NEUROENDOCRINE TUMORS OF THE GI AND PANCREATOBILIARY TRACT

Neuroendocrine lesions of the GI tract have various facets, and the following discussion will be provided based on the degree of neuroendocrine differentiation and its biologic significance:

1. Incipient neuroendocrine neoplasia: Dysplasia/Tis
2. True neuroendocrine tumors
   a. Well differentiated NETs (carcinoids, pancreatic neuroendocrine neoplasms)
   b. High-grade neuroendocrine carcinoma
3. Hybrid tumors
   a. Mixed tumors
      i. Mixed adenocarcinoma-neuroendocrine carcinoma
      ii. Mixed neuroendocrine tumor and non-neuroendocrine carcinoma
      iii. Duodenal gangliocytic paraganglioma
   b. Incidental neuroendocrine proliferations in epithelial tumors
   c. Chimeric tumors (neoplasms with incomplete neuroendocrine differentiation)
      i. Goblet cell carcinoids, conventional type
      ii. Dedifferentiated goblet cell carcinoids (adenocarcinoma ex-GCC)
4. Others
   a. Non epithelial- origin tumors with NE differentiation (small blue cell tumors), induced or aberrant NE differentiation; secondary (metastatic) neuroendocrine neoplasms

I. INCIPIENT NEOPLASIA

Early (precursor type) neuroendocrine lesions in the tubular GI tract
In the GI tract, there are neuroendocrine cell proliferations that can be considered as early (precursor type) lesions. This phenomenon is quite well recognized in the stomach where there is a spectrum of enterochromaffin-like cell (ELC) proliferations that occur in the setting of hypergastrinemia (usually compensatory to atrophic gastritis-related hypochlorhydria). In this condition, the trophic effects of the gastrin hormone seem to lead to a spectrum of ELC proliferations ranging from what is termed as hyperplasia (which can be diffuse, linear or nodular), to dysplasia, and finally to full-blown carcinoid. Naturally, it is difficult to draw lines between the different grades of this process, but some arbitrary criteria have been proposed. For microscopic proliferations, if there is nodular growth of ELCs which measure >150 microns, or if there are conglomerate of nodules, signs of “microinfiltration” and/or new stroma, then the lesion is proposed to be classified as “dysplasia or Tis”.

In a case with minute focus of ELC proliferation, it is important to look for signs of atrophy in the background, and investigate the clinical setting to determine the clinical significance of the lesion. It is also important to keep in mind that the frank carcinoids that arise in this setting may be biologically different and more indolent then sporadic carcinoids.
Another example of incipient NET occurs in the pancreas, in patients with MEN syndrome, but occasionally also without any syndromic background. These patients often develop numerous microscopic nodules that are regarded as “dysplastic” (‘Tis’). Larger ones (< 0.5 cm) are referred as “microadenoma”. With these lesions as well, as in the gastric ELC proliferations, it is often difficult where the “hyperplasia” stops and true autonomous proliferation of neoplasm takes over.

IIA. WDNETS (WELL DIFFERENTIATED NEUROENDOCRINE TUMORS; I.E., CARCINOIDs AND PANNETS)

Terminology/concept
Well differentiated neuroendocrine tumor is now established as the main diagnosis for the conventional carcinoids and what used to be called islet cell tumors, also endorsed by WHO-2010, and the “NET” societies of North America (NANETs) and Europe (ENETs). WDNET category fundamentally corresponds to grade 1 and 2 of the current grading/classification (based on Ki67 labeling index of < 3 and < 20%, respectively; or mitotic activity of < 2/10HPF or < 20/10HPF), although it is becoming increasingly clear that some WDNETs may fall into the lower end of the grade 3 category.

Diagnostic Pitfalls
Morphologically, WDNETs are fairly characteristic and relatively easy to recognize. They are typically composed of uniform, round cells, with fair amount of cytoplasm and “salt-and-pepper” chromatin. The latter is probably the most specific diagnostic feature of these tumors. The cells grow in nests, but may also form trabecular, acinar or rosette-like growth pattern. The main clues for the diagnosis are uniformity of the cells, the chromatin pattern, regularity of the nuclear membranes and plasmacytoid cells, which becomes very helpful in cytologic smears.

Limited nature of the biopsy or the presence of preservation artifact sometimes leads to misdiagnoses, especially considering that WDNETs can be located at the base of the mucosa and thus may be under-represented in the biopsy.

Some of the morphologic variations that commonly lead to diagnostic problems include tubular pattern and intraluminal mucin formation. In particular, *ampullary glandular psammomatous carcinoids (ampullary somatostatinoma)* and some appendiceal cases often have tubule formation and intraluminal mucin. However, intracytoplasmic mucin is almost non-existent.

More problematic are the rare variants that are under-recognized. These are significantly more common in pancreatic or sporadic gastric (type-3) cases. For example, although most PanNETs show diffuse, stroma-poor, sheet-like growth pattern, some show abundant stromal sclerosis and may exhibit tubule formation, mimicking invasive ductal adenocarcinoma. Pleomorphic variant is also often mistaken as a high-grade adenocarcinoma, although it is prognostically not different than even a regular PanNET. Clear cell or lipid-rich variants are often misdiagnosed as carcinomas from adrenal, liver or kidney. Oncocytic variant of these tumors have single prominent nucleoli, and combined with the abundant cytoplasm, these...
can closely mimic hepatocellular carcinomas when they metastasize to the liver. Therefore it is important to recognize these variations in the theme.

**Biologic behavior and reporting**

WDNETs are now established as low-grade indolent malignant neoplasia, perhaps with the exception of the incipient examples discussed above. Therefore, they are documented as any other cancer type. Stage, grade and other parameters are reported separately. The clinicopathologic determinants of biologic behavior and clinical outcome are discussed below. Naturally, these are also the parameters to be documented in the surgical pathology report, whenever feasible.

A. **Clinical setting.** This is best exemplified in the stomach, where the type A cases arising due to hypergastrinemia, often in the background of autoimmune gastritis, typically follows a benign clinical course, in contrast, the type C (sporadic) are often fairly aggressive.

B. **Cell type.** Hormonal activity (cell type) is known to correlate with behavior especially for PanNETs. For example, clinically functioning insulinomas pursue an indolent clinical course in 90% of cases and had even been classified as “benign”. In contrast, most other syndromic PanNETs result in recurrence or metastases in 50-70% of cases. It is possible that the favorable outcome of patients with insulinomas is due, at least in part, to the relatively small size at which these tumors are typically detected relative to other syndromic PanNETs. On the other hand, duodenal gastrinomas often result in metastases even when the primary measures less than 1 cm. Therefore, both the cell biology and the stage with which they present seem to be a factor. In daily practice, the cell type determination is based mostly on the clinical findings.

C. **Primary location.** For example, most appendiceal classical WDNETs are asymptomatic, and similarly, most rectal ones are also non-metastatic.

D. **Stage.** Just as in any other malignancy, with recent studies, stage is proving to be the most powerful predictor of outcome. Each site has its own staging. There are also different staging schemes proposed for a given site. In the US, the TNM put forth by AJCC/UICC and endorsed by the College of American Pathologists is in wider use, although the ones proposed by the ENETS may prove to have more validity.

E. **Grade.** There is now overwhelming consensus that NETs ought to be graded and staged separately. The grading system put forth by ENETS and now endorsed by the WHO-2010 classifies NETs into 3 categories based on either mitotic activity (per 10 HPF) or Ki-67 index (percent of cells): NET-1 as <2, NET-2 as 2-20, and NET-3 as >20. This is applied regardless of the location. Naturally, as any other semi-quantitative analysis in surgical pathology, this grading system is subject to challenges created by heterogeneity, false positivity by extrinsic factors, methodologies, instruments, operators and others. For Ki67 counting, eye-ball ing which was considered acceptable until recently, has now been shown to be too unreliable. The preferable approach is the manual or digital count of captured image of the hot-spot. Guidelines are unclear for cases that fall into the 2-3 % range, but most authors seem to place those into grade 2. Grading is employed also for metastatic tumors; there are multiple studies showing the value of grading metastatic tumors. With the current cut-offs of 2 and 20, if the Ki67 count is performed accurately, it invariably proves to be higher than the mitotic count, perhaps negating the need for the more tedious mitotic count. Of note, studies are showing that Ki67 may also be valuable in limited cytologic specimens (cell blocks). For mitotic count, as always, it is imperative to correct for one’s microscope’s field area which can vary
greatly, sometimes two folds or more. Many authors believe the cut-off of 5% may prove to be more valuable in the future than the 2% currently in use. More importantly, the 20% cut-off appears to lump two prognostically distinct groups into one category. It is becoming clear that those with > 55% Ki67 are much more aggressive and closer to true high-grade neuroendocrine carcinoma than those with Ki67 20-50%, which are highly proliferative version of ordinary WDNETs. We classify the former as high-grade neuroendocrine carcinomas, and the latter as WDNETs that are grade-3 by proliferation index.

F. Adjunct prognosticators: As in any other malignancy, perineural and vascular invasion are generally regarded as signs of aggressiveness, although their specific association with clinical outcome has not yet been established. They are nevertheless reported routinely. Although the prognostic value of necrosis has not yet been established, we also report it routinely as well.

Reporting of WDNETs at metastatic site
The diagnostic term to be used for a metastatic WDNET is problematic. In the current WHO-2010 classification, the word “carcinoma” has become synonymous with Grade-3 (high grade neuroendocrine carcinomas). Therefore, designating a metastatic WDNET as a “carcinoma” can be misleading. For this reason, we report the WDNETs as a NET, and make comment indicating that this is not a high-grade neuroendocrine carcinoma.

Another question that comes up is their diagnostic evaluation. Consensus is to perform Ki67 index and grading even in these metastatic tumors. In terms of determining the site of origin, this is often achieved with fair amount of success through careful clinical evaluation, octreotide scan (which has very high success rate), radiologic examination, serologic analysis. Immunohistochemical markers appear to be of limited value. Recent studies have shown that CDX2 is significantly more common in GI than pancreatic ones and isl-1 shows the opposite pattern. Additionally, Pax8 is commonly present in pancreatic and rectal, but seldom expressed in ileal ones. However, these markers ought to be used cautiously and should not be regarded as the sole determinant of the primary site.

IIB. HIGH-GRADE NEUROENDOCRINE CARCINOMAS

High-grade neuroendocrine carcinomas, defined with the same criteria employed for their pulmonary counterparts, are relatively rare but can occur in essentially any component of the GI tract from esophagus to distal colon. In the esophagus, they are more common than WDNETs of this site. Ampullary tumors seem to be more prone to have neuroendocrine component.

As in other organs, while some HG-NECs are akin to pulmonary small cell carcinomas, others are similar to large-cell NECs. Regardless, they are characterized (and also defined) by high mitotic activity and/or necrosis. Many HG-NECs are associated with conventional adenomas and/or ordinary adenocarcinomas, but occurrence of a WDNET component is exceedingly uncommon. There is emerging evidence that, as in other sites, the tumors with prominent neuroendocrine component are quite aggressive. In fact, it seems to be justified to recognize even a small HGNEC component in other carcinoma types. They appear to respond better to small cell chemotherapy protocols rather than the GI-adenocarcinoma protocols.
In the WHO-2010, HGNECs are unfortunately regarded synonymous as Grade-3 NETs (defined as Ki67 and mitosis > 20). However, it is becoming clear that a small subset of WDNETs (ordinary carcinoids or PanNETs) also show Ki67/mitosis above 20. Recent studies illustrate that it is those NET-3 cases with Ki67 > 55 % which typically also have the characteristic HGNEC morphology that behave as HGNECs, whereas, those with 20-55% Ki67 index are more characteristically of the ordinary WDNET morphology and have a corresponding intermediate prognosis between grade-2 WDNETs and true HGNECs.

Needless to say, HGNECs ought to be distinguished from other types of high grade malignancies (melanomas, basaloid carcinomas, medullary carcinomas, etc), for which, the criteria (and challenges and controversies) applicable in any other organ system are also valid here.

III. HYBRID TUMORS

Some of the endocrine lesions in the GI-tract exhibit a hybrid of neuroendocrine and non-endocrine elements.

The main example is the so-called MANECs, i.e., “mixed adenocarcinoma-neuroendocrine carcinomas”. In the vast majority of these the NE component is high-grade, and thus it may be appropriate to classify these as HGNECs with mixed adenocarcinoma component, since any HGNEC component seems to be significant, with the current data in hand.

There are few hybrid tumors in which the NE component is well-differentiated. Mixed acinar and neuroendocrine tumors of the pancreas is the prototype of this phenomenon. Scattered endocrine cells are present in 40 % of acinar cell carcinomas. “Mixed” is arbitrarily defined as those with at least 25% of the second tumor type. In these tumors, it is important to recognize the acinar component as it is the main determinant of biologic behavior.

Duodenal gangliocytic paraganglioma is a peculiar tumor of unknown origin that exhibits a mixture of: (a) ganglion-like cells (b) spindle cells of nerve sheath type, and (c) epithelioid (paraganglioma-like or carcinoid-like) elements. Carcinoid-like elements may predominate and lead to a mis-diagnosis. It is usually seen in late adulthood. The tumor is typically located in the peripancreatic duodenum. Although generally regarded as benign, some cases have lymph node metastasis, but even these cases have indolent behavior.

Chimeric tumor: Goblet cell carcinoid, conventional type
Goblet cell carcinoid (GCC) has been reported under different names including, adenocarcinoid, mucinous carcinoid, intermediate type of carcinoid, crypt cell carcinoma, amphicrine (endo-exocrine) neoplasia, composite tumor and microglandular carcinoma. Despite the confusion caused by its synonym “adenocarcinoid”, which had led for these tumors to be mistaken as mixed adenocarcinoma-neuroendocrine carcinoma, and consequently the false belief that this tumor type can occur elsewhere, it is now widely acknowledged that this tumor type is fairly specific to the appendix.

Although GCCs are classified under the generic category of “carcinoids” (or neuroendocrine neoplasia), it is becoming increasingly clear that they ought to be regarded somewhat separately from ordinary carcinoids (WDNETs). They appear to be amphicrine tumors, presumably arising from pluripotent stem cells with divergent neuroendocrine and goblet-cell
(mucinous) differentiation. The only justification to maintain the name GCC at this point is that they behave more like carcinoids, especially if they are low stage.

Mean age of the patients with GCCs is mid-50's (as opposed to mid-30's for classical carcinoids). Clinical diagnosis of GCC is seldom made preoperatively. Most of the patients present with signs and symptoms of an acute appendicitis due to luminal obstruction. The tumor cells proliferate sparsely and do not form nodules. The appendiceal wall thickens diffusely with fibrous proliferation, leading to contraction of the appendiceal lumen, which is the cause of the appendicitis. Interestingly, in most cases, there is no well-defined mass. The layers of the appendix are often preserved, with the tumor clusters often laying innocuously in an unaffected wall.

Classical GCC has a very distinctive morphology. The hallmark of this tumor is widely separated well-formed and round glandular units that are highly similar to colonic crypts, intervened by smooth muscle or stroma without any significant desmoplastic reaction. The glands are composed entirely of goblet-like cells with their nuclei strikingly well polarized at the periphery. Paneth cells can be seen. Rare examples have conventional (nested) WDNET component. Extension into the muscle and serosa is common, but the mucosa is characteristically spared, except for the areas of apparent connection between tumor nest and the base of the crypts. Diffuse infiltration into the peri-appendiceal fat and perineural invasion is seen in most cases.

Dual (amphicrine) phenotype of the classical GCC is evidenced both at ultrastructural and immunohistochemical levels, exhibiting both abundant intracellular mucin as well as neurosecretory granules which can be highlighted by chromogranin and synaptophysin stains.

The distinctive pathologic features of GCC are also translated into in its clinical behavior. In contrast with appendiceal carcinoids that metastasize in < 5% of cases, conventional GCCs metastasize in 15 to 30% of cases. The most common route of metastasis is through lymphatic vessels, trans-coelomic and intraperitoneal invasion whereas hematogenous metastasis to the liver or other distant organs is rare. The ovary is the most common target site of metastasis of GCC followed by abdominal carcinomatosis.

**Dedifferentiated GCC (adenoca ex-GCC)**

It is becoming increasingly clear that GCCs come to clinical attention in two highly different settings: 1) Tumors localized to the appendix, clinically presenting with appendicitis type picture. Recent literature indicates that such cases have fairly high cure rate and indolent behavior with protracted clinical course. 2) Advanced tumors presenting with “peritoneal carcinomatosis” type picture, typically involving peritoneal surfaces, ovaries and the uterus (occurring predominantly in females). Gynecologic pathology experts are typically exposed to the widely disseminated examples of this entity, and have published it under the heading of “ovarian metastases of appendiceal tumors with goblet cell carcinoid-like and signet ring cell patterns” and documented a highly aggressive behavior for this tumor type. In another study presented in abstract form these tumors were designated as “dedifferentiated” GCC and shown to have rapid abdominal intraabdominal dissemination, in particular to gynecologic organs, and mostly avoiding the liver. Tang et al have classified these tumors as “adenocarcinoma ex GCC” on the basis of the histologic features of the tumor at the primary site. This adenocarcinoma ex GCC group was further divided into signet ring cell and poorly differentiated adenocarcinoma types.
Regardless of the name, the common denominator to these tumors is that they exhibit, in addition to conventional GCC pattern, some areas with high-grade cytologic features and/or mixed patterns including cord-like infiltration, goblet (signet-ring) cells in cords or individual cells, non-mucinous microglandular pattern, ordinary intestinal pattern or extravasated mucin.

IV. OTHERS

Focal neuroendocrine differentiation can be seen in essentially any epithelial tumor of the GI tract. Scattered endocrine cells (chromogranin positive cells) are not uncommon. These are generally neglected unless they form more diffuse areas or are collaborated with histologic (H&E) findings such as nested growth pattern. There are no established guidelines (with proven clinical relevance) as to when these focal changes are to be recognized as significant and made into the diagnosis.

One peculiar phenomenon that also needs to be noted here is the emergence of neuroendocrine features subsequent to therapy in conventional adenocarcinomas. For example, rectal adenocarcinomas that receive neo-adjuvant chemo/radio therapy often show prominent neuroendocrine features including chromogranin positivity.

Along the same lines, in the setting of graft-versus-host disease or similar patterns of injury such as mycophenolate that leads to disappearance of glands, the remaining epithelium is often rich in neuroendocrine cells. In some cases these residual NE cells form cords and minute nests.

REFERENCES


25. Graham RP, Shrestha B, Caron BL, Smyrk TC, Grogg KL, Lloyd RV, Zhang L. Islet-1 is a sensitive but not entirely specific marker for pancreatic neuroendocrine


