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Heterogeneity of duodenal neuroendocrine tumors: natural history and prognostic factors in a large cohort prospectively followed-up

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ABSTRACT

Background and aims. The natural history, clinical characteristics and optimal management of duodenal neuroendocrine neoplasms (dNENs) are unclear. Present multicenter study was aimed at analyzing the natural history of patients with dNENs. The secondary goal was to evaluate the overall (OS) and progression-free survival (PFS), according to the histological features, stage at initial diagnosis, and other possible prognostic parameters.

Methods. This is a retrospective analysis of patients with histologically confirmed diagnosis of dNENs, managed at five Italian tertiary referral Centers in Italy.

Results. From 2000 to 2017, 108 patients were included in this study. Seventy-one patients had G1, 21 G2, 4 G3 dNENs (12 Ki-67 not available). Fifty-four patients showed metastases at diagnosis, and 20 patients developed metachronous metastases. Thirty patients had a functioning dNEN (14 metastatic). Fifty-seven patients had the dNEN surgically resected, 16 endoscopically, 23 metastatic, received medical therapy + surgery or endoscopy. Seven patients underwent liver-directed therapies, and one patient had PRRT. Median OS was 187 months. During a median follow-up of 76 months, 20 patients died (19 of disease-related causes). At Cox's multivariate proportional hazard regression, grading and age were the only variables independently related to OS. Median PFS was 170 months. Grading and staging at the initial diagnosis were independently related to PFS. No differences in terms of OS and PFS were observed between patients treated surgically or endoscopically.

Conclusions. dNENs prognosis may be highly variable. These tumors can be metastatic in up to 50% of cases at the time of first diagnosis and can develop metastases thereafter. Functioning neoplasms express high metastatic potential. Nuclear imaging should be performed to exclude distant metastases in all dNENs. Endoscopy and surgery play a primary role in the management of the disease. Further prospective studies are needed.

INTRODUCTION

Gastro-entero-pancreatic neuroendocrine neoplasms with a specific focus on duodenal neuroendocrine neoplasms: an overview

Epidemiology

Gastro-entero-pancreatic neuroendocrine neoplasms (GEP-NENs) are quite rare neoplasms, characterized by heterogeneous biological behavior and clinical course. However, their global incidence has been hugely increasing due to both improvement in diagnostic techniques and better disease awareness [1]. GEP-NENs include both carcinoid tumors, which arise from the endocrine (enterochromaffin) cells of the gastrointestinal (GI) tract, and pancreatic NENs (PNENs). These neoplasms include functioning tumors, which secrete a variety of peptide hormones, and non-functioning tumors, which are often metastatic at the time of diagnosis. Even if the majority of GEP-NENs are well-differentiated, low-grade tumors, others show a more aggressive behavior with a dismal prognosis.

Duodenal neuroendocrine neoplasms (dNENs) are rare tumors, which represent up to 3% of all duodenal tumors [2] and 2–3% of all gastrointestinal (GI) tumors [3]. The incidence of dNENs has significantly increased from 0.027/100,000 in 1983 to 1.1/100,000 in 2010, this increase possibly reflecting the greater utilization of upper GI endoscopy [4,5].

DNENs are usually present in the 6th decade of age and there is a slight male predominance (1.5/1 in the latest SEER data set) [6]. In 50–70% of the cases, dNENs are well-differentiated forms (G1 according to WHO 2010), whilst poorly differentiated tumors are rare (less than 3%)[7]. They include functioning (i.e., gastrinoma (48%) and somatostatinoma (44%)) and non-functioning serotonin (28%) and calcitonin (9%)-containing tumors, rare duodenal gangliocytic paragangliomas, and high-grade poorly-differentiated neuroendocrine carcinomas [8,9]. DNENs arise more frequently in the first (58%) and second part (33%) of the duodenum, whilst tumors located in the ampulla of

Vater (approximately 20%) are often considered as a separate entity because of their clinical behavior, which is more similar to pancreatic tumors [10–12]. They are usually small (>75% of them range from 1.2 to 1.5 cm up to 2 cm), limited to the mucosa and submucosa, although regional lymph node metastases are reported to be present in 40–60% of cases at first diagnosis[7,9]. Liver metastases occur in less than 10% of patients [13]. In cases of multiple dNENs, multiple endocrine neoplasia type 1 (MEN1) should be suspected [14,15,16]. MEN1 occurs in 20–30% of all dNEN patients with Zollinger–Ellison syndrome (ZES) [17].

Clinical presentation

The clinical manifestations of GEP-NENs are heterogeneous as these malignancies may be asymptomatic or may cause non-specific or obstructive symptoms, particularly in those cases where metastases are already present at the first diagnosis. However, functioning tumours show typical syndromes which are the consequence of hormonal hypersecretion.

Patients with gastro-intestinal (GI) NENs are often asymptomatic, although these neoplasms might be responsible for non-specific symptoms, which are often confused with irritable bowel syndrome (abdominal pain/discomfort, change in bowel movements). Moreover, intestinal NENs can cause obstructive symptoms due to a local fibrotic reaction, thus their prompt diagnosis with consequent surgical resection of the primary tumour is needed. Intestinal tumours with liver metastases can be responsible for the typical carcinoid syndrome, present in 18% of patients with jejunal-ileal carcinoids[18] and characterized by flushing, diarrhea, abdominal pain and more rarely from tearing, profuse sweating, telangiectasia, cardiac fibrosis, cutaneous manifestations[19]. It depends on the release of serotonin, which is not any more metabolized in the liver, together with others molecules (tachykinins, prostaglandins, bradykinin) [20].

Patients with pancreatic neuroendocrine pancreatic neoplasms (pNENs) are heterogenous with varying tumor biology and clinical presentation. Non-functioning tumours contribute 60% of all

pNENs. Functioning pNENs with specific clinical syndromes include: 1. Insulinoma, which is characterized by hypoglicemia-related symptoms; 2. Zollinger-Ellison syndrome which includes diarrhea, recurrent peptic ulcers, gastro-esophageal reflux symptoms, pain; 3. Verner-Morrison syndrome (VIPoma syndrome), characterized by diarrhea, hypokaliemia, hypochloridia; 4. Glucagonoma which is characterized by the so-called 4D syndrome consisting of diabetes, dermatitis, deep vein thrombosis, and depression; 5. Somatostatinoma which includes diarrhea, diabetes mellitus, cholelithiasis; 6. ACTH-producing pNEN which is characterized by the Cushing syndrome.[21,22]

Duodenal NENs, when non-functioning, may be discovered incidentally during upper GI endoscopy (up to 33% of the cases) usually performed for non-specific symptoms, especially dyspepsia[16,23]. The most commonly presented symptoms are: pain, jaundice, nausea/vomiting, bleeding, anemia, diarrhea and duodenal obstruction [14,16]. Gastrinomas are neuroendocrine neoplasms located in the duodenum (70%), pancreas (25%) and rarely (5%) in other sites (stomach, liver, ovary, lung), secreting gastrin, which is responsible for the development of clinical ZES. Other common symptoms are: persistent pain in the upper abdomen, nausea, vomiting, weight loss and GI bleeding [24,25]. Carcinoid syndrome rarely occurs (<4%) in dNENs and usually as a consequence of the presence of liver metastases. Other rare presentations are: Cushing's syndrome, acromegaly due to growth hormone-releasing hormone-secreting tumors, somatostatinoma syndrome, insulinoma, glucagonoma or the development of polycythemia vera (PV) [3].

Jaundice, bile duct dilation, vomiting and diarrhea are often associated with NENs located in the proximity of the ampulla of Vater [21]. Peri-ampullary NENs are more frequently associated with von Recklinghausen's disease (18%) and the presence of somatostatin immuno-reactivity (25–100%), although a frank clinical somatostatinoma syndrome is very rare in this setting[10].

Diagnosis

Histopathology

Gastroenteropancreatic (GEP) NENs are classified according to the grade of differentation and proliferation index. Particularly they are named neuroendocrine tumours (NETs) when they are well differentiated (WD) whereas neuroendocrine carcinomas (NECs) when they are poorly differentiated (PD). Gastroenteropancreatic NENs were classified in four categories, including NETs G1 (WD with <3% Ki-67), NETs G2 (WD with 3-20% Ki-67), NETs G3 (WD with > 20% Ki-67) and NECs (PD with > 20% Ki-67) in accordance with the 2019 WHO classification [26].

To date, four histopathological subtypes of dNENs have been recognized[8]:

1. gangliocytic paragangliomas, which typically show a peculiar admixture of an epithelial endocrine (paraganglioid) component, mostly expressing pancreatic polypeptide and somatostatin, with gangliocytic and spindle cell components, better stained by synaptophysin or somatostatin and by S-100 antibodies, respectively. Most gangliocytic paragangliomas are confined to mucosasubmucosa, with or without angio-invasion. In the series from Vanoli et al., most gangliocytic paragangliomas were well-differentiated low-grade (G1) tumors.

2. gastrinomas, which are typically G1 tumors, characterized by well-defined gyriform trabeculae, often coupled with vascular pseudorosettes, with histochemical and/or immunochemical evidence of gastrin hypersecretion in the tumor tissue or blood.

3. ampullary-type somatostatin-producing tumors, which are characterized by extensive reactivity for somatostatin (involving 60–100%) with strong preference for the ampullary-papillary region. These neoplasms usually present with tubular-acinar structures, with or without intraluminal psammoma bodies, in a moderately defined solid or trabecular background of fairly large cells with abundant granular eosinophilic cytoplasm. The proliferative index is usually low (G1) but

microinvasion (especially lympho-invasion), high local lymph node metastatic rate and signs of pancrea pancreatobiliary duct obstruction are frequently observed.

4. ordinary non-functioning NENs, which usually show prominent staining with general neuroendocrine expression (CgA and synaptophysin) and reactivity to a variety of hormones either alone or variably admixed. Gastrin cells are often the dominant cell type in such NENs. Non-functioning NENs are predominantly localized in the first part of the duodenum and are typically small G1 (82%) mucosal-submucosal tumors with a trabecular or microlobular structure.

Biochemical markers

Biochemical markers are evaluated in the blood, urine or other body fluids and are usually elevated in the presence of a tumor [27]. There are generic and specific biochemical markers for GEP-NENs.

Specific biomarkers are secreted by specialized neuroendocrine cells by functioning NEN and are responsible for specific GEP-NEN associated clinical syndrome. Specific markers include 5-Hydroxyindole acetic acid (5-HIAA), insulin, gastrin, vasoactive intestinal peptide (VIP), glucagon, growth hormone-releasing hormone (GHRH), calcitonin, adrenocorticotrophic hormone (ACTH), corticotropin-releasing hormone (CRH).

Chromogranin A (CgA), which is an acidic glycoprotein of 439 amino acids and a molecular mass of 48 kDa, secreted by neurons and neuroendocrine cells, belongs to the granin family[28]. CgA is found throughout the diffuse neuroendocrine system and has shown an overall sensitivity of 96% and 75% in functioning and non-functioning NENs, respectively, and a specificity ranging from 68% to 100%[29,30]. However, CgA is generally considered a sensitive neuroendocrine marker, whereas its specificity might decrease (up to 68%), as it can be falsely positive in several conditions. CgA seems to be an accurate marker to detect tumor recurrence/progression of GEP-NENs and CgA levels should be always measured at first diagnosis and repeated during follow-up, particularly in those patients with baseline impaired levels[31,32,33].

Chromogranin A (CgA) levels should be measured whenever there is a suspicion of dNENs as an elevated CgA level occurs in 56–100% of dNENs [23]. In addition, the functional tumors may also require specific relevant assay estimations (e.g., gastrin, somatostatin, pancreatic polypeptide). For patients with multiple dNENs, clinical characteristics of MEN1 syndrome, and/or a positive family history of MEN1, genetic tests for the presence of any germinal menin gene mutation should be performed [7].

Imaging

Conventional radiological techniques including abdomen ultrasound (US), computed tomography (CT) scan and magnetic resonance imaging (MRI) are useful to localize both the primary tumour and possible metastases. PET/CT with ^{68}Ga-labeled somatostatin analogues **(SSAs)** has shown the highest sensitivity for localizing NENs, and also a high specificity. According to several studies the sensitivity varied from 86 to 100% and the specificity from 79 to 100%[34], except insulinomas, in which case the sensitivity was only 25%[35]. PET/CT with ^{68}Ga-labeled SSAs is therefore the method of choice to fully stage and localize the extent of disease in patients with NENs, except for insulinoma[36].

In the specific setting of dNEN, in order to fully assess the extent of disease and detect any possible distant metastases, both conventional imaging (i.e., ultrasound, CT scan, magnetic resonance MRI and SRS (particularly, PET-CT with 68Ga-DOTA-peptides) should be performed, although they may misdiagnose the dNENs because of their small diameter [14]. If there is any suspicion of bone metastases on conventional imaging, MRI of the spine or bone scintigraphy, or better 68Gallium-PET should be performed [7].

Endoscopy

Digestive tract endoscopy allows to identify and to diagnose, by targeted biopsy, mucosal and submucosal NENs located in all the sites of the digestive tract reachable from the endoscope. The diagnosis of small bowel NENs may be challenging with upper and lower GI endoscopy and their diagnosis has improved with the advent of capsule endoscopy (CE) and double balloon enteroscopy (DBE), which allow for direct visualization of the entire small bowel. CE and DBE may be complementary and show a similar diagnostic yield even if their role in routine staging needs further clarification, also considering the lack of data on potential procedural risks of these methods in NENs [37,38]. Endoscopic ultrasound (EUS) is the modality of choice for diagnosing PanNENs and for the loco-regional staging of gastric, duodenal, pancreatic and rectal NETs. In the setting of PanNENs it has demonstrated higher accuracy in tumour detection than other imaging modalities with sensitivity ranging up to 94%. The sampling adequacy rate of EUS-fine needle aspiration has been reported to be of 83–93%, with an overall complication rate of about 1–2% [39,40].

Upper GI endoscopy with biopsy is the most sensitive modality to detect the primary dNEN and obtain the histopathologic sample, which is always necessary to establish the diagnosis. The minimal tests to support the diagnosis are: immunohistochemistry for CgA and synaptophysin. The mitotic count and the Ki-67 proliferation index should be performed in all cases. In the case of gastrinoma, specific lesions associated with gastric hypersecretion, such as multiple gastric and duodenal ulcers, or severe reflux esophagitis may be detected by upper endoscopy[7]. Endoscopic ultrasound (EUS) with optional fine-needle aspiration/biopsy should be performed in order to locally stage the disease by assessing the extent of intramural invasion, and in all cases of non-diagnostic endoscopy[39,41].

Treatment options

Local/locally advanced stage

Each patient with a GEP NEN or suspicious for that should be referred as soon as possible to a referral center for NENs.

For patients presenting a pure G1-G2 GEP NEN at a local or locally advanced radically resectable stage an upfront surgical approach should be proposed.

For patients presenting a pure G3 GEP NEC at a local or locally advanced radically resectable stage a chemotherapy +/- radiotherapy should be discussed integrated with a possible surgical approach and its timing.

For patients presenting a pure G3 GEP NET at a local or locally advanced radically resectable stage an upfront surgical approach versus an upfront medical treatment should be discussed within the multi-disciplinary team.

Advanced stage

A variety of therapeutic options exist for metastatic neuroendocrine disease. These options include surgery, loco-regional therapies, such as transcatheter arterial embolization (TAE) or chemoembolization (TACE) or loco-regional radiotherapy or radioembolization, medical therapy including chemotherapy, biotherapy with SSAs and interferon (IFN), molecular targeted therapies and the systemic peptide receptor radionuclide therapy. In selected patients with diffuse liver-only metastases, liver transplantation (OLT) may be evaluated[42].

Treatment of duodenal neuroendocrine neoplasms

Endoscopic resection is increasingly performed instead of surgery, even if the therapeutic approach in this specific setting is not fully standardized. Because of the different treatment strategies,

dNENs are classified into ampullary and non-ampullary. Surgical resection is generally recommended for ampullary dNENs regardless of the tumor size due to its anatomical location and more aggressive behavior compared to the non-ampullary type [16]. Conversely, the therapeutic approach in non-ampullary forms depends on the size of the tumor. The latest European Neuroendocrine Tumor Society (ENETS) guidelines proposed that surgical resection should be recommended for non-ampullary dNENs >20 mm in diameter[16], but the value of surgical resection has not yet been established in non-ampullary tumors ≤ 20 mm in diameter.

Aim of the study[43]

Despite clear differences, in the current guidelines dNEN management is treated along with either gastric or, if functioning, pancreatic NENs[16,21]. As a result, their natural history, clinical characteristics, treatment, and prognosis are still poorly understood.

Therefore, present multicenter study first analyzed the natural history of patients with dNENs. The secondary goal was to evaluate the overall (OS) and progression-free survival (PFS), according to the histological features, stage at initial diagnosis, and other possible prognostic parameters.

Patients and Methods

Study Population

Patients with a histologically confirmed diagnosis of dNENs, as diagnosed and treated from January 2000 to January 2017, at five referral Centers in Italy (Gastroenterology and Endoscopy Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milano, University of Milan; the Digestive and Liver Diseases Department, University ''La Sapienza'' of Rome, Sant'Andrea Hospital; the Division of Endocrinology, Department of Clinical Medicine and Surgery, University ''Federico II'' Naples; the Department of Medical and Surgical Sciences, Bologna University's St. Orsola-Malpighi Hospital; the Pancreatic Surgery Unit, Pancreas Translational and Clinical Research Center, San Raffaele Scientific Institute, University ''Vita-Salute'' Milan) were identified from the database and retrospectively were analyzed.

All consecutive patients who met the following inclusion criteria were enrolled: age >18 years; histologically confirmed dNEN; availability of (1) histology (according to the WHO classification), (2) clinical data with a minimum 3-month follow-up after diagnosis, (3) morphologic imaging techniques, such as ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), (4) nuclear medicine imaging, (5) endoscopy parameters, (6) biochemical data [Chromogranin A (CgA), gastrin, somatostatin, neuron specific enolase (NSE)]. The exclusion criteria were: histologic finding of MANECs (mixed adenoneuroendocrine carcinomas); age < 18 years; nonadherence to the written, informed consent to participate in the study; the use of experimental drugs during the 2 months preceding inclusion in this study. The tumors were staged according to the TMN stage scoring system and classified on the basis of their immunohistochemical characteristics according to the WHO 2010 classification, as: dNENs of grade G1 (Ki-67 B 2%), G2 (Ki-67 3–20%), and G3 (Ki-67[20%)[44,45]. The tumors were classified as functioning or nonfunctioning neoplasms. All neoplasms were diagnosed by pathological examination, formalin-fixed, and routinely processed. Sections of the tumors were immunostained

for CgA, NSE, synaptophysin (SYN), and the Ki-67 proliferative index, using the MIB-I antibody. Functioning tumors were defined as those in which distinct clinical symptoms and excessive levels of specific hormones (i.e., gastrin or somatostatin) were present together with the evidence of predominant or exclusive immunoreactivity to specific hormones. Germline mutations for multiple endocrine type-1 neoplasia (MEN-1) syndrome were tested when clinically suspected, based on the coexistence of dNEN with another major feature of MEN-1 (i.e., primary hyperparathyroidism or pituitaryadenoma) or in all cases of gastrinoma.

After the initial diagnosis, regular clinical, biochemical, upper gastro-intestinal (GI) endoscopy, and imaging follow-up examinations were performed for all the cases, at least once a year (range 3–12 months). Conventional radiological imaging, endoscopy, and nuclear medicine imaging (somatostatin receptor scintigraphy or Gallium-68 PET) were performed when clinically indicated. Endoscopic ultrasound was generally performed to assess the degree of wall invasion before resection, in case of > 1-cm lesions. After enrollment, the type of treatment received by each patient was classified as: (1) radical surgery, (2) endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD), (3) follow-up alone, and (4) medical therapy.

Morphological imaging was used to evaluate the objective responses of the disease (i.e., tumor size) according to the criteria released by the Italian Trials in Medical Oncology Group19 as: complete or partial response (with a tumor size decrease > 50%), stable (< 50% decrease or < 25% increase), and progressive (increase >25%).

Data Collection

All the data were prospectively collected at the Center where the patient had been observed. A single, computerized data sheet was created and for each patient the following data were entered: age, gender, date of diagnosis, age at diagnosis, type of treatment, pathological features, tumor size and localization, number of lesions, time of follow-up, grading according to WHO 2010, staging

according to the TNM system, presence/absence of MEN-1 syndrome, functioning/nonfunctioning tumor, OS, PFS.

Statistical Analysis

Continuous variables were reported as median (range); categorical variables were reported as count (percentage).

All data were checked for distribution normality by the Kolmogoroff–Smirnoff test. The differences between groups were assessed with the Mann–Whitney test and Kruskal–Wallis test as appropriate.

The analysis of the predictive factors of metastatic disease was performed by univariate and multivariate analysis using logistic regression. Predictive factors were expressed as odds ratio (OR) and 95% confidence interval (95% CI). The forward stepwise method was used to build a multivariate model after the inclusion of all variables. OS was calculated from the date of dNEN diagnosis to the patient's death or the end of data collection. PFS was defined as the time interval between diagnosis and disease progression or the patient's death. The survival curves were estimated using the Kaplan–Meier method, and the log-rank test was used for comparing the survival curves between patient groups. The Cox univariate–multivariate regression model was used to analyze the possible association between the variables of interest (TNM staging, Ki-67 index, age, gender, primary tumor size and localization, number of lesions, presence of any functioning tumor, and presence of any MEN-1 syndrome) and both the risk of death and the risk of progression. The best multivariate model was identified by using a stepwise forward method (entry criterion: $P < 0.05$; removal criterion: $P > 0.1$). The estimated hazard ratios (HR)—as derived from the Cox models—were reported along with the pertinent 95% confidence intervals (CIs). A P value < 0.05, two-sided, was considered statistically significant. The analyses were carried out by software: Graph Pad Prism version 6.00 (GraphPad Software, San Diego, CA) and MedCalc version 17.9.5 (MedCalc Software bvba, Ostend, Belgium).

Results

In the study period, 108 patients (69 males, 39 females, median age 59.5 [range 18–87] years) were diagnosed and treated at the referral institutions. The patients' characteristics are detailed in **Table 1.**

Thirty patients had a functioning dNEN (25 gastrinomas, 5 somatostatinomas), of whom 14 were metastatic. Sixteen patients (14.8%) had MEN-1 syndrome, of whom ten had a functioning dNEN (9 Zollinger-Ellison's syndrome, 1 somatostatinoma) and six had a nonfunctioning dNEN. Seventy-one patients had G1, 21 had G2, and 4 had G3 dNENs according to the WHO 2010 classification.18

For 12 patients, the Ki-67 index was not available. dNENs were single in 84 and multiple in 24 patients (median number of lesions 3, range 2–37). Localization was: bulbar in the majority (44 patients), periampullary in 38, and located in the second portion of duodenum in 24 (not specified in 2 cases). The median diameter was 12 (range 3–130) mm. Twenty-three of 108 patients were incidentally diagnosed. Overall, 54 patients (50%) showed metastases at diagnosis: 12 in the liver, 37 in lymph nodes, and 5 presented both. Twenty patients (18.5%) also developed new metachronous metastases, during the follow-up period, of whom 5 de novo and 15 who were already metastatic at diagnosis. During the entire study period, a total of 59 patients (54.6%) showed metastatic disease. Fifty-seven patients (52.7%) had the dNEN surgically resected, 16 (14.8%) underwent endoscopic treatment, and 23 (21.3%), who were metastatic, received a combination of medical therapy (somatostatin analogues [SSA] and chemotherapy [CT]) with either surgery or endoscopy. Among the 57 patients who had undergone surgical therapy, 4 patients showed metastatic disease not detected preoperatively by the combination of morphological and functional imaging. Seven patients underwent liver-directed therapy. Peptide receptor radionuclide therapy (PRRT) was performed only in one patient. The results of the logistic regression for predictive factors of metastatic disease are reported in **Table 2**.

At univariate analysis grading (OR 5.44; P = 0.003), size (OR 1.03; P = 0.04), functioning status (OR 2.45; P = 0.04) and age (OR 0.97; P = 0.04) were related to the presence of metastases. The location of the primary tumor, number of lesions, gender, and presence of MEN-1 syndrome were not significantly related to the presence of metastases.

At multivariate analysis, age (OR 0.96; P = 0.02), grading (OR 7.7; P = 0.0009), and functioning status (OR 5.8; P = 0.003) were the variables independently related to metastatization. Over a median 76-month follow-up (range 7–211), 20 patients (18.5%) died, of whom 19 of disease-related causes. Survival analysis showed a median 187-month OS. Median PFS was 170 months with 21 patients experiencing progression. No differences in terms of both OS and PFS were observed between the patients treated surgically versus endoscopically.

At Cox's multivariate proportional hazard regression, grading (HR 29.6, CI 3.05–288.3, P = 0.0037 for G3 neoplasia) and age (HR 1.07 CI 1.01–1.13, P = 0.008) were the only variables independently related to OS. As concerns PFS, grading [31.29 (5.8–167.13); P = 0.0001 for G3 neoplasia] and staging at the initial diagnosis [HR 4.35, CI 1.23–15.40, P = 0.02 for staging IV] were independently related to PFS (**Table 3A, B**).

Discussion

The present multicentric study[43] confirmed that dNENs are heterogeneous tumors characterized by highly variable prognosis, according to the previously available data. dNENs often have been considered similar to gastric NENs, because they are usually small, well-differentiated, and indolent. However, they can be metastatic in up to 54.6% of cases, as observed in the present series, either at diagnosis or thereafter.

This observation should be taken into account, also on consideration that the median diameter of the lesions in this series was 12 mm, which is very small. In view of their potential aggressive behavior, and given that dNENs tend to spread to the submucosal layer even during the early stages of the disease, surgical resection has been suggested as the preferred treatment modality over endoscopic treatment. Moreover, the duodenal wall is thinner than the gastric wall, and this is possibly a reason to consider first towards surgery versus endoscopic approach, due to the risk of perforation[46–54]. Surgery often is suggested as the treatment of choice because of the risk of lymph node metastases with these tumors and the poor detection rate of conventional imaging for micrometastases[46]. Conversely, the endoscopic resection of dNENs is increasingly performed instead of surgery and it has proved to be safe and effective only for lesions of B 10 mm size, confined to the submucosal layer, with no lymph node involvement or distant metastasis[55–59]. In a recent series of 38 dNEN patients diagnosed over a 5-year period, no recurrence was observed over a mean 17-month follow-up period, and endoscopic submucosal dissection (ESD) achieved a higher rate of radical excision than endoscopic mucosal resection (EMR)[59]. Therefore, both surgery and endoscopy play a primary role in the management of the disease for curative purposes, even if the efficacy of endoscopic treatment in comparison to surgical therapy has not been systematically evaluated. In our multicenter study, we did not observe any significant difference in terms of both OS and PFS between the patients surgically versus endoscopically treated. The majority of patients endoscopically treated had been incidentally diagnosed and exhibited smaller lesions and lower

stages. Obviously, this is a retrospective study, so this data cannot be considered as a base of evidence-based equivalence between the two treatments. However, surgery should be performed for tumors > 1 cm and/or involving the muscolaris propria or in those with positive margins after endoscopic resection[16].

Because of the high metastatic potential observed in the present study, in case of dNENs, an initial complete staging should be performed before surgery or endoscopic treatment, including nuclear medicine imaging, to exclude distant metastases. Moreover, because of the heterogeneity of these neoplasms and the risk of local and distant metastases (also observed during the study), long-term follow-up is necessary after their initial endoscopic or surgical resection.

The present series has showed that functioning neoplasms express higher metastatic potential and appear to be more aggressive compared with nonfunctioning forms, differently from the pancreatic NENs[21]. In our series, we have defined the tumors as ‘‘functioning’’ only in the presence of distinct clinical symptoms and excessive levels of specific hormones (i.e., gastrin or somatostatin) together with the evidence of predominant or exclusive immunoreactivity to specific hormones. According to these criteria, we observed that 14 of 30 functioning dNENs (25 gastrinomas, 5 somatostatinomas) were metastatic. This finding is in line with the recent study by Vanoli et al.[8] who identified five types of NENs with distinct clinicopathological profiles among more than 200 neoplasms arising in the duodenal tract. They showed that the ampullary-type, somatostatin-producing tumors and gastrinomas presented with high rates of local infiltration (especially lympho-invasion and deep duodenal wall/pancreatic tissue invasion) and lymph node metastases, whereas the non-functioning forms had significantly lower and more size-dependent local invasive potential. Besides the functioning status, age and grading were the variables independently related to metastatization. Furthermore, grading and age were the only variables independently related to OS, and grading and staging at initial diagnosis were independently related to PFS. Based on these observations, the identification of subgroups of patients with a potentially more aggressive disease

(e.g., elderly patients with specific histological characteristics or presence of a clinical syndrome) who deserve a more aggressive therapeutic approach (i.e., surgery) should be the ultimate goal.

Finally, in this series we have observed 16 patients (14.8%) with MEN-1 syndrome. This is an interesting finding as the association with MEN-1 has been historically better defined for pancreatic NENs[17]. Thus, one should keep in mind that also in cases of dNENs, either functioning or nonfunctioning, MEN-1 syndrome should be considered[15]. This is in line with previous observations in which MEN-1 has reportedly occurred in 20–30% of all patients with dNENs with Zollinger-Ellison's syndrome (ZES)[15]: Vanoli et al. found 7 MEN-1 cases among 20 gastrinoma patients in their retrospective study (i.e., 35% of gastrinomas but only approximately 4% of the entire cohort)[8]. In our series, the patients with MEN-1 syndrome presented gastrinomas and also nonfunctioning dNENs (a somatostatinoma was observed in one case).

A possible limitation of this study is a degree of unavoidable variability among the monitoring or treating methods because of its multi-institutional retrospective nature. Nevertheless, from this multicenter study, we can derive worthwhile information about this kind of NENs. In fact, the present study has depicted dNENs as heterogeneous tumors that can exhibit an aggressive behavior with distant metastases, despite their small size, more frequently than previously described. Therefore, careful disease staging, including nuclear medicine testing, and a radical treatment approach should be considered. Follow-up should be extended to a lifelong horizon.

Further prospective studies are needed to better define standardized guidelines dedicated to dNENs, including optimal patient treatment and management and effective follow-up intervals.

Table 1. Baseline characteristics of patients with duodenal neuroendocrine neoplasms (dNENs).

Characteristics	**N [%]**
Number of patients	108 [100]
Age (years), median (range)	59.5 (18–87)
Gender (M/F)	69/39
Location	
Bulb	44 [41]
Peripapillary	38 [35]
Descending duodenum	24 [22]
NA	2 [2]
Grading	
G1	71 [66]
G2	21 [19]
G3	4 [4]
NA	12 [11]
Diameter (mm), median (range)	12 [3–130]
Functioning (gastrinoma/somatostatinoma)	30 (25/5) [28]
Non-functioning	78 [72]
Single	84 [78]
Multiple	23 [21]
Stage (I, II, III, IV)	41[38], 13 [12], 37[34], 17 [16]
Primary type of treatment	
Surgery	57 [53]
Endoscopy	16 [15]

Systemic therapy Liver directed therapy PRRT	23 [21] 7 [6] 1 [1]
MEN-1	16 [15]

Table 2. Results of the logistic regression for predictive factors (covariates) at diagnosis of the development of metastatic disease at univariate and multivariate analyses.

Covariate	**Univariate *P*-value**	**OR (95% CI)**	**Multivariate *P*-value**	**OR (95% CI)**
Age	0.04*	0.97 (0.94–0.99	0.02*	0.96 (0.92–0.99)
Grading	0.003*	5.44 (1.78–16.56)	0.0009*	7.72 (2.31–25.83)
Size	0.04*	1.03 (1.00–1.07)	0.36	1.01 (0.98–1.05)
Functioning status	0.04*	2.45 (1.00–6.03)	0.0036*	5.87 (1.78–9.38)
Site primary	0.09	1.55 (0.92–2.62)	-	-
Number of the lesions	0.39	0.94 (0.8–1.08)	-	-
Gender	0.49	1.31 (0.59–2.91)	-	-
MEN-1	0.88	1.08 (0.37–3.14)	-	-

A p-value <0.05 was considered as statistically significant (*).

Table 3. Covariates in relation to the overall survival (A) and progression-free survival (B).

A) Overall survival

Covariate	Univariate *P*-value	HR (95% CI)	Multivariate *P*-value	HR (95% CI)
Grading (G2)	0.32	1.86 (0.54–6.30)	-	-
Grading (G3)	0.0001*	44.26 (10.81–181.38)	0.0037*	29.6 (3.05–288.3)
Age	0.0002*	1.06 (1.02–1.10)	0.008*	1.07 (1.01–1.13)
Number of lesions	0.041*	0.35 (0.06–1.94)	0.51	0.69 (0.23–2.05)
Size	0.46	1.0 (0.99–1.02)	-	-
Stage	0.73	1.2 (0.47–2.86)	-	-
Site primary	0.47	1.9 (0.61–6.50)	-	-
Functioning status	0.99	1.0 (0.37–2.069)	-	-
Gender	0.47	0.68 (0.24–1.91)	-	-
MEN-1	0.15	0.29 (0.03–1.19)	-	-

B) Progression-free survival

Covariate	Univariate *P*-value	HR (95% CI)	Multivariate *P*-value	HR (95% CI)
Grading (G2)	0.04*	3.07 (1.03–9.16)	0.47	1.64 (0.43–6.28)
Grading (G3)	0.0001*	39.29 (9.70–159.08)	0.0001*	31.29 (5.8–167.13)
Age	0.24	1.01 (0.98–1.05)	-	-
Number of lesions	0.68	0.92 (0.64–1.33)	-	-
Size	0.63	0.99 (0.97–1.01)	-	-
Stage (stage IV)	0.0007*	6.40 (2.24–18.29)	0.02*	4.35 (1.23–15.40)
Site primary	0.87	0.91 (0.35–2.35)	-	-
Functioning status	0.12	2.11 (0.83–5.35)	-	-
Gender	0.45	1.42 (0.58–3.47)	-	-
MEN-1	0.78	1.18 (0.34–4.07)	-	-

A p-value <0.05 was considered as statistically significant.

Ancillary study - Risk of Pre-Operative Understaging of Duodenal Neuroendocrine Neoplasms: a Plea for Caution in the Treatment Strategy.

ABSTRACT

Background and aim. Pre-treatment staging is the milestone for planning either surgical or endoscopic treatment in dNENs. Herein, a series of surgically treated dNEN patients was evaluated to assess the concordance between the pre and post-surgical staging.

Methods. Retrospective analysis of patients with a histologically confirmed diagnosis of dNENs, who underwent surgical resection observed at eight Italian tertiary referral Centers. The pre-surgical TNM stage, based on radiological and functional imaging, was compared to the pathological TNM stage, after surgery.

Results. From 2000 to 2019, 109 patients were included. Sixty-six patients had G1, 26 a G2, 7 a G3 dNEN (Ki-67 not available in 10 patients). In 46/109 patients (42%) there was disagreement between the pre- and post-surgical staging, being it understaged in 42 patients (38%), overstaged in 4 (3%). As regards understaging, in 25 patients (22.9%), metastatic loco-regional nodes (N) resulted undetected at both radiological and functional imaging. Understaging due to the presence of distal micrometastases (M) was observed in 2 cases (1.8%). Underestimation of tumor extent (T) was observed in 12 patients (11%); in three cases the tumor was understaged both in T and N extent.

Conclusions: Conventional imaging has a poor detection rate for loco-regional nodes and micrometastases in the pre-surgical setting of the dNENs. These results represent important advice when local conservative approaches such as endoscopy or local surgical excision are considered and it represents a strong recommendation to include endoscopic ultrasound in the preoperative tools for a more accurate local staging.

Aim of the study

Endoscopic resection is increasingly performed instead of surgery, even if the therapeutic approach in this specific setting is not fully standardized[23]. However, dNENs are characterized by highly variable prognosis, and, despite the small size, can be metastatic in up to 55% of cases, either at diagnosis or thereafter[43]. Therefore, given their potential aggressive behavior, and taken into account that dNENs tend to spread or probably even rise to the submucosal layer and that the duodenal wall is thinner than the gastric wall, surgical resection has been suggested as the preferred treatment modality over endoscopic treatment at least for lesions > 2 cm and/or when dNENs invade the submucosal layer [60]. Moreover, pre-treatment staging is the basis for planning either surgical or endoscopic treatment. However, the accuracy in detecting loco-regional nodes and micrometastases at conventional imaging might represent an issue, and data focused on this topic are few and scanty. Furthermore, in those patients who are endoscopically treated, complete pathological staging is not possible, exposing them to a potential risk of understaging and under-treatment Moreover, it's not clear if a possible pre-surgical understaging might have a negative impact on disease-specific survival and disease-free survival. The current study aimed to evaluate the concordance between pre- and post-surgery staging in a series of patients affected by dNEN who underwent surgical resection and to analyze whether a pre-surgical understaging could influence the survival.

Patients and Methods

Study design.

A retrospective analysis of a multi-center prospectively-collected database was performed. All consecutive dNEN patients, surgically treated between 2000 and 2019, at eight Italian tertiary referral centers (Department of Pathophysiology and Organ Transplant University of Milan; Gastrointestinal and Hepato-Pancreatic Surgery and Liver Transplantation Unit, National Health Institute, Milan; Department of Surgery 1 Pancreatic and Endocrine Digestive Surgical Unit, University of Padua, Padua; the Pancreatic Surgery Unit, Pancreas Translational and Clinical Research Center, San Raffaele Scientific Institute, University ‘‘Vita-Salute’’ Milan; the Department of Medical and Surgical Sciences, Bologna University’s St. Orsola-Malpighi Hospital; Humanitas Clinical and Research Center - IRCCS, Humanitas University, Rozzano, Milan; the Digestive and Liver Diseases Department, University ‘‘La Sapienza’’ of Rome, Sant’Andrea Hospital; Division of Gastroenterology, San Gerardo Hospital, University of Milano-Bicocca School of Medicine, Monza; the Division of Endocrinology, Department of Clinical Medicine and Surgery, University ‘‘Federico II’’ Naples) were included. All the data were retrieved at the center where each patient had been diagnosed and followed-up. The study inclusion criteria were: age >18 years; histological diagnosis of dNEN of any grade and stage, surgical treatment of the primary tumor, availability of (1) complete histopathological examination of the surgical specimen (according to the WHO classification 2010), (2) clinical data with a minimum 3-month follow-up after diagnosis, (3) pre-surgery morphologic imaging techniques, such as ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), (4) nuclear medicine imaging (Gallium68 PET/Octreoscan©), (5) biochemical data [Chromogranin A (CgA), gastrin, somatostatin, neuron-specific enolase (NSE)]. The exclusion criteria were: histologic finding of MANECs (mixed adenoneuroendocrine carcinomas); age <18 years; non-adherence to the written, informed consent to participate in the

study; the use of experimental drugs during the 2 months preceding inclusion in this study; pregnancy or breastfeeding status.

Data collection

The tumor characteristics analyzed comprised: the site and the size of the primary tumor, number of lesions, grade, and stage (localized, regional, distant, and unknown). The patient's characteristics included the age at first diagnosis, the presence of genetic syndrome, i.e. multiple endocrine neoplasia (MEN)-1, the presence of functioning neoplasms.

Data regarding the clinical history, date, and age at diagnosis, treatments received, clinical and biochemical parameters, conventional radiological imaging, endoscopy examinations, and nuclear medicine imaging (somatostatin receptor scintigraphy or Gallium-68 PET) were recorded and evaluated at each referral center. The type of surgical intervention, i.e. pancreaticoduodenectomy, total pancreatectomy, duodenectomy +/- lymphadenectomy, has been recorded for all the patients.

As regards the follow-up, each patient had at least a yearly medical check-up after the diagnosis of dNEN. Data regarding the mean follow-up, disease-specific survival (DSS), and progression-free survival (PFS) were collected.

The data collection was closed in November 2019.

Clinico-pathological evaluation of NEN

The histological/cytological specimens were examined by an experienced pathologist. Neoplasms were classified according to the WHO 2019 classification[61] and staged according to the current European Neuroendocrine Tumor Society (ENETS) TNM clinical staging [45]. Whenever enough

material was available, the Ki-67 proliferative index was assessed on primary tumor and/or on biopsies obtained from liver metastases.

The tumors were also classified as functioning or non-functioning neoplasms, based on the presence of both distinct clinical symptoms and excess levels of specific hormones (e.g. gastrin, somatostatin).

Statistical analysis

Continuous variables were reported as median (range); categorical variables were reported as count (percentage). All data were tested for distribution normality by the Kolmogorov-Smirnoff test. The differences between groups were assessed with the Mann-Whitney test and the Kruskal-Wallis test as appropriate.

Disease-specific survival (DSS) was calculated from the date of dNEN diagnosis to the patient's death or the end of data collection. Progression-free survival (PFS) was defined as the time interval between diagnosis and disease progression/relapse or the patient's death. The univariate-multivariate Cox regression model was used to analyze the possible association between come covariates (age, gender, grading, staging, type of surgery, size of the primary tumor, functioning status, presence of MEN-1 syndrome) and the risk of death. The best multivariate model was identified by using a stepwise forward method (entry criterion: $P < 0.05$; removal criterion: $P > 0.1$). For all the fitted Cox models, the proportional hazard assumption was checked and found to be met. The estimated hazard ratios (HR) – as derived from the Cox models – were reported along with the pertinent 95% confidence intervals (CIs). A P value <0.05, two-sided, was considered statistically significant. The analyses were carried out by SAS/STAT® release 9.2 software (SAS Institute Inc., Cary, N.C., USA), Graph Pad Prism version 6.00 (GraphPad Software, San Diego, California, USA) and MedCalc version 17.9.5 (MedCalc Software bvba, Ostend, Belgium).

The agreement between radiological findings and pathological one was expressed with Cohen's kappa coefficient, where kappa indicates a slight agreement when values range between 0.01 – 0.20, fair agreement for values between 0.21 – 0.40, a moderate agreement between 0.41 – 0.60, a substantial agreement between 0.61 – 0.80, and almost perfect or perfect agreement when between 0.81 – 1.00.

RESULTS

From 2000 to 2019, 109 patients were included in the study. The patients' characteristics are detailed in **Table 1**.

The mean follow-up of the entire cohort was 75 months (range: 3-288 months) and the median age at diagnosis was 59 years (range 18-81-years). As concerned grading, 66 patients had G1 (60.5%), 26 a G2 (24.5%), and 7 a G3 (6%) dNENs according to the WHO 2019 classification[61], whereas the Ki-67 was not available in 10 patients (9%). Sixty-one out of the 109 patients (56%) underwent a pancreaticoduodenectomy, 34 patients (31%) underwent a duodenectomy, 10 patients (9%) a duodenectomy with lymphadenectomy, 4 patients only a total pancreatectomy (4%). Overall, 25 patients (23%) were diagnosed at stage I, 19 patients (17%) at stage II, 47 patients (43%) at stage III, 19 at stage IV (17%).

Signs of gastric metaplasia associated with the presence of dNEN were reported in 14 patients (12.8%).

Pre- and post-operative staging concordance

In 46 out of 109 patients (42%) there was disagreement between the radiological/functional pre-surgical and pathological surgical staging, being the disease understaged in 42 patients (38%) and overstaged in 4 (3%), with only a slight agreement with pathological findings (Cohen's k: 0.16) and without differences between CT and MRI; Gallium PET, performed in 67 out of 109 patients (61.4%), was only slightly more accurate than CT, even if even Gallium-68 PET had 5 false-negative results, missing five primary dNENs). As regards understaging, in 25 patients (22.9%), metastatic loco-regional nodes (N) resulted undetected at both radiological (CT and/or MRI), and functional imaging. Understaging due to the presence of distal micrometastases (M), i.e. metastases not detected by imaging but visible at surgical exploration, was observed in 2 cases (1.8%), in

which both CT and Octreoscan® did not detect liver involvement, which instead was present at surgical exploration.

Underestimation of tumor extent (T) was observed in 12 patients (11%): in 6 cases the primary tumor was visible neither at endoscopy nor at CT and functional imaging, whereas in 6 cases the tumor was visible, but underestimated in size (being 5 mm the median difference). In three cases the tumor was understaged both in T and N extent. Endoscopic ultrasound (EUS) was performed in 11 patients (10%) only and detected the primary tumor in 80% of the cases, but detected 100% of node involvement.

As depicted in **Figure 1**, the disease resulted to be understaged particularly in dNENs at an advanced stage (i.e. III and IV), whereas in stage II dNENs the disease was overstaged in the pre-surgical setting.

Long-term outcomes after surgery

After a median follow-up of 26.5 months (range 3-342 months), 20 patients (18.3%) showed disease recurrence after surgery, with lymph node metastases in 8 cases (40%), liver lesions in 9 (45%), both liver and lymph node metastases in 2 cases (10%), anastomotic recurrence in one patient (5%). Seven patients (35%) had a G1 neoplasm, 9 (45%) a G2, 4 (20%) a G3. Five patients (20%) were classified as stage I disease at pre-surgical CT scan, while 7 patients (35%) were at stage II and one patient (5%) at stage III. Seven out of these 20 patients (35%) showed synchronous metastases detected on conventional CT scans performed before surgery, thus being classified as stage IV disease. The median DSS of the entire cohort was 187 months (95%CI: 120-187), whilst the median PFS was 156 months (95%CI: 120-181). Recurrent disease was treated with somatostatin analogs (SSAs) in all the patients. Besides SSAs, six patients (5%) underwent surgical removal of the hepatic or lymph-node metastases, three patients (3.3%) with liver metastases

underwent trans-arterial chemo-embolization (TACE), three (3.3%) peptide receptor radio-targeted therapy (PRRT), six (5%) chemotherapy, one patient only was treated with everolimus.

At Cox univariate analysis, the DSS resulted related to grading (OR for G3 =13.2, 95% CI 4.30-40.44, p=0.0001), understaging (OR for patients being understaged = 2.70, 95% CI 1.3-5.57, p=0.007), post-surgical staging (for stage IV OR= 3.93, 95% CI 1.2-12.84, p=0.02), and age (OR= 1.04, 95%CI 1.01-1.07, p=0.004). At Cox multivariate analysis, DSS resulted independently related to advanced grade, i.e. G3 (OR=13.2, 95%CI: 4.30-40.43, p=0.0001) only (**Table 2**).

At Cox univariate analysis PFS was significantly related to the grade of dNEN (OR for G2= 3.23, 95% CI:1.41-7.35, OR for G3=22.30, 95% CI: 7.19-69.69, p<0.0001), pre-surgical advanced stage (for stage IV OR=4.50, 95% CI: 1.84-11.08, p=0.001), pathological surgical staging (for stage IV OR= 3.16, 95% CI: 1.42-6.99, p=0.004), and presence of metastases at diagnosis (OR 0.46, 95% CI 0.22-0.98, p=0.045). At Cox multivariate analysis, only advanced grade (G3) (OR= 28.14, 95% CI: 7.42-106.71, p<0.0001) and post-surgical stage IV (OR=3.6, 95% CI: 1.33-9.91, p=0.01) were independently related to PFS (**Table 2**).

DISCUSSION

According to the present series, conventional and functional imaging is associated with a high-risk of understaging of dNENs. Of note, in 42% of the whole cohort, there was disagreement between the pre- and post-surgical staging, being it understaged in 42 patients and overstaged in 4. This should be taken into account in the treatment decision making since limited resections including endoscopic resection or conservative surgical procedures without proper lymphadenectomy, might not be radical due to the relevant risk of undetected micrometastases.

There is controversy regarding the management of dNENs and endoscopic resection is increasingly performed instead of surgery. Surgery is generally recommended for ampullary dNENs [62] and lesions > 2 cm in size. However, the type of surgical resection is still not clearly defined. Milanetto et al. [63] suggested that ampullary dNEN < 2 cm, which are mostly G1–G2 neoplasms should be treated with a less invasive surgery (i.e. ampullectomy/excision).

Furthermore, another matter of concern for the selection of patients with dNENs amenable to endoscopic resection is the risk of metastatic spread. Current ENETS consensus guidelines recommend endoscopic mucosal resection for tumors less than 1 cm as they seem to have a low rate of nodal disease [16]. According to some authors, endoscopic resection of dNENs has proved to be safe and effective for lesions of < 10 mm size, confined to the submucosal layer, with no lymph node involvement or distant metastasis [55,56,57,58,59,64]. Conversely, routine radical resection with lymphadenectomy is recommended for tumors greater than 2 cm, given a high risk of nodal involvement [4,14]. However, the management of tumors between 1 and 2 cm is still debated and largely based on tumor location and the presence of nodal involvement on imaging. According to our recent multicentre study, dNENs are quite aggressive tumors and can be metastatic in more than 50% of the cases independently of tumor diameter[43]. Furthermore, according to other studies, more than 10% of patients with dNENs smaller than <1cm in size develop lymph node metastases [41,65]. Again, Gamboa and colleagues [66], reported that resection was associated with improved median

overall survival compared to no resection in all sizes, thus suggesting the need for a radical surgical approach for all dNENs despite the size of the primary tumor. Park et al.[67], observed that non-bulb location, tumor size >10 mm, invasion beyond the submucosa, WHO grade 2, and lymph vascular invasion represent risk factors for lymph node metastasis.

The accuracy in detecting loco-regional nodes and micrometastases at conventional imaging is, then, of paramount importance, even if no specific data are available regarding the detection rate of micrometastases in this setting. Low accuracy of tumor detection has been reported in previous series[62]. Furthermore, conventional radiology might not be accurate in the detection of small dNENs, which are generally located at the submucosal layer. Conversely, both functional imaging (particularly the Gallium68 PET scan) and EUS has been reported to be extremely sensitive for both the detection of smaller tumors [68,69] and the local staging (depth of intestinal wall invasion, local lymph node metastases) [39].

In the present study, metastatic loco-regional nodes (N) resulted undetected at both radiological (CT or MRI) and functional imaging in 22.9% of the cases, the presence of distal micrometastases (M) was understaged in 1.8%, whilst underestimation of tumor extent (T) was observed in 12.8% of the cases.

Although the pre-surgical understaging is not related to survival, a significant correlation between this variable and DSS was found at the univariate analysis, but this was not confirmed at the multivariate analysis nor it resulted significant as a prognostic factor of recurrence. A possible explanation of the lack of statistically significant results at the multivariate analysis might be related to the fact that the pre-surgical staging is not a variable independent of the initial disease stage. In fact, the disease resulted to be understaged particularly in dNENs at advanced stages (i.e. III and IV), whereas in dNENs at stage II the disease was overstaged. Of note, the pathological staging was found to be predictive of PFS and, even if it didn't reach a statistical significance at univariate

analysis, we observed a trend to impact also on DSS, which highlights both the risk of understaging in the pre-surgical setting based on radiology only and the relevance of the surgical contribution.

Of note, signs of gastric metaplasia associated with the presence of dNEN were reported in almost 13% of the cases, thus representing a remarkable percentage. Gastric metaplasia, which is defined as the replacement of the surface, foveolar, and glandular epithelium in the oxyntic or antral mucosa by the intestinal epithelium, is considered to be a pre-cancerous lesion. The pathogenesis of gastric cancer might be driven by inflammation towards intramucosal cancer and invasive form [70]. In detail, the first histologic change in the cascade is active chronic inflammation, followed by complete metaplasia, incomplete metaplasia, and low- and high-grade dysplasia, up to carcinoma [70]. Although no clear-cut data are available regarding the potential relationship between gastric metaplasia and dNEN occurrence, our findings might suggest possible common underlying etiopathogenic mechanisms, which, however, require to be further clarified.

Possible limitations of this study are represented by its retrospective design and the variability among the diagnostic or treating methods due to its multi-institutional nature. Furthermore, in the present series, only a small percentage of patients (namely 10%) underwent EUS in the pre-surgical setting, which might represent another possible limitation. In fact, EUS has been recognized to be an accurate technique in both the diagnosis of pancreatic and duodenal NENs also of small size and in the staging phase for the detection of loco-regional lymph node metastases[21,39,41]. As our results highlight the risk of dNEN understaging, particularly regarding the N status, one can speculate that EUS should be always performed to stage all dNENs despite the small size. On the other hand, we can derive valuable information about this kind of NENs from the current multicenter study.

In summary, conventional and functional imaging of dNENs is characterized by a high-risk of understaging mainly due to the presence of nodal and distant micrometastases. These results represent a sign of warning for conservative approaches such as endoscopy or local surgical excision. A possible strategy of improvement may include the routinely use of EUS in the

preoperative phase although its value should be validated in prospective studies. The identification of prognostic factors of tumor growth and metastatization would be of great help to select for more conservative management (i.e. endoscopic treatment rather than surgery) only those patients with clearly defined benign and slow-growing tumors. Based on our previous [8] and present results, we might suggest a radical surgical approach for functioning neoplasms, which seem to express high metastatic potential. Further prospective studies are needed to better define standardized guidelines dedicated to dNENs, particularly to delineate specific prognostic factors in order to identify those patients who deserve a more radical approach.

Figure legend.

Figure 1. Figure shows pre- and pathological staging of duodenal neuroendocrine neoplasms (dNENs). The disease resulted to be understaged particularly in dNENs at an advanced stage (i.e. III and IV), whereas in stage II dNENs the disease was overstaged in the pre-surgical setting.

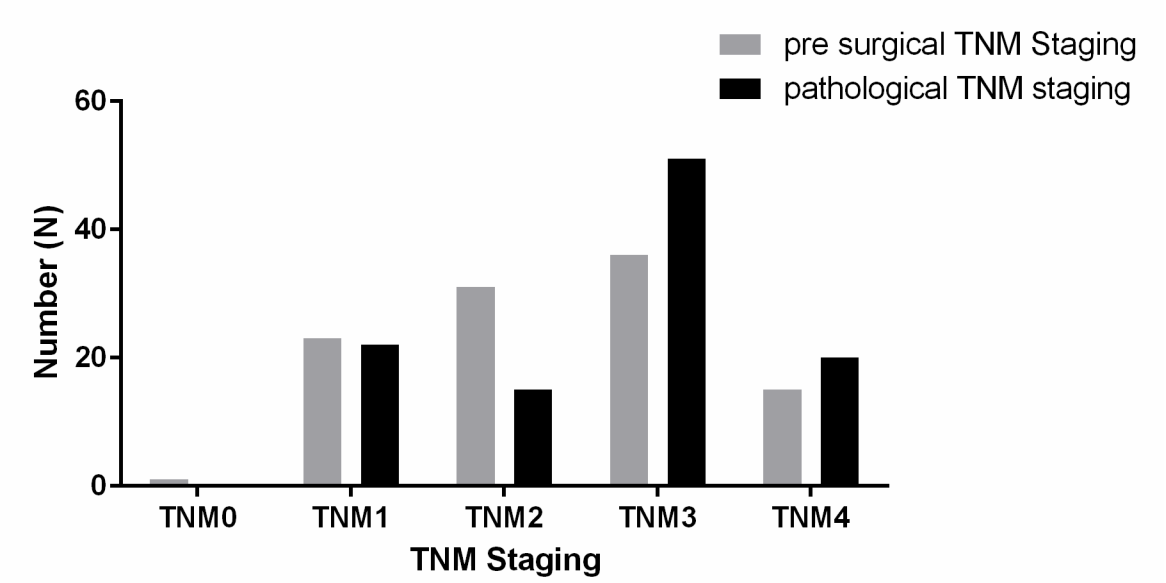

Table 1. Baseline characteristics of patients with duodenal neuroendocrine neoplasms (dNENs).

Characteristics	**N [%]**
Number of patients	109 [100]
Age (years), median (range)	59 (17–81)
Gender (M/F)	68/41
Location	
Bulb	36 [33]
Periampullary	41 [37.7]
Descending duodenum	24 [22]
NA	8 [7.3]
Grading	
G1	66 [60.5]
G2	26 [23.8]
G3	7 [6.4]
NA	10 [9.1]
Diameter (mm), median (range)	15 [1.5–130]
Functioning (gastrinoma/somatostatinoma)	32 (27/5) [29.3]
Non-functioning	78 [70.7]
Single	93 [85.3]
Multiple	16 [14.6]
Stage	
I	25 [22.9]
II	19 [17.4]
III	47 [43]
IV	19 [17.4]
Type of surgery	
Pancreaticoduodenectomy	61 [56]
Total pancreatectomy	4 [4]
Duodenotomy + enucleation	34 [31]
Partial Duodenectomy +	10 [9]

lymphadenectomy	
MEN-1	18 [16.5]

Table 2. Univariate and multivariable analysis related to the disease specific survival (A) and progression-free survival (B).

A) Disease specific survival

Covariate	**Univariate** ***P*-value**	**OR (95% CI)**	**Multivariate** ***P*-value**	**HR (95% CI)**
Grading (G3)	**0.0001**	13.2 (4.30-40.44)	**0.0001**	13.2 (4.30-40.43)
Age	**0.004**	1.04 (1.01-1.07)	0.05	1.03 (0.99-1.06)
Stage IV	**0.02**	3.77 (1.23-11.55)	0.87	0.85 (0.11-6.09)
Size	0.17	1.01 (0.98-1.04)	0.27	1.01 (0.98-1.04)
Under-staging	**0.007**	2.70 (1.3-5.57)	0.25	1.81 (0.66- 4.95)
Pre-surgical staging	0.59	4.4 (1.95-10.56)	0.40	1.47 (0.58-3.69)
Pathological staging	**0.02**	3.93 (1.2-12.84)	0.54	1.5 (0.40-5.60)
Type of surgery (DCP)	0.61	0.79 (0.18-3.45)	0.29	2.62 (0.43-15.7)
Metastases at diagnosis	0.09	0.53 (0,25-1,11)	**0.02**	4.74 (1.23-18.28)
Functioning status	0.07	0.47 (0.20-1.11)	0.83	0.89 (0.29-2.65)
Gender	0.28	0,65 (0.29-1.45)	0,40	1.40 (0.62-3.16)
MEN-1	0.05	0.23 (0.05-0.97)	0.13	0.20 (0.02-1.65)

B) Progression-free survival

Covariate	**Univariate** ***P*-value**	**OR (95% CI)**	**Multivariate** ***P*-value**	**HR (95% CI)**
Grading (G2)	**<0.0001**	3.23 (1.41-7.35)	0.06	2.55 (0.94-6.91)
Grading (G3)	**<0.0001**	22.30 (7.19-69.69)	**<0.0001**	28.14 (7.42-106.71)
Stage (stage IV)	**0.0006**	4.50 (1.91-10.565)	**0.01**	3.6 (1.33-9.91)
Metastases at diagnosis	**0.045**	0.46 (0.22-0.98)	0.17	0.38 (0.09-1.52)
Under-staging	0.08	1.94 (0.93-4.05)	0.32	1.79 (0.55- 5.74)
Pre-surgical staging	**0.001**	4.5 (1.84-11.08)	0.53	1.82 (0.27-11.99)
Pathological staging	**0.004**	3.16 (1.42-6.99)	0.07	9.45 (0.81-110.28)
Type of surgery (DCP)	0.24	0.49 (0.11-2.23)	0.05	2.96 (0,97- 9,04)
Age	0.40	1.01 (0.98-1.03)	0.70	1.0 (0.97-1.03)
Size	0.97	0.99 (0.98-1.01)	0.12	0.97 (0.94-1.0)
Gender	0.83	0.92 (0.43-1.94)	0.22	1.82 (0.69-4.77)
Functioning status	0.44	0.72 (0.31-1.67)	0.90	1.07 (0.34-3.35)
MEN-1	0.10	0.44 (0.15-1.30)	0.11	0.33 (0.08-1.28)

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