# Neuroendocrine Neoplasms of the Gastrointestinal Tract

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## List of Frequently Asked Questions



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**1. What are neuroendocrine neoplasms?**

With the exception of paragangliomas, neuroendocrine neoplasms are generally epithelial tumors with neuroendocrine differentiation. This category encompasses two discrete neoplasms: well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs). They can arise from numerous sites in the body, including the respiratory system, the pancreas, and the luminal gastrointestinal tract.

**2. How are neuroendocrine tumors (NETs) defined, and how are they diagnosed?**

NETs are well-differentiated neuroendocrine neoplasms, composed of cells with features similar to those of normal neuroendocrine cells. They are uncommon tumors, accounting for less than 1% of gastrointestinal malignancies, though they are increasing in incidence and prevalence. They are typically arranged in nests or trabecula, with rarer architectural patterns including broad sheets or pseudoglandular structures. The tumor cells have uniform cytological features, with moderate eosinophilic granular cytoplasm and round-to-ovoid nuclei with smooth nuclear membranes, finely granular (so-called salt-and-pepper) chromatin, and indistinct nucleoli.

Chromogranin A and synaptophysin are immunohistochemical markers of neuroendocrine differentiation that can be used to confirm a diagnosis of NET, though if the morphological features are classical, they are not needed. Chromogranin A is the most specific marker for neuroendocrine differentiation but is not very sensitive. Rates of positivity vary based on anatomical location, with the highest expression at over 80% in NETs of the tubular gastrointestinal tract proximal to the colon, and somewhat lower sensitivities in the colon and rectum, in the range of 40–60%. Synaptophysin is less specific for NETs, as its expression can be seen in other tumors, such as glomus tumors.

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Although the histological features of NETs are similar at all sites throughout the body, the clinical features, tumor biology, and prognosis of NETs are site-specific. Depending on their cell of origin, NETs may secrete bioactive amine or peptide hormones that can cause clinical symptoms and syndromes. Although typically indolent compared to carcinomas, they frequently present at an advanced stage of disease. NETs have historically been referred to as "carcinoid tumors," though this term is technically only applicable to a subset of NETs and may lead to confusion and so is now discouraged. They have also historically been divided embryologically into foregut (gastric, pulmonary, duodenal, and pancreatic) tumors, midgut (jejunal, ileal, appendiceal, and cecal) tumors, and hindgut (colonic and rectal) tumors, as proposed by Williams and Sandler in 1963. Tumors grouped by this classification share some clinical and histological features. Midgut NETs have a propensity for nested architecture and brightly eosinophilic cytoplasmic granules and are frequently associated with the carcinoid syndrome. On the other hand, foregut and hindgut NETs commonly show trabecular architecture. This classification also correlates well with differential expression of transcription factors, which can be used to determine the site of origin of metastatic NETs of unknown primary.
References: [1–4]

### 3. How are neuroendocrine carcinomas (NECs) defined, and how are they diagnosed?

NECs are poorly differentiated, high-grade malignant neoplasms. They are classically divided into two types, small cell carcinoma (SCC) and large cell neuroendocrine carcinoma (LCNEC), based on their morphological features, though they frequently show mixed features. In SCC, cells are arranged in a diffuse, sheet-like pattern, though organoid or rosette-like arrangements may focally be present. The cells are small to intermediate sized with scant cytoplasm, though a minority of cells is allowed to have more moderate cytoplasm. They have a high nucleus-to-cytoplasm ratio with round to fusiform nuclei. Confluent necrosis, numerous apoptotic cells, and abundant mitotic figures are present (Fig. 17.1). If the morphological pattern is classical and easily discernible, confirmatory immunohistochemical staining may not be necessary.

LCNECs have variable growth patterns, including diffuse, nested, or trabecular patterns. They are composed of large polygonal tumor cells with abundant eosinophilic cytoplasm, coarse or vesicular chromatin, and prominent nucleoli. Given their similar cytomorphological appearance to other carcinomas, evidence of glandular or squamous differentiation must be excluded, and immunohistochemical confirmation with two markers of neuroendocrine differentiation (synaptophysin and chromogranin A) is recommended.

In one study, gastrointestinal NECs arising from squamous mucosa (esophagus and anus) were more frequently SCC, while those arising from glandular mucosa were more likely to be LCNEC or showed mixed morphological features.
Reference: [5]

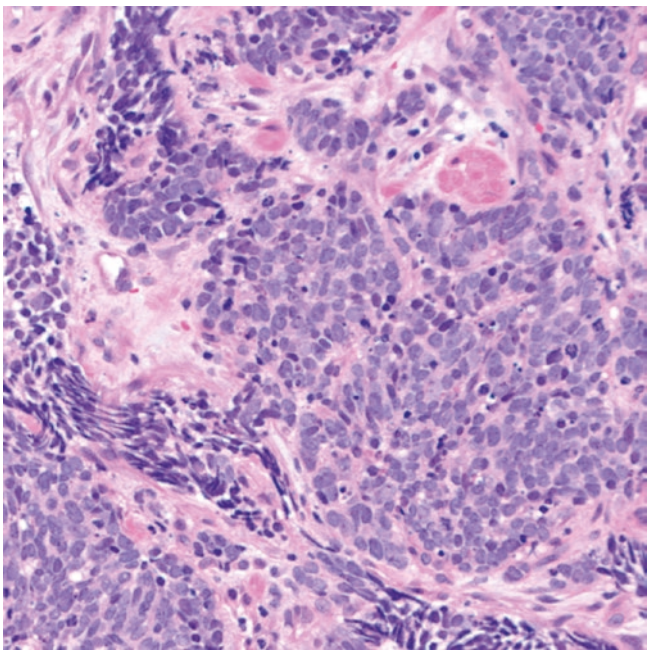

**Fig. 17.1** Small cell carcinoma of the rectum. Tumor cells show fine nuclear chromatin, inconspicuous nucleoli, nuclear molding, readily identifiable mitotic figures, and abundant apoptotic cells

### 4. How can NETs be differentiated from NECs?

Distinguishing high grade (G3) NETs from NECs is primarily accomplished by recognizing the classical morphological patterns, as described in the preceding sections. However, in certain situations, this differentiation may be difficult, such as when an NET demonstrates a more prominent infiltrative or sheet-like growth pattern than usual or contains foci of necrosis, leading to the tumor potentially being mistaken for a NEC.

Small biopsies or biopsies with extensive crush artifact can also be problematic. Though difficult to interpret, Ki-67 immunohistochemistry can be useful for making the distinction in these circumstances, as NECs typically show a Ki-67 proliferative index of >50% whereas G3 NETs are less likely. However, borderline cases do exist because NECs may occasionally show a Ki-67 index of 20–50%. Thus, Ki-67 cannot be reliably used to distinguish a NEC from a G3 NET. Immunohistochemical staining for somatostatin receptor may also be helpful, as NETs typically express somatostatin receptor, while NECs show lower expression or lack it entirely. In addition, NECs may show loss of RB expression or aberrant p53 expression (either overexpression or complete absence) due to mutations of these genes, which may aid in the differential diagnosis. In some instances, definite determination between NET and NEC may be impossible, especially on core biopsy: the

clinical course ultimately determines the nature of a neuroendocrine neoplasm.
References: [6, 7]

## 5. How are NETs graded?

Until recently, grading schemes, including the World Health Organization (WHO) 2010 classification, divided neuroendocrine neoplasms into three grades (G1, G2, and G3) based on the mitotic count and Ki-67 index, in which NECs composed the G3 category. In 2017, after multiple published studies demonstrated different behaviors of pancreatic neuroendocrine neoplasms with high proliferative activity in combination with the degree of differentiation, a new scheme was adopted that uniformly applied to all gastrointestinal neuroendocrine neoplasms (Table 17.1). In this system, NETs are graded as G1, G2, and G3, whereas NEC are considered high grade, by definition.

**Table 17.1** Summary of grading of neuroendocrine tumors at all sites of the gastrointestinal tract

| Grade | Mitotic count (per 2 mm$^2$)[a] | Ki-67 proliferative index (%)[b] |
|---|---|---|
| Grade 1 (low grade) | <2 | <3 |
| Grade 2 (intermediate grade) | 2–20 | 3–20 |
| Grade 3 (high grade) | >20 | >20% |

[a]At least 10 mm$^2$ should be counted in most mitotically active areas. Only clearly identifiable mitotic figures should be counted. The number of high-power fields (40×) needed for 10 mm$^2$ depends on microscopes used. For example, 42 high-power fields need to be counted for a microscope with a field diameter of 0.55 mm at 40×
[b]A minimum of 500 tumor cells needs to be counted

Once the diagnosis of NET is established based on the morphological features previously outlined, grade is assigned based on the mitotic activity and Ki-67 proliferative index (Fig. 17.2), which is considered the most reliable predictor of prognosis. The mitotic count is assessed over 10 mm$^2$ of contiguous high-power fields and averaged to express the number of mitoses per 2 mm$^2$. The Ki-67 proliferative index is calculated by counting 500–2000 cells with all stained nuclei of any intensity and any pattern counted as positive. Both the mitotic count and Ki-67 proliferative index should be evaluated in "hot spot" regions with the highest proliferative activity. In the event that the mitotic rate and Ki-67 pro-

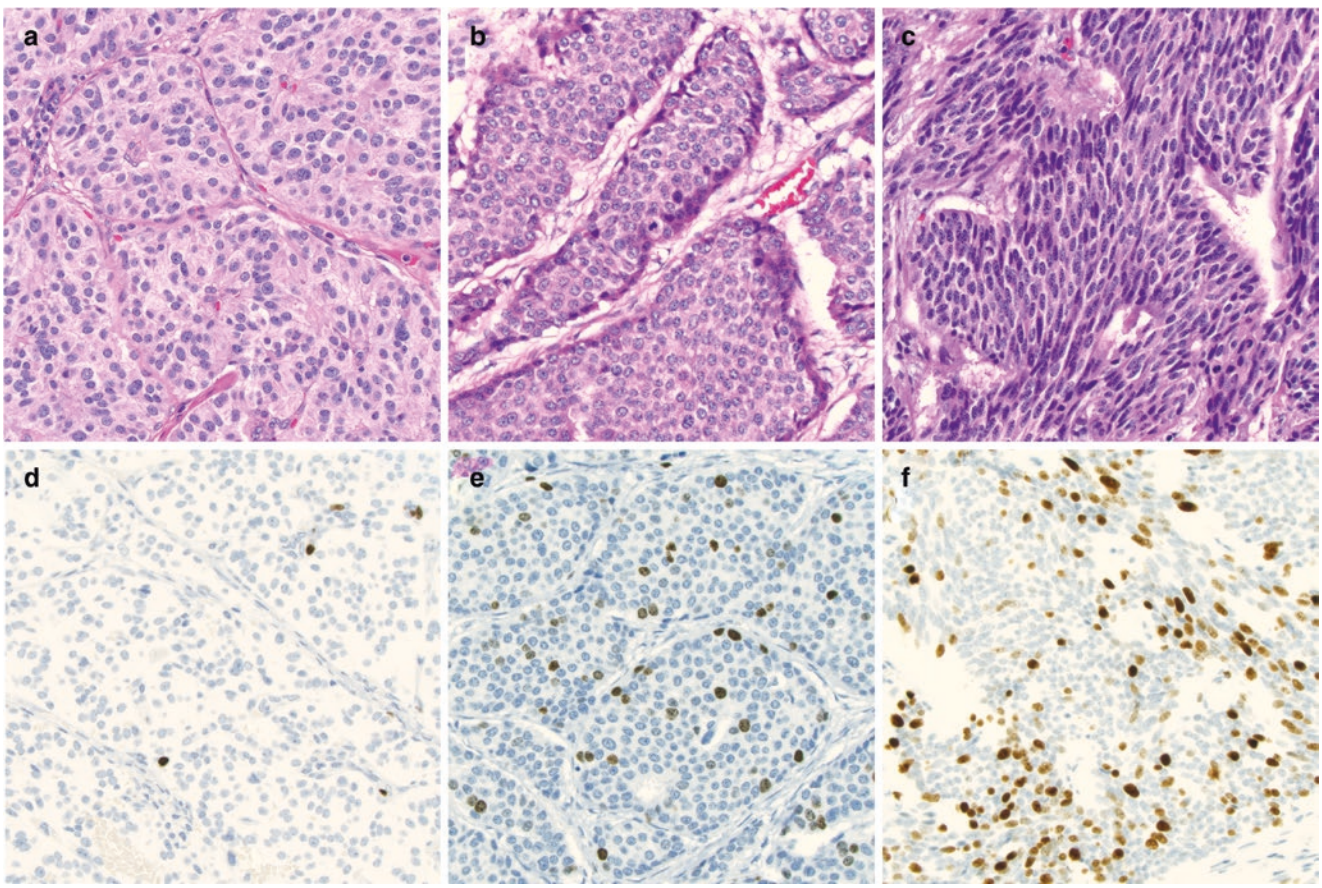


**Fig. 17.2** Grading neuroendocrine tumors (NETs). (**a**) A grade 1 duodenal NET with no mitotic figures in a representative high-power field. (**b**) A grade 2 ileal NET with a mitotic figure in this representative high-power field. (**c**) A grade 3 small intestinal NET with multiple mitotic figures in this representative high-power field. (**d**) The grade 1 NET from **a** showing a Ki-67 proliferative index of <1%. (**e**) A representative high-power field of the grade 2 NET from **b** showing a Ki-67 proliferative index of 5–10%. (**f**) A representative high-power field of the grade 3 NET from **c** showing a Ki-67 proliferative index of >20%

liferative index lead to different grade assignments, the higher of the two is considered the final grade.

For assessment of the Ki-67 proliferative index, it was thought until recently that an overall "eyeball" assessment was acceptable. However, this gestalt visual inspection method has been shown to have very low accuracy, especially when considering borderline cases. In a recent comparison of four different counting methods including eyeballing, a visual cell count through a microscope, a manual count of a camera-captured printed image, and an automated cell count via a digital image analysis system, the manual cell count on a printed image was found to have the highest accuracy and lowest interobserver variability, making it now the preferred method for evaluating the Ki-67 proliferative rate. Ki-67 immunostaining also highlights inter- and intratumoral heterogeneity in primary and metastatic tumors and throughout the course of disease. Therefore, it is recommended to perform Ki-67 immunostaining not only on primary NETs but also on metastases. It has also been shown that Ki-67 staining on NET core biopsies provides a reliable proliferative index for prognostication of NET metastasis to the liver.
References: [8–11]

**6. How are neuroendocrine neoplasms staged?**

Staging of NETs varies by site throughout the gastrointestinal tract. In general, both size and depth of invasion play a part in the primary tumor stage. See Table 17.2 for a detailed explanation of staging for primary NETs at each primary site in the gastrointestinal tract. NECs are staged using the staging systems for other primary carcinomas at each primary site of the gastrointestinal tract and do not use the staging systems for NETs.

**7. What are differential diagnostic considerations for neuroendocrine neoplasms?**

NETs have a fairly characteristic appearance. Other tumors with trabecular or organoid arrangement and bland nuclei may be encountered in the gastrointestinal tract, though they are exceedingly rare. Metastatic polygonal cell tumors, such as hepatocellular carcinoma, adrenocortical carcinoma, or renal cell carcinoma, typically have greater nuclear irregularities, though a history of these neoplasms and immunohistochemistry are helpful. Glomus tumors very rarely involve the gastrointestinal tract (mostly in the stomach) and have similar trabecular architecture, though not typically the same densely collagenized stroma. If an NET has a prominent pseudoglandular pattern, it may be mistaken for adenocarcinoma, though adenocarcinoma usually has greater nuclear atypia.

The differential for NECs is broad. Sarcomas of the tubular gastrointestinal tract should be considered, particularly including gastrointestinal stromal tumor and malignant gastrointestinal neuroectodermal tumors in this location. Poorly cohesive LCNEC may be mistaken for large cell lymphomas. Broad-spectrum keratins may be useful to exclude these diagnoses, particularly on small biopsies.

Before diagnosing NEC, other poorly differentiated carcinomas, such as poorly differentiated squamous cell carcinoma or poorly differentiated adenocarcinoma, must be excluded. The presence of more than occasional intracytoplasmic mucin and significant nuclear pleomorphism/atypia favors the diagnosis of adenocarcinoma or mixed adenoneuroendocrine carcinoma (see below).

**8. Are there any site-specific markers for gastrointestinal NETs that can be used to identify the site of origin of neuroendocrine neoplasms of unknown primary?**

Some immunohistochemical markers may aid in the identification of the primary site of metastatic NETs of unknown origin. Most of these antibodies are directed against transcription factors and are most helpful for identifying the broad embryological categories of primary tumors as outlined by Williams and Sandler.

Midgut NETs (those of the jejunum, ileum, cecum, and appendix) are typically strongly positive for homeobox protein CDX2. They are usually negative for transcription termination factor 1 (TTF1), insulin gene enhancer protein islet-1 (ISL1), and paired box proteins 6 and 8 (PAX6 and PAX8).

Hindgut NETs (mainly composed of rectal NETs, though the rare colonic NETs are included) are typically strongly and diffusely positive for special AT-rich sequence-binding protein 2 (SATB2). However, the majority of appendiceal NETs show similarly strong and diffuse SATB2 positivity. Jejunal and ileal NETs may show SATB2 positivity, though staining is typically weak and patchy. Rectal NETs may also show strong positivity for CDX2, like midgut NETs.

Duodenal and rectal NETs have a unique profile among gastrointestinal NETs that is similar to pancreatic NETs. PAX6, PAX8 (polyclonal), and ISL1 are typically positive in duodenal and rectal NETs.

Immunohistochemical stains are not useful for determining the site of origin of NECs. Gastrointestinal tract primary NECs may show TTF1 positivity, and CDX2 may be positive, regardless of the site of origin.
References: [4, 12–15]

**9. What is the utility of immunohistochemistry for hormone products in diagnosing NETs?**

While the secretory products of normal neuroendocrine cells distributed throughout the gastrointestinal tract are well known, correlation of immunohistochemical staining and serum measurements is less precise. Many NETs show ectopic hormone secretion and may show immunohistochemical positivity for multiple hormones, though only one may be detected in serum, or the NET may be entirely nonfunctional. Immunohistochemical staining may also rarely be negative for products that have been measured to be elevated in serum, which may be due to abnormal proteins not recognized by the antibodies. Immunohistochemical staining for hormone products may be useful in select circumstances, such as con-

**Table 17.2** Summary of staging of neuroendocrine tumors at different sites of the gastrointestinal tract

| Stage | Stomach | Duodenum | Ampulla | Jejunum and ileum | Appendix | Colon and rectum |
|---|---|---|---|---|---|---|
| pT1 | Invades the lamina propria or submucosa and ≤1 cm in size | Invades the lamina propria or submucosa and ≤1 cm in size | Confined to sphincter of Oddi and ≤1 cm in size | Invades the lamina propria or submucosa and ≤1 cm in size | ≤2 cm in size | Invades lamina propria or submucosa and pT1a: <1 cm in size pT1b: 1–2 cm in size |
| pT2 | Invades muscularis propria or >1 cm in size | Invades muscularis propria or >1 cm in size | Invades duodenal submucosa or muscularis propria or >1 cm in size | Invades muscularis propria or >1 cm in size | >2 cm and ≤4 cm in size | Invades muscularis propria or >2 cm |
| pT3 | Invades subserosal tissue | Invades pancreas or peripancreatic tissue | Invades pancreas or peripancreatic tissue | Invades subserosal tissue | >4 cm or Invades subserosal tissue or Invades mesoappendix | Invades subserosal tissue |
| pT4 | Invades serosa/ visceral peritoneum or Invades adjacent organs or structures | Invades serosa/ visceral peritoneum or Invades adjacent organs or structures | Invades serosa/ visceral peritoneum or Invades adjacent organs or structures | Invades serosa/ visceral peritoneum or Invades adjacent organs or structures | Invades serosa/ visceral peritoneum or Invades adjacent organs or structures | Invades serosa/ visceral peritoneum or Invades adjacent organs or structures |
| pN1 | Regional lymph node metastases | Regional lymph node metastases | Regional lymph node metastases | Regional lymph node metastases in <12 lymph nodes | Regional lymph node metastases | Regional lymph node metastases |
| pN2 | Not applicable | Not applicable | Not applicable | Regional lymph node metastases in ≥12 lymph or Mesenteric masses (>2 cm in size) | Not applicable | Not applicable |
| pM1a | Metastasis confined to liver | Metastasis confined to liver | Metastasis confined to liver | Metastasis confined to liver | Metastasis confined to liver | Metastasis confined to liver |
| pM1b | Metastasis to ≥1 extra-hepatic site | Metastasis to ≥1 extra-hepatic site | Metastasis to ≥1 extra-hepatic site | Metastasis to ≥1 extra-hepatic site | Metastasis to ≥1 extra-hepatic site | Metastasis to ≥1 extra-hepatic site |
| pM1c | Metastasis to liver and Metastasis to ≥1 extra-hepatic site | Metastasis to liver and Metastasis to ≥1 extra-hepatic site | Metastasis to liver and Metastasis to ≥1 extra-hepatic site | Metastasis to liver and Metastasis to ≥1 extra-hepatic site | Metastasis to liver and Metastasis to ≥1 extra-hepatic site | Metastasis to liver and Metastasis to ≥1 extra-hepatic site |

Adapted from Amin et al.

firming that an NET is the source of hormone elevations or as an aid for diagnosis of uncommon NET variants (e.g., using somatostatin immunostaining to confirm the diagnosis of a duodenal somatostatinoma).
Reference: [16]

### 10. What are mixed adenoneuroendocrine carcinomas?

Mixed adenoneuroendocrine carcinomas (MANECs) are tumors composed of both morphologically recognizable adenocarcinoma and NEC components. Each component must account for at least 30% of the neoplasm as a whole, as defined by the WHO in 2010. La Rosa and coauthors have recently proposed changing this terminology to "mixed neuroendocrine-nonneuroendocrine neoplasms" (MiNENs) to more completely encompass this heterogeneous group. The MiNEN category incorporates all grades of neuroendocrine neoplasms (both NET and NEC) combined with different subtypes of carcinoma, including adenocarcinoma-NEC, squamous cell carcinoma-NEC, and adenocarcinoma-NET. MiNEN is the currently recommended terminology by WHO. Again, to qualify the diagnosis, both neuroendocrine and nonneuroendocrine components should be morphologically and immunohistochemically recognizable and each constitutes ≥30% of the neoplasm. Preinvasive precursor lesions, such as adenoma, should not be considered part of MiNEN.

While MANECs are rare as a whole, at least one-third of NECs will show some component of adenocarcinoma after thorough sampling. The NEC component of mixed tumors is

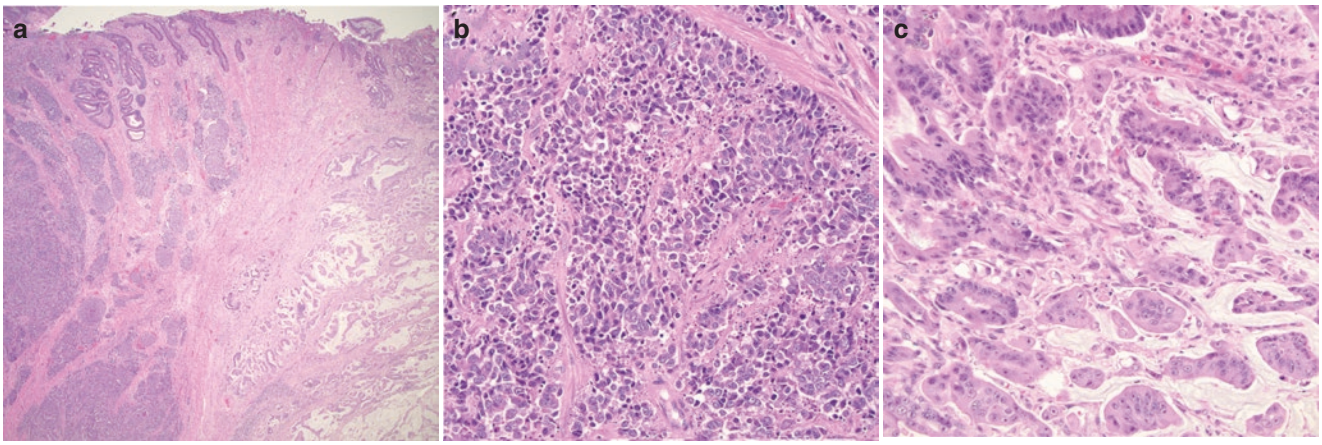


**Fig. 17.3** Mixed adenoneuroendocrine carcinoma (MANEC). (**a**) A tumor in the right colon composed of a high-grade neuroendocrine carcinoma (NEC) component (left) and conventional and mucinous adenocarcinoma component (right) admixed and in close proximity, diagnostic of MANEC. (**b**) The NEC component shows high nucleus-to-cytoplasm ratios, markedly irregular nuclear contours, variably prominent nucleoli, numerous mitotic figures, and patchy necrosis. (**c**) The adenocarcinoma component shows infiltrative glands and cell clusters floating in mucin pools

often present in the deeper portions of the tumor, and sampling bias is frequent in biopsy samples. The NEC component of MANEC is more frequently of LCNEC or mixed morphology than SCC alone (Fig. 17.3). While the NEC component is most likely to metastasize to lymph nodes and the liver, both components may be present, with adenocarcinoma alone being the metastatic component in a very small subset of cases.

Although, by definition, at least 30% of both components are required to be labeled as a MANEC, smaller components of NEC may drive the prognosis. In such cases, it is advisable to diagnose adenocarcinoma with a focal NEC component and state the percentage of the NEC component. In adenocarcinoma or other types of carcinoma without morphological features of neuroendocrine differentiation, patchy expression of neuroendocrine markers may be seen that is of unclear prognostic significance. These lesions should not be diagnosed as MANEC.
References: [5, 17–20]

### 11. What is gastric neuroendocrine cell hyperplasia, and how is it differentiated from gastric NET?

Neuroendocrine cell hyperplasia refers to enterochromaffin-like (ECL) cell proliferation in the stomach, especially occurring in a background of autoimmune (atrophic) gastritis. ECL cells are predominantly present in the gastric body and fundus. They are seen at the bases of the gastric pits, though scattered cells can be present in the neck region. Under normal physiological conditions, gastrin secreted by antral G cells stimulates the ECL cells to release histamine, which in turn stimulates parietal cells to secrete hydrochloric acid. In autoimmune gastritis, anti-parietal cell or anti-intrinsic factor antibodies lead to widespread destruction of parietal cells and subsequently decreased hydrochloric acid production (hypochlorhydria or achlorhydria). This loss of hydrochloric acid removes critical negative feedback on antral G cells, leading to hypergastrinemia, which in turn leads to ECL cell proliferation.

The spectrum of ECL proliferation ranges from simple hyperplasia to gastric NETs, as described by a landmark publication from Solcia and coauthors in 1988. Linear ECL cell hyperplasia is defined as at least two groups of five or more adjacent neuroendocrine cells lining a gastric pit per millimeter. This finding is not easily appreciated on routine hematoxylin and eosin staining and is best visualized with immunohistochemical (chromogranin) staining. Micronodular ECL cell hyperplasia consists of clusters of five or more neuroendocrine cells, bounded by a basement membrane, measuring <150 μm (or less than the diameter of a gastric pit). These clusters may be grouped or scattered throughout the mucosa. When five or more of these clusters aggregate, it is termed adenomatoid hyperplasia. Dysplasia occurs when these micronodules fuse with concomitant loss of basement membrane or an individual micronodule becomes >150 μm. An intramucosal aggregation of ≥500 μm or new stroma formation signifies microinvasion, and these lesions are designated as micro-NETs. These lesions are frequently undetected at endoscopy. Larger lesions (≥5 mm) or lesions of any size that invade the muscularis mucosae or submucosa are invasive NETs.
Reference: [21]

### 12. How are gastric NETs classified?

Gastric NETs are subclassified into four subtypes. Each type has a different mechanism of tumorigenesis, different clinical behavior, and different management implications (see Table 17.3 for a summary).

The majority (70–80%) of gastric NETs are type 1 NETs. These are derived from the ECL cells of the gastric body and are associated with autoimmune gastritis, in which ECL cell hyperplasia leads to dysplasia and to NET development (Fig. 17.4). The diagnosis of type 1 NETs is made by identifying the histological features of NET in background of atro-

**Table 17.3** Gastric neuroendocrine tumor types

| | Type 1 | Type 2 | Type 3 | Type 4 |
|---|---|---|---|---|
| Associated disorder | Autoimmune (atrophic) gastritis | Zollinger-Ellison syndrome, MEN1 | None | Dysfunctional parietal cells |
| Background mucosa | ECL cell hyperplasia, parietal cell atrophy | ECL cell hyperplasia, parietal cell hyperplasia | Normal | ECL cell hyperplasia, parietal cell hyperplasia |
| Site | Body, fundus | Body, fundus | Throughout the stomach | Body, fundus |
| Serum gastrin | Elevated | Elevated | Normal | Elevated |
| Hydrochloric acid secretion | Achlorhydria | Hyperchlorhydria | Normal | Achlorhydria |

*MEN1* multiple endocrine neoplasia type 1, *ECL* enterochromaffin-like

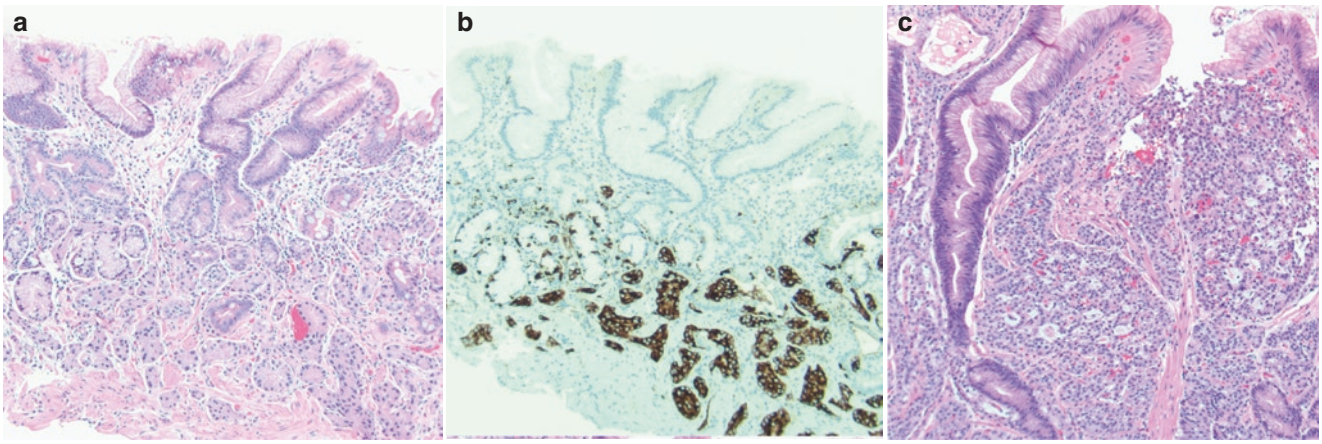


**Fig. 17.4** Type 1 gastric neuroendocrine tumor arising in a background of autoimmune (atrophic) gastritis. (**a**) Gastric body mucosa showing a lymphoplasmacytic infiltrate, parietal cell atrophy, pyloric metaplasia, and intestinal metaplasia, all typical features of autoimmune (atrophic) gastritis. (**b**) Chromogranin A immunostain highlights neuroendocrine cell dysplasia consisting of confluent and irregularly shaped neuroendocrine cell nests. (**c**) A large, well-circumscribed neuroendocrine cell aggregate arising in a background of autoimmune (atrophic) gastritis, the size and confluent growth pattern of which qualify it as a type 1 gastric NET

phic metaplastic gastritis and ECL cell hyperplasia. These type 1 NETs have an indolent clinical course, with only rare reports of regional lymph node or distal metastases. Endoscopic surveillance with endoscopic resection is the typical management for these tumors, though definitive resection is suggested for the rare cases that are >2 cm in size, show lymphovascular invasion, invade the muscularis propria, or have a Ki-67 proliferative index indicating intermediate grade (G2).

Type 2 gastric NETs are caused by gastrinomas seen with Zollinger-Ellison syndrome. Zollinger-Ellison syndrome-associated gastrinomas are classically found in the "gastrinoma triangle," the anatomical triangle formed by the junction of the cystic duct with the common hepatic duct, the transition from the second to the third portion of the duodenum, and the head of the pancreas. However, contemporary studies reveal that type 2 gastric NETs account for only a minority of Zollinger-Ellison syndrome diagnoses (see the section on duodenal NETs below). Most patients with type 2 gastric NETs have multiple endocrine neoplasia (MEN) syndrome, type I (MEN1). Whether sporadic or syndromic, these gastrinomas cause gastrin hypersecretion with resultant proliferation of body and fundus ECL cells. The metastatic potential of type 2 gastric NETs is low, though marginally higher than type 1 gastric NETs. The diagnosis is based on identification of the morphological features of an NET (frequently multiple) in a background of normal mucosa or mucosa with parietal cell hyperplasia. Serum measurements reveal hypergastrinemia and hyperchlorhydria (pH <2).

Type 3 gastric NETs arise sporadically. They have morphology typical of NETs and arise in a background of normal mucosa and normal serum gastrin levels. They are commonly larger than 1 cm at the time of diagnosis, with consequently higher rates of metastasis and worse overall survival (75–80% at 5 years, compared to 90–95% for type 1 gastric NETs).

There are rare reports to suggest a type 4 gastric NET that consists of multiple small lesions arising in a background of parietal cell hyperplasia and hypertrophy. The parietal cells display vacuolated cytoplasm and harbor structural abnormalities that prevent the hydrochloric acid from being secreted. Consequently, achlorhydria, hypergastrinemia, and ECL cell hyperplasia ensue, leading to the development of these NETs. References: [22–31]

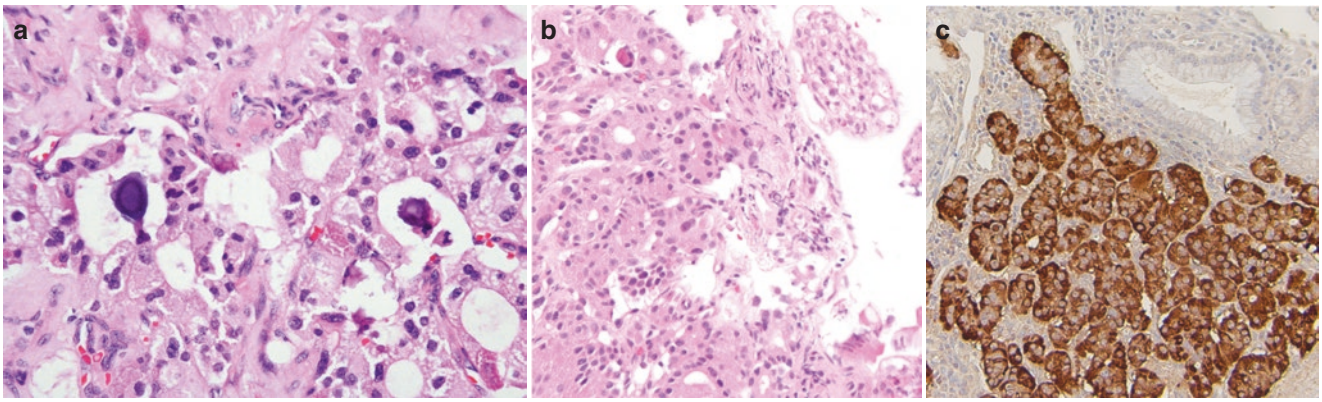


**Fig. 17.5** Duodenal somatostatinoma. (**a**) Typical morphology of a duodenal somatostatinoma with prominent pseudoglandular architecture and psammomatous calcification. (**b**) Strong and diffuse immunoreactivity to somatostatin demonstrated in tumor cells

**13. What types of NETs occur in the duodenum?**

In addition to conventional NETs, the duodenum and periampullary area give rise to several particular types of NET, including some that are virtually exclusive to these locations.

Somatostatinomas are primarily located at the ampulla and in the duodenum (26% of duodenal NETs) with a lesser proportion of biologically distinct tumors in the pancreas. They arise from the somatostatin-producing D cells. They are characterized by prominent pseudoglandular/tubular architecture. The lumens of these structures may contain densely eosinophilic proteinaceous secretions. Psammomatous calcifications can be numerous throughout the tumor and are found in up to 68% of duodenal somatostatinomas. PAS with diastase predigestion highlights the secretions as brightly fuchsinophilic and also highlights a microvillous brush border on the pseudoglandular structures. Because of these peculiar morphological features, somatostatinomas may be mistaken for adenocarcinomas. The lack of significant cytological atypia and the presence of psammomatous calcifications should be an indication of the correct diagnosis, which can be confirmed by immunohistochemistry. Synaptophysin and chromogranin A immunostains are usually positive, and somatostatin immunostaining is diffusely positive (Fig. 17.5). Unlike pancreatic somatostatinomas that frequently present with somatostatin syndrome (the symptom constellation including cholelithiasis, diabetes mellitus, weight loss, and diarrhea), duodenal somatostatinomas are almost always asymptomatic upon discovery or only present due to biliary obstruction at the ampulla. The majority of duodenal somatostatinomas are sporadic, but a large subset (up to 43%) are associated with neurofibromatosis type 1 (NF1). These NF1-associated somatostatinomas have a particular predilection for the ampulla. Rare cases are also associated with MEN type 1, in which cases the somatostatinomas are often small, incidentally discovered upon resection for treatment of Zollinger-Ellison syndrome, and may be associated with somatostatin cell hyperplasia. Duodenal/ampullary somatostatinomas average 1.8 cm and may have lymph node metastases, though liver metastases or death from disease are rare.

Gastrin cell neoplasms predominantly arise in the proximal duodenum, with far fewer, biologically distinct tumors arising in the pancreas. Sporadic duodenal gastrin cell neoplasms are predominantly unifocal and may be secretory or nonsecreting. Gastrin cell neoplasms with secretory activity are termed "gastrinomas" and, with sufficient gastrin secretion, are responsible for Zollinger-Ellison syndrome, in which excessive gastrin secretion leads to gastric parietal cell and ECL cell hyperplasia with subsequent gastric and duodenal ulcers. Gastrinomas can be very small, with 74% of duodenal gastrinomas being smaller than 1 cm. And despite their small size, 60–80% of gastrin cell neoplasms have lymph node metastases at presentation. Between 25% and 33% of gastrinomas arise in the setting of MEN1. These are almost always secretory, located in the duodenum rather than the pancreas, and unlike sporadic gastrinomas are most commonly multifocal and arise in a background of duodenal gastrin cell hyperplasia. Morphologically, both sporadic and syndromic gastrin cell neoplasms show morphological features typical of gastrointestinal NETs, with predominantly trabecular architecture and fibrotic stroma. Patchy pseudoglandular formation may be seen, though this is not typically a prominent feature as in somatostatinomas, and a small subset of syndromic gastrinomas may have associated psammomatous calcifications. MEN1-associated gastrinomas have a much higher rate of lymph node and liver metastases than sporadic tumors (75% versus 6% and 20% versus 0, respectively, in one large series). Some authors propose that 28% of gastrinomas may be lymph nodal primary tumors based on an inability to localize a primary tumor and superior prognosis after resection compared to cases with known duodenal or pancreatic primaries. However, extensive tissue examination with correlative hormone immunohistochemistry has shown that duodenal gastri-

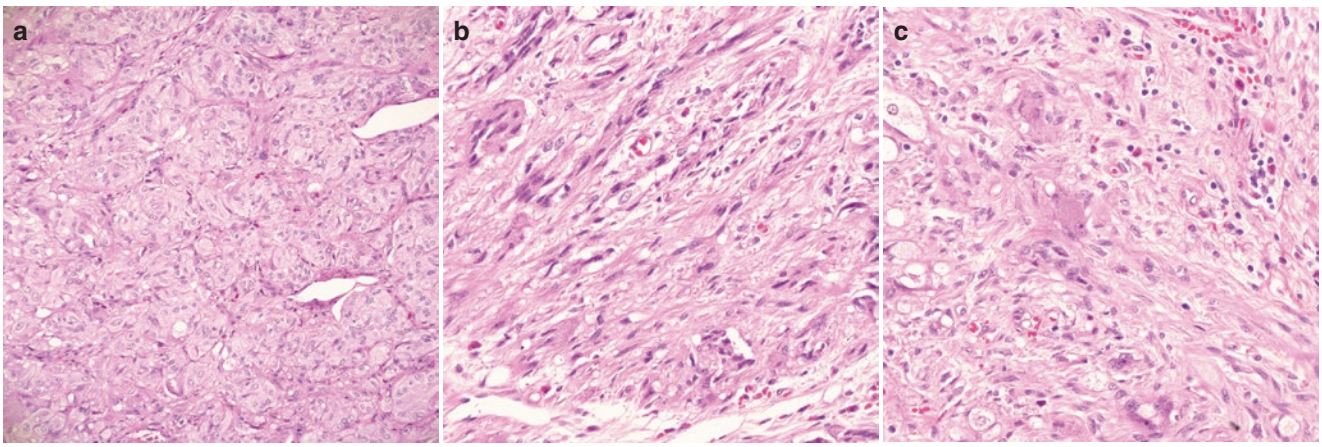


**Fig. 17.6** Gangliocytic paraganglioma. (**a**) An area of prominent epithelioid component, arranged in trabecula. (**b**) Another area of the same mass predominantly showing a spindle cell component. (**c**) Scattered ganglion cells interspersed in the spindle cell area

nomas even <1 mm in size are capable of producing much larger lymph node metastases. This would explain why surgery removing bulky peripancreatic and/or periduodenal lymph nodal disease without an identifiable primary improves prognosis and suggests that many (if not all) of these instances are due to microscopic, unidentifiable gastrointestinal primary tumors.

Gangliocytic paraganglioma is a rare, unique tumor nearly exclusive to the second part of the duodenum and ampulla. It is composed of an admixture of three cell types (Fig. 17.6). Epithelioid cells are arranged in nests, trabecula, or pseudoglands, with palely eosinophilic cytoplasm and ovoid nuclei with fine chromatin and inconspicuous nucleoli. Ganglion cells may be clustered or scattered and are characterized by abundant eosinophilic cytoplasm with Nissl bodies and round nuclei with prominent nucleoli. Bland spindle cells are the third cell type and may be arranged haphazardly or in fascicles. The three components may be present in variable amounts and may not all be evident on small biopsies. Neuroendocrine markers, such as synaptophysin and chromogranin A, or pancytokeratin can highlight the epithelioid cells, which are also frequently positive for pancreatic polypeptide and somatostatin. Ganglion cells are highlighted by synaptophysin and neurofilament. Spindle cells are positive for S100 protein and neurofilament. Depending on which component is most prevalent in the sampled tissue, differential diagnostic considerations include NET, ganglioneuroma, paraganglioma, schwannoma, leiomyoma, and gastrointestinal stromal tumor. Before diagnosing any of these in the duodenum/ampulla, one should make a careful examination for the three components of gangliocytic paraganglioma, with immunohistochemistry applied as necessary to highlight the components. Up to 11% of gangliocytic paragangliomas may have metastases to regional lymph nodes, though only up to 1% may have liver metastases, and there is only a single reported case of death from disease. Extension into the submucosa or sphincter of Oddi and size >3.1 cm are associated with a significantly increased risk of lymph node metastases. No histological features have been shown to predict metastasis or aggressive behavior, and no necrosis or significant cytological atypia is typically seen, even in cases with metastases.

References: [32–42]

### 14. What are the characteristics of ileal NETs?

While the ileum is a common site for NETs, this category also includes biologically similar tumors that occur in the jejunum and cecum (the "midgut carcinoids" as originally proposed by Williams and Sandler). They are predominantly derived from ECL cells and are characterized morphologically by solid nests of cells with brightly eosinophilic cytoplasmic granules (Fig. 17.7). They are typically strongly and diffusely positive for CDX2.

Ileal NETs are graded similarly to other gastrointestinal NETs (see Question 5). However, detailed multivariate analysis has suggested that a Ki-67 proliferative rate cutoff of 5%, rather than 3%, may better predict aggressive tumor behavior. Recent studies using modern imaging and endoscopic techniques indicate up to 54% of small intestinal NETs are multifocal at presentation, though this feature does not seem to have an impact on survival or recurrence.

Although typically small, with an average size of 1.8 cm, ileal NETs are frequently metastatic at the time of diagnosis. Tumors <2 cm can produce significant mesenteric tumor deposits, including some many times larger than the primary tumor. Recent studies indicate that these tumor deposits are more prognostically significant even than lymph node metastases. This led to the addition of an N2 stage in the eighth edition of the *AJCC Cancer Staging Manual* for jejunal and ileal NETs, which includes tumors with ≥12 positive lymph nodes or mesenteric masses measuring >2 cm (Table 17.2). However, more recent investigation indicates that the num-

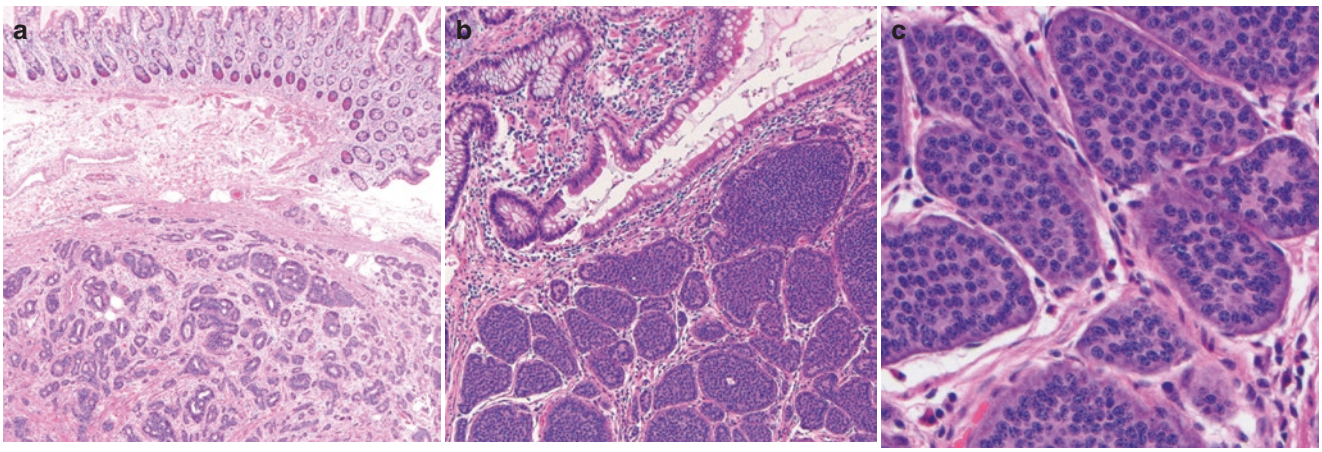


**Fig. 17.7** Typical morphological features of midgut neuroendocrine tumor. (**a**) An ileal NET presenting as a submucosal proliferation with nests, trabecula, cords, and pseudoglandular architecture set in fibrotic stroma. (**b**) Predominantly nested architecture. (**c**) High magnification showing a moderate amount of eosinophilic granular cytoplasm, round nuclei, stippled chromatin, and inconspicuous nucleoli

ber, rather than size, of mesenteric deposits has a greater impact on prognosis.

Serotonin secretion is typically subclinical until a large metastatic tumor burden, particularly in the liver, leads to the carcinoid syndrome. Carcinoid syndrome appears in 20–30% of patients with metastases and is composed of diarrhea, flushing, and bronchoconstriction. Of patients with carcinoid syndrome, 25–50% develop carcinoid heart disease, in which effects of elevated serum serotonin lead to right heart endocardial fibrosis, subsequent tricuspid and pulmonic valve dysfunction, and right heart failure. Significant mesenteric and retroperitoneal fibrosis is also associated with advanced disease and significant serum serotonin elevation in rare cases, which can lead to adhesions, obstruction, and ischemia.
References: [4, 12, 43–52]

### 15. What are the characteristics of appendiceal NETs?

Prognostication is particularly important in appendiceal NETs, as they are frequently identified incidentally at the time of laparoscopic appendectomy, prompting the question of whether additional intervention is necessary. In addition to histological grading and staging, which are broadly important in all gastrointestinal NETs, tumor size, location, depth of invasion, and histology play key roles in management decisions.

Size is a particularly clear indicator of whether a right hemicolectomy with appropriate lymphadenectomy is warranted or not. For NETs <1 cm, appendectomy with clear margins is curative. NETs >2 cm have a risk of lymph node metastases ranging from 25% to 40%, and right hemicolectomy is recommended. For NETs measuring between 1 and 2 cm with a reported lymph node positivity rate of up to 10%, the necessity of a right hemicolectomy is less clear.

In these intermediate-sized NETs, the location of the tumor can provide additional guidance. Tumors limited to the appendiceal tip or otherwise clearly completely resected may be cured with appendectomy alone. >3 mm infiltration of tumor into the mesoappendix has also been suggested to reflect more aggressive biology and a higher probability of lymph-vascular invasion, suggesting consideration for right hemicolectomy. While less well documented, intermediate histological grade (G2) and the presence of lymphovascular invasion have also been suggested to confer a worse prognosis and warrant consideration of right hemicolectomy.

While the majority of appendiceal NETs derive from enterochromaffin (EC) cells, a subset is derived from the glucagon-like peptide-, pancreatic polypeptide-, and peptide YY-secreting L cells that are quantitatively less abundant in the appendix than in more distal parts of the gastrointestinal tract. L cell NETs are typically incidental findings at appendectomy. If detected grossly, they may appear as <1 cm nodules near the distal tip of the appendix. Histologically, they are composed of cords, thin trabecula, or tubular structures. They have no or minimal mitotic activity. While synaptophysin is positive by immunohistochemistry, most widely available chromogranin immunohistochemical stains (which stain for chromogranin A) are negative. Positivity for glucagon is a helpful adjunct to confirm the diagnosis. Due to their small size and minimal invasion at the time of diagnosis, L cell NETs have an indolent prognosis, when compared to EC NETs of similar size.

L cell NETs composed exclusively or predominantly of a tubular pattern have historically been described as "tubular carcinoids" (Fig. 17.8). The currently recommended terminology by WHO is "tubular NET" for this lesion. The tubular structures may show inspissated mucin, which is a diagnostic pitfall that may lead these to be mistaken for adenocarcinoma or goblet cell adenocarcinoma, though no intracellular mucin is present. They have a similar immunohistochemical profile to other L cell NETs. Tubular carcinoids described in the literature are uniformly benign. Please see Chap. 8, Question 9, for more discussion on this topic.
References: [51, 53–55]

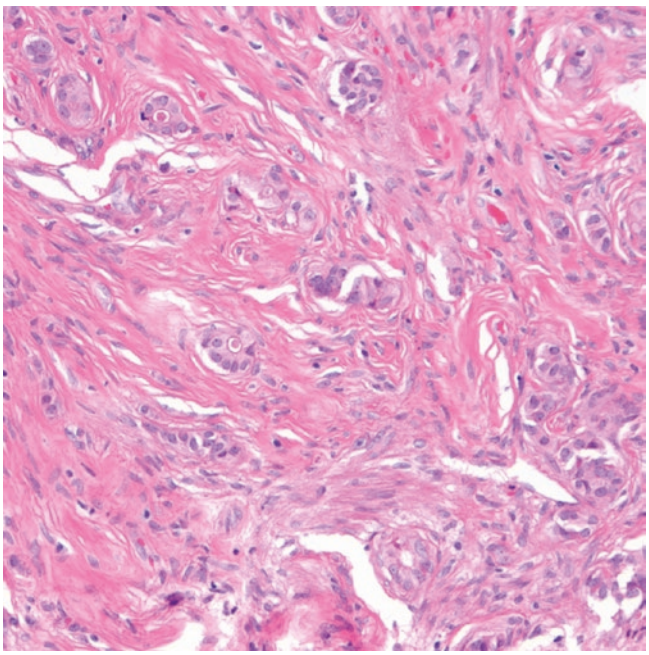

**Fig. 17.8** An L cell neuroendocrine tumor of the appendix with predominantly tubular architecture (so-called "tubular carcinoid" or "tubular NET")

### 16. What are the characteristics of rectal NETs?

Rectal NETs can be subdivided into two types based on histological pattern and secretory products: serotonin-producing EC cell NETs and glucagon-like peptide-, pancreatic polypeptide-, and peptide YY-producing L cell NETs.

L cell NETs are characterized by predominantly trabecular or ribbon-like architecture and are the type most commonly found in the rectum (Fig. 17.9). In contrast, EC cell NETs predominantly show solid nests. However, staining for pancreatic polypeptide and serotonin does not correlate well with the morphological pattern, and immunohistochemical staining is not mutually exclusive between these two NET types. L cell rectal NETs are regarded as of uncertain malignant potential, in comparison to other gastrointestinal NETs, which are generally considered malignant. In one recent study, non-L cell immunophenotype and large tumor size (>1 cm) were associated with tumor grade and stage, both of which were independently poor prognostic indicators, although small L cell NETs may have lymph node metastases.

Only 30% of rectal NETs are positive for chromogranin A. Large majorities show diffuse, moderate to strong staining for ISL1 (89%) and polyclonal PAX8 (79%), similar to pancreatic NETs. CDX2 positivity is reported in only approximately 30% of rectal NETs, with CDX2-positive cases often showing patchy and weak staining. In contrast, rectal NETs often show strong and diffuse positivity for SATB2. Rectal NETs also display high levels (97%) of positivity for prostatic acid phosphatase.

References: [12, 15, 56, 57]

### 17. Are there any characteristic molecular features of NETs?

NETs have only a small number of recurrent molecular alterations, with the most common mutation being in *CDKN1B* in approximately 10% of small intestinal NETs. This mutation most frequently coexists with chromosome 18 loss of heterozygosity, the single most common genomic alteration in small intestinal NETs at 55%. Amplifications on chromosomes 4, 5, and 20 are also well described. New evidence suggests that methylation may play a key role in driving NET biology, as tumors with high methylation have a worse prognosis than tumors with chromosome 18 losses and low methylation rates.

In NETs, greater understanding of molecular mechanisms has led to recent treatment innovations. NETs have long been noted to have a rich capillary network, and targeting the vascular endothelial growth factor pathway has been shown to extend progression-free survival in a clinical trial. Inhibition of the mammalian target of rapamycin (mTOR) pathway, affecting both proliferation and angiogenesis, has also shown promise against gastrointestinal NETs in a late phase clinical trial. Recent studies have shown that grade 3 NETs and NECs have a high expression of programmed death-ligand 1 (PD-L1) on tumor cells and tumor-infiltrating lymphocytes, suggesting that these tumors could be promising targets for immunotherapeutic agents involved in PD-L1 blockade.

Microsatellite instability has been detected in 12–15% of NECs and MANECs, highly correlated with extensive gene methylation (CpG island methylator phenotype), a rate similar to that in adenocarcinomas of the gastrointestinal tract. *BRAF* V600E mutations are frequent in these microsatellite-unstable NECs and MANECs. The microsatellite-unstable subset of NECs and MANECs is also associated with a significantly better prognosis, with a median survival of 60 months in microsatellite-unstable carcinomas compared to 5.5 months in microsatellite-stable carcinomas.

A greater understanding of the genomic landscape of NECs and MANECs has led to strong evidence that the neuroendocrine component of these carcinomas is derived from glandular adenomas or adenocarcinomas, rather than from NETs. Loss of heterozygosity at the same loci for *APC* and *TP53* genes has been demonstrated in the adenocarcinoma and NEC components of MANECs. In sequencing studies, identical *BRAF*, *KRAS*, and *TP53* mutations are frequently seen in the adenocarcinoma and NEC components of colorectal MANECs. Gastric MANECs have shown similar alterations. Alterations in these genes are common in typical colorectal adenocarcinomas and suggest derivation from glandular dysplasia/neoplasia. The NEC components of gastrointestinal MANECs then typically show additional mutations, most commonly of the retinoblastoma (*RB*) gene or related genes.

References: [58–68]

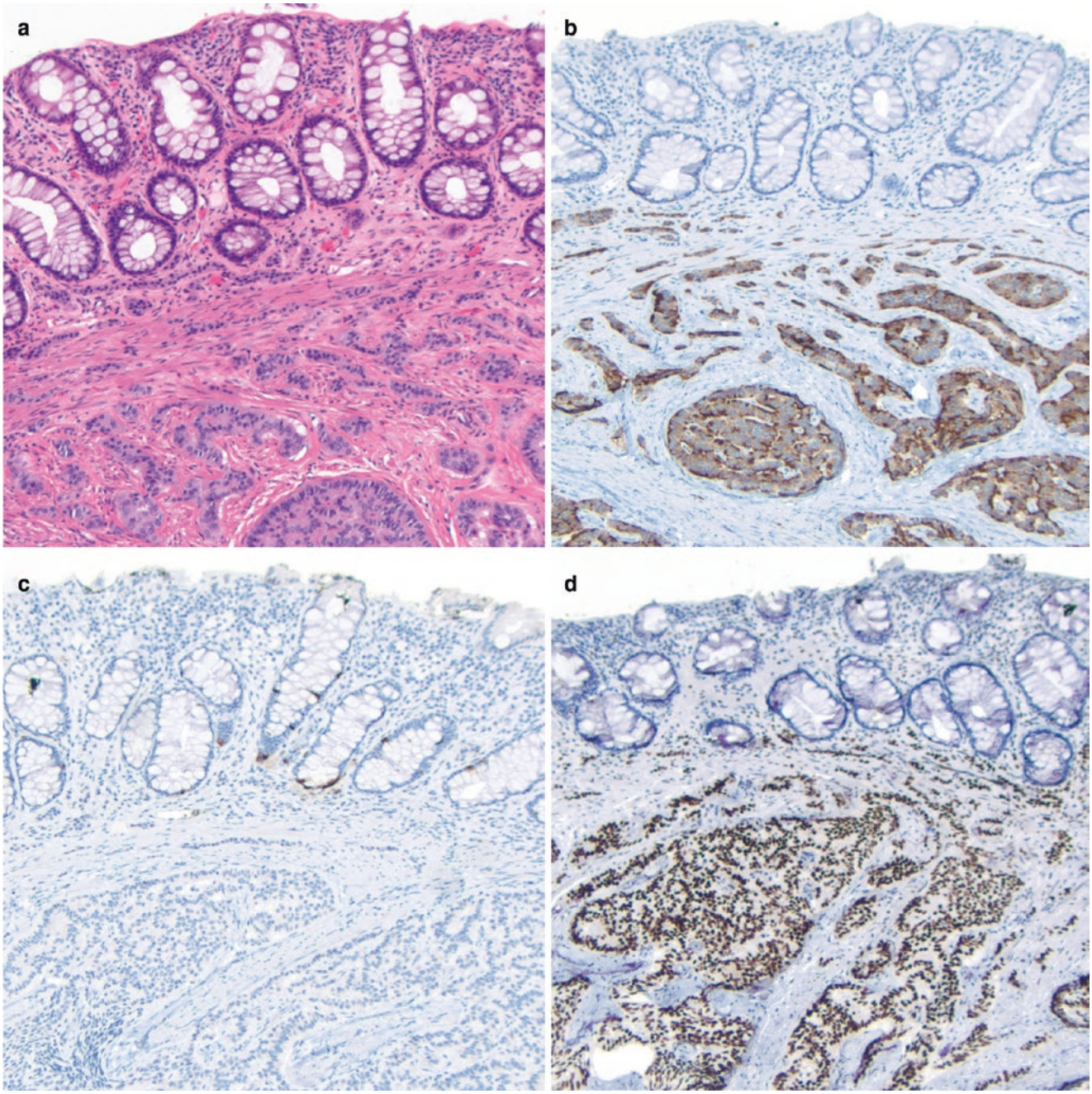


**Fig. 17.9** Typical features of rectal neuroendocrine tumor. (**a**) A rectal NET presenting as a predominantly submucosal proliferation of neuroendocrine cells arranged in cords, trabecula, and nests. (**b**) Strong and diffuse synaptophysin positivity seen in tumor cells. (**c**) Negativity for chromogranin A. (**d**) Strong and diffuse nuclear ISL1 positivity

## Case Presentation

### Case 1

A gastric biopsy shows a monotonous, nested epithelioid cellular proliferation in the deep lamina propria. The cells have a nested architecture, moderate amount of cytoplasm, round nuclei with inconspicuous nucleoli, and stippled chromatin, typical of an NET (Fig. 17.10). Synaptophysin and chromogranin A are positive, confirming neuroendocrine differentiation.

But is this proliferation an NET, neuroendocrine cell hyperplasia, or dysplasia? Neuroendocrine cell hyperplasia is characterized by linear arrangements of neuroendocrine cells present within gastric pits but increased in number. Nodular neuroendocrine cell hyperplasia consists of small

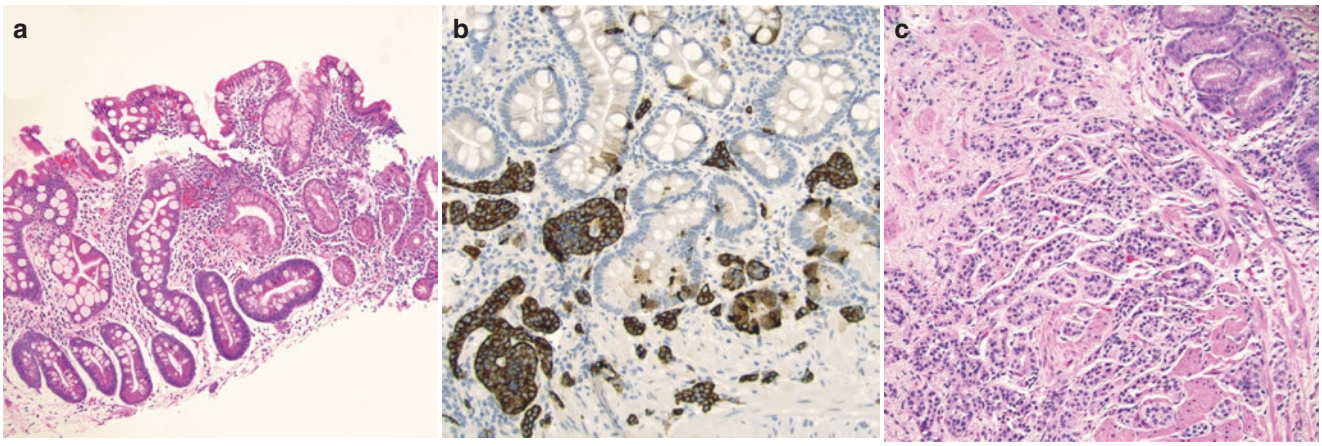


**Fig. 17.10** (Case 1) (**a**) Atrophic gastric corpus mucosa with extensive intestinal metaplasia, pyloric metaplasia, lamina propria lymphoplasmacytic infiltrate, and parietal cell atrophy. (**b**) Nodular neuroendocrine cell hyperplasia and dysplasia consistent with autoimmune (atrophic) gastritis. (**c**) NET with confluent growth and infiltration into the submucosa

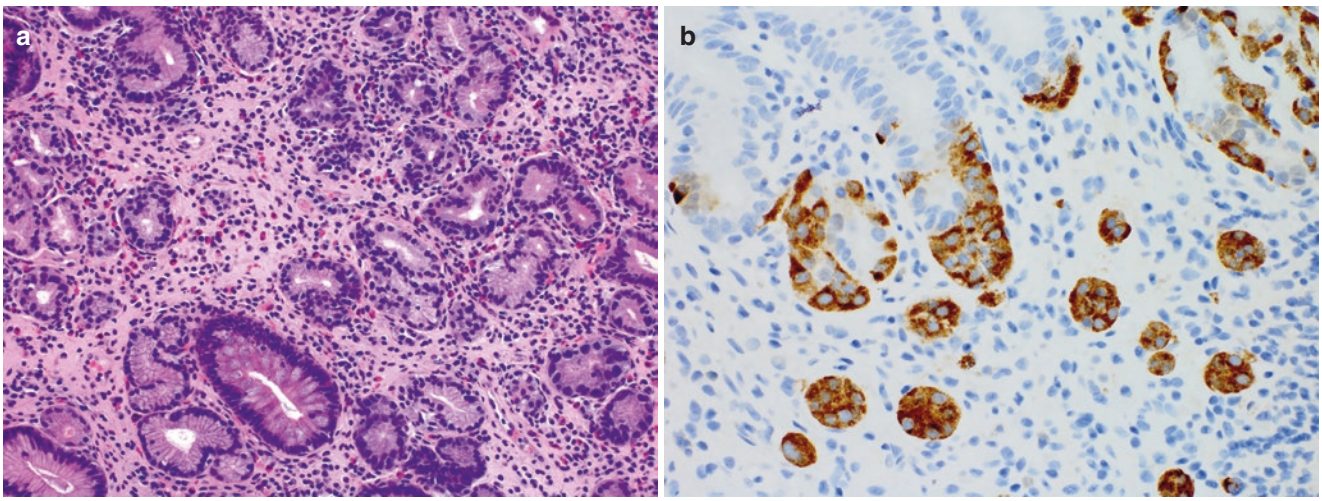


**Fig. 17.11** (Case 1) Additional biopsies of the gastric body further showing features of atrophic gastritis (**a**) and linear and nodular neuroendocrine cell hyperplasia as highlighted by chromogranin immunostain (**b**). The findings support the diagnosis of low-grade gastric NET, type 1

aggregates of five or more neuroendocrine cells. Adenomatoid hyperplasia is characterized by five or more of these nodules aggregated near each other. Finally, dysplasia occurs when these neuroendocrine nests become confluent and/or enlarged to ≥150 μm. The proliferation in this case is a discrete mass with new stroma formation and measures >500 μm, all features of NET.

Biopsies of the background mucosa of the gastric antrum show mild chronic inactive gastritis. The background corpus mucosa shows moderate chronic gastritis, patchy intestinal and pyloric metaplasia, and marked parietal cell atrophy. The findings of corpus-predominant inflammation, metaplasia, and parietal cell atrophy in the background mucosa raise the possibility of autoimmune (atrophic) gastritis. Careful microscopic examination reveals no *Helicobacter* organisms (confirmed by immunohistochemistry) and a gastrin immunostain on the sections labeled as coming from the gastric corpus confirm a lack of G cells, thereby confirming that the biopsies are from atrophic body mucosa and not antral mucosa. A chromogranin A immunostain highlights linear and nodular neuroendocrine cell hyperplasia, further suggesting the possibility of autoimmune (atrophic) gastritis (Fig. 17.11).

The lack of identified *Helicobacter* organisms and the pattern of corpus-predominant chronic inactive gastritis rather than antral-predominant chronic active gastritis exclude *Helicobacter*-associated gastritis. The presence of parietal cell atrophy rather than parietal cell hyperplasia excludes Zollinger-Ellison syndrome (and type 2 gastric NET). Altogether, the background features are strongly suggestive of a type 1 gastric NET: an NET arising in the background of autoimmune atrophic gastritis. This case is reported with a note to correlate with serological testing for anti-parietal cell and anti-intrinsic factor antibodies to confirm the diagnosis of autoimmune gastritis and

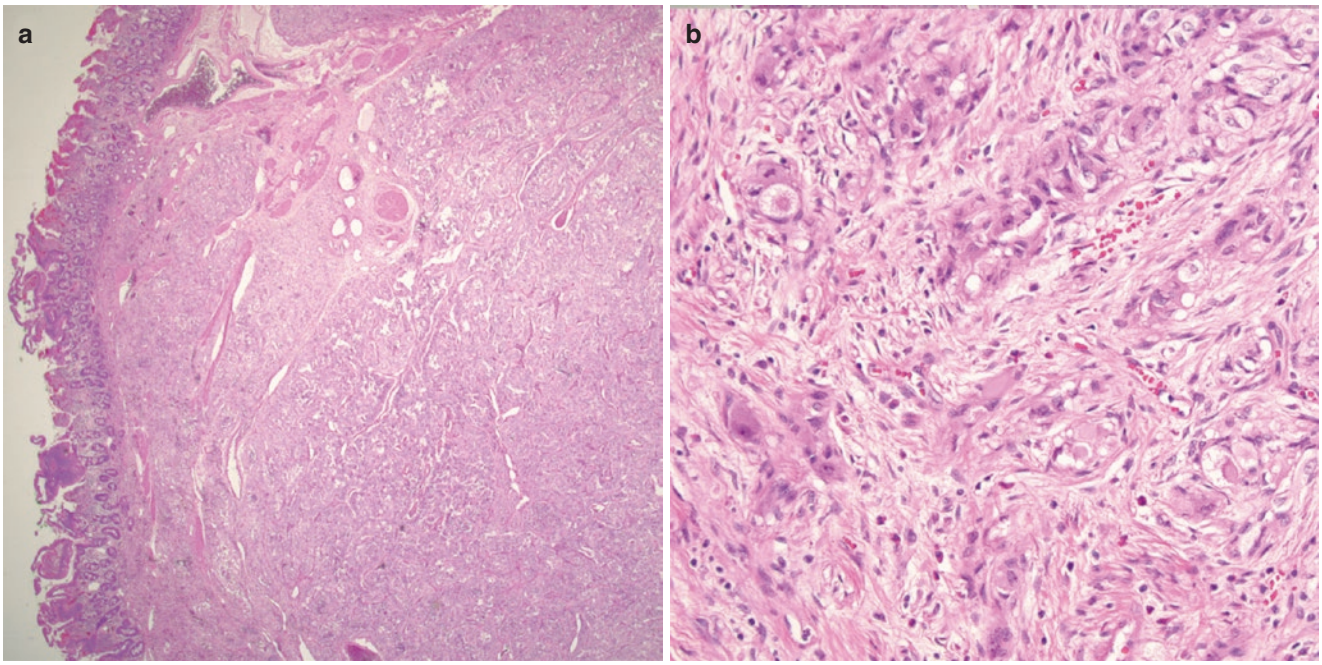


**Fig. 17.12** (Case 2) (**a**) Low-power examination showing a nested proliferation in the submucosa. (**b**) High-power examination revealing an admixture of nests of epithelioid cells, scattered ganglion cells, and intervening spindle cells, diagnostic of gangliocytic paraganglioma

report the high likelihood that this NET represents a type 1 gastric NET. This information is important to convey to the clinician, as type 1 NETs have indolent biology with lower risks of nodal and distant metastases than type 2 and type 3 gastric NETs, and endoscopic surveillance with mucosal resection may be an adequate treatment.

## Case 2

A mucosal biopsy from the ampulla of Vater shows an epithelioid cellular proliferation in the lamina propria. Careful inspection shows rare ganglion cells intimately admixed and scattered with spindle cell areas (Fig. 17.12). This characteristic triphasic morphology is diagnostic of gangliocytic paraganglioma.

The second part of the duodenum and the ampulla of Vater are the classical location for gangliocytic paraganglioma, and, though very rare, this diagnosis should always be kept in mind from biopsies of nodules, polyps, or masses in this area. In the current case, careful examination of the hematoxylin- and eosin-stained sections readily reveals all three of the characteristic cells. However, in small biopsies, one or more of the cell types may be sparse or entirely absent (unsampled). Thus, a high index of suspicion at this site is necessary. Immunohistochemistry may be used to highlight rare cells: a practical panel may include pancytokeratin to highlight the epithelioid cells, synaptophysin to highlight the ganglion cells, and S100 protein to highlight the spindle cells.

If the characteristic morphology is not recognized, these tumors can easily be misdiagnosed as ganglioneuromas (if an epithelioid component is not identified), paragangliomas (if the ganglion cell component is not identified), or spindle cell neoplasms (schwannomas, smooth muscle tumors, or even gastrointestinal stromal tumors, if epithelioid and ganglion cell components are not identified). If the epithelioid cell component is prominent, the fact that these frequently stain positively for synaptophysin and chromogranin A makes NET a frequent misdiagnosis. Furthermore, the same location is notable for several unique NETs, such as somatostatinoma and gastrinoma.

While gangliocytic paragangliomas typically behave in an indolent fashion, even with lymph node metastases, it is important to exclude some of the above entities with decidedly more aggressive prognoses. NETs at this location may also suggest wider syndromes, such as the association of somatostatinomas with NF1 and of gastrinomas with MEN1.

## Case 3

A small intestinal resection is performed for a submucosal/intramural tumor. The tumor is composed primarily of trabecula and nests of epithelioid cells with a moderate amount of cytoplasm, finely granular chromatin, and inconspicuous nucleoli (Fig. 17.13). Synaptophysin and chromogranin A immunostains are diffusely positive, confirming neuroendocrine differentiation. Mitotic figures are readily

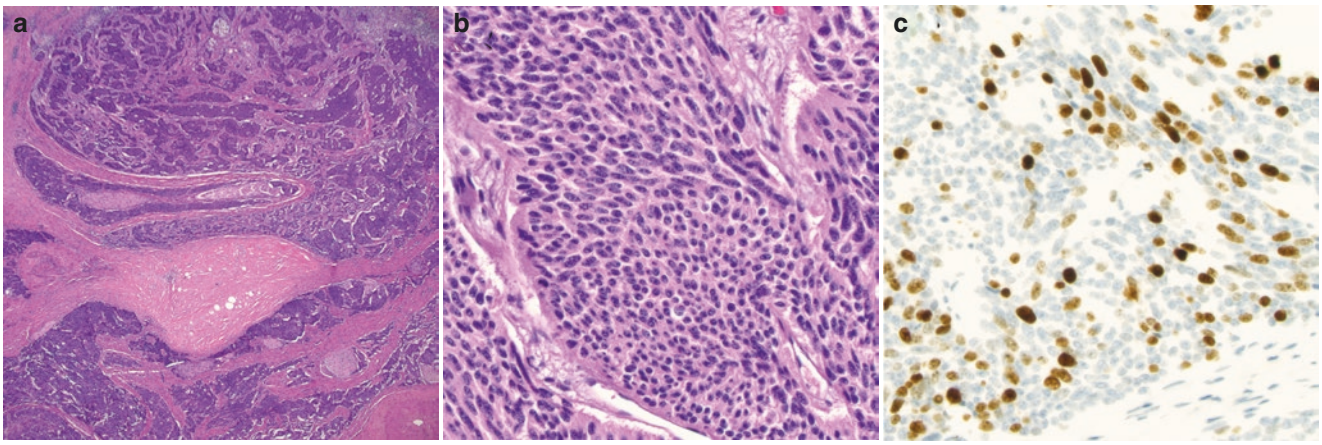


**Fig. 17.13** (Case 3) (**a**) Low-power view showing nested and trabecular architecture with significant stromal fibrosis and perineural invasion. (**b**) Although the tumor cells are somewhat spindled in this case, they have moderate cytoplasm, inconspicuous nucleoli, and finely granular chromatin, all features of an NET. (**c**) Although well-differentiated morphologically, the tumor shows a Ki-67 proliferative rate in excess of 20%

identified, and a count of 10 $mm^2$ reveals 22 mitotic figures per 2 $mm^2$. Ki-67 immunostain shows 30% of 1300 tumor cells counted staining positively in the area of highest positivity.

Now that the diagnosis of a neuroendocrine neoplasm is established, the primary question becomes: Is this a high-grade (G3) NET or a NEC? Both NETs and NECs may have mitotic rates >20 per 2 $mm^2$, and both may have Ki-67 proliferative indexes of >20%. Differentiating the two resides in the morphological appearance. The tumor described above shows classical features of an NET. NECs are, by definition, poorly differentiated showing morphological features not seen in the current case. SCCs show a high nucleus-to-cytoplasm ratio, nuclear molding, smooth chromatin, necrosis, and abundant apoptosis. LCNECs show vesicular or coarse chromatin, prominent nucleoli, abundant cytoplasm, and necrosis. Immunohistochemistry may be helpful as supportive evidence. NECs are more likely to show aberrant staining for p53, loss of RB protein, and negative staining for somatostatin receptor 2.

Although high-grade NETs appear to be rare in the gastrointestinal tract, reports from other organs indicate a prognosis intermediate between G2 NETs and NECs. More investigation is necessary as to the implications of this diagnosis in the tubular gastrointestinal tract, but accurate diagnosis is the first step toward determining prognostic differences and differences in therapeutic efficacy.
Reference: [6]

## Case 4

A portion of terminal ileum is resected for a large mesenteric mass and lymphadenopathy. Gross examination reveals an 11.5 cm well-circumscribed mass near the root of the mesentery, multiple enlarged lymph nodes, and a 1.5 cm submucosal mass with focal overlying mucosal ulceration. Microscopic examination of the submucosal mass shows features typical of an NET, including trabecular architecture, moderate cytoplasm, eosinophilic cytoplasmic granules, finely granular chromatin, and inconspicuous nucleoli predominantly involving the submucosa with extension through the muscularis propria and to the serosal surface. Identical features are seen in abundant representative sections of the mesenteric mass (Fig. 17.14). Five lymph nodes are positive, though the large mesenteric mass shows no obvious residual lymphoid tissue after extensive sampling. Chromogranin A and synaptophysin immunostains confirm the diagnosis of NET. Mitotic rate and Ki-67 proliferative index are both low, indicating low grade (G1).

Can such a small ileal tumor produce such a large mesenteric mass? Should the possibility of an occult, unsampled primary be considered? Small intestinal primary NETs even <2 cm may produce significant lymphadenopathy and mesenteric masses. In a case like this, there is no reason to doubt that the sampled submucosal mass represents the primary. Should there be any doubt, positive CDX2 immunostaining in the mesenteric mass, combined with negative TTF1, PAX6, PAX8, ISL1, and/or SATB2, would be consistent with an ileal primary.

But what is the nature of this large mesenteric mass? Is it a large nodal metastasis, lymphovascular invasion, or perineural invasion? No surrounding residual lymph nodal tissue or vascular smooth muscle is identified, excluding classification as a lymph node metastasis or lymphovascular invasion. While small entrapped vessels and nerves may be seen, the size and gross configuration (well circumscribed) of this lesion best qualifies it as a mesenteric mass rather than perineural invasion or lymphovascular invasion. In the current version of the AJCC staging manual, mesenteric masses >2 cm in size qualify as N2 disease. The finding of the large mesenteric mass in this case upstages the tumor to pT4N2.
Reference: [49]

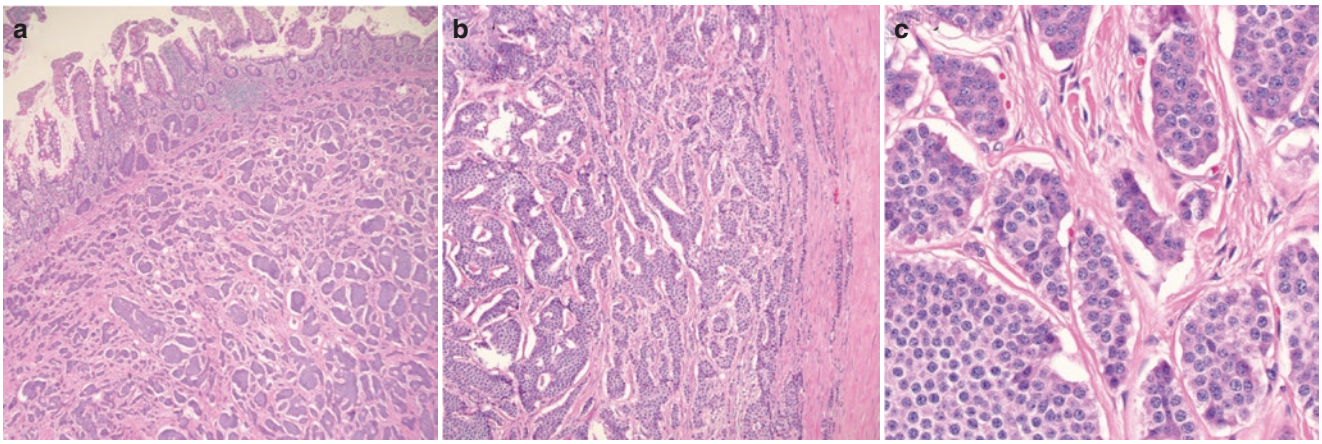


**Fig. 17.14** (Case 4) (**a**) A small intestinal tumor composed of nests, cords, and trabecula of tumor cells set in fibrotic stroma filling the submucosa. (**b**) A discrete mesenteric mass with a thick fibrotic capsule present, which is separate from the mural tumor in part A. There is no discernible lymphoid tissue or surrounding vascular smooth muscle to indicate a lymph node metastasis or vascular invasion. (**c**) High-power examination showing the classical features of an NET, including moderate cytoplasm and round nuclei with fine granular cytoplasm and inconspicuous nucleoli

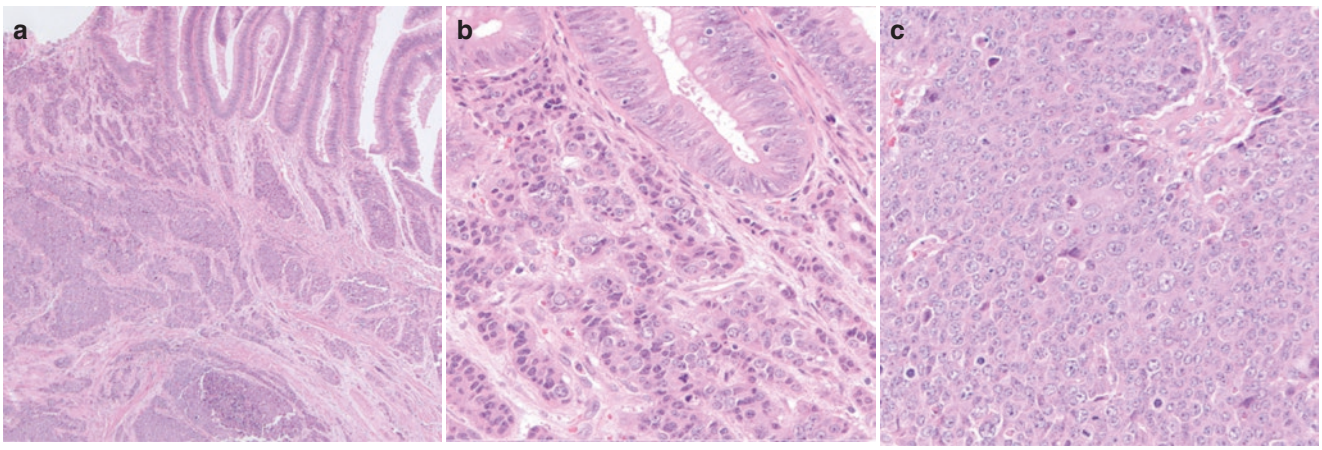


**Fig. 17.15** (Case 5) (**a**) The top portion of the photomicrograph shows villiform surface projections lined by low-grade neoplastic epithelium overlying solid nests and trabecula of more poorly differentiated cells. (**b**) High-power examination showing glandular epithelium with scattered apical mucin-containing cells and elongated, hyperchromatic, and pseudostratified nuclei. The glandular component is directly adjacent to invasive carcinoma arranged in trabecula and cords, typical morphology for neuroendocrine neoplasms. (**c**) High-power examination of the invasive component showing neoplastic cells with a high nucleus-to-cytoplasm ratio, irregular nuclear contours, vesicular chromatin, variably prominent nucleoli, and brisk mitotic activity. With confirmation of neuroendocrine differentiation by immunohistochemistry, this component is labeled as NEC. The entire tumor can thus be labeled as a mixed neuroendocrine-nonneuroendocrine neoplasm (MiNEN)

## Case 5

An abdominoperineal resection is performed for a low rectal mass. Histological examination shows that the superficial half of the mass is composed of villous adenoma with pseudostratified columnar cells having apical intracellular mucin and elongated, hyperchromatic nuclei. Immediately deep to the villous adenoma are nests and trabecula of cells with high nucleus-to-cytoplasm ratios, vesicular chromatin, and variably prominent nucleoli (Fig. 17.15). Mitotic figures are abundant. Due to the nested and trabecular architecture, immunostains for synaptophysin and chromogranin A are performed, which are diffusely positive in the portion of the tumor with this architecture. The villous component is negative for both immunostains. Ki-67 proliferative index in the neuroendocrine component is 80%.

What is the explanation for half of the tumor showing positivity for neuroendocrine markers? Is this NET or NEC? Although the nested and trabecular architecture of the tumor may raise the possibility of NET, high-power examination reveals nuclear atypia, a high nucleus-to-cytoplasm ratio, and chromatin patterns suggestive of LCNEC. This tumor would be best classified as NEC arising in a villous adenoma.

The recent proposal by La Rosa and colleagues creates the expansive category of mixed neuroendocrine-nonneuroendocrine neoplasms (MiNEN), which captures all tumors composed of ≥30% each of neuroendocrine and nonneuroendocrine elements. This classification includes neuroendocrine neoplasms of all types (NET, LCNEC, and SCC) and all grades and includes any glandular component, whether invasive or adenomatous. While broad, this proposal best reflects the heterogeneity of these mixed neoplasms and has been adopted by the current WHO tumor classification. In the current case, the designation of MiNEN best fits the histological findings and appropriately flags the potentially aggressive nature of the lesion.
Reference: [18]

## References


1. Dasari A, Shen C, Halperin D, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. JAMA Oncol. 2017;3(10):1335–42.
2. Kimura N, Pilichowska M, Okamoto H, Kimura I, Aunis D. Immunohistochemical expression of chromogranins A and B, prohormone convertases 2 and 3, and amidating enzyme in carcinoid tumors and pancreatic endocrine tumors. Mod Pathol. 2000;13(2):140–6.
3. Chejfec G, Falkmer S, Grimelius L, et al. Synaptophysin. A new marker for pancreatic neuroendocrine tumors. Am J Surg Pathol. 1987;11(4):241–7.
4. Williams ED, Sandler M. The classification of carcinoid tumours. Lancet. 1963;1(7275):238–9.
5. Shia J, Tang LH, Weiser MR, et al. Is nonsmall cell type high-grade neuroendocrine carcinoma of the tubular gastrointestinal tract a distinct disease entity? Am J Surg Pathol. 2008;32(5):719–31.
6. Tang LH, Basturk O, Sue JJ, Klimstra DS. A practical approach to the classification of WHO grade 3 (G3) well-differentiated neuroendocrine tumor (WD-NET) and poorly differentiated neuroendocrine carcinoma (PD-NEC) of the pancreas. Am J Surg Pathol. 2016;40(9):1192–202.
7. Wang Y, Wang W, Jin K, et al. Somatostatin receptor expression indicates improved prognosis in gastroenteropancreatic neuroendocrine neoplasm, and octreotide long-acting release is effective and safe in Chinese patients with advanced gastroenteropancreatic neuroendocrine tumors. Oncol Lett. 2017;13(3):1165–74.
8. Amin MB, Edge SB, Greene FL, et al., editors. AJCC Cancer staging manual. 8th ed. Chicago, IL: American College of Surgeons; 2017. p. 351–406.
9. Reid MD, Bagci P, Ohike N, et al. Calculation of the Ki67 index in pancreatic neuroendocrine tumors: a comparative analysis of four counting methodologies. Mod Pathol. 2015;28(5):686–94.
10. Klöppel G, La Rosa S. Ki67 labeling index: assessment and prognostic role in gastroenteropancreatic neuroendocrine neoplasms. Virchows Arch. 2018;472:341–9.
11. Yang Z, Tang LH, Klimstra DS. Effect of tumor heterogeneity on the assessment of Ki67 labeling index in well-differentiated neuroendocrine tumors metastatic to the liver: implications for prognostic stratification. Am J Surg Pathol. 2011;35(6):853–60.
12. Bellizzi AM. Assigning site of origin in metastatic neuroendocrine neoplasms: a clinically significant application of diagnostic immunohistochemistry. Adv Anat Pathol. 2013;20(5):285–314.
13. Li Z, Yuan J, Wei L, et al. SATB2 is a sensitive marker for lower gastrointestinal well-differentiated neuroendocrine tumors. Int J Clin Exp Pathol. 2015;8(6):7072–82.
14. Mohanty S, Bhardwaj N, Lugo H, et al. Diagnostic utility of SATB2 in determining the site of origin of well-differentiated neuroendocrine tumors. Pancreas. 2018;47(3):348.
15. Koo J, Zhou X, Moschiano E, De Peralta-Venturina M, Mertens RB, Dhall D. The immunohistochemical expression of islet 1 and PAX8 by rectal neuroendocrine tumors should be taken into account in the differential diagnosis of metastatic neuroendocrine tumors of unknown primary origin. Endocr Pathol. 2013;24(4):184–90.
16. Uccella S, La Rosa S, Volante M, Papotti M. Immunohistochemical biomarkers of gastrointestinal, pancreatic, pulmonary, and thymic neuroendocrine neoplasms. Endocr Pathol. 2018;29(2):150–68.
17. Klimstra DS, Capella C, Arnold R, et al. Neuroendocrine neoplasms of the colon and rectum. In: Bosman FT, Carneiro F, Hruban RH, Theise N, editors. WHO classification of tumours of the digestive tract. 4th ed. Lyon, France: IARC Press; 2010. p. 714–177.
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(4):284–311.
19. De Mestier L, Cros J, Neuzillet C, et al. Digestive system mixed neuroendocrine-nonneuroendocrine neoplasms. Neuroendocrinology. 2017;105(4):412–25.
20. Li Y, Yau A, Schaeffer D, et al. Colorectal glandular-neuroendocrine mixed tumor: pathologic spectrum and clinical implications. Am J Surg Pathol. 2011;35(3):413–25.
21. Solcia E, Bordi C, Creutzfeldt W, et al. Histopathological classification of nonantral gastric endocrine growths in man. Digestion. 1988;41(4):185–200.
22. Scherübel H, Cadiot G, Jensen RT, Rösch T, Stölzel U, Klöppel G. Neuroendocrine tumors of the stomach (gastric carcinoids) are on the rise: small tumors, small problems? Endoscopy. 2010;42(8):664–71.
23. Gladdy RA, Strong VE, Coit D, et al. Defining surgical indications for type I gastric carcinoid tumor. Ann Surg Oncol. 2009;16(11):3154–60.
24. Lupinacci RM, Dias AR, Mello ES, Kondo A. Minute type I gastric carcinoid with regional lymph node metastasis. Int J Surg Pathol. 2013;21(2):169–72.
25. Grozinsky-Glasberg S, Thomas D, Strosberg JR, et al. Metastatic type 1 gastric carcinoid: a real threat or just a myth? World J Gastroenterol. 2013;19(46):8687–95.
26. Modlin IM, Kidd M, Latich I, Zikusoka MN, Shapiro MD. Current status of gastrointestinal carcinoids. Gastroenterology. 2005;128(6):1717–51.
27. Hung OY, Maithel SK, Willingham FF, Farris AB 3rd, Kauh JS. Hypergastrinemia, type 1 gastric carcinoid tumors: diagnosis and management. J Clin Oncol. 2011;29(25):e713–5.
28. Borch K, Ahrén B, Ahlman H, Falkmer S, Granérus G, Grimelius L. Gastric carcinoids: biologic behavior and prognosis after differential treatments in relation to type. Ann Surg. 2005;242(1):64–73.
29. Ooi A, Ota M, Katsuda S, Nakanishi I, Sugawara H, Takahashi I. An unusual case of multiple gastric carcinoids associated with diffuse endocrine cell hyperplasia and parietal cell hypertrophy. Endocr Pathol. 1995;6(3):229–37.
30. Abraham SC, Carney JA, Ooi A, Choti MA, Argani P. Achlorhydria, parietal cell hyperplasia, and multiple gastric carcinoids: a new disorder. Am J Surg Pathol. 2005;29(7):969–75.
31. Nakata K, Aishima S, Ichimiya H, et al. Unusual multiple gastric carcinoids with hypergastrinemia: report of a case. Surg Today. 2010;40(3):267–71.
32. Garbrecht N, Anlauf M, Schmitt A, et al. Somatostatin-producing neuroendocrine tumors of the duodenum and pancreas: incidence, types, biological behavior, association with inherited syndromes, and functional activity. Endocr Relat Cancer. 2008;15(1):229–41.

33. Tanaka S, Yamasaki S, Matsushita H, et al. Duodenal somatostatinoma: a case report and review of 31 cases with special reference to the relationship between tumor size and metastasis. Pathol Int. 2000;50(2):146–52.
34. Anlauf M, Perren A, Meyer CL, et al. Precursor lesions in patients with multiple endocrine neoplasia type 1-associated duodenal gastrinomas. Gastroenterology. 2005;128(5):1187–98.
35. Williams GT. Endocrine tumours of the gastrointestinal tract-selected topics. Histopathology. 2007;50(1):30–41.
36. Donow C, Pipeleers-Marichal M, Schröder S, Stamm B, Heitz PU, Klöppel G. Surgical pathology of gastrinoma. Site, size, multicentricity, association of multiple endocrine neoplasia type 1, and malignancy. Cancer. 1991;68(6):1329–34.
37. Anlauf M, Garbrecht N, Henopp T, et al. Sporadic versus hereditary gastrinomas of the duodenum and pancreas: distinct clinicopathological and epidemiological features. World J Gastroenterol. 2006;12(34):5440–6.
38. 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(3):277–87.
39. Chen Y, Deshpande V, Ferrone C, et al. Primary lymph node gastrinoma: a single institution experience. Surgery. 2017;162(5):1088–94.
40. Anlauf M, Enosawa T, Henopp T, et al. Primary lymph node gastrinoma or occult duodenal microgastrinoma with lymph node metastasis in an MEN1 patient: the need for a systematic search for the primary tumor. Am J Surg Pathol. 2008;32(7):1101–5.
41. Okubo Y, Yoshioka E, Suzuki M, et al. Diagnosis, pathological findings, and clinical management of gangliocytic paraganglioma: a systematic review. Front Oncol. 2018;8:291.
42. Li B, Li Y, Tian XY, Luo BN, Li Z. Malignant gangliocytic paraganglioma of the duodenum with distant metastases and a lethal course. World J Gastroenterol. 2014;20(41):15454–61.
43. Burke AP, Thomas RM, Elsayed AM, Sobin LH. Carcinoids of the jejunum and ileum: an immunohistochemical and clinicopathologic study of 167 cases. Cancer. 1997;79(6):1086–93.
44. Panzuto F, Campana D, Fazio N, et al. Risk factors for disease progression in advanced jejunoileal neuroendocrine tumors. Neuroendocrinology. 2012;96(1):32–40.
45. Choi AB, Maxwell JE, Keck KJ, et al. Is multifocality an indicator of aggressive behavior in small bowel neuroendocrine tumors? Pancreas. 2017;46(9):1115–20.
46. Gangi A, Siegel E, Barmparas G, et al. Multifocality in small bowel neuroendocrine tumors. J Gastrointest Surg. 2018;22(2):303–9.
47. Gonzalez RS, Liu EH, Alvarez JR, Ayers GD, Washington MK, Shi C. Should mesenteric tumor deposits be included in staging of well-differentiated small intestine neuroendocrine tumors? Mod Pathol. 2014;27(9):1288–95.
48. Fata CR, Gonzalez RS, Liu E, Cates JM, Shi C. Mesenteric tumor deposits in midgut small intestinal neuroendocrine tumors are a stronger indication than lymph node metastasis for liver metastasis and poor prognosis. Am J Surg Pathol. 2017;41(1):128–33.
49. Woltering EA, Bergsland EK, Beyer DT, et al. Neuroendocrine tumors of the jejunum and ileum. In: Amin MB, Edge SB, Greene FL, et al., editors. AJCC Cancer staging manual. 8th ed. Chicago, IL: American College of Surgeons; 2017. p. 375–87.
50. Gonzalez RS, Cates JMM, Shi C. Number, not size, of mesenteric tumor deposits affects prognosis of small intestinal well-differentiated neuroendocrine tumors. Mod Pathol. 2018;31(10):1560–6.
51. Pape UF, Perren A, Niederle B, et al. ENETS Consensus Guidelines for the management of patients with neuroendocrine neoplasms from the jejuno-ileum and the appendix including goblet cell carcinomas. Neuroendocrinology. 2012;95(2):135–56.
52. Daskalakis K, Karakatsanis A, Stålberg P, Norlén O, Hellman P. Clinical signs of fibrosis in small intestinal neuroendocrine tumours. Br J Surg. 2017;104(1):69–75.
53. Iwafuchi M, Watanabe H, Ajoka Y, Shimoda T, Iwashita A, Ito S. Immunohistochemical and ultrastructural studies of twelve argentaffin and six argyrophil carcinoids of the appendix vermiformis. Hum Pathol. 1990;21(7):773–80.
54. Burke AP, Sobin LH, Federspiel BH, Shekitka KM, Helwig EB. Goblet cell carcinoids and related tumors of the vermiform appendix. Am J Clin Pathol. 1990;94(1):27–35.
55. Warkel RL, Cooper PH, Helwig EB. Adenocarcinoid, a mucin-producing carcinoid tumor of the appendix: a study of 39 cases. Cancer. 1978;42(6):2781–93.
56. Koo J, Mertens RB, Mirocha JM, Wang HL, Dhall D. Value of islet 1 and PAX8 in identifying metastatic neuroendocrine tumors of pancreatic origin. Mod Pathol. 2012;25(6):893–901.
57. Kim JY, Kim KS, Kim KJ, et al. Non-L-cell immunophenotype and large tumor size in rectal neuroendocrine tumors are associated with aggressive clinical behavior and worse prognosis. Am J Surg Pathol. 2015;39(5):632–43.
58. Karpathakis A, Dibra H, Pipinikas C, et al. Prognostic impact of novel molecular subtypes of small intestinal neuroendocrine tumor. Clin Cancer Res. 2016;22(1):250–8.
59. Yao JC, Phan A, Hoff PM, et al. Targeting vascular endothelial growth factor in advanced carcinoid tumor: a random assignment phase II study of depot octreotide with bevacizumab and pegylated interferon alpha-2b. J Clin Oncol. 2008;26(8):1316–23.
60. Pavel ME, Hainsworth JD, Baudin E, et al. Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. Lancet. 2011;378(9808):2005–12.
61. Cavalcanti E, Armentano R, Valentini AM, Chieppa M, Caruso ML. Role of PD-L1 expression as a biomarker for GEP neuroendocrine neoplasm grading. Cell Death Dis. 2017;8(8):e3004.
62. Sahnane N, Furlan D, Monti M, et al. Microsatellite unstable gastrointestinal neuroendocrine carcinomas: a new clinicopathologic entity. Endocr Relat Cancer. 2015;22(1):35–45.
63. La Rosa S, Marando A, Furlan D, Sahnane N, Capella C. Colorectal poorly differentiated neuroendocrine carcinomas and mixed adenoneuroendocrine carcinomas: insights into the diagnostic immunophenotype, assessment of methylation profile, and search for prognostic markers. Am J Surg Pathol. 2012;36(4):601–11.
64. Vortmeyer AO, Lubensky IA, Merino MHJ, et al. Concordance of genetic alterations in poorly differentiated colorectal neuroendocrine carcinomas and associated adenocarcinomas. J Natl Cancer Inst. 1997;89(19):1448–53.
65. Karkouche R, Bachet JB, Sandrini J, et al. Colorectal neuroendocrine carcinomas and adenocarcinomas share oncogenic pathways. A clinico-pathologic study of 12 cases. Eur J Gastroenterol Hepatol. 2012;24(12):1430–7.
66. Scardoni M, Vittoria E, Volante M, et al. Mixed adenoneuroendocrine carcinomas of the gastrointestinal tract: targeted next-generation sequencing suggests a monoclonal origin of the two components. Neuroendocrinology. 2014;100(4):310–6.
67. Woischke C, Schaaf CW, Yang HM, et al. In-depth mutational analyses of colorectal neuroendocrine carcinomas with adenoma or adenocarcinoma components. Mod Pathol. 2017;30(1):95–103.
68. Takizawa N, Ohishi Y, Hirahashi M, et al. Molecular characteristics of colorectal neuroendocrine carcinoma; similarities with adenocarcinoma rather than neuroendocrine tumor. Hum Pathol. 2015;46(12):1890–900.