



PATHOLOGICA

Journal of the Italian Society of Anatomic Pathology
and Diagnostic Cytopathology,
Italian Division of the International Academy of Pathology

REVIEW

- 1 Inflammatory myofibroblastic tumour: a rare entity with wide differential diagnosis
S.M. Gilani, P.J. Kowalski

ORIGINAL ARTICLE

- 7 Combined hepato-cholangiocarcinoma arising in a gallbladder intracystic papillary neoplasm. A new view on so-called "hepatoid adenocarcinoma of the gallbladder"
J.B. Dettoni, W. Andrauss, N.D. Theise, M.S. Rocha, E.S. Mello, C.H.L. Rocha, T.E.F. Pfiffer, V.A.F. Alves

CASE REPORTS

- 14 A rare case of transmural endometriosis in primary adenocarcinoma of the rectum
M. Falleni, D. Bauer, E. Opocher, L. Moneghini, G.P. Bulfamante
- 16 Collision tumour of the breast composed of Merkel cell carcinoma and invasive ductal carcinoma: a case report
D. Nedved, C. Connor, P. Sharma, M. O'Neil
- 22 Intestinal tuberculosis: a diagnostically-challenging case misdiagnosed as Crohn's disease at colorectal biopsy
M. Onorati, D. Morganti, M. Bocchi, E. Colombo, G. Petracco, P. Uboldi, F. Di Nuovo
- 26 Primary tumour of the round ligament of the liver: a case presentation
J.A. Solarana Ortíz, J.E. Placencia Gilart, M. Rodríguez Diéguez, Z. Miranda Moles, M. Pullés Labadié, J.C. Lau Cuza, S. Corpas Fuster
- 29 Metastasizing pleomorphic adenoma of the submandibular gland: a case report
S. Miladi, S. Mestiri, W. Kermani, S. Ziadi, B. Sriha, K. Bouzouita, M. Mokni
- 32 Fibroadenoma in an ectopic vulvar breast gland: a common neoplasm in an uncommon site
A. Ayadi-Kaddour, A. Khadhar, M. Mlika, E. Braham, O. Ismail, D. Zegal, F. El Mezni

01

Vol. 106 March 2014



SIAPEC-IAP

Società Italiana di Anatomia Patologica e Citopatologia Diagnostica,
Divisione Italiana della International Academy of Pathology

PACINI
EDITORE
MEDICINA



PATHOLOGICA

Journal of the Italian Society of Anatomic Pathology
and Diagnostic Cytopathology,
Italian Division of the International Academy of Pathology

Editor-in-Chief

Marco Chilosi, Verona

Associate Editor

Roberto Fiocca, Genova

Managing Editor

Roberto Bandelloni, Genova

Scientific Board

R. Alaggio, Padova
G. Angeli, Vercelli
M. Barbaresi, Trento
C.A. Beltrami, Udine
G. Bevilacqua, Pisa
M. Bisceglia, S. Giovanni R.
A. Bondi, Bologna
F. Bonetti, Verona
C. Bordini, Parma
A.M. Buccoliero, Firenze
G.P. Bulfamante, Milano
G. Bussolati, Torino
A. Cavazza, Reggio Emilia
G. Cenacchi, Bologna
P. Ceppa, Genova
C. Clemente, Milano
M. Colecchia, Milano
G. Collina, Bologna
P. Cossu-Rocca, Sassari
P. Dalla Palma, Trento
G. De Rosa, Napoli
A.P. Dei Tos, Treviso
L. Di Bonito, Trieste
C. Doglioni, Milano
V. Eusebi, Bologna
G. Faa, Cagliari
F. Facchetti, Brescia
G. Fadda, Roma
G. Fornaciari, Pisa
M.P. Foschini, Bologna
F. Fraggetta, Catania
E. Fulcheri, Genova
P. Gallo, Roma
F. Giangaspero, Roma
W.F. Grigioni, Bologna

G. Inghirami, Torino
L. Leoncini, Siena
M. Lestani, Arzignano
G. Magro, Catania
A. Maiorana, Modena
E. Maiorano, Bari
T. Marafioti, Londra
A. Marchetti, Chieti
D. Massi, Firenze
M. Melato, Trieste
F. Menestrina, Verona
G. Monga, Novara
R. Montironi, Ancona
B. Murer, Mestre
V. Ninfo, Padova
M. Papotti, Torino
M. Paulli, Pavia
G. Pelosi, Milano
G. Peffinato, Napoli
S. Pilieri, Bologna
R. Pisa, Roma
M.R. Raspollini, Firenze
L. Resta, Bari
G. Rindi, Parma
M. Risio, Torino
A. Rizzo, Palermo
J. Rosai, Milano
G. Rossi, Modena
L. Ruco, Roma
M. Rugge, Padova
M. Santucci, Firenze
A. Scarpa, Verona
A. Sidoni, Perugia
G. Stanta, Trieste
G. Tallini, Bologna
G. Thiene, Padova
P. Tosi, Siena
M. Truini, Genova
V. Villanacci, Brescia
G. Zamboni, Verona
G.F. Zannoni, Roma

Editorial Secretariat

G. Martignoni, Verona
M. Brunelli, Verona

Governing Board SIAPEC-IAP

President:

G. De Rosa, Napoli

President Elect:

M. Truini, Genova

General Secretary:

L. Molinaro, Torino

Past President:

C. Clemente, Milano

Members:

M. Basciu, Monza
A. Bondi, Bologna
G. Boffi, Napoli
F. Crivelli, Busto Arsizio
A. Fassina, Padova
R. Fiocca, Genova
A.M. Florena, Palermo
A. Marchetti, Chieti
D. Massi, Firenze
G. Mazzoleni, Bolzano
L. Resta, Bari
G. Tinacci, Firenze

Associate Members

Representative:

T. Zanin, Genova

Copyright

Società Italiana di Anatomia
Patologica e Citopatologia
Diagnostica, Divisione
Italiana della International
Academy of Pathology

Publisher

Pacini Editore S.p.A.
Via Gherardesca, 1
56121 Pisa, Italy
Tel. +39 050 313011
Fax +39 050 3130300
info@pacinieditore.it
www.pacinimedica.it

01

Vol. 106 March 2014



SIAPEC-IAP

Società Italiana di Anatomia Patologica e Citopatologia Diagnostica,
Divisione Italiana della International Academy of Pathology

PACINI
EDITORE
MEDICINA

Information for Authors including editorial standards for the preparation of manuscripts

Pathologica is intended to provide a medium for the communication of results and ideas in the field of morphological research on human diseases in general and on human pathology in particular. The Journal welcomes contributions concerned with experimental morphology, ultrastructural research, immunocytochemical analysis, and molecular biology. Reports of work in other fields relevant to the understanding of human pathology may be submitted as well as papers on the application of new methods and techniques in pathology. The official language of the journal is English.

Authors are invited to submit manuscripts according to the following instructions by E-mail to:

pathologica@pacinieditore.it, landreazzi@pacinieditore.it

Lisa Andreazzi - Editorial Office

Pathologica - c/o Pacini Editore S.p.A.

Via Gherardesca 1, 56121 Pisa, Italy - Tel. +39 050 3130285

The files containing the article, illustrations and tables must be sent in attachment and the following statement of Authors must also be enclosed: separate letter, signed by every Author, stating that the submitted material has not been previously published, and is not under consideration (as a whole or partly) elsewhere, that its content corresponds to the regulations currently in force regarding ethics research and that copyright is transferred to the Publisher in case of publication. If an experiment on humans is described, a statement must be included that the work was performed in accordance to the principles of the Declaration of Helsinki (1975, rev. 2000) and Authors must state that they have obtained the informed consent of patients for their participation in the experiments and for the reproduction of photographs. As regards the studies performed on laboratory animals, Authors must state that the relevant national laws or institutional guidelines have been observed. The Authors are solely responsible for the statements made in their article. Authors must also declare if they got funds, or other forms of personal or institutional financing from Companies whose products are mentioned in the article.

Editorial standards for the preparation of manuscripts

The article, exclusively in English, should be saved in .RTF format. Do not use, under any circumstances, graphical layout programmes such as Publisher™, Pagemaker™, Quark X-press™, Adobe Indesign™. Do not format the text in any way (avoid styles, borders, shading ...); use only character styles such as italics, bold, underlined. Do not send the text in PDF. Text and individual tables must be stored in separate files.

When submitting a manuscript Authors should consider the following points/items:

- a) Title of the work.
- b) Names of the Authors and Institute or Organization to which each Author belongs.
- c) Section in which the Authors intend the article to be published (although the final decision rests with the Editor-in-Chief).
- d) Name, address, telephone number and E-mail address of the Author to whom correspondence and galley proofs should be sent.
- e) 3-5 key words.
- f) Abstract, less than 250 words and subdivided into the following sections: Introduction, Method(s), Results, Discussion. In the Introduction section, the aim (or the aims) of the article must be clearly summarised (i.e., the hypothesis Authors want to verify); in the Method(s) section, the Authors must report the context of the study, the number and the kind of subjects under analysis, the kind of treatment and statistical analysis used. In the Results section Authors must report the results of the study and of the statistical analysis. In the Discussion section Authors must report the significance of the results with regard to clinical implications.
- g) References must be limited to the most essential and relevant, identified in the text by Arabic numbers and listed at the end of the manuscript in the order in which they are cited. The format of the references should conform with the examples provided in *N Engl J Med* 1997;336:309-15. The first six authors must be indicated, followed by "et al". Journals should be cited according to the abbreviations reported on Pubmed. Examples of correct format for bibliographic citations:

Journal articles: Jones SJ, Boyede A. *Some morphological observations on osteoclasts*. *Cell Tissue Res* 1977;185:387-97.

Books: Taussig MJ. *Processes in pathology and microbiology*. Oxford: Blackwell 1984.

Chapters from books: Vaughan MK. *Pineal peptides: an overview*. In: Reiter RJ, ed. *The pineal gland*. New York: Raven 1984, pp. 39-81.

- h) Acknowledgements and information on grants or any other forms of financial support must be cited at the end of the references.
- i) Notes to the text, indicated by an asterisk or similar symbol, should be shown at the bottom of the page.

Tables must be limited in number (the same data should not be presented twice, in both text and tables), typewritten one to a page, and numbered consecutively with Roman numbers.

Illustrations: Send illustrations in separate files from text and tables.

Software and format: preferably send images in .TIFF or .EPS format, resolution at least 300 dpi (100 x 150 mm). Other possible formats: .JPEG, .PDF, .PPT (Powerpoint files). Please do not include, when possible, illustrations in .DOC files. Insert an extension that identifies the file format (example: .Tif; .Eps).

Drugs should be referred to by their chemical name; the commercial name should be used only when absolutely unavoidable (capitalizing the first letter of the product name and giving the name of the pharmaceutical firm manufacturing the drug, town and country).

The editorial office accepts only papers that have been prepared in strict conformity with the general and specific editorial standards for each survey. The acceptance of the papers is subject to a critical revision by experts in the field, to the implementation of any changes requested, and to the final decision of the Editor-in-Chief.

Authors are required to correct and return (within 3 days of their mailing) only the first set of galley proofs of their paper. Authors may order reprints, at the moment they return the corrected proofs by filling in the reprint order form enclosed with the proofs.

Applications for advertisement space should be directed to:

Pathologica - Pacini Editore S.p.A., Via Gherardesca, 56121 Pisa, Italy

Tel. +39 050 3130217 - Fax +39 050 3130300

E-mail: mmori@pacinieditore.it

Subscription information

Pathologica publishes four issues per year. The annual subscription rates for non-members are as follows:

Italy € 105,00; all other countries € 115,00. This issue € 20,00 for Italy, € 25,00 abroad.

Subscription orders and enquiries should be sent to: *Pathologica* - Pacini Editore S.p.A. - Via Gherardesca, 56121 Pisa, Italy E-mail: abbonamenti@pacinieditore.it - On line subscriptions: www.pacinimedica.it

Subscribers' data are treated in accordance with the provisions of the Legislative Decree, 30 June 2003, n. 196 – by means of computers operated by personnel, specifically responsible. These data are used by the Publisher to mail this publication. In accordance with Article 7 of the Legislative Decree n. 196/2003, subscribers can, at any time, view, change or delete their personal data or withdraw their use by writing to Pacini Editore S.p.A. - Via A. Gherardesca 1, 56121 Pisa, Italy.

Photocopies, for personal use, are permitted within the limits of 15% of each publication by following payment to SIAE of the charge due, article 68, paragraphs 4 and 5 of the Law April 22, 1941, n. 633. Reproductions for professional or commercial use or for any other other purpose other than personal use can be made following a written request and specific authorization in writing from AIDRO, Corso di Porta Romana, 108, 20122 Milan, Italy (segreteria@aidro.org – www.aidro.org).

The Publisher remains at the complete disposal of those with rights whom it was impossible to contact, and for any omissions.

Journal printed with total chlorine free paper and water varnishing.

Printed by Pacini Editore, Pisa, Italy - April 2014

CONTENTS

REVIEW

Inflammatory myofibroblastic tumour: a rare entity with wide differential diagnosis

S.M. Gilani, P.J. Kowalski

Inflammatory myofibroblastic tumour (IMT) is a rare, distinctive mesenchymal neoplasm. Grossly, it appears as a circumscribed mass with a rubbery to firm cut surface. Microscopically, it is characterized by a spindle cell proliferation within a myxoid stroma with admixed plasma cells, lymphocytes and eosinophils. Immunohistochemical staining is usually positive for vimentin, smooth muscle actin (SMA) and anaplastic lymphoma kinase (ALK). ALK gene rearrangement is present in approximately 50-70% IMTs. The standard treatment is surgical resection, and it is essential to differentiate IMT from benign and malignant mimickers so that appropriate therapy may be provided. Clinical and radiological follow-up is required to detect recurrence.

ORIGINAL ARTICLE

Combined hepato-cholangiocarcinoma arising in a gallbladder intracystic papillary neoplasm. A new view on so-called "hepatoid adenocarcinoma of the gallbladder"

J.B. Dettoni, W. Andrauss, N.D. Theise, M.S. Rocha, E.S. Mello, C.H.L. Rocha, T.E.F. Pffiffer, V.A.F. Alves

A 62-year-old female presented with abdominal pain, weight loss of 20 kg in the prior 6 months, and a palpable mass in the right upper quadrant during physical exam. Standard liver tests, including screening for hepatitis B and C and alpha-fetoprotein were negative or within normal limits. Computerized tomography depicted a transmural gallbladder tumor infiltrating into the adjacent liver with an irregular ill-defined mass occupying segments IV-V-VI, measuring 13.0 x 9.2 x 8.5 cm, with a solid-cystic component and heterogeneous captation of endovenous contrast media. Complete surgical resection of the neoplasm was achieved through an extended cholecystectomy and excision of hepatic segments IV, V and VI, with an uneventful follow-up 18 months until now. Morphological and immunohistochemical assessment favored a diagnosis of combined hepatocellular-cholangiocarcinoma arising in a gallbladder intracystic papillary neoplasm with invasive carcinoma. This case raises the hypothesis that the so-called "hepatoid adenocarcinoma of the gallbladder" may presently be better understood as a neoplasm derived from hepatobiliary stem/progenitor cells. Such cells have been recognized in the canals of Hering, in peribiliary glands within the liver and in the extrahepatic biliary tree, and in gallbladder mucosa.

CASE REPORTS

A rare case of transmural endometriosis in primary adenocarcinoma of the rectum

M. Falleni, D. Bauer, E. Opocher, L. Moneghini, G.P. Bulfamante

Intestinal endometriosis of the rectum and sigmoid colon, occurring in up to 34% of pelvic endometriosis, mimics a wide number of conditions that are difficult to differentiate from inflammatory or malignant diseases. Herein we report the first case of transmural endometriosis concomitant with advanced primary rectal adenocarcinoma, presenting with obstructive symptoms. Correct diagnosis based on morphological identification and immunohistochemical characterization of the two entities is crucial for treatment.

Collision tumour of the breast composed of Merkel cell carcinoma and invasive ductal carcinoma: a case report

D. Nedved, C. Connor, P. Sharma, M. O'Neil

We report a case of a 71-year-old female with a palpable breast mass. Pathologic evaluation of the breast mass showed a unique collision tumour with a high-grade invasive and in-situ ductal carcinoma component and a high-grade neuroendocrine carcinoma component. The neuroendocrine component turned out to be Merkel cell carcinoma (MCC), with immunohistochemical confirmation. To the

best of our knowledge, this is the first case report of a collision tumour with ordinary ductal carcinoma and MCC in the breast.

Intestinal tuberculosis: a diagnostically-challenging case misdiagnosed as Crohn's disease at colorectal biopsy

M. Onorati, D. Morganti, M. Bocchi, E. Colombo, G. Petracco, P. Uboldi, F. Di Nuovo

The clinical presentation of two different digestive diseases such as Crohn's disease and intestinal tuberculosis may be so similar to induce a delay in correct diagnosis and appropriate treatment (immune suppression versus antibiotic therapy). Herein, we describe the case of a young man from Eastern Europe who came to our observation complaining of clinical symptoms initially misdiagnosed as an inflammatory bowel disease. It is important to keep in mind the possibility of an active tubercular disease, particularly in patients coming from countries endemic for the disease. Morphological findings of sarcoid-like granulomas at biopsy is not enough for a conclusive diagnosis of Crohn's disease, and tuberculosis should be ruled out on the basis of clinical information, laboratory tests and radiological imaging.

Primary tumour of the round ligament of the liver: a case presentation

J.A. Solarana Ortíz, J.E. Placencia Gilart, M. Rodríguez Diéguez, Z. Miranda Moles, M. Pullés Labadié, J.C. Lau Cuza, S. Corpas Fuster

A 40-year-old Caucasian female patient presented to the outpatient General Surgery ward in "V. I. Lenin" Teaching Hospital complaining of a recurrent mesogastric pain that had lasted for 3 months. Physical examination showed a palpable mass confined to that area. She was then admitted with diagnosis of an abdominal tumour. Diagnostic work-up revealed that the process involved the round ligament of the liver, which is an exceptional localization, which motivated us to publish this case after surgical treatment by excision, having also taken into account the results of histopathology which revealed a PEComa, confirmed by immunohistochemistry. After reviewing the available literature, the low incidence of these lesions, as well as the unusual histological variety, makes the present case one of interest.

Metastasizing pleomorphic adenoma of the submandibular gland: a case report

S. Miladi, S. Mestiri, W. Kermani, S. Ziadi, B. Sriha, K. Bouzouita, M. Mokni

Pleomorphic adenoma (PA), originally called mixed tumour, is the most common neoplasm of the salivary glands. It is usually a benign, slow-growing and well-circumscribed tumour. However, PA may occasionally give rise to metastases that usually occur after a previous recurrence. These tumours display benign histological features in both primary tumours and metastases. Such tumours have been termed metastatic PA or metastatic mixed tumours. We report a case of metastatic PA of the submandibular gland with metastasis to the cervical lymph nodes.

Fibroadenoma in an ectopic vulvar breast gland: a common neoplasm in an uncommon site

A. Ayadi-Kaddour, A. Khadhar, M. Mlika, E. Braham, O. Ismail, D. Zegal, F. El Mezni

Ectopic breast tissue is defined as glands located outside of the breast. It can be found anywhere along the milk line extending from the axilla to the groin, and can occur in the vulva. Ectopic breast tissue should be excised because it may develop benign or malignant pathologic processes. Less than 40 cases of fibroadenoma in the vulva have been reported in the literature. We report a case of a 37-year-old woman presenting a solitary vulvar mass. The mass was excised completely, and histology demonstrated an ectopic breast fibroadenoma. This is one of the few reports on the benign pathologies of vulvar mammary glands.

Inflammatory myofibroblastic tumour: a rare entity with wide differential diagnosis

S.M. GILANI, P.J. KOWALSKI

Department of Pathology, St. John Hospital & Medical Center, Detroit, USA

Key words

Myofibroblastic tumour • Anaplastic lymphoma kinase • Surgical resection • Follow-up

Summary

Inflammatory myofibroblastic tumour (IMT) is a rare, distinctive mesenchymal neoplasm. Grossly, it appears as a circumscribed mass with a rubbery to firm cut surface. Microscopically, it is characterized by a spindle cell proliferation within a myxoid stroma with admixed plasma cells, lymphocytes and eosinophils. Immunohistochemical staining is usually positive for vimentin,

smooth muscle actin (SMA) and anaplastic lymphoma kinase (ALK). ALK gene rearrangement is present in approximately 50-70% IMTs. The standard treatment is surgical resection, and it is essential to differentiate IMT from benign and malignant mimickers so that appropriate therapy may be provided. Clinical and radiological follow-up is required to detect recurrence.

Introduction

Inflammatory myofibroblastic tumour (IMT) is a rare mesenchymal proliferation. Much of what today is encompassed by IMT includes many lesions historically called inflammatory pseudotumour, plasma cell granuloma, omental-mesenteric myxoid hamartoma, etc. A rearrangement of the anaplastic lymphoma kinase (ALK) gene at chromosome 2p23 is present in approximately 50% of IMTs, suggesting that this entity is a neoplastic, as opposed to a reactive, process^{1,2}. IMT occurs in many body sites, but has been most frequently reported in the viscera, soft tissue and lungs of children and adults. IMTs have a tendency for local tissue infiltration, occasional rapid growth, and local recurrence. IMT may be confused with not only neoplasms (malignant fibrous histiocytoma, dendritic cell neoplasms and myxoid fibrosarcoma), but also with benign, inflammatory conditions (inflammatory pseudotumour and nodular fasciitis).

Clinical features and imaging

IMT may arise at any an anatomical site, with the lungs and retroperitoneum as the most common primary locations³. Yu et al. found that IMT is one of the most

common paediatric lung tumours⁴. Other sites include gastrointestinal tract, omentum, mesentery, head and neck, central nervous system, orbit, spleen, soft tissues and genitourinary system (including bladder and prostate)^{3,5}. Involvement of the colon and intestines is very rare, and may produce intestinal obstruction or intussusception^{6,7}. IMT associated with Sjögren's disease, systemic lupus erythematosus and non-Hodgkin lymphoma of the lung has been reported^{7,8}. Associated laboratory findings include elevated erythrocyte sedimentation rate (ESR), variable white blood cell count (WBC), anaemia, thrombocytosis and hypergammaglobulinemia².

Imaging studies, especially magnetic resonance imaging (MRI), may aid in identifying a mass lesion in involved tissues, however, a diagnosis of IMT cannot be made solely on radiologic findings. Positron emission tomography (PET) scanning is helpful in evaluating the presence of local invasion, in assessing the aggressiveness of the lesion and in monitoring patient response after surgical resection⁹.

Pathological features

IMTs usually present as a multilobular or well-circumscribed mass, with rubbery to firm cut surface, depending

Correspondence

Syed M. Gilani, Department of Pathology, St. John Hospital & Medical Center, Detroit, MI 48236, USA - E-mail: magilani@hotmail.com

on the amount of myxoid stroma present. Focal haemorrhage, necrosis and calcifications may be present. The tumour size is variable ranging from 1 to 17 cm with a mean diameter of 6.4 cm¹⁰.

IMTs have three classical architectural patterns. The most common is a proliferation of bland spindle or stellate-shaped cells distributed haphazardly within a variably myxoid stroma (Fig. 1) with admixed lymphocytes, plasma cells, neutrophils and eosinophils (Fig. 2). Less commonly, densely packed stromal cells are arranged in a storiform pattern with intermingled inflammatory cells. The third rarest pattern resembles a desmoid tumour. In one study, it was demonstrated that all cases (100%) of IMTs showed infiltrative borders¹⁰. Regardless of the pattern, the spindle-shaped myofibroblasts lack cytological atypia and nuclear pleomorphism. The myofibroblasts have eosinophilic to amphophilic

cytoplasm and nuclei with open, vesicular chromatin (Fig. 3). Mitotic activity is variable but generally minimal (1-2/high power field) and without atypical forms. Calcifications and foci of metaplastic bone may be seen. The morphologic spectrum varies from classic appearing IMT to those with an atypical histologic appearance. Atypical morphological features include ganglion-like cells, abundant cellularity, nuclear pleomorphism, atypia and mitotic figures (typical and atypical). Historically, these features were thought to portend poor prognosis, but currently there is no evidence to support this³. The rare epithelioid or round cell morphologic variant of IMT with aggressive clinical behaviour has been described in the literature. This pattern shows neoplastic cells that are round to epithelioid in appearance, disposed in a myxoid background with admixed inflammatory infiltrates. A less prominent spindle cell component and foci of ne-

Fig. 1. Haematoxylin-eosin-stained section highlighting proliferation of spindle cells distributed evenly in the myxoid appearing stroma with background inflammatory cells (original magnification x 100).

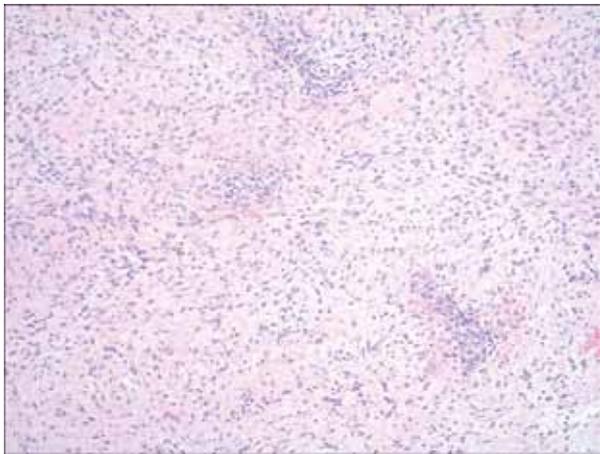


Fig. 3. Haematoxylin-eosin-stained section showing the myofibroblasts with abundant, eosinophilic to amphophilic, fibrillary cytoplasm. There are background lymphocytes, plasma cells, neutrophils and eosinophils (original magnification x 400).

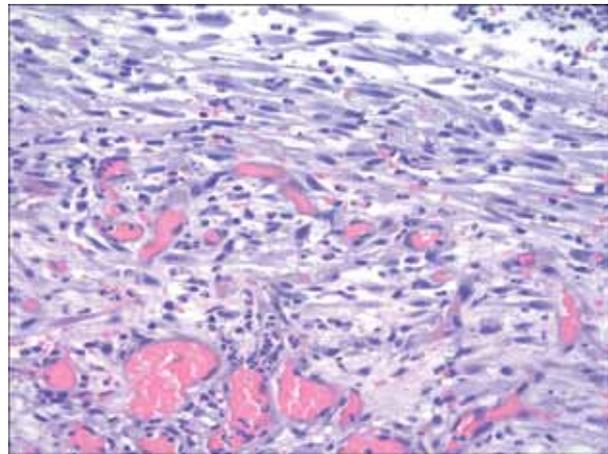


Fig. 2. Haematoxylin-eosin-stained section showing the admixture of spindle cells, plasma cells and lymphocytes (original magnification x 200).

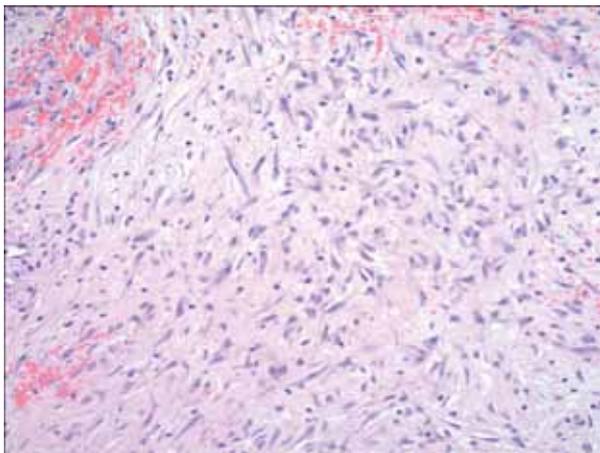
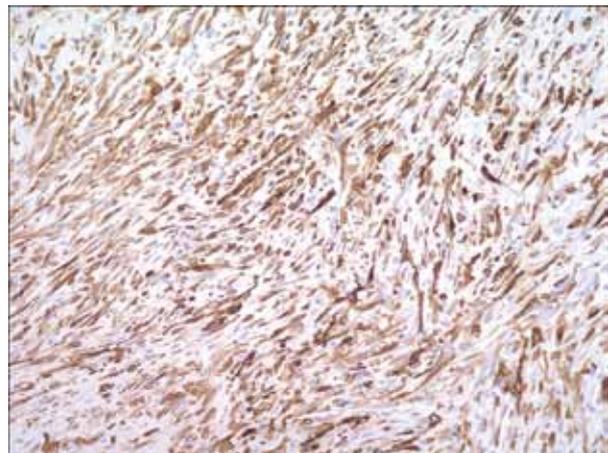


Fig. 4. Immunohistochemical staining with smooth muscle actin (SMA) demonstrating diffuse staining of myofibroblasts. (Original magnification x 200).



crisis can be seen. The mitotic activity is variable. Immunohistochemically, neoplastic cells exhibit predominantly a nuclear membrane staining pattern and, less frequently, cytoplasmic expression of ALK¹¹.

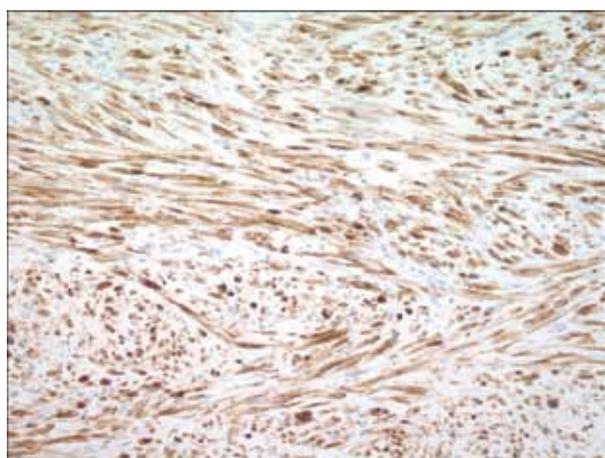
The evaluation of IMT on frozen sections is a diagnostic challenge as it can mimic various reactive and neoplastic lesions. Immunohistochemically, the myofibroblastic spindle cells are frequently immunoreactive for vimentin, smooth muscle actin (SMA) (Fig. 4), muscle-specific actin and less frequently for desmin, cytokeratin and CD68. Cytoplasmic immunoreactivity with anaplastic lymphoma kinase (ALK) in 50-70% of cases has also been demonstrated in IMT (Fig. 5)⁹. ALK expression has also been reported in other non-IMT mesenchymal tumours (malignant peripheral nerve sheath tumour, rhabdomyosarcoma, leiomyosarcoma and malignant fibrous histiocytoma) which raise the possibility that a subset of mesenchymal neoplasms harbour a common mutation, although the exact mechanism is not clear¹². IMT can show variable cellular atypia, nuclear pleomorphism, elevated mitotic activity with atypical mitotic figures, overexpression of p53 and aneuploidy, but these findings have no proven correlation as a predictor of aggressive behaviour¹². Overexpression of Ki-67 is usually seen in highly proliferative lesions.

Ultrastructurally, the spindle cells that compose IMTs exhibit a poorly-developed Golgi apparatus and rough endoplasmic reticulum, as well as abundant extracellular collagen consistent with myofibroblastic and fibroblastic differentiation^{12,13}.

Genetics and biology

Recent genetic findings have played a crucial role in conceptualizing IMT as a neoplastic process, while the importance of molecular findings regarding biologic behaviour remains uncertain. IMTs have been shown to exhibit cy-

Fig. 5. Immunohistochemical staining with anaplastic lymphoma kinase (ALK) showing diffuse staining of myofibroblasts. (Original magnification x 200).



togenetic clonal rearrangement of the anaplastic lymphoma kinase (ALK) gene on chromosome 2p23^{14,15}. This abnormality can be detected by conventional cytogenetics, fluorescence in-situ hybridization (FISH) and reverse transcription polymerase chain reaction in approximately 50% of IMTs¹⁶⁻¹⁸. A fusion gene involving tropomyosin (TPM) N-terminal coiled domains and ALK C-terminal domain may play a key role in pathogenesis¹⁹. Other fusion genes, including tropomyosin 3 gene (TPM3), tropomyosin 4 gene (TPM4), SEC31 homolog A (*S. cerevisiae*) (SEC31L1), ran-binding protein 2 (RANBP2), cysteinyl-tRNA synthetase (CARS) and clathrin heavy chain gene (CTLC), have also been reported^{3,19,20}. Immunohistochemical staining with ALK and p80 are often overexpressed, both of which are indicators of the 2p23 abnormality²¹⁻²³. However, the immunohistochemical staining pattern of ALK gene is variable depending on the type of the fusion gene. This can be cytoplasmic in TPM3, TPM4 and SEC31L1 fusion, while RANBP2 fusion products exhibit nuclear expression¹¹. Some authors suggest that RANBP2 gene fusion may be associated with IMTs having an aggressive clinical course^{11,18}.

In one study, Coffin et al. demonstrated patients with ALK-negative IMTs are typically older adults, whose tumours have increased frequency of metastases and atypical mitoses. In contrast, ALK-positive IMTs were associated with younger age and increased frequency of local recurrences^{3,22}. However, morphologic evaluation cannot reliably predict ALK status in any individual case. ALK-negative tumours pose the additional problem of being more difficult to separate from alternative diagnoses, including both inflammatory and neoplastic entities. ALK-negative cases are, in practicality, considered most likely neoplastic, based upon similar histology with ALK-positive tumours. However, this is controversial and reflects a lack of understanding regarding the full impact of ALK-negativity. Molecular abnormalities, in association with a better understanding of biologic potential, may eventually supplant histologic classification for ALK-negative tumours.

The presence of an ALK abnormality also raises the question as to its significance as a prognostic factor in IMT. While ALK chromosome translocations {t(1;2)(q25;p23)}, along with the expression of other markers, may have prognostic significance in anaplastic large cell lymphoma (ALCL), this cytogenetic correlation is as yet unclear in IMT^{24,25}.

The ALK positive IMT patients may receive targeted therapy. Crizotinib, an ALK inhibitor, in conjunction with surgery may be useful in ALK positive cases that are complicated by local recurrence whereas ALK negative IMT are nonresponsive to crizotinib²⁶.

Differential diagnosis

The differential diagnosis of IMT is quite broad and, depending on the anatomic site, includes inflammatory pseudotumour, dendritic cell neoplasms, low-grade sar-

comas, sarcomatoid variant of ALCL, low grade myofibroblastic sarcoma and nodular fasciitis. These entities can share some common histopathological findings. However, their clinical behaviour is highly variable. *Inflammatory pseudotumour* (IPT) is a non-neoplastic inflammatory lesion that is not limited to myofibroblasts. In the past, some have considered IPT and IMT to be synonymous terms but according to review of the current literature, molecular and immunophenotypic findings have helped to separate these entities^{1 14 18}. While the diagnoses of IMT and IPT have been used interchangeably, IPT can now be regarded as a heterogeneous group of lesions, composed of various amounts of inflammatory cells and fibrosis that may mimic a neoplasm on imaging studies, but it is not neoplastic²⁷. IMT and IPT share certain histologic features; however a high mitotic count and proliferative index, cytologic atypia, positive ALK immunoreactivity and rapid recurrence after initial resection are supportive of a diagnosis of IMT. A *dendritic cell neoplasm* consists of uniform, oval-to-spindle cells with smooth nuclear membranes and background lymphocytes. Follicular dendritic cells stain positive for CD21 and CD35, and interdigitating dendritic cell tumours are immunoreactive with CD1a. Low-grade sarcomas like *myxoid fibrosarcomas* are characterized by a proliferation of bland appearing spindle cells with focal atypia and a background that is more fibrous than myxoid. *Sarcomatoid variant of ALCL* shares some of the histologic features such as storiform pattern and myxoid stroma with IMT but, like ALCL, is T-cell in origin which is not true for IMT¹⁷. *Nodular fasciitis* is characterized by fibroblasts arranged in short bundles and fascicles. The intervening matrix is rich in mucopolysaccharides and the spindle cells are bland, without significant atypia. Some chronic inflammatory cells may be seen in the stroma. In cases with lung involvement, all of the above diagnoses and, in addition, *solitary fibrous tumour* may potentially be considered in the differential diagnosis. *Solitary fibrous tumours* consist of fibroblast-like cells in a patternless arrangement, with hypocellular- and hypercellular areas separated by thick, hyalinized collagen with cracking artefact and a haemangiopericytoma-like vascular pattern. These tumours usually stain positively for CD34 and CD99.

The differential diagnosis is variable according to the site of presentation. *Desmoid tumours* may be considered in the differential diagnosis, especially if IMT is involving the abdominal region. *Desmoid tumours* show a myofibroblastic proliferation set within a variable amount of collagen. A subset of desmoid tumour is associated with beta catenin mutation²⁷. They have less inflammation compared to IMT and do not express ALK.

IMT can involve the urinary bladder, and in these cases, a *postoperative spindle cell nodule* (PSCN), a pseudosarcomatous myofibroblastic proliferation, is also a diagnostic consideration. The pathogenesis of PSCN is uncertain, as is its classification as a neoplastic or reactive process^{28 29}. However, the presence of a prior history of instrumentation and injury with a resulting spindle cell myofibroblas-

tic proliferation favours a reactive process. Grossly, it appears as a sessile nodule with ulceration. Histologically, it shows a proliferation of spindle cells with an admixture of inflammatory cells, hemosiderin-laden macrophages and extravasated red cells. Mitotic activity is usually variable with absence of atypical mitosis. Focal areas of necrosis with surrounding inflammatory cells can be seen corresponding to the previous surgery site. IMT share many histologic features with PSCN which makes differentiation difficult on morphologic evaluation alone. However, the history of recent instrumentation, proximity to the instrumentation site and bland fascicular growth pattern, facilitates its recognition.

If IMT is suspected in the paediatric age group, fibrosarcoma and leiomyosarcoma may be included as diagnostic considerations. The characteristic histologic appearance and immunohistochemical staining pattern of each entity helps to differentiate it from IMT. *Fibrosarcoma* usually shows an infiltrative growth pattern with malignant spindle cells arranged in herringbone fashion. The cells show cytologic atypia, pleomorphism and abundant mitoses. They can demonstrate variable expression for smooth muscle actin (SMA) and vimentin, while they are negative for S-100 and CD-34. *Leiomyosarcomas* are smooth muscle tumours and are characterized by a malignant spindle cell proliferation arranged in interweaving fascicles. They demonstrate expression for SMA and desmin.

Treatment

Surgical resection is the mainstay of treatment of IMT in order to prevent recurrence and to rule out malignancy. Systemic corticosteroids aid in improving a patient's overall clinical condition and promote regression of disease³⁰. Bertocchini et al. emphasizes the role of adjuvant anti-inflammatory drugs and chemotherapy in the resolution of the clinical symptoms³¹. Some authors have also suggested a role for post-operative radiotherapy³².

Prognosis

IMT is a neoplastic proliferation of myofibroblasts that usually follows an indolent and benign clinical course. There is a potential for recurrence and persistent local growth, similar in some respects to fibromatosis. The rate of local recurrence is variable for different sites of the body, including 25% for abdominal and pelvic region. Lu et al. described a case of IMT in a 14 year old with high mitotic index, recurring 20 days after complete resection³³. Metastasis to other sites is very rare with only a few cases having been reported^{34 35}. Hagenstad et al. reported a case of abdominal IMT with metastasis to bone marrow³⁵. In a review of 46 cases, Montgomery et al. described 2 patients with IMT and co-existing sarco-

matoid carcinoma of the bladder, while one patient with malignant IMT, having positive ALK gene rearrangement, showed morphologically sarcomatoid features. That patient did not respond to chemotherapy, showed multiple recurrences and died within a few months due to metastatic disease⁵. Sarcomatoid transformation and rapidly progressive disease leading to metastasis suggest malignant IMT⁵. However, in a study of 59 IMT, Coffin et al showed 6 cases with metastatic disease with negative ALK by immunohistochemistry. Of those 6 cases, 3 were with atypical morphologic features³. In a series of 11 cases, Mariño-Enríquez et al. described 2 cases of IMT with epithelioid morphology showing distant metastasis and, overall, 5 patients dying due to aggressive disease¹¹. These findings raise the possibility of malignant behaviour with IMT, but this is unclear and needs further study.

References

- Griffin CA, Hawkins AL, Dvorak C, et al. *Recurrent involvement of 2p23 in inflammatory myofibroblastic tumors*. *Cancer Res* 1999;59:2776-80.
- Brooks JK, Nikitakis NG, Frankel BF, et al. *Oral inflammatory myofibroblastic tumor demonstrating ALK, p53, MDM2, CDK4, pRb, and Ki-67 immunoreactivity in an elderly patient*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005;99:716-26.
- Coffin CM, Hornick JL, Fletcher CD. *Inflammatory myofibroblastic tumor: comparison of clinicopathologic, histologic, and immunohistochemical features including ALK expression in atypical and aggressive cases*. *Am J Surg Pathol* 2007;31:509-20.
- Yu DC, Grabowski MJ, Kozakewich HP, et al. *Primary lung tumors in children and adolescents: a 90-year experience*. *J Pediatr Surg* 2010;45:1090-5.
- Montgomery EA, Shuster DD, Burkart AL, et al. *Inflammatory myofibroblastic tumors of the urinary tract: a clinicopathologic study of 46 cases, including a malignant example inflammatory fibrosarcoma and a subset associated with high-grade urothelial carcinoma*. *Am J Surg Pathol* 2006;30:1502-12.
- Tanaka A, Hirabayashi K, Sadahiro S, et al. *Inflammatory myofibroblastic tumor of the ascending colon in adults manifested by positive fecal occult blood test*. *Gastrointest Endosc* 2010;71:214-6.
- Shlopov BV, French SW. *Inflammatory myofibroblastic tumor of the lung with unique histological pattern and association with Sjögren's disease and systemic lupus erythematosus*. *Exp Mol Pathol* 2011;91:509-14.
- Salgado-Sánchez E, Flores-Flores J, Pérez-Toriz MU, et al. *Myofibroblast tumor*. *Rev Gastroenterol Mex* 2003;68:219-21.
- Dietrich S, Egerer G, Ho AD, Kasper B. *Chylous ascites in a patient with inflammatory myofibroblastic tumor*. *Case Rep Oncol* 2009;2:144-9.
- Coffin CM, Watterson J, Priest JR, et al. *Extrapulmonary inflammatory myofibroblastic tumor: a clinicopathological and immunohistochemical study of 84 cases*. *Am J Surg Pathol* 1995;19:859-72.
- Mariño-Enríquez A, Wang WL, Roy A, et al. *Epithelioid inflammatory myofibroblastic sarcoma: An aggressive intra-abdominal variant of inflammatory myofibroblastic tumor with nuclear membrane or perinuclear ALK*. *Am J Surg Pathol* 2011;35:135-44.
- Li XQ, Hisaoka M, Shi DR, et al. *Expression of anaplastic lymphoma kinase in soft tissue tumors: an immunohistochemical and molecular study of 249 cases*. *Hum Pathol* 2004;35:711-21.
- Hussong JW, Brown M, Perkins SL, et al. *Comparison of DNA ploidy, histologic and immunohistochemical findings with clinical outcome in inflammatory myofibroblastic tumor*. *Mod Pathol* 1999;12:279-86.
- Chen ST, Lee JC. *An inflammatory myofibroblastic tumor in liver with ALK and RANBP2 gene rearrangement: combination of distinct morphologic, immunohistochemical, and genetic features*. *Hum Pathol* 2008;39:1854-8.
- Coffin CM, Dehner LP, Meis-Kinblom JM. *Inflammatory myofibroblastic tumor, inflammatory fibrosarcoma, and related lesions: an historical review with differential diagnostic considerations*. *Semin Diagn Pathol* 1998;15:102-10.
- Freeman A, Geddes N, Munson P, et al. *Anaplastic lymphoma kinase (ALK 1) staining and molecular analysis in inflammatory myofibroblastic tumours of the bladder: a preliminary clinicopathological study of nine cases and review of the literature*. *Mod Pathol* 2004;17:765-71.
- Su LD, Atayde-Perez A, Sheldon S, et al. *Inflammatory myofibroblastic tumor: cytogenetic evidence supporting clonal origin*. *Mod Pathol* 1998;11:364-8.
- Gleason BC, Hornick JL. *Inflammatory myofibroblastic tumours: where are we now?* *J Clin Pathol* 2008;61:428-37.
- Lawrence B, Perez-Atayde A, Hibbard MK, et al. *TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors*. *Am J Pathol* 2000;157:377-84.
- Bridge JA, Kanamori M, Ma Z, et al. *Fusion of the ALK gene to the clathrin heavy chain gene, CLTC, in inflammatory myofibroblastic tumor*. *Am J Pathol* 2001;159:411-5.
- Ma Z, Hill DA, Collins MH, et al. *Fusion of ALK to the Ran-binding protein 2 (RANBP2) genes in inflammatory myofibroblastic tumor*. *Gene Chromosomes Cancer* 2003;37:98-105.
- Cessna MH, Zhou H, Sanger WG, et al. *Expression of ALK 1 and p80 in inflammatory myofibroblastic tumor and its mesenchymal mimics: a study of 135 cases*. *Mod Pathol* 2002;15:931-8.
- Coffin CM, Patel A, Perkins S, et al. *ALK1 and p80 expression and chromosomal rearrangements involving 2p23 in inflammatory myofibroblastic tumor*. *Mod Pathol* 2001;14:569-76.
- Morris SW, Kirstein MN, Valentine MB, et al. *Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma*. *Science* 1995;267:316-7.

Conclusion

Inflammatory myofibroblastic tumour is a neoplasm with intermediate biologic behaviour that very rarely presents with advanced features such as local invasion, recurrence and malignant transformation with distant metastasis³⁵. The use of ALK immunohistochemistry and molecular studies including cytogenetics are important diagnostic tools. IMT may be suspected preoperatively due to haematologic abnormalities and radiologic findings, but tissue from a biopsy or surgical resection is required for adequate and accurate histologic confirmation. Careful histopathologic evaluation of the lesion is necessary so as not to mistake IMT for other benign and malignant conditions. Complete surgical resection, if possible, is the recommended treatment. Follow-up is required to detect recurrence and prevent complications.

- ²⁵ Cheng L, Foster SR, MacLennan GT, et al. *Inflammatory myofibroblastic tumors of the genitourinary tract--single entity or continuum?* J Urol 2008;180:1235-40.
- ²⁶ Butrynski JE, D'Adamo DR, Hornick JL et al. *Crizotinib in ALK-rearranged inflammatory myofibroblastic tumor.* N Engl J Med 2010;363:1727-33.
- ²⁷ Escobar C, Munker R, Thomas JO, et al. *Update on desmoid tumors.* Ann Oncol 2012;23:562-9.
- ²⁸ Hirsch MS, Dal Cin P, Fletcher CD. *ALK expression in pseudosarcomatous myofibroblastic proliferations of the genitourinary tract.* Histopathology 2006;48:569-78.
- ²⁹ Harik LR, Merino C, Coindre JM, et al. *Pseudosarcomatous myofibroblastic proliferations of the bladder: a clinicopathologic study of 42 cases.* Am J Surg Pathol 2006;30:787-94.
- ³⁰ Diop B, Konate I, Ka S, et al. *Mesenteric myofibroblastic tumor: NSAID therapy after incomplete resection.* J Visc Surg 2011;148:e311-14.
- ³¹ Bertocchini A, Lo Zupone C, Callea F, et al. *Unresectable multifocal omental and peritoneal inflammatory myofibroblastic tumor in a child: revisiting the role of adjuvant therapy.* J Pediatr Surg 2011;46:17-21.
- ³² Ong HS, Ji T, Zhang CP, et al. *Head and neck inflammatory myofibroblastic tumor (IMT): evaluation of clinicopathologic and prognostic features.* Oral Oncol 2012;48:141-8.
- ³³ Lu CH, Huang HY, Chen HK, et al. *Huge pelvi-abdominal malignant inflammatory myofibroblastic tumor with rapid recurrence in a 14-year-old boy.* World J Gastroenterol 2010;16:2698-701.
- ³⁴ Debelenko LV, Arthur DC, Pack SD, et al. *Identification of CARS-ALK fusion in primary and metastatic lesions of an inflammatory myofibroblastic tumor.* Lab Invest 2003;83:1255-65.
- ³⁵ Hagenstad CT, Kilpatrick SE, Pettenati MJ, et al. *Inflammatory myofibroblastic tumor with bone marrow involvement. A case report and review of the literature.* Arch Pathol Lab Med 2003;127:865-7.

Combined hepato-cholangiocarcinoma arising in a gallbladder intracystic papillary neoplasm. A new view on so-called "hepatoid adenocarcinoma of the gallbladder"

J.B. DETTONI¹, W. ANDRAUSS², N.D. THEISE³, M.S. ROCHA⁴, E.S. MELLO¹, C.H.L. ROCHA⁵, T.E.F. PFIFFER⁵, V.A.F. ALVES¹

¹ Department of Pathology, University of São Paulo School of Medicine, São Paulo, Brazil; ² Discipline of Liver Transplantation, Department of Gastroenterology, University of São Paulo School of Medicine, São Paulo, Brazil; ³ Department of Pathology, Beth Israel School of Medicine, New York, USA; ⁴ Department of Radiology, University of São Paulo School of Medicine, São Paulo, Brazil; ⁵ Discipline of Clinical Oncology, Instituto do Câncer do Estado de São Paulo, Brazil

Key words

Gallbladder • Combined hepato-cholangiocarcinoma • Neoplasms • Stem-cell

Summary

A 62-year-old female presented with abdominal pain, weight loss of 20 kg in the prior 6 months, and a palpable mass in the right upper quadrant during physical exam. Standard liver tests, including screening for hepatitis B and C and alpha-fetoprotein were negative or within normal limits. Computerized tomography depicted a transmural gallbladder tumor infiltrating into the adjacent liver with an irregular ill-defined mass occupying segments IV-V-VI, measuring 13.0 x 9.2 x 8.5 cm, with a solid-cystic component and heterogeneous captation of endovenous contrast media. Complete surgical resection of the neoplasm was achieved through an extended cholecystectomy and excision of

hepatic segments IV, V and VI, with an uneventful follow-up 29 months until now. Morphological and immunohistochemical assessment favored a diagnosis of combined hepatocellular-cholangiocarcinoma arising in a gallbladder intracystic papillary neoplasm with invasive carcinoma. This case raises the hypothesis that the so-called "hepatoid adenocarcinoma of the gallbladder" may presently be better understood as a neoplasm derived from hepatobiliary stem/progenitor cells. Such cells have been recognized in the canals of Hering, in peribiliary glands within the liver and in the extrahepatic biliary tree, and in gallbladder mucosa.

Introduction

The so-called "hepatoid adenocarcinoma" is a rare malignant tumor recognized along the biliary tract and other extra-hepatic niches, depicting morphologic and immunophenotypic resemblance to hepatocellular carcinoma (HCC) which is essential for its identification. The present case report as well as some recently published case reports provide evidence that hepatoid adenocarcinomas of the gallbladder and extrahepatic biliary tree are derived from biliary progenitor cells with bidirectional differentiation towards hepatocytes and cholangiocytes, thus supporting a novel view that the so-called "hepatoid adenocarcinomas" might be better understood as a combined hepatocellular-cholangiocarcinoma (cHCC-CC) arising in the biliary tree.

Materials and methods

CASE REPORT

A 62-year-old Caucasian female presented with abdominal pain and weight loss of 20 kg in the prior 6 months, accompanied by diarrhea after food intake and weakness. Past medical history included hypertension, Chagas disease and type II diabetes mellitus, without evidence of previous hepatobiliary disease. She denied smoking or alcohol consumption. During physical exam, a palpable mass was found at right upper quadrant without jaundice. Standard liver tests, including screening for hepatitis B and C, were within normal limits or negative. She was anaemic (haemoglobin 9.8 g/dl) and her white blood cell count was increased ($20.7 \times 10^3/l$). Laboratory tests for metabolic profile and serum alpha-

Correspondence

Venancio Avancini Ferreira Alves, Department of Pathology, University of São Paulo School of Medicine, Av. Dr. Arnaldo, 455, room 1103, 01246-903 - São Paulo - SP - Brasil - E-mail: venancio@uol.com.br

Fig. 1. Abdominal enhanced CT: A. Axial image: Heterogeneous mass in liver segments IV and V. B. Coronal image: Irregular and heterogenous transmural gallbladder mass in continuity with infiltration of adjacent liver parenchyma. C. Coronal image (more posterior than B): Mass predominantly in the gallbladder lateral wall.



fetoprotein (AFP = 2.8 ng/ml) were normal. Computerized tomography (CT) showed a transmural gallbladder tumor as an irregular ill-defined mass infiltrating liver segments IV, V and VI, measuring 11.6 x 9.8 x 8.6 cm. The tumor had solid and cystic components with heterogeneous captation of endovenous contrast media. A gallstone was also found (Fig. 1).

Complete surgical resection of the neoplasm was achieved through an extended cholecystectomy and excision of hepatic segments IV, V and VI. The outcome was uneventful, with normal laboratory values and no residual tumor on image. The patient did not receive any adjuvant therapy, chemotherapy or radiotherapy. After 29 months of follow-up, the patient remains free of tu-

mor recurrence. Based on the clinical observations and gross findings, the tumor was considered to have arisen in the gallbladder and infiltrating surrounding liver.

Results

PATHOLOGY

Grossly, the specimen showed an ill-defined mass with infiltrative borders, measuring 12.0 x 9.0 x 8.5 cm, with whitish, firm areas intermingled with extensive necrosis. The tumor clearly appeared to be primarily located in the gallbladder mucosa wall, depicting progressive thickening of gallbladder wall with mucosal ulceration

Fig. 2. Microscopic findings: A-B: calculous chronic cholecystitis with cholesterosis and papillary formations (H&E 50x and 100x); C-D: Transition with intracystic papillary neoplasm with areas of dysplasia and papillary formation (H&E 50x and 100x); E-F: Transition of high grade dysplasia with solid, "glassy cell, poorly differentiated cholangiocarcinoma", invading through the gallbladder wall and adjacent liver parenchyma (H&E 50x and 100x).

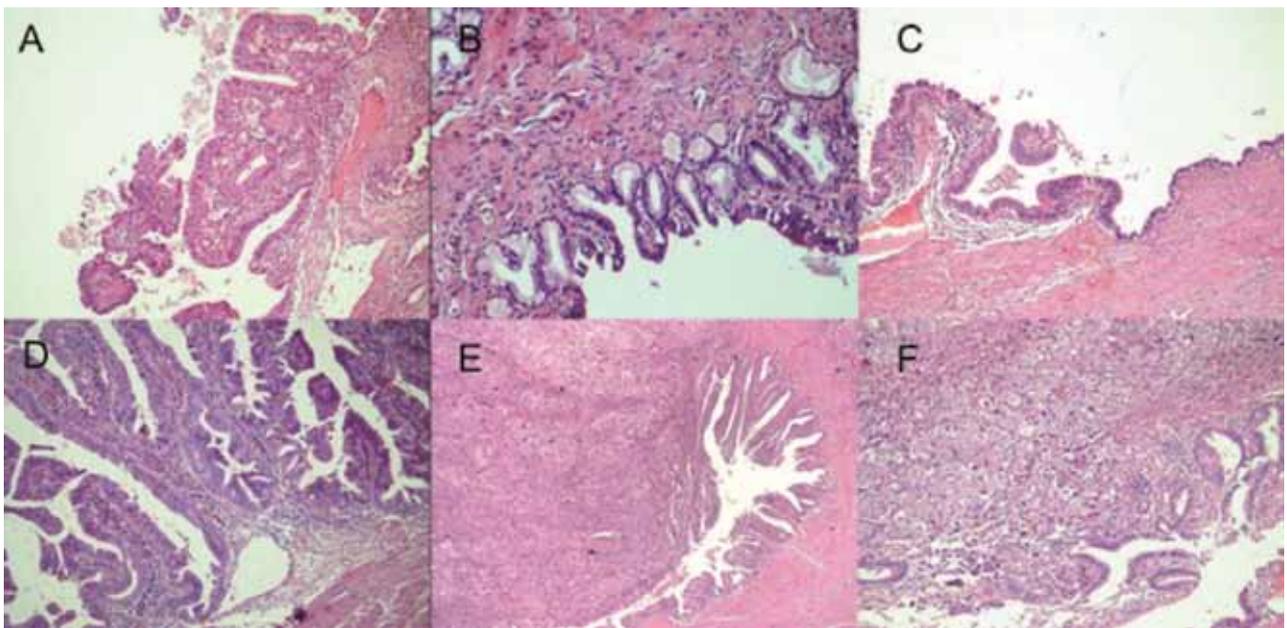
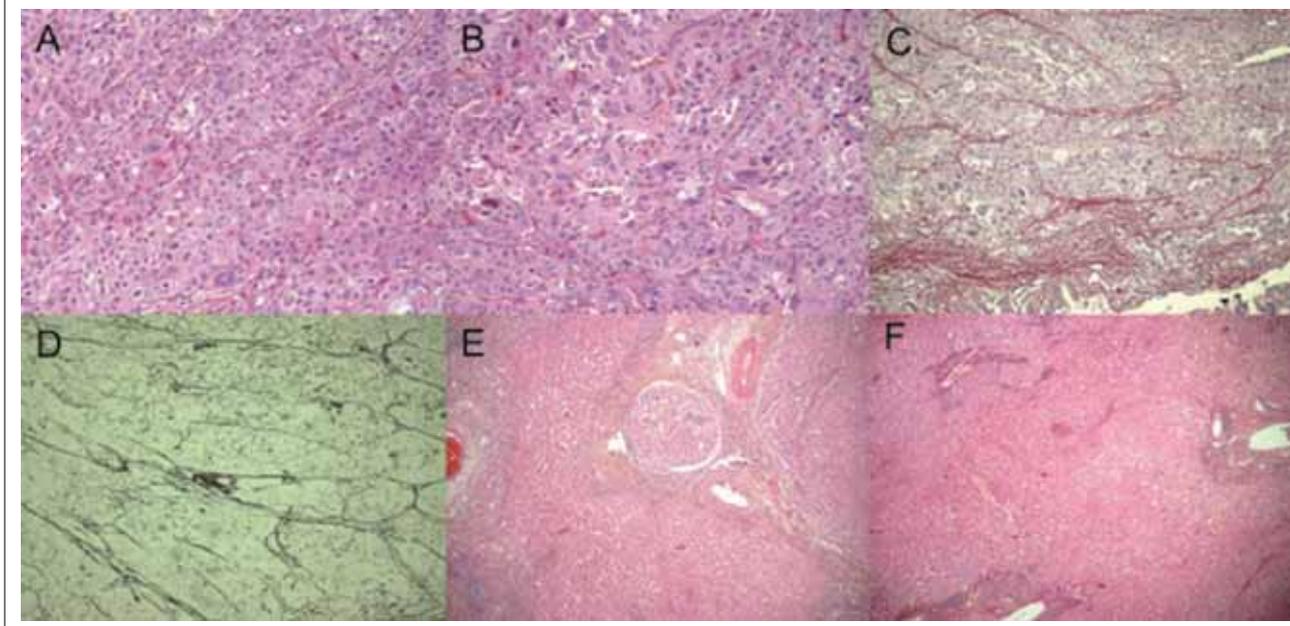


Fig. 3. Microscopic findings: A-B: Solid, trabecular or pseudo-acinar carcinoma with a predominance of poorly differentiated polygonal hepatocytes with central, variably atypical vesicular nuclei with large nucleoli. Dispersed small cells, resembling “oval cells”, are seen. (H&E 50x and 100x); C: Sirius red stain (SR 50x); D: Reticulin (50x); E: Vascular microinvasion (H&E 50x); F: The surrounding liver was non-cirrhotic/fibrotic and without features of biliary disease or hepatitis (H&E 50x).



and infiltration into surrounding liver parenchyma. The adjacent gallbladder wall was thickened and had a grey-white appearance. The lumen contained a mixed stone and the mucosa had an irregular appearance. The remaining liver parenchyma showed a grossly normal architecture without grossly evident fibrosis or cirrhosis. There was no macroscopic evidence of vascular invasion. Microscopically, the gallbladder mucosa showed a background of calculous chronic cholecystitis and cholesterosis within which there was an intracystic papillary neoplasm, extending into Rokitansky-Aschoff sinuses. There was high grade biliary intra-epithelial neoplasia (BilIN-3) that was continuous with a solid component, of poorly

differentiated cholangiocarcinoma, composed of intermingled small and large cells, extensively invading the gallbladder wall and adjacent liver parenchyma (Fig. 2). Deeper in the liver (Fig. 3), the tumor showed poorly differentiated, but hepatocyte-like cells growing in solid, trabecular or pseudo-acinar patterns typical of HCC admixed with small cells, resembling hepatobiliary stem/progenitor cells. Mitotic figures were frequent and necrosis was multifocal. No bile production was seen, but hyaline globules were noticed within the cytoplasm of larger cells (Fig. 3). The normal architecture of the surrounding liver was preserved, except for focal mild sinusoidal dilatation and lymphocytic infiltration probably

Tab. I. Immunohistochemistry panel (C - canalicular; M - membranous; SC - small cell).

Antibody	Papillary Adenocarcinoma	Solid/sclerosing	“Conventional HCC”	Glandular hyperplasia (DR)
K8	3+	3+	2+ (sc 3+)	3+
K7	3+	3+	1+ (sc 1+)	3+
K19	3+	2+	1+ (sc 1+)	3+
Hep Par 1	1+	1+	3+	1+
pCEA	C+M	C+M	canalicular	C+M
Arginase	0	0	0	0
Lactoferrin	0	1+	0	2+
CDX2	0	0	0	0/1 goblet
MUC1	3+	3+	1+	3+
MUC2	1+	0	0	2+
MUC5AC	1+	0	0	2+
CD56	0	0	0	2+
CD133	0	0	0	2+
Vimentin	0	0	0	0
EGFR	0	2+	2+	0

due to the nearby space occupied by the lesion. Features of chronic hepatitis, metabolic disease or chronic biliary tract disease were not seen.

IMMUNOHISTOCHEMISTRY

Immunohistochemical staining for hepatocellular, biliary, endothelial, stem/progenitor cell and other differentiations were performed; the summary of results is

provided in Table I and in Figures 4 and 5. Muc-1 was diffusely positive in non-neoplastic gallbladder mucosa, in intracystic papillary neoplasia and in its invasive component. As the tumour invaded liver parenchyma, the components with hepatocellular morphology depicted lower and more focal reactivity to Muc-1. On the other hand, HepPar-1 was diffusely positive in HCC areas and focal in the intraepithelial papillary component,

Fig. 4. Immunohistochemistry panel: A-B. MUC-1 diffusely positive in intraepithelial and invasive papillary neoplasia and in solid, poorly differentiated cholangiocarcinoma. Hepatocellular component depicted more focal reactivity. C-E. HCC areas diffusely positive for Hep Par-1 and only focally reactive in papillary cholangiocarcinoma. F-G. HCC positive for pCEA on canalicular structures. Papillary and solid components of cholangiocarcinoma presented circumferential membranous and cytoplasmic reactivity. H. CD34 showing "diffuse capillarization" in HCC areas.

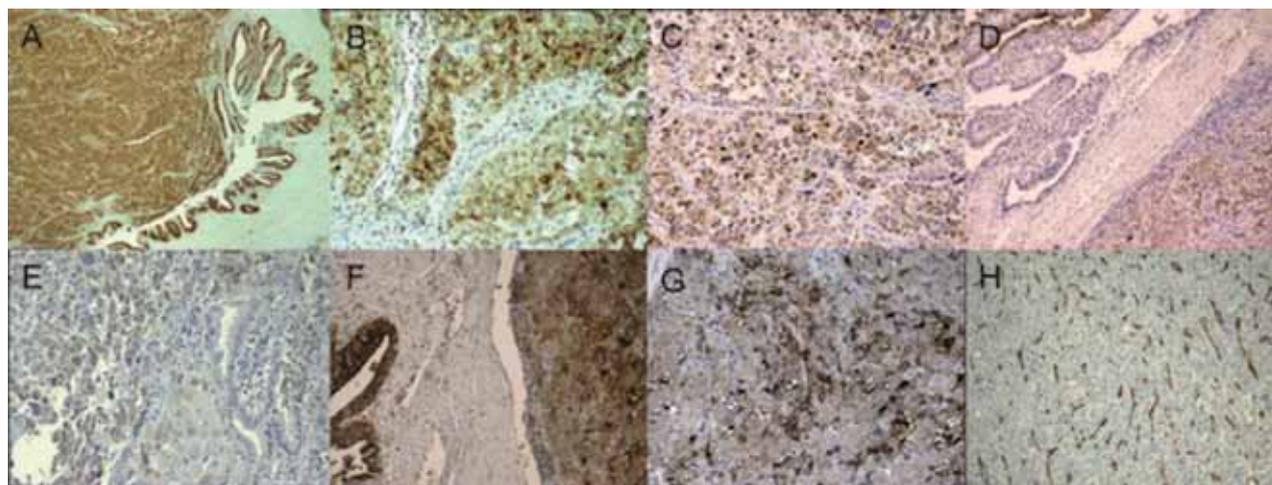
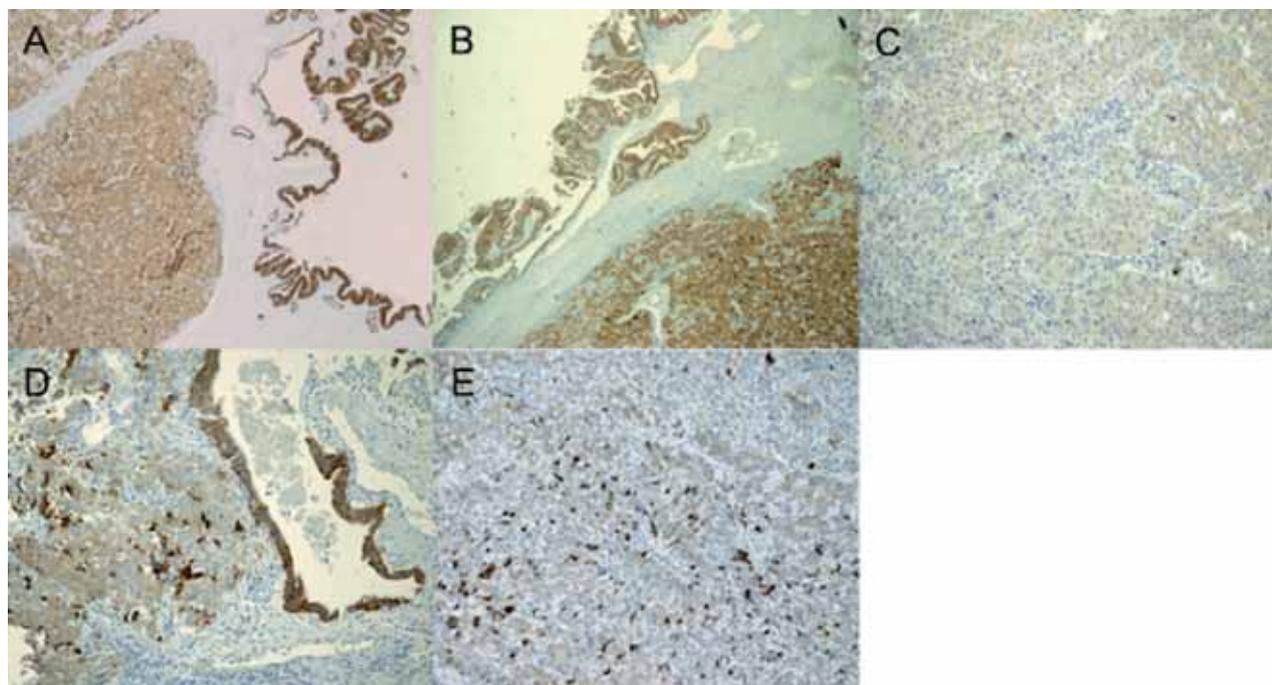


Fig. 5. Keratin markers: A. Keratins 8/18 (K8/18) positive in all components; B-E. K7 (B and C) and K19 (D and E) depicted a double cell population in cholangiocarcinoma, with stronger reactivity in small cells. In contrast, HCC areas were either negative or only focally reactive for K7 and for K19.



but negative in areas of invasive cholangiocarcinoma. Focal immunoreactivity was found in the intraepithelial papillary component. As expected, HCC areas were positive for pCEA on cell membranes, especially marking canalicular structures, while both papillary and solid components of cholangiocarcinoma had circumferential membranous and cytoplasmic reactivity. CD34 reactivity in endothelial membranes showed “sinusoidal capillarization” in areas of HCC (Fig. 4).

CAM5.2 stain for keratins 8/18 (K8/18) and stains for keratins 7 and 19 (K7, K19) were positive in all components of cholangiocarcinoma, with stronger reactivity in small cells. As expected, contrasting with these cholangiocellular areas, the HCC areas, while positive for K8/18, were either negative or only focally reactive for K7 and K19 (Fig. 5). Immunostaining for hepatobiliary stem/progenitor cell markers (CD56, CD133) was negative. Other markers (see Tab. I) were also negative.

Discussion

The radiological, morphological and immunohistochemical findings in the present case favour a diagnosis of cHCC-CCa arising in an intracystic papillary biliary neoplasm from the gallbladder. Histomorphology further suggests “stem cell features” within the mixed tumour. The smooth transition of the *in situ* papillary lesion of the gallbladder to solid, poorly differentiated cholangiocarcinoma (with an admixture of small and large cholangiocytes), and then to extensive areas of conventional appearing HCC, is closely similar to previous reports of hepatoid adenocarcinoma of the gallbladder, thus leading us to suggest the hypothesis that “hepatoid adenocarcinomas” of gallbladder, extra-hepatic biliary tract and pancreas might be better understood as cHCC-CCa with a probable origin in hepatobiliary stem/progenitor cells. Allen and Lisa first described the features of cHCC-CC in 1949¹ as an infrequent hepatic neoplasm defined as a tumour containing components of both HCC and cholangiocarcinoma intimately admixed, as seen in the present case. Dual differentiation towards hepatocytes and bile duct epithelia coexist in this neoplasm² and, although a few several studies regarding the clinical and pathological features of cHCC-CC are now available, information about clinical outcome after hepatic resection is still contradictory. There is a lack of data regarding treatment of advanced cHCC-CC. Even the larger case series are unable to guide systemic therapy³. The feeling that cHCC-CC may have an unfavourable behaviour compared to ordinary HCC might derive from the fact that most of patients with cHCC-CC had high histological grade tumours and advanced stage disease at the time of presentation^{4,6}. The presence of gross portal invasion and thrombosis has been related to worse prognosis of patients with cHCC-CC⁴. In the present case, the papillary component showed features of intra-cholecystic papillary-tubular neoplasm (ICPN), which has recently been reported to be a more indolent gallbladder adeno-

carcinoma, even when invasive⁷. Although no gross vascular invasion was noticed in the present case, micro-invasion was detected in portal vein branches (Fig. 3). Classification of cHCC-CC has been a matter of controversy and thus, reports like the present and other series of cases can add clinical and morphological clues that are potentially useful for further understanding these intriguing neoplasms. While some studies propose that these neoplasms are a cholangiocarcinoma reproducing the pattern of small biliary ducts⁸, others consider they could be a subtype of HCC or derive from hepato-biliary stem/progenitor cells⁹. According to the current WHO classification, tumour cells of cHCC-CC with predominantly prominent features of hepato-biliary stem/progenitor cells are regarded as cHCC-CCs “with stem cell features”, which have further been subdivided into three subtypes: typical, intermediate cell and cholangiolocellular subtype^{9,10}. Genetic studies of cHCC-CC found gene expression profiles favouring activation of hepatobiliary stem/progenitor cells, and genetically closer to CC than to conventional HCC¹¹.

Hepato-biliary stem cells have been progressively detected in different niches along the biliary tree in the normal human liver. Initial studies based on three-dimensional analysis of injured human and murine livers identified the canal of Hering as a stem cell niche^{12,13}, though others have been postulated. *In vivo* identification of stem cell niches by label-retaining cell assay has been performed, confirming the canal of Hering as one such niche, but also supporting others previously hypothesized: isolated cholangiocytes within the small to medium bile ducts themselves, peribiliary hepatocytes and periductal null cells¹⁴. Activation of stem cells in one or more of these niches gives rise to transient amplifying cells, with bipotent, hepatobiliary differentiation and rapid proliferation; these are considered hepatobiliary progenitor cells. In humans, these have been called the “intermediate hepatobiliary cell” component of the ductular reaction; in rodents, they are referred to as oval cells¹²⁻¹⁴. Given the impossibility (so far) to reliably distinguish stem cells from their progenitor cell progeny on the basis of morphology and immunophenotype, they are usually described in disease or malignancy as “stem/progenitor cells”, reflecting the continuity of their immunophenotypic, cytologic and functional characteristics along a spectrum. The immunohistochemical profiles confirm mixed biliary and hepatocytic immunophenotypes: OV-6, K19, K7, HepPar 1, epithelial cell adhesion molecule (EpCAM), neural cell adhesion marker (NCAM-1, CD56) and stem cell markers including CD117 and CD34, amongst others¹⁷. Ductular reactions display this spectrum, although with disease specific variations, in a variety of disorders including alcoholic and non-alcoholic fatty liver disease, cholestatic liver disease, chronic hepatitis, submassive necrosis and in acute-on-chronic events^{18,19}. Microarray analysis demonstrates up-regulation of genes related to liver stem/progenitor cells in a ductular reaction in subjects with alcoholic hepatitis. These genes include KRT7,

SOX9, EpCAM, KRT19, PROM1, CD44 and others²⁰. In neoplasms, stem/progenitor cell phenotypes have been reported in both benign and malignant tumours²¹⁻²⁶. The two most common primary liver carcinomas are HCC and cholangiocarcinoma; in most cases these are easily distinguished on the basis of morphology; however, in poorly differentiated tumours or in tumours with mixed phenotypes, immunohistochemistry is often necessary for complete evaluation. Hepatocytic markers include HepPar-1, Arginase-1 and the canalicular pattern of immunostaining with CD10 or polyclonal anti-CEA. Although not specific, K7, K19 and mucins (especially Muc-1, Muc-5AC and Muc-6) are more frequently found in cholangiocarcinoma or in cHCC-CC. However, HepPar-1 is not fully specific, since it may be present in gallbladder epithelium and occasionally even in other adenocarcinomas from the respiratory tract, gynaecological tumours, digestive tract, genitourinary tract and neuroendocrine tumours²⁷⁻²⁹. In the case reported herein, we found HepPar-1 positivity in tumour cells and in gallbladder intra-epithelial neoplasm. With regard to ordinary cholangiocarcinoma, recent histological and molecular characterization highlights the heterogeneity of this cancer emerging at different sites of the biliary tree with different macroscopic or morphological features³⁰. Considering the current case, recent reports ascribe the origin of mixed carcinomas of the gallbladder to hepatobiliary progenitor cells²¹⁻³¹. Moreover, additional stem cell niches have been identified in peribiliary glands of the extrahepatic and large intra-hepatic bile ducts as well as in the gallbladder³². These sites could be sources of malignantly transformed, tumour generating stem/progenitor cells. We also raise the possibility that previously-reported cases of the rare hepatoid adenocarcinoma of the gallbladder are also evidence of malignant transformation of gallbladder hepato-biliary stem/progenitor cells³³.

Differential diagnoses of hepatoid adenocarcinomas in this setting include HCCs or cHCC-CCs invading the gallbladder. Immunohistochemically, the component with hepatoid features diffusely expresses HepPar-1, pCEA and CD10, but not K7, whereas the well-differentiated tubular-papillary adenocarcinoma is immunoreactive for K7, but not for AFP, HepPar-1, pCEA or CD10³².

In conclusion, detailed morphologic and immunohistochemical assessment of this case suggests that the so-called "hepatoid adenocarcinoma of the gallbladder" may presently be better understood as part of the spectrum of cHCC-CC, and that these may arise in the gallbladder, and not only in the liver itself. As to whether the neoplasm is derived from malignant transformation of hepato-biliary stem/progenitor cells, this case does not settle the question. On the one hand, hepatobiliary stem cells have been identified in niches within the gallbladder mucosa and peribiliary glands³², and thus they could undergo malignant transformation. On the other hand, the present finding of the fact that the tumour arising in a pre-existing biliary in situ neoplasm in continuity

with a progressively invasive that then gives rise to adenocarcinoma further as it invades, which in turn displays hepatocellular phenotypes and morphology when growing into the liver environment, may also support the possibility that some of these tumours are derived from altered differentiation of a primarily adenocarcinoma, intracystic papillary biliary neoplasm (ICPN). The possible role of the hepatic environment in modulating such differentiation is particularly intriguing and raises questions about the role of microenvironment in the development and differentiation of biliary malignancies.

References

- Allen RA, Lisa JR. *Combined liver cell and bile duct carcinoma*. Am J Pathol 1949;25:647-55.
- Ishak KG, Anthony PP, Sobin LH. *Histological typing of tumors of the liver* - 2nd ed. Berlin: Springer 1994, pp. 20.
- Zhan Q, Shen BY, Deng XX, et al. *Clinical and pathological analysis of 27 patients with combined hepatocellular-cholangiocarcinoma in an Asian center*. J Hepatobiliary Pancreat Sci 2012;19:361-9.
- Park H, Choi KH, Choi SB, et al. *Clinicopathological characteristics in combined hepatocellular-cholangiocarcinoma: a single center study in Korea*. Yonsei Med J 2011;52:753-60.
- Goodman ZD, Ishak KG, Langloss JM. *Combined hepatocellular cholangiocarcinoma. A histologic and immunohistochemical study*. Cancer 1985;55:124-35.
- Wang J, Wang F, Kessinger A. *Outcome of combined hepatocellular and cholangiocarcinoma of the liver*. J Oncol 2010;2010. pii 917356.
- Adsay V, Jang KT, Roa JC, et al. *Intracholecystic papillary-tubular neoplasms (ICPN) of the gallbladder (neoplastic polyps, adenomas, and papillary neoplasms that are ≥ 1.0 cm): clinicopathologic and immunohistochemical analysis of 123 cases*. Am J Surg Pathol 2012;36:1279-301.
- Nakanuma Y, Xu J, Harada K, et al. *Pathological spectrum of intrahepatic cholangiocarcinoma arising in non-biliary chronic advanced liver diseases*. Pathol Int 2011;6:298-305.
- Theise ND, Nakashima O, Park YN, et al. *Combined hepatocellular-cholangiocarcinoma*. In: Bosman FT, Carneiro F, Hruban RH, et al, eds. *WHO classification of tumours of the digestive system*. 4th ed. Lyon: IARC Press 2010, pp. 225-227.
- Park HS, Bae JS, Jang KY, et al. *Clinicopathologic study on combined hepatocellular carcinoma and cholangiocarcinoma: with emphasis on the intermediate cell morphology*. Korean Med Sci 2011;26:1023-30.
- Cazals-Hatem D, Rebouissou S, Bioulac-Sage P, et al. *Clinical and molecular analysis of combined hepatocellular-cholangiocarcinomas*. J Hepatol 2004;41:292-8.
- Theise ND, Saxena R, Portmann BC, et al. *The canals of Hering and hepatic stem cells in humans*. Hepatology 1999;30:1425-33.
- Paku S, Nagy P, Kopper L, et al. *2-acetylaminofluorene dose-dependent differentiation of rat oval cells into hepatocytes: confocal and electron microscopic studies*. Hepatology 2004;39:1353-61.
- Kuwahara R, Kofman AV, Landis CS, et al. *The hepatic stem cell niche: identification by label-retaining cell assay*. Hepatology 2008;47:1994-2002.
- Roskams TA, Theise ND, Balabaud C, et al. *Nomenclature of the finer branches of the biliary tree: canals, ductules and ductular reactions in human livers*. Hepatology 2004;39:1739-45.
- Gouw AS, Clouston AD, Theise ND. *Ductular reactions in human liver: diversity at the interface*. Hepatology 2011;54:1853-63.

- ¹⁷ Zhang L, Theise N, Chua M, et al. *The stem cell niche of human livers: symmetry between development and regeneration*. Hepatology 2008;48:1598-607.
- ¹⁸ Tan J, Hytioglou P, Wieczorek R, et al. *Immunohistochemical evidence for hepatic progenitor cells in liver diseases*. Liver 2002;22:365-73.
- ¹⁹ Richardson MM, Jonsson JR, Powell EE, et al. *Progressive fibrosis in nonalcoholic steatohepatitis: association with altered regeneration and a ductular reaction*. Gastroenterology 2007;133:80-90.
- ²⁰ Sancho-Bru P, Altamirano J, Rodrigo-Torres D, et al. *Liver progenitor cell markers correlate with liver damage and predict short-term mortality in patients with alcoholic hepatitis*. Hepatology 2012;55:1931-41.
- ²¹ Vadlamani I, Brunt EM. *Hepatocellular progenitor cell tumor of the gallbladder: a case report and review of the literature*. Modern Pathology 2005;18:864-70.
- ²² Kim H, Park C, Han KH, et al. *Primary liver carcinoma of intermediate (hepatocyte-cholangiocyte) phenotype*. J Hepatol 2004;40:298-304.
- ²³ Uenishi T, Hirohashi K, Shuto T, et al. *Primary liver cancer with dual expression of hepatocyte and bile duct epithelial markers*. Hepatogastroenterology 2002;49:1092-4.
- ²⁴ Roskams T, De Vos R, Desmet V. *Undifferentiated progenitor cells in focal nodular hyperplasia of the liver*. Histopathology 1996;28:291-9.
- ²⁵ Libbrecht L, De Vos R, Cassiman D, et al. *Hepatic progenitor cells in hepatocellular adenomas*. Am J Surg Pathol 2001;25:1388-96.
- ²⁶ Tickoo SK, Zee SY, Obiekwe S, et al. *Combined hepatocellular-cholangiocarcinoma: a histopathologic, immunohistochemical, and in situ hybridization study*. Am J Surg Pathol 2002;26:989-97.
- ²⁷ Kakar S, Muir T, Murphy LM, et al. *Immunoreactivity of HepPar 1 in hepatic and extrahepatic tumors and its correlation with albumin in situ hybridization in hepatocellular carcinoma*. Am J Clin Pathol 2003;119:361-6.
- ²⁸ Yan BC, Gong C, Song J, et al. *Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms*. Am J Surg Pathol 2010;34:1147-54.
- ²⁹ Lugli A, Tornillo L, Mirlacher M, et al. *Hepatocyte paraffin 1 expression in human normal and neoplastic tissues: tissue microarray analysis on 3940 tissue samples*. Am J Clin Pathol 2004;122:721-7.
- ³⁰ Cardinale V, Carpino G, Reid L, et al. *Multiple cells of origin in cholangiocarcinoma underlie biological, epidemiological and clinical heterogeneity*. World J Gastrointest Oncol 2012;4:94-102.
- ³¹ Paniz Mondolfi AE, Slova D, Fan W, et al. *Mixed adenoneuroendocrine carcinoma (MANEC) of the gallbladder: a possible stem cell tumor?* Pathol Int 2011;61:608-14.
- ³² Cardinale V, Wang Y, Carpino G, et al. *The biliary tree-a reservoir of multipotent stem cells*. Nat Rev Gastroenterol Hepatol 2012;9:231-40.
- ³³ Sakamoto K, Monobe Y, Kouno M, et al. *Hepatoid adenocarcinoma of the gallbladder: case report and review of the literature*. Pathol Int 2004;54:52-6.

A rare case of transmural endometriosis in primary adenocarcinoma of the rectum

M. FALLENI¹, D. BAUER¹, E. OPOCHER², L. MONEGHINI¹, G.P. BULFAMANTE¹

¹ Division of Pathology, Department of Health Sciences, University of Milan, A.O. San Paolo, Milan, Italy; ² Hepato-biliary Surgery, Department of Health Sciences, University of Milan, A.O. S. Paolo, Milan, Italy

Key words

Endometriosis • Colon carcinoma • Rectal carcinoma

Summary

Intestinal endometriosis of the rectum and sigmoid colon, occurring in up to 34% of pelvic endometriosis, mimics a wide number of conditions that are difficult to differentiate from inflammatory or malignant diseases. Herein we report the first case of transmural

endometriosis concomitant with advanced primary rectal adenocarcinoma, presenting with obstructive symptoms. Correct diagnosis based on morphological identification and immunohistochemical characterization of the two entities is crucial for treatment.

Case report

A 50-year-old female with no significant past clinical history presented with abdominal pain and obstruction symptoms. After diagnostic workup indicative of rectal obstructive cancer with liver metastasis, she underwent proctosigmoidectomy and hepatic segmentectomy. A rectal vegetating mass with luminal stenosis infiltrated the full thickness of the intestinal wall. Multiple white nodules ranging from 0.6 to 3 cm in greatest dimension were found in the hepatic specimen. At the microscopic level, glandular structures from the subserosal tissue to the luminal intestinal surface were observed. At higher magnification, moderately differentiated malignant glands with necrotic areas were intermingled with cystic glands composed of focally ciliated cells, with minimal atypia and no mitotic activity, often surrounded by loose connective tissue. The lesion was tested for cytokeratin 7 (OV-TL 12/30), cytokeratin 20 (K20.8), CEA(II-7), CDX2 (AMT28), oestrogen (1D5) and progesterone (636) receptor immunoreactivity. Neoplastic glands showed strong immunoreactivity only for cytokeratin 20, CDX2 and CEA, and were documented in all intestinal layers and in perivisceral fat. The second glandular component immunostained for cytokeratin 7 and for oestrogen and progesterone receptors antigens; it was pre-

dominantly sited in the subserosal fat and in the intestinal muscular walls, but also in the submucosa and in the deeper portion of mucosa. A diagnosis of colonic adenocarcinoma of the classic type, associated with transmural endometriosis implants, was made. Metastasis of the adenocarcinomatous component to locoregional lymph nodes and in the hepatic specimen, and to the salpinges one year later, were found. At gynaecological controls, the patient was negative for atypical endometrial hyperplasia and/or endometrial carcinoma. No mutations in codons 12 and 13 of KRAS or in codon 600 of BRAF were found in either the colonic or metastatic lesions.

Discussion

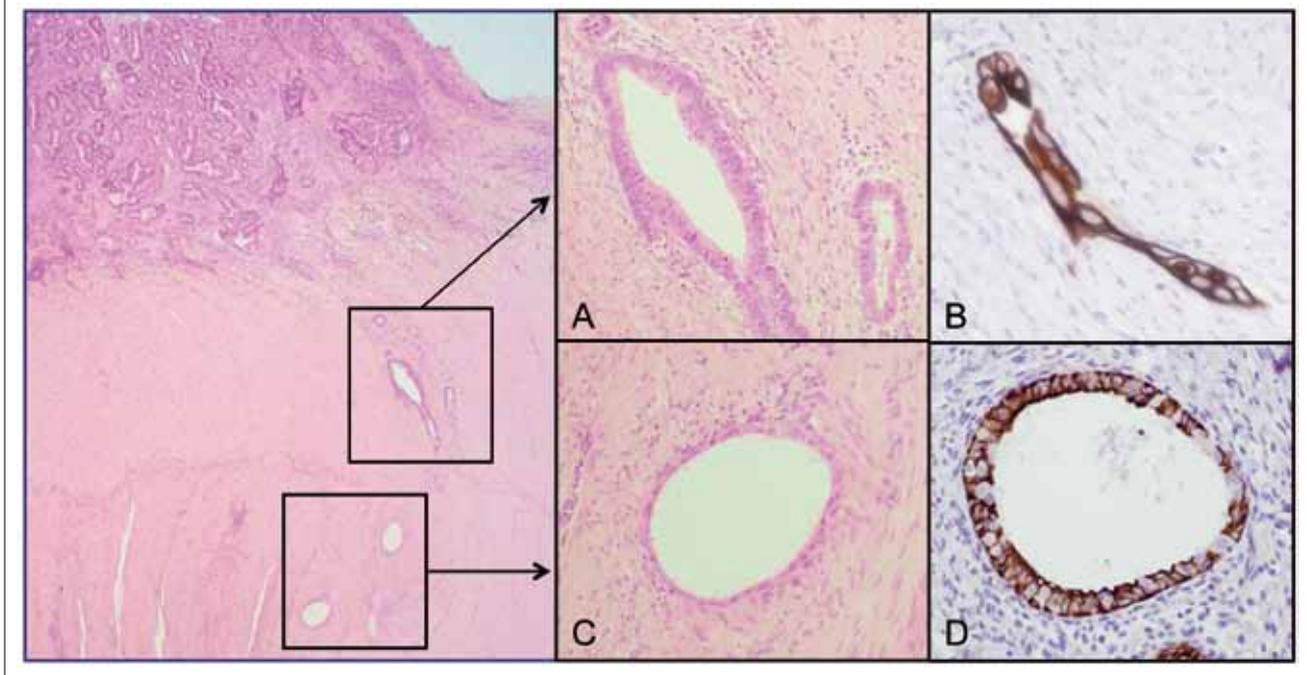
Differential diagnosis of the intestinal lesion is broad. Clinical and histological aspects of intestinal endometriosis may be confusing and pathologists should be aware of diagnostic pitfalls.

Obstructive diseases of the recto-sigmoidal tract to be considered are: ischaemic disease, diverticular disease, Crohn's disease and malignancies; in pre/peri-menopausal women, intestinal endometriosis, reported to occur in up to 34% of pelvic endometriosis, should be kept in mind¹⁻⁶. Malignant transformation of endometriosis is

Correspondence

Laura Moneghini, Division of Pathology, A.O. San Paolo, Milan, Italy - Tel. +39 02 81844606 - Fax +39 02 503 23168 - E-mail: laura.moneghini@ao-sanpaolo.it

Fig. 1. Glandular proliferation throughout muscular intestinal layers of the large bowel (haematoxylin and eosin, 2X). Double glandular components of the lesion with colorectal carcinoma glands (A: haematoxylin and eosin, 20X; B: cytokeratin 20 immunoreactivity, 20X) and endometriotic implants (C: haematoxylin and eosin, 20X; D: cytokeratin 7 immunoreactivity, 20X).



uncommon, but intestinal endometrioid or clear cell adenocarcinomas, and endometrial stromal sarcomas have been reported²⁻⁵.

Architectural glandular pattern of endometriosis can resemble those of ischaemia, inflammatory bowel diseases and/or ulcerative inflammation and infiltrating adenocarcinoma. At higher magnification, the presence of endometrial stroma surrounding bland glands is extremely useful to recognize endometriosis, but it is not always present, even in serial sections. Cellular atypia, mitotic activity and necrosis are useful to identify malignant structures. Intestinal endometriosis and its possible

malignant transformation should always be kept in mind in fertile and post-menopausal women with intestinal symptoms.

This case, presenting with intestinal obstructive symptoms, shows that endometriosis can be associated with colon cancer. Correct diagnosis, strictly depending on immunohistochemical characterization of the lesion, is crucial for treatment. As colon cancer staging guides oncological treatment, it is extremely important not to misinterpret the concurrent endometriosis as colonic neoplastic proliferation, thus overstaging and overtreating the patient.

References

- 1 Kelly P, McCluggage WG, Gardiner KR, Loughrey MB. *Intestinal endometriosis morphologically mimicking colonic adenocarcinoma*. *Histopathology* 2008;52:510-4.
- 2 Chen KT. *Endometrioid adenocarcinoma arising from colonic endometriosis mimicking primary colonic carcinoma*. *Int J Gynecol Pathol* 2002;21:285-8.
- 3 Leiserowitz GS, Gumbs JL, Oi R, et al. *Endometriosis-related malignancies*. *Int J Gynecol Cancer* 2003;13:466-71.
- 4 McCluggage WG, Desai V, Toner PG, et al. *Clear cell adenocarcinoma of the colon arising in endometriosis: a rare variant of primary colonic adenocarcinoma*. *J Clin Pathol* 2001;54:76-7.
- 5 Chen CW, OU JJ, Wu CC, et al. *High-grade endometrial stromal sarcoma arising from colon endometriosis*. *Int J Colorectal Dis* 2007;22:1551-3.
- 6 Yantiss RK, Clement PB, Young RH. *Neoplastic and pre-neoplastic changes in gastrointestinal endometriosis: a study of 17 cases*. *Am J Surg Pathol* 2000;24:513-24.

Collision tumour of the breast composed of Merkel cell carcinoma and invasive ductal carcinoma: a case report

D. NEDVED¹, C. CONNOR², P. SHARMA³, M. O'NEIL¹

Departments of ¹Pathology; ²Surgery, and ³Internal Medicine; University of Kansas, Kansas City, KS, USA

Key words

Merkel cell carcinoma • Collision tumour with invasive breast carcinoma

Summary

We report a case of a 71-year-old female with a palpable breast mass. Pathologic evaluation of the breast mass showed a unique collision tumour with a high-grade invasive and in-situ ductal carcinoma component and a high-grade neuroendocrine carcinoma com-

ponent. The neuroendocrine component turned out to be Merkel cell carcinoma (MCC), with immunohistochemical confirmation. To the best of our knowledge, this is the first case report of a collision tumour with ordinary ductal carcinoma and MCC in the breast.

Case presentation

A 71-year-old Caucasian female presented to her primary care physician with a palpable right breast mass. The patient was unsure how long the mass had been present and had never had a mammogram. She did not report any prior history of hormone replacement therapy or family history of breast cancer. Clinical breast exam revealed a palpable mass in the upper outer quadrant with mild skin retraction over the mass. Ultrasonography demonstrated a hypoechoic, heterogenous solid mass with irregular borders, measuring 1.5 cm in its greatest dimension, and the result of a diagnostic mammogram was compatible with that of the ultrasound. Needle core biopsy revealed a high grade neuroendocrine neoplasm associated with high-grade ductal carcinoma in situ (DCIS) with comedo necrosis. Immunohistochemistry demonstrated the neuroendocrine component to be positive for cytokeratin (CK) 8 (CAM 5.2), synaptophysin, neurofilaments, chromogranin A, and CK 20. In particular, CK 20 showed characteristic dot-like positivity. CK 7 and thyroid transcription factor-1 (TTF-1) were negative in the neuroendocrine component. Ki-67/MIB-1 immunostaining demonstrated a proliferative index of approximately 90% in the neuroendocrine component. In contrast, the DCIS was positive for CK7 and CK8 and

negative for CK 20, neurofilaments, chromogranin A and synaptophysin. The DCIS component showed 95% and 75% positivity for oestrogen (ER) and progesterone receptor (PGR), respectively. MCC was negative for ER and PGR. A diagnosis of Merkel cell carcinoma (MCC) associated with high grade DCIS of the breast was made. Lumpectomy with sentinel lymph node biopsy was planned. At the time of the surgical procedure, one month after the ultrasound was performed, it was noted that the palpable mass measured approximately 5 cm in size and the sentinel lymph node frozen section was positive for metastatic tumour consistent with MCC. Thus a right modified radical mastectomy was performed. The mastectomy specimen demonstrated a palpable mass measuring 4.5 cm in its greatest dimension adjacent to a previous biopsy site cavity. On permanent sections, histologic examination revealed a MCC (comprising 80% of the overall tumour bed) intimately admixed with high grade comedo DCIS (Fig. 1) (approximately 20% of the overall tumour bed) and a small 2 mm focus of poorly differentiated invasive ductal carcinoma (Fig. 2). Immunohistochemistry was repeated on the entire tumour mass and showed identical results to the needle biopsy sample (Figs. 3-5). In addition, the invasive component showed an identical immunophenotype to the DCIS. HER-2/neu immunohistochemistry showed 1+ staining

Correspondence

Maura O'Neil, Department of Pathology & Laboratory Medicine, MS 3045, 3901 Rainbow Blvd., Kansas City, KS 66160, USA - Tel. (913) 588-7103 - Fax (913) 588-8780 - E-mail: moneil@kumc.edu

Fig. 1. Image showing two tumours: the solid growth pattern of blue cells with vesicular nuclei of the MCC is seen on the left (asterisk) while a more eosinophilic large duct filled with DCIS with comedo necrosis is seen on the right (arrow). (haematoxylin and eosin).

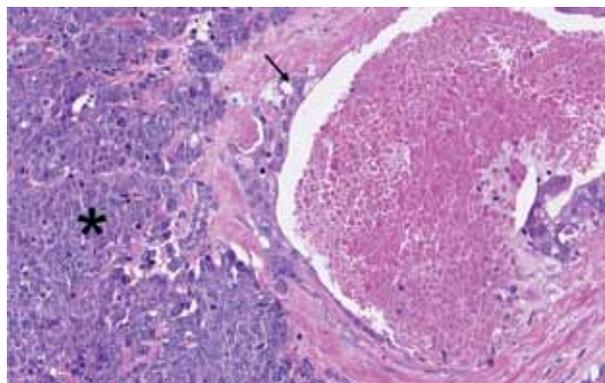
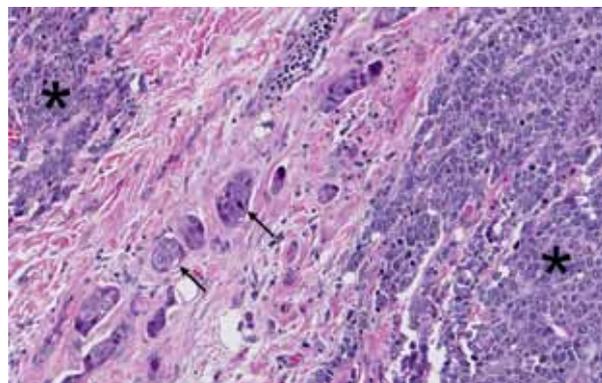


Fig. 2. This image shows scattered malignant epithelial nests of the invasive ductal carcinoma component (arrows) intimately admixed with MCC on both sides (asterisks). (haematoxylin and eosin).



of the invasive and in-situ ductal carcinoma and no staining of the MCC component. Subsequent fluorescence in situ hybridization for HER-2 detected no amplification in any of the components. Despite clinical suspicion, the overlying skin was free of tumour. Two additional separate satellite tumour nodules of MCC (each measuring <0.5 cm and located 2.0 cm from the main tumour mass) were present. Of the 23 lymph nodes submitted, six were

positive for metastatic MCC, and none were positive for metastatic breast carcinoma.

Follow-up

Post-operative staging with computed tomography (CT) scan of the chest, abdomen and pelvis and a positron emis-

Fig. 3. A: MCC featured by solid sheets of basophilic monomorphic cells with a high mitotic count, and high nuclear:cytoplasmic ratio (Hematoxylin and Eosin). **B:** MCC showing diffuse, strong positivity for CK 8/CAM 5.2 (immunoperoxidase). **C-D:** Diffuse strong cytoplasmic positivity for both chromogranin (C) and neurofilament proteins (D) with dot-like features (immunoperoxidase).

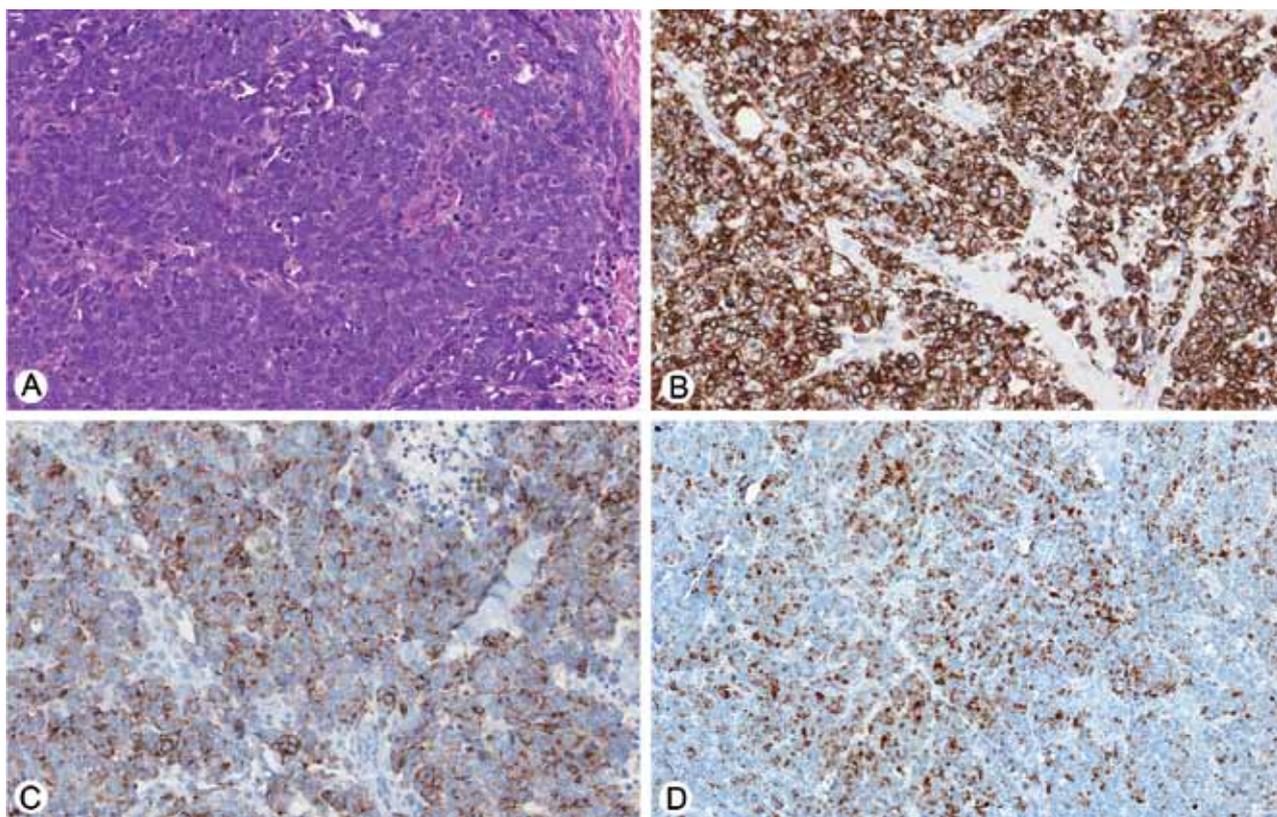


Fig. 4. A: In-situ and invasive ductal carcinoma showing strong positivity for CK 7. **B:** MCC is negative for CK 7. (immunoperoxidase).

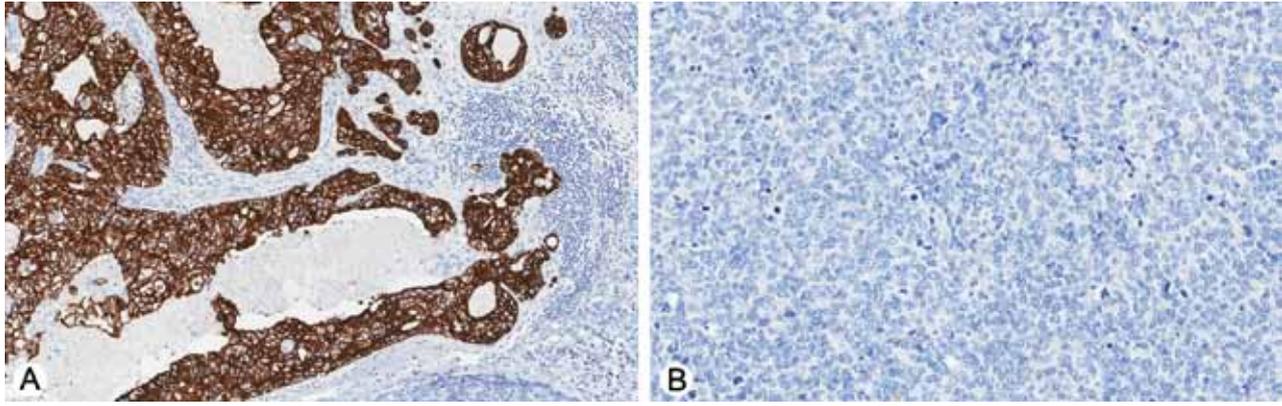
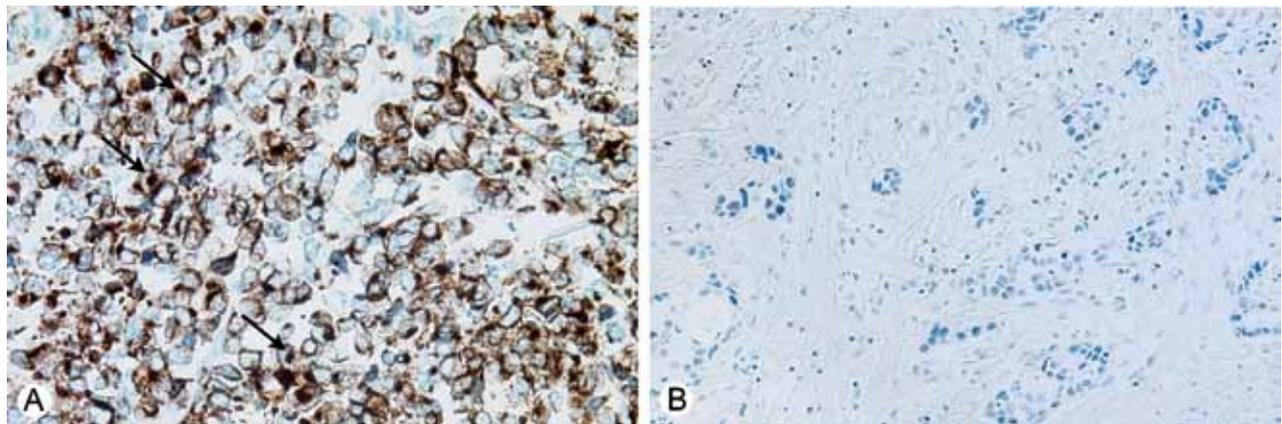


Fig. 5. A: CK 20 immunohistochemical stain depicting the paranuclear dot-like positivity (indicated by arrows) in the MCC tumour component. **B:** The invasive ductal carcinoma is negative for CK 20. (immunoperoxidase).



sion tomography (PET) scan demonstrated multiple metastatic tumour nodules within the liver. Biopsy revealed metastatic MCC. Systemic chemotherapy with etoposide and carboplatin was recommended. However, the patient developed postmastectomy chest wall flap necrosis and chest wall wound healing problems, and thus chemotherapy could not be started and radiation therapy could not be recommended. Therefore, after an octreotide scan was obtained which showed activity in the liver lesions, therapy with Sandostatin LAR Depot was initiated. Unfortunately within the next two weeks the patient developed rapidly progressive liver disease, acute cholecystitis, local chest wall recurrence, renal failure and declining physical status. The patient and family opted for palliative measures and the patient succumbed to her disease just four months after her initial presentation. For completeness and due to the particularly aggressiveness of MCC in this case, while preparing this manuscript, we studied the immunohistochemical expression of p63, since this marker has been shown to correlate with prognosis and survival. The MCC component showed focal weak p63 staining with Ventana 790-4509 clone 4A4.

Discussion

Merkel cell carcinoma (MCC) is an aggressive cutaneous tumour of older Caucasian individuals with a predilection for sun exposed areas¹, which was first described by Toker in 1972 as trabecular carcinoma of the skin². Exceptional cases outside the skin, such as those primarily arising on mucosal surfaces of the oral cavity³, rhinopharynx⁴, conjunctiva⁵, and vagina⁶ have been recorded. MCC is occasionally seen also in a lymph node (mainly inguinal) in the absence of a primary skin tumour, either as an occurrence secondary to the regression of the cutaneous primary⁷ or as a tumour primarily arising from intranodal neuroendocrine cells⁸. A handful of cases of primary MCC of the breast have been reported as well⁹⁻¹⁴. Microscopically, MCC is composed of nests and strands of monomorphic cells with a high mitotic count and a high nuclear: cytoplasmic ratio¹⁵. Immunohistochemical studies are generally required for diagnosis and for distinguishing MCC from other mimickers. In this particular case, the main histopathologic dif-

ferential diagnostic considerations included neuroendocrine carcinoma of the breast (so-called carcinoid of the breast, Cubilla-Woodruff type), invasive ductal carcinoma of breast with neuroendocrine features, metastatic small cell (neuroendocrine) carcinoma from other sites (mainly the lung), and primary small cell carcinoma of the breast, now qualified as pulmonary type, which occasionally was called Merkel-like carcinoma of the breast in the past. Generally, MCC will stain positive for CK20 (in a characteristic paranuclear, dot-like pattern), chromogranin A, synaptophysin, neuron-specific enolase, and CK8/CAM 5.2, and is negative for TTF-1 and CK 7¹⁵. Neuroendocrine carcinoma of the breast (so-called [primary] carcinoid tumour) is also positive for neuroendocrine markers, such as chromogranin A, synaptophysin, and neuron-specific enolase, but does not show positivity for CK20. Neuroendocrine differentiation can also occur in invasive breast carcinoma NOS and in some special types, such as mucinous carcinoma and solid papillary carcinoma: these tumours will characteristically stain positive for CK7 and negative for CK20 and neurofilament proteins (the converse is true for most MCC)¹⁶. Metastatic neuroendocrine carcinoma, large cell type and small cell type from another site to the breast is usually positive for TTF-1 as well as negative for both CK20 and neurofilament proteins, while the overwhelming majority of MCC are negative for TTF-1 and positive for both CK20 and neurofilament proteins¹⁷. Primary small cell carcinoma of the breast, of which around 50 cases have been recorded in the literature, is often positive for TTF-1 and usually negative for CK20¹⁸.

Recent studies revealed the presence of a novel polyomavirus in Merkel cell carcinoma, aptly named Merkel cell polyomavirus (MCPyV). Initially found in eight of 10 MCC patients by Feng et al.¹⁹, that research was expanded to include 39 more patients with MCC who also were positive for the same polyomavirus²⁰. However, the clinical importance of MCPyV infection on prognosis, detection, and treatment has yet to be firmly established. There is a reported diagnostic role of immunohistochemistry for MCPyV which is highly specific. However, sensitivity for MCPyV is not high, ranging from 18-67% in limited case series²¹⁻²⁴. MCPyV positivity was initially claimed to correlate with worse prognosis and shorter survival, even though this concept was not confirmed by further studies which, while not disclosing any statistically significant difference in survival between MCPyV positive and negative tumors on one hand, on the other discovered that p63 is a better immunohistochemical marker for prognosis in MCC²⁵. In our case, an immunohistochemical antibody stain for MCPyV was performed, using clone CMB2B4, directed against the Merkel cell virus large T-antigen, and was negative (courtesy of Dr. Dominic Spagnolo, of PathWest Laboratory Medicine, Western Australia). p63 in our case showed focal faint positivity.

The recommended treatment for MCC is usually surgery (wide local excision of primary tumour along with regional lymph node dissection). Radiation therapy can be effective as an adjunct, and chemotherapy is employed for metastatic tumours. In one case with metastatic MCC Sandostatin was employed with good clinical response after receptors for somatostatin were demonstrated using OctreoScan²⁶. Along this line, in our case a course of Sandostatin was administered for three consecutive days with the plan for injections every three weeks with hope for long-term treatment. Unfortunately, the patient developed liver failure, thought to be of multifactorial aetiopathogenesis due to both tumour burden in the liver and octreotide toxicity. Shortly thereafter, the patient developed acute renal injury, secondary to dehydration, and the patient was started on hospice.

Reported cases of primary MCC of the breast have occurred in patients 52 to 93 years old, with one male patient documented^{9-11 13 14}. Tumour size at presentation ranged from 1.7 to 10 cm, and rapid growth was reported in most cases. One case demonstrated metastasis into the spinal canal compressing the medullary cord and causing paralysis⁹. Another case (relevant to the male patient) reported no lymph node or distant metastases during a follow-up period of 5 years¹⁰ [follow-up information received from one of the authors, Ahmed Al-zaraa, who was contacted for the purpose of this publication], whereas another case reported lymph node metastases in 14 of 22 lymph nodes¹³.

The unique feature in our case is that the MCC is intermixed with a high-grade comedo DCIS and invasive ductal carcinoma of the breast.

Second concurrent malignancies in the same as well as different anatomic sites are not uncommon in patients with MCC. Brenner et al.²⁷ reported 25% of patients diagnosed with MCC had a second malignancy diagnosed either previously or shortly thereafter. Gass et al.²⁸ reported that as many as 70% of patients with MCC had a second primary malignant tumour. The majority of the second malignancies were additional cutaneous tumours, such as melanoma, squamous cell carcinoma and basal cell carcinoma. A minority of patients presented with non-cutaneous malignancies including colorectal, haematological and breast tumours (lobular carcinoma). It has been proposed that the second neoplasm itself may represent a predisposing immunocompromised condition for development of MCC or both tumours may be the result of a generalized immunocompromised state^{27 28}.

However, while concurrent MCC with a second neoplasm is common (around 40% of cases), collision tumours between MCC and other malignancies are quite rare. A collision tumour is a generic term for the extremely uncommon coexistence or merging of two histologically distinct malignant tumours within the same mass. Reported collision tumours with MCC include squamous cell carcinoma, either in situ or invasive²⁹, chronic lymphocytic leukaemia involving the skin³⁰

and melanoma³¹. Additionally, mixed MCC showing squamous or eccrine foci of differentiation (biphenotypic differentiation), or squamous, glandular and melanocytic foci (tripartite differentiation) have been also recorded²⁹, attesting to the origin of MCC from a multipotential stem cell of ectodermal derivation, but even heterologous (leiomyosarcomatous, rhabdomyoblastic, fibrosarcomatous) differentiation or dedifferentiation have been reported both in primary and metastatic MCC³²⁻³⁴.

In conclusion, to the best of our knowledge and based on a search of the literature, a collision tumour with ductal breast carcinoma (either ordinary or special type) and MCC has not previously been reported.

References

- ¹ Heath M, Jaimes N, Lemos B, et al. *Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features*. J Am Acad Dermatol 2008;58:375-81.
- ² Toker C. *Trabecular carcinoma of the skin*. Arch Dermatol 1972;105:107-10.
- ³ Yom SS, Rosenthal DI, El-Naggar AK, et al. *Merkel cell carcinoma of the tongue and head and neck oral mucosal sites*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:761-8.
- ⁴ Wu KN, McCue PA, Berger A, et al. *Detection of Merkel cell carcinoma polyomavirus in mucosal Merkel cell carcinoma*. Int J Surg Pathol 2010;18:342-6.
- ⁵ Kase S, Ishijima K, Ishida S, et al. *Merkel cell carcinoma of the conjunctiva*. Ophthalmology 2010;117:637.e1-2.
- ⁶ Coleman NM, Smith-Zagone MJ, Tanyi J, et al. *Primary neuroendocrine carcinoma of the vagina with Merkel cell carcinoma phenotype*. Am J Surg Pathol 2006;30:405-10.
- ⁷ Tarantola TI, Vallow LA, Halyard MY, et al. *Unknown primary Merkel cell carcinoma: 23 new cases and a review*. J Am Acad Dermatol 2013;68:433-40.
- ⁸ Eusebi V, Capella C, Cossu A, et al. *Neuroendocrine carcinoma within lymph nodes in the absence of a primary tumor, with special reference to Merkel cell carcinoma*. Am J Surg Pathol 1992;16:658-66.
- ⁹ Cusick L, Refsum S. *Merkel cell carcinoma of the breast: report of a case and review of the literature*. Ulster Med J 2004;73:137-8.
- ¹⁰ Alzaraa A, Thomas G, Vodovnik A, et al. *Merkel cell carcinoma in a male breast: a case report*. Breast J 2007;13:517-9.
- ¹¹ Marullo M, Cancellieri A, Lemma G, et al. *Merkel cell tumour: a case report and literature review*. G Chir 2004;25:395-7.
- ¹² Asioli S, Dorji T, Lorenzini P, et al. *Primary neuroendocrine (Merkel cell) carcinoma of the nipple*. Virchows Arch 2002;440:443-4.
- ¹³ Sananes N, Meyer C, Straub P. *Merkel cell carcinoma of the breast: ST-scan and histologic finding*. Breast J 2010;16:429-31.
- ¹⁴ Monteagudo B, Cabanillas M, Cainzos T, et al. *Merkel cell carcinoma of the breast*. Actas Dermosifiliogr 2009;100:151-62.
- ¹⁵ Schrama D, Becker JC. *Merkel cell carcinoma - pathogenesis, clinical aspects and treatment*. J Eur Acad Dermatol Venereol 2011;25:1121-9.
- ¹⁶ Boyd A, Hayes B. *Metastatic small cell neuroendocrine carcinoma of the breast*. J Cutan Pathol 2012;39:1042-6.
- ¹⁷ Ralston J, Chiriboga L, Nonaka D. *MASH1: a useful marker in differentiating pulmonary small cell carcinoma from Merkel cell carcinoma*. Mod Pathol 2008; 21:1357-62.

Conclusion

Merkel cell carcinoma represents a highly deadly skin tumour, usually affecting sun-exposed areas and immunocompromised hosts. A unique case of a collision tumour with MCC and high-grade invasive and in-situ ductal carcinoma presenting as a breast mass is presented herein. The particularly rapid progression of local and systemic disease in this patient is consistent with the known unfavourable prognosis of MCC. Pathologists should be aware of the possibility of such an occurrence as the prognosis and management of both invasive ductal carcinoma with neuroendocrine differentiation and primary neuroendocrine carcinomas of the breast are markedly different from those of MCC.

- ¹⁸ Christie M, Chin-Lenn L, Watts MM, et al. *Primary small cell carcinoma of the breast with TTF-1 and neuroendocrine marker expressing carcinoma in situ*. Int J Clin Exp Pathol. 2010;3:629-33.
- ¹⁹ Feng H, Shuda M, Chang Y, Moore PS. *Clonal integration of a polyomavirus in human Merkel cell carcinoma*. Science 2008;319:1096-100.
- ²⁰ Kassem A, Schöpflin A, Diaz C, et al. *Frequent detection of Merkel cell polyomavirus in human Merkel cell carcinomas and identification of a unique deletion in the VP1 gene*. Cancer Res 2008;68:5009-13.
- ²¹ Andres C, Belloni B, Puchta U, et al. *Is Merkel cell polyomavirus also prevalent in non-Merkel cell carcinoma (MCC) tumors of sun exposed skin? A study of 66 patients*. J Cutan Pathol 2010;37:28-34.
- ²² Duncavage EJ, Zehnbauer BA, Pfeifer JD. *Prevalence of Merkel cell polyomavirus in Merkel cell carcinoma*. Mod Pathol 2009;22:516-21.
- ²³ Paik JY, Hall G, Clarkson A, et al. *Immunohistochemistry for Merkel cell polyomavirus is highly specific but not sensitive for the diagnosis of Merkel cell carcinoma in the Australian population*. Hum Pathol 2011;42:1385-90.
- ²⁴ Busam KJ, Jungbluth AA, Rektman N, et al. *Merkel cell polyomavirus expression in Merkel cell carcinomas and its absence in combined tumors and pulmonary neuroendocrine carcinomas*. Am J Surg Pathol 2009;33:1378-85.
- ²⁵ Hall BJ, Pincus LB, Yu SS, et al. *Immunohistochemical prognostication of Merkel cell carcinoma: p63 expression but not polyomavirus status correlates with outcome*. J Cutan Pathol 2012;39:911-7.
- ²⁶ Cirillo F, Filippini L, Lima GF, et al. *Merkel cell tumor. Report of case and treatment with octreotide*. Minerva Chir 1997;52:1359-65.
- ²⁷ Brenner B, Sulkes A, Rakowsky E, et al. *Second neoplasms in patients with Merkel cell carcinoma*. Cancer 2001;91:1358-62.
- ²⁸ Gass J, Rytina S, Greenberg D, et al. *Multiple primary malignancies in patients with Merkel cell carcinoma*. J Eur Acad Dermatol Venereol. 2010;24:601-3.
- ²⁹ Kohler S, Kerl H. *Merkel cell carcinoma*. In: LeBoit PE, Burg G, Weedon D, et al, eds. *World Health Organization Classification of Tumors. Pathology and Genetics: Skin Tumors*. Lyon: IARC Press 2006, pp. 272-273.
- ³⁰ Barroeta JE, Farkas T. *Merkel cell carcinoma and chronic lymphocytic leukemia (collision tumor) of the arm: a diagnosis by fine-needle aspiration biopsy*. Diagn Cytopathol 2007;35:293-295.

- ³¹ Forman SB, Vidmar DA, Ferringer TC. *Collision tumor composed of Merkel cell carcinoma and lentigo maligna melanoma*. J Cutan Pathol 2008;35:203-6.
- ³² Cooper L, Debono R, Alsanjari N, Al-Nafussi A. *Merkel cell tumour with leiomyosarcomatous differentiation*. Histopathology 2000;36:540-3.
- ³³ Adhikari LA, McMalmont TH, Folpe AL. *Merkel cell carcinoma with heterologous rhabdomyoblastic differentiation: the role of immunohistochemistry for Merkel cell polyomavirus large T-antigen in confirmation*. J Cutan Pathol 2012;39:47-51.
- ³⁴ Tan KB, Murali R, Karim RZ, et al. *Merkel cell carcinoma with fibrosarcomatous differentiation*. Pathology 2008;40:314-6.

Intestinal tuberculosis: a diagnostically-challenging case misdiagnosed as Crohn's disease at colorectal biopsy

M. ONORATI¹, D. MORGANTI², M. BOCCHI², E. COLOMBO², G. PETRACCO¹, P. UBOLDI¹, F. DI NUOVO¹

¹ Pathology Unit, Garbagnate Milanese, AO "G. Salvini" Garbagnate Milanese, Italy;

² Gastroenterology Unit, Garbagnate Milanese, AO "G. Salvini" Garbagnate Milanese, Italy

Key words

Intestinal tuberculosis • Crohn's disease • Diagnosis

Summary

The clinical presentation of two different digestive diseases such as Crohn's disease and intestinal tuberculosis may be so similar to induce a delay in correct diagnosis and appropriate treatment (immune suppression versus antibiotic therapy). Herein, we describe the case of a young man from Eastern Europe who came to our observation complaining of clinical symptoms initially misdiagnosed as an inflammatory bowel

disease. It is important to keep in mind the possibility of an active tubercular disease, particularly in patients coming from countries endemic for the disease. Morphological findings of sarcoid-like granulomas at biopsy is not enough for a conclusive diagnosis of Crohn's disease, and tuberculosis should be ruled out on the basis of clinical information, laboratory tests and radiological imaging.

Introduction

Crohn's disease and intestinal tuberculosis (TBC) have a different aetiology, but share several common clinical and histological features, raising diagnostic problems. These two diseases require different therapeutic approaches, immunosuppressive in Crohn's disease while antibiotic in tuberculosis, with possible injury to the patient if prolonged immunosuppressive treatment is administered during an on-going bacterial infection. If a correct diagnosis of intestinal tuberculosis may be suspected in areas of high tubercular endemic rate, this is not the case in geographical areas such as Europe, especially Western Europe, where tuberculosis is rarely seen. We describe the case of a 28-year-old man admitted to Garbagnate Milanese Hospital for recurrent abdominal pain lasting one year, accompanied by nausea and vomiting. In the absence of complete clinical data, he was misdiagnosed as suffering from Crohn's disease.

Case report

A 28-year-old male immigrant from Moldova was admitted to our hospital in Garbagnate Milanese for a colonoscopy, requested by his physician because of recurrent abdominal pain of one year duration, accompanied by nausea and vomiting. He did not complain of bowel disorders, but of a progressive weight loss, about 14 kg from the beginning of symptoms. He had no significant past medical history except for acute bronchitis one year before. On colonoscopy, the left and transverse colon appeared to be macroscopically normal, while multiple discrete longitudinal ulcerations with fibrin deposits were seen along the entire ascending colon. In the most proximal portion of the right colon these ulcerations merged with one another. The caecum appeared macroscopically normal, except for prominent signs of mucosal inflammation and narrowing of the ileocecal valve, so that the ileum was not explored. The biopsies of lesions were fixed in formalin and stained with haematoxylin and eosin. Microscopically, samples taken from rectum, sigmoid and left colon showed mild to moderate chronic and acute inflammation, with eo-

Acknowledgements

The authors wish to thank Dr. Paolo Fociani and Prof. Piero Tosi for their expertise and fruitful discussion.

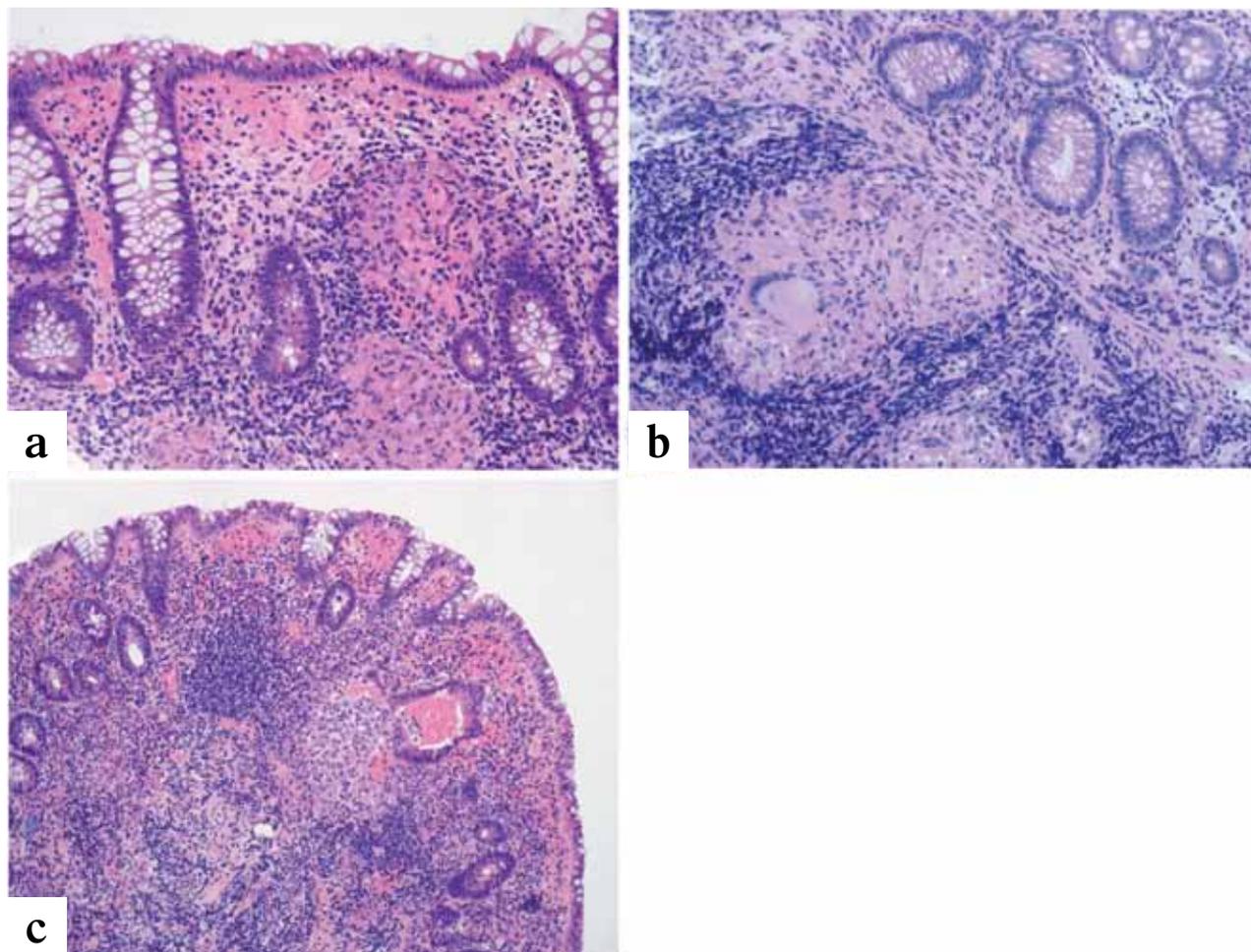
Correspondence

Monica Onorati, Pathology Unit, Garbagnate Milanese, AO "G. Salvini", viale Carlo Forlanini 121, 20024 Garbagnate Milanese, Italy - Tel. +39 02 994302631-2 - E-mail: monica.onorati@libero.it

sinophilia, glandular distortion and scattered foamy histiocytes in the lamina propria. Samples taken from the proximal segments showed a pattern of severe chronic and acute inflammation, with eosinophilia and scattered erosions or frank ulcers, with foci of acute cryptitis, rare cryptic microabscesses and a mild degree of glandular distortion. A prominent histological feature was the presence of sarcoid-like granulomas without necrosis (Fig. 1). In consideration of this last characteristic and clinical data, a histological diagnosis of idiopathic chronic inflammatory bowel disease, suggesting active Crohn's disease, was made. Treatment with prednisone 1 mg/kg and mesalazine 3 g/day was started. The patient had a partial improvement of his general condition, followed by a subsequent slow worsening. Physical examination of the abdomen was normal, except for a slight pain in the right groin, without any further weight loss. Steroid treatment was maintained. Two weeks later the patient was examined again. He reported 1-2 bowel movements a day, with solid stools and no trace of blood. He also claimed moderate weakness, slight abdominal pain and occasional nausea. Slow tapering of

the steroid treatment was continued without reducing the dose of mesalazine. A few days later the patient returned because of vomiting and hyporexia. Meanwhile, he had spontaneously withdrawn steroid treatment. He reported progressive weight loss. The gastroenterologist consulted decided to hospitalize the patient for further investigation and therapeutic adjustment. During hospitalization steroid treatment was re-started, with a modest and temporary improvement of general conditions, followed by the reappearance of abdominal pain, nausea and vomiting. Due to the initial diagnosis and dyspeptic symptoms, an oesophagogastroduodenoscopy was also performed, which revealed a wide ulcer at the beginning of the descending duodenal segment. Biopsy samples, taken to rule out a duodenal localization of Crohn's disease, were consistent with a peptic ulcer. Moreover, a cytological examination of a brushing from distal oesophagus disclosed mycosis. Based on these findings, treatment with a biological drug was discussed by clinicians. Before the administration of infliximab, Mantoux and Quantiferon tests, together with HBV, HCV, HIV viral markers and a chest X-ray were performed. All

Fig. 1 a, b, c. Submucosal non-necrotizing granulomas in colonic biopsy specimens. The granulomas are often poorly formed and show a sarcoid-like aspect with scattered giant cells.
 (A: Haematoxylin-eosin (H&E); Original Magnification (O.M.): 20x; B: H&E; O.M.: 40x; C: H&E; O.M.: 10x)



these exams were normal, except for the chest X-ray, which revealed scattered nodular lesions consistent with possible tubercular disease. Laboratory tests showed a neutrophilic leucocytosis and a slight increase in erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). A chest and abdomen computed tomography CT was performed, which disclosed multiple pulmonary nodular lesions, with a diameter ranging from 6 to 23 mm, mainly peripherally and subpleurally placed, in upper right pulmonary lobe and lower and upper left lobes. Nodular calcifications and excavations were also seen. Blurred and soft hyperdense spots, adjacent to the nodules, compatible with tubercular lesions, were seen subpleurally, without any feature of pleural or pericardial effusion. Oval shaped lymph nodes were present in the Baretz's space and in pre- and subcarenal positions, with a maximum size of 15 mm. CT of the abdomen showed a thickening of the colonic walls, more marked in caecum and ascending colon, and of the last ileal loop as well as the ileocecal valve. The pneumologist called for consultation decided to perform a bronchoscopy, which showed a hyperaemic, friable and easily bleeding bronchial mucosa. A bacteriological culture performed on a smear from bronchoaspirated material revealed the presence of alcohol-acid resistant bacteria, while on laboratory tests an increase in biochemical indexes of inflammation was seen. Steroid treatment was withdrawn and the patient was promptly transferred to a specialized bronchopneumological ward, where further investigations confirmed a pulmonary tuberculosis. Treatment with rifampicin, isoniazide, pyrazinamide and ethambutol was started. The biopsies taken on the first colonoscopy were revised by a pathologist, but on all samples, accurately re-examined with Ziehl-Neelsen staining, no acid-fast bacteria were demonstrated. One month after discharge the patient appeared to be in good general conditions, symptomless and with a progressive recovery of body weight. A subsequent MRI of the upper and lower abdomen, performed with and without contrast, did not show any wall thickening or any other abnormal finding in any of the explored colic segments. Upon further clinical examination, performed three months later, the patient was in good health with a complete recovery of body weight and normal laboratory tests.

Discussion

Crohn's disease and intestinal TBC share several clinical and histological features, thus determining problems of differential diagnosis. The case herein represents an example of an insidious differential histological diagnosis due to the absence of complete clinical data. If a correct diagnosis can be relatively easier to suspect in areas of high tubercular prevalence, this is not the case in geographical areas such as Europe, especially Western Europe, where tuberculosis is rarely seen. In our case, correct diagnosis was achieved only after an unfavourable clinical course and an unsatisfactory therapeutic re-

sponse, which prompted consideration for treatment with a biological drug. Routine pre-treatment chest X-ray was the pivotal finding that prompted us to re-evaluate the patient. In fact, the absence of acid-fast bacteria in the revised histological samples, taken on the first colonoscopy, influenced subsequent diagnostic and therapeutic behaviour, and it was only the chest X-ray, performed when administration of a biological treatment was considered, that allowed for correct diagnosis. Even if a chest X-ray may be non diagnostic in almost half of cases^{1,2}, since less than 50% of patients affected from intestinal tuberculosis show clear-cut radiological marks of a pulmonary involvement, in our case it was the finding of nodular pulmonary lesions on a chest-X ray which induced us to perform a CT scan, which confirmed the presence of nodular opacities coherent with a tubercular disease. Crohn's disease and TBC seem to share a reduced constitutional immunity, since only 5-10% of patients infected by *Mycobacterium tuberculosis* develop a clinically active form of the disease². NOD2 (nucleotide binding oligomerization domain) and Toll-like (TLRs) receptors, for instance, may have a role in the early inducing phase of both the diseases², and individual changes in biological interactions between the host and bacteria can weaken the effectiveness of the immune response. Apart from epidemiological considerations, a diagnosis of Crohn's disease relies upon anamnestic, clinical, endoscopic and biopsy criteria and on the exclusion of a mycobacterial infection²⁻⁴. Cigarette smoking is an environmental factor associated with both diseases, probably due to a direct action on pulmonary macrophages or dendritic cells². The variety of extraintestinal features in inflammatory bowel diseases is well known, but its importance is weakened by the fact that extrapulmonary sites may also be involved in tuberculosis². Tuberculin reaction as a diagnostic criterion in patients with ileocolic manifestations of the disease is hampered by possible cross-reactions with the Calmette-Guérain bacillus (BCG), by the local prevalence of mycobacterial species different from the tubercular ones and by diffusion of latent forms of tubercular infections². From a clinical point of view, both diseases may have an insidious onset, with chronic abdominal complaints and systemic symptoms, and raise problems of differential diagnosis with lymphoma, adenocarcinoma and infectious diseases, such as for example amebiasis, actinomycosis or *Yersinia enterocolitica*¹. Accurate colonoscopy, with examination of the last ileal loop and multiple biopsies, is of pivotal importance in the evaluation of every patient with suspected Crohn's disease or intestinal tuberculosis. In the majority of cases of intestinal tuberculosis, the ileocaecal tract is affected. However, in almost 20% of patients a segmentary involvement of colon is seen in the absence of any lesion in the ileocaecal loop². In 44% of cases, the so-called "skip lesions", involving two or more colonic segments alternating with macroscopically normal tracts, may be seen². Fistulae may be seen both in Crohn's disease and intestinal tuberculosis¹. In an Indian endoscopic series including 43

patients³, colonic mucosal ulcerations were present in 70% of cases, followed by mucosal nodules (56%), ulcerations associated to nodularities (44%), distortion of caecum and ileocaecal valve (40%), luminal narrowing (23%), polypoid lesions (14%) and bridging fibrotic stripes (7%). Together with the endoscopic picture, histologic examination is an important diagnostic criterion, particularly difficult to be correctly interpreted in areas of high tubercular prevalence. In intestinal tuberculosis, the pathognomonic marker caseous granuloma, together with the presence of acid-fast bacteria, can be found in less than 30% of cases^{2,4}, and a bacterial culture is unequivocally positive in only 20% of cases and requires a lengthy time for results^{2,3}. Lesions such as confluent, multiple and/or large granulomas, ulcerations surrounded by bands of epithelioid histiocytes, submucosal granulomas and signs of severe mucosal inflammation can be seen almost exclusively in intestinal TBC². On the contrary, in Crohn's disease single granulomas and signs of mucosal distortion at a distance from the granulomas tend to prevail². Nonetheless, several biopsy samples from all colonic segments, either macroscopically involved or seemingly normal, are of paramount importance. In a retrospective study from India⁴, biopsy samples taken from 61 anatomic sites of 20 patients suffering from intestinal TBC were examined and compared to biopsy samples taken from 112 anatomic sites of 20 patients suffering from intestinal Crohn's disease. In patients with intestinal TBC, granulomas tended to be multiple, of greater size, confluent and often contain areas of caseous necrosis. Ulcerations appeared to be surrounded by clusters of epithelioid histiocytes and presented brisk submucosal inflammation. In contrast, in cases of Crohn's disease granulomas appeared to be more scattered, smaller and associated with foci of active colitis and diffuse signs of chronic inflammation, even in areas of normal endoscopic appearance. Among diagnostic imaging techniques, the role of barium X-ray examination has been declining, while contrast-enhanced CT, ultrasound examination and MRI are increasingly performed. Using these techniques, accurate evaluation of

intestinal parietal alterations, mesenteric lesions and local lymph-nodes network can be made². A possible role of laparoscopy in the differential diagnostic work-up between intestinal tuberculosis and Crohn's disease has not yet been validated by systemic studies². Anti-*Saccharomyces cerevisiae* antibodies (ASCA), which can be positive in 60-80% of patients suffering from Crohn's disease, are not useful in differential diagnosis⁵. In a recent study from the USA⁶, the problem of differential diagnosis between Crohn's disease and intestinal tuberculosis has been approached by comparing several parameters in two groups, each with 53 patients, suffering from one or the other disease. Together with past history and clinical data, histological findings were re-examined, with particular attention to ulcerations, architectural changes, kind of damage to crypts and type of granulomas and inflammation. A multivariate analysis identified, as possible independent predictive factors of intestinal tuberculosis, compared to Crohn's disease, four diagnostic criteria, namely presence of blood in faeces, weight loss, involvement of the sigmoid colon and presence of a focally-active colitis. However, the authors cautioned that these data need to be confirmed in further studies before being implemented in clinical practice. In our case, the point completely ignored in the initial work up of the patient was geographical origin, since some areas of Eastern Europe have a higher tubercular prevalence compared to Western Europe. The fact that the histologic samples which were revised after correct diagnosis did not show acid-fast bacteria could be related to scanty biopsy sampling. A further cause of the diagnostic delay was the lack of a chest X-ray on the occasion of the first hospital admission. This was performed only in a later phase of the clinical course, when it was decided to administer a biological treatment, and was of crucial importance in achieving correct diagnosis and starting effective treatment.

On the basis of our experience, a correct diagnosis can usually be made only through careful clinical history, accurate physical examination and complete diagnostic testing.

References

- Loh KWJ, Bassily R, Torresi J. *Crohn's Disease or tuberculosis?* J Travel Med 2011;15:1-3.
- Epstein D, Watermeyer G, Kirsch R. *Review article: the diagnosis and management of Crohn's disease in populations with high-risk rates for tuberculosis.* Aliment Pharmacol Ther 2007;25:1373-88.
- Alvares JF, Devarbhavi, Makhija P, et al. *Clinical, colonoscopic and histological profile of colonic tuberculosis in a tertiary hospital.* Endoscopy 2005;37:351-6.
- Pulimood AB, Ramakrishna BS, Kurian G, et al. *Endoscopic mucosal biopsies are useful in distinguishing granulomatous colitis due to Crohn's disease from tuberculosis.* Gut 1999;45:537-41.
- Makharia GK, Sachdev V, Gupta R, et al. *Anti Saccharomyces cerevisiae antibody does not differentiate between Crohn's disease and intestinal tuberculosis.* Dig Dis Sci 2007;52:33-9.
- Makharia GK, Srivastava S, Goswami P, et al. *Clinical, endoscopic, and histological differentiations between Crohn's disease and intestinal tuberculosis.* Am J Gastroenterology 2010;105:642-51.

Primary tumour of the round ligament of the liver: a case presentation

J.A. SOLARANA ORTÍZ¹, J.E. PLACENCIA GILART¹, M. RODRÍGUEZ DIÉGUEZ², Z. MIRANDA MOLES²,
M. PULLÉS LABADIÉ², J.C. LAU CUZA³, S. CORPAS FUSTER⁴

¹Department of General Surgery; ²Department of Gastroenterology; ³Department of Anesthesiology; ⁴Department of Pathology. Hospital "Vladimir I. Lenin", Holguín, Cuba

Key words

Mesogastric • Tumour • Round ligament of the liver

Summary

A 40-year-old Caucasian female patient presented to the outpatient General Surgery ward in "V. I. Lenin" Teaching Hospital complaining of a recurrent mesogastric pain that had lasted for 3 months. Physical examination showed a palpable mass confined to that area. She was then admitted with diagnosis of an abdominal tumour. Diagnostic work-up revealed that the process involved the round ligament of the liver, which is an

exceptional localization, which motivated us to publish this case after surgical treatment by excision, having also taken into account the results of histopathology which revealed a PEComa, confirmed by immunohistochemistry. After reviewing the available literature, the low incidence of these lesions, as well as the unusual histological variety, makes the present case one of interest.

Introduction

The round ligament of the liver is part of its means of fixation¹. Tumours can grow in any cells of the liver, for example in the parenchyma, in the epithelium of the biliary ducts, in the vascular structures and in any mesenchymatous tissue. Tumours in the round ligament are very rare²⁻⁶. In the last 5 decades few cases have been reported worldwide.

Case report

A 40-year-old white female patient, from a rural area, who had been apparently healthy, presented to the outpatient General Surgery's ward at "Vladimir Ilich Lenin" Provincial Teaching Hospital, in Holguín province, Cuba, complaining of a recurrent mesogastric pain which had lasted for 3 months. The pain was of variable intensity and insidious, without a well defined radiation, and with no accompanying symptoms. She had some relief with analgesics.

On examination an abdominal mass was found, palpable in both the mesogastrium and the right hypochondrium, which measured approximately 8 centimetres, rounded in shape with regular borders, flat surface, hard and movable. On palpation there was no tenderness. She was hospitalized with the presumptive diagnosis of an abdominal tumour. On investigation, laboratory studies, including liver function tests, were within normal limits. Chest X-ray showed no particular pleuropulmonary lesions. The abdominal ultrasound revealed a 60 by 50 cm well defined rounded heterogeneous image of refringent contours, with multiple calcifications of small size, located in the mesogastric area, to the right of the middle line (Fig. 1) but, with no defined origin of the tumour. Abdominal CT showed a very dense image in the abdominal cavity, 40-50 HUs, 71 by 56 mm, homogeneous, with inner dotted calcifications, that produced, after the injection of contrast medium, an intense non-homogenous enhancement, with a pedicle that adhered to the abdominal wall at the level of the umbilical scar, displacing adjacent intestinal structures, with a well defined flat contour. (Fig. 2)

Correspondence

Joaquin Solarana Ortiz, Department of General Surgery, Hospital V I Lenin. Ave. Lenin N°2. Holguín, Cuba - Tel. +5324-489122 - E-mail: margarita@hvil.hlg.sld.cu

Fig. 1. Ultrasonographic image showing the tumour with undefined origin.



Fig. 2. CT image after injection of contrast medium showing non-homogeneous enhancement of the tumour.



Fig. 3. Surgical specimen. The tumour and round ligament weighed 200 gm.

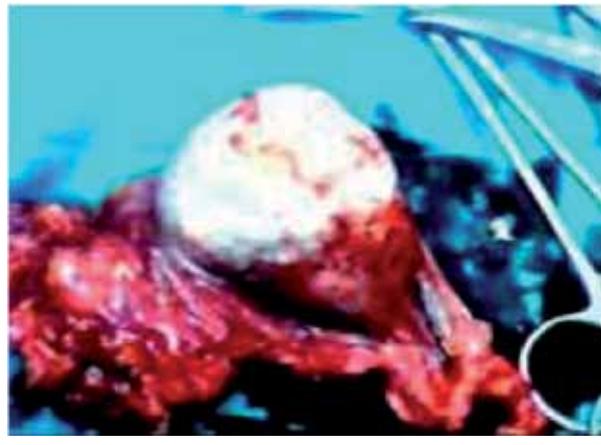
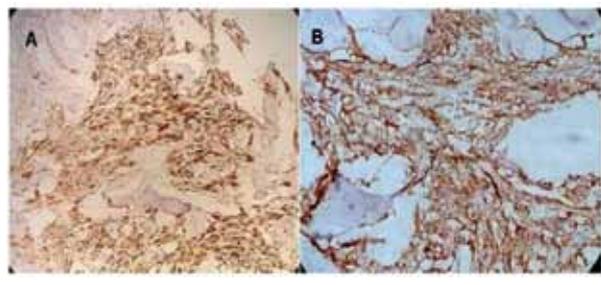


Fig. 4. Immunohistochemical features. A: Positivity for HMB-45 immunostaining. B: Positivity for Caldesmon. S-100 and CK proteins were negative.



A laparoscopy revealed a 7 cm round formation, in the round ligament of the liver, with whitish areas in the lower part and areas of dark red colour with vascular appearance. These vessels were mostly dilated in the upper part of this structure. It was concluded that it was a tumour of the hepatic round ligament.

In August 2012 she was treated surgically. The tumour was identified and removed by laparotomy. It was a well encapsulated, 8 to 10 cm round, hard tumour, with flat and regular borders, and intense red colour contrasting with whitish areas with calcifications. It had several dilated superficial vessels; its pedicle extended up to the umbilical scar.

The removal of the tumour and the round ligament were carried out completely, including segments of the peritoneum, aponeurosis and muscle, which were in direct relationship with the neoplasm. The entire abdominal cavity was examined, and no metastasis in the liver or any neighbouring organ were revealed, with no intra-abdominal adenopathies. (Fig. 3).

Histopathology was consistent with a perivascular epithelioid cell tumour (PEComa) of the round hepatic ligament, sclerosing variant, immunohistochemically positive for caldesmon, HMB-45 and Ki67, negative for CK and S100, in less than 10% of the neoplastic cells. (Fig. 4).

Discussion

Primary tumours of the round ligament of the liver are extremely rare⁷. The main clinical features are unspecified symptoms, for instance, abdominal pain; an epigastric or mesogastric palpable mass can be found. Its diagnosis may be reached as an incidental discovery during preoperative work-up or upon laparotomy.

Several histological subtypes have been described sporadically in the medical literature⁸, benign or malignant in nature. In the year 2000, Folpe⁹ described a new member of the family of PEComas, the clear cell myomelanocytic tumour of the falciform ligament/ligamentum teres, which show predilection for children and young adults.

Metastasis of gastrointestinal cancers can be found at this site, particularly mucinogen appendiceal neoplasm, peritoneal mesothelioma, and rarely breast cancer and hepatocarcinoma¹⁰⁻¹².

The PEComa in our case had a variable immunohistochemical expression for smooth muscle and melanocytic markers. At present, their natural history is often unforeseeable¹³; some cases have been described with just a few or some symptoms, while others have shown more alarming symptoms like abdominal pain with bleeding¹⁴.

The PEComas are mesenchymal neoplasms that include the clear cell “sugar” tumour of the lung and extra pulmonary sites, angiomyolipoma, clear cell myomelanocytic tumour of the falciform ligament/ligamentum teres, and rarely lymphangiioleiomyomatosis-like tumours¹⁵. They affect predominantly female patients and young adults. They have been identified in several anatomical locations such as the liver, uterus, vulva, rectum, heart, breast, urinary bladder, abdominal wall and pancreas¹⁶. In 1992, Bonetti proposed the concept of PEComa, and the term was introduced for the first time by Zamboni 4 years later. In the year 2003, after initial scepticism, the WHO defined PEComas as mesenchymal tumours. A current hypothesis is that these neoplasms derive from undifferentiated cells of the neural crest with smooth muscle and melanocytic phenotype, a second is that they have a myoblastic origin from smooth muscle and a third involves a pericytic origin. Histology with immunostaining is usually necessary to make a correct diagnosis^{17,18}. These tumours express positive staining for myogenic and melanocytic markers such as HMB45, HMSA-1, MelanA/Mart1, MIB-1, CD31 and micro-ophthalmia transcription factor (Mitf), smooth muscle actin (SMA)

and desmin. Immunoreactivity for vimentin is usually unremarkable, being negative for protein S-100, cytokeratin-AE1/AE3, cytokeratin-5 and CD30.

About 100 PEComas have been reported, 38 from the uterus. Those of the liver are extremely rare and only a small number of cases have been reported so far.

Surgical resection represents the only healing procedure for a primary PEComa, because chemotherapy and radiotherapy have not demonstrated significant benefits¹⁹. Only a few recent clinical studies have reported encouraging results related to therapy, after the oral administration of the inhibitor mTOR in patient with metastasis²⁰. Recently, Folpe and colleagues have suggested malignancy criteria including a size bigger than 5 cm, high mitotic count of more than 1 per 50 high-power fields and necrosis.

The patient was discharged 48 hours after surgery, and has had an uneventful follow-up up to date.

Conclusions

Primary PEComas of the round ligament of the liver are rare, difficult to diagnose, and are usually found during laparotomy or for a medical check-up for other conditions; diagnostic laparoscopy can be of enormous help for making a correct diagnosis and to choose appropriate surgery. As far as we know, this is the first case of a PEComa of the round ligament of the liver reported from Cuba.

References

- Beyer L, Delperio J R, Chetaille B, et al. *Solitary fibrous tumor in the round ligament of the liver: a fortunate intraoperative discovery*. Case Rep Oncol 2012;5:187-94.
- Yamaguchi J, Azuma T, Fujioka H, et al. *Leiomyosarcoma occurring in the ligamentum teres of the liver: a case report and a review of seven reported cases*. Hepatogastroenterology 1996;43:1051-6.
- Shanbhogue AK, Prasad SR, Takahashi N, et al. *Somatic and visceral solitary fibrous tumors in the abdomen and pelvis: cross-sectional imaging spectrum*. Radiographics 2011;31:393-408.
- Park MS, Araujo DM. *New insights into the hemangiopericytoma/solitary fibrous tumor spectrum of tumors*. Curr Opin Oncol 2009;21:327-331.
- Korkolis DP, Apostolaki K, Aggeli C, et al. *Solitary fibrous tumor of the liver expressing CD34 and vimentin: a case report*. World J Gastroenterol 2008;14:6261-4.
- Perini MV, Herman P, D'Albuquerque LA, et al. *Solitary fibrous tumor of the liver: report of a rare case and review of the literature*. Int J Surg 2008;6:396-9.
- Park HS, Kim YK, Cho BH, et al. *Pedunculated hepatic mass*. Liver Int 2011;31:541.
- Korkolis DP, Apostolaki K, Aggeli C, et al. *Solitary fibrous tumor of the liver expressing CD34 and vimentin: a case report*. World J Gastroenterol 2008;14:6261-4.
- Folpe AL, Goodman ZD, Ishak KG, et al. *Clear cell myomelanocytic tumor of the falciform ligament/ligamentum teres*. Am J Surg Pathol 2000;24:1239-46.
- Sugarbaker PH, Bijelic L. *The porta hepatis as a site of recurrence of mucinous appendiceal neoplasms treated by cytoreductive surgery and perioperative intraperitoneal chemotherapy*. Tumori 2008;94:694-700.
- Prete FP, Di Giorgio A, Alfieri S, et al. *Breast-cancer metastasis in the round ligament of the liver*. Lancet Oncol 2007;7:354.
- Poilblanc M, Coquaz S, Welschbillig K, et al. *A case report of stromal tumor in the ligamentum teres hepatis*. Gastroenterol Clin Biol 2007;30:469-70.
- Sotiropoulos GC, Moskalenko V, Lang H. *Clinical challenges and images in GI. Image 2. Perivascular epithelioid cell (PEC) tumor of the ligamentum teres*. Gastroenterology 2009;136:2065, 2416.
- Priola AM, Priola SM, Cataldi A, et al. *Acute abdomen as an unusual presentation of hepatic PEComa. A case report*. Tumori 2009;95:123-8.
- Folpe AL, Kwiatkowski DJ. *Perivascular epithelioid cell neoplasms: pathology and pathogenesis*. Hum Pathol 2010;41:1-15.
- Armah HB, Parwani AV. *Perivascular epithelioid cell tumor*. Arch Pathol Lab Med 2009;133:648-54.
- Martignoni G, Pea M, Reghellin D, et al. *Molecular pathology of lymphangiioleiomyomatosis and other perivascular epithelioid cell tumors*. Arch Pathol Lab Med 2010;134:33-40.
- Peng J, Yu B, Wang J, et al. *An unresectable malignant perivascular epithelioid cell tumor resistant to multiple combined chemotherapies: a unique case report*. J Pediatr Hematol Oncol 2010;32:136-8.
- Wagner AJ, Malinowska-Kolodziej I, Morgan JA, et al. *Clinical activity of mTOR inhibition with Sunitinib in Malignant Perivascular Epithelioid Cell Tumors: targeting the pathogenic activation of mTORC1 in tumors*. J Clin Oncol 2010;28:835-40.

Metastasizing pleomorphic adenoma of the submandibular gland: a case report

S. MILADI¹, S. MESTIRI¹, W. KERMANI², S. ZIADI¹, B. SRIHA¹, K. BOUZOUITA², M. MOKNI¹

¹ Department of Pathology, F. Hached Hospital, 4000 Sousse, Tunisia; ² Department of Oto-Rhino-Laryngology, F. Hached Hospital, 4000 Sousse, Tunisia

Key words

Pleomorphic • Adenoma • Metastasizing • Submandibular • Gland

Summary

Pleomorphic adenoma (PA), originally called mixed tumour, is the most common neoplasm of the salivary glands. It is usually a benign, slow-growing and well-circumscribed tumour. However, PA may occasionally give rise to metastases that usually occur after a previous recurrence. These tumours dis-

play benign histological features in both primary tumours and metastases. Such tumours have been termed metastatic PA or metastatic mixed tumours. We report a case of metastatic PA of the submandibular gland with metastasis to the cervical lymph nodes.

Introduction

Pleomorphic adenoma is the most common neoplasm of the salivary glands. It is usually a benign, slow-growing and well-circumscribed tumour. However, a few of these tumours metastasize to distant sites without undergoing malignant transformation¹. In the 2004 WHO classification of salivary glands tumours, the term “Malignant Mixed Tumours” includes three subtypes: carcinoma ex pleomorphic adenoma (CEPA), carcinosarcoma (or true malignant mixed tumour) and the metastatic mixed tumours². We report a case of multiple cervical lymph node metastasis originating from a histologically benign pleomorphic adenoma of the submandibular gland.

Case report

A 38-year-old woman, native of Sudan, with a history of recurrent pleomorphic adenoma of the submandibular gland, resected the first time 20 years ago, presented with a slowly growing and painless swelling of the left submandibular gland.

Both physical examination and cervical ultrasound (Fig. 1) revealed the presence of multiple asymptomatic lymph nodes on both sides of the neck (submandibular, upper and midjugular chains) that were not fixed to

underlying tissues or the overlying skin. Parotid glands were normal.

Under general anaesthesia, biopsies followed by frozen sections of the submandibular lump and cervical lymph nodes were obtained, showing in both locations a typical pleomorphic adenoma with no evidence of extracapsular growth, atypia, vascular or perineural involvement at histological examination.

Total resection of the submandibular gland with bilateral neck dissection at level II was performed.

On gross examination, the submandibular gland measured 4 cm in diameter, showed a nodular white lesion that measured 1.3 cm. We received 13 jugular lymph nodes and 6 submandibular lymph nodes.

Microscopically, the submandibular gland tumour displayed histopathological features consistent with a MPA (Figs. 2 and 3). It was composed of tubules, ductal structures, trabeculae and small aggregates. The ducts were lined with an inner layer of cuboidal cells and an outer layer of flat myoepithelial cells. A distinctive hyaline and fibromyxoid stroma was present. Margins of the surgical specimen were free of tumour. Immunostaining for oestrogen receptors (6F11, Novocastra, 1/40) and progesterone receptors (PgR 636, DAKO, 1/40) was negative.

On the 19 lymph nodes received, 10 were metastatic and showed the same biphasic proliferation described previ-

Correspondence

Mestiri Sarra, Department of Pathology, Farhat Hached Hospital, 4000 Sousse, Tunisia - E-mail: mestirisarra 75@yahoo.fr

Fig. 1. Cervical ultrasound shows an enlarged lymph node.

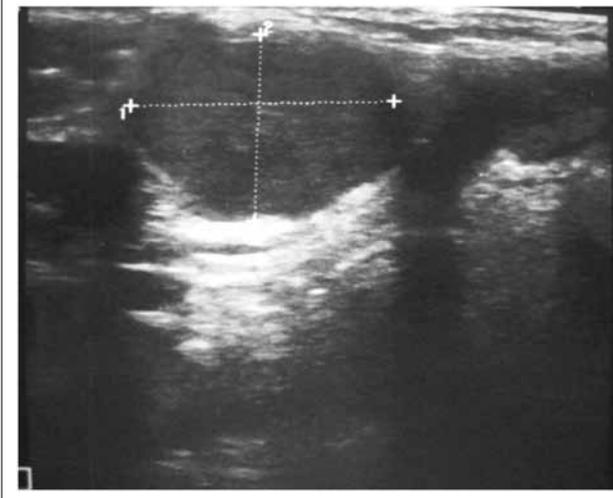
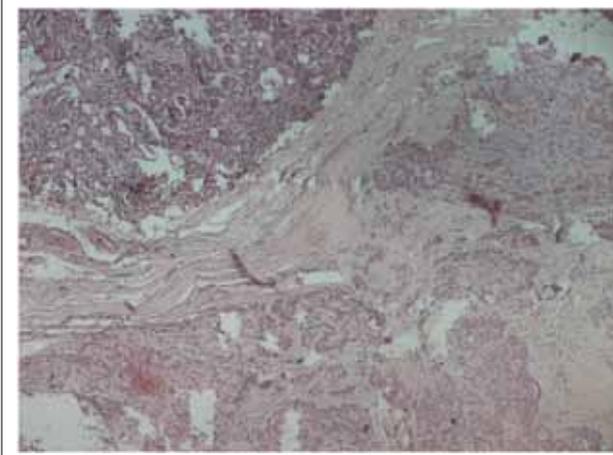


Fig. 2. H.E x 40: A submandibular gland partially replaced by a well circumscribed tumor showing both epithelial and mesenchymal differentiation.



ously (Fig. 4). The histological features were consistent with a diagnosis of metastasizing pleomorphic adenoma. Chest radiography was within normal limits. The post-operative course was uneventful. The patient remains asymptomatic on follow-up at 8 months.

Discussion

Metastasizing pleomorphic adenoma (MPA) was first described in 1942, and up to 80 cases have since been reported³. Benign MPA is distinguished from truly malignant pleomorphic tumours (such as carcinomas arising from pleomorphic adenomas) by its benign histological features. MPA of the salivary glands is a rare neoplasm that raises controversy over its nomenclature and its true biological nature. These neoplasms were termed in early reports as metastatic benign mixed tumours, a term that

Fig. 3. H.E. x 100: The epithelial component consists of well formed ductal structures, lined by regular cells. The mesenchymal component is fibrous, with regular fibroblasts.

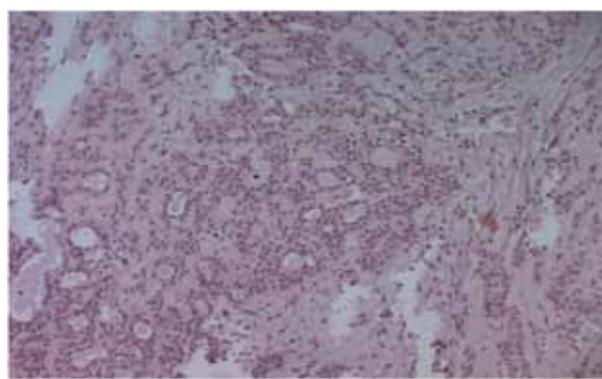
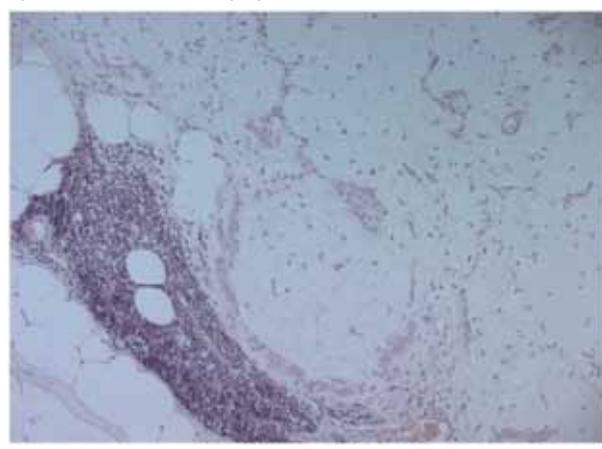


Fig. 4. H.E. x 200: Lymph node parenchyma replaced by a biphasic cellular proliferation, without cytonuclear atypia, leaving a peripheral rim of residual lymphoid tissue.



is confusing, since the terms benign and metastatic are paradoxical². They have also been termed metastasizing mixed tumours (MZMTs). In the last WHO classification of salivary gland tumours², they have been included in the category of malignant epithelial tumours and classified as metastasizing pleomorphic adenoma.

There is no age or sex predilection. The average age is of presentation is 41 years^{4,5}. A literature search using PubMed retrieved 50 cases of MPA reported during the last three decades. Primary tumour sites were the parotid gland (33 cases), submandibular gland (11 cases) and minor salivary glands (6 cases all located in the soft palate). There was no case of MPA reported in the sublingual gland. These tumours usually present with multiple local recurrences⁶. The interval between diagnosis of primary pleomorphic adenoma and metastases varies between 3 and 52 years after the occurrence of the primary tumour^{6,7}. Bone is the most common site for metastases, followed by regional lymph nodes and lung. Metastatic deposits have also been discovered in the oral cavity, skin, liver, retroperitoneum, kidney and central nervous system. The metastases occur either simultaneously with

an episode of recurrent pleomorphic adenoma or many years after a recurrence^{6,8}. In our case, both the primary lesion and the cervical nodal metastasis presented simultaneously, almost 20 years after the occurrence of the initial tumour.

The mechanism underlying the metastatic behaviour of MPA is still uncertain. As most MPA occurred after surgical treatment of primary or recurrent lesions of the salivary gland, one hypothesis suggests that surgical manipulation may cause tumour cell dislodgement, haematogenous spread and subsequent implantation at distant sites. More probably, the metastatic potential of PA is the result of key genetic alterations that cause histological and biological progression. Nevertheless, no recurrent cytogenetic abnormalities have been reported^{3,9}.

Histopathological examination of pleomorphic adenoma from the primary site and from the metastasis usually shows a typical biphasic pattern with a chondromyxoid stroma without any sign of malignancy. These tumours present more frequently with focal absence of the capsule, and with satellite nodules. Some authors suggest that the presence of both mitotic activity and an infiltrative growth pattern in the primary tumour could be predictive of the metastatic potential of PA^{10,11}.

There are a few cases in the literature documenting the expression of oestrogen and progesterone receptors in MPA. Larbcharoensub et al.¹² found expression of progesterone receptors in the mesenchymal component of MPA. The present findings suggest that expression of hormonal receptors may play a role in the development of MPA, and also raise the possibility that these tumours could respond to hormone therapy. In our case, oestrogen receptors and progesterone receptors were negative. MPA remains an aggressive disease. The percentage of

patients dying of metastatic disease is quite high (22%), and therefore these neoplasms should not be considered as benign in spite of their benign histological features. Some authors prefer to classify metastatic PA as low-grade malignant salivary gland neoplasms. Unfortunately, there is no clinical, histopathological or molecular findings that can predict an eventual metastatic course of PA^{2,11}.

According to Nouarej et al.¹³, in a virtual series of all identified cases of MPA reported in the literature over almost 50 years, found that independent prognostic factors were the occurrence of metastasis in a single or multiple site, and the interval between the initial tumour and the occurrence of metastasis.

Patients presenting with metastatic lesions in multiple sites had significantly worse prognosis compared with patients whose metastases were in multiple sites. Patients presenting with MPA within 10 years of their initial primary tumour had a significantly worse prognosis compared with those whose metastases were detected more than 10 years after the initial PA¹⁴.

Treatment of MPA is mainly surgical. It usually consists of local tumour resection, and metastasectomy, irrespective of the distant site. Metastasectomy confers a significant survival advantage over non-operative treatment^{2,6,14}. Adjuvant radiotherapy is sometimes indicated.

Conclusion

Metastasizing pleomorphic adenoma is a very rare neoplasm that is actually considered as a malignant tumour. It probably complicates the course of an incompletely resected pleomorphic adenoma. Therefore, the quality of the primitive tumour resection is crucial.

References

- 1 Steele NP, Wenig BM. *Sessions RBA case of pleomorphic adenoma of the parotid gland metastasizing to a mediastinal lymph node*. Am J Otolaryngol 2007;28:130-3.
- 2 Barnes L, Eveson JW, Reichart P, et al. *Head and Neck Tumors*. In: *WHO Classification of Tumors*. Lyon: IARC Press 2005.
- 3 Reiland MD, Koutlas IG, Gopalakrishnan R, et al. *Metastasizing pleomorphic adenoma presents intraorally: a case report and review of the literature*. J Oral Maxillofac Surg 2012;70:e531-40.
- 4 Speight PM, Barrett AW. *Salivary gland tumours*. Oral Dis 2002;8:229-40.
- 5 Batsakis JG. *Malignant element tumor*. Ann Otol Rhinol Laryngol 1982;91:1342-3.
- 6 Gnepp DR. *Malignant element tumours of the salivary glands: a review*. Pathol Annu 1993;28:279-328.
- 7 Som PM, Shugar JMA, Sacher M, et al. *Benign and malignant parotid pleomorphic adenomas: CT and MRI studies*. J Comput Assist Tomogr 1988;12:65-6.
- 8 Czader M, Eberhart CG, Bhatti N, et al. *Metastasizing mixed tumor of the parotid: initial presentation as a solitary kidney tumor and ultimate carcinomatous transformation at the primary site*. Am J Surg Pathol 2000; 24:1159-64.
- 9 Wenig BM, Hitchcock CL, Ellis GL, et al. *Metastasizing mixed tumor of salivary glands. A clinicopathologic and flow cytometric analysis*. Am J Surg Pathol 1992;16:845-58.
- 10 Ioffe OB. *Metastasizing Pleomorphic Adenoma of the Salivary Gland*. Arch Pathol Lab Med 2008;132:1445-7.
- 11 Gnepp DR. *Malignant element tumours*. In: *Surgical Pathology of the Salivary Glands*. Philadelphia, Pa: WB Saunders 1991, pp. 350-368.
- 12 Larbcharoensub N, Karnsombut P, Tungkeeratchai J, et al. *Expression of hormonal receptor in patients with metastasizing pleomorphic adenoma of the major salivary gland; a clinicopathological report of three cases*. J Med Assoc Thai 2009;92:1250-5.
- 13 Nouraej SA, Ferguson MS, Clarke PM, et al. *Metastasizing pleomorphic salivary adenoma*. Arch Otolaryngol 2006;132:7-11.

Fibroadenoma in an ectopic vulvar breast gland: a common neoplasm in an uncommon site

A. AYADI-KADDOUR¹, A. KHADHAR¹, M. MLIKA¹, E. BRAHAM¹, O. ISMAIL¹, D. ZEGAL², F. EL MEZNI¹

¹Department of Anatomopathology, Abderrahmen Mami Hospital, Ariana 2080, Tunisia; ²Department C of Gynecology and Obstetrics, Maternity and Neonatology, Tunisian Medical Center La Rabta, Tunis, Tunisia

Key words

Vulva • Ectopic breast • Adenofibroma

Summary

Ectopic breast tissue is defined as glands located outside of the breast. It can be found anywhere along the milk line extending from the axilla to the groin, and can occur in the vulva. Ectopic breast tissue should be excised because it may develop benign or malignant pathologic processes. Less than 40 cases of fibroad-

enoma in the vulva have been reported in the literature. We report a case of a 37-year-old woman presenting a solitary vulvar mass. The mass was excised completely, and histology demonstrated an ectopic breast fibroadenoma. This is one of the few reports on the benign pathologies of vulvar mammary glands.

Introduction

Supernumerary breasts are a known entity with reported incidence of 1-6 %¹. This is attributed to the failure of regression of milk line remnants during embryogenesis. Although rare, an increasing number of cases of ectopic breast tissue in the vulva have been described since Hartung first reported a fully formed mammary gland in the vulva in 1872, which is known to develop a variety of pathologic changes^{2,3}. Ectopic breast fibroadenoma of the vulva is extremely rare, although this entity should not be overlooked since some may become neoplastic.

Case report

A 37-year-old woman was referred to our institution complaining of a 6-month history of vulvar mass with progressive growth. Previous medical and familial history was not contributory to the present illness. Upon physical examination, a solitary pedunculated softy mass located on the right labium majus was observed. The patient was admitted and underwent surgical intervention. The entire mass was completely removed. Grossly, a smooth mass measuring 7 x 3.5 x 2.5 cm,

covered by skin, was obtained. Cut surface showed a yellowish-white nodule, well delimited, measuring 4 cm (Fig. 1). Microscopic examination revealed a benign fibroepithelial lesion showing proliferation of both the stromal and the glandular component with a few ducts being dilated. The epithelium was double layered with a cuboidal luminal layer and a basal myoepithelial layer. The fibrosis area was abundant. The tumour was circumscribed and separated from the coat by connective tissue. Morphologically, the appearance was consistent with a fibroadenoma of the mammary gland (Figs. 2, 3). Immunohistochemical staining was performed. Immunoreactivity of oestrogen and progesterone receptors was detected in tumour tissue.

Discussion

Vulvar fibroadenoma is one of the mammary-like fibro epithelial lesions with controversial histogenesis. The traditional theory regarding the occurrence of supernumerary breast tissue is that it is an embryological mammary remnant. Foushee and Pruitt suggest that fibroadenoma arise in ectopic breast tissue⁴. However, Vander Putt states that many lesions previously reported as associated with the supernumerary mam-

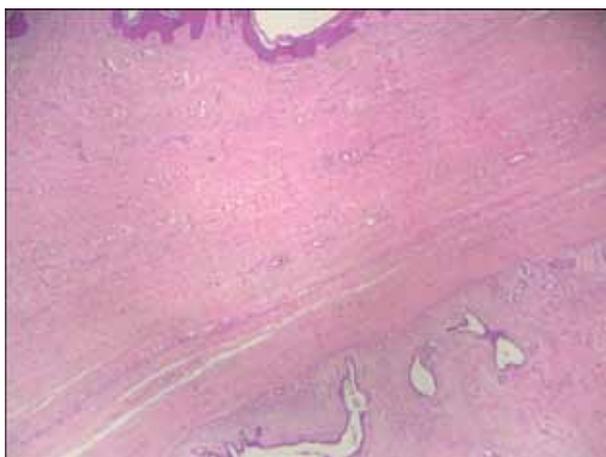
Correspondence

Aïda Ayadi-Kaddour, Department of Pathology, Abderrahmen Mami Hospital, 2080 Ariana, Tunisia - Tel. +216 22551641 - E-mail: kaddour1fr@yahoo.fr

Fig. 1. Excised gross subcutaneous nodule. The cut surface revealed a 4 cm white nodule.

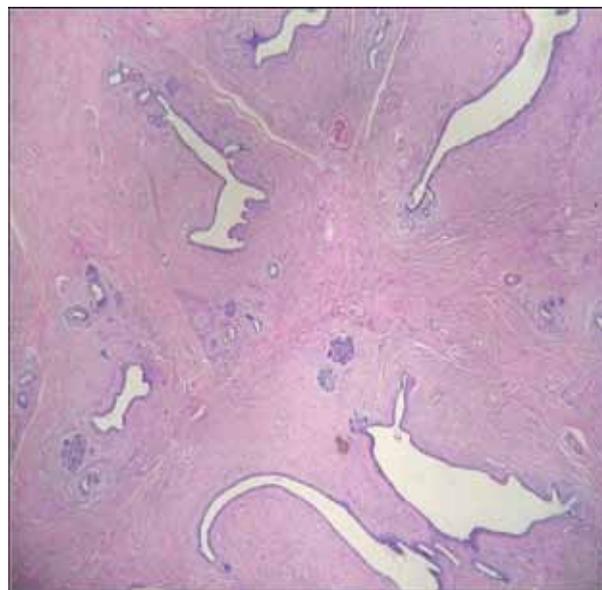


Fig. 2. Histologic section showed well-limited tumour proliferation separated from the epidermal coat by connective tissue.



mary glands are in fact derived from the mammary-like anogenital glands⁵. As a rule, vulvar breasts do not attract attention until they become enlarged or active (pregnancy, puberty, lactation). Ectopic tissue may be associated with malignancies and other congenital abnormalities such as pyloric stenosis, epilepsy and especially urinary tract abnormalities⁶. Renal malformations and renal adenocarcinoma are the most common abnormalities seen in patients with polymastia⁶. Aberrant tissue is capable of behaving in the same way as normally situated breasts, and responds to hormonal influences at puberty, during the menstrual cycle and during pregnancy and lactation. Both benign and malignant pathologic processes may occur. Average patient age at moment of diagnosis is 38.7 years (range,

Fig. 3. Histologic section showed mammary lobules, and ducts showing proliferation of both the stromal and glandular component with a few ducts being dilated.



20-60 years)^{7,8}, and the average tumour size is 3 cm (range, 0.8-6 cm)³. Biopsy of the vulvar mass is important for differential diagnosis, and to make an appropriate surgical decision in case of ectopic breast tissue. If it is malignant, a wider excision with lymph node dissection must be considered. If it is benign, then simple excision is sufficient. Diagnosis is based on histological findings in association with immunohistochemical features. Fibroadenoma originating from an ectopic breast should be taken into consideration in differential diagnosis of vulvar mass.

Conclusion

Vulvar heterotopic breast is rare and can present a challenge for both the clinician and the surgical pathologist in making a correct diagnosis. Histopathological confirmation is mandatory to exclude the possibility of another tumour, including malignancy, and to eliminate a neoplastic process in the ectopic breast tissue.

References

- 1 Mahajan NN, Choudhri L. *Fibroadenoma of ectopic breast tissue in the vulva. A case report.* *Obstet Gynecol India* 2010;60:529-30.
- 2 Zhang J, Chen Y, Wang K, et al. *Prepubertal vulvar fibroma with a coincidental ectopic breast fibroadenoma: report of an unusual case with literature review.* *Obstet Gynecol* 2011;37:1720-5.
- 3 Dhaoui A, Nfoussi H, Kchir N, et al. *Vulvar lactating adenoma associated to a fibroadenoma: common neoplasms in an uncommon site.* *Pan Afr Med J* 2012;13:47.
- 4 Hassim AM. *Bilateral fibroadenoma in supernumerary breasts of the vulva.* *J Obstet Gynec Brit Commonw* 1969;76:275-7.

- ⁵ Atwal GSS, O'Connor SR, Clamp M, et al. *Fibroadenoma occurring in supernumerary breast tissue*. *Histopathology* 2006;50:511-30.
- ⁶ Lucas EW Jr, Branton P, Mecklenburg FE, et al. *Ectopic breast fibroadenoma of the vulva*. *Obstet Gynecol* 2009;114:460-2.
- ⁷ Kazakov DV, Spagnolo DV, Kacerovska D, et al. *Lesions of anogenital mammary-like glands: an update*. *Adv Anat Pathol* 2011;18:1-28.
- ⁸ Carter JE, Mizell KN, Tucker JA. *Mammary-type fibroepithelial neoplasms of the vulva: a case report and review of the literature*. *J Cutan Pathol* 2008;35:246-9.