General Features and Classification. Pancreatic neuroendocrine tumors (PanNETs) account for roughly 2-4% of clinically detected pancreatic neoplasms. Males and females are equally affected. PanNETs may arise at any age\(^1\), but most occur between the ages of 30 and 60 yrs; those arising in patients with multiple endocrine neoplasia 1 (MEN1) syndrome occur at a younger age.

The majority of neuroendocrine tumors are in the well differentiated category, in the sense that they retain the organoid architecture typical of neuroendocrine organs and have a relatively low proliferative rate (less than 20 mitoses per 10 high power fields). PanNETs measuring less than 0.5 cm are designated *neuroendocrine microadenomas* and are regarded to be biologically benign. Most clinically relevant neuroendocrine tumors fall in the category of *well differentiated PanNETs* and are low to intermediate grade malignancies. A small group of primary pancreatic neoplasms qualify as *poorly differentiated neuroendocrine carcinomas* due to their diffuse architecture, high proliferative rate, and abundant necrosis. Finally, there are rare mixed neuroendocrine neoplasms that contain elements with ductal or acinar differentiation (e.g., *mixed ductal neuroendocrine carcinoma*, *mixed acinar neuroendocrine carcinoma*).

The well differentiated PanNETs are also subclassified based on the presence and type of associated clinical syndrome due to inappropriate hormone secretion. PanNETs are designated as insulinoma, glucagonoma, gastrinoma, etc. if the patient exhibits characteristic clinical findings. PanNETs without a clinical syndrome are termed “nonfunctional”, although most of these PanNETs also exhibit some evidence of hormone production if serum peptide levels are measured or immunohistochemistry is employed to detect them\(^2\). A nonfunctional PanNET that is documented to produce a specific hormone may be designated based on the corresponding cell type (e.g., “\(\alpha\) cell neoplasm”, “\(\beta\) cell neoplasm”, etc.), but they should not be labeled as functional tumors in the absence of the appropriate clinical syndrome\(^3\). It should be noted that rare PanNETs have been described that exhibit expression of pancreatic polypeptide (PP) by immunohistochemistry, associated with elevations in serum PP levels\(^4\). Although these neoplasms have been designated “PPomas”, there is no specific clinical syndrome associated with PP hypersecretion, so PPomas are technically nonfunctional PanNETs. However, PP cell PanNETs are still designated as PPomas and classified with the functional group, largely for historical reasons. Approximately one third of all PanNETs are nonfunctional\(^3, 5\)\(^7\); in MEN1 patients with multiple PanNETs, a greater proportion of the individual tumors are nonfunctional\(^8\), but most MEN1 patients have at least one PanNET that is functional\(^3, 9\). Insulinomas are the most common functional tumors (45%), followed by gastrinomas (20%), glucagonomas (13%), VIPomas (10%), and somatostatinomas (5%). PanNETs producing other unusual syndromes (Cushing’s syndrome, carcinoid syndrome, acromegaly, etc.) occur but are rare.

PanNETs may be associated with a number of genetic syndromes, most importantly MEN1 and von Hippel-Lindau (VHL) syndromes\(^10, 11\). In these cases, the genetic abnormality underlying the syndrome plays a role in the development of the PanNETs, which often are...
The pathologic features of these PanNETs are generally similar to those occurring sporadically, although the PanNETs in VHL syndrome patients may have clear cell features.

**Pathologic Features.** Grossly, PanNETs are generally circumscribed, solid masses composed of tan, uniform, fleshy parenchyma. Larger PanNETs are multinodular, with fibrous septa dividing the neoplasm. Cystic change is a less common phenomenon, usually in the form of a single central locule lined by a thin rim of neoplastic cells. Most PanNETs have an expansile growth pattern, and small neoplasms may be completely surrounded by a fibrous capsule. It is common to find invasive growth, however, including extension into the adjacent pancreatic parenchyma or peripancreatic soft tissues.

Histologically, PanNETs have characteristic features of well differentiated neuroendocrine-type neoplasms at both the architectural and cytologic levels. Cells usually are arranged in regular nests, ribbons, or trabecula, and it is common for more than one pattern to be found in different regions of a single neoplasm. The nuclei are usually round to oval and uniform, although scattered enlarged nuclei are not uncommon. The chromatin is coarsely granular and clumped, imparting the classic “salt and pepper” appearance. Nucleoli may be inconspicuous, although some PanNETs have easily identifiable or even prominent nucleoli.

Although the architectural and cytologic features described above are key to recognizing PanNETs, there are many variations in histology that may cause diagnostic dilemmas. The stroma varies considerably in amount. Some PanNETs have nearly no collagen within the neoplasm, whereas other examples contain abundant, hyalinized or amyloid-like stroma. Calcifications may be found, including psammoma bodies. The quantity and appearance of the cytoplasm also varies. A moderate amount of amphophilic to basophilic cytoplasm is typical, but PanNETs with oncocytic cytoplasm or clear cell change have been described. Some PanNETs have scant cytoplasm, and the resulting high nucleus-to-cytoplasm ratio may cause confusion with small cell carcinomas or primitive neuroectodermal tumors. The nuclear morphology also may vary; PanNETs with marked nuclear pleomorphism have been reported (“pleomorphic PanNETs”), and these cases are commonly confused with ductal adenocarcinomas or other high grade neoplasms. In these instances, the nuclear atypia is not generally accompanied by an increased proliferative rate and does not appear to have adverse prognostic significance.

The mitotic rate is an important measure of aggressiveness in PanNETs. Well differentiated PanNETs are defined to have less than 20 mitotic figures per 10 high power fields (hpf); neoplasms with 20 or more mitoses per 10 hpf are considered poorly differentiated (high grade) neuroendocrine carcinomas (see below). In many PanNETs, mitotic figures are nearly undetectable, a search of 30 to 50 hpf (or more) being required to find even a single mitotic figure. Some PanNETs have a higher proliferative rate, and the finding of 2 or more mitoses per 10 hpf places a PanNET in a worse prognostic category. Necrosis is also variably present; most commonly it is accompanied an increase in proliferative rate and signifies a more aggressive PanNET.

Glands may also be found in PanNETs and may take several forms. In some cases, the neoplastic neuroendocrine cells form lumina. Although these gland-forming PanNETs may be mistaken for adenocarcinomas, the cells lining the lumina retain neuroendocrine differentiation. In other cases, non-neoplastic ductules are entrapped within PanNETs. The neoplastic neuroendocrine cells are often closely juxtaposed to the ductules, but they are cytologically distinct. Finally, true mixed ductal-neuroendocrine carcinomas occur (see Mixed Neuroendocrine Carcinomas, below).
Immunohistochemistry. Once the diagnosis of PanNET is suspected based on the histologic features, there are several methods to confirm the diagnosis. Immunohistochemical expression of general neuroendocrine markers including chromogranin, synaptophysin, and neural cell adhesion molecular (CD56) is detectable in essentially all PanNETs\(^\text{(23)}\). Synaptophysin expression is commonly more diffuse, and some PanNETs may demonstrate only focal staining for chromogranin. Expression of peptides such as insulin, glucagon, PP, somatostatin, gastrin, or vasoactive intestinal polypeptide is common, and most functional PanNETs can be shown to produce the appropriate peptide by immunohistochemistry\(^\text{(2)}\). In addition, minor cell populations producing a variety of other peptides are commonly detectable\(^\text{(24-26)}\). Nonfunctional PanNETs also contain a variety of peptide cell types, usually (but not inevitably) comprising less than 25% of the total cell population.

Many PanNETs also immunolabel for glycoproteins such as carcinoembryonic antigen (CEA) and CA19.9\(^\text{(20, 27, 28)}\), especially those with gland formation. Focal acinar differentiation may also be present in scattered cells that label for trypsin or chymotrypsin\(^\text{(28, 29)}\). A subset of PanNETs expresses CD99, as do normal islet cells. Labeling of PanNETs with the proliferation marker MIB1 demonstrates a relatively low proliferative rate in keeping with their low mitotic rate. Generally 1-5% of the nuclei are labeled, but some examples demonstrate labeling of up to 20% of cells\(^\text{(3)}\).

Cytologic Findings. Fine needle aspiration (FNA) is a sensitive technique for the preoperative diagnosis of PanNETs\(^\text{(30, 31)}\). Aspirates of PanNETs produce relatively cellular smears with a clean background. The cells are arranged in clusters and individually. Nuclei are round to oval and uniform, and the characteristic neuroendocrine chromatin pattern is often present. The nuclei are eccentrically located, producing a plasmacytoid configuration to the cells. Neuroendocrine differentiation may be documented by immunohistochemical labeling for chromogranin or synaptophysin to confirm the diagnosis\(^\text{(32)}\).

Molecular Genetic Features. Recent cytogenetic and molecular studies have identified many chromosomal alterations in PanNETs. Interestingly, activation of oncogenes does not appear to play a major role in the development of these tumors. In PanNETs arising in patients with MEN1 or VHL syndromes, the genetic defect responsible for the syndrome is involved in the pathogenesis of the pancreatic neoplasms\(^\text{(12, 13, 33)}\). PanNETs arising in MEN1 patients show a germ line mutation in the *menin* gene on chromosome 11q13 coupled with a somatic (acquired) loss of the normal copy of this gene. Studies on sporadic PanNETs have also detected relatively common losses at 11q13 or elsewhere on the short arm of chromosome 11 (70%), but specific *menin* gene mutations are only present in approximately 20% of the neoplasms, suggesting involvement of another tumor suppressor gene\(^\text{(34-37)}\). The VHL gene is usually normal in sporadic PanNETs\(^\text{(38)}\). Most of the genes targeted in the development of ductal adenocarcinomas of the pancreas are not targeted in PanNETs\(^\text{(39-42)}\). In particular, *KRAS*, *p53*, *p16*, and *SMAD4* are not mutated in most PanNETs, although the *p16* gene is inactivated via hypermethylation of the promotor in 40% of cases\(^\text{(43, 44)}\).

Some of the genetic alterations in PanNETs are more commonly detected in larger or higher stage neoplasms, suggesting that there is continuing genetic progression in PanNETs that parallels clinical progression. Fewer gains and losses of genetic material are seen in smaller PanNETs (less than 2 cm)\(^\text{(45)}\). In fact, some data suggest that smaller PanNETs may represent poly- or oligoclonal proliferations from which more aggressive monoclonal neoplasms may arise\(^\text{(46)}\).
Natural History and Prognostic Considerations. The natural history of PanNETs is highly variable. Small neoplasms without adverse prognostic features (see below) are readily curable by surgical resection. Many insulinomas fall into this category, since they generally measure less than 2 cm when detected. Most other functional and all nonfunctioning PanNETs are usually larger at diagnosis, and the outcome is much less favorable. Approximately 50-70% of these neoplasms will recur or metastasize, and up to 30% of patients already have metastatic disease at first presentation. The five year survival after surgical resection for nonfunctioning PanNETs is 65%, but the ten year survival is only 45%.

Despite the high rate of metastasis, relatively long survival is typical. Because the disease progresses slowly, many patients live for several years or even over a decade following the appearance of metastases. Unfortunately, metastatic PanNETs are relatively resistant to chemotherapy, and cure is unlikely after metastases develop.

One of the most controversial aspects of PanNETs is the predicting their clinical behavior. For many years, attempts were made to separate PanNETs into benign and malignant categories; recently, a borderline malignant potential category was proposed as well. Because some PanNETs that demonstrate malignant behavior have deceptively bland histologic features, it was felt that few pathologic parameters accurately stratify PanNETs, and only the finding of locally invasive growth, large vessel invasion, or distant metastases could be considered absolute criteria of malignancy. Even with these criteria, however, some “malignant” PanNETs do not recur after resection and some “benign” PanNETs lacking these features ultimately prove lethal. Several different grading schemes have been proposed for PanNETs. Based in part on the Capella classification of neuroendocrine neoplasms, the 2004 WHO classification separated PanNETs into two general groups: “well-differentiated endocrine tumors” and “well-differentiated endocrine carcinomas”. In this classification, well-differentiated endocrine tumors were confined to the pancreas (or have only local extension into peripancreatic tissues) whereas well-differentiated endocrine carcinomas had either gross local invasion or metastases. Within the well-differentiated endocrine tumor category, those PanNETs that measured less than 2 cm in diameter, had less than 2 mitoses per 10 hpf (or a Ki67 labeling index less than 2%), and demonstrated no perineural or vascular invasion were predicted to have “benign behavior”; those that either were greater than 2 cm in diameter, had 2-10 mitoses per 10 hpf (or have a Ki67 index greater than 2%), or had perineural or vascular invasion were considered to have “uncertain behavior”. This classification has proven complicated and confusing, and has several other shortcomings, including the lack of prognostic stratification for high stage tumors and the implication that the diagnosis should change (from “tumor” to “carcinoma”) when metastases develop. The newly developed 2010 WHO classification is much simpler. It is based on the European Neuroendocrine Tumor Society proposal for grading all gastroenteropancreatic neuroendocrine tumors, which separates the tumors into “well differentiated neuroendocrine tumors” and “poorly differentiated neuroendocrine carcinomas” using a mitotic rate cut point of 20 mitoses per 10 hpf, or a Ki67 labeling index of 20%. Within the well differentiated group, PanNETs are graded as G1 (<2 mitoses per 10 hpf and <3% Ki67) and G2 (2-20 mitoses per 10 hpf and 3-20% Ki67); the poorly differentiated neuroendocrine carcinomas are regarded as G3. An alternative system uses a different mitotic rate (<2 versus 2-50 mitoses per FIFTY hpf) and the presence of necrosis to separate low grade from intermediate grade PanNETs. In addition to this grade-based classification, TNM staging of PanNETs can now be performed using the same parameters applied for exocrine type carcinomas of the pancreas.

Other studies have focused on defining additional prognostic factors for resected PanNETs. As expected from the grading parameters mentioned above, general features of prognostic significance in PanNETs include tumor size, mitotic rate, presence of necrosis, extrapancreatic invasion, and vascular invasion, in addition to the presence of nodal or distant metastases.
metastases\textsuperscript{(3, 20, 52)}. Peptide production detected in the serum or by immunohistochemistry is not a prognostic factor for nonfunctional PanNETs\textsuperscript{(20)}. Nuclear pleomorphism is also not a useful predictor\textsuperscript{(19)}; however, some studies have demonstrated a correlation between overall nuclear grade and prognosis\textsuperscript{(20)}. Other factors reportedly predictive of more aggressive behavior include loss of progesterone receptor expression\textsuperscript{(53, 54)}, aneuploidy\textsuperscript{(55)}, increased Ki67 or PCNA labeling index\textsuperscript{(56)}, loss of heterozygosity (LOH) of chromosome 17p13\textsuperscript{(8)}, LOH of chromosome 22q\textsuperscript{(57)}, increased fractional allelic loss\textsuperscript{(58)}, upregulated CD44 isofrom expression\textsuperscript{(59)}, and immunohistochemical expression of cytokeratin 19\textsuperscript{(60)}.

**Differential Diagnosis.** The pathologic differential diagnosis for PanNETs includes acinar cell carcinoma, pancreatoblastoma, solid-pseudopapillary neoplasm, and ductal adenocarcinoma. Acinar cell carcinoma and pancreatoblastoma exhibit acinar differentiation and demonstrate well-formed acinar structures and granular, eosinophilic cytoplasm\textsuperscript{(61, 62)}. Pancreatoblastomas also have distinctive squamoid nests and a hypercellular stromal component. Both acinar cell carcinoma and pancreatoblastoma consistently produce pancreatic exocrine enzymes and can be distinguished from PanNETs by immunohistochemical labeling for trypsin and chymotrypsin, which are usually diffusely expressed. However, both acinar cell carcinoma and pancreatoblastoma may also contain a minor component of neuroendocrine cells, so focal labeling for chromogranin and synaptophysin may be found. Solid-pseudopapillary neoplasms have many histologic similarities with PanNETs but can be distinguished by the presence of pseudopapillae, nuclear grooves, aggregates of foamy tumor cells and histocytes, and collections of large hyaline globules\textsuperscript{(63)}. By immunohistochemistry, solid-pseudopapillary neoplasms do express CD56 and often also synaptophysin, but they are never positive for chromogranin. The hyaline globules of solid-pseudopapillary tumors stain for alpha-1-antitrypsin, and there is consistent positivity for CD10 and nuclear accumulation of β-catenin. Solid-pseudopapillary neoplasms express vimentin but are negative or only focally positive for keratin. Pancreatic ductal adenocarcinomas generally are not difficult to distinguish from PanNETs, with the exception of PanNETs that exhibit gland formation. Even in such PanNETs, the glands are found within larger nests of cells, in contrast to the individual infiltrating glands of ductal adenocarcinomas, and intracellular mucin is not present. Ductal adenocarcinomas usually also have a higher mitotic rate and more significant nuclear pleomorphism.

**High Grade Neuroendocrine Carcinoma.** High grade neuroendocrine carcinomas are extremely rare in the pancreas. These neoplasms are related to small cell carcinomas\textsuperscript{(64, 65)}, and a metastasis from sites such as the lung has to be excluded before an example can be accepted as primary in the pancreas. Most patients are older adults. The neoplasms are often large and metastatic at diagnosis, so resected examples are few. Histologically, high grade neuroendocrine carcinomas may be composed of either small or large cells. The neoplastic cells grow in diffuse sheets and have a markedly infiltrative growth pattern. There is often little nesting or other architectural patterns. The principle feature that separates this group from well differentiated PanNETs is the proliferative rate. More than 20 mitoses per 10 hpf should be found according to the new WHO classification, and often the rate is 50 or more. In addition, there is abundant necrosis. A diagnosis of small cell carcinoma may be rendered for a high grade neuroendocrine carcinoma when there are predominantly cells with minimal cytoplasm and fusiform nuclei with a granular chromatin pattern and inconspicuous nucleoli. In other high grade neuroendocrine carcinomas the cells are larger, with moderate amounts of cytoplasm, the nuclei are round, and nucleoli are prominent. These large cell neuroendocrine carcinomas must be distinguished from poorly differentiated carcinomas lacking neuroendocrine differentiation, so
immunohistochemical staining for chromogranin or synaptophysin must be performed to confirm the diagnosis. High grade neuroendocrine carcinomas of the pancreas are highly aggressive neoplasms, with a natural history equal to or more virulent than that of ductal adenocarcinomas.

**Mixed Neuroendocrine Carcinomas.** Minor neuroendocrine elements are relatively common in predominantly exocrine pancreatic neoplasms. Thus, it should not be surprising that rare neoplasms exist in which both neuroendocrine and exocrine components are significantly represented. These “mixed” neoplasms have been arbitrarily defined to contain more than 25% of each component, and neuroendocrine, acinar, and ductal lines of differentiation may all be represented. Reported mixed neoplasms that contain a neuroendocrine component include mixed ductal-neuroendocrine carcinoma, mixed acinar-neuroendocrine carcinoma, and mixed acinar-neuroendocrine-ductal carcinoma (66-70). In most reported examples, the exocrine elements predominate. The different cell types are usually intimately intermixed, and immunohistochemical labeling is often necessary to detect the various lines of differentiation. It should be noted that ductal differentiation in the form of lumen formation or expression of glycoproteins such as Ca19.9 is common in conventional PanNETs (32) and does not constitute sufficient evidence for a diagnosis of mixed ductal-neuroendocrine carcinoma. Rather, a separate distinct gland-forming component with intracellular mucin production should be found combined with the neuroendocrine elements. Most reported mixed pancreatic neoplasms have demonstrated aggressive clinical behavior, paralleling that of the more aggressive exocrine component.

**References**


57. Wild A, Langer P, Celik I, Chaloupka B, Bartsch DK. Chromosome 22q in pancreatic endocrine tumors: identification of a homozygous deletion and


SAM Questions.

1. Which of the following statements about pancreatic neuroendocrine tumors is CORRECT?
   a. **Prognosis depends on the proliferative rate of the tumor.**
   b. Oncocytic cytoplasm is characteristic of those occurring in patients with von Hippel Lindau syndrome.
   c. The architectural pattern of the tumor is the most important factor predicting outcome.
   d. Functioning tumors are usually benign.
   e. No staging system exists for pancreatic neuroendocrine tumors.

   The proliferative rate is a very strong predictor of prognosis in pancreatic neuroendocrine tumors. Clear cell change is reported in cases affecting von Hippel-Lindau patients, not oncocytic cytoplasm. The architectural pattern is not related to prognosis. Most functioning tumors are insulinomas, which are usually very indolent and curable by resection, but no neuroendocrine tumors larger than 0.5 cm are considered benign, and all other types of functioning tumors are equally aggressive as nonfunctioning neuroendocrine tumors. The AJCC now includes neuroendocrine tumors in the same TNM staging system used for other pancreatic neoplasms.

2. Which of the following immunohistochemical markers, when positive, favors a diagnosis of pancreatic neuroendocrine tumor rather than solid pseudopapillary neoplasm?
   a. Synaptophysin.
   b. Chymotrypsin.
   c. **Chromogranin.**
   d. CD56.
   e. Cam5.2.

   Most PanNETs express chromogranin, synaptophysin, CD56, and Cam5.2 by immunohistochemistry. Solid pseudopapillary neoplasms can also express synaptophysin, CD56, and Cam5.2, but they never express chromogranin. Neither expresses chymotrypsin.

3. Which of the following statements factors in pancreatic neuroendocrine tumors confers an adverse prognosis?
   a. Immunoreexpression of glucagon.
   b. Serum elevation of insulin levels.
   c. Nuclear pleomorphism.
   d. **Immunoreexpression of CK19.**
   e. Clear cytoplasm.

   The stage and grade of PanNETs are the most important prognostic factors. Immunoreexpression of CK19 has been correlated with a poorer prognosis in several studies. No specific peptide staining or serum elevations are strong predictors of outcome, although insulinomas generally have a favorable prognosis, probably because they are detected while at an early stage. Neither nuclear pleomorphism nor clear cell change are prognostically relevant.