel invasion. The tumour was mainly composed of monomorphic, spindled-to-ovoid cells, with eosinophilic/clear cytoplasm and bland, round-to-ovoid nuclei, arranged in sheets focally merging into cords. Focally, well delimited nests or glands composed of monomorphic epithelioid cubic cells with round nuclei, cytologically similar to the spindled/ovoid cells, were detected; nearby, epithelioid cells blended with spindled ones forming ill-defined clusters. Neither mitotic activity nor necrosis were observed. There were also intercellular hyaline deposits, blood vessels, interspersed giant multinucleated floret cells and haemosiderin. The three retrieved perigastric lymph nodes were unremarkable. Neoplastic cells expressed vimentin and CD10 (mostly in the spindle-cell component), cytokeratins AE1/AE3 (in the epithelioid component and, focally, in the neighbor cell clusters) and CD56. Tumour harbored a *MALAT1-GLI1* fusion gene. The histological slides from the previously resected "paraganglioma" were revised, revealing morphological and immunophenotypical features overlapping with the current tumour, except for the lack of an epithelioid component; of note, a *MALAT1-GLI1* fusion gene was found also in this case.

Discussion. The present tumour is the relapse of a disease first presented at 74 years of age. Interestingly, the cord-like arrangement of mesenchymal, cytokeratin-negative neoplastic cells (reported only once in the non-epithelial component of gastroblastoma) was only focal at relapse but constituted the majority of the primary tumour, in the absence of an epithelial, cytokeratin-positive component, that was present only in the recurring neoplasm. Therefore, in its first presentation, the herein reported lesion strongly resembled most of the extragastric "malignant epithelioid neoplasms with *GLI1* gene rearrangements" reported by Antonescu and colleagues. Of note, *MALAT1* was the fusion partner of *GLI1* in only 1 case out of the 6 studied in that series.

Conclusions. The herein reported case not only consti-

tutes a solid example of gastroblastoma, an exceedingly rare neoplasm, but also 1) sensibly extends the known age range of this tumour, 2) is the fifth case confirmed by *MALAT1-GLI1* fusion gene detection and 3) could be the hitherto missing link between gastroblastoma and "malignant epithelioid neoplasms with *GLI1* gene rearrangements".

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IMMUNOHISTOCHEMICAL ANALYSIS OF SOME AUTOPHAGY-RELATED PROTEINS IN USUAL AND RARER HISTOLOGIC TYPES OF GASTRIC CARCINOMA

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Objective. Autophagy represents a lysosome-mediated intracellular degradation and recycling pathway, which may play different roles in tumorigenesis (Cao et al., 2016, Bortink and Gorski, 2017; Caruso et al., 2018; Ieni et al., 2019); moreover, in advanced neoplasms, it may promote the cancer cell survival, contributing to cancer progression (Galluzzi et al., 2015; Ieni et al., 2019). However, autophagy-related proteins (ARP) are effectors able to initiate, promote and complete the formation of double-membrane autophagosomes, and they can be immunohistochemically evaluated in order to establish "the autophagy signature" in malignancies (Cao et al., 2016, Bortink and Gorski, 2017). Although ultrastructural analysis has been considered the traditional method to identify autophagy, we thought it would be of interest to analyze a cohort of advanced usual and rarer histologic types of gastric carcinoma with some ARP, such as LC3A/B, Beclin-1, activating molecule in Beclin-1-regulated autophagy protein-1 (AMBRA-1), ULK-1 and p62 in order to verify any potential relationship among immunohistochemical ARP signature, clinico-pathological parameters and overall survival. Through immunohistochemistry, autophagy (A-IHC) is diagnosed when at least two out of all tested proteins are positive in gastric neoplastic samples.

Materials and methods. From the archives of our Department, a cohort of 65 surgically resected gastric carcinomas was collected; 32 were tubular adenocarcinomas, 18 poorly cohesive carcinomas (PCC), while 15 cases were represented by rare histotypes, such as 9 hepatoid adenocarcinomas (HAS) and 6 mitochondrion-rich ones (MRC). Clinical and pathological parameters such as age, gender, tumor site, pTNM stage, grade and clinical course regarding all cases of gastric carcinomas (WHO 2010) were available. 5-micron thick sections obtained from corresponding tissue-blocks were deparaffinized, then washed in descending alcohol scale, treated by 3% hydrogen peroxide for 10 min, washed again in deionized water three times and incubated with normal sheep serum to prevent unspecific adherence of serum proteins for 30 min at room temperature. Subsequently, sections were washed with deionized water and incubated for 30 min at 37 °C with commercially obtained primary polyclonal rabbit anti-human antisera against Beclin-1 (working dilution 1:250; Abcam, Cambridge, MA, USA), AMBRA1 (working dilution 1:250; Abcam, Cambridge, MA, USA), ULK-1 (working dilution 1:200; Abcam), p62 (working dilution 1:2000; Abcam) and LC3A/B (working dilution 1:100; Abcam). After the incubation with secondary antibody as well as PAP complexes, the site of immunoreaction was revealed by diaminobenzidine tetrahydrochloride and counterstained with Mayer's hematoxylin. Negative controls were obtained omitting the specific antisera and substituting PBS for the primary antibody. The cytoplasmic immunostaining intensity was rated as follows: 0, negative; 1, weak; and 2, strong. The percentage of positively stained cells was graded as follows: grade 0, 0–5%; grade 1, >5–25%; grade 2, >25–50%; grade 3, >50–75%; and grade 4, >75–100% for all antibodies. The immunohistochemical staining samples were independently scored by two pathologists. Statistical evaluation was performed using the SPSS version 13.0 software package (SPSS, Inc., Chicago, IL, USA). The association between ARP expression and clinicopathological features (age, gender, tumour site, pTNM stage, grade and Ki-67 LI) was analyzed using the Chi-square ($Ð^2$) or Fisher exact test. Cancer-specific survival analysis was performed by the Kaplan–Meier method, and for comparison of the survival curves, the Mantel–Cox log-rank test was used. A multivariate analysis (Cox regression model) was utilized to determine the independent effects of variables on overall survival. A value less than 0.05 was considered statistically significant.

Results. LC3A/B, Beclin 1, ULK-1 and p62 were expressed both in the cytoplasm and in the nucleus of the adenocarcinomatous elements, while AMBRA-1 was preferentially localized in the nucleus. Interestingly, the sensibility and specificity of LC3A/B and Beclin-1 ranged from 81.25% to 93.75%, with high efficiency (90.63%) for Beclin-1 in adenocarcinoma histotype, while Beclin-1 and p62 exhibited the highest efficiency (94,27%) in HAS. Regarding other histotypes (PCC and MRC), the immunoreactivity of tested ARP was inhomogeneous and inconstant. A statistically significant correlation among different ARP and clinicopathological parameters such as grade, stage, clinical course and Ki-67 LI was revealed; in detail, this relationship regarded LC3A/B and Beclin-1 in usual histotypes, while Beclin-1 and p62 emerged as significant in rarer ones. Finally, in multivariate survival analysis grade, histotype as well as the abovementioned ARP appeared as independent significant variables.

Conclusions. Our results suggested a peculiar so-called "autophagy signature" for different histotypes occurring in gastric malignancies. These data pointed out the action of different ARP pathways, which deserves further investigations. In fact, main challenges remain still unresolved, such as differences in staining techniques, scoring systems and cut-off points as well as

bias due to limited samples size. Therefore, standardized approaches for the prognostic-predictive evaluation of ARP expression are needed to translate their possible value in the associated targeted therapeutics.

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MULTIFOCAL HEPATIC ANGIOSARCOMA MISDIAGNOSED AS HEPATIC PELIOSIS: A CASE REPORT AND LITERATURE REVIEW

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Background and aim. Hepatic angiosarcoma is a malignant mesenchymal neoplasm composed of highly atypical, pleomorphic endothelial cells, which diffusely grow along liver sinusoids and other preformed vascular channels, replacing normal endothelial cells[1]. Herein we report the case of a multifocal hepatic angiosarcoma initially misdiagnosed as hepatic peliosis basing on radiological features, along with a brief literature review. The aim of this report is to present the clinical, radiological and pathological findings of this rare entity and to highlight the mandatory integration of clinical and pathological features to reach the correct diagnosis.
Methods. A 73 years-old man with jaundice underwent a laparoscopic biospy of multiple hepatic lesions with radiological features suggestive for peliosis. Three days after the procedure the patient died of severe liver failure and an autopsy was performed. Twenty-four tissue samplings from the liver were obtained. Tissues were fixed in 10% formalin solution and embedded in paraffin blocks. Four-micrometer-thick sections were obtained and stained with hematoxylin and eosin for microscopic examination. An immunohistochemical panel including CD 31 (clone JC/70A, DAKO, dilution 1:50), CD34 (clone QBEND/10, Novacastra, dilution 1:200), ERG (clone EPR3864, Abcam, dilution 1:200) was performed.
Results. Grossly, the liver had irregular external surface and revealed blood filled cavities and grey-brown spongy areas surrounded by normal parenchyma at cut examination. Microscopically, multiple cavernous spaces covered by highly atypical endothelial cells were observed. Moreover, nodular areas made up of spindle cells surrounded by dilated sinusoids filled with neoplastic cells were seen. The atypical cells were intensely positive for endothelial markers, such as CD-31 and CD-34.
A chronic occupational vinyl chloride exposure was retrospectively discovered. Based on these clinical data and the morphologic features, the diagnosis of multifocal hepatic angiosarcoma was made.
Conclusion. Due to its rarity, hepatic angiosarcoma's diagnosis may result extremely difficult, representing a potential radiological and histological pitfall. Integration of pathological and radiological findings with patients' clinical and occupational history is the key for avoiding wrong interpretations and reaching the correct diagnosis.

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IRON PILL-INDUCED GASTRITIS: AN UNDER-RECOGNIZED AND RARE CLINICO-PATHOLOGICAL ENTITY

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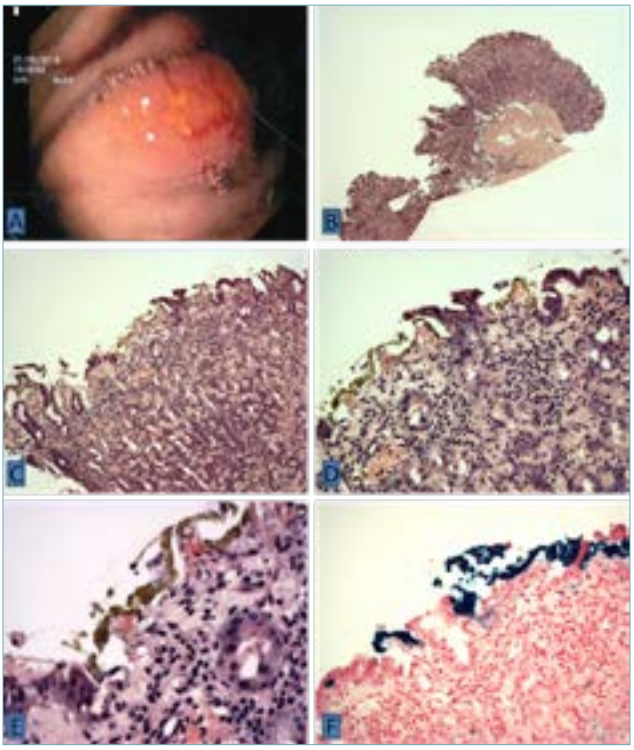
Aims. Oral iron supplements are widely used in the treatment of iron-deficiency anemia, most commonly in the form of ferrous sulphate tablets (1). While gastrointestinal adverse outcomes that can result from an iron overdose have been well described in the literature, injury from standard therapeutic oral iron, named iron pill-induced gastritis, is a less well-known and under-recognized condition (2; Tab. I). Despite its rarity, this

Tab. I. The table summarizes the case reports of iron pill-induced gastritis reported to date in the literature with a well-characterized histology.

Authors	Year of publication	Sex and age of the patients	Site of the biopsy	Endoscopic appearance
Ji Hongxiu and Yardley JH	2004	Female, 48 y/o	Gastric antrum	Normal endoscopic appearance
Zhang X, Ouyang J et al	2009	Male, 76 y/o	Gastric body	2,5 cm2 pale and villous appearing flat lesion
Hashash JG, Proksell S et al	2013	Male, 59 y/o	Gastric body	6-mm superficial gastric ulcer
Meliţ LE, Mărginean CO et al	2017	Female, 14 y/o	Gastric antrum	Multiple erosions
Sunkara T, Caughey ME et al	2017	Female, 46 y/o	Gastric antrum	Multiple erosions and a non-bleeding ulcer



entity can lead to potentially fatal complications, such as severe upper digestive hemorrhage (3). Clinical manifestations are nonspecific, represented by abdominal pain, nausea and vomiting. Endoscopic features are not pathognomonic, including erosions, erythema, flat black dots and yellowish-brown discoloration of the gastric mucosa (3). The histopathologic exam is crucial for the diagnosis, revealing large clumps of coarse, somewhat fibrillar extracellular brownish material on the surface and luminal aspect of the gastric mucosa, often associated with reactive gastritis and erosions (4). Here we report a case of iron pill-induced gastritis with a well-characterized histology.

Materials and methods. Our patient is an asymptomatic 83-year-old female with history of heart failure and iron deficiency anemia. She was receiving 80 mg of ferrous sulphate by mouth once daily. Because of persistent anemia (hemoglobin level of 8.8 g/dL), she underwent an upper gastrointestinal endoscopy. Endoscopic examination revealed a small superficial erosion along the greater curvature of the gastric body (Fig. A). The stomach was otherwise normal in appearance. Several biopsies of the erosion were obtained.

Results. Histopathological analysis revealed a reactive gastritis pattern with microerosions of the superficial epithelium and chronic inflammation in the lamina propria (Fig. B, hematoxylin-eosin, original magnification X2,5; Fig. C, hematoxylin-eosin, original magnification X10). In addition brown, coarse and crystalline material was identified in the extracellular and most superficial aspect of the biopsies (Fig. D, hematoxylin-eosin, original magnification X20; Fig. E, hematoxylin-eosin, original magnification X40). Prussian blue iron stain confirmed that the material was iron (Fig. F, original magnification X20). No *Helicobacter pylori* organisms were identified in the biopsies. Taking together, the morphological features were consistent with iron pill-induced gastritis. The patient was started on proton pump inhibitors and instructed to discontinue her oral iron tablets.

Conclusions. We report a case of iron pill-induced gastritis with a well-characterized histology. Iron-pill induced gastritis is a rare clinico-pathological entity that can carry a potentially life-threatening complications. The recognition of iron-induced histological changes by pathologists can alert clinicians to this under-recognized and easily correctable pathological process.

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APOPTOTIC COLOPATHY: FIRST SEARCH FOR THE RIGHT DRUG

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Background. 'Apoptotic colopathy' is a general term indicating a histologic gastrointestinal pattern dominated by inflammation with apoptosis in cryptic and superficial epithelium. Despite these typical features, apoptotic colitis is an injury pattern common to many medical conditions. These include graft versus host disease (GvHD), therapy-induced diseases, especially immunosuppressive drugs as mycophenolate mofetil, checkpoint inhibitor therapies, antimetabolites and tumour necrosis factor-α (TNF-α) and infections, particularly Cytomegalovirus (CMV) and Adenovirus. Moreover the clinical management of these conditions is completely different. Evidence demostrates that Idelalisib, a phosphatidylinositol-3 kinase δ inhibitor (PI3Kδ), approved for the treatment of patients with relapsed chronic lymphocytic leukemia/small cell lymphoma, follicular lymphoma and indolent non-Hodgkin›s lymphoma, has also been associated with gastrointestinal symptoms.

Case report. A 74-year-old woman presents to medical attention with abdominal pain and diarrhea. She had a history of indolent lymphoma since 2011, treated with R-CHOP and Rituximab with remission until recurrence in 2018. In October 2018, she was started on Idelalisib in monotherapy, due to follicular lymphoma relapse with multiple localizations of the disease, included iliac crest and pleura.

Left-side colonoscopy performed in August 2019 shows multiple diverticula with gross inflammation from the rectum to the descending colon, in the absence of other evident lesions. During endoscopy random rectal biopsies are collected.

Microscopically, they reveal multifocal cryptitis with

neutrophils, also organized in micro abscesses, crypt rupture and erosions on the surface epithelium associated with intraepithelial lymphocytosis (CD3+) and regenerative aspects as globet cell depletion. On such inflammatory background, a striking large number of epithelial apoptosis at the crypt base is also observed.

Discussion. Idelalisib, on the basis of the few and small reported series, may cause gastrointestinal symptoms like diarrhea closely mimicking autoimmune enteritis, GvHD, inflammatory bowel diseases (IBD), infectious colitis and celiac disease. The pathophysiology of Idelalisib-induced diarrhea and colitis is unclear. Its main mechanism of action is a strong inhibition of PI3Kδ (Phosphatidyl Inositol 3, 4, 5 triphosphate, isoform delta), primarily expressed in hematopoietic cells, hence a suppression of proliferation, chemotaxis, motility, adhesion and survival of B cells and a promotion of apoptosis in various cell lines.

As results of recent studies, diarrhea is a well-known side effect of Idelalisib. Patients on this anti-neoplastic therapy manifesting colitis should therefore undergo colonoscopy to evaluate the extent of damage and to exclude other causes, even if it rarely happens.

The histologic features observed in colonic biopsies are reported to be a 'triad' of epithelial cell apoptosis, intraepithelial lymphocytosis and neutrophilic cryptitis. Milder degrees of interstitial inflammatory infiltrate, resembling lymphocytic colitis, can be present, but not typically. Intestinal biopsies usually show an aspecific pattern characterized by increased intraepithelial lymphocyte number with right-to-left gradient, apoptosis and acute inflammation in glands and crypts.

Thus histological diagnosis may be challenging: awareness of the microscopic features of Idelalisib-associated enterocolitis, together with the clinical history and the therapeutic protocol is essential to distinguish Idelalisib-associated apoptotic colopathy from its potential mimics.

MALIGNANT NEUROECTODERMAL TUMOR OF THE ILEUM: A CASE REPORT

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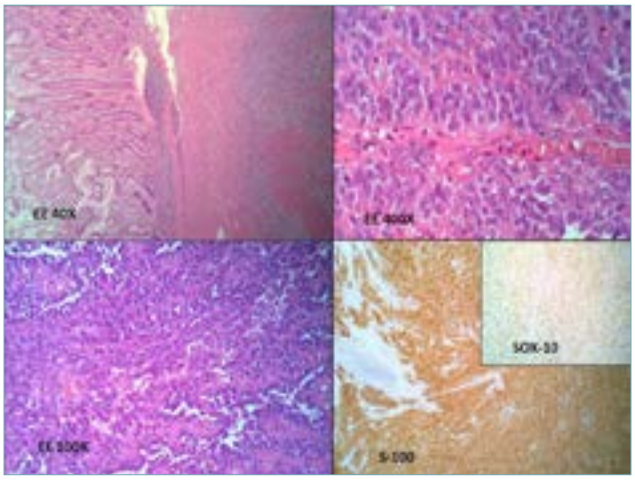
Introduction and objectives. Clear cell sarcoma (CCS) is a rare, malignant mesenchymal neoplasm of unknown cell origin, whose immunohistochemical and ultrastructural features overlap with those of malignant melanoma, and is classically associated with deep soft tissues of lower extremities of young adults. Recent studies have shown that CCS has a specific genetic profile, as it lacks BRAF mutations and shows a recurrent chromosomal translocation t (12;22)(q13;q12), resulting in the fusion of the EWS gene on 22q12 with the ATF-1 gene on 12q13. In addition to the tendons and aponeuroses of the distal extremities, rare examples in other locations including the ear, penis, retroperitoneum, pleura, mediastinum, bone and visceral organs have been described[1]. Furthermore in recent years, gastrointestinal neoplasms bearing morphologic, immunohistochemical and molecular features similar to CCS, but without evidence of melanocytic differentiation have been reported and first designated as clear cell



sarcoma-like tumors of the gastrointestinal tract [2]. Later they were renamed malignant gastrointestinal neuroectodermal tumors (GNET) [3,4]. We report a case of GNET for its rarity, and to underline the difficulties encountered in the differential diagnosis.

Materials and methods. A 68-year-old-man with autoimmune thyroiditis and chronic duodenitis in his past medical history was visited at Hospital "San Giacomo", Novi Ligure (AL), because he was suffering from recurrent melena and iron-deficiency anemia for about three years. Before admission, gastroscopy and colonoscopy, were both unremarkable; analogously, clisma TC of the small bowel was negative. A subsequent abdominal computed tomography (CT) scan revealed a 54 mm. segmental thickening involving the wall of the ileum. A 10cm-long segment of small bowel with an abnormal stricture was removed and an umbilical hernia plastic remodelling was performed too.

Results. Macroscopically, the resected small bowel specimen showed a grey-white solid to cystic firm mass of 8 cm in its maximum dimension, involving the full thickness of the intestine with penetration into the mesenteric adipose tissue. Microscopically, a cellular population was observed prevalently composed of sheets and vague nests of monomorphic, small to medium-sized, polygonal to slightly elongated cells, containing vesicular nuclei with small nucleoli, surrounded by variable amounts of eosinophilic to clear cytoplasm. Scattered, unevenly distributed fascicular, pseudoalveolar, pseudopapillary and pseudovascular arrangements of neoplastic elements with focal abortive rosette-like structures and pseudoglandular spaces were also seen. There were areas of haemorrhagic necrosis and focal osseous metaplasia. There was neoplastic involvement of the resection margin on the serosa. The mitotic rate was low (0-1/10 HPF). The immunophenotype characterization of the neoplasm revealed positivity for vimentin, S-100 protein, CD56 and SOX-10, focal expression of synaptophysin. There was no immunoreactivity for AE1/AE3 keratin, CD34, CD117, DOG-1, MHT cocktail (MART-1, HMB-45 and tyrosinase), smooth-muscle actin, desmin, chromogranin. Ki67 score was about 20% with an unhomogeneous expression. Interfase fluorescence in situ hybridization was consistent with the translocation 22q12 involving the EWSR1 gene region. Based on the morphological features, immu-

nophenotype and molecular genetic findings the tumor was diagnosed as a GNET. Surveillance imaging at 6 months post-operatively showed progression to disseminate intrabdominal disease with widespread peritoneal metastasis. The patient was treated with chemotherapy (olaratumab combined with doxorubicin for about 12 months and then, ifosfamide) but died of disease 26 months following primary resection.

Conclusions. This case represents a very rare example of GNET and imposes a differential diagnosis with GIST, monophasic synovial sarcoma, primary or metastatic malignant melanoma, PEComa, epithelioid variant of MPNST, metastatic seminoma, and especially, CCS ("true" clear cell sarcoma)[3]. The last condition represents the most difficult differential diagnosis for similar histologic and immunohistochemical findings and shared molecular translocations. In equivocal cases, the total lack of expression of melanocytic markers and evidence of neural differentiation might be helpful for a diagnosis of GNET, rather than CCS [3,4]. However, a combined approach utilizing immunohistochemistry and/or ultrastructural and molecular analyses plays a fundamental role for proper identification of these neoplasms and for separating them from their mimics.

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A CASE OF LANGERHANS CELL HISTIOCYTOSIS IN LIVER

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Objective. Under the name "Langerhans cells histiocytosis" there is a group of rare proliferative disorders of histiocytes and other immune cells which infiltrate and proliferate within a tissue. The pathogenesis remains unclear, but some authors suggest that Langerhans cell histiocytosis in some cases arises from a genetic mutation of B-RAF (V600E) occurring in a myeloid progenitor cell. It affects predominantly children, with a heterogeneous clinical presentation, depending on the organ affected. The evolution of this pathology is variable, and ranges from a self-healing disease to chronic recurrences, and from single to multiple lesions.

Materials and methods. A 60-year-old female came to our department of epatogastroenterology claiming an abdominal pain not responding to the pharmacotherapy prescribed by her family doctor. An ultrasonography was performed, and showed a slightly enlarged liver, with diffuse dishomogeneity of parenchyma. Blood tests were all in the normal range. It was decided to perform a computed tomography (CT) scan with contrast agent. The CT pointed out multiple hypodense areas located in the liver, focal adipose tissue inclusion and areas of perilesional hypervascularization as sign of liver parenchyma damage. Subsequently, ultrasound-guided core needle biopsy (US-CNB) was performed.

Results. Morphologically hepatic CNB slides showed hepatic parenchyma almost completely substituted by middle size cells population characterized by nuclei with membrane irregularity, indented, with incision, with disperse chromatin, and slightly eosinophil cytoplasms. A mixed infiltrate composed by lymphocytes, plasma cells, histiocytes and numerous eosinophil granulocytes were also present around the lesions. There was a moderate fibrosis, with disappearing of the portal spaces. The remaining parenchyma resulted substantially normal, showing only a slight inflammatory infiltrate in the portal spaces. Immunohistochemistry showed positivity for CD1a and S100, and negativity for CD68 in the middle size cells described population.

Conclusions. A diagnosis of Langerhans cell histiocytosis was rendered. After histological diagnosis of Langerhans cell histiocytosis, the mutational state of N-RAS and B-RAF genes by NGS was also investigated, that resulted wild type for canonical mutations.

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PATOLOGIA ENDOCRINA

A RARE THYROID LESION: THYROLIPOMA

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Background. The presence of mature fat tissue in thyroid gland is an uncommon phenomenon, sometimes we can find it adjacent to the capsule and along the fibrous tissue septa, or around blood vessels in the sub-capsular area, but it is never intermixed with follicles. In pathological conditions we can find mature fat infiltrating thyroid gland as sometimes occurs in follicular adenomas or even in malignant lesions such as papillary carcinoma. Thyroid fat-containing lesions are extremely rare and not always benign.

Case Presentation: We report a case of 58 years old man who undergo surgery for a multinodular goiter, showing multiple nodules of up to 2 cm. Laboratory exams revealed moderate primitive hyperthyroidism. Ultrasonogram revealed diffusely enlarged thyroid gland with

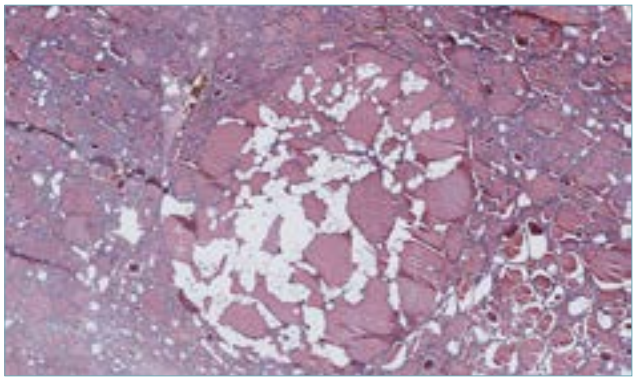

Fig. 1. Thyrolipoma: clusters of mature adipocytes in a thyroid goitrous nodule.

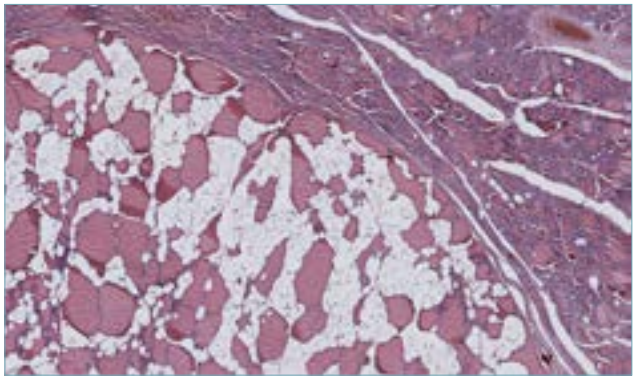

Fig. 2. Adipose tissue in the inter-follicular stroma without any cytological atypia. The nodule is completely enveloped by thin fibrous capsule.

altered echotexture for the presence of multiple nodules both in the right lobe, in the left one and in the isthmus. Technetium-99m scan revealed hyperfunctioning area in the inferior part of the right lobe, so the diagnosis was pre- toxic adenoma of the right lobe with functional inhibition of the rest of the gland. Fine needle aspiration cytology was performed suggesting benignity (Thy 2). A total thyroidectomy was performed under general anesthesia. Microscopic examination revealed multiple nodules composed of variable size and colloid filled follicles, separated by fibrous septa with congested blood vessels, foci of hemorrhage and hemosiderin pigment. Analysis showed the presence of mature adipocytes without any cytological atypia in the inter-follicular stroma of a well circumscribed nodule completely enveloped by thin fibrous capsule (Fig 1-2). A histopathological diagnosis of collid-cystic goiter with a thyrolipoma of the left lobe was made.

Conclusions. This case has been reported because of its rare occurrence. Fatty tissue infiltration or fatty masses may be isoechoic and cannot be differentiated from normal thyroid tissue on ultrasonogram. We can use for the diagnosis also fine needle aspiration cytology, but only the histological exam can reveal the nature of the fat-containing lesion, so thyroidectomy is the only treatment that can be considered.

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TTF-1/P63 POSITIVE POORLY DIFFERENTIATED NSCLC: HISTOGENETIC HYPOTHESIS FROM THE BASAL RESERVE CELL OF THE TERMINAL RESPIRATORY UNIT

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Introduction. TTF-1 is expressed in the alveolar epithelium and in the basal cells of the epithelium of the distal bronchioles [1]. It is considered the most sensitive and specific marker to define the adenocarcinoma originating from the "Terminal Respiratory Unit (TRU) [2,3]. TTF-1, p63 and more recently p40(ΔNp63) [4] are useful for typify the majority of non small cell cancers respectively as adenocarcinoma or squamous cell carcinoma. Poorly differentiated non small cell carcinomas (PD-NSCLC) with co-expression of both TTF-1 and p63 [5,6] in the same cells are rare. We described 10 cases and suggest an histogenetic hypothesis of their origin.

Methods. We report 10 case of peripheral PD-NSLC. Immunohistochemistry was performed by using TTF-1 (8G7G3/1), p63(4A4), p40(BC28), CK5/6(D5/16B4) and CK7(SP52), ALK(D5F3). Mutational analysis of EGFR, BRAF and all-RAS, genes was performed by using Real-Time PCR.

Results. All the cases were poorly differentiated carcinomas consisting of solid nests, formed by cells with large, eosinophilic cytoplasm with an apparent tendency to keratinization. Six of them showed CK7+/ CK5/6- immunostaining; the other ones showed a misleading squamous morphology and positive immmunostaining for CK5/6 but didn't stain fo p40. All the cases were diagnosed as "PD-NSLC, favour adenocarcinoma" in keeping with the presence of TTF1 expression and p40 negative immunostaining.

A "wild type" genotype of EGFR was evidenced in all the cases. No mutations of ALK and BRAF were found. Nuclear P63 and TTF1 co-expression was evidenced in the basal cells of the non neoplastic distal terminal bronchioles.

Conclusions. PD-NSCLC with co-expression of p63 and TTF-1 could take origin from the basal cells of the distal bronchioles of the TRU, potentially able to differentiate towards different histogenetic lines.

We hypothesize that they could be a kind of "basal-type tumors", with more aggressive clinical and morphological features, as the better known "basal-type" cancer of the breast.

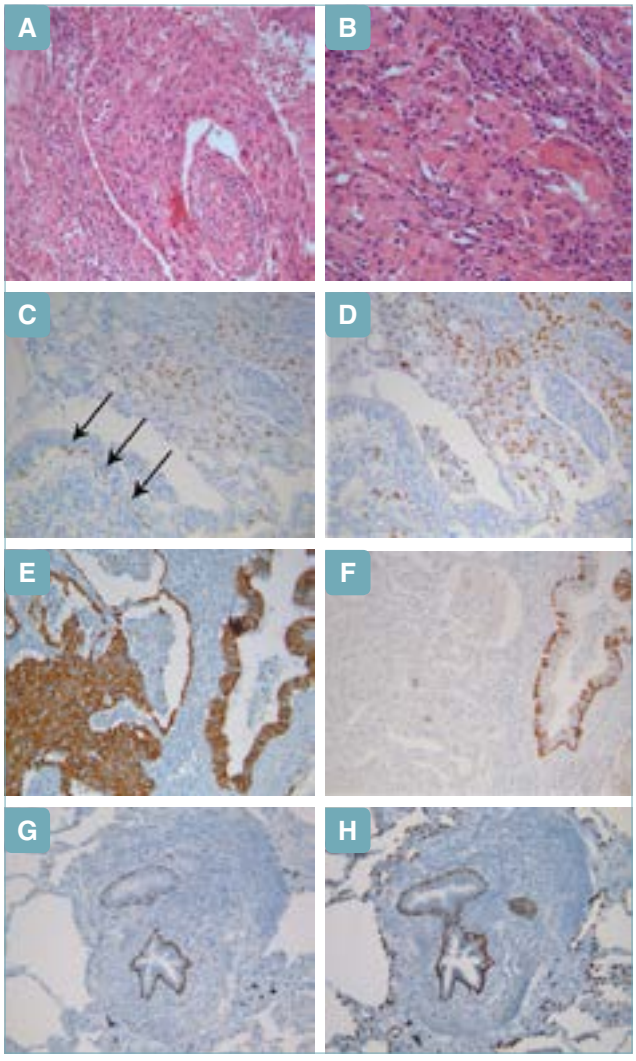

Fig. 1. (A-B) Neoplastic solid nests, formed by cells with large, eosinophilic cytoplasm with an apparent tendency to keratinisation **(C-D-E-F)** Positive immunostaining for p63**(c)** TTF-1**(d)**, CK7**(e)** and negative immunostainig for CK5/6 **(f)** in neoplastic infiltrating areas. CK5/6 and p63 stained positively in the basal layer of the terminal bronchiolar epithelium *(C, arrows ; E, right side).***(G-H)** Basal reserve cells of the terminal bronchioles positive for p63**(g)** and TTF-1 **(h).**

All the patients had an aggressive clinical course with an overall survival less than 5 years.

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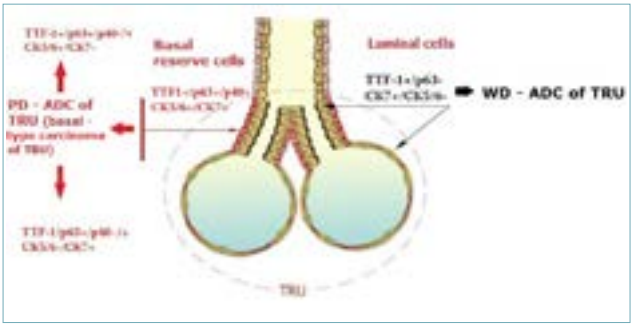

Fig. 2. At right the scheme illustrates the origin of WD-ADC from the superficial epithelial cells of the alveolar e bronchiolar lining of the TRU (in yellow). At left the hypothesis of the origin of solid, TTF1+/p63+/p40+/- PD-ADC from the basal reserve cells of the TRU (in red).

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IMMUNOHISTOCHEMICAL TESTING FOR HBME IN THYROID CYTOLOGICAL SMEARS

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Background. HBME-1 (Hector Battifora mesothelial-1 Antigen) is a valuable marker for distinguishing thyroid malignancies from benign thyroid lesions. It labels thyroid papillary carcinoma and follicular carcinoma (with cytoplasmic and membranous staining) but not normal thyroid.

Several studies have shown that HBME-1 is more useful than Galectin-3 and CK19 in the assessment of thyroid malignancy in histological samples, because it is absent in non-neoplastic diseases and in normal tissue (ref. 1-2-3).

Immunohistochemical examination of samples obtained by cell block method yields excellent results but in our experience, getting a cell block with adequate thyroid cellularity is sometimes difficult. Liquid based cytology is more expensive and it is not available in every laboratory, So the use of smeared fine needle thyroid cytology is still very frequent.

Nevertheless, when the material of fine needle aspiration has been smeared on more slides and these are observed after routine cytological stainings, sometimes only few smears contain an adequate number of thyreocytes, and no other smears are available to perform immunohistochemistry.

Aim. We performed HBME-1 on thyroid cytological smears previously stained with hematoxylin and eosin (H&E) and we compared the results with the ones obtained on their matched histological specimens, with the aim of evaluate the usefulness of HBME-1 immunocytological assay on cytological, H&E stained, smeared samples.

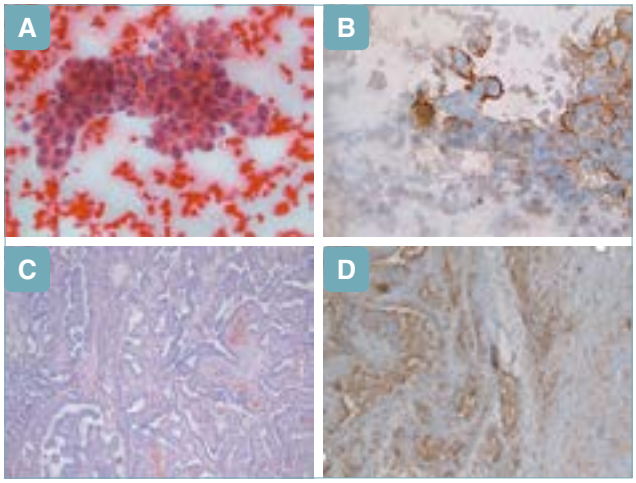

Fig. 1. Papillary carcinoma positive for HBME-1 immunostaining. (A-B) Cytological smear with papillary cluster that showed focal positive immunostaining for HBME-1(fig. 1.E&E; fig. 2: HBME-1); (C-D) Histological specimen: the neoplastic area showed strong, diffuse, positive immunostaining for HBME-1 (fig. 4 left side; fig. 3: E&E).

Methods. we performed a retrospective study on a total of 22 cases of cytological smears with adequate cellularity of patients of whom immunohistochemical HBME-1 results were available on thyroid resection specimens
They consisted of 8 cases of papillary carcinoma (6 smears performed on thyroid nodules, 2 smears obtained on needle aspiration of metastatic cervical lymph node), 4 case of NIFTP, 10 cases of non-neoplastic conditions (5 nodular goiter cases and 5 Hashimoto thyroiditis cases).
Immunohistochemistry with anti-HBME1 was performed on Citofix fixed, H&E stained, cytological samples (with Ventana BenchMark XT automated slide-staining system) without prior bleaching.
Results. On cytological smears HMBE1 staining was negative in 10/10 non neoplastic cases and 4/4 NIFTP. It was expressed in 8/8 cases of papillary carcinoma, with a focal specific (cytoplasmic and membranous) staining in many neoplastic cells, but some clusters of neoplastic cell showed only weak and incomplete staining or stained negatively for HBME-1. Immunostaining on the matched histological cases showed strong, complete and specific positivity in 8/8 cases of papillary carcinoma, a faint incomplete staining in 2/4 case of NIFTP and negative expression in non-neoplastic cases.
Conclusions. HBME-1 cytological results on smears are weaker and focal than the ones obtained on matched histological samples. However, HBME-1 showed to be useful to support morphological interpretation of thyroid cytological smear when we take in account even weak and incomplete membranous positivity, even if it is not present in all the neoplastic cells. In this way, the results are overlapping with the ones obtained on matched histological specimens, in which, HBME-1 positive immunostaining was stronger and more diffuse. We can hypothesize that alcohol –based fixation, that is routinely used to fix cytological smears, could affect the immunohistochemical results (ref. 4). Further studies are necessary to assess if the use of formalin based fixation of thyroid cytological samples could improve the immunohistochemical results of HBME-1 without damage the observation of the cytological details.

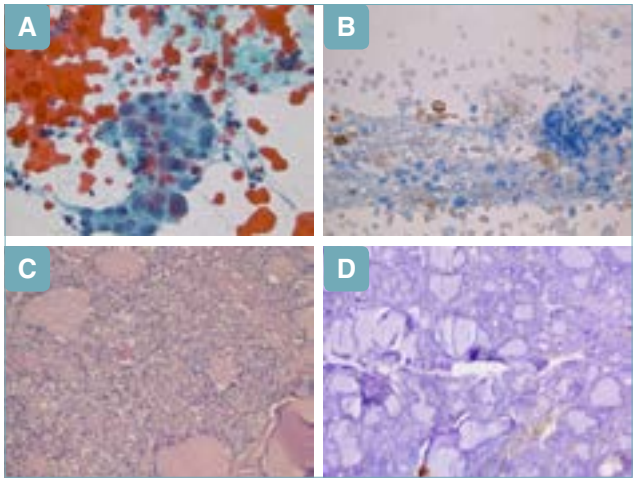

Fig. 2. Multinodular goiter negative for HBME-1 immunostaining. (A-B) Cytological smear (Fig 1: Papanicolau; Fig. 2. HBME-1); (C-D): Histological specimen (fig 1: E&E; 2: HBME-1).

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FOLLICULAR GLOMERULOID CARCINOMA OF THYROID, A RARE NEOPLASIA WITH PECULIAR HISTOCHEMICAL FEATURES

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Objectives. The most recent revision of the World Health Organization (WHO) Classification of Tumours of Endocrine Organs[1] introduced a new variant of Follicular Thyroid Carcinoma (FTC). It is characterized by a "glomeruloid" architectural pattern of growth. We present a case of micro-invasive glomeruloid FTC with Alcian Blue positive mucinous stromal degeneration in the foci of capsular micro-invasion. To our knowledge this the second case of glomeruloid FTC reported[2,3] and the first case in which Alcian Blue staining was used to highlight capsular invasion. Moreover, RAS mutation was investigated to further support that this is a variant of follicular carcinoma.
Materials and methods. The surgical specimen was fixed in neutral, buffered 10% formalin, and paraffin-embedded sections were stained with Haematoxylin and Eosin and with Alcian Blue staining. The immunohistochemical staining was carried out with BenchMark

XT automated slide staining system (Ventana Medical Systems, Tucson, AZ) according to the manufacturer's instructions, by using the following antibodies: TTF1, Galectin-3, CK19, HBME-1, 34betaE12, p53, Ki67.
Genomic DNA was extracted from 10-µm paraffin-embedded tumor sections. Slides were microscopically examined and tumor areas were marked and carefully dissected under microscopic observation. Dissected material was deparaffinized in xylene, washed in ethanol, and rehydrated. DNA extraction was performed using the QIAamp Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.
Results. Grossly, the nodule measured 2,5 cm and appeared solid, encapsulated, well-defined, orange-greyish with a central hemorrhagic area. The histologic examination of the nodule showed a partially encapsulated lesion with a microfollicular pattern of growth. Follicles were often empty of colloid and lined by cuboidal cells, without nuclear crowding and pseudostratification. The presence of epithelial tufts growing within the follicles, sometimes with a fibro-vascular core, thus mimicking the appearance of renal glomerulus, was occasionally detected (Fig. 1a, 1b). Nuclei were small, round and oval, with evenly distributed chromatin and absent or, rarely, inconspicuous nucleoli. Nuclear features of papillary carcinoma were not detected. The capsule was thin and, after careful searching, a few foci of capsular micro-invasion were found. Interestingly, mucinous stromal material was evidenced at the periphery of the lesion, in foci of micro-invasion (Fig. 1c, 1d, 1e) and at the interface between the tumor and the surrounding parenchyma, where the capsule was absent (Fig. 2a, 2b). Mucinous stromal material stained positively with Alcian Blue staining (Fig. 1f, 2c). It was absent where the capsule was intact (Fig. 2d) and in the central areas of the lesion (Fig. 1). The immunohistochemical assay showed positive immunostaining for TTF1 and negative immunostaining for HBME-1, Gal-3, 34betaE12, CK19, p53. Ki67 stained positively in 5% of nuclei.
Cytogenetic analysis showed a mutation in exon 2 of N-RAS. The mutation in codon 61 consisted of a CAA to CGA change that leads to a substitution of glutamine by an arginine (p.Q61R), detected by a real-time, allele-specific amplification essentially as described by Castro et al [4].
Conclusions. The absence of true papillae, of papillary nuclear changes and the negative immunostaining for HBME-1, Galectine 3 and CK19 suggest the diagnosis of follicular neoplasia, in keeping with cytogenetic analysis, showing N-RAS mutation [5].
It is important to be aware of the existence of this variant of FTC because glomeruloid bodies can mimick true papillae, from which they differ due to the lack of typical nuclear features. On the other hand, because of the bland nuclear features, glomeruloid bodies may be underestimated, mainly when they are scanty. Therefore, some cases of glomeruloid carcinoma could be underdiagnosed.
We emphasize that, near the foci of capasular micro-invasion, where the capsule was absent, stromal mucinous degeneration was present at the interface between the tumor and the surrounding parenchyma. No extracellular mucin deposition was found in the inner part of the proliferation, in contrast with the with extensive mucin deposition sometimes reported in follicular adenomas[6,7]. This feature might thus evidence the advancing front of the neoplasia and we emphasize the usefulness of Alcian Blue staining to highlight the foci of capsular micro-

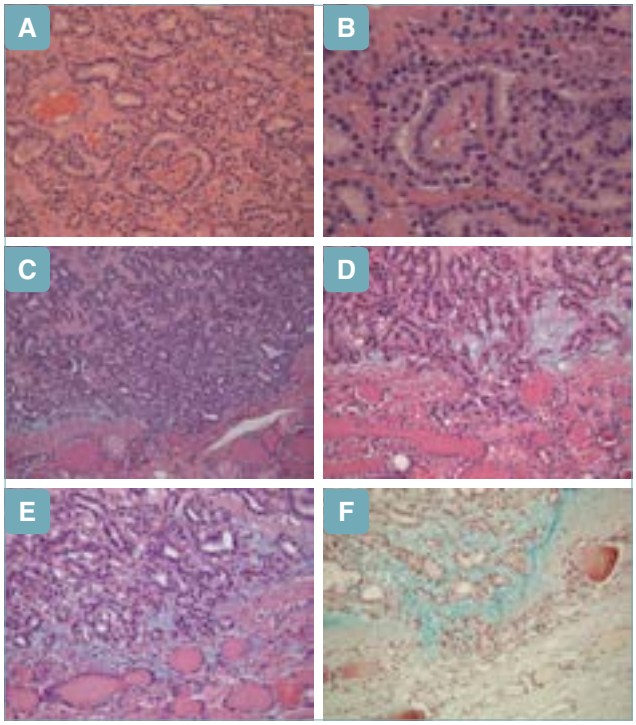

Fig. 1. Neoplasia with microfollicular pattern of growth. The follicles are lined by cuboidal cells, lacking nuclear crowding and overlapping. Rare epithelial tufts grow within the follicles, with a fibro-vascular core, mimicking the appearance of renal glomerulus (a, b), foci of capsular micro-invasion (c, d, e) with stromal mucinous material staining positively with Alcian Blue staining (f).
a, b, c, d, e = H-E staining; a= 200x; b=400x, C= 100x, d=200x, e=200x
f = Alcian Blue staining, 200x

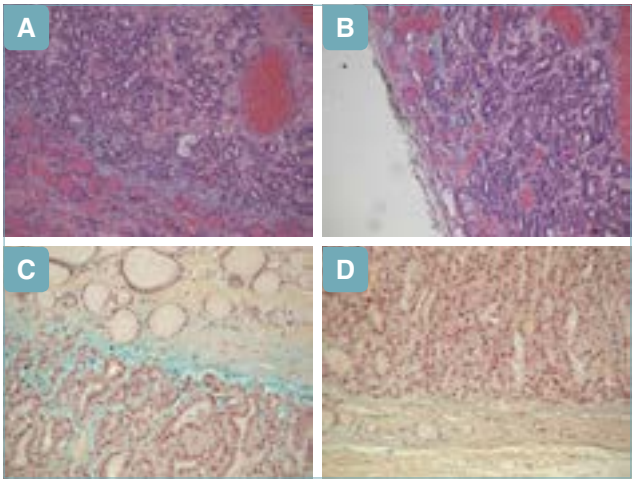

Fig. 2. Stromal mucinous material (a, b) positive for Alcian Blue staining (c) at the interface between the tumor and the surrounding parenchyma, in areas in which the capsule was absent. Stromal mucinous material was absent where the capsule was intact (d).
a, b: H-E staining, 200x; c, d: Alcian blue staining, 200x.

invasion and its absence where the capsule was intact. This feature has not been reported in previous literature so far. We could hypothesize that enzymes secreted by the tumor, such as some metalloproteases, could induce stromal degradation, thus facilitating the invasion[8,9]. This hypothesis needs to be further investigated on a larger number of cases, as well as to confirm if this is a typical feature of "glomeruloid FTC".
In keeping with the above-described features we diagnosed this case as "minimally invasive follicular carcinoma with glomeruloid features".
The prognosis of the patient was excellent after two years follow-up, as expected for a minimally invasive follicular carcinoma[10], but a longer follow-up is necessary.

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PATOLOGIA FETOPLACENTARE

EVALUATION OF PLACENTAL LESIONS IN A CONSECUTIVE SERIES, ACCORDING TO THE AMSTERDAM CRITERIA: A PILOT STUDY

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Objectives. In 2016, the Amsterdam Placental Workshop Group Consensus Statement was published to homogenise the criteria both of tissue sampling and of histological evaluation of the placental lesions. In particular, the stringent Consensus criteria allowed to provide suitable tools (i.e. the number of samples, the number of inflammatory foci) in order to avoid the frequent errors referable to the overestimation/underestimation of chronic villitis.
No recent data have been published regarding the study of consecutive series of placental lesions taking into account the criteria set out in the Consensus.
The aim of the study is to analyze 50 consecutive placentas according to the Amsterdam Criteria with particular attention to the research of villitis, not macroscopically detectable but with potentially relevant clinical implications.
Materials and methods. Fifty placentas obtained from consecutive unselected singleton deliveries (July 2019) at the Obstetrics and Gynecology Unit of the Buzzi Hospital, ASST Fatebenefratelli Sacco, Milan, were included in the study.
Histological sampling and analysis on formalin fixed and paraffin embedded E&E sections was performed by pathologists with experience on the field.
From each placenta, 7 or 8 paraffin blocks were obtained depending on the thickness of the parenchyma at the insertion of the umbilical cord (according to the Consensus Criteria regarding the optimal range for detecting villitis). Immunohistochemistry to phenotype the inflammatory infiltrate was performed if necessary.
The comparison between histological and clinical outcome was carried out collegially between pathologists and clinicians.
Results. The histological diagnoses were the following: 6 cases of chronic villitis with unknown aetiology, (12%); 7 acute inflammatory pathologies (14%), in particular 5 chorionitis and 2 chorioamnionitis; 3 cases of maternal vascular malperfusion (6%); 5 chorangiosis (10%); 3 cases of delayed villous maturation (6%); 1 chronic deciduitis (2%); 25 placentas (50%) without significant histological changes.
Of the 50 pregnant women, 29 were clinically healthy. The remaining 21 had diseases with eventually obstetric interest: 10 cases of thyroid disease, 3 cases of gestational diabetes, 2 cases of oligo-anidramnios, 2 cases of poliidramnios, 1 case of alterations in coagulation factors, 2 cases of gestational hypertension, 1 case with outcomes of previous abdominal surgery.
Histological diagnosis and clinical data were compared. Considering placentas from healthy patients (n° 29), 19 had no significant histological alterations, whereas 10 had different types of lesions: 2 high-grade chronic villitis, 2 low-grade chronic villitis, 2 chorionitis, 1 maternal vascular malperfusion, 1 chorangiosis, 2 cases of delayed villous maturation
Considering placentas from "pathological" patients (n° 21), 6 had no detectable_significant histological alterations, whereas 15 had different types of lesions: 2 high grade chronic villitis, 2 chorioamnionitis, 3 chorionitis, 1 chronic deciduitis, 2 case of maternal vascular malperfusion, 4 chorangiosis, 1 delayed villous maturation.
Conclusions. In our series, 6 placentas (12%) presented aspects of chronic villitis. Our percentage of chronic villitis is greater than reported in the literature in the pre-Consensus era. In particular, 2 high grade chronic villitis were detected in placentas otherwise not subjected to histological examination because of clinically healthy women. It is possible that our larger sampling could explain these results, leading to an increase in the detection of lesions not visible macroscopically but with potential relevance in the management of future pregnancies.
The main bias of this study in the low number of samples. But our preliminary data, when extended to a higher number of cases, could provide further interesting results in helping clinical practice.

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PATOLOGIA GINECOLOGICA

HISTOLOGICAL AND CLINICAL CHARACTERIZATION OF AN EXCEPTIONAL CASE OF BILATERAL OVARIAN MALACOPLAKIA

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Goals. Malacoplakia is a rare inflammatory disorder of the mononuclear-phagocytes, characterized by the infiltration of tissues by histiocytes with pathognomonic intracytoplasmic bodies. Malacoplakia generally affects the urinary or gastrointestinal tract. Gynecological involvement is rare and only round twenty cases of malacoplakia of the female genital tract have been reported. We hereby describe the seventh case of ovarian malacoplakia and discuss its role as mimicker of malignancy.
Materials and methods. A 41-year-old woman presented to our gynecology department with a slow growing bilateral multilocular solid ovarian lesion, measuring 10 x 13 cm on the right ovary and 8 x 4 cm on the left. Because of the clinical suspicion of malignancy, the patient underwent surgical exploration. Frozen section lead to a preliminary diagnosis of benignancy. Therefore, bilateral salpingo-oophorectomy and resection of an intestinal segment due to the presence of hard adhesions between the ovaries and the small bowel, was performed. The histological slides of the lesions were obtained from formalin-fixed, paraffin-embedded (FFPE) tissue blocks, cutting each slide 3 µmthick. The slides were then stained with hematoxylin and eosin (HE) and with antibodies against CD68/PGM1, CK7, CK20, EMA, Inhibin, Calretinin, Ki67, (Agilent/Dako, Santa Clara, California USA), CD163 (Cell Marque/Millipore Sigma, Rocklin, California, USA). Histochemistry with von Kossa, and Perls were made in our laborarory.
Results. At gross examination, the pathological specimen consists of a large yellowish-brown soft mass, completely replacing the ovaries. Microscopy revealed a diffuse histiocytic infiltration made of large, mononucleated, foamy or slightly eosinophilic histiocytes, multinucleated giant-cells of both Langhans's and Touton's type and several rounded medium-sized histiocytes containing the so called "Michaelis-Gutman bodies" (MGB) (Fig. 1, A-B). The histiocytes stain positive for CD163, CD68/PGM1 (Fig. 1C). Some histiocytes also contained calcium and iron deposits as demonstrated by von Kossa and Prussian blue stain respectively (Fig. 1D). Ki67 stained around 1-5% of the histiocytes. During the surgical procedures, a tubal-ovarian abscess was also identified. Cultural examinations isolated Escherichia coli. The pathological findings lead to a definitive diagnosis of ovarian malacoplakia. Two years after surgery the patient is alive and in good clinical condition without any sign of relapse.
Conclusion. International literature reports approximately 500 described cases of malacoplakia. Gynaecological manifestation of malacoplakia is most commonly related to endometrium and cervix involvement. To the best of our knowledge, the worldwide literature describes only six other cases of ovarian malacoplakia and just one of these, displays bilateral involvement of the ovaries [4-9]. The most frequent presenting symptoms were abdominal discomfort, presence of a palpable mass or fever and the treatment included salpingo-oophorectormy in 4/6 cases. Three mechanisms have been hypothesized: the first postulates a pathogenic role for microorganisms with Escherichia coli as the most commonly detected (up to 70% of cases), even if a clear-cut evidence of pathogenicity has never been provided. The other two pathogenetic hypotheses implicate an altered immune response or an abnormal macrophage phagocytic activity, in the accumulations of histiocytes within tissues. Differential diagnosis of malacoplakia is a poorly investigated subject with high clinical and pathological impact. At a clinical level, malacoplakia often presents as a mass that may be symptomatic, depending on size and location. Consequently, it is frequently excided in the suspicion of malignancy. Recognition of the benignancy of the malacoplakia at frozen section allow to correctly address diagnosis on FFPE samples and to reduce the time to treatment. A part from solid neoplasia, non-oncological differential diagnosis should include tuberculosis and inflammatory disorders (e.g. Crohn's Disease). In conclusion, we highlight the clinical and pathological relevance of differentiating malacoplakia in differential diagnosis especially in gynecological-oncology. As it may mimic malignancy,

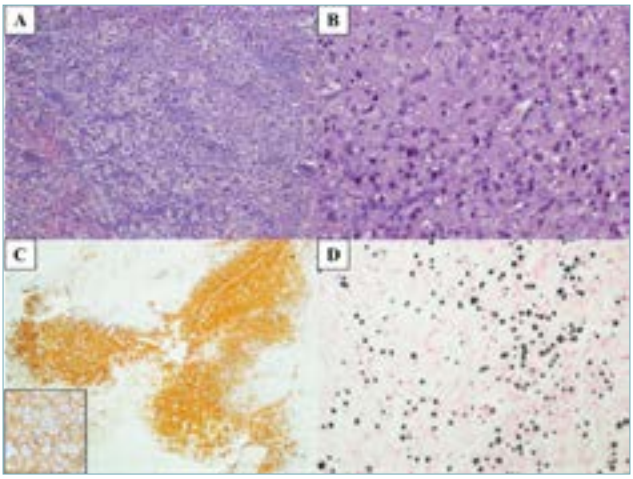

Fig. 1. Histopathological feature of our case. A: HE, 10x; B: HE, 40x; C: CD163; D: Von Kossa stain. See the main text for further descriptions.

misinterpretation of the clinical-pathological picture may result in overtreatment (e.g. unnecessary surgeries and chemotherapy). Even if ethiopathogenesis of malacoplakia is still hard to decipher literature surely indicated it as a benign disorder who may self-heal or benefit of antibiotics, cholinergic agents or focused resection only.

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MORPHOLOGICAL AND IMMUNOCYTOCHEMICAL FEATURES OF PRIMARY CHORIOCARCINOMA OF THE UTERUS: A CASE REPORT

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Choriocarcinoma is a highly malignant epithelial tumor originating in trophoblast. Usually present within a hydatyform mole, it primarily occurs during the fertile period and is extremely rare after menopause [1]. Choriocarcinoma is a biphasic proliferation of trophoblast and syncytiotrophoblast, with morphology similar to primitive trophoblast of the placental previllous stage; chorial villi are absent in this tumor type. Choriocarcinoma shows variable clinical signs and symptoms, the most frequent are abnormal uterine bleedings [2].

We report a case of Choriocarcinoma of the uterus in a 43 years old female. A 43-year-old female patient whit anamnesis of two at-term deliveries, presented to the clinicians with an abdominal pain; an abdominal computer-tomography showed a 7 cm mass located in the body of the uterus; for this reason a total hysterectomy was performed.

The tissue was routinely processed in a formalin-fixed, paraffin-embedded material.

Histologic study of the sections stained with E/E showed myometrium infiltrated by bulky and multi-nuclear cytotrophoblast elements, with hyperchromic and atypical nuclei interspersed with syncytiotrophoblast cells. The chorionic villi were absent and areas of necrosis and hemorrhages were also observed. The neoplasm infiltrated more than half of the myometrium (Fig. 1). Representative sections were cut for morphological examination and tissue blocks submitted for immunohistochemistry methods that were used to confirm the diagnosis. The lesion showed positivity for beta-HCG and panCK and Ki67=80%.

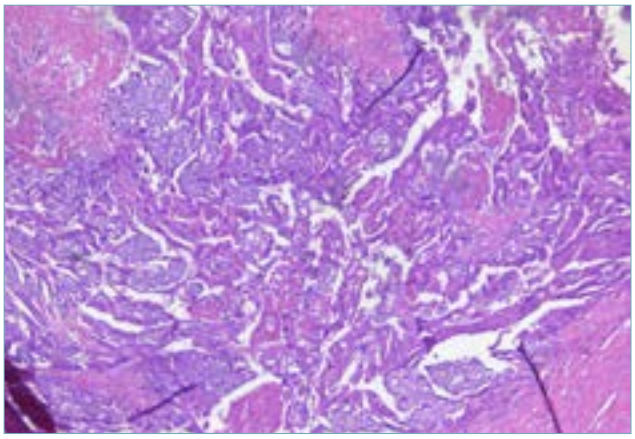

Fig.1. E/E 5x

These morphological and IHC findings were compatible with a choriocarcinoma.

Choriocarcinoma is a biphasic proliferation of trophoblast and syncytiotrophoblast, with a morphology similar to primitive trophoblast of the placental preventative stage. One of its histological characteristics is the absence of chorionic villi [2]. Its incidence is one case per 20,000 to 25,000 pregnancies in western countries [3]. There are two types of choriocarcinoma: gestational and non-gestational choriocarcinomas. Gestational (GC) (derived from the placenta) and non-gestational (NGC) choriocarcinomas are trophoblastic diseases originated from abnormal proliferation of trophoblastic cells in uterus or in other sites (ovary, lung, stomach etc.). These rare tumors share similar morphology and pathological features and differ on chemotherapy response, genetic origin and prognosis [4]. Immunohistochemical analysis is useful for classification
of choriocarcinoma. Immunoreactivity for fraction human chorionic gonadotropin beta confirms its
diagnosis. The OCT-3/4, CD-30 and AFP markers are expressed in undifferentiated pluripotential cells, including germ cells AE1/AE3 are a combination of 2 antibodies to cytokeratin; your staining is usually positive in this neoplasm [5-6]. As far as staging is concerned, the FIGO published in 2002 a classification that correlates the old anatomical classification system with a score based on the risk factors defined by the WHO (age, previous pregnancy, interval between the last pregnancy and chemotherapy, beta-hCG dosage, number of metastases, site of metastasis). According to this system tumors can be classified into two categories: low risk if the score is less than 6; high risk if the score is equal to or greater than 7 [7]. The preferred therapy for these types of tumors is surgery followed by adequate chemotherapy.

In conclusion a combination of histopathologic appearance of the tumor, and immunohistochemical studies will often help to distinguish choriocarcinoma from other neoplasms.

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OVARIAN YOLK SAC TUMOR IN OLDER WOMEN: A CASE REPORT

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Objectives. Ovarian yolk sac tumors (YSTs) are rare malignant germ cell tumors generally found in young females. They are the second most common type of ovarian germ cell tumor and occur very rarely after menopause. The majority of YSTs in postmenopausal patient are associated with epithelial ovarian carcinoma and appear to be associated with a poor outcome. They are less responsive to the chemotherapy currently used for ovarian germ cell tumors. Serologic and immunohistochemical alfa-fetoprotein (AFP) expression characterizes this tumor. This report presents a case of YST diagnosed in a 60-year-old patient accompanied by high-grade serous carcinoma (HGSC) diagnosed in our hospital in May 2019.
Materials and methods. A 60 years-old woman presented with abdominal tenderness and sensation of a mass. CT imaging and positron emission tomography-computed tomography (PET-CT) revealed complex solid and cystic pelvic mass located in the bilateral adnexa, behind uterus, with multiple metastases on mesentery and peritoneum. The preoperative AFP was 2550 ng/ml (normal 0-20 ng/ml), CA125 was 90 U/ml (normal 0-35 U/ml). The previous laparoscopic exploration revealed complex, solid and cystic pelvic mass located in the bilateral adnexa with involvement of the omentum, ileocecal surface, anterior rectal wall, pelvic and diaphragmatic peritoneum.
Patches of biopsies were performed and diagnosed as HGSC. The search for the somatic and germinal mutations of BRCA1-2 genes was negative. The patient underwent a primary cytoreductive surgery with total abdominal hysterectomy, bilateral salpingo-oophorectomy, right colectomy, cholecystectomy, splenectomy, resection of rectum-sigma, pelvic and diaphragmatic peritonectomy, omentectomy and metastatic tumor resection without macroscopic residual disease. The FIGO stage was IIIC.
Results. Macroscopically the right ovarian tumor was 5x5x4 cm with cystic and solid components; the left ovarian tumor was 7x5x5 cm and predominantly cystic. Microscopic examination showed a bilateral HGSC. The left ovary in some areas exhibited atypical cells of various shapes: one consisted of aggregates of small epithelial-like polygonal cells with clear cytoplasm and large vesicular or pyknotic nuclei (solid pattern); the other consisted of alveolar, gland-like cavities lined by cuboidal epithelial-like cells with large, prominent nuclei and surrounded by myxomatous stroma. Some of these spaces are lined by small papillary projections protruding into the lumen (glandular-alveolar pattern). Finally, a cribriform and tubular pattern was observed. Hyaline globules are conspicuous, both within the cells and in the stroma. Therefore this tumor showed features consistent with YST. Immunohistochemically the germ cells were positive for SALL4, AFP, EMA, focally for CDX2, and were negative for P53, napsinA, progesterone and estrogen receptor, CD99, TTF1, BerEP4. In the HGSC component, P53 and CK 7 were diffusely positive, AFP and SALL4 negative.
Conclusions. We reported a rare case of ovarian mixed YST and HGSC in postmenopausal female. The diagnosis of YST in elderly patients is difficult and can be overlooked especially when the YST is limited. Adequate sampling is very important. YST tumor in postmenopausal woman is less responsive to traditional germ-cell chemotherapy. It has been hypothesized that the YST components of these tumors may originate from epithelial cells not germ cells through a process of metaplasia/retrodifferentiation and through a molecular pathway different from germ cell-tumor in younger patients. In our case, the expression of epithelial markers (EMA) by the YST component supported this hypothesis.
The histogenesis may cause a more aggressive behavior of the tumor regardless of stage of presentation and a more aggressive behavior compared to that occurring in young women. At the current date our patient is performing the third cycle of post-operative adjuvant cisplatin-based chemotherapy. No ideal chemotherapy has yet been established for this type of tumor: further investigations are necessary for specific chemotherapy based on the histogenesis.

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ENDOMETRIAL STROMAL TUMOR (SARCOMA) WITH LIMITED MIOMETRIAL INFILTRATION: A RARE CASE WITH PELVI-PERITONEAL METASTASES

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Aims Endometrial stromal tumors (EST) are rare endometrial neoplasms arising from endometrial stroma, that account for less than 2% of all uterine tumors. Based on morphological features, the last (2014) WHO classification system recognizes four categories: i) endometrial stromal nodule (ESN); ii) low-grade endometrial stromal sarcoma (LGESS); iii) high-grade endometrial stromal sarcoma (HGESS); and iv) undifferentiated uterine sarcoma (UUS). However there is an intermediate neoplasm with overlapping morphological features between ESN and LGESS, for which the descriptive term "*endometrial stromal tumor (EST) with limited infiltration"* has been proposed. Given its rarity, the prognostic information is limited, even if a benign clinical course is expected in the majority of cases (1). We herein report on a case of a 46-year-old woman who presented with multiple LGESS peritoneal metastases, developed after ten years from surgical excision of an uterine nodule, histologically consistent with an "*EST with limited infiltration*". This paper, showing the metastatic potential of this tumor supports the hypothesis that this lesion is a true low-grade sarcoma for which the term "*Low-grade endometrial stromal sarcoma (LGESS) with limited infiltration*" seems to be more appropriate.

Materials and Methods In 2009, a 36-year-old woman underwent nodulectomy for a suspected uterine leiomyoma. Histological examination was "*cellular leiomyoma*". In 2018 the patient presented with multiple, symptomatic peritoneal lesions, highly suspicious for pelvic and peritoneal endometriosis. All macroscopically evident nodules were surgically removed, accordingly. Surgical specimens were submitted for histological examination in neutral-buffered 10% formalin, dehydrated using standard techniques and embedded in paraffin. Immunohistochemical studies were performed with the labeled streptavidin-biotin peroxidase detection system using the Dako automated immunostainer (Dako autostainer link 48, Glostrup, Denmark). The following antibodies were tested: CD10, α-SMA, desmin, h-caldesmon, ERG, CD31, S-100 protein, CD34, β-catenin, EMA, cytokeratins (AE1/AE3 clone).

Results. Grossly, multiple tumor nodules, ranging from a few millimeters to 3 cm, with infiltrative borders were seen. The cut surface showed solid masses, whitish in color and firm in consistency, without necrosis. Histological examination showed hypercellular lesions composed of uniform, small-sized round cells with oval to spindle nuclei, closely packed and focally arranged with a whorled pattern around arteriole-like vessels. The neoplastic cells resembled those of the proliferative endometrial stroma. Some nodules contained, as additional feature, the presence of a few number of scattered endometrial cystically dilated glands scattered throughout the stromal component. Mitotic activity was low and necrosis was absent. The nodules showed widespread infiltration, often with a tongue-like pattern, of the peritoneal fibro-fatty tissue. Immunohistochemically the stromal component was diffusely stained with vimentin, CD10, estrogen and progesterone receptors and only focally with alpha-smooth muscle actin. Based on morphological and immunohistochemical features, the diagnosis of "*metastatic LGESS with endometrioid gland differentiation*" was rendered (2). Differential diagnosis mainly revolved around peritoneal endometriosis. This benign condition was ruled out on the basis of a diffuse infiltrative, often, tongue-like growth pattern, and a few number of endometrial glands limited to a few nodules. Total histerectomy was performed, but no tumor was found in surgical specimen. A second histological revision of all slides of the previously excised uterine cellular leiomyoma was performed, and the diagnosis was changed into "*endometrial stromal tumor (EST) with limited infiltration*". This diagnosis was based on the evidence of a tumor with the characteristics of a stromal nodule in which, however, it was possible to identify 3 finger-like projections into the adjacent myometrium, one exceeding 3 mm (up 4 mm). Non vascular invasion was seen. Taken together all the clinical and pathological data, the final diagnosis was "*multiple peritoneal metastases from EST with limited stromal infiltration*". The patient is still being treated with hormone therapy, using megestrol acetate 160 mg/die. After 10 months of follow-up, the patient is well and disease-free.

Conclusions The present paper emphasizes the importance of recognizing the so-called "*endometrial stromal tumor with limited infiltration*", as this lesion has the capability of metastasize in the form of multiple peritoneal nodules, after 10 years from surgical excision of the primary uterine mass. As previously suggested, it seems to be more appropriate to consider this tumor as a low-grade sarcoma with low metastatic potential, for which the term "*Low-grade endometrial stromal sarcoma with limited infiltration*" should be used (2).

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PATOLOGIA MAMMARIA

ABERRANT E-CADHERIN IMMUNOHISTOCHEMICAL EXPRESSION AND CDH1 MUTATIONS IN PLEOMORPHIC LOBULAR BREAST CANCER

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Background. Pleomorphic lobular carcinoma (PLC) is a rare histological subtype of invasive lobular carcinoma, characterized by more aggressive nature compared to the classical invasive lobular carcinoma (ILC). It is well known for its large size, high-grade cytological features, high incidence of distant metastasis, presence of lympho-vascular invasion and advanced stage

at diagnosis. Although the morphologic features of the pleomorphic lobular carcinoma are well described, it often eludes accurate pathologic characterization. The loss of membranous expression of E-cadherin (ECAD), a transmembrane glycoprotein that mediates cell-to-cell adhesion, is the defining immunohistochemically feature of lobular differentiation in breast carcinoma. Aberrant ECAD staining in ILC, ranging from 0% to 23.5% in different series, could be misleading for classification purposes. The main aims of this study were: to accurately explore for the first time the molecular genetic alterations of CDH1 (ECAD) gene in a series of PLC cases and to compare the obtained results with the presence of aberrant expression of ECAD, with clinico-pathological features and prognostic/predictive markers status.

Materials and methods. Fifty-eight patients diagnosed with PLC between 2010-2016 at the Pathology Section of Padua University were included in our study. For each case, slides were re-examined by two expert pathologists. Tumor size, grade, stage, LVSI, margin status (when appropriate), nodal status, histological subtype, presence/absence and extension of associated pleomorphic carcinoma in situ were recorded. All clinical characteristics of the included patients were provided, as well. For each case, estrogen receptors (ER), progesterone receptors (PgR), proliferation index (Mib1), human epidermal growth factor (HER2) and ECAD were re-evaluated and scored according to the newest guidelines (ASCO 2018). If not available, immunostaining for ECAD was performed. For molecular analyses, CDH1 analysis from each tissue sample was carried out by standard procedures of exon-by-exon amplification of the whole genomic region and flanking intron borders, followed by direct bidirectional Sanger sequencing on an AB3130 xl Sequencer (Applied Biosystems/Thermofisher, Foster City, CA, USA). Sequence data were aligned and analysed using the CodonCode Aligner software. Ten cases diagnosed with classic lobular invasive carcinoma and ten cases diagnosed with invasive ductal carcinoma were included as negative and positive controls, respectively.

Results. Aberrant ECAD expression, with a cytoplasmic granular pattern, was found in 8 out of 58 (14%) cases. Six of them (6/7;86%) tested by molecular analysis also have a pathogenetic mutation (- and 1 was non-mutated ($p<0.0001$). Among the 50 PLC cases with completely ECAD negative by immunostaining: 3 showed a ECAD variant of uncertain significance (VUS) and one case was non-mutated, the remaining cases being non mutated. No statistically significant relation was found between aberrant E-Cadherin staining or CDH1 mutations and clinico-pathological variables examined.

Conclusions. PLC is an uncommon variant of invasive lobular carcinoma, characterized by its poor prognosis. Even if our cohort is small, a statistically significant correlation between aberrant ECAD staining and CDH1 mutations was demonstrated for the first time in PLC by Sanger sequencing method. However, since most studies include a small number of cases and molecular data regarding this histological type are still incomplete, additional larger series are needed to better clarify ECAD peculiar phenotype and molecular alterations in PLC and their possible relation with clinico-pathological parameters.

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AXILLARY DISSECTION VS NO AXILLARY DISSECTION IN BREAST CANCER PATIENTS WITH POSITIVE SENTINEL LYMPH NODE: A SINGLE INSTITUTION EXPERIENCE.

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Background. Axillary surgery of breast cancer patients is undergoing a paradigm shift, as axillary lymph node dissections (ALND) usefulness is being questioned in the treatment of patients with tumor-positive sentinel lymph node biopsy (SLNB). The aim of this study is to investigate the overall survival (OS) and relapse free-survival (RFS) of patients with positive SLNB treated with ALND or not.

Methods: In this retrospective cohort study, we investigated 617 consecutive patients with cN0, pN1(sn) operable breast cancer undergoing mastectomy or conservative surgery at Sant'Anna Hospital, Turin, Italy between December 2004 and October 2014; 416 patients underwent ALND and 211 were managed expectantly. Survival and recurrence curves were estimated using the Kaplan-Meier method. Cox proportional hazards regression analysis was used to take account of the lack of randomization.

Results. After a median follow-up of 84.4 months there was no significant difference in OS and RFS between the two groups. The incidence of local breast recurrence and axillary recurrence in SLNB only group and ALND group were low and not significant (respectively 4.2% and 1.4%). OS was 87.8% in ALND group and 94.8% in SLNB only group (log rank p 0.07). The RFS was 82.3% in ALND group and 87.1% in SLNB only group (log rank p 0.51).

CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF MALE BREAST CARCINOMA

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Introduction. Male breast carcinoma (MBC) is an uncommon neoplasm accounting for less than 1% of all breast cancers. European prevalence is of 1/100.000. The etiology remains unknown given the rarity of pathology. In this study, we reviewed the clinicopathological and immunohistochemical characteristics of this entity.

Material and Methods. We retrospectively collected 35 cases of MBC at the our Department between 2010 and 2019.

Discussion and Conclusions. The mean age at diagnosis was 65-years. All patients presented with a palpable mass. They all underwent surgery and only five of them had neoadjuvant-chemotherapy. Twenty-seven patients had mastectomy and eight had lumpectomy. Twenty seven patients underwent axillary dissection. Macroscopy examination revealed a cystic cavity with endoluminal papillary lesion in three cases and a firm nodule measuring from 1,5 to 8 cm in 32 cases. Histopathology found invasion carcinoma of no special type (NST) in 27 cases while the other cases were: two intracystic papillary carcinomas with invasion, one micro-invasive carcinomas, one invasive lobular carcinoma, one mixed invasive NST and lobular carcinoma, one apocrine carcinoma, one mucinous carcinoma, and one neuroendocrine carcinoma. Angiolymphatic and perineural invasion were found respectively in 18 and 15 cases. Tumours were associated with ductal carcinoma in situ in 11 cases, with gynecomastia in seven and with a Paget disease in four. Tumour grade was mostly G2. Axillary status was pN+ in 18 patients. Estrogen and progesterone receptors were respectively positive in 22 and 26 patients. HER2 was over-expressed in only two patients. MBC's diagnosis is often made at advanced stages. Consequently, patients were submitted to more aggressive treatments, with poor clinical responses. In summary the main points are: 1) rare entity for which biology is still unclear; 2) surgery is considered standard of care; 3) tamoxifen is the mainstay of treatment; 4) chemotherapy in ER-, PgR-; 5) Trastuzumab recommended over-expression of HER2; 6) Aromatase inhibitors in combination with surgical or medical orchidectomy.

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Tab. I. The table shows the histological features evaluated in the CNB specimen with their frequencies in FA and PTs.

Histological features on CNB	Excision diagnosis				
		Benign PT	Borderline PT	Malignant PT	P-value
Stromal cellularity					0,047*
Mild (10)	6 (60%)	4 (40%)	0	0	
Moderate (13)	5 (38,46%)	6 (46,15%)	1 (7,69%)	1 (7,69%)	
Marked (2)	0	0	1 (50%)	1 (50%)	
Stromal cell pleomorfism					0,017*
Mild (14)	9 (64,29%)	4 (28,57%)	1 (7,14%)	0	
Moderate (4)	0	3 (75%)	0	1 (25%)	
Marked (2)	0	0	1 (50%)	1 (50%)	
Absent (6)	3 (50%)	3 (50%)	0	0	
Mitosis					0,003**
None (16)	11 (68,75%)	4 (25%)	1 (6,25%)	0	
Present (9)	0	6 (66,67%)	1 (11,11%)	2 (22,22%)	
Periglandular stromal condensation					0,038*
Absent (20)	10 (50%)	9 (45%)	1 (5%)	0	
Present (5)	1 (20%)	1 (20%)	1 (20%)	2 (40%)	
Stromal Overgrowth					0,002**
Absent (22)	11 (50%)	9 (40,91%)	2 (9,09%)	0	
Present (3)	0	1 (33,33%)	0	2 (66,67%)	
Fragmentation					0,017*
Absent (14)	9 (64,29%)	4 (28,57%)	0	1 (7,14%)	
Present (11)	2 (18,18%)	6 (54,55%)	2 (18%)	1 (9,09%)	
Fat entrapment					0,195
Absent (21)	10 (47,62%)	9 (42,86%)	1 (4,76%)	1 (4,76%)	
Present (4)	1 (25%)	1 (25%)	1 (25%)	1 (25%)	
Stromal Distribution					0,616

▶

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Histological features on CNB	Excision diagnosis				
		Benign PT	Borderline PT	Malignant PT	P-value
Absent (9)	4 (44,44%)	3 (33,33%)	1 (11,11%)	1 (11,11%)	
Present (16)	7 (43,75%)	7 (43,75%)	1 (6,25%)	1 (6,25%)	
Epithelial Hyperplasia (UDH)					0,434
Absent (20)	9 (45%)	8 (40%)	1 (5%)	2 (10%)	
Present (5)	2 (40%)	2 (40%)	1 (20%)	0	
Growth Pattern					0,595
Pericanalicular (2)	0	1 (50%)	1 (50%)	0	
Intracanalicular(17)	9 (52,94%)	7 (41,18%)	0	1 (5,88%)	
Mixed (5)	2 (40%)	2 (40%)	1 (20%)	0	
Leaf-like Pattern					0,002**
Absent (14)	10 (71,43%)	3 (21,43%)	0	1 (7,14%)	
Present (6)	0	4 (66,67%)	1 (16,67%)	1 (16,67%)	
Not Evaluable (5)	1 (20%)	3 (60%)	1 (20%)	0	
PASH					0,506
Absent (19)	9 (47,37%)	7 (36,84%)	1 (5,26%)	2 (10,53%)	
Present (6)	0	6 (66,67%)	1 (11,11%)	2 (22,22%)	
Myxoid Changes					0,067
Absent (5)	3 (60%)	2 (40%)	0	0	
Present (20)	8 (40%)	8 (40%)	2 (10%)	2 (10%)	
Multinucleated Giant Cells					0,010*
Absent (23)	11 (47,83%)	10 (43,48%)	2 (8,70%)	0	
Present (2)	0	0	0	2 (100%)	
Lesional Edge					0,186
Espansive (0)	0	0	0	0	
Infiltrative (1)	0	0	1 (100%)	0	
Not Evaluable (24)	11 (45,83%)	10 (41,67%)	1 (4,17%)	2 (8,33%)	
Dimensions of tumor					0,140
Age					0,031*

FINE-NEEDLE ASPIRATION BIOPSIES OF BREAST IN THE CORE-BIOPSY ERA. WHERE ARE WE AND WHERE ARE WE GOING TO?

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Aim. Fine needle aspiration (FNA) is a safe and economic diagnostic tool to guide the management of patients with breast lesions. However, the use of core needle biopsy is increasingly frequent due to the different reasons. In particular, a dedicated cytopathologist who ensure an appropriate management of the aspirated material avoiding inadequate or sub-optimal cytological samples is not always available. Moreover, a standardized reporting system for breast cytology is still lacking. This results in a poor reproducibility among different cytopathologist, which in turn may cause misunderstanding between cytopathologist and clinician, leading to an inappropriate treatment of the patients. To overcome these issue, in 2016 the International Academy of Cytology (IAC) introduced a five-tiered reporting system, identifying five categories with different risk of malignancy (ROM) and managements: inadequate (C1), benign (C2), atypical, probably benign (C3), suspicious (C4) and malignant (C5).[1] Our Institution has a 40-year experience in breast FNA and starting from 2010, we have adopted a 5-tiered classification similar to the one proposed by the IAC.[2,3] The aim of this study is to compare our contemporary experience, with both our historical experience (1976-1984/1985-1988) and literature data, including the cytological series in which a four-tiered reporting system was adopted.

Material and methods. Electronic data on n=4625 breast FNA performed between January 2010 and December 2017 were retrieved. In n=1771 (38,29%) histological diagnosis was available.

Results. Out of n=4625 FNA, n=888 were C1 (19,2%), n=1708 were C2 (36,93%), n=499 were C3 (10,79%), n=218 were C4 (4,71%) and n=1312 were C5 (28,37%). [Fig. 1] Out of n=1771 surgical patients, n=128 previously had C1 FNA, n=261 C2, n=289 C3, n=164 C4 and n= 929 C5. A final histological diagnosis of malignancy was made in n=1213 (68,49%) patients, and n=530 (29,93%) patients had a benign histology. The risk of malignancy (ROM) was 49,22% for C1, 11,49% for C2, 25,26% for C3, 78,66% for C4 and 98,82% for C5.[Fig. 2]

Conclusions. C5 ROM was 98.82%, comparable to both literature and our historical data.[4] Moreover, ROM associated with the "suspicious" category is higher in 5-tiered studies, thanks to the introduction of C3 category, that led to the reclassification of mild atypical lesions as "atypical, probably benign", avoiding the overtreatment of these patients. The ROM of C2 cases may be biased by the limited histological follow-ups available

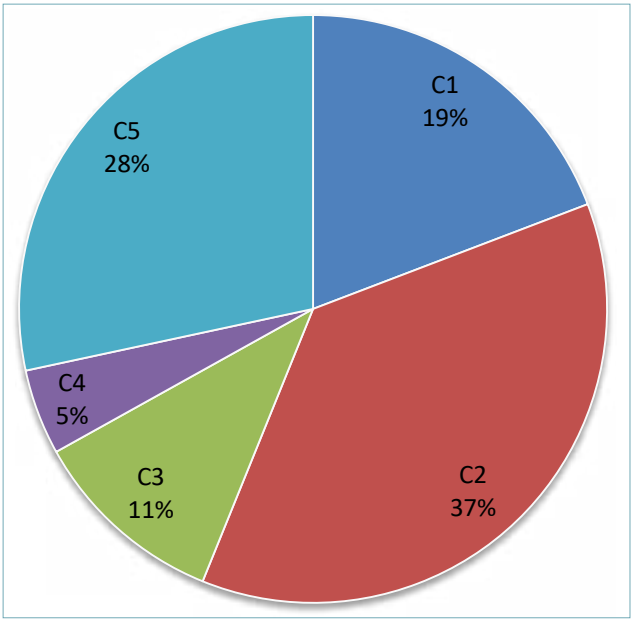

Fig. 1. Distribution of FNA in our institution (2010-2017).

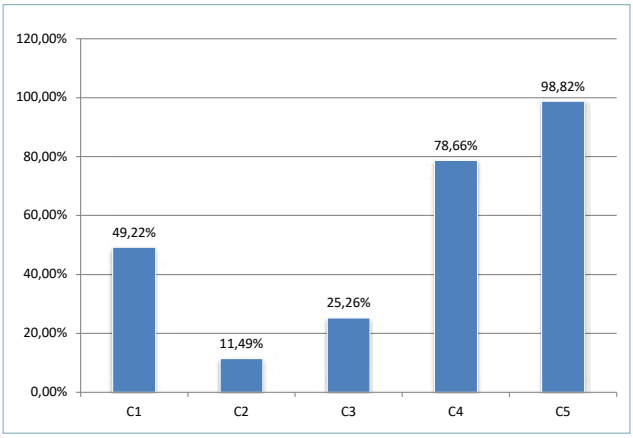

Fig. 2. Risk of malignancy (ROM) in our institution (2010-2017).

in both our and other series, related to the selection for surgery of patients with high risk clinical and radiological features. Our findings show how cytology can still represent a valuable tool for the diagnosis and correct management of clinically and radiologically worrisome breast lumps.

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DIAGNOSTIC VALUE OF MAMMARY NEEDLE CORE BIOPSY IN THE DIFFERANTIAL DIAGNOSIS BETWEEN FIBROEPITHELIAL LESIONS

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Background. Fibroepithelial lesions of the breast represent a group of heterogeneous biphasic neoplasms, characterized by the proliferation of epithelial and stromal components of the terminal duct-lobular unit (TDLU). Fibroadenoma (FA) is the most common benign tumor in women while phyllodes tumor (PT) is less common but more often found in older women and tends to be larger. FAs are benign tumors so they can be eligible for conservative treatment. PTs, on the other hand, must be distinguished into benign, borderline ad malignant, they can recur locally and give distant metastases, so they require more radical intervention.
Aim: Our purpose was to evaluate histological parameters and highlight the most useful to make the proper diagnosis between fibroadenoma and phyllodes tumor on core needle biopsies (CNB) specimens.
Materials and methods. We performed a retrospective analysis of 25 cases of fibroepithelial lesions arrived at the Operative Unit of Anatomic Pathology of the University of Palermo. Each lesion underwent an excisional biopsy, so histopathological features were evaluated on both CNB and excisional biopsy. The features evaluated were: *stromal cellular changes* (stromal cellularity, stromal cell pleomorfism, mitotic count, periglandular stromal condensation), *stromal architectural changes* (stromal overgrowth, fragmentation of the cores, fat in stroma, stromal distribution), *epithelial cellular changes* (epithelial hyperplasia, apocrine metaplasia), *epithelial architectural changes* (growth pattern, leaf-like or epithelial fronding), *miscellaneous features* (pseudoangiomatous stromal hyperplasia (PASH), myxoid and cystic changes, multinucleated giant cells, lesional edge).
Results. The results of Spearman correlation test showed that stromal cellularity (rho=0,400; p=0,047), stromal cell pleomorphism (rho=0,0472; p=0,017), stromal overgrowth (rho=0,587; p=0,002), presence of mitosis (rho=0,571; p=0,003), number of mitoses x 10 HPF (rho=0,656; p=0,0001); periglandular stromal condensation (rho=0,417; p=0,038); phylloid/leaf-like pattern (rho=0,598; p=0,002), fragmentation of the specimen (rho=0,474; p=0,017), the presence of multinucleated giant cells (rho=0,505; p=0,010) and patient's age (rho=0,432; p=0,031) correlated with worsen lesions.
Conclusions. This study consolidates the current understanding of pathobiology and clinical behavior of these tumors and emphasizes how key histological features such as stromal cellularity, stromal cell pleomorphism, presence of mitosis, stromal overgrowth, phylloid/leaf-like pattern, periglandular stromal condensation, fragmentation and the presence of multinucleated giant cells can be helpful in the diagnosis of phyllodes tumors on CNB specimen.

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SHORT-TIME AMPLIFICATION OF BREAST CIRCULATING TUMOUR CELLS FOR FURTHER CYTOLOGICAL INVESTIGATION

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While tissue/cellular biopsy has a central role in cancer diagnosis, recently less invasive, blood-based diagnostic techniques based on circulationg tumor cells are being developed for more rapid cancer screening. Circulating molecular biomarkers, generally useful to monitor the course of neoplastic disease, cannot be employed for primary diagnosis due to their poor specificity. Convenient enrichment methodologies are hence necessary to improve the efficiency to isolate and characterize specific diagnostic biomarkers. We conducted an observational prospective project:" CHARACTEX" on patients with previous cancer diagnosis and on healthy subjects. We apply optimized, previously described protocols, to isolate non-haematological cell populations after short time in vitro-expansion (≤14dys), promoting those cells which are able to proliferate and reach a sufficiently high number for further characterization and analysis. Here we show the cytological presentation of circulating breast cancer cases enrolled in this study, stained with Diff-Quik and integrated by immunocytochemical stains. Our results suggest an intriguing diagnostic role of this methodology and its potential applications in the personalized follow up, in monitoring response to therapy and prognostic evaluation of breast cancer.

RADIATION-ASSOCIATED ANGIOSARCOMA (RAA) OF THE BREAST: A CASE REPORT

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Background. Breast angiosarcoma is a rare disease accounting for approximately 1% of soft tissue sarcomas and 0.04% of malignant neoplasm of the breast. It can occur as a primary form without a known precursor or as a secondary form associated with radiotherapy. Adjuvant radiotherapy has a significant role in preventing local recurrence in women treated with conservation

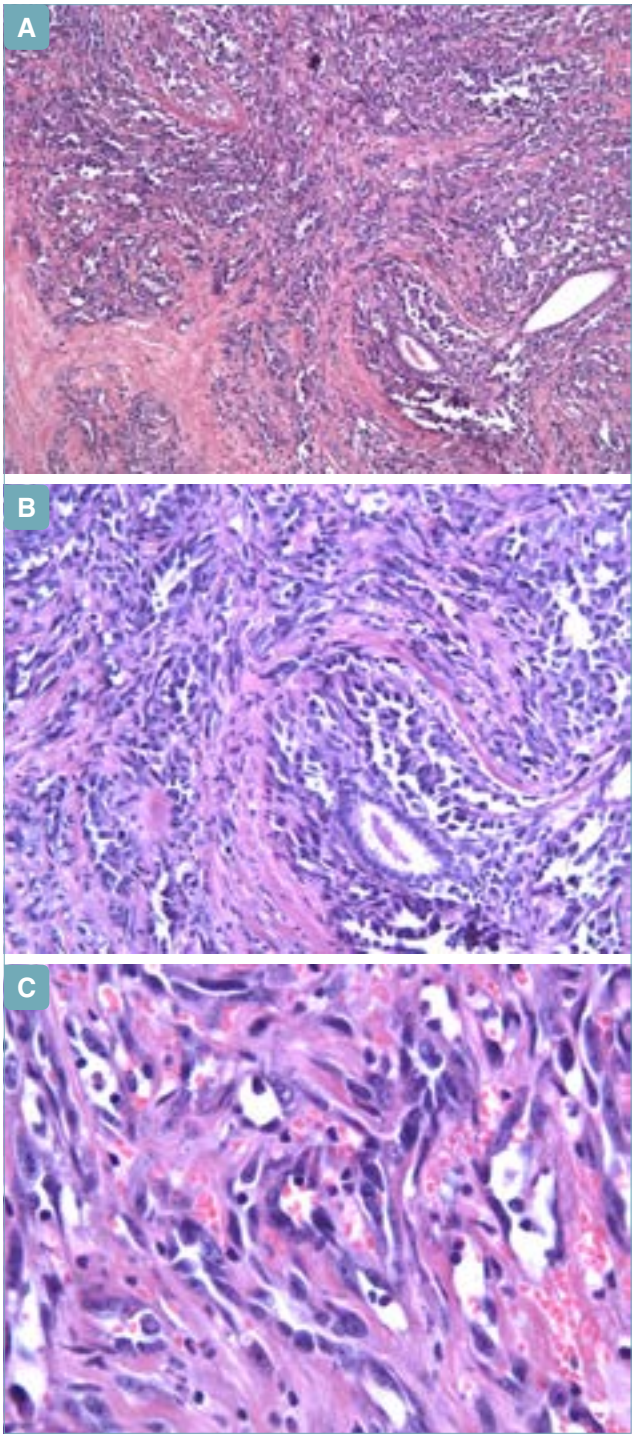

Fig. 1. a) EE 10x; b) EE 20x and c) EE 40x.

therapy for early stage breast carcinoma or multicentric tumors. Postradiation angiosarcoma usually affects the dermis of the breast within the radiation field and may occasionally develop in the breast parenchyma. Compared with the latency of other radiation-associated sarcomas, the latency for breast radiation-associated angiosarcoma is relatively short with a median of 7 years. The risk of developing secondary angiosarcoma does not outweigh the benefit of treatment; therefore, radiation therapy continues to be a mainstay modality in the treatment of breast cancer patients. Early detection is essential because angiosarcomas are associated with a poor prognosis. Wide surgical resection is the standard treatment for these tumors.

Methods. Our patient was 74 years-old woman with a history of stage IA invasive ductal carcinoma of right breast diagnosed in december 2011, wich was estrogen receptor positive, progesterone receptor positive, HER-2neu negative with very low Ki67 (≤5%). She underwent quadrantectomy and axillary lymphadenectomy and received adjuvant whole breast external beam radiation with a standar dose of 50.4 Gy in 25-28 fractions / 5 times a week (1.8 / 2 Gy / fraction) and hormonal therapy.

Seven years after surgery, she presented with a palpable mass with faint purple discolouration in the skin at the site of the previous scar. Mammographically, the appearance of this tumor is not specific. It looks like an ill-defined, no calcified mass. On ultrasuond, it appears as a heterogeneous, hyperechoic, hypervascular mass that is associated with disruption of the normal breast architecture. An agobiopsy/tru-cut was performed with the diagnostic category B1 for the detection of only stroma. Therefore a lumpectomy was decided. Macroscopically, the nodule measured 6x5x3 cm and it has a hemorrhagic appearance. Hystologically, tumor is composed of solid sheets of anaplastic spindle cells forming complex vascular channels in hyaline stroma. Necrosis, hemorrhage (blood lakes) and readily identifiable mitoses were present. On the periphery of the nodule atrophic mammary gland with mild aspecific periductal inflammation was displaced (Fig. 1).

On immunohistochemical examination, the tumor cells stained strongly for vimentin and typical endothelial markers (CD31, Factor VIII, CD34), while they were negative for CKAE1/3CK19, E-Cadherin, Gata3, p63, Actin Muscolar HHF35 and Desmin. The mitotic index was ≥50mitosis/10HPF and Ki67-labeling index has been reported ≥ 60% (Fig. 2).

On the basis of data morphological and clinical history a diagnosis of RAA was made.

A completion right mastectomy was performed with histological finding of neoplastic residual areas both near and far from the surgical bed, indicative of multifocality.

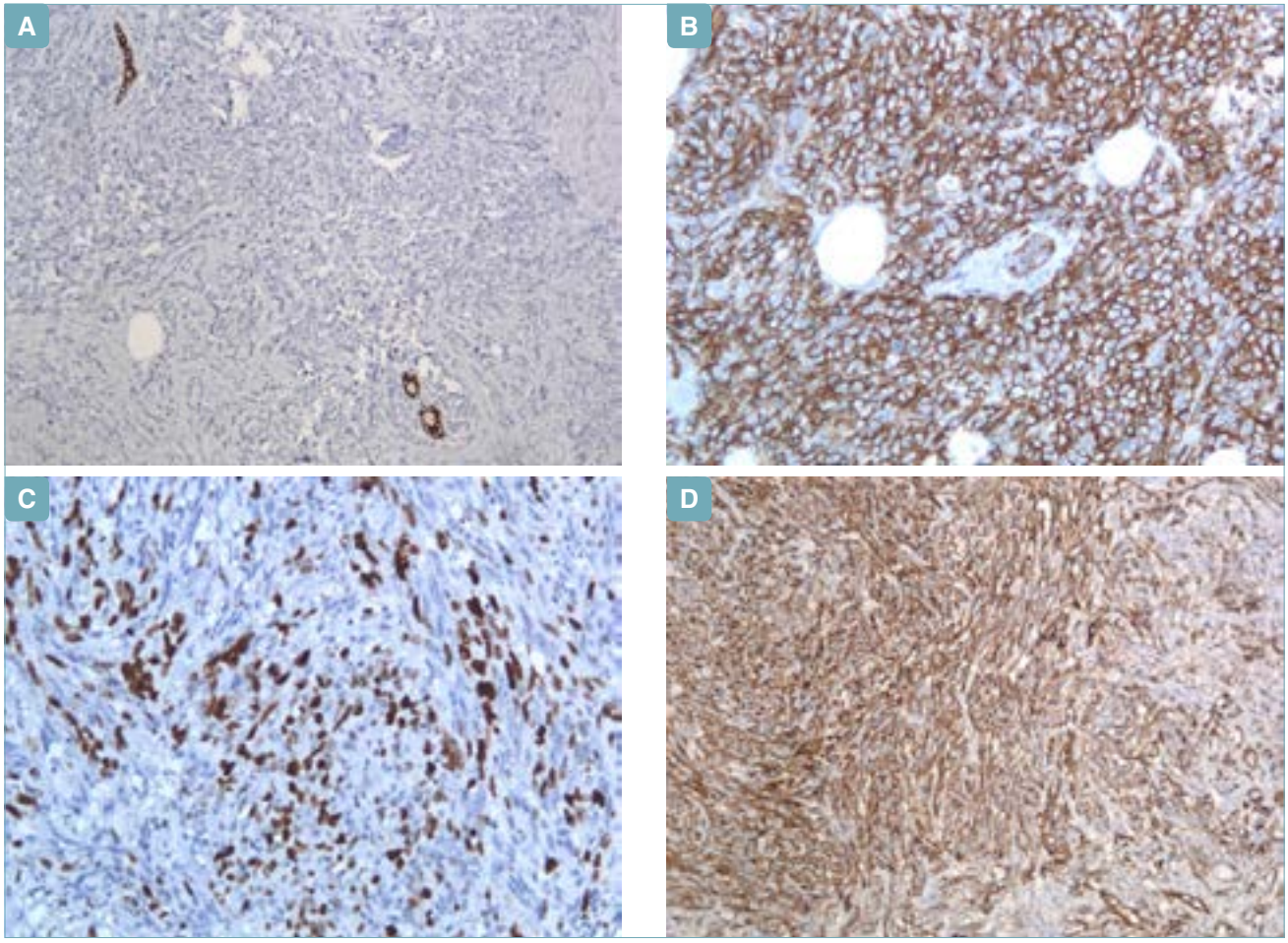

Fig. 2. a) CK 19; b) CD 31; c) Ki-67 and d) Fattore VIII.

Discussion. Angiosarcoma (AS) of the breast in an aggressive vascular tumor. It appear de novo (primary angiosarcoma) or as complication of radiotherapy (RAA) or chronic lymphedema (Stewart-Treves symdrome). RAA is a rare but severe long-term complication in the breast-preserving management of breast cancer, treated with breast-conserving surgery and radiotherapy. Contray to what occurs in primary AS of the breast, which usually appears in women aged 30-50 years, breast RAA affects older women (with a median age of of 67-71 years) about seven years after radiotherapy as treatment of primary breast cancer (the median onset latency varies from 5-10 years). The former is localized in the parenchyma, the latter predominantly in dermal and subcutaneous layers of the skin of radiated fields. The pathogenesis of RAA is poorly understood. It is believed to be related to irreversible DNA damage induced by radiation resulting in genome instability and by direct tumor induction by radiation through mutations of relevant cancer-related genes. Molecular studies in radiation-associated sarcomas have revealed common inactivation of the p53 pathway and expression and amplification of the MYC oncogene. In addition to the direct oncogenic effect of ionizing radiation, prolonged cellular stimulation during repair of tissue damage resulting from radiation-induced ischemic change may have a role in the development of angiosarcoma. The prognosis is poor with a high rate of relapse and an incresed tendency to metastasis. RAA of the breast is treated with simple mastectomy and/or wide, local excision. Axillary dissection is not necessary. Adjuvant/neoadjuvant radiation therapy for RAA is usually used to improve local control. There are conflicting data regarding the effectiveness of chemotherapy. Future treatment of the disease with antiangiogenesis therapy and vascular targeting agents is currently being evaluated in clinical trials.

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EXTENSIVELY CALCIFIED NODULE MASQUERADING METAPLASTIC BREAST CARCINOMA IN A 68-YEAR-OLD WOMAN.

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Background and objectives. Metaplastic breast carcinoma (MBC) is a rare subtype of breast cancer accounting for 0.2-5% of all invasive breast carcinomas (1). The term encompasses a heterogeneous spectrum of neoplasms characterized by differentiation of the neoplastic epithelial component into squamous cells and/or mesenchymal elements, including spindle, chondroid, osseous and rhabdomyoid cells. There is no consensus on the percentage of the metaplastic component within a tumor to define MBC (2). It can be associated with a conventional in situ or invasive mammary carcinoma and some studies demonstrate clonality between these components (3,4). No consistent immunophenotype is clearly identified, thus probably indicating the morphological and molecular heterogeneity of this tumor encompassing biologically different tumor subtypes (5) and the need to use a wide panel of antibodies when diagnosing a MBC (2). More than 90% of MBC are ER, PR and HER2 negative (6,7). Distant metastases, preferentially involving brain and lungs, can be detected in the absence of lymphnode metastases. MBC shows lower response rates to conventional adjuvant chemotherapy and a worse clinical outcome than other forms of triple-negative breast cancers (2, 8,9). Herein we describe a case of mixed epithelial and mesenchymal MBC occurring in a 68-year-old woman and clinically presenting as an extensive calcified nodule.

Methods. A 68-year-old woman presented with a palpable left breast lump. Mammography revealed a well circumscribed extensively calcified nodule, 1.5x1 cm in size, located in the lower-inner quadrant, scored as R3. Biopsy identified a spindle cell proliferation in a background of bone trabeculae, scored as B3. She underwent nodule resection followed by sentinel lymph node biopsy and mastectomy.

Results. The epithelial component is represented by ductal carcinoma in situ (DCIS), nuclear grade G2/G3, mixed with an atypical spindle cell component arranged in a storiform pattern and a large mesenchymal component composed of immature bone trabeculae. The DCIS component was positive for high-molecular-weight basal cytokeratins CK14, CK5/6 and 34betaE12 and for p63, thus showing a basal-like immunophenotype. The spindle cell component was negative for cytokeratins and for p63. Both the two components were negative for estrogen and progesterone receptor and HER2. Sentinel lymph node was negative.

She was treated with chemotherapy and is alive without evidence of disease at 15 months of follow-up.

Conclusion. The case of MBC we describe is composed of a combined in situ and invasive epithelial component with extensive mesenchymal bone formation. As reported in the largest MBC series our case was hormone receptors and HER2 negative.

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LOW-GRADE DUCTAL CARCINOMA IN SITU OF THE BREAST WITH RADIAL SCAR-LIKE SCLERO-ELASTOTIC STROMA: A RADIOLOGICAL AND HISTOLOGICAL MIMICKER OF MALIGNANCY

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Aims. Carcinoma in situ of the breast usually manifests as casting-type calcifications on mammography and ultrasound examination, but several other unusual forms of presentation can be observed. We herein report an unusual case of low-grade ductal carcinoma in situ with extensive sclero-elastotic stroma which imparted tumor pseudo-infiltrative margins, mimicking, on radiological and histological examination, an invasive carcinoma.
Material and methods. A 48-year-old woman underwent radiological screening for breast carcinoma. At ultrasound examination the patient showed a lesion measuring 2 cm in greatest diameter, with irregular margins; at mammographic examination the lesion appeared as a radio-opacity mass with finger-like margins and suspicious calcifications, highly suggestive of invasive carcinoma (BI-RADS 5). A tru-cut biopsy was performed, accordingly. After rendering the diagnosis of low-grade cribriform ductal carcinoma in situ (p63 focally retained) with associated sclero-elastotic stroma, a quadrantectomy was performed due to the radiological suspicion of malignancy.
Results. Histological examination showed a lesion with finger-like infiltrative margins, composed of low-grade cribriform ductal carcinoma in situ with diffuse and marked sclero-elastotic stromal reaction. Frequently the neoplastic ducts showed an architectural distorsion with a pseudoinfiltrative growth pattern. However p63 stained a variable number of myoepithelial cells, ruling out the possibility of an invasive carcinoma. Benign epithelial lobules and ducts with the proliferative changes as seen in radial scar, were lacking both at the periphery and in the center of the lesion. The diagnosis of "*low grade cribriform ductal carcinoma in situ with radial scar-like sclero-elastotic stroma*" was rendered.
Conclusions. We herein present a challenging case of a low-grade cribriform ductal carcinoma in situ, radiologically and histologically mimicking an invasive carcinoma. The diagnostic difficulty was due to a diffuse and marked sclero-elastotic reaction of the stroma, which imparted lesion an infiltrative growth pattern. The main differential diagnosis was radial scar, a benign lesion characterized by a fibro-elastotic core from which ducts and lobules, with variable changes ranging from typical ductal hyperplasia to ductal/lobular in carcinoma in situ, radiate. In our case no benign epithelial component was found both at the periphery or in the center of the lesion. However the possibility that the present case was originally a radial scar which entirely underwent malignant transformation (carcinoma in situ) cannot be completely ruled out. Nevertheless the present case emphasizes the rare possibility that low-grade ductal carcinoma in situ may present infiltrative margins at mammography, highly suggestive of an invasive carcinoma. The histological basis of this unusual finding is a radial scar-like sclero-elastotic reaction of tumor stroma. Pathologists should be aware of this possibility to avoid confusion with radial scar or invasive carcinoma.

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PROGNOSTIC ROLE OF TCF1/WNT PATHWAY EXPRESSION IN PRIMARY BREAST CANCER PATIENTS

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Background. The highly conserved Wingless (Wnt) signaling pathway regulates different cellular processes as cell proliferation, motility, and stem cell renewal[1]. The signaling activation needs both the frizzled receptors (FZDs) and the low density lipoprotein receptor-related protein (LRP) 5 and 6 co-receptors. One of its downstream transcription factors is T-cell factor (TCF) and lymphoid enhancer factor (LEF), TCF1, which is linked to transcription of genes cancer-related[2-3]. Aberrant Wnt/β-catenin signaling has been implicated in tumorigenesis and cancer progression in many cancers including breast cancer (BC)[4]. Furthermore, β-catenin expression has proven to be a significant prognostic factor in BC[5]. Our aim was to evaluate the expression of some of the majoir Wnt pathway components: β-catenin, FZD4, LRP5, LRP6 and TCF1 proteins, and their impact on patients'clinical outcome.
Methods. We evaluated 220 primary BC samples by immunohistochemistry on tissue microarrays. All statistical evaluations were performed with the Prism version 5.00 software package (GraphPad Software, San Diego, CA, USA), with the statistical significance set at $p < 0.05$. Univariate Cox regression and Kaplan Meier analyses of disease free survival (DFS) and overall survival (OS) were performed to evaluate the prognostic significance of marker expression.
Results. Univariate analysis revealed that the subgroup of patients with low β-catenin expression had a better 5-year % DFS compared to patients with high β-catenin expression 92% vs 67% ($p<0.0001$). Patients with high FZD4 expression had a worse 5-years % DFS compared with patients with low FZD4 expression (71% vs 91% $p=0.0001$). A worse 5-years % DFS for patients with high respect to low LRP5 expression (70% vs 93%; $p<0.0001$) and for patients with high respect to low LRP6 expression (68% vs 95%; $p<0.0001$) was also ob-

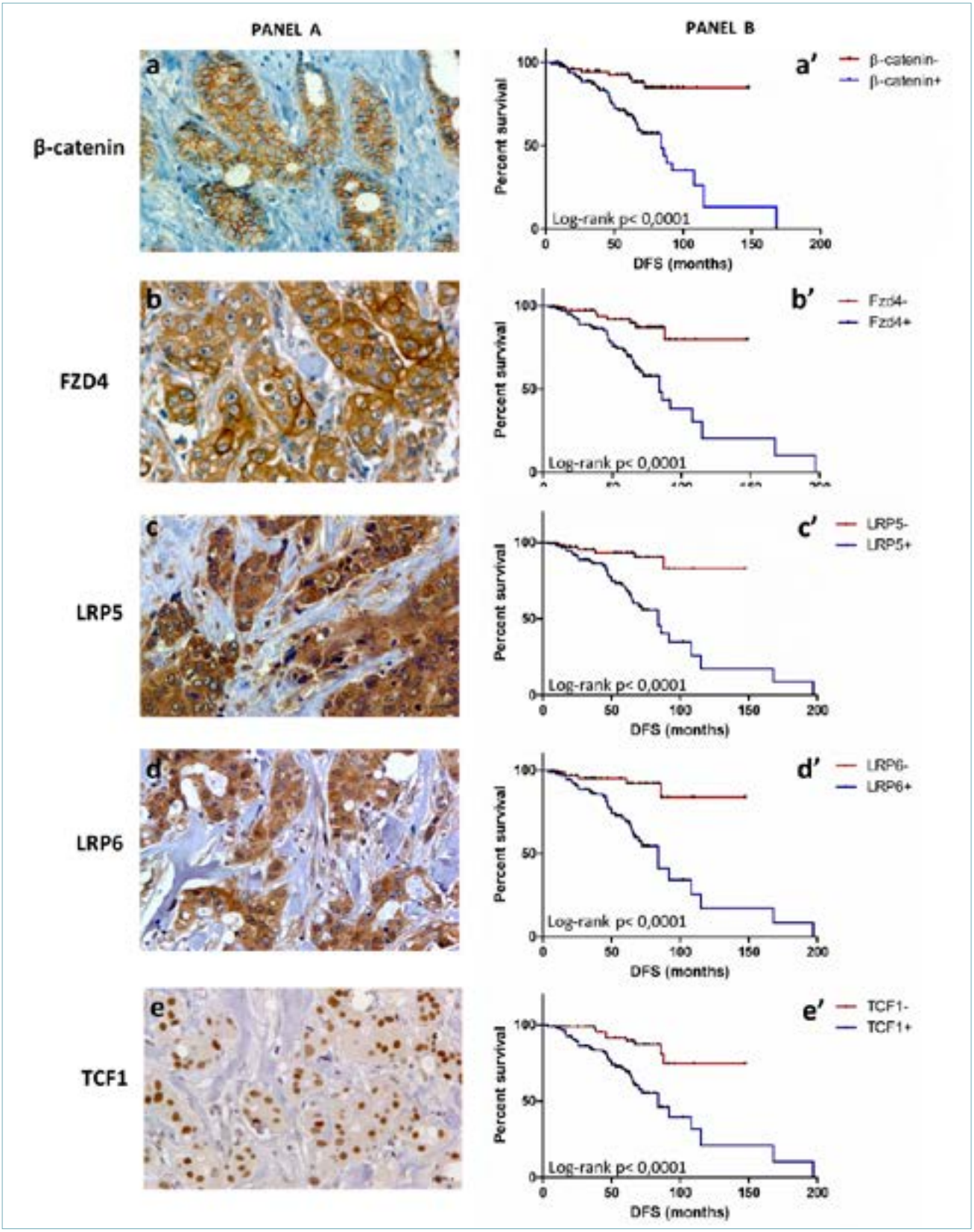

Fig. 1. Representative images of immunohistochemical staining in BC tissues. The panel A displays the representative expression of molecular biomarkers in tumor zone: a) membranous and cytoplasmic β-catenin expression; b) cytoplasmic FZD4 over-expression; c) and d) cytoplasmic LRP5 and LRP6 over-expression; e) nuclear TCF1 expression (original magnification, ×400). Survival analyses. The panel B shows DFS curves for patients with a') β-catenin-versus β-catenin+ expression (p<0.0001); b') FZD4- versus FZD4+ expression (p<0.0001) ; c') LRP5- versus LRP5+ (p<0.0001); d') LRP6-versus LRP6+ (p<0.0001); e') TCF1- versus TCF1+ (p<0.0001).

served. Further, a worse 5-years % DFS was found for patients with high respect to low TCF1 expression (68% vs 92%; p<0.0001). Kaplan-Meier analysis confirmed the univariate data and it showed a poor DFS in the subgroup of patients with high β-catenin expression, high FZD4 expression, high LRP5 and LRP6 expression and in the subgroup of patients with high TCF1 expression (p<0.0001 for each group) (Fig. 2a-e). In multivariate analysis, TCF1 and β-catenin expression were independent prognostic variables of worse DFS (p=0.009 and p=0.027, respectively).

Conclusions. We first identified TCF1 as independent prognostic factor of poor outcome in primary BC patients, indicating it as a new potential biomarker for BC patients management. Moreover, we showed that the over-expression of the Wnt pathway proteins was associated to a worse DFS. These results suggest the possible capability of these proteins to identify more aggressive phenotypes.

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THE ASSOCIATION OF NHERF1 EXPRESSION AND TUMOR INFILTRATING LYMPHOCYTES INFLUENCES PROGNOSIS OF BREAST CANCER PATIENTS

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Background. Breast cancer (BC) is an heterogeneous disease, and patients with apparently similar clinico-pathological characteristics in clinical practice show different outcome. In this scenario, the need to find new biological markers for a more precise characterization of the disease for prognosis and therapeutic purposes has became mandatory. During the last ten years our group have focused own studies on Na^+/H^+ exchanger regulatory factor 1 (NHERF1), a scaffold protein involved

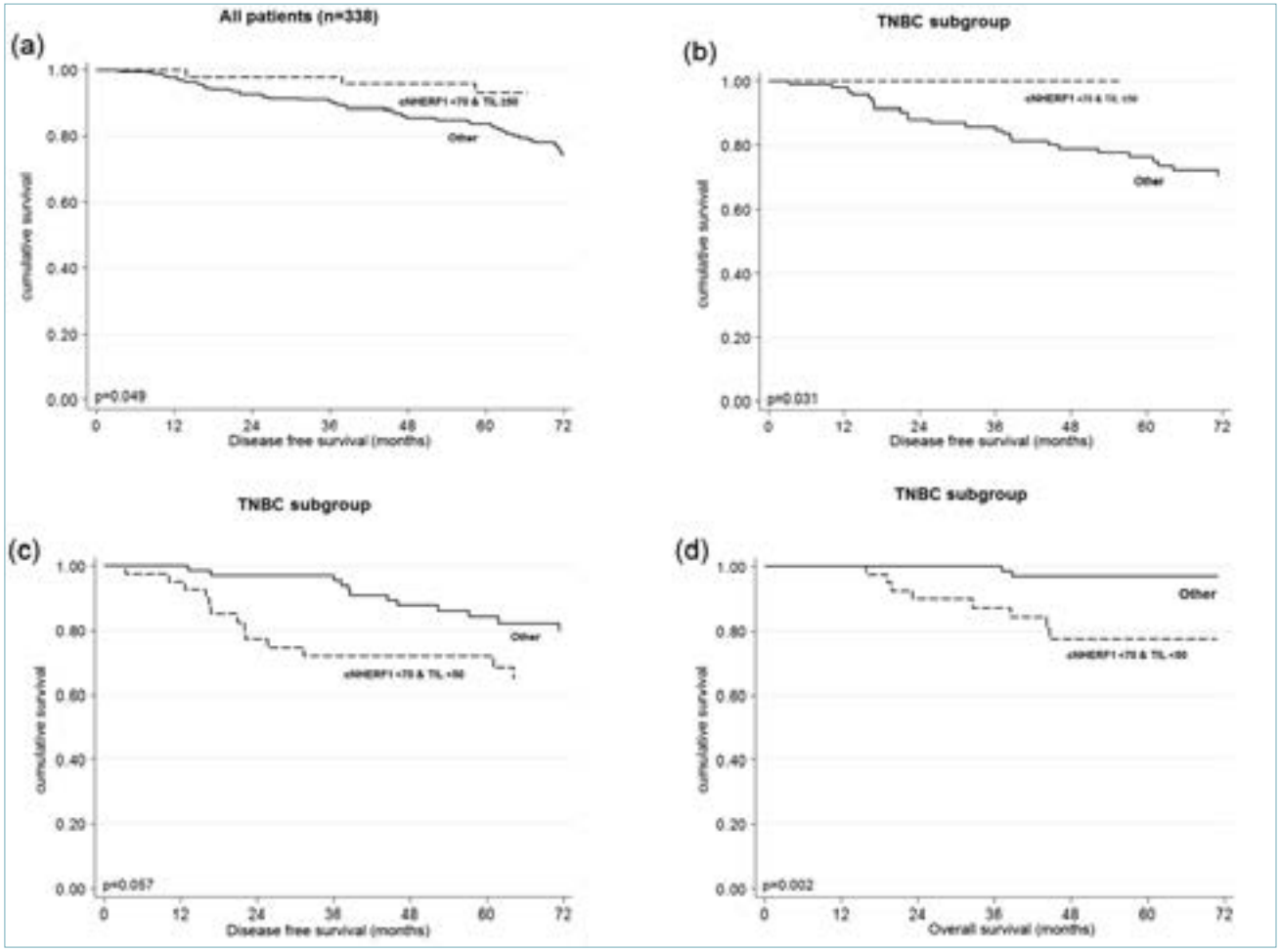

Fig. 1. Kaplan-Maier curve analysis and log rank test. (a) Kaplan-Maier curve for Disease Free Survival according to cNHERF1<70% TIL ≥50% (cNHERF1-/TILs+) versus Others in All patients; (b) Kaplan-Maier curve for Disease Free Survival according to cNHERF1<70% TIL ≥50% (cNHERF1-/TILs+) versus Others in TNBC subgroup; (c) Kaplan-Maier curve for Disease Free Survival according to cNHERF1<70% TIL < 50% (cNHERF1-/TILs-) versus Others in TNBC subgroup; (d) Kaplan-Maier curve for Overall Survival according to cNHERF1<70% TIL < 50% (cNHERF1-/TILs-) versus Others in TNBC subgroup.

in many human cancers, including BC. Our previous results showed the prognostic significance of nuclear NHERF1 (nNHERF1) expression in BC, and that the loss of nNHERF1 was associated with poor DFS [1]. Recently, the study of tumor microenvironment has acquired more and more importance in many tumors [2] and it was observed that tumor infiltrating lymphocytes (TILs) can predict response to chemotherapy and improved prognosis in BC patients [3].

Objectives. Little is known about the involvement of NHERF1 in the immunotherapeutic field. This study evaluated in primary BCs and in the triple negative breast cancers (TNBCs), NHERF1 expression, the level of TILs, and their association respect to the prognosis and the clinical outcome of patients.

Material and methods. NHERF1 expression was assessed by immunohistochemistry in 338 BC samples by an automated staining instrument; the analysis of TILs was examined using hematoxilyn and eosin stained slides, according to International TILs Working Group 2014 [4]. NHERF1 and TILs analysis was carried out in relation to disease-free survival (DFS) and overall survival (OS) of patients with a median follow-up time of 72 months.

Results. Multivariate analysis identified TILs as an independent prognostic factor for DFS in the entire cohort and in the TNBC subgroup (HR, 0.32; 95% CI, 0.12-0.87; *P* =0.026, and HR, 0.22;95% CI, 0.06-0.80; *P* =0.022, respectively). Univariate and survival analysis by Kaplan–Meier method revealed that patients with cytoplasmic (c) $NHERF1^{-}/TILs^{+}$ expression had better DFS than other patients (*P* =0.049), and this result was also found in the TNBC subgroup (*P* =0.031) (Fig. 1, a and b). Moreover, TNBC patients with $cNHERF1^{-}/TILs^{-}$ expression had a worse DFS and OS than other patients (*P* =0.057 and *P* =0.002, respectively) (Fig. 1, c and d)

Conclusions. Interestingly, we found an association between cNHERF1 expression and TIL levels that could be useful to identify BC and particularly TNBC patients with different prognosis and clinical outcome.

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PATOLOGIA MOLECOLARE

BRAF AND *RAS* MUTATIONS IN DIFFERENTIATED THYROID CANCER OF PATIENTS WITH LOW-RISK TO INTERMEDIATE-RISK TREATED WITH RADIOIODINE THERAPY

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Background. Differentiated thyroid cancer (DTC), including papillary (PTC) and follicular thyroid carcinoma (FTC), account for >90% of all thyroid cancers. In recent years, the incidence of DTC is rapidly rising in many areas of the world and PTC represents the prevalent histotype. In particular, PTC with a maximum diameter up to 10 mm has increased faster than other types of PTC and the appropriate management of this microcarcinoma (PTMC) has become a crucial issue. In fact only a relatively small number of PTMCs have aggressive behavior. Therefore various criteria have been proposed to select DTC patients eligible for 131-radioiodine therapy (RaIT) with either ablative or adjuvant purpose after thyroidectomy. The guidelines of American Thyroid Association (ATA) stratify DTC patients in low, intermediate and high risk of disease recurrence and/or persistence for therapeutic decision-making on the basis of clinicopathological parameters. However there is still controversy about this issue. Several somatic molecular alteration have been associated to DTC and point mutations in *BRAF*, *KRAS*, *NRAS* or *HRAS* represent genetic markers enable definitive diagnosis of malignancy. In addition, $BRAF^{V600E}$ is the most common mutation in PTC and it is associated with aggressive clinicopathological features. Moreover, has been documented that $BRAF^{V600E}$ mutation is associated with loss of radioiodine uptake by silencing of thyroid iodine-handing genes and impairing the sensitivity to RaIT.

Objective. The aim of the present study was to evaluate the incidence of *BRAF* and *RAS* mutations in DTC with ATA low-risk to intermediate-risk features as well as to assess the role of mutational status in guiding postoperative RaIT.

Material and Methods. Sixty six patients (17 M, 49 F; mean age 51.1 yrs) with DTC who showed ATA low-risk to intermediate risk features after thyroidectomy and received RaIT between April 2017 and June 2019 were included in the study. Patients were followed up with clinical examination including instrumental and laboratory analysis every 6 months; all patients with a follow-up period <12 months were not considered. Tissue samples were formalin-fixed and paraffin-embedded (FFPE). At histopathological examination 52 cases were PTCs (23 pT1a, 20 pT1b, 9 pT2), 10 FTCs (5 pT1a, 1 pT1b, 4 pT2) and 4 Hurthle cell carcinoma (2 pT1b, 2 pT2). After micro-dissection of tumour cells DNA extraction was performed by QIAamp DNA FFPE Tissue (Qiagen). Mutational analysis was performed by real-time PCR utilizing the Thyroid Cancer Mutation Analysis Kit (EntroGen). This kit allows the detections of 16 different mutations in the following genes: *BRAF* (V600E); *KRAS* (G12A, G12D, G12R, G12V, G12C, G12S, G13D); *NRAS* (Q61H (CAC), Q61H (CAT), Q61L, Q61K, Q61R); *HRAS* (G12V, G13R, Q61R). Statistical analysis was performed using χ^2 test or Fisher's exact test; P values <0.05 was considered statistically significant.

Results. Point mutations were found in 36/66 (45.5%) DTCs, including 35/52 (67.3%) PTCs and 1/10 (10%) FTC. In PTC group 30 (57.7%) patients showed mutations in *BRAF*, 2 (3.8%) patients in *NRAS* and 3 (5.8%)

patients in *HRAS*; no concomitant mutations were identified. No significant correlation was documented between *BRAF* mutational status and sex, age, pT stage or tumor multifocality. In FTC group only one *NRAS* mutation (10%) was detected.
The follow-up time of 42 DTC patients ranged from 12 to 25 months (median 18.5); eight patients presented persistence of disease, while 34 showed no evidence of disease. No significant association between mutational status and clinical response to RaIT was documented. In addition, no significant correlation between *BRAF* mutational status and response to RaIT was found in 32 patients with PTC.
Conclusions. Our preliminary results suggest that $BRAF^{V600E}$ mutation may not predict a poor therapeutic efficacy of RaIT in PTC patient with low-risk to intermediate-risk features.

COMPARISON OF IMMUNOHISTOCHEMISTRY EXPRESSION OF CLONES ROS1: D4D6 AND SP384. OUR EXPERIENCE.

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ROS1 rearrangement characterizes a small subgroup of non-small cell lung cancer, and is associated with young non-smoking patients, usually with adenocarcinoma histotype.
The *ROS1* gene is located on chromosome 6 (6q22) and encodes for a tyrosine kinase protein (TK) belonging to the insulin receptor family;(1) the expression of SLC34A2/CD74-ROS protein fusion has been found in primary lung cancer.
Although rearrangement of the *ROS1* gene is present in about 2% of NSLCs, the response to treatment with specific inhibitors (crizotinib) shows significantly better survival than conventional therapy.(2)
As a result, the attention of the *ROS1* test labs is growing.
Most of the *ROS1* fusion genes have been detected mainly in FISH. However, is a very long and laborious method, it is therefore necessary to use an equally effective but faster screening test.
The up-grade of the ASCOCAP 2018 guidelines recommends the use of IHC as a screening test in patients with pulmonary adenocarcinoma; however, cases with ROS1 IHC positive should be confirmed by a molecular or cytogenetic method.(3)
Although until 2018 only the monoclonal antibody D4D6 (Cell-Signaling) was commercially available; a new monoclonal antibody, SP384 (Ventana-Roche), was introduced at the IASLC in Toronto in September 2018.
In the our pilot study performed in the Complex Operativ Unit of Pathology, Monaldi Hospital, we have compared the specificity and sensitivity of these monoclonal antibodies D4D6 and SP384.
The model is based on a retrospective study of tissue samples positive for *ROS1*, evaluated with both IHC and FISH method, from Jenuary 2017 to July 2019.

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$P16^{INK4A}$ EXPRESSION IN HR-HPV NEGATIVE DIFFERENTIATED VULVAR INTRAEPTHELIAL NEOPLASIA (DVIN): A PRELIMINARY STUDY IN A SELECTED COHORT

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Background. Vulvar Intraepithelial Neoplasia (VIN) is the precursor lesion of Vulvar Squamous Cell Carcinoma (VSCC). There are two distinct types of vulvar intraepithelial neoplasia, which differ in their clinical presentation, aetiology, pathogenesis and histological/immunophenotypical features. The first type, driven by high-risk human papilloma virus infection, usually occurs in young women and has been termed usual VIN (uVIN). The second type, not related to viral infection, occurs in postmenopausal women with chronic skin conditions as lichen sclerosus and lichen simplex chronicus and is termed differentiated VIN (dVIN). Human papillomavirus (HPV)-associated carcinomas are of basaloid or warty type, whereas tumors unrelated to HPV are usually keratinizing and differentiated. The incidence of dVIN is lower than uVIN.
In contrast to uterine cervix cancer, where the HPV is found in over 95% of the cases, vulvar cancer presents an overall rate of HPV infection of only 30-40% of the cases.
Aim. Classification of vulvar intraepithelial neoplasia is made on morphology and does not involve HPV molecular testing because of no benefit found in terms of cost effectiveness, as reported in literature (1). Routinely, $p16^{INK4a}$ protein expression is the surrogate marker helpful to distinguish between HPV-related VIN from HPV-no related VIN. In fact, p16 negative expression supports dVIN diagnosis while negativity supports uVIN diagnosis.
However there are cases in which dVIN morphology supported by HPV negative molecular test do not correlate with p16 negative expression.
The purpose of this study was to investigate $p16^{INK4a}$ protein expression in a cohort of selected older women with a morphologic dVIN diagnosis and HPV negative test.
Methods. Seventeen selected female patients were involved in the study (from 2013 to 2019): 10 VIN 1, 1 uVIN 1 with adjacent CIN1 (used as positive control), 1 VIN 1-2, 2 VIN 3 and 3 VSCCs. Mean age were 66 years old (from 39 to 86 y.o.). HPV DNA Test were assessed with Hybrid Capture 2 (Digene, Gaithersburg, MD). P16INK4a immunoreaction was detected by CinTec Histology Kit and was assessed on all cases.
Results. Sixteen out of 17 selected cases resulted negative to HPV DNA Test, only the case of uVIN 1 with adjacent CIN1 (used as positive control) was positive to

High-Risk HPV DNA test. About p16 immunoreaction, it was observed in 3 out of 10 VIN1, in the only one VIN 1 with adjacent CIN1, in the only one VIN 1-2 (Fig. 1), in 1 out of 2 VIN 3 and in 1 out of 3 VSCCs (Tab. I).

Discussion. In our selected cohort, 63% of dVIN cases were negative to $p16^{INK4a}$ expression and this result was concordant with the HPV DNA test data, whereas 37% of dVIN cases which were positive for $p16^{INK4}$a expression but negative for HPV infection.

This data could mean that in these dVIN samples the protein overexpression were not linked to viral infection but to other molecular mechanisms involved in the carcinogenesis process. In fact it must be remembered that $p16^{INK4a}$ is a cyclin-dependent kinase-4 inhibitor which has an anti-proliferative effect during regular cell cycle progression, so a $p16^{INK4a}$ overexpression without HPV infection could mean a worse prognosis, because p16 expression could be the consequence of the increase of mutations load in the neoplastic unregulated proliferating cells. Indeed in recent target sequencing study (2) were reported there were several mutations which prove that multiple pathway can contribute to the development of dVIN through the loss of cell proliferation control.

Conclusions. According literature (2, 3), our preliminary data showed that the higher rate of dVIN were negative to p16 and HPV but, at the same time, the presence the p16 protein were detected also in HPV negative dVIN, so we supposed that p16 identified "proliferating" lesions with a probably worse prognosis. In this hypothesis, probably p16INK4a positivity could detect among the low grade dVIN the "proliferating" lesion which could have a more rapid evolution in Vulvar Squamous Cell Carcinoma.

However, based on these results, we would to like to this study increasing the number of the involved cases as well as to test new molecular markers.

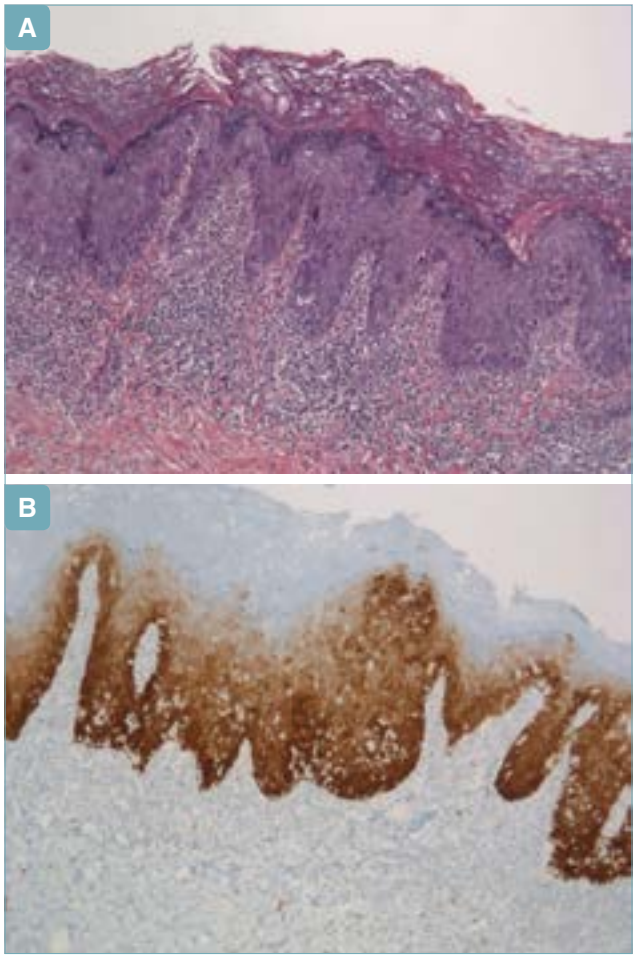

Fig. 1. a) EE 10; b) p16INK4a 10x

Tab. I. Distribution of p16 expression and HPV infection in the cohort.

	P16 positive	P16 negative	HPV DNA positive	HPV DNA negative	Total
VIN 1	3	7	-	10	10
uVIN1+CIN1	1	-	1	-	1
VIN 1-2	1	-	-	1	1
VIN 3	1	1		2	2
VSCCs	1	2		3	3
Total	7	10	1	16	17

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A HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY POINTS OUT FERRITINOPHAGY AS PATHOGENETIC MECHANISM IN KERATOCONUS

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Keratoconus (KC) is a corneal dystrophic, non-inflammatory disease characterized by gradual thinning and acquisition of a conical shape that cause a severe impairment of vision. The KC etiology is not fully understood but it appears multifactorial probably arising because of genetic predisposition and environmental insults (1). It typically occurs in adolescents and progresses to fourth decade of life. The incidence of KC in the European population is estimated around 1per 2000 and recent studies have shown that incidence and annual prevalence are increasing (2,3). It is possible to hypothesize the involvement of autophagic dysfunction and in particular ferritinophagy as crucial part of the mechanism.

Objective. To evaluate the possible contribution of autophagy and ferritin degradation as pathological factors in the development of KC.

Materials and methods. We carried out an immunohistochemical and histochemical analysis on the epithelial

layer of corneas taken from KC patients that underwent keratoplasty surgery and corneas explanted from corpses as controls. On corneal sections we studied immunoreactivity for LC3B, and ferritin (heavy chain) and performed the PERLS staining for free Fe^{3+} detection.
Results. Our analysis showed a different distribution of immunoreactivity between KCs and controls. In KCs LC3B and ferritin distribution is almost superimposable and increased in the basal layer. The PERLS staining shows particularly in KCs, the presence of free iron throughout the corneal epithelium as probable index of ferritin degradation.
Conclusions. These findings support the hypothesis that ferritin degradation is upregulated in KC and it creates a microenvironment in which Fenton's reaction take place generating ROS and triggering the cell dead process of ferroptosis (4). The oxidative attack to the corneal epithelium causes its degradation contributing to the KC pathogenesis.

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PATOLOGIA NEFROLOGICA

NEW IMAGING APPROACH ALLOWS FOR BETTER EVALUATION OF PARENCHYMAL INVOLVEMENT BY OXALATE CRYSTALS DEPOSITION IN KIDNEY BIOPSY

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Background. Oxalate nephropathy is a rare but likely underestimated cause of end stage renal disease (ESRD). Oxalate crystal deposition can be secondary to inherited oxalate metabolism abnormalities (primary oxalate nephropathy) or, more frequently, to secondary causes, including intestinal malabsorption (i.e. after bariatric surgery) or increased oxalate intake. When history of kidney stones and/or classical risk factors for enteric hyperoxaluria are present, the diagnosis is easy. Nevertheless, sometimes the clinical onset is confusing, and the diagnosis belated. Even when a kidney biopsy is performed, recognition and characterization of oxalate crystals can be challenging and observation of H&E sections under polarized light is needed. Even when this is done, quantification of the degree of parenchymal involvement by oxalate deposition can be difficult in FFPE sections.
Case description. A 50-year-old female with ESRD due to enteric hyperoxaluria secondary to bariatric surgery,

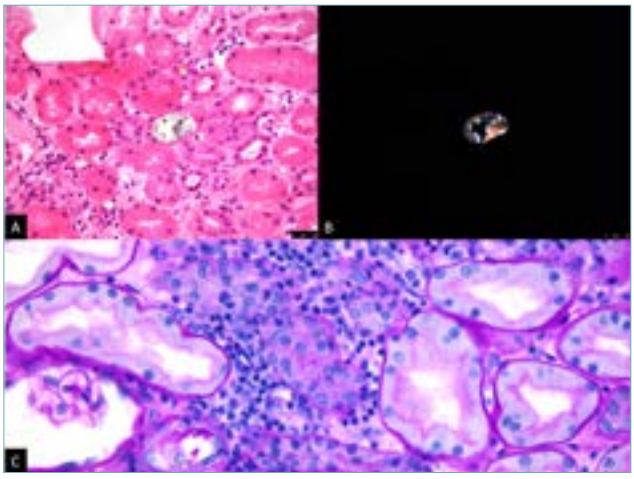

Fig. 1. First kidney biopsy. Panels A and B show birefringent intratubular oxalate crystal (H&E, 400x). Focal intense interstitial inflammation and moderate tubulitis were also observed, in tubules with no crystal deposition (Panel C, PAS, 400x).

underwent deceased donor kidney transplant. Cytotoxic crossmatch was negative, and the patient showed no donor specific antibodies at the time of the transplant. Induction therapy included basiliximab and maintenance immunosuppression was based on tacrolimus, prednisone and mofetil mycophenolate. The patient had immediate graft function with creatinine dropping to 2.0 mg/dl in 5 days; however, on day 6 after transplant she experienced reduction of urinary output and an increase in creatinine. A first kidney biopsy (Fig. 1) showed focal intratubular birefringent crystals deposition, compatible with oxalate crystals, moderate interstitial inflammation and tubulitis, indicative of acute cell mediated rejection Banff 1A. Glomeruli were unremarkable. Patient was started on pulse steroids and daily dialysis for oxalate removal, but creatinine did not improve, and a second biopsy was obtained on day 10 after transplant. Immediately after the biopsy was performed, 1 mm thick fresh kidney core was observed under standard bright field microscope using white light and polarized light (Fig. 2).

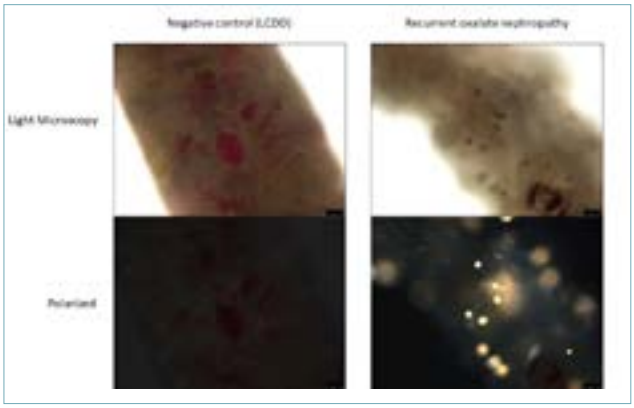

Fig. 2. Second biopsy. Fresh kidney biopsy needle cores observed under bright field and polarized light. Left panels show the biopsy of a patient with light chain deposition disease (LCDD) and no signal under polarization; right panels show intense and diffuse birefringence from oxalate crystals at different levels of the core (magnification 100x) in a patient with oxalate nephropathy.

Polarization revealed the presence of birefringent oxalate crystals in the entire thickness of the core. A biopsy from a patient with diagnosis of light chain deposition disease was observed using the same technique and showed no birefringent structures.
Conclusions. Observation of fresh kidney biopsy core under polarized light using bright field microscopy might improve our ability to estimate the three-dimensional parenchymal involvement in cases of nephropathy due to birefringent crystals deposition (including oxalate, 2,8 dihydroxyadenine and other rare crystals). The method is non-destructive and can be quickly applied before fixation and paraffine inclusion.

PROLIFERATIVE GLOMERULONEPHRITIS WITH MONOCLONAL IGG DEPOSITS (PGNMIGD): A CASE REPORT

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Background. Monoclonal gammopathies of renal significance (MGRS) represent a wide family of diseases characterized by kidney injury mediated by a monoclonal protein (MP), and with no evidence of underlying hematologic malignancies. Tissue damage can be due to direct MP parenchymal deposition (i.e. AL amyloidosis, Light Chain Deposition Disease, etc) or indirect action of the MP (i.e. C3 glomerulopathy secondary to MP). In MGRS, virtually any kidney structure can be involved and damaged by the MP. In 2004, Nasr et al. (JASN) described a new pattern of proliferative glomerulonephritis, histologically mimicking proliferative/membranoproliferative glomerulonephritis (MPGN), characterized by glomerular deposits of monoclonal IgG. Frequently, the MP cannot be demonstrated in patient's serum. Proliferative glomerulonephritis with monoclonal IgG deposits (PGNMIGD) are extremely rare and the diagnosis can be challenging.
Case description. A 76 year old Caucasian male, with past medical history significant for laryngeal cancer in 2017, gout and hypertension, was found to have nephrotic range proteinuria (12g/day with albuminuria 7g/day), nephrotic syndrome (hypoalbuminemia, anasarca, severe hypercholesterolemia) and increased creatinine (3 mg/dl, no previous value available). Viral serologies as well as autoimmune screening were negative, and a small IgG lambda monoclonal component was found. The patient underwent kidney ultrasound guided needle kidney biopsy (Fig. 1). Glomeruli showed diffuse MPGN lesions, with nodular mesangial expansion, mesangial and intracapillary hypercellularity and diffuse global reduplication of the basement membranes. On immunofluorescence, IgG and C3 deposits were observed in glomerular basement membranes and mesangium (Fig. 2). These deposits showed monotypic phenotype with kappa light chains and gamma-3 heavy chains restriction (Fig. s 3 and 4). The patient underwent a bone marrow biopsy, showing a small plasma cell clone (<5%) positive for lambda light chains.

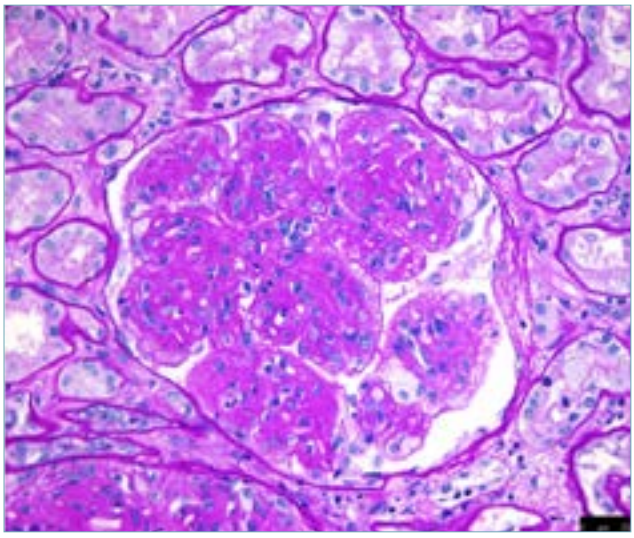

Fig. 1. PAS slide showing a glomerulus with MPGN pattern, nodular mesangial expansion, hypercellularity and glomerular basement membranes reduplication (400x).

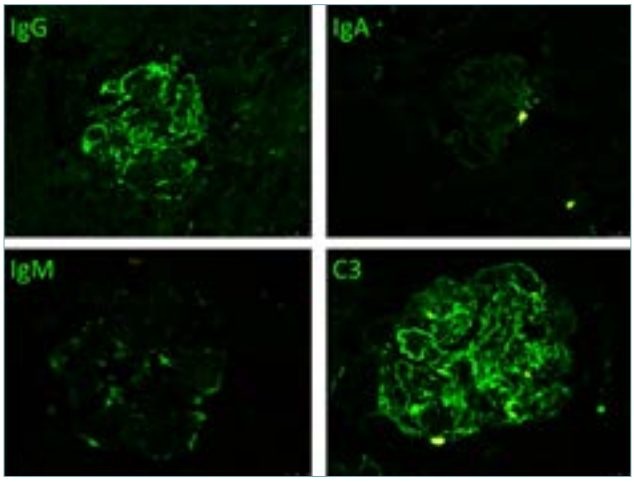

Fig. 2. Immunofluorescence shows basement membranes and mesangial deposits of IgG and C3, with negative IgA and trace IgM anti sera (400x).

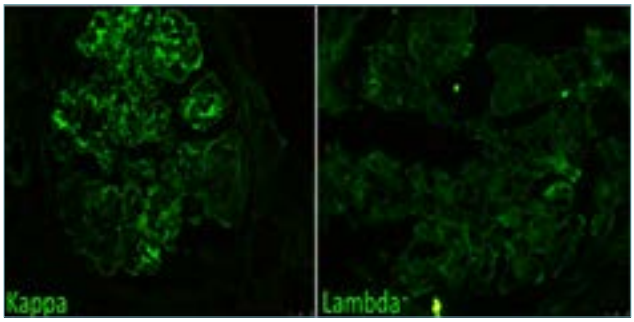

Fig. 3. Immunofluoresce. Immune deposits are positive for kappa light chains only (400x).

Conclusions. PGNMIGD represents a rare form of MGRS, histologically identical to MPGN. The diagnosis is challenging and can be missed if no specific tissue test for kappa and lambda light chain is performed (in

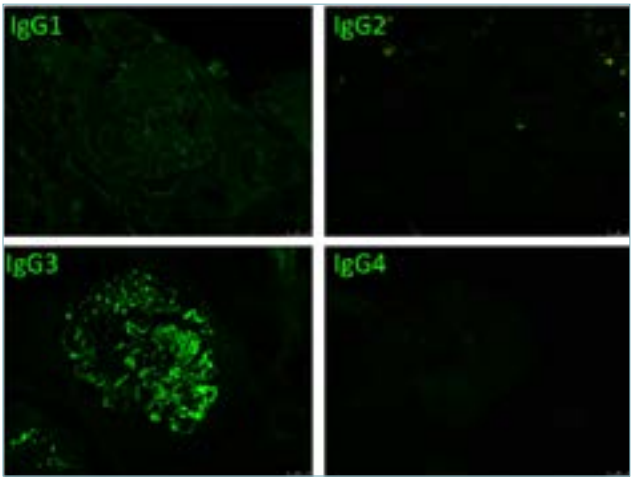

Fig. 4. The deposits only stain for IgG3 subclass. Immunofluorescence, 400x.

this case the biopsy would have been classified as MP-GN, immune-complex mediated). Moreover, circulating MP coherent with the one observed in the kidney can be completely undetectable, as seen in our case.

PATOLOGIA PEDIATRICA

UNUSUAL MORPHOLOGY OF RHABDOID TUMOR IN PEDIATRIC AGE: DIAGNOSTIC ROLE OF INI1

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Aims

Malignant rhabdoid tumor (MRT) is a highly aggressive neoplasm that usually occurs in the central nervous system and kidney of infants and children. Histologically it is tipically composed of large polygonal cells with eccentric vescicular nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm containing juxta nuclear hyaline-like inclusions or globules. We report four diagnostically challenging cases of pediatric renal and extra-renal MRTs which showed, as unusual feature, the presence of a small round cell component. The aim of this study is to emphasize the diagnostic role of INI-1 to support the diagnosis of MRT with unusual morphology, particularly from small biopsies.

Material and methods

Case 1. A 4-year-old girl presented with a voluminous abdominal mass which, on CT scan, sited in the retroperitoneum without renal involvement. A wide incisional biopsy (1.5 cm in greatest diameter), consisting of myxoid material with foci of hemorrhage, was performed for pathological examination. The case had been diagnosed as "small round cell tumor, consistent with Ewing sarcoma (EWS)/PNET". A combination of radiotherapy and chemotherapy for non-rhabdo soft tissue sarcomas was administered with poor response. The patient died 9 months after the initial diagnosis.

Case 2. A 5-month-old girl presented paraparesis of lower limbs. CT scan showed a paravertebral mass extending from the thorax to the abdominal cavity and invading the vertebral canal, causing spinal cord compression. An incisional biopsy (small fragmented tissues) was available for histological examination. The diagnosis of "malignant tumor, consistent with atypical/large cell Ewing sarcoma" was made. A combination of radiotherapy and chemotherapy for non-rhabdo soft tissue sarcomas was administered with poor response. The patient died 12 months after the initial diagnosis.

Case 3. A 4-month-old girl presented a paravertebral mass which, on CT scan, extended to thorax, clinically suspicious for neuroblastoma. An incisional biopsy (small fragmented tissues) was available for histological examination. The diagnosis of "small cell malignant rhabdoid tumor" was made. The patient underwent to radio and chemotherapy for malignant rhabdoid tumor and after two months from diagnosis is still alive.

Case 4. A 8-month-old girl presented a renal mass which, on CT scan, was suspicious for nephroblastoma. An incisional biopsy (small fragmented tissues) was available for histological examination. The diagnosis of Wilms tumor was made and chemotherapy for nephroblastoma was administered. Radical nephrectomy was performed.

Results

Case 1. The tumor showed the presence of lacunar-like spaces around neoplastic cells, likely due to artefact retraction, imparted them a chondrocytic-like appearance. Only focally were neoplastic cells with larger size as well as spindle-shaped cells with nuclei containing finely dispersed chromatin, and one or two small evident nucleoli. Mitotic activity was 5 mitoses per 10 high-powered fields. Notably, even after a meticulous search of the whole tumor tissue, large rhabdoid cells were lacking. The neoplastic cells expressed diffusely vimentin, CD99 and α-smooth muscle actin. The diagnosis of Ewing sarcoma was made.

Case 2. Tumor was composed of closely packed, medium-sized round cells, without intervening stroma with moderate amount of pale cytoplasm and vesicular nuclei containing one or two prominent nucleoli. Mitotic activity was 8 mitoses per 10 high-powered fields. No unequivocal rhabdoid cell was identified. The neoplastic cells expressed focally CD99 and cytokeratins and diffusely vimentin and smooth muscle actin. An atypical/large-cell variant of Ewing sarcoma (EWS)/PNET was suspected. In both cases (1 and 2) the molecular analyses failed to confirm the most common translocations, i.e., EWS-FLI-1 (type 1 and 2) and EWS-ERG translocations, seen in approximately 85% and 10% of cases, respectively, of this tumor family. When revising the cases, patient age and tumor site led us to consider the possibility of MRT with unusual morphology. Accordingly, we immunohistochemically tested anti-INI1 protein antibody. The absence of INI1 expression in both tumors supported the diagnosis of "MRT with predominant small cell component".

Case 3. Tumor was composed of small- to medium-sized round cells, occasionally with rhabdoid features, embedded in an abundant myxoid stroma. Neoplastic cells had vesicular nuclei containing one or two prominent nucleoli. Mitotic activity was 7 mitoses per 10 high-powered fields. Necrosis was seen. Based on morpho-

logical features, a wide panel of antibodies was tested including vimentin, CD99, LCA, NB84, cyclin D1, WT1, CD56, myogenin, desmin, S-100 protein, cytokeratins, α-smooth muscle actin, NUT and INI1. The neoplastic cells showed a diffuse staining for vimentin and focal staining for CD99. No immunoreactivity was observed for INI1 while endothelial cells and lymphocytes present within tumors displayed nuclear immunoreactivity, and they served as an internal positive control. Then the diagnosis of MRT was rendered.

Case 4. On incisional biopsy, renal tumor was composed of small-sized round cells, only occasionally with rhabdoid features, embedded in myxoid stroma. Focally lacunar-like spaces around neoplastic cells, likely due to artefact retraction, imparted them a chondrocytic-like appearance. A wide panel of antibodies was tested, the neoplastic cells showed nuclear WT1 staining. According to pathological examination and immunohistochemical profile, the diagnosis of "Wilms' tumor" was made. After chemotherapy, radical nephrectomy was performed. Pathological examination of whole mass revealed medium- to large-sized neoplastic cells with abundant intensely eosinophilic cytoplasm, vesicular nuclei containing one or two prominent nucleoli, mixed with a minority of small-sized round cells, embedded in myxoid matrix. Mitotic activity was 8 mitoses per 10 high-powered fields. Necrosis was seen. Neoplastic cells were nuclear expression positive for EMA, pancytokeratins, vimentin, WT1 (nuclear staining), but they lacked INI1 nuclear expression. Based on pathological and immunohistochemical features the diagnosis of "malignant rhabdoid tumor with a minority of small round cell component" was rendered.

Conclusions. The present study emphasizes the possibility that small biopsies from renal and extra-renal MRT may exhibit cells with unusual morphology, especially unexpected small round cells. Accordingly, we suggest to include routinely INI1 protein in the immunohistochemical panel when evaluating malignant pediatric tumors, particularly from small biopsy samples.

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PATOLOGIA PLEUROPOLMONARE

COMPARISON OF 3 IMMUNOHISTOCHEMISTRY ASSAYS FOR DETECTION OF ROS1 REARRANGEMENTS IN A SERIES OF 158 CONSECUTIVES SURGICALLY-RESECTED ADENOCARCINOMAS OF THE LUNG

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Background and aim. ROS1 rearrangements characterize about 1-2% of lung adenocarcinomas and represent a molecular target for specific therapy. Immunohistochemistry is a rapid and cost-effective screening test to detect ROS1 rearrangements, although positive cases require confirmation by FISH or other extractive molecular methods.

Methods. The diagnostic accuracy of 3 primary antibodies anti-ROS1, namely clone EPMGHR2 (Abcam), clone SP384 (Ventana) and clone D4D6 (Cell Signaling Technology) was evaluated in a series of 158 consecutive and surgically-resected adenocarcinomas of the lung. All investigations were performed in an automated immunostainer (ULTRA, Ventana). All positive cases and 57 randomly-selected negative cases were re-tested by FISH technique using the ROS1 dual color break-apart probe (ZytoVision). Positive staining was quantified by H-score, then recording the percentage (0-100) and intensity (0-3) of immunoreactivity in tumor cells (cytoplasm and/or membrane localization). Cases were considered positive with EPMGHR2 and D4D6 using a H-score > 150, while SP384 positivity was quoted when at least >30% of tumor cells with 2+/3+ of intensity were recorded.

Results. Only 2 adenocarcinomas (1.2%) showed ROS1 positivity at IHC and FISH. All 3 clones displayed complete sensitivity, while specificity ranged from 96.3% (EPMGHR2) to 97.5% (clone SP384) and 99.3% (D4D6). Of note, 6, 4 and 1 false positive cases were detected with EPMGHR2, SP384 and D4D6, respectively. Most interesting, 49 cases (31%) showed borderline immunoreactivity with EPMGHR2 and 27 cases (17%) with SP384, whereas no indeterminate results were noted with D4D6.

Conclusion. The IHC screening to detect ROS1 rearrangements in lung cancer is effective, but pathologists should consider that the clone of anti-ROS1 primary antibody may affect specificity, with clone D4D6 showing the best performance.

CYTOMATRIX FOR A RELIABLE AND SIMPLE CHARACTERIZATION OF LUNG CANCER STEM CELLS FROM MALIGNANT PLEURAL EFFUSIONS

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Background. Cancer Stem cells (CSCs) are a subpopulation with the properties of extensive self-renewal, capability to generate differentiated cancer cells and resistance to therapies [1-3]part of Springer Nature.

Cancer stem cells (CSCs. We have previously shown that Malignant Pleural Effusions (MPEs) from patients with Non Small Cell Lung Cancer (NSCLC) represent a valuable source of cancer cells that can be grown as 3D spheroids enriched of stem-like features, which depend on the activation of the YAP-TAZ/Wnt-bcatenin/SCD1 axis [4]the enzyme involved in monounsaturated fatty acids synthesis, has a role in several cancers. We previously demonstrated that SCD1 is important in lung cancer stem cells survival and propagation. In this article, we first show, using primary cell cultures from human lung adenocarcinoma, that the effectors of the Hippo pathway, Yes-associated protein (YAP.

Methods. An innovative support, called CytoMatrix, we used for the characterization of limited amounts of cancer cells isolated from MPEs of patients with NSCLC. CytoMatrix is an innovative support originally designed to permit an easy and rational management of the biological material from needle aspirates. The powerful feature of this support is the ability to permit a rapid and reliable diagnosis even in absence of large amounts of material. In line with this concept, we thought that CytoMatrix could be a useful tool for the cytological evaluation of tumor cells directly isolated from lung MPEs in order to obtain a rapid and complete phenotypic characterization. CytoMatrix peculiarity is to efficiently restrain small amounts of biological material inside its three-dimensional structure. The porous support is provided into a plastic bio-cassette allowing to make easy the following steps of classical immunocytochemistry (ICC) technique, such as formalin fixation, paraffin inclusion and microtome cut.

Conclusion: Our results show that this synthetic matrix allows an easy and fast characterization of several epithelial cellular markers. The use of Cytomatrix to study CSCs sub-populations confirms that SCD1 protein expression is enhanced in 3D spheroids as compared with 2D adherent cell cultures. YAP/TAZ nuclear-cytoplasmic distribution analyzed by Cytomatrix in 3D spheroids is highly heterogeneous and faithfully reproduces what observed in tumor biopsies. Our results confirm and extend the robustness of our workflow for the isolation and phenotypic characterization of primary cancer cells derived from lung MPEs and underscore the role of SCD1. In this regards the possibility to analyse in depth the structure of tumoroids embedded into Cytomatrix in normal conditions and after drug testing will open up new promising perspectives.

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PULMONARY SCLEROSING PNEUMOCYTOMA, A RARE TUMOR OF THE LUNG

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Introduction. Pulmonary sclerosing pneumocytoma (PSP), formerly known as pulmonary sclerosing hemangioma, is a rare pulmonary tumor initially described by Liebow et al., in 1956, as a tumor with marked sclerosis and vascularization. PSP is usually seen in adults over 50 years old, with a female to male ratio of 5: 1. PSP has been shown to be the primitive epithelial origin, most likely from type II alveolar pneumocytes by immunohistochemical markers. The essential feature of the PSP is the presence of cuboidal surface cells and stromal round cells, both of which are thought to be neoplastic. In the 2015 World Health Organization (WHO) classification, “miscellaneous tumors” have been switched to “adenomas”.

We are presenting this PSP case, it is rare, benign disease, which might be confused with malignities to radiological investigations, and that can present diagnostic difficulties to the intraoperative examination and to the definitive diagnosis.

Case report. A 38-year-old female had no complaints, no smoking or tuberculosis history. She was suffering from intestinal polyposis. She was not using any medication. Contrast-enhanced Thorax CT showed a well-defined, hypodense soft-tissue lesion in the size of mm 55, closely located in the anterior segment of the right upper lobe and in the upper segment of the right lower lobe, in contiguity with the adjacent mediastinic pleura. In PET-CT, a mass of soft tissue with a lobulated contour with evidence of increased FDG uptake (SUV max 3,5) was detected in these locations. Furthermore, PET-paratracheal adenopathy was present (SUV max 3,7). The patient was referred to thoracic surgery who proceeded to a thoracotomy. Frozen sample was sent during thoracotomy.

The resected specimen contained a yellowish tumor, easily enucleable, with central necrosis, of 5,5x5,0 cm. The tumor progressed expansively in the lungs, was encapsulated and was peripheral and subpleural. Histological observation showed clear margins with respect to the surrounding parenchyma, with expansive growth, a pattern of solid and papillary growth, and with a wealth of vascular structures with aspects of cicatricial sclerosis of the stroma and sometimes papillae. There were a dual population of cuboidal surface cells overlaying stromal round cells with little nuclear atypia, and very low mitotic index.

Immunohistochemistry analysis of the resected lesion showed positive (+) with TTF-1, CK-7, Progesteron-Receptor and Vimentina; negative with PAX-8, CD10, CD31, CD34 and Estrogen-Receptor.

Discussion. PSP is a benign tumor with low prevalence. Patients are usually asymptomatic and it is detected coincidentally. A cough, chest pain and hemoptysis may also occur. Although PSP is often solitary, well-defined, round or oval, homogeneous nodule or mass, there is no definitive diagnostic radiographic finding. However, there are also cases of metastases (<1%) to the lymph

nodes, pleura, and bones. Patients may present with a mass lesion of up to 7 cm through 73% of the lesions are below 3 cm. Often the tumor consists of superficial cuboidal and round interstitial cells with a combination of various patterns. If the papillary component of the sclerosing pneumocytoma in the biopsy material is predominant, the diagnosis may be difficult. In addition, both superficial cuboidal cells and round interstitial cells are positively immunoreactive for TTF1. TTF1 is used in the diagnosis of lung adenocarcinoma and may be misleading for PSP. The differential diagnosis must be made with carcinoids, papillary renal cell carcinoma, breast cancer and thyroid carcinoma.
Conclusion. The treatment of PSPs is based on surgical resection. Rarely, these tumors can lead to hemoptysis or airway obstruction and respiratory failure. Recently, different treatment approaches have been suggested. It is appropriate that each patient is assessed in a multidisciplinary fashion. Radiotherapy was also suggested as an alternative treatment for inoperable patients.

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ONE-YEAR EXPERIENCE OF A CENTRALISED LABORATORY IN PD-L1 EXPRESSION ASSESSEMENT ON ROUTINE SAMPLES IN NON-SMALL CELL LUNG CANCER

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Aim. Programmed death ligand-1 (PD-L1) immunohistochemically (IHC) evaluation is a predictive biomarker to define response to immunotherapy.[1-4] Our centralized laboratory received routine non-small cell lung cancer (NSCLC) samples for PD-L1 IHC evaluation from different centers. In this study, we reviewed our data in order to assess the rate of PD-L1 positive and negative NSCLC cases in our diagnostic practice.[5]
Material and methods. We performed the IHC for the evaluation of PD-L1 expression by using a validated 22C3 laboratory developed test. We carried out the analysis on n = 211 prospectively routine NSCLC samples, obtained from n = 10 different institutions. According to literature data, PD-L1 expression was assessed by using the tumor proportion score (TPS) and reported by using a three tiered cut-point system of PD-L1 expression: <1, 1%-49% and ≥50%.
Results. In 91.5% (193/211) of samples showed adequacy for PD-L1 evaluation (more than 100 viable neoplastic cells). On the overall, 62.7% (121/193), 17.6% (34/193), and 19.7% (38/193) of samples showed a TPS of <1%, 1%-49%, and ≥50%, respectively. Of note, no significant differences were evaluated in PD-L1 expression between different histotypes and site of sampling. Conversely, a statistically significant difference was associated to the type of samples. In particular, cytological samples showed more frequently a TPS<1% and less often featured a TPS>50% than histological samples (surgical resections and biopsies).
Conclusions. We showed data on IHC PD-L1 expression from routinely collected NSCLC samples of a centralized laboratory.[5] This data can better clarify the percentage of PD-L1 positive and negative cases, and underlined the necessity of a better standardization of PD-L1 testing in clinical practice.

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CIRCULATING TUMOR CELLS USEFUL FOR CYTOLOGICAL DIAGNOSIS AND FOR GUIDING THERAPY DECISION IN A CASE OF NSCLC

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The progressive lengthening of the average life and the incidence of neoplasms in older subjects impose a reflection on the diagnostic and therapeutic means to be used in this segment of the population. The frequent coexistence of chronic pathologies, in fact, not only conditions the choice of anticancer

treatments but limits, upstream, the use of the most invasive diagnostic procedures. We present the case of an advanced pulmonary neoplasm in a female patient aged 86, with an important cardiovascular comorbidity. Hospitalized for sudden respiratory failure, the patient performed a highresolution CT scan that showed a voluminous nodular formation of about 4.3 x 3.5 cm, strongly suspected for primitiveness, occluding the bronchial branches for the middle lobe and innumerable secondary nodularity on both lungs. Multiple mediastinal lymph-adenopathy were evident, the major ones having a maximum short axis of about 2 cm in the aortic-pulmonary window and in the Barety loggia and a modest bilateral basal free pleural effusion. The investigation confirmed a severe cardiomegaly. The patient's clinical conditions did not allow for the execution of endoscopic or needle-borne trans pleural samples and therefore it was decided to proceed with the collection of tumour cells by peripheral blood sampling (Malara et al 2018). From the collected material, some slides were set up. Staining with the quick diff showed the presence of numerous large tumour elements, with morphological characters (large pleomorphic and nucleated nuclei) and immunophenotypic (CKAE1 / AE3 +, TTF1 +) consistent with pulmonary adenocarcinoma. The investigation with PD-L1 showed an intense membrane positivity in more than about 90% of the circulating neoplastic elements predicting efficacy response to immune checkpoint PD-1/ PDL1inhibitors.

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POTENTIAL PROGNOSTIC ROLE OF INDOLEAMINE 2,3-DIOXYGENASE 2 IN HUMAN NON-SMALL CELL LUNG CANCER: AN IMMUNOHISTOCHEMICAL STUDY

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Aims. Non-small cell lung cancer (NSCLC) is nowadays one of the primary causes of cancer death[1]. Although many studies both have improved therapeutical approach to NSCLC and have increased the knowledge on the interactions between tumors and host immune system, the relationships among molecules of tumoral microenvironment and NSCLC progression still remain to be entirely understood. In particular, indoleamine 2,3-dioxygenase 2 (IDO2) is a recently discovered molecules[2], and it could be a promising biomarker of cancer progression, because IDO2 seems to be involved in tryptophan metabolism[3] and is overexpressed in some human carcinomas[4-7]. The aim of the present study is to elucidate the role of IDO2 in a NSCLC series, analyzing its correlations with clinical-pathological parameters, other molecules of tumoral microenvironment and prognosis.

Materials and methods. One hundred ninety-one NSCLC surgical specimens, formalin-fixed, paraffin-embedded, were assessed for immunohistochemical expressions of IDO2, indoleamine 2,3-dioxygenase 1 (IDO1) and programmed cell death ligand 1 (PD-L1); the immunolabelings were evaluated on neoplastic cells, according to a previously used H Score[8] obtained by the sum of the intensity of the stain and the percentage of the tumoral cells labeled. Thereafter, the scores obtained were collapsed in a binary scoring system, encompassing a low expression and a high expression class. Furthermore, tumor-infiltrating lymphocytes (TILs) density and TILs localization were evaluated. Afterwards, the data was statistically correlated with clinical-pathological parameters, disease free and overall survivals.

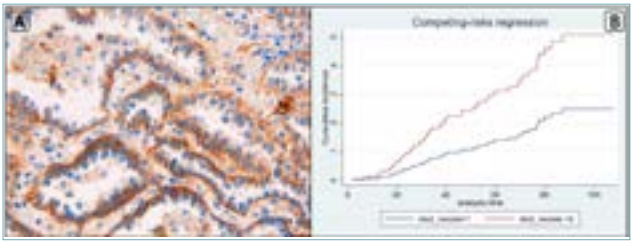

Fig. 1. A. IDO2 high expression in an adenocarcinoma, original magnification 400X. B. Relationship between IDO2 expression and rate of death from NSCLC. Red line: IDO2 high expression class; blue line: IDO2 low expression class; analysis time: months.

Results. Briefly, an increased expression of indoleamine 2,3-dioxygenase 2 (IDO2) is associated with high level of PD-L1, both in adenocarcinomas (p=0.035; Fig. 1A) and in squamous cell carcinomas (p=0.012), and with an intratumoral/mixed tumor-infiltrating lymphocytes (TILs) localization.

Furthermore, a worst overall survival was registered in patient with tumoral overexpression of IDO2, both in the univariate and in the multivariate analysis (p=0.028 and p=0.024, respectively; Fig. 1B).

Conclusions. The present study suggests a close association between IDO2 high expression and molecules participating in NSCLC microenvironment. Moreover, the correlation among IDO2 and NSCLC prognosis should give rise to further studies both for a better understanding of the potential role of IDO2 as tumoral biomarker and for an extensive investigation about potential combined therapeutics approaches for this cancer type.

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SPREAD THROUGH AIR SPACES (STAS): HISTOLOGICAL FEATURES AND CLINICOPATHOLOGICAL CORRELATIONS IN A SERIES OF LUNG ADENOCARCINOMAS

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Introduction. Tumor spread through air spaces (STAS) has recently been recognized as a pattern of invasion in lung adenocarcinoma (1). However, the diagnostic morphologic criteria for STAS, its frequency and significance are still debated.

Aim. The purpose of this study was to investigate the frequency, morphologic patterns and extent of STAS in a series of resected pulmonary adenocarcinomas (ADC). To better understand the clinical impact of this pattern of invasion, we correlated the features of STAS with clinical findings and ADC subtypes.

Methods. Between January 2018 and May 2019 we analyzed 81 consecutive cases of resected lung ADC. There were 45 wedge resections, 34 lobectomies, 1 bilobectomy and 1 pneumonectomy. Tumor stage ranged between I and III (sec. AJCC 2017). In all cases, the interface between the neoplasia and the adjacent lung parenchyma was circumferentially sampled for histologic examination. Tumor STAS was defined as tumor cells (> 1 cell, forming either micropapillary structures, or solid nests, or present as isolated cells) spreading within air spaces beyond the tumor edge, without direct connection to the main tumor mass (2-5).

For each case we assessed presence of tumor STAS, its radial and circumferential extension and the number of neoplastic cells in the largest alveolar cluster. Based on the extent of circumferential extension, we divided our cases into "low STAS" (involving 1/3-2/3 of the tumor circumference) and "high STAS" (involving the entire tumor circumference).

Results. Noticeably, tumor STAS was observed in the majority of our cases (66/81, 81%). The most frequent morphologic pattern was micropapillary (38/66, 58%) either as the only or as the prevalent mode of alveolar spread. The number of neoplastic cells ranged between 5 and 20 per cluster in over half of cases (35/66, 53%). The radial extension of STAS ranged from 0.2 to 11 mm (median: 1.95 mm). "High" STAS was observed in in 26% of cases (17/66). Interestingly, cases with "high" STAS were significantly correlated with a radial extension of neoplastic cells above the median value (p= 0.002).

The presence and type of STAS were related to the specific growth pattern of ADC: it was rare (2/66, 0.3%) in tumors with a predominantly lepidic growth, while it was present in 100% of cases with a micropapillary growth pattern. The latter was also associated with an increased frequency of "high" STAS (9/10, 90%) as compared to other growth patterns.

As compared with tumors without STAS, tumors with STAS showed more frequently poor differentiation (48% vs 20%), higher T (T3-T4: 20% vs 12%), lymphovascular invasion (29% vs 6%) nodal metastases (35% vs 13%) and higher stage at diagnosis (stage III: 29% vs 13%, stage II: 24% vs 13%, stage I: 47% vs 73%).

Discussion. STAS is a unique pattern of invasion of lung ADC and currently represents an underestimated entity. Moreover, despite the numerous definitions of STAS in literature (6) a real consensus to define tumors with STAS and consequently to accurately assess the frequency of this phenomenon and its clinical significance has not been established.

Our study represents an attempt to investigate this process and to evaluate its extension (7, 8). According to our preliminary results, the presence of STAS identifies a disease with a more aggressive biological behavior. Accordingly, STAS should be evaluated in routine histology reports and considered in the future as a part of tumor staging (3, 9, 10).

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MULTIPLEX FLUORESCENCE IN SITU HYBRIDIZATION TO DETECT ANAPLASTIC LYMPHOMA KINASE AND ROS PROTO-ONCOGENE 1 RECEPTOR TYROSINE KINASE REARRANGEMENTS IN LUNG CANCER CYTOLOGICAL SAMPLES

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Aims. Several predictive biomarkers of response to specific inhibitors have become mandatory for the therapeutic choice in non-small-cell lung cancer (NSCLC). ALK- and ROS-rearranged patients represent distinct molecular subsets of NSCLCs sensitive to specific tyrosine kinase inhibitors (TKIs), therefore their identification is clinically essential. Both IHC and FISH have been approved for ALK-R detection, whereas FISH represents the gold standard for ROS1-R detection based on FDA approval in lung cancer therapy with specific TKIs. Currently, ALK and ROS1 FISH tests in lung cancer patients are performed separately on two different slides using specific Break Apart Probes. In most lung cancer patients, the biological materials available to morphological and molecular diagnosis are exclusively cytologic samples and minimum tumor wastage is necessary. Multiplex FISH to detect simultaneously ALK and ROS1 gene rearrangements on a single slide could be useful in clinical practice to save cytologic samples for further molecular analysis.

Previous studies analyzed the diagnostic performance of multiplex ALK/ROS1 FISH approach compared to the classical FISH assay to detect separately the status of two oncogenes in formalin-fixed paraffin-embedded samples obtained from lung surgical resection or excisional biopsy.

To the best of our knowledge, until now, no study has analyzed the performance of multiplex ALK/ROS1 FISH in lung cytological samples. In this study, we aim to validate diagnostic performance of multiplex ALK/ROS1 FISH approach in lung adenocarcinoma cytologic serie compared to classic single break apart probes.

Methods and materials. We retrospectively reviewed archival cell blocks of lung adenocarcinoma with a sufficient amount of available material. Cytological samples included in the present study were characterized by a moderate (between 100 and 500 representative cells in the sample) or high cellularity (more than 500 representative cells in the sample). A series of 61 lung adenocarcinoma cytological specimens enriched in tumors harboring ALK and ROS1 rearrangements was collected. Study population was composed of 6 ALK-positive, 2 ROS1-positive and 53 ALK/ROS1-wild type. ALK and ROS1 rearrangement was previously assessed by both classic FISH test using single break apart probes and immunohistochemistry. FISH was performed using ZytoLight SPEC ALK Dual Color Break Apart Probe and ZytoLight SPEC ROS1 Dual Color Break Apart Probe. ICC was performed using the anti-ALK (D5F3, Ventana) antibody and the rabbit primary monoclonal antibody anti-ROS1 (D4D6, Cell Signaling Technology,). All specimens were analyzed by multiplex FISH assay using FlexISH ALK/ROS1 DistinguISH Probe Zytovision.

The concordance of results obtained from FlexISH ALK/ROS1 DistinguISH Probe was evaluated in relation to the ALK and ROS1 status of the specimen previously determined.

Results. The dual ALK/ROS1 FISH probe test results were fully concordant with the results of previous single ALK and ROS1 FISH tests on two different slides. 6 ALK-positive and 2 ROS1-positive were confirmed through multiplex FISH test, without false-positive and false-negative results. Multiplex ALK/ROS1 FISH test results agreed with IHC staining results.

In our experience, the interpretation of FlexISH ALK/ROS1 DistinguISH Probe should be performed in two steps: i) in the first time the assessment of the ALK and ROS1 gene status evaluating exclusively green/orange fusion signals: if no split signals are detected the report will be ALK and ROS1 wild type; if the split signals are detected the second step will be require. ii) in the second step the analysis of aqua wavelength spectrum should be performed to discriminate between ALK-R and ROS1-R.

Conclusion. Multiplex ALK/ROS1 FISH test is a useful tool to detect simultaneously ALK- and ROS1-rearrangements on a single slide in cytological specimens with a small amount of biomaterial. FlexISH ALK/ROS1 DistinguISH Probe ensuring a reasonable approach for the simoultaneous detection of two therapeutic target since an easy interpretation of the test is feasible for the experts in the field, not closely linked to automatic scanning. ALK/ROS1 Dual FISH assay should be used into daily clinical practice in order to improve a simultaneous detection of dual targets on a single slide, resulting in a biomaterial preservation. In conclusion, multitargets FISH approach in NSCLC could improve the molecular diagnosis in terms of time, costs, but above all the spending review of samples in cases with a limited number of neoplastic cells available for extensive molecular investigation.

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PATOLOGIA SPERIMENTALE

THE CLINICOPATHOLOGICAL AND PROGNOSTIC SIGNIFICANCES OF C1Q EXPRESSION IN GLIOMAS: A BIOINFORMATICS ANALYSIS

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Introduction. The complement system represents an important component of the inflammatory response and acts as a functional bridge between the innate and adaptive immune response. The contribution of the complement component C1q in the pathophysiology of brain cancers has been recently considered in light of its well-known involvement in carcinogenesis. Brain malignancies arise from cells of the CNS and are classified according to the tissue of phylogenetic origin. Gliomas represent the most common and aggressive form of brain tumours in adults. They derive from glial cells that help to support the functions of the other main brain cells type, the neurons (1). These are a heterogeneous group of diseases with multiple subtypes (1, 2). Glioblastoma multiforme (GBM) is the most common and fatal form of a primary brain tumour, accounting for approximately 60% of all glioma cases (3), whereas grade-II and -III gliomas are the second most common type of glioma in adults (~30%) (3). C1q molecule, together with other complement components, can be locally produced within the CNS by microglia and astrocytes, rendering it an attractive player in primary brain tumour development (4). The role of C1q in gliomas microenvironment is still poorly characterized and it is still quite puzzling whether it exerts a beneficial or a harmful activity for cancer progression. In the present study we performed a bioinformatics analysis aimed at investigating if C1q can serve as a potential prognostic marker for gliomas.

Methods. The expression levels of *C1qA*, *C1qB* and *C1qC* genes in gliomas were analysed using Oncomine analysis. Available genomics data from The Cancer Genome Atlas project was used for Kaplan–Meier survival analysis to generate survival probability plots, using UALCAN analysis.

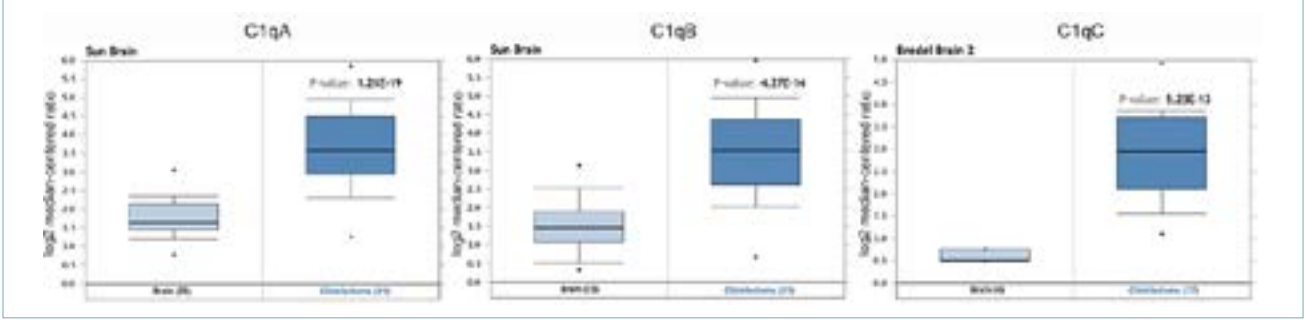

Fig. 1.

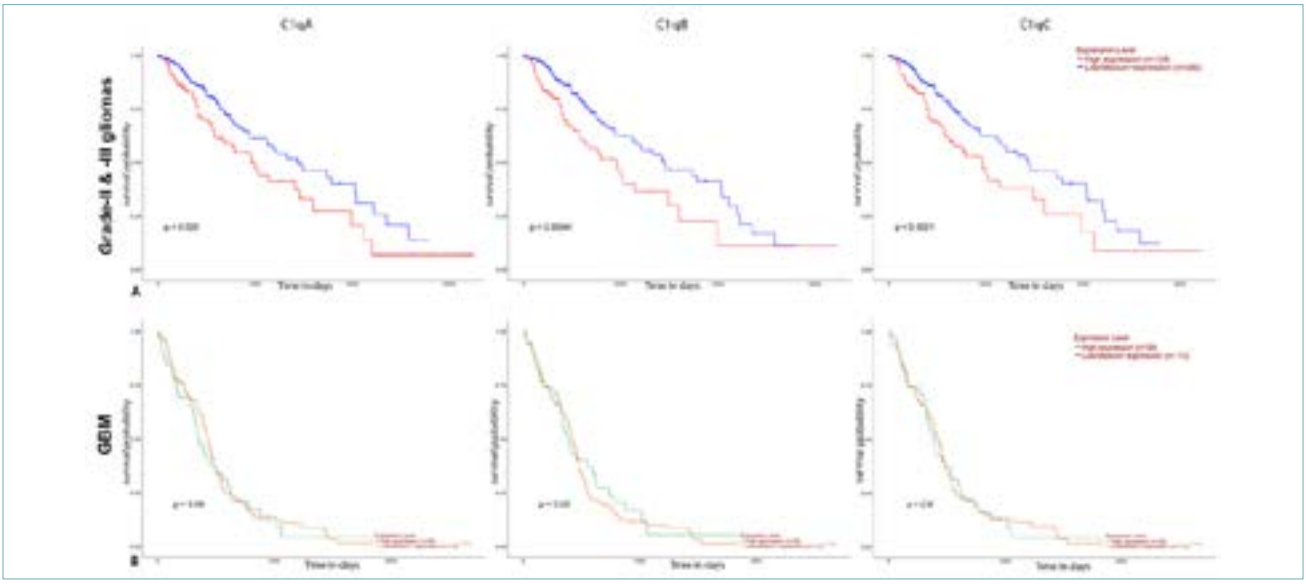

Fig. 2.

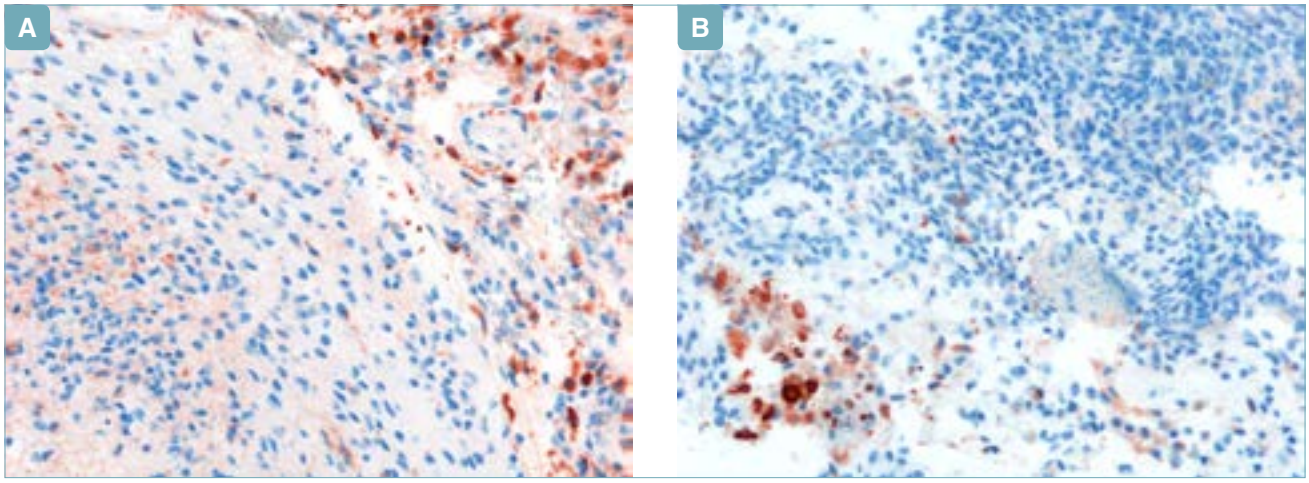

Fig. 3.

Results. From the analysis performed on several datasets using Oncomine, we showed a significantly higher mRNA expression levels for *C1qA*, *C1qB* and *C1qC* chains were detected in gliomas (different histotypes and grades) as compared to normal brain tissue (Fig. 1). We observed a positive correlation between the mRNA expression of *C1qA*, *C1qB* and *C1qC* mRNA polypeptide chains and the unfavorable prognosis only in gliomas grade-II and -III, where the survival probability is indeed reduced (P <0.05) (Fig. 2). No correlation was observed in glioblastoma multiforme (Fig. 2). By immunohistochemical approaches we detected a high deposition of C1q in the tumor microenvironment of both in grade-II and -III gliomas and in GBMs examined (Fig. 3a glioma, 3b glioblastoma multiforme; 20x Magnification). Moreover, in double immunocytochemical experiments we demonstrated that CD68 positive infiltrating cells are actively synthesizing C1q in the tumor micro-environment. CD68 expression is characteristic of tumor-associated macrophages, whose enrichment in glioma has been associated with poor prognosis (5).

Conclusion. In our study C1q expression was significantly correlated with poor survival probability in gliomas grade-II and -III while this is not the case for GBM. These data altogether underline how complex, multifaceted and still poorly understood is the role C1q can exert on tumor progression, and how the very same molecule can differentially affect the outcome depending on the biological context it comes to act.

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PATOLOGIA TESTA COLLO

MIXED ODONTOGENIC TUMORS: A CASE REPORT

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Background. Odontogenic tumors (OT) represent a heterogeneous group of lesions ranging from hamartomas to benign and malignant tumors. They arise from ectomesenchymal and/or epithelial tissues involved in odontogenesis [(1)] and depending on the tooth germ tissue of origin, they are classified as epithelial, ectomesenchymal or mixed tumors [(2,3)]. The latest (4th) edition of the WHO Classification of Odontogenic and maxillofacial bone lesions, introduced significant changes in the classification of these lesions, particularly in the chapter of mixed odontogenic tumors, adding a new entity (the *primordial odontogenic tumor*) and removing four other ones including the odontoameloblastoma and ameloblastic fibro-odontoma (AFO).

Case presentation. We report a case of a 14-years-old male patient, with a lesion in the left mandibular angle, clinically compatible with odontoma. Histopathological examination of the incisional biopsy revealed proliferating odontogenic epithelium arranged in islands, cords, follicles and interconnecting strands in a highly hypercellular myxoid stroma (ameloblastoma-like) and odontoma-like elements: dentin containing dentinal tubules, enamel space and tissue resembling pulp. This morphological features are compatible with a mixed odontogenic tumor with areas consistent with ameloblastic fibro-odontoma associated with complex odontoma, formerly called odontoameloblastoma/ameloblastic odontoma.

Discussion. Odontoameloblastoma was introduced in the 1971 WHO classification and described as a mixed

odontogenic tumor including an ameloblastomatous component and odontoma-like features [4,5]. In the current WHO Classification of Odontogenic and maxillofacial bone lesions, odontoameloblastoma was deleted because it was believed to be not a true mixed tumor but an ameloblastoma associated with an odontoma.
Ameloblastic fibro-odontoma was defined as "a neoplasm composed of proliferating odontogenic epithelium in a cellular ectomesenchymal tissue with varying degrees of inductive changes and dental hard tissue formation" [6]. At present, it is considered part of the spectrum of histological changes seen in developing odontomas. However, this lesion does not always present clinical-pathological features that support hamartomatous nature: on the contrary, it can behave like a true tumor. [7] In fact, despite AFO and odontoma share some characteristics as the age of occurrence, the site of presentation, the clinical association with an impacted tooth, AFO may recur after an inadequate surgical removal, especially in cases of large lesions or when an aggressive behavior against the surrounding bone is present.
Conclusion. Odontogenic tumors are lesions characterized by a wide range of biological behavior and histological presentations; pathologists still dispute their pathogenesis and the clinical and histological features of diagnostic and prognostic value. Our case report aims to highlight the critical issues in the diagnostic framework of odontogenic lesions with combined aspects, underlining the importance of emphasizing the presence of an AFO component, since it is associated to greater aggressive potential, and not just making a tout-court diagnosis of odontoma.

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ZOLEDRONATE MODULATES PD-L1 EXPRESSION IN PERIODONTAL LIGAMENT STEM CELLS

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BRONj (Bisphosphonate-Related Ostenonecrosis of the Jaws) is a condition characterized by necrosis and exposition to the oral cavity of the maxillofacial bones, associated with use of bisphosphonate therapy. It represents an emerging disease and the most feared avverse effect in odontological field. (1 RB)
BRONJ is considered a multifactorial disease involving environmental and genetic factors, drug related risk, comorbility, but also local factors such as dental diseases, dental surgery (e.g., tooth extraction, implantology), oral trauma, periodontitis, and poor dental hygiene.
The occurrence is associated with the increasing prescription of bisphosphonate (BP) for the osteoporosis prevention, oncological/hematological and dysmetabolic diseases.
The dental MSCs, neural crest-derived (ecto)mesenchymal cells, contribute to damaged bone repairing. Among them, the periodontal ligament stem cells (PDLSCs) are able to regenerate periodontal tissues, such as cementum and alveolar bone (2). In our study, we investigated the effects of zoledronate on PDLSCs and their potential role in the BRONj occurrence. In particular we characterized for the first time in PDLSCs the expression of PD-L1, a protein considered a checkpoint of immune system (3).
Mesenchimal cells have been isolated from periodontal ligaments (hPDL) obtained from impacted third molars of 3 healthy donors aging between 18 and 25 years. Mesenchimal phenotype has been demonstrated by the cytofluorometric analisys (CD90, CD73, CD105 positivity and CD14, CD34 e CD45 negativity). The cells have been cultured in MesemPro medium (Invitrogen) until the fourth passage and then used for the following tests.
The PD-L1 protein expression was evaluated on periodontal ligament stem cells (PDLSCs) by IHC and Western Blotting. The protein level was assessed in basal condition and after a treatment with different micromolar concentration of zoledronate from 48 to 120 hours.
From the results, it appeared that zoledronate it's able to modulate PD-L1 expression. This preliminary result encourage to investigate the role of PDLSCs in the immune response triggered during the BRONj onset.

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ORAL CANCER SCREENING BY DIAGNOSTIC CYTOPATHOLOGY AND DNA HPV TESTING

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Objectives. The survival rate for squamous cell carcinoma of the oral cavity (OSCC) remains low, as it is of-

ten diagnosed late due to no reliable diagnostic method of selecting people with high risk of transformation. In presence of precancerous lesions, liquid based oral cytology alone can provide useful information (sensitivity, specificity and PPV have been reported to be as high as 94.7%, 98.9% and 95.9%) and gives good first level screening results (1-2). Conversely, there are no reliable test in order identify subjects with high risk of malignant transformation in absence of precancerous lesions. Although the most important risk factors are tobacco and alcohol, a potential role of oral HPV infection in the onset of OSCC, could represent an adjunctive predictive factor (3).

Methods. We are currently screening apparently normal subjects with a first level method, i.e. liquid-based cytology combined with investigation with DNA-HPV test. Samples were obtained by the cytobrush on the most commonly involved sites for oral carcinoma (floor of the mouth, tongue, gums and cheek lining).

Results. One hundred and forty two subjects were enrolled, 68 males and 74 females: 141/142 had normal cytology results, 1 had a low-grade oral lesion (OIN 1) and 5 had HPV-DNA test positive (the HR-HPV types were 16, 31, 53 and 16, 31 in two females; one male had HR-HPV (52) and two males had low risk HPV, 62 and CP6108 respectively: in these five cases the cytology was normal or with keratosis).

Conclusions. Combining liquid based diagnostic oral cytology and tests for HPV infection seems able to select a subgroup of patients with potential predictive factors for OSCC development and thus requiring proper follow-up schedule even in absence of visible oral lesions (4-5).

Relevance. The prospective evaluation of healthy subjects with oral HPV infection could give important information about its role in the development of OSCC. It is also important to choose the appropriate HPV test in as much as many HPV commercial kits were originally developed for cervical carcinoma screening. To date, the classification of high and low risk HPV genotypes has been based on cervical cancer evidence, but this nomenclature could be misleading in the presence of OSCC. However, in case of a proven causal role of HPV, a combination of oral cytology and tests for HPV infection could represent a significant diagnostic step forward early diagnosis of OSCC, as yet demonstrated with the experience in uterine cervical carcinoma screening with PAP test and DNA-HPV.

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ADULT LARYNGEAL HEMANGIOMA: A RARE CLINICO-PATHOLOGICAL ENTITY

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Aims. Hemangiomas are benign blood vessel tumors occurring most commonly as cutaneous lesions of the head and neck (1), rarely involving the larynx, especially in adults (Tab. I). Adult laryngeal hemangiomas are more frequent in men and usually present with glottic or supraglottic masses (2). Symptoms include hoarseness, dyspnea, dysphagia and upper airway foreign body sensation (1). Grossly, hemangiomas are bluish red, clearly defined exophytic submucosal masses (2), diagnosed primarily by physical examination through flexible laryngoscopy (3). Complete microlaryngoscopical excision is usually used for treatment (2). Pathological examination, revealing the typical morphological features, is essential to form a definitive diagnosis (4). Histologically, hemangiomas are characterized by a growth phase with hypercellularity and endothelial proliferation (3); on the basis of morphology, they are classified as capillary, cavernous (venous) and arteriolovenous (mixed) hemangiomas. Capillary hemangiomas are composed of small, compressed vascular spaces, while cavernous hemangiomas show dilated vascular spaces with thinned smooth muscle walls. Arteriolovenous (mixed) hemangiomas contain thick-walled arterial vessels in addition to thin-walled vessels (1). Here we report a case of adult laryngeal hemangioma, to date the thirtieth case reported in the literature with a well-characterized histology.

Materials and methods. Our patient is a 16-year-old boy who presented to department of otorhinolaryngology with the complaint of a lump in the throat associated with persistent dysphagia (both for liquids and solids). Laryngoscopic examination revealed a 5 cm red-colored submucosal mass in the right supraglottic region, involving the vallecula, the pharyngoepiglottic fold, the aryepiglottic fold and the false vocal cord (Fig. A). Hypomobility of the right vocal cord was observed. The lesion showed compression of the right piriform sinus. A complete resection was achieved through microlaryngoscopic CO2 laser excision. Post-operatively a video laringoscopy revealed no recurrence of the lesion (Fig. F) and the subsequent follow-up showed total relief of symptoms.

Results. The specimen submitted to pathology was red colored tissue fragments which the largest was 4 cm in diameter. Histopathological analysis revealed that some tissue fragments were covered with squamous epithelium showing normal maturation and acanthosis without cytological atypia (Fig. B, hematoxylin-eosin, original magnification X2,5). The others fragments were superficially ulcerated and covered with fibrin. Large numbers of vessels of various shapes and sizes, including thin-walled veins and thick-walled arteries were observed in subepithelial areas (Fig. C, hematoxylin-eosin, original magnification X2,5; Fig. D-E, hematoxylin-eosin, original magnification X10). Blood-filled vessels were separated by scant connective tissue. Taking together, the morphological features were consistent with arteriolovenous laryngeal hemangioma.

Conclusions. We report a case of adult hemangioma of

Tab. I. The table summarizes the cases of adult laryngeal hemangioma reported to date in the literature.

Authors	Year of publication	Sex and age of the patients	Place of the lesion	Pathological type
Kimmelman et al.	1979	Male, 32 y/o	Vocal cord	Cavernous hemangioma
Sataloff et al.	1995	Male, 35 y/o	Vocal cord	Capillary hemangioma
Bielamowicz et al.	2000	Male, 28 y/o	Vocal cord	Cavernous hemangioma
Lomeo et al.	2000	4 cases, nos	Glottic area	Hemangioma, nos
Yilmaz et al.	2003	Male, 41 y/o	Vocal cord	Cavernous hemangioma
Egeli et al.	2005	Male, 15 y/o	Vocal cord	Capillary hemangioma
Lucioni et al.	2006	6 Males, 27-50 y/o	Supraglottis	Cavernous hemangioma
Erkan et al.	2007	Male, 24 y/o Male, 33 y/o	Vocal cord	Cavernous hemangioma
Prasad et al.	2008	Female, 35 y/o	Vocal cord	Cavernous hemangioma
Iriz et al.	2009	Male, 40 y/o	Vocal cord	Cavernous hemangioma
Yu-Hsing Lin	2010	Male, 57 y/o	Aryepiglottic fold	Cavernous hemangioma
Akhtar et al.	2012	Male, 61 y/o	Supraglottis	Capillary hemangioma
Ibrahimov et al.	2013	Male, 45 y/o	Aryepiglottic fold	Cavernous hemangioma
Feng Lin et al.	2015	Male, 16 y/o	Subglottis	Capillary hemangioma
Wang W-H et al.	2015	Female, 58 y/o	Aryepiglottic fold	Cavernous hemangioma
Wang Xurui et al.	2015	Male, 61 y/o	Arytenoid cartilage and aryepiglottic fold	Cavernous hemangioma
Karatayli-Ozgursoy et al.	2015	Male, 43 y/o	Vocal cord	Hemangioma, nos
Prieto-Frias	2018	Male, 56 y/o	Aryepiglottic fold	Cavernous hemangioma
Saraydaroglu et al.	2019	Male, 58 y/o Female, 62 y/o	Piriform sinus and subglottis	Cavernous and capillary hemangioma

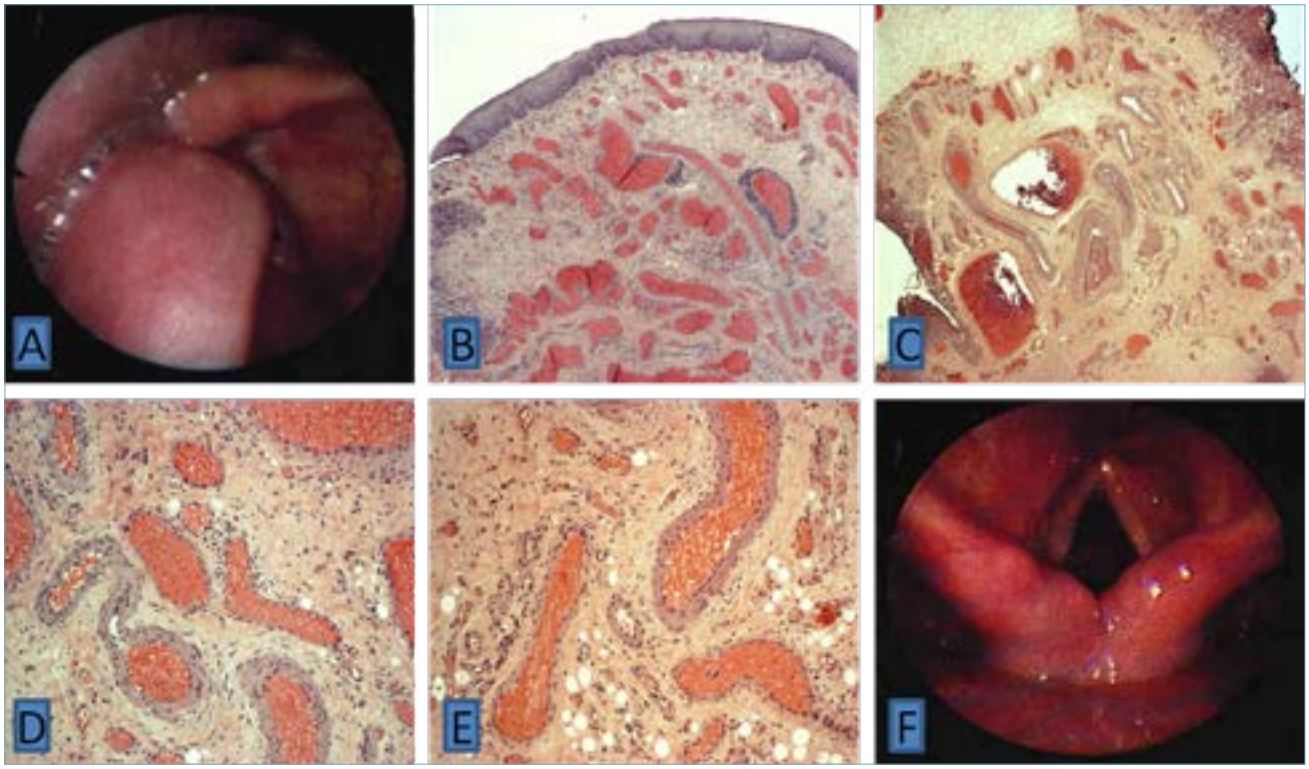

Fig. 1.

the larinx, to date the thirtieth case reported in the literature with a well-characterized histology. Despite the rarity of the lesion, hemangioma should be considered in the differential diagnosis of patients presenting with a submucosal mass in the larynx, in order to perform a careful histological analysis which is therefore crucial for a definitive diagnosis.

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QUALITÀ E SICUREZZA

QUALITY OF CONSULTATION/INTERFACE BETWEEN PATHOLOGISTS AND FORENSICS FOR HISTOPATHOLOGY EXAMINATION AFTER THE FORENSIC AUTOPSY

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Background. Debate remains to how much the magnitude of histopathological examination and quality of procedures may be of benefit to medico-legal purposes after forensic autopsies. We sought to address the question after reviewing consultation cases requested to pathologists from forensics in a real word routine practice.
Materials and methods. We audited and reviewed consultants performed in the Pathology Unit at University of Verona by medical pathologists based on requests received from medico-legal/coroner pathologists, along 2015-2018.
Results. 493 forensic autopsies have been performed. Fifty-two consultants have been requested to pathologists (11%). Gross analysis was requested in 22/52 (42%) cases and histopathology was performed on single organs in 15/52 cases (29%) primary on lung and heart, whereas systematic hollow and parenchymatous multi-organ analysis was performed in 14/52 (27%) cases. Bone-marrow sampling was studied in only 2/52 (4%). Immunohistochemical profiling was needed in 16/52 (31%), special stains in 9/52 (21%) and molecular analysis in 4/52 (8%). The major requests for immunohistochemical analysis (>75%) were posed to rule out neoplastic tissue or histotyping neoplasia (i.e. pancreatic lesions vs adenocarcinoma, 1 case; confirming mesothelioma, 5 cases; atypical infiltration of bone-marrow suspicious for neoplasia, 2 cases; adrenal-gland neoplasia, 1 case; benign solid-cystic hepatic nodules vs colangiocarcinoma, 1 case). In the remaining cases the analysis was needed to characterize inflammatory tissues (and rule out lympho-proliferative diseases) by using primarly CD20, CD3, CD68, CD15 panel of antibodies, on brain (meningitis vs reactive flogistic infiltration of inflammatory cells post-trauma, 1 case), heart (myocarditis, 4 cases) and colonic bowel (pre-existing chronic bowel inflammatory diseases), 1 case. Most common special stains needed (>90% of requests) were Alcian-Pas&PAS (on lung tissue for DAD diagnosis), Grocott (lung tissue to rule out fungal infection) and Masson's Trichrome analysis (again heart and lung tissue). Molecular analysis was performed to test the ALK gene predictive oncological biomarker by FISH technique (two cases) and to verify an exchange of bioptic material in between two consecutive patients (female/male) by using the X/Y chromosome probes (two case). Focusing on technical processes, standard methodology on pre-analytical procedures was changed in 10/52 cases (19%) to answer critical issues posed by forensics: Perls special staining for asbestosis fiber research were tested only after re-sampling of the pulmonary parenchyma due to the need of 30µm tickness tissue lung parenchyma (4 cases) and the Oil Red O analysis was performed after daily water washing, in order to remove formalin liquid from piece of lung tissue up to 48-72hrs (4 cases). These changes were followed to avoid potential false-negative results during interpretation.
Conclusion. Consultation/interface between pathologists and forensics for histopathology examination, after forensic autopsies usually does happen in 11% of forensic autopsies stratified as follows: 1) gross revaluation analysis needed in 42% of cases, with re-sampling needed in most cases; 2) immunohistochemical analysis needed in 31% and special stains in 21% of cases; 3) molecular analysis in 8% of cases. Notably lack of systemic sampling of bone marrow was revealed with absence of information potentially useful for forensics. Overall, growing relationships at any pathological level are welcome and needed between medical and forensic/coroner pathologists; relationship must be planned to re-inforce education, standardization and quality sharing to answers deeper critical forensic questions. Rather than to point the issue "necessary vs unnecessary histopathological examinations" in the forensic practice or to enchance the term "discretion" when dealing with tissue sampling, the evidence-based approaches highlighted by Audit in the real world routine practice may help and improve guidelines and quality of the histopathology examination in forensics.
Key words: autopsy, histopathology, consultation, forensics, pathologists.

RISK MANAGEMENT IN SURGICAL PATHOLOGY UNIT

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The Pathologist has an absolutely relevant role in patients' healthcare and curative workflows as well as a greatly important relevance in the prevention, diagnosis, treatment selection choices and follow up options of all diseases. The mission of the pathologist can be summarized in providing the oncologist or clinician all the necessary information to make a diagnosis, address therapeutic and follow-up options, identify factors predictive of response to therapy and prognostics factors. Therefore, the Pathologist can be considered the business owner of the clinical and therapeutic process since his/her competence can be applied to all the phases of it. More specifically he/she knows all the expectations of internal and external customers as well as manages the inter-relationships among all the different stakeholders of the healthcare system. The Pathologist can manage and monitor the trend of this process, guarantee the integrity and quality control of the lab, the best allocation of resources and instruments and decide and put in

place all eventual corrective and improvement actions. The question would be: which features are necessary and fundamental of an Anatomic Pathology Unit? The results provided by a pathology lab should be correct, mistaken-proof, timely provided and deployed within the timelines appropriate for the patients' therapeutic management as well as in a format allowing a correct interpretation and application within the diagnostics and therapeutic process. Based on these premises, risk management in Anatomic pathology is a must and should be deployed considering multifactorial and complex considerations, based on the multiple task and activities that compose the Pathologist's job. A pathology unit is based on dynamic workflows and laborious activities with multiple critical phases, depending on human intervention factors and high-technology-based instruments. Risk management is therefore an indispensable tool to prevent mistakes and manage all possible risky events in the context of a multi-professional environment with process automation and interconnected task activities. Risk management is based on all positive improvement and corrective actions to optimize the outcome of healthcare services, in order to guarantee the security of the patient. Given the intrinsic characteristics and multiple activities performed by the Pathologist, it is not possible to imagine that there is only one and unique ideal instrument to manage the risk. In order to reduce critical steps we need to refer to applications tailored for the specific professional context and organizational features of the job so that all the different workflow phases of the biological material are properly covered and supported. The principle tools and instruments available for risk management are: *Incident Reporting, Root Cause Analysis, Failure Mode and Effect Analysis* and *Quality Certification (UNI EN ISO 9001:2015, risk-based thinking)*. They represent milestones of risk management and can be applied to the different phases of the professional activity of the Pathologist. Quality certification of an Anatomic Pathology service, *UNI EN ISO 9001:2015*, is a complex path involving processes control but specifically oriented to the outcome, interpreted as the customers satisfaction for the product and/or service provided, being both patients and clinicians asking for the service, the ultimate customers. Quality in Anatomic Pathology is a synergistic effort between *Good Clinical Practice* and *Good Laboratory Practice*, the former aiming at the appropriateness of the request and the meaningful sample representation; the latter being focused on the quality of the activities done by the personnel of the lab as well as its instrumentation. All the involved professionals, independently from their qualifications and rank, participate to the workflow and provide the valuable contribution and therefore an active collaboration is fundamental as well as training and *lifelong-learning* must be constantly guaranteed to secure an up to date operational excellence. Each contributor can put in place, beyond the skills required for the role and consequent behavior implied (*Skill based behaviours*, meaning the automatic behaviors during routine tasks) strategies to improve the system deploying a behavior based on the rules *(Ruled based behaviour)* identifying the right behavior tailored for the situation or apply a *Knowledge based behavior,* that allows to overcome an unknown or ambiguous situation, after preparing a strategic plan. In practice, risk management in Anatomic Pathology means monitoring critical steps and inadequate behaviors that can happen in the three phases of the workflow: pre-analytical phase (from patient sampling to histological sample preparation), analytical phase (specimen description, microscopic diagnosis, report preparation), post-analytical phase (report signing out, clinical interpretation). Risk management can be translated in the adoption of tracking systems that allow to monitor all the phases of the product preparation from the sampling rooms (eg ambulatory and surgical rooms) to the transport, sampling preparation and archive so that every step is tracked, the single operator of each phase identified and information can be checked and accessed during and after the entire process. The principle aim is to track the entire workflow and monitor it, given the high number of samples and multiple phases involved, from sampling to archiving and beyond (eg consulting request, revision, retrieval of cases for research publications, etc). The procedures of tracebility of the materials received by a pathology department are IT-based and can include bar code instruments, radiofrequency tools, administrative software to manage the data warehouse and all the information regarding the patient together with the formalities of the preparation of the report electronically signed. This last feature is quite an advantage since allows the real-time access to the reports and allow their correct interpretation and utilization.

To maintain and improve the risk management system it is desirable to check periodically and look for non-compliance events, adverse events, near miss situations and study the root causes to improve the diagnostic activity and mapping the most risky activities in a proactive manner. It is important to put in place and socialize with the culture of the diagnostic mistake in order to recognize it in advance or in real time, reduce the risks related to its latency, study the error and analyze the gaps that made it possible to improve the management and prevention of future mistakes. A further indicator of the quality of diagnostics activity of a pathology unit is the outcome of all the diagnosed cases asked for an external consultation from patients and clinicians to the pathologist.

This "second opinion" procedure is a clinical practice that further protect the patient since a second pathologist is asked to review independently the slides and express an autonomous diagnostics judgement. In conclusion the Pathologist diagnostics profession is fundamental in the process of providing the patient the right and most appropriate therapeutic treatment. Fundamental characteristics of this profession are correctness and precision. Consequently professional risk management is a compulsory feature that guarantees deployment of appropriate diagnostics workflows for therapeutic options choices and also protect all the professionals in the execution of their jobs. Finally, we would like to underline that the culture of risk management is mandatory in Pathology Unit to positively develop and apply processes based on guidelines, good clinical and laboratory practice and tracking procedures to guarantee a more secure, efficient and sustainable healthcare system.

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RECURRENCE OF TUMORAL CALCINOSIS: A CASE REPORT

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Aim. Tumoral calcinosis (TC), also called Teutschlander disease, is a relative rare disorder characterized by calcium salts accumulation in iuxta-articular soft-tissues, producing solitary or multiple painless periarticular masses[1,2]. Large joints such as the hip, shoulder, and elbow are usually involved. This entity most commonly presents in the first two decades of life. Approximately one-third of patients with TC shows familial inheritance[3,4]. To date, there are few studies presenting computed tomography (CT) and magnetic resonance (MR) imaging characteristics of TC with radio-pathologic correlation[5]. We report the unusual case of an adult patient without familiar inheritance, who had undergone surgery for elbows TC during the first decade of life and presented left hip recurrence in the seventh decade. We describe CT and MR findings with histopathological correlation on surgical specimen.
Material and metods. A 64 years-old male patient was referred to our institution for swelling of the left gluteus and hip. He had a history of elbow recurring TC in the first decade of life that needed five surgical procedures. No other manifestations of calcinosis occurred during the following decades. He didn't referred TC in the other family members. However, family history was positive for multiple primary malignancies (MPM) because his father had died at the age of 60 of colon carcinoma, which was diagnosed simultaneously to a bladder tumor. The patient had a mild hyperphosphatemia, without haematic value alterations of 1,25-dihydroxyvitamin D or parathyroid hormone. Renal function was normal.
Results. Radiography showed a grossly periarticular calcified mass around the left hip joint and the upper thigh. At contrast-enhanced (CE) multidetector computed-tomography (MDCT) a grossly calcified lobular mass was visible with large cystic areas and fluid-fluid levels inside (CT sedimentation sign). A peripheral enhancement of cystic areas was observed after CE administration, without pathologic solid portions. At MRI the lesion had inhomogeneous diffuse low signal intensity on T1-weighted sequences, while it presented alternating signal patternson T2-weighted sequences with low intensity areas and cystic components with fluid-fluid levels (MRI sedimentation sign). After CE administration, fibrous septa surrounding cystic and calcified areas enhanced, producing a "web" or "cobblestone" pattern. No nodular enhancement was reported, neither involvement of surrounding muscolar and bone structures, vessels and nerves. Patient underwent surgery. The mass was resected, measuring 62 cm in the largest diameter. Pathologic evaluation confirmed the diagnosis of TC, showing grossly calcifications and cysts with chalky material inside (precipitated calcium salt), surrounded by inflammatory reaction and fibrosis. No signs of malignancy were found and no part of the mass was seen to make direct contact with the underlying bone.
Conclusions. Our patient the disease showed an unusual course: after a typical onset in the first decade of life, disease had been silent until the seventh decade, when it recurred with a left hip active growth lesion. Surgical excision is a well documented treatment, but recurrences after surgery due to poor circumscription are common, particularly in metabolically active lesions[5]. Recurrences can also occur several years after intervention[6]. Until now, however, only few reports in literature have described MRI findings in TC recurrences[5]. To conclude, TC can occur with different clinical and biochemical patterns. We report an unusual relapse with a metabolically active lesion in an elderly after a long period of quiescence. Differential diagnosis can be difficult in unusual clinical settings, but a careful evaluation of imaging findings with histopathological correlation on surgical specimen and biochemical data can suggest the correct diagnosis.

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PERINEURIOMA: A BIZARRE SOFT TISSUE TUMOR: A CASE REPORT

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Introduction. Perineuriomas of soft tissue are nearly always benign peripheral nerve sheath tumors composed entirely of perineurial cells. Intraneural and mucosal types also exist. Soft tissue perineuriomas are typically not associated with nerve and are variably whorled; malignant soft tissue perineurioma is a rare variant of malignanr peripheral nerve sheath tumour displaying perineural differentiation.
These tumours are rare (about 200 cases have been reported), are slightly more common in females than

males (ratio 2.1) and occur over a wide age range, with a peak in adolescence or early adulthood. Soft tissue perineuriomas are located in the deep soft tissue and are grossly unassociated with nerve.
We reporte a case repor of perineurioma of soft tissue in a male of 29 years old.
Clinical Case. Male subject, 29 years old, who has had pain and swelling in his left leg for several months. Instrumental examinations are carried out (images in the final work!) That frame this lesion as a possible tumor of peripheral nerve / perineuroma sheaths. The lesion appears to extend for about 6 cm.
On the macroscopic examination, a oval formation is described, with a smooth surface, of whitish color, of 5x4x3 cm. Bray is described as a compact part within which there are yellow-brownish areas and a cystic formation with a gelatinous, amber content of 0.8 cm of maximum diameter. The sections were included in paraffin, set up and colored with hematossiln-eosin. At high magnification power, we describe tumor cells that are slender spindle cells with wavy or tapering nuclei, indistict nucleoli, and characteristic delicate bipolar cytoplasmic processes. The stroma is collagenous, with focal myxoid matrix. Mitotic activity is absent. These tumor cells show a predominantly storiform growth pattern, and occasionally show degenerative nuclear atypia, including pleomorphic and multinucleate cells. Immunoistochemical profile: positivity of EMA (Epitelial membrane antigen) in tumor cells (like normal perineurial cells); reaction for CD34 and S-100 protein are negative. Focal positivity for Claudin-1 and GLUT-1.
Comment. The presented case fully reflects the characteristics of the perineuroma which are described by the latest WHO classification. The size and topography of the lesion are within the average. The secret for a correct diagnosis of certainty of this nosographic entity remains the immunohistochemistry for EMA, which reflects the perineural origin of the tumor.

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UNDISCLOSING POTENTIAL OF HISTOPATHOLOGICAL ANALYSIS OF SYNOVIUM IN THE THE MANAGEMENT OF PATIENTS WITH INFLAMMATORY JOINT DISEASES. A SINGLE CENTRE CROSS-SECTIONAL STUDY

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Aim. The Krenn's histopathological synovitis score evaluates inflammatory changes of synovium assessing eother the width of lining layer, the stromal density and the inflammatory infiltrate. For each domain four semiquantitative levels (normal "0". mild "1". moderate "2". severe "3.") exist; their addition results in a final score varying from 0 to 9. On this basis osteoarthritis-related low-grade synovitis (i.e. ≤ 4) can be distinguished with good accuracy from high-grade synovitis (i.e. > 5), which is associated to rheumatic diseases. The synovitis score has potential to enable a risk stratification of high-grade synovitis (i.e. progression risk and sensitivity for biologicals) [1, 2]. Here we describe a multidisciplinary single centre experience in managing patients with rheumatic diseases twith the contribution of histopathological synovitis score.
Methods. In this cross-sectional study we analyzed histopathological specimina from patients with rheumatic disease who underwent routine ultrasound guided synovial biopsy from January 2019 to June 2019 in a dedicated biopsy service of a Rheumatology Unit.
After proper fixation in formaldehyde and inclusion in paraffin, all specimina were stained with hematoxylin and eoxin and then assessed by means ok Krenn's Synovitis score. We also recorded patients' demographic and clinical characteristics as well as ultrasound features of biopsied joints. Spearman's correlation was used to determine whether association existed between ultrasound features a synovitis score. We also determined the impact of hystopathological findings on rheumatologists' clinical decision in managing patients.
Results. Eleven rheumatic patients with knee arthritis (6/11 women. 54.54%). mean age (± SD) of 47.72 ± 12.63 years underwent ultrasound-guided knee biopsy. Five out of 11 patients had rheumatoid arthritis (45.45%). 5/11 patients had psoriatic arthritis (45.45%) whereas 1 out of 11 patients had undifferentiated arthritis (9.09%).Of these procedures. a specimen good for histopathological analysis was obtained for 7 out of 11 patients (63.63%). We recognized n. 4 low grade synovitis (57.14%) and n.3 low grade synovitis (42.85%). Synovitis score was strongly related with the sonographic ultrasound power doppler score of synovitis ($r = 0.93$. $p = 0.002$). In 5 cases out of 7 (71.42%) the histopathological report was able to guide the therapeutic choice, thus allowing personalized treatment.
Conclusions. Despite the small-sized cohort, our preliminary results confirm that Krenn's synovitis is a valuable tool in the real-world management of patients with inflammatory joint disease.

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UROPATOLOGIA

INCIDENTAL FINDING OF PARATESTICULAR AGGRESSIVE ANGIOMYXOMA IN 72 YEARS-OLD MONORCHID MALE

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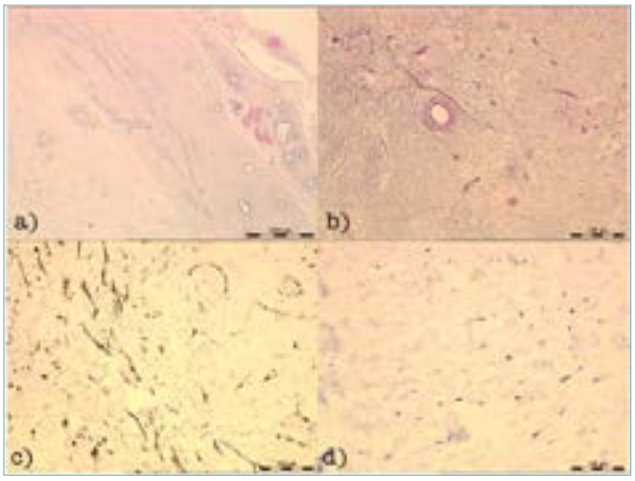

Fig. 1. a) Remnants of rete testis and efferent ductules, hematoxylin eosin 2,5x. b) hypocellular lesion with characteristic fusiform and stellate cells, hematoxylin eosin 10x. c) Positive staining for Actin 1A4, 20x. d) Positive staining for Progesterone receptors, 20x.

Objectives. Aggressive angiomyxoma (AA) is a rare mesenchymal myxoid neoplasm usually found in fertile females and occurring very rarely in males, with potential of local recurrence. This case report describes an uncommon case of incidental finding of paratesticular aggressive angiomyxoma in asymptomatic, criptorchid male patient.
Matherials and methods. The patient is a 72 year old male followed by the Urology Department for urothelial carcinoma (G3) and benign prostatic hyperplasia since 2017. He had an appendectomy in childhood and was cryptorchid since birth. He never went under any evaluation regarding the cryptorchidism. In December 2018 the patient underwent an abdominal contrast CT scan that showed a focal stricture of final third of left ureter with grade I hydronephrosis. In the left iliac fossa a 7.7x5.6 cm oval formation with fluid density and thin walls without contrast-enhancement was found, described as possible mucocele. Physical examination did not reveal any abdominal mass. The left hemiscrotum was occupied by adipose tissue that mimicked the testicle and its normal ovoid firm surface. Intraoperatively the abdominal mass had intimate relationship with the sigma with no signs of invasion, and complete excision was performed.
Results. Macroscopically the mass was ovoid, ivory colored, pseudocapsulated with an adjacent 6 cm tubuliform structure. On cut surface the color was brownish yellow, myxoid. Microscopic examination revealed a paucicellular lesion with small number of fusiform cells with fine chromatin and indistinct nucleolus, immersed in myxocollagenous stroma containing blood vessels of various calibre, mostly ectasic. Some vessel showed signs of hyalinization or medial thickening, but mostly they were thin-walled. There was no cellular atypia and exceptional mitotic Fig. s. Minimal rete testis and efferent ductules remnants were observed at the periphery of the mass. Immunohistochemical study revealed positive staining for CD34, Desmin, Actin HHF35, Actin 1A4, and was negative for S100, Estrogen, but positive for Progesterone receptors.
Discussion. Aggressive angiomyxoma is a rare benign, mesenchymal, myxoid neoplasm usually found in females and occurring very rarely in males, with a 6,6:1 ratio. The adjective of aggressive, attributed to the tumour, is based on its potential of local recurrence, common after surgical excision (about 72% of the cases) and the aspect of blood vessels immersed in myxoid stroma, although its metastatic potential is very low. [1,2] Histological differential diagnosis includes angiomyofibroblastoma, cellular angiofibroma, and myxoid variant of solitary fibrous tumor[3]. Wide local excision is currently standard of care, and, generally, there is no need for any neoadiuvant/adiuvant therapies, although hormonal therapy could facilitate the excision in large tumours that express estrogen or progesterone receptors because of their hormone-related growth[4,5].

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PROSTHETIC FLORID HISTIOCYTIC REACTION MIMICKING LYMPH NODE METASTASIS

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Introduction. The dissection of lymph node is an essential part of the staging of cancer. Frequently in this condition, the lymph nodes show a nonspecific reactive hyperplasia with sinus histiocytosis, characterized by an increase of macrophages and sinus cells. In peculiar conditions, the normal lymph node architecture could be replaced by massive histiocytic reaction mimicking a metastasis.
Material and methods. We report a case of a 80-year-old Caucasian male underwent to cysto-prostatectomy with regional lymphadenectomy for a previous diagnosed of poorly differentiated high grade urothelial carcinoma.
Results. The histological diagnosis of cancer was confirmed; the pathological evaluation of the lymph nodes for staging evidenced a massive and abnormal infiltration of the obturator lymph nodes by large size cells in the sinuses and in the inter-follicular regions. These cells were characterized by abundant, finely granular, eosinophilic cytoplasm with oval, eccentric nuclei, occasionally with oncocytic morphology, suspect for metastasis. However, their metastatic nature has been excluded by negative CKAE1/AE3-immunostain, opposite to CD68-positive immunostain confirming the histiocytic nature. Moreover, high magnification showed rare scattered dusty cytoplasmic brown material. Looking at previous medical history, the patient performed a polyethylene/cement prosthetic knee joint replacement eleven years before; so these histological features could

represent florid histiocytic reaction to deteriorated prosthetic material.

Conclusions. The prosthetic devices are mainly composed by metal, polyethylene and/or cement, and these could deteriorate over time after joint replacements. Occasionally, this process induces a foreign body reaction in draining lymph nodes usually characterized by granulomatous giant cells reaction, rarely by massive and abnormal sinus histiocytosis with disruption of the nodal architecture mimicking a neoplastic process and this represents a diagnostic pitfall. It is important to keep in mind this peculiar condition overall, when we are dealing with cancer staging, such in our case.

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PRAJA2 EXPRESSION IN BENIGN UROTHELIAL NEOPLASMS AND UROTHELIAL CARCINOMA

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Introduction and objectives. The urinary bladder cancer is the seventh most common type of cancer worldwide with a significant prevalence in males (4:1), with a median age of 65 years. The urothelial carcinoma accounts for about 80-90% of all bladder cancers. Most of the patients are diagnosed either with a non-invasive or early invasive form of this disease, that also has a high risk of a reoccurrence (50-70%) and progresses in only 15-25% of cases[1]. It is yet unknown though what exactly triggers the progression of this tumour.The E3 ubiquitin protein-ligase praja2 is an enzyme, found in various mammalian tissues. It controls the stability of protein kinases and participates in the ubiquitin–proteasome system (UPS). A dysregulation of the UPS plays an important role in the control of cell metabolism, growth and survival, favouring its malignant turnover [2].

Our aim was to analyse the expression of praja2 in the muscle invasive and muscle non-invasive urothelial carcinoma of the bladder and compare it to its expression in benign urothelial neoplasms.

Materials and methods. At the ''Federico II'' University of Naples 21 records were selected from the files of the histopathology units. All of them focused on the patients with a **high grade (G3)** of muscle non-invasive urothelial carcinoma, which progressed into muscle invasive carcinoma within 6 months or more. One of the selected patients (4.8%) was female, the rest of them (95,2%) were males. The average age of the patients at the time of diagnosis was 73,1 years.

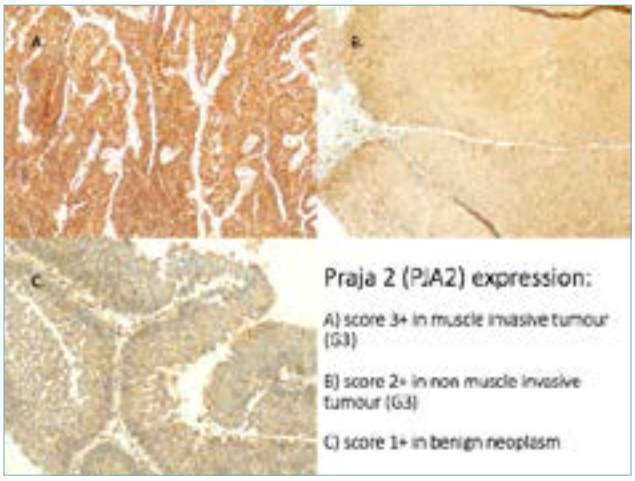

Fig. 1.

2 tissue microarrays (TMA) from the representative areas of each tumour were generated: the first TMA contained 15 evaluable samples from 13 different patients with **muscle non-invasive** specimen. The second TMA was composed of 26 samples from 21 patients with **muscle invasive** specimen.

The immunohistochemical staining with PJA2/Praja2 antibody was performed.

The expression level of PJA2 was evaluated using a score from 0 (negative) to 3+ (very strong and diffuse positivity).

In the first TMA 27% of tumours were scored as (3+), 60% as (2+) and 13% as (1+). In the second TMA 46% of tumours were scored as (3+), 35% as (2+) and 19% as (1+).

Praja2 expression was analysed in 16 TMA samples containing benign urothelial lesions. The majority (69%) of cases were scored as 1+, 25% were scored as 2+ and 1 case (6%) was negative (0).

Results and conclusions. The praja2 was expressed in all high-grade (G3) urothelial carcinoma samples with more than 80% of them showing strong positivity for this enzyme (39% scored as 3+ and 44% scored as 2+). The praja2 expression in benign urothelial lesions was significantly weaker (only 25% scored as 2+ and 0 cases scored as 3+). We hypothesize that this enzyme is involved in urothelial carcinoma proliferative pathways and is contributing to its growth.

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SMALL CELL (NEUROENDOCRINE) CARCINOMA ARISING FROM A URINARY BLADDER DIVERTICULUM

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Introduction. Bladder tumors arising within a diverticulum are uncommon and pose a unique diagnostic and therapeutic challenge. Small cell carcinoma of the urinary bladder is a rare, aggressive, poorly differentiated neuroendocrine neoplasm accounting for 0,3-0,7% of bladder tumors. Few protocols encounter small cell neuroendocrine carcinoma with detailed clinico-pathological features among clinical trials. Small cell carcinoma detected in a bladder diverticulum has been described in few patients in the literature. We present a new case of this rare entity disease.
Material and methods. A 40-year-old woman presented to our Hospital with a gross hematuria and abdominal pain. Chest and abdominal CT scan revealed a solid tumor (*5.5 x 4.5 cm*) within a bladder diverticulum located in the right lateral bladder wall with a multiple lymphadenopathy (*aortic, celiac and iliac lymph-nodes*). Cystoscopy confirmed this finding and transurethral resection of the tumor was performed. The histopathology assessment showed a highly cellular poorly differentiated carcinoma, with no mixed histology that invaded the detrusor muscle. At morphology, tumor showed nests of small round malignant cells with pyknotic round to oval nuclei and evenly dispersed "*salt and pepper chromatin*". The Azzopardi phenomenon and foci of necrosis were also observed. The mitotic rate was high *(>10 mitotic Fig. s/10 HPF*). In immunohistochemical staining tumor cells expressed neuroendocrine markers (*CD56, NSE, synaptophysin and chromogranin A*) and strongly positivity for CKAE1/AE3 (*dot-like perinuclear pattern*), p53, Ki-67 (*from 60 to 90% nuclei*), and focally *(<50% of cells*) for CK34B12, CK7, CK20, TTF-1, CD117 (*c-Kit*) and p63. A diagnosis of small cell (*neuroendocrine*) carcinoma (*2016 WHO classification*), with a clinical stage T2 grade 3 was made. The treatment of bladder small carcinoma requires a multidisciplinary approach and after the discussing with the patient the possible therapeutic approach, neoadjuvant chemotherapy prior to radical cystectomy, was decided. The patient was treated with cisplatin (*75 mg/mq ev*) and etoposide VP16 (*100 mg/mq ev*) every 21 days for 3 cycles. The treatment has been well tolerated and toxicity was absent. TC scan control after 4 months showed a sensible reduction of the solid tumor in the bladder diverticulum and the lymph-nodes involvement. In the next month the patient will be submitted to one radical cystectomy with lymph-nodes dissection.
Discussion and Conclusions. Bladder tumors originating within a diverticulum are also uncommon and challenging. They occur mainly as a result of increased intravescical pressure secondary to bladder outlet obstruction or may infrequently result from congenital disarrangement of muscle fibers at the ureterovescical junction. Contrary to the normal bladder wall, lack of muscle fibers in the diverticulum makes it difficult to stratify the tumors into superficial and invasive bladder cancer. In addition, the paucity of muscle fibers beyond the mucosa theoretically allows the tumor to invade earlier and more easily than in a normal bladder wall containing thick muscle. Small cell carcinoma of the bladder occurs more often in elderly males and prognosis is poor. Tumor cells are strongly positive for CKAE1/AE3, CAM 5.2, p53 and focally for CK34B12, CK7, CK20, TTF-1, c-Kit and p63. Most cases are immunoreactive for neuroendocrine marker such as diffusely for CD56, NSE and focally for synaptophysin and chromogranin. Patients with neuroendocrine tumors benefit to neoadjuvant chemotherapy, as evidenced by better overall survival and lower rates of non-organ-confined disease at the time of radical cystectomy. For tumors with micropapillary differentation, sarcomatoid differentiation, or adenocarcinoma, neoadjuvant chemotherapy decreased the frequency of non-organ-confined disease at the time of radical cystectomy. However, this favorable effect did not translate into a statistically significant overall survival benefit for these patients, potentially due to the aggressive tumor biology.
The management of small cell (*neuroendocrine*) carcinoma of the bladder is not standardized and requires a multidisciplinary consultation. For us the recognition of this rare entity should enable better detailed tumour clustering when designing clinical trials using drugs targeting patient affected by small cell neurondocrine phenotype of urothelial carcinoma.

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HISTIOCITIC SARCOMA: FIRST DESCRIPTION OF UNEXPECTED NEOPLASM OF THE BLADDER

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Objective. Histiocytic sarcoma (HS) is a very rare malignant lymphohaematopoietic neoplasm reported in the last edition of WHO classification as a malignant proliferation of cells exhibiting morphological and immunophenotypic features of mature histiocytic lineage. Despite its unknown etiology, HS has been associated with haematolymphoid malignancies (particularly low-grade B-cell lymphomas) and germ cell tumors. HS affects people of all ages but most cases occur in adults. It often presents in lymph nodes, skin (sometimes as primary presentation) and other extranodal sites, including the gastrointestinal tract, superficial and deep soft tissue, lungs, and nasal cavity. The bladder localization of HS has been reported only in another patient with a previous history of Diffuse Large B-cell Lymphoma (DLBCL). The aim of our report is to describe the first case, to the best of our knowledge, of primary bladder localization of HS without prior or metachronous history of lymphoproliferative neoplasm.
Materials and methods. A 68-year-old male with a previous history of hypertension presented with recurrent episodes of hematuria, back pain, complaints of fever, fatigue, decreased appetite and weight loss. Laboratory findings revealed a neutrophilic predominant leukocytosis and anemia. Urine cytologies were negative for urothelial malignant cells, but CT abdomen showed a 4 cm

heterogeneous intravescical mass, located at the posterior and left wall of the urinary bladder, that was confirmed by cystoscopy. The patient underwent transurethral resection (TUR) of the tumor that revealed a highly undifferentiated neoplasm comprised of highly atypical large cells with eosinophilic to vacuolated cytoplasm and hemophagocytosis. A wide panel of immunohistochemistry was set up and erroneously it oriented towards a sarcomatous origin for the tumor cells. On the basis of these results, a cystoprostatectomy was performed. Histologically, the neoplasm cells were obscured by a prominent inflammatory infiltrate consisting of small lymphocytes, plasma cells, benign histiocytes neutrophils and eosinophils. Occasionally haemophagocytosis was observed in association with vascular invasion. The neoplasm infiltrated the external muscular layer of bladder and consisted of epithelioid and pleomorphic cells with abundant eosinophilic cytoplasm often with some fine vacuoles. These malignant cells show large, round to oval nuclei, sometimes placed at the periphery with vesicular chromatin. In consideration of this unusual neoplasm histological pattern, it have been necessary a large immunohistochemistry determination. The immunohistochemical panel showed positive in tumoral cells including: CD68 (KP1 and PGM1), CD31, CD4, CD43, LCA, MPO and CD45. Negative stain was observed for GATA3, EMA, p63, ALK, CAM5.2 and S-100. The neoplastic cell showed a proliferative index Ki67 about 90%. Considering morphology and immunohistochemical staining profile, a diagnosis of HS was achieved. Shortly the patient developed pulmonary and liver metastasis and a recurrent mass in the pelvic wall occurred. His conditions got worse and died 2 months following the histologic diagnosis.

Results. HS is an extremely rare aggressive haematological neoplasm with very few numbers of reported series. These neoplasms are almost impossible to diagnose only by morphology because they share similar histologic feature with other neoplasm like epithelioid sarcoma, melanoma and carcinoma. For these similarities the use of immunohistochemistry markers is essential to provide a correct diagnosis of HS that shows the expression of specific histiocytic markers, as CD68 and lysozime. As it is reported in last WHO of 2017, the patients affected by this neoplasm has a wide age range, but most of the cases occurs in adult and some studies had found that there is a male predilection. The clinical presentation of this neoplasm is usually as localized mass, in rare case the neoplasm can present as disseminated diseases. In literature cases of brain, mediastinum, uterine cervix, choroid and liver localization have been reported. There is also a case report of HS in a patient with history of kidney transplant with multiple masses in native kidneys and liver. In WHO of 2017 is reported an association with metachronous or prior low-grade lymphoma (commonly follicular lymphoma), but also with chronic lymphocytic leukaemia/small lymphocytic lymphoma. The prognosis of patients affected by HS is very poor and it depends on stage and primary localization, the only treatment agreement seem to be surgical excision followed by chemotherapy and/or radiotherapy, but there are only few data.

Conclusions. To the best of our knowledge there is only one other report of bladder involvement by HS in a 80 years old man with a previous history of DLBCL. In our patient there is no previous history of lymphoma, transplantation or other neoplasm, so this is the first described case of primary HS in bladder without previous or metachronous lymphoma.

HS are very rare tumours and represented than 1% of all haematological malignancies, histological diagnosis is very difficult and cannot be diagnosed without auxilio of immunohistochemestry.

The use of histiocytic lineage such as CD68 (PGM1 and KP1) as diagnostic marker of HS must be considered for differential diagnosis in this very rare and difficult cases.

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ZINNER SYNDROME: A WOLFFIAN CONUNDRUM. A SYSTEMATIC REVIEW

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Background. Zinner's Syndrome consists in an aberrant development of the mesonephric duct and in the absence of the ureteric bud during embryogenesis, that leads to ipsilateral renal agenesis and atresia of the ejaculatory duct, which subsequently progresses to cystic dilation of seminal vesicles. Often It is considered to be the male counterpart of Mayer-Rokitansky-Kuster-Hauses syndrome seen in females.

The close embryologic relationship between the genital and urinary tracts could explains the developmental aberrations leading to this anomaly: an insult occurring before the 7th gestation week leads to maldevelopment of the distal part of the mesonephric duct producing atresia of both ejaculatory duct and the ureteric bud.

Materials and methods. Scientific literature is limited to case reports and small series; Pubmed research for Zinner Syndrome discosed a total of 90 items. The research has been restricted to English written full papers for the period 1995-2019. A total of 32 papers have been reviewed considering 48 patients including two cases of our observation.

Results. The syndrome is usually diagnosticated between the 3rd and 4th decades the main symptoms of presentation were urinary (n=18), pelvic/abdominal pain (n=12), infertility/ejaculatory impairment (n=9) while fever (n=2) and incidental finding (n=8) were rarer. In 8 cases other variable malformation were associated and in 2 other cases also other urologic malignancies were present. 45 cases out of 48 underwent to surgery at the time of the diagnosis or for exacerbation of the symptoms. The two cases of our observation were both in the 4th decades come to medical attentention for lower back pain and as occasional finding. The pathologic exami-

nation of the surgical specimens showed a dysplastic structures including a large cystic mass, morphologically and immunophenotipically of possible mesonefric derivation (GATA3+, PAX8+, CK7+, focally PSA+ and focally PSAP+) and with a prevalent tubule-cystic component surrounded by smooth muscle fibers (SMA+; h-Caldesmon+) arranged in helical bundles. One case also showed minimal differentiation in renal direction including scant tubulo-glomerular structures.

Conclusions. Zinner's syndrome is considered rare condition in general presenting the 3rd- 4th decades of life for the vague and aspecific urological symptoms; just a smaller population is diagnosed in pediatric age as occasional finding during screening imaging. The pathological examination of the surgical specimens appear crucial for correct diagnosis of the syndrome. The histological features are poorly covered in literature and may represent a surgical, pathological and embriological conundrum. A thorough histological examination with a limited panel of immunohistochemistry markers is required for a correct diagnosis considering the existence of other associated malformation and the risk of malignancy,

POST-RADIATION ANGIOSARCOMA OF THE BLADDER: A TRUE PATHOLOGICAL DIAGNOSTIC CHALLENGE. TWO CASE REPORTS AND LITERATURE REVIEW

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Aims. Primary angiosarcomas of the bladder are rare tumors of middle-aged and elderly men that typically present with locally advanced disease. The association of angiosarcoma with therapeutic radiation has been previously described. Microscopically it consists of atypical anastomosing vascular channels in most reported cases. It is not uncommon for this tumor to dedifferentiate and form sheets of primitive cells, spindle cells, epithelioid cells, or a mixture of these cell types. Awareness of the histopathologic spectrum of angiosarcoma is important for the correct diagnosis.

Material and methods. We herein report two new cases of this neoplasm, both occurred after a pelvic irradiation for prostatic adenocarcinoma, highlighting the clinical presentation, histological features, immunophenotypic profile, and treatment. At the best of our knowledge, one of the two cases is the first described for which the diagnosis of angiosarcoma was made with a concurrent metastatic disease in the bone-marrow biopsy.

Results. The sections from the transurethral resection chips of the bladder, in both patients, showed a densely fibrotic subepithelial connective tissue with extension of the sclerotic tissue within the muscolaris propria. The mucosa was extensively ulcerated with an evident granulation tissue immediately beneath, intermixed with a focal areas of proliferation of small and medium sized vessels sometimes anastomizing, lined by a plumped endothelial lining without frank atypia. The superficial urothelium, where present, showed focal reactive atypia. Immunohistochemical positivity for vascular markers, including CD31, CD34, ERG, and factor VIII lead us toward the right diagnosis.

Conclusion. Angiosarcomas can occasionally involve the urinary tract. Primary angiosarcomas of the bladder are rare tumors of middle-aged and elderly men that typically present with locally advanced disease. (1,2). Pathologists should have a high index of suspicion in elderly men presenting with hematuria and a urinary bladder mass (3,4). The association of angiosarcoma with therapeutic radiation has been previously described (5). The absence of a history of radiotherapy does not exclude angiosarcoma. The broad pathologic spectrum of primary bladder angiosarcomas could make a right diagnosis extremely difficult, particularly on limited biopsy material (6). The pathology is that of typical angiosarcoma in only half of the cases. Awareness of the histopathologic spectrum of angiosarcoma is important for the correct diagnosis, especially when dealing with small biopsy specimens. The most useful morphological characteristics to raise one's suspicion of an angiosarcoma are the presence of blood-filled spaces of variable size and shape lined by atypical cells. This "classic pattern" with typical cytology was the most common pattern reported in primary bladder angiosarcomas (6). However, this finding may be focal and overlooked. Moreover almost half of the cases had a solid growth component, and roughly one third had either spindled or epithelioid cytology. Hence, immunohistochemical stains are often crucial to confirming the diagnosis of angiosarcoma. In atypical cases, testing with immunohistochemistry is essential. Immunohistochemical positivity for vascular markers, including CD31, CD34, ERG, and factor VIII should lead toward the right diagnosis(6,7). Most cases are negative for cytokeratin but focal positive cytokeratin stain is commonly seen and should not be interpreted as sufficient evidence of epithelial origin (6). Evidence of c-Myc amplification, as seen in the current case report, may be supportive, as it is found in over half of the post-radiation angiosarcoma (7).

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XP11 TRANSLOCATION RENAL CELL CARCINOMA DIAGNOSED IN BONE METASTASIS IN ADULT PATIENT: DESCRIPTION OF TWO CASES

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Objectives. Xp11 translocation renal cell carcinoma (RCC) is a pathological entity recently included in the last WHO in the MiT family group. According to the last datas this neoplasm is far more common among young patients (up to 40% of pediatric RCCs), than among adults (1.6-4% of adult RCCs). Morphologically the neoplasm can present with several patterns at the same time, frequently showing both solid and papillary architecture. This feature creates considerable diagnostic difficulties, as the neoplasm can mimic the papillary RCC, the clear cell RCC and the clear cell papillary RCC, particularly in cytology, where the diagnostic material is not abundant like that of histological preparations. Furthermore, a particular aggressiveness in adults and the elderly is characteristic of this neoplasm.
Materials and methods. We describe two cases of adult patients with bone metastases from cancer of unknown primary origin, the first (Case A) with a cytological diagnosis, and the second (Case B) with histological diagnosis of Xp11 translocation RCC. First patient was a 56-year-old woman with numerous metastases from unknown primitive neoplasia. The patient was subjected to CT-guided fine-needle aspiration cytology (FNAC) of the mass involving the iliac bone for typing the primary lesion. Second patient, a previously healthy woman of 50 years old with pain in the right shoulder, who showed at magnetic resonance imaging (MRI) an osteolitic lesion of the proximal humerus.
Results. The smears of the Case A patient, as well as the cell-block set-up, showed a clear cell neoplasm with alternation of eosinophilic elements, arranged in nests or pseudo-glandular structures. The immunocytochemistry performed on the cell-block showed diffuse and intense positivity for CD10, PAX8 and TFE3, supporting the diagnosis of Xp11 translocation RCC. Case B patient underwent biopsy, who showed diffuse infiltration from a neoplasia composed by cells arranged in primarily solid and papillary patterns, with abundant clear to eosinophilic cytoplasm and prominent nucleoli. The immunohistochemical analysis performed showed positivity for RCC, CD10 and PAX8, while CK7, Trombomodulin, Uroplakin III and GATA3 were negative. Also TFE3 break-apart FISH was performed exhibiting TFE3-gene translocation, so a diagnosis of Xp11 translocation RCC was performed.
Conclusions. The diagnosis of Xp11 translocation renal cell carcinoma in metastasis is very rarely described. Moreover, the cytologic findings of this neoplasm are very limited in the literature and its cytological features remains poorly understood. Although the histological aspect of the neoplasm is well known, this tumor is often underestimated as a rare pathology, and even more rarely diagnosed on metastasis. In conclusion, in adult patients with renal mass, with a rapidly progressive clinical history, presenting cytologically or histologically a variable morphology, with clear cells arranged in solid or papillary pattern, it is necessary to consider this family of neoplasms among the diagnostic hypotheses.

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BLADDER EPICHECK IN HIGH-RISK POPULATION OF BLADDER CARCINOMA

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Background/Aim. Bladder carcinoma (BCa) represents the most expensive cancer due to its high rate of recurrence. The main issue of BCa lies in the long follow-up and in the absence of urinary assays currently able to replace and overcome, in terms of diagnostic accuracy, the current gold-standard of follow-up (based on cystoscopy and cytology), in particular in high-risk population. Bladder EpiCheck is a new urinary test that analyzes DNA methylation biomarkers in order to identify high-risk urothelial cancer.
Materials and methods. A prospective, blinded, single-center, non-randomized phase-2 study was carried out. Urine for testing was collected before standard-of-care cystoscopy/TURBT. Cytology and Bladder Epicheck analysis were performed by two different blinded experienced urocytopathologist.
The inclusion criteria were: patients with high risk BCa (high grade, T1, Carcinoma in situ) in follow-up or as first diagnosis, able to produce 10 ml of urine, and able to consent.
We recruited 170 consecutive patients: 133 patients with history of high risk BCa (60 not treated in the last 3 months and 73 recently treated), 10 with a new diagnosis of high risk BCa; the latter were compared to 27 consecutive subjects undergoing endoscopic evaluation for macrohematuria or positive cytology.
Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of Bladder EpiCheck test were evaluated and compared to cytology and cystoscopy results, taking the confimatory pathology as the reference standard; when absent, the reference standard was considered positive if there was a clinical decision to start oncologic treatment.
Results. In case of first diagnosis, the diagnostic accuracy appeared comparable among the three approaches, with 100% of sensitivity and NPV rates for all of them.
In case of previously (> than 3 months) treated patients, overall sensitivity and NPV of Epicheck (90.5% and 91.1%) appeared higher than cytology (82.3% and 46.4%) and cystoscopy (83.3% and 68.1%); the same in case of recent treatment (95.6% and 97.3% for EpiCheck, 82.6% and 88.6% for cytology, 52.6% and 75.0% for cystoscopy, respectively).
In patients with CIS, sensitivity and NPV did not differ between Epicheck (88.0% and 87.0%, respectively) and cytology (88.0% and 84.2%, respectively) in previously treated patients, while the differences were higher in case of recent treatment (100% and 100% for Epicheck vs. 81.8% and 86.7% for cytology, respectively).
Conclusions. The Bladder EpiCheck test showed very high diagnostic values, higher than the currently gold standard assessment. The test might clinically improve

the BCa management in terms of reduced number of inconclusive/suspicious reports of cytology and endoscopy, reduced number of further examinations, reduced associated patient and economic burdens. The non-invasive and not expensive Bladder EpiCheck should be incorporated in standard BCa follow-up setting.

CILIATED CELL METAPLASIA IN RENAL PELVIS: AN ANUSUAL FINDING

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Objectives. Ciliated epithelial cells have been rarely observed within urothelial lining. In the literature only three reports [1-3] have described this phenomenon, though was never associated with urothelial carcinoma, nor outside the urethra. Herein we illustrate the finding of ciliated pseudostratified columnar cells into the urothelium lining the renal calyx, adjacent to an area of urothelial invasive carcinoma.
Materials and methods. A 82 years old male with history of nephrolithiasis underwent total nephrectomy for a 7,5 x 4 cm mass in the renal pelvis involving the calyces and determining hydronephrosis. Representative samples from the surgical specimen were fixed in 10% buffered formalin and 5-µ paraffin sections were stained with haematoxilin-eosin.
Results. Histological examination revealed an invasive high grade urothelial carcinoma (G3). The urothelial lining adjacent to the lesion was replaced by a layer of pseudostratified columnar ciliated cells covering a linear extension of 5 mm. The cells showed PAS positive vacuoles in the apical cytoplasm.
Conclusions. The presence of ciliated epithelia within urothelial lined system is not well characterized, due to the low number of reported cases. The significance of such phenomenon is still unknown: it could be either a developmental abnormality or a metaplastic change. Further investigations are necessary.

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PRIMARY WHIPPLE'S DISEASE OF THE CNS PRESENTING WITH INTRACEREBRAL MASS LESION: A CASE REPORT

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Objectives. Whipple's disease (WD) is a rare, chronic and systemic illness characterized by intestinal involvement but including also a variety of other organs, especially the lymphatic system and the heart. CNS is a major site of involvement in extraintestinal WD and carries a poor prognosis with a mortality rate of approximately 25% within 4 years of diagnosis. Only a small number of cases of isolated CNS form of WD without intestinal manifestation are reported. Men are affected much more often than women and the mean age of onset approaches 50 although the age range extends from childhood to senility. This rare, chronic, multisystemic infectious disorder is caused by the bacterium Tropheryma Whipplei (TW) wich is related to the family of Actinomyces. It is a weakly gram-positive rod shaped bacillus which is not acid fast-positive. It is 1-2 millimicrons in length and has a thick wall, the inner layer of which stains with PAS reagents and thus accounts for the PAS staining pattern of bacilli and their remnants within macrophages. The mechanism by which this agent traverses the blood brain barrier and escapes host defense remains unknown. Successful antibiotic treatment had been reported: patients with WD who are treated with antibiotics get better with the disappearance of bacilli. We report a rare case, with only a small number of cases previously described, of WD confined to the CNS illustrating the diagnostic difficulties in this context.
Material and methods. A of 27-year-old male was admitted in our hospital in May 2018 with recent history of increasingly severe headaches, vomiting, drowsiness. He had no rash, fever, lymphadenopathy or other abnormality on general examination. Routine investigation were all normal. No immunological abnormalities were evident in our patient. An MRI scan showed a high signal mass lesion in the right temporo-insulo-frontal lobe with infiltrative portions that displaced the midline structures and partially enhanced following intravenous gadolinium administration.
Glioma was suspected on the imaging results. Excision of right cerebral lesion was performed by open craniotomy. The tissue was fixed and processed with standard methods for light microscopy.
Results. The histological diagnosis of low-grade astrocytoma was suggested according to the diagnosis of glioma on MRI. During the follow-up, in November 2018, MRI showed a right cerebellar lesion that misured 1 cm, with mass effect on the vermian and paravermian structures. The lesion displaced the IV ventricle to the left. Following the appearance of the new cerebellar lesion, the patient asked for a second opinion at a center of high specialization. On that occasion the diagnosis of WD was suspected. PAS staining highlighted and confirmed the presence of granular, foamy PAS-positive macrophages. Microscopically, a gliovascular tissue was observed with a remarkable number of large astrocytes and perivascular cuffs of mononuclear cells, as well as smaller collections of foamy macrophages and small nodules or granulomas scattered in grey matter of the cerebral cortex. Those granulomas have been shown to contain strongly positive PAS staining macrophages surrounded by large astrocytes. The number of astrocytes was increased but there was no evidence of moderate/severe atypia, mitosis, necrosis, and the Ki-67 index was 2%. The foamy cells were positive in the immunohistochemical investigations for CD68 (PGM1), negative for GFAP and S100. The liquor examination was normal, including the culture test and Borrelia's research. Negative the search for TW with PCR on brain tissue included in paraffin wax, liquor,

feces and urine. The patient started antibiotic therapy. At the next check MRI did not confirm the previous cerebellar lesion.

Conclusions. We report a rare case of WD of SNC presented with symptoms of rapidly evolving raised intracranial pressure in the absence of other systemic symptoms with mass lesion on CT and MRI. It was diagnosed as glioma on the neuroimaging results and initially confirmed by histological examination. The appearance, after a short time, of a new lesion in the cerebellar site, an unusual event in a low-grade astrocytoma, led to a revision of the case and of the brain biopsy at a referential histopathological laboratory center, where WD diagnosis of the CNS was done. Brain imaging techniques are not diagnostic. On clinical ground it can be appreciated that the differential diagnosis of WD of the CNS encompasses a large slice of neurology. The brain biopsy is essential for the diagnosis of CNS WD. It is particularly difficult when the patient does not show symptoms outside the CNS and the neuroradiological images show expansive-infiltrative brain lesions as in our case, suggestive of neoplasia. Reactive astrocytosis can be mistaken for low-grade glial neoplastic proliferation. Granulomatous diseases, including sarcoid granulomas and tubercolosis can cause diagnostic confusion. The presence of foamy PAS-positive macrophages, scattered between astrocytic proliferation or aggregates in small granulomas, must make the pathologist think of WD of the CNS.

The diagnosis should be confirmed with a PCR assay against Tropheryma Whipplei performed on brain tissue or CSF. It is now the diagnostic method of choice, although its limitations have not yet been defined. In conclusion, although the WD confined to the CNS is very rare, it is necessary for the clinician and for the pathologist to include it among the differential diagnoses of neoplastic diseases and not.

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