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Review

Neuroendocrine neoplasms of the biliary tree, liver and pancreas: a pathological approach

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Summary

Neuroendocrine neoplasms of the pancreatobiliary tract and liver are a heterogeneous group that encompass a spectrum of entities with distinct morphological, biological and clinical features. Although in the various anatomical sub-sites of this region they show specific characteristics, these tumors, as a whole, share several etiological and clinical aspects. This review systematically addresses NENs arising in the extrahepatic bile ducts, gallbladder, liver and pancreas, with the principal aim of pinpointing essential diagnostic and classification issues. In addition, the section on hepatic NENs has been expanded to include metastatic disease of unknown primary site.

Key words: neuroendocrine neoplasms, pancreas, liver, biliary tract

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Introduction

Neoplastic diseases of the pancreatobiliary tract and liver are rare but potentially life-threatening. Although in the various anatomical sub-sites of this region they show specific biological and morphological features, these tumors, as a whole, share several etiological and clinical aspects. Among pancreatobiliary and hepatic malignancies, neuroendocrine neoplasms (NENs) are among the rarest subtypes. This review will systematically address NENs arising in the extrahepatic bile ducts and gallbladder, in the liver and pancreas, with the principal aim of highlighting essential diagnostic and classification issues. In addition, the paragraph on hepatic NENs has been expanded to include metastatic disease of unknown primary site.

Neuroendocrine neoplasms of the biliary tree and liver

The liver and the intra- and extra-hepatic bile tract (including gallbladder) are the rarest sites of occurrence for NENs, if metastatic lesions are excluded. Here we will revise the available knowledge about primary NENs of the biliary tract and liver and discuss the diagnostic management of metastatic NENs in the liver.

Primary NENs of the extrahepatic bile ducts (EHBD) and of the gallbladder (GB)

Due to the rarity of these entities, clinicopathological studies on large series are lacking. Indeed, even retrospective analyses of large databases and national tumor registries, as well as literature meta-analyses, are poorly affordable, as the nomenclature in this field is often confusing and the distinction between neuroendocrine tumors (NETs) and neuroendocrine carcinomas (NECs) is not well defined [1,2]. However, if we consider well documented case reports and case series, as well as reviews and meta-analysis on specific types of NENs, we can summarize the features of NETs and NECs in these sites.

Overall, these neoplasms represent 0.2 to 2% of all NENs, and around 2% of all primary malignancies arising in EHBD and GB [3,4]. Gallbladder NENs most commonly arise in women (M:F ratio = 1:4), whereas only a slight female prevalence (M:F ratio = 2:3) is seen in patients with NENs of EHBD [3]. All ages can be affected, and rare pediatric cases are also described [5]. In these sites, NECs are much more common than NETs and mostly occur in the gallbladder, followed by the common distal bile duct and the common hepatic duct.

Although some authors have suggested that NENs of the biliary tree may arise from neuroendocrine cells of post-inflammatory metaplastic mucosa [6], the most likely hypothesis is that they derive from an epithelial precursor that may also give rise to glandular neoplastic proliferation, in analogy to other gastroenteropancreatic NENs [7]. Independently from the specific mechanism of cancerogenesis, the main risk factor for gallbladder NENs is the presence of cholelithiasis and cholecystitis [7], whereas no specific etiology has been identified for NENs of the EHBD. As concerns the pathogenetic mechanisms, little is known about the molecular pathways underlying the development of GB and HEBD NETs, but alterations in tumor-related genes involved in local adenocarcinomas, such as *TP53*, *KRAS* and *RB1*, seem not to be present in these neoplasms [8]. An association with von Hippel-Lindau syndrome (VHL) has been proposed for EHBD NETs, as two cases have been reported in VHL patients [9,10].

For NECs of these sites, a very recent paper reporting the largest series of GB NECs published until now, showed frequent loss of *Rb1*, hyperexpression of *p16*, and no mutation of *BRAF* in these cases [11]. In addition, *TP53* point mutation has been found in a case [12], while the presence of microsatellite instability and alterations of genes involved in the *ERBB* pathway (*HMCN1* and *CDH10*) were reported in a case [13]. These results, together with the evidence that GB and EHBD NECs are frequently mixed with invasive and pre-invasive non-neuroendocrine components, and can be called mixed neuroendocrine/non-neuroendocrine neoplasms (MiNENs) [11,12,14-17], suggest that also in these sites NEC may share common pathogenetic pathways with autoctonous adenocarcinomas, in analogy to MiNENs of other digestive and extra-digestive locations [18,19].

Macroscopically, tumor masses have a mean diameter of 2.2 to 3 cm [2,17] in the EHBD, whereas GB NENs are larger (mean diameter 3.5 to 5.6 cm) [11,20,21]. In the gallbladder, the most commonly involved subsite is the fundus[11]. The histopathological appearance of GB and EHBD NETs is similar to NETs of other anatomical sites, with well differentiated neuroendocrine morphology [8], and NECs may be of small or large cell type, and can be found in the context of a MiNEN, mixed with an adenocarcinoma or a papillary neoplasm [11,17,22]. At immunohistochemistry, pan-cytokeratins and general neuroendocrine markers (synaptophysin and chromogranin A) are expressed [11,17,22], with variable patterns between NETs and NECs, as described in other sites (Fig. 1) [23]. Cytokeratin 7 has been reported to be consistently expressed in GB NECs, in contrast with NECs of other digestive sites [17]. CD117 immunostain, which has also been reported to be positive in NECs of other sites, is found in a significant fraction of GB NEC and may represent an additional marker in the differential diagnosis with poorly differentiated adenocarcinoma and with NET and a putative therapeutic target [11]. A number of hormonal products have been found in neoplastic cells of a subset of NETs, mainly gastrin and serotonin [2], whereas no data regarding transcription factors and other site-specific markers is available, apart from the nonspecific expression of TTF1 in a subset of NECs [11]. Ki-67-related labelling index is far above 20% in NECs, whereas in NETs it may vary from up to 3% (NET G1) to more than 20% (NET G3), through cases in which it ranges between more than 3% but less than 20% [24].

The clinical presentation of EHBD and GB NENs varies according to their site. EHBD NENs become evident due to jaundice and other signs of cholestasis, whereas GB NENs are most often asymptomatic and are occasionally diagnosed during abdominal imaging for nonspecific symptoms or after cholecystectomy for cholecystitis [11,17]. Very rare cases become evident with symptoms of hormone hypersecretion or other paraneoplastic syndromes [25]. The prognosis of EHBD and GB NENs heavily depends on their morphological characterization. Patients with NECs of small and large cell type have a poor outcome, with an overall survival (OS) at 5 years of 19%, and AJCC stage strongly influences prognosis [11,22]. In contrast,

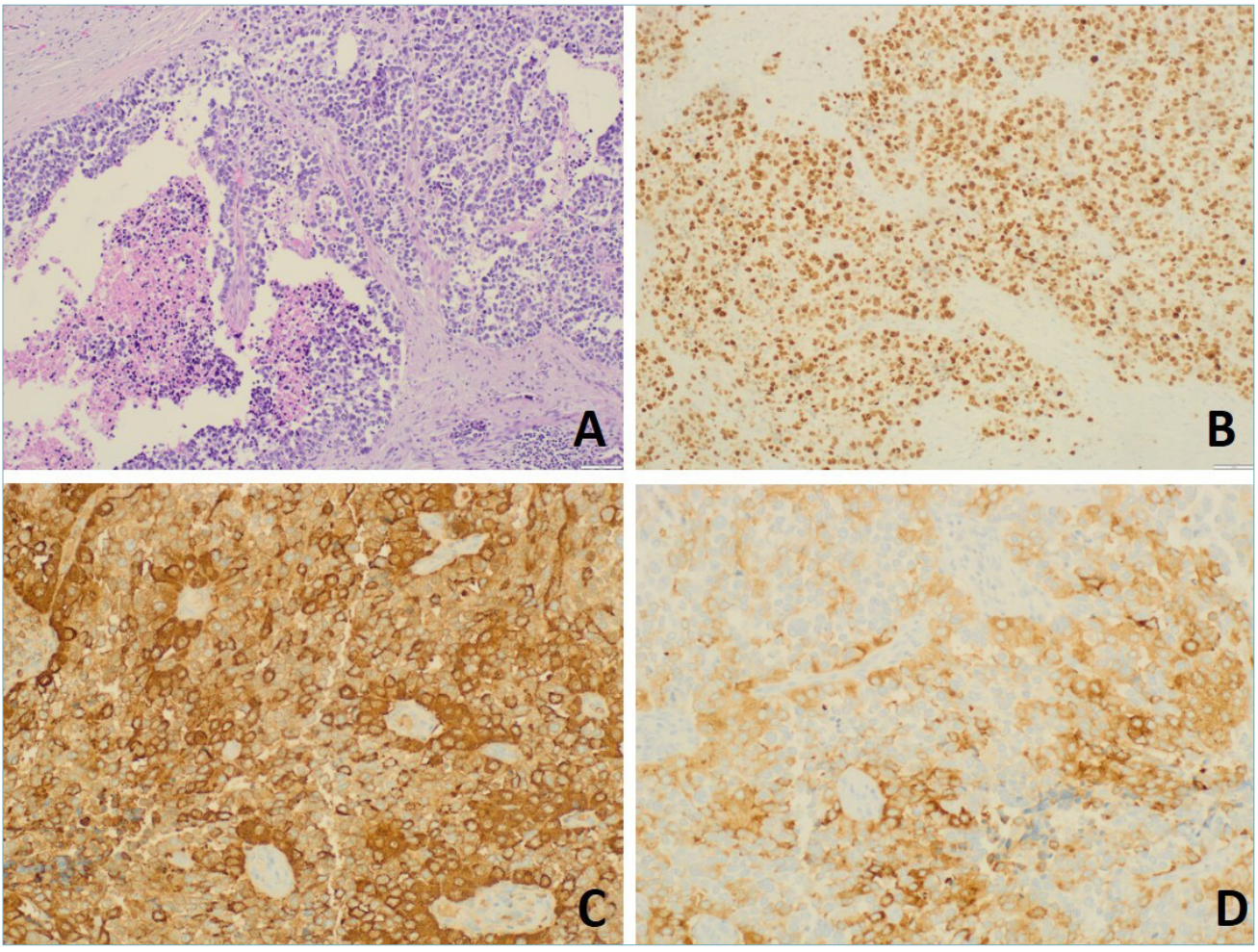

Figure 1. Small cell neuroendocrine carcinoma of the gallbladder. Solid growth of poorly differentiated small cells with zonal necrosis (A). Ki-67-related index is higher than 80% (B) and tumor cells are immunoreactive for synaptophysin (C) and chromogranin A (D). (Hematoxylin-eosin and immunoperoxidase, original magnification x100).

NETs bear a better prognosis than adenocarcinomas of the same anatomical sites, with a 10-year OS of 36% for gallbladder NETs and 80% for EHBD NETs [26]. The distinction of NENs from adenocarcinomas of the same sites is of paramount importance for establishing a correct treatment, in particular for NECs, which are often diagnosed in advanced stages and benefit from adjuvant platinum-bases chemotherapy [11].

Primary NENs of the liver

Primary hepatic NENs (H-NENs) are exceedingly rare, representing, as a whole, less than 1% of all resected primary neoplasms of the liver [27]. The distinction from metastases from other primary site is important to establish the treatment and the prognosis of patients [28,29].

Among H-NENs, NETs are reported to be slightly less frequent than NECs [27,30], and these latter are nearly always associated with a non-neuroendocrine component (hepatocellular carcinoma, HCC) in a MiNEN [18,27]. No definite sex prevalence has been reported and mean age at diagnosis in in the adulthood (around 50 years), with very rare cases under the age of 40 [30,31]. A meta-analysis of 69 cases showed that the most common intrahepatic location was the right lobe, in which half of the cases was detected [31].

The histogenesis of hepatic NENs is controversial and it has been proposed that they may derive from ectopic intrahepatic pancreatic tissue [32], but it is more conceivable that NETs arise from progenitor cells in intrahepatic bile ducts, whereas NECs may follow a common pathogenetic pathway with hepatocellular carcinoma [33]. Definitive data on genetic features of H-NENs are lack-

ing, due to their rarity. Only single cases were studied, revealing loss of one copy each of chromosomes 3 and 18, and gain of 1q in a NET G2 metastatic to the orbit [34], whereas *TP53* mutations, associated or not with *EGFR* and other well-known cancer related genes were found in two cases of NEC [35,36].

Macroscopically, H-NENs are expanding masses in the liver parenchyma, with a tannish cut surface that, in NECs, may show areas of necrosis and hemorrhage. NETs are reported to be larger (mean diameter around 5 cm) than NECs (mean diameter around 3 cm) [27]. The microscopic appearance of H-NETs is mainly of a trabecular or pseudo-glandular growth of neoplastic cells with well differentiated morphological features [27]. General neuroendocrine markers are well expressed, as well as cytokeratins 7, 18, and 19, but HepPar-1, which is a site specific antibody for hepatocellular neoplasms is not expressed [27]. Based on Ki-67-related proliferative index NETs may be graded, but until now only G1 and G2 H-NETs have been reported [33]. H-NECs are mainly of the small cell subtype, but also large cell variant has been reported [33]. Both subtypes have overlapping histopathological features with NECs in other anatomical sites and are typically found in MiNENs, combined with HCC [33]. However, occasional MiNENs with a cholangiocarcinoma component have been described [37]. Compared with NH-NETs, H-NECs show a lower expression of general neuroendocrine markers and of cytokeratins. HepPar-1 is consistently negative also in NECs [27]. As a whole, there are no specific morphological or immunohistochemical characteristics that may support the diagnosis of a primary H-NEN versus a metastasis and the practicing pathologist should always be aware that a primary H-NEN is always a diagnosis of exclusion, after careful consideration of all clinical and radiological information.

The clinical presentation of H-NENs may include nonspecific abdominal symptoms, such as abdominal discomfort or diarrhea, but a significant proportion of cases is asymptomatic [30]. Serum liver tests are mostly in the normal range and circulating tumor markers have no diagnostic value [30]. Symptoms of hormone hypersecretion (Zollinger-Ellison syndrome, Cushing syndrome and hypercalcemia due to gastrin, adrenocorticotroph hormone, and parathyroid hormone, respectively) have been reported [38-40]. The most important prognostic parameters is the distinction between NETs and NECs and the possibility of radical surgery [30].

NENs metastatic to the liver

Virtually all NENs have metastatic potential, and 90% of symptomatic patients with symptomatic NENs have synchronous metastases at diagnosis, and up to 20% of the cases present as metastasis from an occult primary [41]. The identification of the primary site is an important step towards the correct management of the patient, particularly for NETs, as the therapeutic approach may vary depending on the primary site and cell type. In addition, even when liver metastases are unresectable, the surgical treatment of the primary NET has been shown to have a positive impact of patient's outcome [42]. Consequently, thorough morphological and immunohistochemical analyses are expected to give important clues to the recognition of the site of origin of a metastatic NET. In contrast, NECs, independent of the primary site, are currently treated with platinum-based regimens, and the role of the pathologist may be limited to the distinction between a visceral NEC and a Merkel cell carcinoma of the skin, because the latter requires wide local excision, sentinel node biopsy and possibly radiotherapy. Of note, most of the diagnostic approaches discussed below have poor reliability in the context of NECs. Irrespective of the primary site, the liver represents the most frequent location of metastatic NENs and liver biopsy is the most common specimen with which the practicing pathologist is faced in the challenge of differential diagnosis.

Digestive NETs, particularly ileal (Fig. 2) and pancreatic NETs, are the major sources of liver metastases among all NETs. However, also thoracic NETs may not infrequently give hepatic secondary localizations. As pure morphological features are frequently too subtle to recognize and are also commonly obscured by crash artifacts in small liver samples, immunohistochemistry turns out to be the corner stone of the differential diagnosis in this setting. The wise use of a step-wise immunohistochemical approach using transcription factors, hormones and other markers, such as carcinoembryonic antigen (CEA), prostate-specific acidic phosphatase (PSAP), and others, may be very helpful to identify the unknown primary site of a metastatic NET [23,41]. In this respect, despite the existence of a wide range of available antibodies for each putative primary site (Tab. I), an algorithmic approach is desirable to avoid waste of time and of financial resources (Fig. 3). In fact, after the initial confirmation of the epithelial and neuroendocrine nature of the proliferation using pan-cytokeratins and general neuroendocrine markers (synaptophysin, chromogranin A and, lately, the new marker INSM1), as well as of its well differentiated morphology, the use of TTF1 and CDX2 may represent an initial step for the triage of the possible primary site. Immunoreactivity for CDX2 points towards a gastroenteropancreatic (GEP) origin, whereas TTF1 is

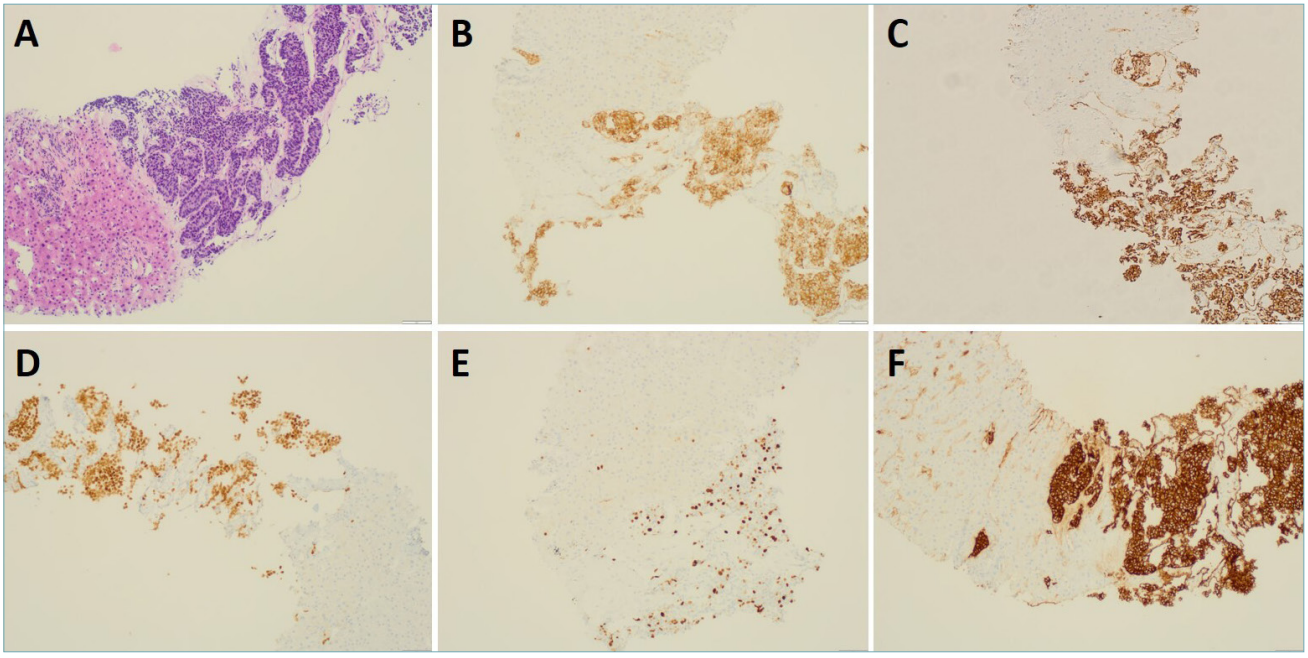

Figure 2. Ileal NET G2 metastatic to the liver (A) strongly immunoreactive for synaptophysin (B), chromagranin A (C) and CDX2 (D). Ki67 proliferation index is about 15% (E) and somatostatin receptor 2A shows strong membranous stain (F) (hematoxylin-eosin and immunoperoxidase, original magnification x100).

Table I. Useful immunohistochemical markers for the identification of the occult primary site of a NEN.

Putative primary site	Transcription factors	Hormones	Other markers
Pituitary	Pit1, SF1, Tpit, ER-α, GATA-2, GATA-3	PRL, GH, TSH, ACTH, FSH, LH, α-SU	
Thyroid	PAX8, TTF1	Calcitonin	CEA, CGRP
Parathyroid	GATA-3	PTH	
Lung	TTF1, OTP	Bombesin, serotonin, calcitonin	
Stomach	CDX2	(Histamine), Serotonin, Ghrelin	v-MAT2
Duodenum	ISL-1, PDX-1, CDX2	Somatostain, Gastrin	
Pancreas	ISL-1, PAX6, PDX-1, CDX2	Insulin, Glucagon, PP, Somatostatin, Gastrin, VIP, ACTH, Serotonin, Calcitonin, others	
Jejunum/Ileum	CDX2	Serotonin	v-MAT1
Appendix	CDX2	Serotonin, Glucagon-like peptides	
Colon-rectum	CDX2	PYY, Glucagon-like peptides, Serotonin	Prostatic acid phosphatase
Paraganglioma	GATA-3	(Catecholamines)	Tyrosine hydroxylase, v-MAT1, v-MAT2

reminiscent of a thoracic primary. The additional use of the transcription factor PDX1, which has been reported to have a certain specificity for pancreatic NETs, may be considered, although, even in expert hands, the immunoreaction with commercially available antibodies may be difficult to evaluate. In CDX2-positive metastases, the employment of antibodies directed against hormonal products like serotonin, pancreatic hormones and glucagon-related peptides may give clues to an ileal, pancreatic, or colo-rectal origin, respectively. In addition, other markers may be of help in confirming, for example, an ileal (substance P), colo-rectal (PSAP), gastric (vesicular monoamine transporter 2, v-MAT2) or duodenal (gastrin, somatostatin) primary. In this setting, one should not forget the importance of using immunohistochemical panels, and not just single antibodies, as no individual marker has absolute sensitivity and specificity in identifying an unknown

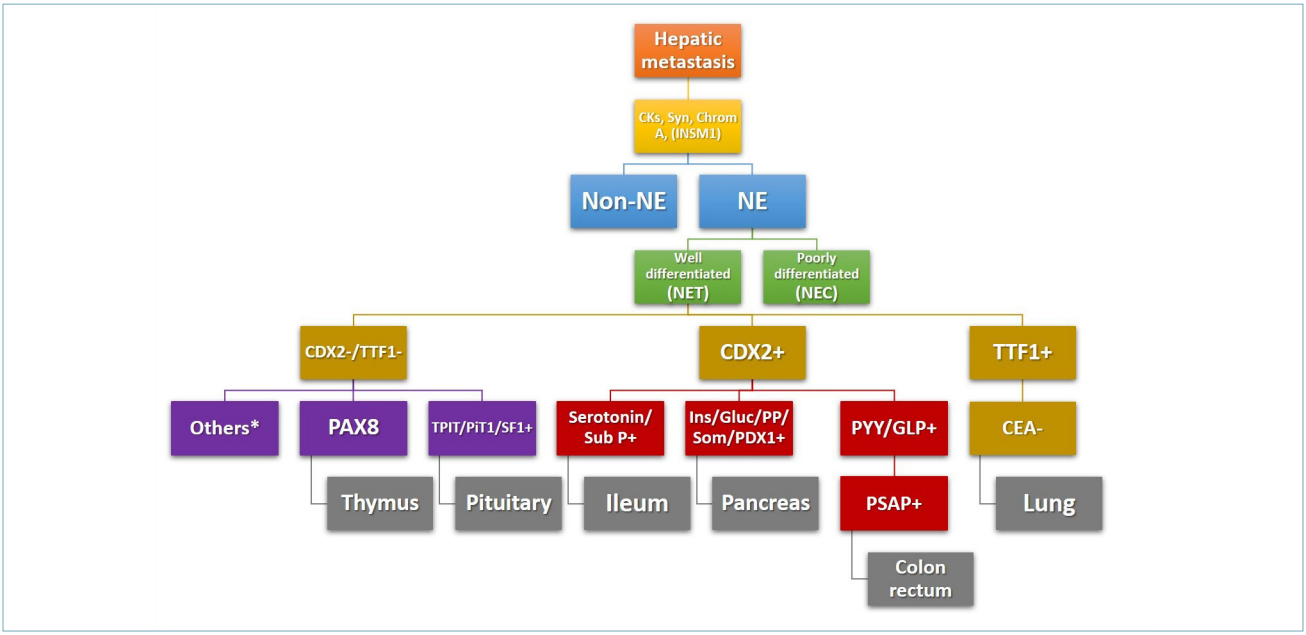

Figure 3. Practical diagnostic algorithm for the identification of the unknown primary site of a metastatic neuroendocrine tumor (NET). CKs, Cytokeratins; NE, Neuroendocrine; Syn, synaptophysin; Chrom A, chromogranin A; Sub P, substance P; Ins, insulin; Gluc, glucagon; Som, somatostatin; GLP, glucagon-like peptides; PSAP, prostate-specific acidic phosphatase; *GATA3 for breast NENs (but it is also positive in gonadotroph pituitary NETs); SSTR2A and SSTR5 to predict avidity to somatostatin analogues-based imaging.

primary. Just as an example, the positive immunostain for TTF1 is not exclusive of lung NETs (carcinoids), as, in the context of NENs, medullary thyroid carcinomas (MTCs) are also TTF1-positive and, in turn, calcitonin expression is not an absolutely affordable marker of MTCs, as also lung NETs may stain positive for this hormone. In such case, a positive stain for CEA favors MTC versus lung NET [41].

Liver metastases from NETs arising in rare sites (extra-GEP and extra-thoracic) are possible findings and even in this case immunohistochemistry may be of help (Tab. I, Fig. 1). For example, the possibility of metastases from pituitary NETs should not be underestimated, particularly in the clinical context of a Cushing syndrome [43]. In this case, the use of pituitary-specific transcription factors may be useful to reach a correct diagnosis. Finally, another useful immunohistochemical tool that can support the clinical search for the unknown primary site of a NET is the immunostain for somatostatin receptors (SSTRs). Indeed, a strong membranous positivity for SSTR2A and/or for SSTR5 has a good correlation with the avidity of the neoplasm for somatostatin analogues-based imaging that can identify the primary NET [44].

In conclusion, the workup of a metastatic NEN represents a critical responsibility of pathologists. It requires careful interpretation of clinical, morphologic and immunohistochemical findings. The use of a panel of approach combining cytokeratins along with anatomic site-related transcription factors, hormones and other biomarkers can assist identifying the origin of the metastatic NEN. The power of this approach is limited in the setting of poorly differentiated NENs (NECs).

Neuroendocrine neoplasms of the pancreas

NENs of the pancreas (PanNENs) are a heterogeneous group of tumors with different histologic, molecular and clinical features. The current World Health Organization (WHO) diagnostic guidelines have refined their classification, which reflects more appropriately the different biological landscapes and prognostic implications [24].

Definition/Terminology. The heterogeneous group of neuroendocrine lesions of the pancreas has been named as pancreatic neuroendocrine neoplasm (PanNEN). The current WHO classification subdivides PanNEN in three main categories: i) pancreatic neuroendocrine microadenoma (lesion < 5 mm); ii) well-differentiated pancreatic neuroendocrine tumor (PanNET),

which includes functional PanNETs (F-PanNENs; tumors with clinical evidence of hormone production, such as insulinoma, glucagonoma, gastrinoma, VIPoma, etc.) and non-functional PanNETs (NF-PanNET); iii) poorly differentiated pancreatic neuroendocrine carcinoma (PanNEC), featuring either small cell or large cell PanNECs [24].

The last WHO classification has subdivided PanNETs into three subgroups of tumors: i) Grade 1 PanNET (PanNET G1): < 2 mitoses/2 mm^2 and a Ki-67 proliferation index < 3%; Grade 2 PanNET (PanNET G2): 2-20 mitoses/2 mm^2 or a Ki-67 proliferation index of 3-20%; Grade 3 PanNET (PanNET G3): > 20 mitoses/2 mm^2 or a Ki-67 proliferation index > 20% 24. This last category represents the main novelty in PanNENs classification, and should be distinguished from PanNEC, which in turn includes small-cell and large-cell carcinoma [24].

A last category to be considered is represented by MiNEN. Both components must represent at least 30% of the total tumor mass, are usually high-grade (G3) and the non-neuroendocrine part is generally represented by acinar carcinoma or ductal adenocarcinoma [18,19,24].

Macroscopic description. Because of their small size, microadenoma are rarely documented during routine sampling. PanNETs are usually brownish lesions, with lobulated or pushing borders and soft to fleshy consistency. The vast majority of PanNETs are encapsulated, at least in part, and sharply demarcated from the adjacent pancreatic parenchyma. Cystic changes are rare but, if present, a unilocular cyst is reported. Conversely, PanNECs generally show infiltrative margins, hard consistency and brownish to whitish color; typically, necrotic areas are reported.

Histopathology. A classic example of neuroendocrine microadenoma is shown in Figure 4, and paradigmatic examples of PanNETs G1, G2 and G3 and of PanNEC are depicted in Figure 5. By definition, neuroendocrine microadenoma is a small and well differentiated neuroendocrine neoplasm. Histologically, NF-PanNETs display a well-differentiated growth pattern, with a spectrum of architectural patterns, including solid-nesting, paraganglioma-like, trabecular, gyriform and glandular aspects[24,45,46]. The stroma is highly vascular, but areas with dense and hyalinized collagen are often present. Most of neoplastic cells are monomorphic, cuboidal and with the classic nuclei showing “salt and pepper” chromatin texture [24,45,46]. They are centrally located and polarized. In addition to these classical aspects, some functional PanNETs may exhibit particular features, although distinctive morphological hallmarks are lacking. The more peculiar histological features for functional PanNETs

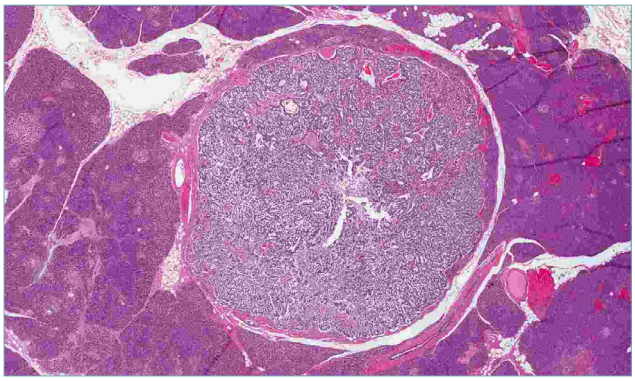

Figure 4. Example of a classical neuroendocrine microadenoma. This category includes small (<0.5 cm) well-differentiated neuroendocrine neoplasms. Original magnification: x20.

are the following: i) insulinoma: trabecular and solid growth patterns, with normal pancreatic ducts often entrapped within tumor mass; a stromal deposit of islet amyloid polypeptide is quite specific but very rare (5% of cases) [47]; ii) gastrinoma: trabecular and glandular growth patterns [48]; iii) glucagonoma: presence of densely arranged trabecular structures with scant stroma [49]; iv) somatostatinoma: psammomatous calcification are quite common, although they are more typical of duodenal location [50]; v) serotonin-producing PanNETs: a trabecular architecture is the most common pattern, and vascular / perineural invasion is frequent even in G1 tumors [51].

PanNECs present distinctive histologic features and have been subdivided in small-cell and large-cell PanNECs[24]. Small-cell PanNECs are characterized by diffuse sheets of cells with scant cytoplasm, round or elongated nuclei and finely granular chromatin. As in the pulmonary counterpart, nuclear moulding may be also present [24,52]. Large-cell PanNECs is a more common subtype, and is composed by round to polygonal large cells with coarse chromatin and prominent nuclei [24,52]. Both small-cell and large-cell PanNECs show necrotic areas, often with a comedo-like appearance.

MiNEN are mixed neuroendocrine-non-neuroendocrine neoplasms, where which each counterpart accounts at least for 30% of the entire lesion [24,53]. The non-neuroendocrine counterpart, which is usually represented by acinar cell carcinoma or ductal adenocarcinoma, reflects its conventional morphology.

Immunohistochemical and molecular markers. As neuroendocrine neoplasms, PanNETs are usually stained by pan-cytokeratins and general neuroendocrine markers such as Synaptophysin and Chromogranin-A, with known variability between NETs and NECs [23,24]. Other markers that may be added to the

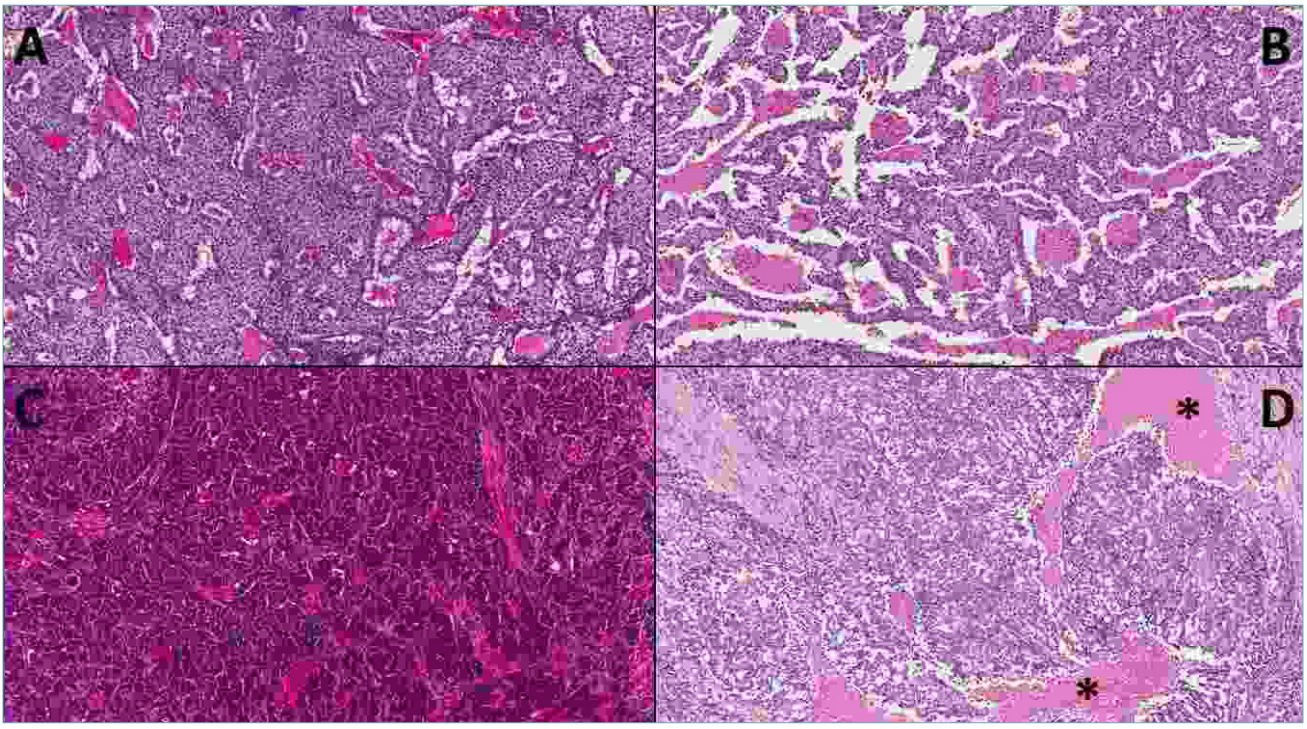

Figure 5. The entire spectrum of PanNET/NEC is shown. A-C: well differentitated PanNETs, as follows: A) PanNET G1, B) PanNET G2, C) PanNET G3; D) PanNEC (black asterisks indicate tumor necrosis). Original magnification: X10.

diagnostic PanNET algorythm, but have low accurancy are: i) CD56, which has a high sensitivity but a low specificity, ii) CD200, which stains in the pancreas both PanNETs and solid pseudopapillary neoplasms (SPNs), iii) Islet-1, whose expression is not restricted to PanNETs but is also commonly found in well and poorly differentiated NENs in extrapancreatic sites [23,24,56]. In the prognostic grading of PanNETs, the evaluation of Ki-67 proliferation index (using clone MIB1) is crucial, as stated before in this review. Ki-67 may be useful also in the differential diagnosis between PanNET G3 vs. PanNEC [52]. In the former, Ki-67 distribution is heterogeneous and usually shows areas with a low (< 20%) proliferation, together with highly proliferating areas, whereas PanNECs displays a more homogeneous staining, usually present in a very high proportion of neoplastic cell nuclei (> 60%). Immunohistochemical stains for hormonal products (both pancreatic and ectopic) may provide additional information for the characterization of both F-PanNENs and NF-PanNENs [23,57], but it has to be noticed that the specific diagnosis of F-PanNET must be based on the hormone-related clinical syndrome rather than immunohistochemical analysis [24].

PanNETs display a mutational profile that includes *MEN1*, *DAXX* and *ATRX* as the most commonly mutated genes, with *DAXX* and *ATRX* mutations being mutually exclusive [58]. Collectively, about 60% of PanNETs carry *MEN1*/*DAXX*/*ATRX* mutations. *DAXX* and *ATRX* mutations have been recently associated with poor prognosis [55]. Furthermore, the biological process of alternative lengthening of telomeres (ALT), which is a telomerase-independent mechanism used by different tumors to maintain the telomere length thus increasing cell replication's potential, is activated in a subset of PanNETs and associated with an increased rate of distant metastases [59]. PanNECs commonly bear *TP53* and *RB1* mutations, which are reflected by abnormal expression patterns of the related proteins, so that the differential diagnosis between PanNET G3 vs. PanNEC can be supported by immunohistochemistry [24,52]. Indeed, an abnormal expression pattern for p53 and the loss of Rb immunostain strongly corroborate a PanNEC diagnosis against a PanNET G3. The genetic differences between PanNET and PanNEC is in keeping with the assumption that they are distinctive and separate tumor entities [45,52].

Differential diagnosis. Besides the importance of the distinction between PanNETs G3 and PanNECs, the main differential diagnoses of PanNENs include pancreatic non-neuroendocrine epithelial malignancies with solid and/or organoid pattern of growth, such

as acinar cell carcinoma (ACC), solid pseudopapillary neoplasm (SPN) and pancreatoblastoma. Morphology and immunohistochemistry are the corner stones of a correct a diagnostic panel of antibodies including CD10, vimentin, β-catenin and LEF1 for SPNs, and Bcl10 and trypsin for acinar cell carcinomas is advisable in selected cases [60,61].

Clinical aspects. PanNETs and PanNECs display distinctive features also under clinical aspects. Most NF-PanNETs are small and located in the pancreatic tail, thus they are identified incidentally and patients do not have specific tumor-related symptoms [24,45]. F-PanNETs present with hormone-related syndromes (e.g., fasting hypoglycemia in insulinomas, diarrhea with dermatitis in glucagonomas, Zollinger-Ellison syndrome in gastrinomas). Patients with PanNEC present more often mass-related symptoms and show rapid clinical progression, requiring prompt cytotoxic chemotherapy, usually with platinum-based regimens [45]. If achievable, complete surgical resection remains the most effective modality for the treatment of PanNENs.

References

1 Ayabe RI, Wach M, Ruff S, et al. Primary Gallbladder Neuroendocrine Tumors: Insights into a Rare Histology Using a Large National Database. Ann Surg Oncol 2019;26:3577-85. https://doi.org/10.1245/s10434-019-07440-6.

2 Michalopoulos N, Papavramidis TS, Karayannopoulou G, et al Neuroendocrine tumors of extrahepatic biliary tract. Pathol Oncol Res 2014;20:765-75. https://doi.org/10.1007/s12253-014-9808-4

3 Modlin IM, Shapiro MD, Kidd M. An analysis of rare carcinoid tumors: clarifying these clinical conundrums. World J Surg 2005;29:92101. https://doi.org/10.1007/s00268-004-7443-z

4 Niu C, Wang S, Guan Q, et al. Neuroendocrine tumors of the gallbladder. Oncol Lett 2020;19:3381-8. https://doi.org/10.3892/ol.2020.11461

5 Tonnhofer U, Balassy C, Reck CA, et al. Neuroendocrine tumor of the common hepatic duct, mimicking a choledochal cyst in a 6-year-old child. J Pediatr Surg 2009;44:E23-5. https://doi.org/10.1016/j.jpedsurg.2009.03.030

6 Albores-Saavedra J, Nadji M, Henson DE, et al. Intestinal metaplasia of the gallbladder: a morphologic and immunocytochemical study. Hum Pathol 1986;17:614-20. https://doi.org/10.1016/s0046-8177(86)80134-4

7 Eltawil KM, Gustafsson BI, Kidd M, et al. Neuroendocrine tumors of the gallbladder: an evaluation and reassessment of management strategy. J Clin Gastroenterol 2010;44:687-95. https://doi.org/10.1097/MCG.0b013e3181d7a6d4

8 Maitra A, Krueger JE, Tascilar M, et al. Carcinoid tumors of the extrahepatic bile ducts: a study of seven cases. Am J Surg Pathol 2000;24:1501-10. https://doi.org/10.1097/00000478-200011000-00005

9 Fellows IW, Leach IH, Smith PG, et al Carcinoid tumor of the common bile duct - a novel complication of von Hippel-Lindau syndrome. Gut 1990;31:728-9. https://doi.org/10.1136/gut.31.6.728

10 Nafidi O, Nguyen BN, Roy A. Carcinoid tumor of the common bile duct: a rare complication of von Hippel-Lindau syndrome. World J Gastroenterol 2008;14:1299-1301. https://doi.org/10.3748/wjg.14.1299

11 Lee SM, Sung CO. Neuroendocrine carcinomas of the gallbladder: a clinicopathologic and immunohistochemical analysis of 34 resected cases. Am J Surg Pathol 2020;44:1308-21. https://doi.org/10.1097/PAS.0000000000001536

12 Sciarra A, Missiaglia E, Trimech M, et al. Gallbladder Mixed Neuroendocrine-Non-neuroendocrine Neoplasm (MiNEN) arising in intracholecystic papillary neoplasm: clinicopathologic and molecular analysis of a case and review of the literature. Endocr Pathol 2020;31:84-93. https://doi.org/10.1007/s12022-020-09605-6

13 Li M, Liu F, Zhang Y, et al. Whole-genome sequencing reveals the mutational landscape of metastatic small-cell gallbladder neuroendocrine carcinoma (GB-SCNEC). Cancer Lett 2017;10;391:20-7. https://doi.org/10.1016/j.canlet.2016.12.027

14 Kaino M, Kaino S, Goma W, et al. A case of mixed neuroendocrine non-neuroendocrine neoplasm of the distal bile duct with biliary intraepithelial neoplasia. Clin J Gastroenterol 2020 Jul 8. Epub ahead of print.

15 Zhang L, Yang Z, Chen Q, et al. Mixed adenoendocrine carcinoma in the extrahepatic biliary tract: A case report and literature review. Oncol Lett 2019;18:1585-96. https://doi.org/10.3892/ol.2019.10502

16 Skalický A, Vištejnová L, Dubová M, et al. Mixed neuroendocrine-non-neuroendocrine carcinoma of gallbladder: case report. World J Surg Oncol 2019;22;17:55. https://doi.org/10.1186/s12957-019-1598-4

17 Zhang L, Wan D, Bao L, et al. Neuroendocrine carcinoma in the extrahepatic biliary tract: A case report and literature review. Medicine (Baltimore) 2018;97:e11487. https://doi.org/10.1097/MD.0000000000011487

18 La Rosa S, Sessa F, Uccella S. Mixed Neuroendocrine-Nonneuroendocrine Neoplasms (MiNENs): unifying the concept of a heterogeneous group of neoplasms. Endocr Pathol 2016;27:284-311. https://doi.org/10.1007/s12022-016-9432-9

19 Uccella S, La Rosa S. Looking into digestive mixed neuroendocrine - nonneuroendocrine neoplasms: subtypes, prognosis, and predictive factors. Histopathology 2020;77:700-17. https://doi.org/10.1111/his.14178

20 Chu H, Zhang C, Shi Y, et al. Gallbladder neuroendocrine carcinoma:a single center experience. Medicine (Baltimore) 2020;99:e21912. https://doi.org/10.1097/MD.0000000000021912

21 Liu W, Chen W, Chen J, et al. Neuroendocrine carcinoma of gallbladder: a case series and literature review. Eur J Med Res 2019;24:8. https://doi.org/10.1186/s40001-019-0363-z

22 Raiker R, Chauhan A, Hasanein H, et al. Biliary tract large cell neuroendocrine carcinoma: current evidence. Orphanet J Rare Dis 2019;14:266. https://doi.org/10.1186/s13023-019-1230-2

23 Uccella S, La Rosa S, Volante M, et al. Immunohistochemical biomarkers of gastrointestinal, pancreatic, pulmonary, and thymic neuroendocrine neoplasms. Endocr Pathol 2018;29:150-68. https://doi.org/10.1007/s12022-018-9522-y

24 Bosman FT, Carneiro F, Hruban RH, et al. WHO Classification of tumours of the digestive system. 5th ed. Lyon: IARC press 2019.

25 Price TN, Thompson GB, Lewis JT, et al. Zollinger-Ellison syndrome due to primary gastrinoma of the extrahepatic biliary tree: three case reports and review of literature. Endocr Pract 2009;15:737-49.

26 Albores-Saavedra J, Batich K, Hossain S, et al. Carcinoid tumors and small-cell carcinomas of the gallbladder and extrahepatic bile ducts: a comparative study based on 221 cases from

the Surveillance, Epidemiology, and End Results Program. Ann Diagn Pathol 2009;13:378-83. https://doi.org/10.1016/j.anndiagpath.2009.08.002

[27] Nomura Y, Nakashima O, Akiba J, et al. Clinicopathological features of neoplasms with neuroendocrine differentiation occurring in the liver. J Clin Pathol 2017;70:563-70. https://doi.org/10.1136/jclinpath-2016-203941

[28] Qiu MJ, Chen YB, Bi NR, et al. Comparative clinical analysis of gastroenteropancreatic neuroendocrine carcinomas with liver metastasis and primary hepatic neuroendocrine carcinomas. Dis Markers 2018;2018:9191639. https://doi.org/10.1155/2018/9191639

[29] Shen YH, Chen S, Zhang WT, et al. Clinical analysis of gastroenteropancreatic neuroendocrine tumor with liver metastasis, compared with primary hepatic neuroendocrine tumor. J Cancer Res Ther 2014;10 Suppl:276-80. https://doi.org/10.4103/0973-1482.151532

[30] Chen RW, Qiu MJ, Chen Y, et al. Analysis of the clinicopathological features and prognostic factors of primary hepatic neuroendocrine tumors. Oncol Lett 2018;15:8604-10. https://doi.org/10.3892/ol.2018.8413

[31] Gravante G, De Liguori Carino N, Overton J, et al. Primary carcinoids of the liver: a review of symptoms, diagnosis and treatments. Dig Surg 2008;25:364-8. https://doi.org/10.1159/000167021

[32] Yu-Ping, X. & Ji-Yao, Y. Primary neuroendocrine carcinoma of the liver. Ultrastruct Pathol 1988;10:331-6. https://doi.org/10.3109/01913128609064197

[33] Klimstra DS. Hepatic neuroendocrine neoplasms. In: WHO Classification of Tumors Editorial Board. Digestive system tumors. 5th ed. Vol 1. Lyon: IARC press 2019.

[34] Rasmussen JØ, von Holstein SL, Prause JU, et al. Genetic analysis of an orbital metastasis from a primary hepatic neuroendocrine carcinoma. Oncol Rep 2014;32:1447-50.

[35] Shastri A, Msaouel P, Montagna C, et al. Primary hepatic small cell carcinoma: two case reports, molecular characterization and pooled analysis of known clinical data. Anticancer Res 2016;36:271-7.

[36] Pastrián LG, Ruz-Caracuel I, Gonzalez RS. Giant primary neuroendocrine neoplasms of the liver: report of 2 cases with molecular characterization. Int J Surg Pathol 2019;27:893-9. https://doi.org/10.1177/1066896919855764

[37] Zheng SL, Yip VS, Pedica F, et al. Intrahepatic bile duct mixed adenoneuroendocrine carcinoma: a case report and review of the literature. Diagn Pathol 2015;10:204. https://doi.org/10.1186/s13000-015-0439-1

[38] Rascarachi G, Sierra M, Hernando M, et al. Primary liver carcinoid tumour with a Zollinger Ellison syndrome - an unusual diagnosis: a case report. Cases J 2009;2:6346. https://doi.org/10.4076/1757-1626-2-6346

[39] Shah NA, Urusova IA, D'Agnolo A, et al. Primary hepatic carcinoid tumor presenting as Cushing's syndrome. J Endocrinol Invest 2007;30:327-33. https://doi.org/10.1007/BF03346308

[40] Kwon HJ, Kim JW, Kim H, et al. Combined hepatocellular carcinoma and neuroendocrine carcinoma with ectopic secretion of parathyroid hormone: a case report and review of the literature. J Pathol Transl Med 2018;52:232-7. https://doi.org/10.4132/jptm.2018.05.17

[41] Uccella S, Asa SL, Mete O. Metastatic neuroendocrine neoplasms of unknown primary site. In: Asa SL, La Rosa S, Mete O, eds. The spectrum of neuroendocrine neoplasia. Cham: Springer 2021. https://doi.org/10.1007/978-3-030-54391-4_16

[42] Capurso G, Bettini R, Rinzivillo M, et al. Role of resection of the primary pancreatic neuroendocrine tumour only in patients with unresectable metastatic liver disease: a systematic review. Neuroendocrinology 2011;93:223-9. https://doi.org/10.1159/000324770

[43] Venable ER, Kerr SE, Lopes MBS, et al. Liver metastases from pituitary carcinomas mimicking visceral well-differentiated neuroendocrine tumors: a series of four cases. Diagn Pathol 2020;15:81. https://doi.org/10.1186/s13000-020-00997-x

[44] Volante M, Brizzi MP, Faggiano A, et al. Somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumors: a proposal of scoring system correlated with somatostatin receptor scintigraphy. Mod Pathol 2007;20:1172-82. https://doi.org/10.1038/modpathol.3800954

[45] Tang LH. Pancreatic neuroendocrine neoplasms: landscape and horizon. Arch Pathol Lab Med 2020 Apr 16. Online ahead of print. https://doi.org/10.5858/arpa.2019-0654-RA

[46] Xue Y, Reid MD, Pehlivanoglu B, et al. Morphologic variants of pancreatic neuroendocrine tumors: clinicopathologic analysis and prognostic stratification. Endocr Pathol 2020;31:239-253. https://doi.org/10.1007/s12022-020-09628-z

[47] Anlauf M, Wieben D, Perren A, et al. Persistent hyperinsulinemic hypoglycemia in 15 adults with diffuse nesidioblastosis: diagnostic criteria, incidence, and characterization of beta-cell changes. Am J Surg Pathol 2005;29:524-33. https://doi.org/10.1097/01.pas.0000151617.14598.ae

[48] Rosentraeger MJ, Garbrecht N, Anlauf M, et al. Syndromic versus non-syndromic sporadic gastrin-producing neuroendocrine tumors of the duodenum: comparison of pathological features and biological behavior. Virchows Arch 2016;468:277-87. https://doi.org/10.1007/s00428-015-1890-9

[49] Hamid QA, Bishop AE, Sikri KL, et al. Immunocytochemical characterization of 10 pancreatic tumours, associated with the glucagonoma syndrome, using antibodies to separate regions of the pro-glucagon molecule and other neuroendocrine markers. Histopathology 1986;10:119-33. https://doi.org/10.1111/j.1365-2559.1986.tb02468.x

[50] Noë M, Pea A, Luchini C, et al. Whole-exome sequencing of duodenal neuroendocrine tumors in patients with neurofibromatosis type 1. Mod Pathol 2018;31:1532-8. https://doi.org/10.1038/s41379-018-0082-y

[51] McCall CM, Shi C, Klein AP, et al. Serotonin expression in pancreatic neuroendocrine tumors correlates with a trabecular histologic pattern and large duct involvement. Hum Pathol 2012;43:1169-76. https://doi.org/10.1016/j.humpath.2011.09.014

[52] Yachida S, Vakiani E, White CM et al. Small cell and large cell neuroendocrine carcinomas of the pancreas are genetically similar and distinct from well-differentiated pancreatic neuroendocrine tumors. Am J Surg Pathol 2012;36:173-84. https://doi.org/10.1097/PAS.0b013e3182417d36

[53] Luchini C, Capelli P, Scarpa A. Pancreatic ductal adenocarcinoma and its variants. Surg Pathol Clin 2016;9:547-60. https://doi.org/10.1016/j.path.2016.05.003

[54] Pelosi G, Zamboni G, Doglioni C, et al. Immunodetection of proliferating cell nuclear antigen assesses the growth fraction and predicts malignancy in endocrine tumors of the pancreas. Am J Surg Pathol 1992;16:1215-25. https://doi.org/10.1097/00000478-199212000-00008

[55] Pelosi G, Bresaola E, Bogina G, et al. Endocrine tumors of the pancreas: Ki-67 immunoreactivity on paraffin sections is an independent predictor for malignancy: a comparative study with proliferating-cell nuclear antigen and progesterone receptor protein immunostaining, mitotic index, and other clinicopathologic variables. Hum Pathol 1996;27:1124-34. https://doi.org/10.1016/s0046-8177(96)90303-2

[56] Agaimy A, Erlenbach-Wünsch K, Konukiewitz B, et al. ISL1 expression is not restricted to pancreatic well-differentiated neuroendocrine neoplasms, but is also commonly found in well and poorly differentiated neuroendocrine neoplasms of extrapancreatic origin. Mod Pathol 2013;26:995-1003. https://doi.org/10.1038/modpathol.2013.40

[57] Uccella S, Sessa F, La Rosa S. Diagnostic approach to neuroendocrine neoplasms of the gastrointestinal tract and pancreas. Turk Patoloji Derg 2015;31 Suppl 1:113-27. https://doi.org/10.5146/tjpath.2015.01319. PMID: 26177322.

[58] Scarpa A, Chang DK, Nones K, et al. Whole-genome landscape of pancreatic neuroendocrine tumours. Nature 2017;543:65-71. https://doi.org/10.1038/nature21063

[59] Marinoni I, Kurrer AS, Vassella E, et al. Loss of DAXX and ATRX are associated with chromosome instability and reduced survival of patients with pancreatic neuroendocrine tumors. Gastroenterology 2014;146:453-460.e5. https://doi.org/10.1053/j.gastro.2013.10.020

[60] La Rosa S, Sessa F, Capella C. Acinar cell carcinoma of the pancreas: overview of clinicopathologic features and insights into the molecular pathology. Front Med (Lausanne) 2015;2:41. https://doi.org/10.3389/fmed.2015.00041. PMID: 26137463; PMCID: PMC4469112.

[61] La Rosa S, Bongiovanni M. Pancreatic solid pseudopapillary neoplasm: key pathologic and genetic features. Arch Pathol Lab Med 2020 Jan 20. https://doi.org/10.5858/arpa.2019-0473-RA. Epub ahead of print. PMID: 31958381.