Chapter

Histopathologic Diagnosis of Neuroendocrine Neoplasms of Head and Neck, Lung and Gastrointestinal Tract

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Abstract

Neuroendocrine neoplasms are classified as epithelial and non-epithelial based on their origin being from epithelial neuroendocrine progenitor cells or derived from the neural crest. The latter are negative for cytokeratin (hence non-epithelial) and mostly result from neoplastic transformation of paraganglia. Here, we are reviewing the most important histologic and immunophenotypic characteristics of neuroendocrine carcinomas as well as the current WHO classification guidelines. The terminology of neuroendocrine neoplasms is confusing due to various classification systems employed for each internal organ. In the lung and GI tract, for example, "neuroendocrine tumors" comprise carcinomas of different degree of differentiation and histologic grade. While in the lung the term refers strictly to low-grade neuroendocrine carcinomas, in the GI tract it comprises both low- and high-grade neuroendocrine carcinomas. Despite concerted efforts to unify the overall classification of neuroendocrine carcinomas across organs, major differences continue to persist.

Keywords: neuroendocrine neoplasms, histopathologic diagnosis, immunohistochemistry, Ki-67, small cell carcinoma

1. Introduction

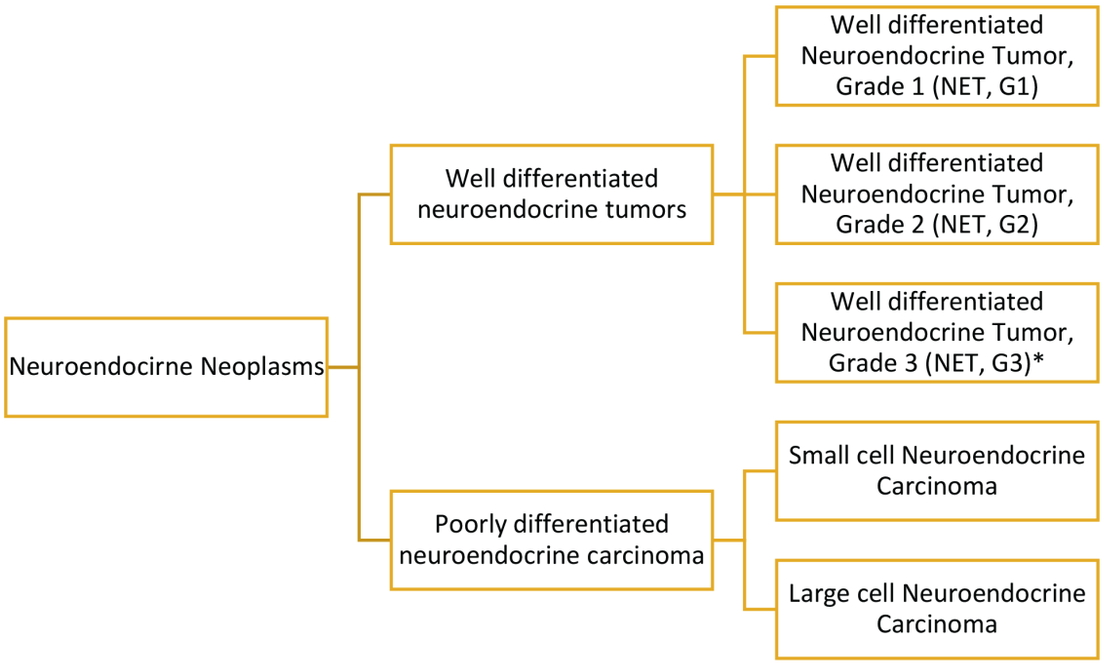
The diagnosis of neuroendocrine neoplasms of the head and neck, lung, ad GI tract share many common features, however, as mentioned, terminology can be confusing among the entities. We aim to provide clarity and simplify the schema for making a histopathologic diagnosis of neuroendocrine neoplasms while maintaining the current WHO guidelines for neuroendocrine tumor classification based on each organ. Misdiagnosing neuroendocrine carcinomas is not unusual and lead to improper therapeutic management. We occasionally come across such cases in our practice. The most common cause for misdiagnosis of a low-grade neuroendocrine tumor of the lung as small cell lung carcinoma (SCLC), for example, is limited tissue obtained as part of a biopsy combined with lack of experience and

inadequate or insufficient use of ancillary studies that can aid in proper classification. We would strongly recommend the use of Ki-67 immunohistochemistry study whenever typical morphologic features of SCLC are not seen in a biopsy specimen (e.g., rather nested growth pattern than large sheets of tumor cells, no or only focal necrosis, mitoses are not readily observed etc.). In general, the pathologist should refrain from classifying a neuroendocrine neoplasm as SCLC if Ki-67 proliferation index is less than 50% overall. Rather, obtaining more tissue for diagnosis may be suggested. The management of typical and atypical carcinoids is entirely different than SCLC, as in the later surgical resection is not an option in most patients. Neuroendocrine neoplasms variably express markers of neuroendocrine differentiation (synaptophysin, chromogranin, neural specific enolase (NSE), CD56 and INSM1), are comprised of tumor cells of epithelial or neuronal/neuroectodermal origin and occur at various anatomic sites throughout the body. Since in poorly differentiated tumors the expression of commonly used neuroendocrine markers like chromogranin and synaptophysin may be lost, the use of second-generation neuroendocrine differentiation biomarkers, especially the INSM1 (nuclear transcription factor that regulates neuroendocrine differentiation) has been shown to be helpful. Within the category of "neuroendocrine neoplasms" these tumors share similar morphologic and cytologic features, though features can vary depending on anatomic site and degree of differentiation. A word of caution regarding use and interpretation of neuroendocrine markers: there are malignancies that are not neuroendocrine but may be misinterpreted as either small cell or large cell neuroendocrine carcinomas due to aberrant expression of above-mentioned markers. In alveolar rhabdomyosarcomas of nasal cavity, for example, in addition to neuroendocrine markers, even unusual expression of cytokeratin may be seen. In such cases, the reviewing pathologist's experience in pattern recognition and keeping an open mind to alternative possibilities, is of most importance. Equally important is good communication among all medical staff involved in patient's care. In larger centers, it is common practice to discuss complex cases in interdisciplinary tumor boards, where all those involved, surgeons, oncologists, radiologists and pathologists, are present, ensuring that the therapy is appropriately tailored to the needs of every patient.

The histopathologic diagnosis of neuroendocrine neoplasms begins with the identification of neuroendocrine morphology. Classification and grading of the neoplasm varies based on the anatomic site. Generally, poorly differentiated neuroendocrine neoplasms are high-grade, with well-differentiated neoplasms falling into the low-grade category. The immunohistochemical profile and expression of neuroendocrine differentiation markers (synaptophysin, chromogranin, INSM1 and CD56) are essential for diagnosis. One should also evaluate for the presence and degree of necrosis. Mitotic count and Ki-67 proliferation index are important, with mitotic activity expressed as mitoses per 2 mm^2 area or 10 high-power fields (hpfs). When evaluating the Ki-67 proliferation index, the count should be performed on "hotspot" regions within the neoplasm, per WHO classification guidelines. When finalizing the pathologic report, the number of mitoses counted within the total area should be included in the final report, either as number of mitoses per 10hpfs or per mm^2. Additionally, in some cases, neuroendocrine tumors may have both neuroendocrine and non-neuroendocrine elements such as squamous cell or adenocarcinoma, encompassing the so-called "mixed" or "combined" malignancies.

2. Head and neck

Head and neck neuroendocrine carcinomas are rare neoplasms, accounting for less than 5% of all head and neck malignancies. With the 5th edition of the WHO head and neck neuroendocrine neoplasms (NEN), classification has been revised to include both the WHO and IARC unified terminology framework. With this classification, the upper aerodigestive tract and salivary gland NENs are included as a group. The classification of neuroendocrine neoplasm (NEN) can be further subclassified into well-differentiated neuroendocrine tumors (NETs) and assigned to proliferative grades 1, 2, and 3, while the poorly differentiated neuroendocrine carcinomas (NECs), including small cell and large cell neuroendocrine carcinoma, are grouped separately, similarly to general classification used for GI tract malignancies. To date, the G3 NET category remains provisional (**Figure 1**, **Table 1**).



2.1 Neuroendocrine tumor (NET)

Neuroendocrine tumors of the head and neck are well-differentiated neoplasms of neuroendocrine differentiation and can arise in the nasal cavity, paranasal sinuses, nasopharynx, oropharynx, larynx, and rarely in salivary glands and the oral cavity. Sinonasal NETs occur within the sinuses and the nasal cavity. The tumors macroscopically appear as polypoid, nodular or exophytic masses that may ulcerate and bleed. Histologically, these tumors are well-differentiated epithelial neoplasms. Architecturally, they are comprised of nests, trabecula, or cords of neuroendocrine cells with monotonous nuclei containing salt-and-pepper finely granular chromatin and moderately abundant cytoplasm, in a background of highly vascular stoma. It is important to assess for the degree of necrosis and mitoses per 2 mm2. The overall Ki-67 proliferation index is generally <20% and can be useful in determining Grade 1 versus Grade 2 NETs. All NETs will show diffuse positivity for neuroendocrine markers (INSM1, synaptophysin, chromogranin-A), keratins (CK7/8), and are negative for TTF-1 in most cases. NETs do not exhibit

Neoplasm	Tumor category	Diagnostic criteria
Well differentiated neuroendocrine neoplasm (Neuroendocrine Tumor, NET)	Well differentiated Neuroendocrine Tumor, Grade 1 (NET, G1)	Necrosis absent AND < 2 mitoses/2 mm^2 Ki-67 < 20%*
	Well differentiated Neuroendocrine Tumor, Grade 2 (NET, G2)	Necrosis present AND/ OR ≥ 2–10 mitoses/2 mm^2 Ki-67 < 20%*
	Well differentiated Neuroendocrine Tumor, Grade 3 (NET, G3)*	SCNEC or LCNEC cytomorphology absent >10 mitoses/2 mm^2 Ki-67 > 20%*
Poorly differentiated neuroendocrine neoplasm (Neuroendocrine Carcinoma, NEC)	Small cell Neuroendocrine Carcinoma (SCNEC)	SCNEC cytomorphology** >10 mitoses/2 mm^2 Ki-67 > 70%
	Large cell Neuroendocrine Carcinoma (LCNEC)	LCNEC cytomorphology*** >10 mitoses/2 mm^2 Ki-67 > 40%

**Provisional criteria applied at this time.*
***Small cell NEC cytomorphology: minimal cytoplasm, hyperchromatic molded nuclei, finely granular chromatin, inconspicuous nucleoli, and cell size smaller than combined diameter of three lymphocytes, prominent apoptotic bodies, and necrosis.*
****Large cell NEC cytomorphology: nested, organoid or trabecular growth with abundant cytoplasm, round nuclei with prominent nucleoli, peripheral palisading, rosette formation, comedo-pattern necrosis.*

Table 1.
Epithelial neoplasms of the upper aerodigestive tract and salivary glands.

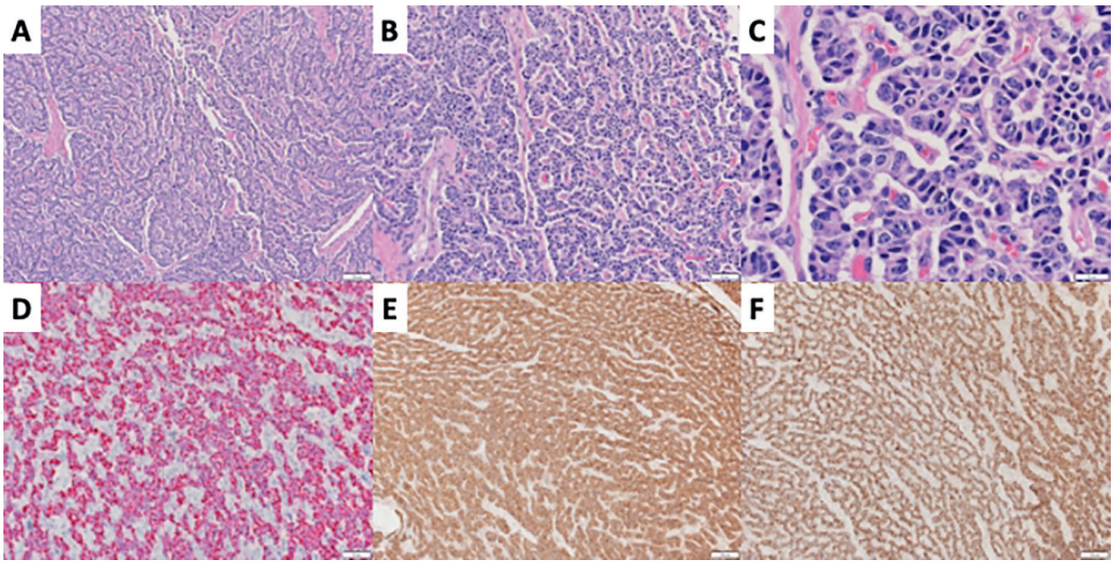


Figure 1.
Low-grade neuroendocrine tumors of lung. Carcinoids are characterized by organoid histologic pattern, frequently nested or trabecular, with no or only focal areas of necrosis (a, 100X; B, 200X). There is mild to moderate cytologic/nuclear atypia and mitoses are either absent or rarely observed (C, 400X). Cytokeratin is diffusely expressed (D, cytokeratin AE1/AE3 immunostain, 200X). These tumors are also diffusely positive for neuroendocrine markers (E, synaptophysin, 100X; F, chromogranin, 100X).

anormal p53 staining and show no loss of Rb. Rare cases of mixed neuroendocrine and non-neuroendocrine tumors have been described in the nasal cavity and larynx.

2.2 Small cell neuroendocrine carcinoma (SCNEC)

SCNEC of the head and neck is a poorly differentiated neuroendocrine carcinoma. By the time of clinical detection, the submucosal SCNECs are generally large, with areas of necrotic tumor or ulceration commonly seen. SNECs are comprised of sheets or nests of epithelial tumor cells with hyperchromatic nuclei, finely granular chromatin, inconspicuous nucleoli, and scant cytoplasm. The tumor cells are small (less than the combined diameter of three lymphocytes), with peripheral palisading, rosettes, or trabeculae often seen and often show nuclear molding. SCNECs have high mitotic counts (required: >10 per $2mm^2$) and necrosis. The SCNEC immunohistochemical profile includes reactivity for at least one cytokeratin (usually low-molecular weight), TTF1 (subset), and variable routine neuroendocrine markers (synaptophysin, chromogranin). Ki-67 proliferation index is >20% (often >70%), though the use of Ki-67 in grading has not yet been standardized and thus it is not established. p16 can be expressed independent of HPV infection. Particular anatomic sites have a higher frequency to occur as a combined lesion, with 5% of laryngeal SCNEC and up to 80% of oropharyngeal SCNEC occurring in combination with another histology, usually squamous cell carcinoma [1, 2].

2.3 Large cell neuroendocrine carcinoma (LCNEC)

LCNEC of the head and neck, like SCNEC, is a poorly differentiated neuroendocrine carcinoma most frequently occurring in the larynx, oropharynx, and sinonasal tract [3]. Of carcinomas arising in the larynx, 80% arise in the supraglottis [4]. Anatomic site LCNECs have independent risk factors, with laryngeal LNCECs associated with tobacco use in greater than 90% of cases, oropharyngeal and sinonasal LCNECs associated with high-risk HPV and smoking history, and the rare nasopharyngeal LCNECs associated with EBV positivity [5, 6]. LCNECs are comprised of nests or sheets of tumor cells with moderately hyperchromatic nuclei, vesicular or speckled chromatin, prominent nucleoli, and abundant cytoplasm. The tumor cells are large (greater than the combine diameter of three lymphocytes), with trabecular or organoid growth and peripheral palisading, rosettes, or comedo necrosis often seen. LCNECs have high mitotic counts (required: >10 per $2mm^2$) and necrosis. The immunohistochemical profile of LCENC is similar to that of other well differentiated neuroendocrine carcinomas and includes reactivity for at least one cytokeratin (usually low-molecular weight), TTF1 (minority of cases), p63 (variable), p40 (variable), and variable routine neuroendocrine markers (synaptophysin, chromogranin). Ki-67 proliferation index is always >20% (>40% in most cases), and while increased, is typically less than that of SCNECs. p16 can be overexpressed independent of HPV status.

3. Lung

Within the lung, neuroendocrine neoplasms are classified as follows: low grade typical carcinoid (TC), intermediate grade atypical carcinoid (AC), and high grade large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC) (**Table 2**). These classifications can be grouped collectively as lung neuroendocrine neoplasms (NENs), with further subclassification of well differentiated typical carcinoid and moderately differentiated atypical carcinoid as neuroendocrine tumors

	Typical carcinoid (TC)	Atypical carcinoid (AC)	LCNEC	SCLC
Mitoses per 2 mm^2	<2	2–10	>10 (median: 70)	> 10 (median: 80)
Necrosis	No	Rare, focal	Yes	Yes
Neuroendocrine morphology	Yes	Yes	Yes	Yes
Ki-67 proliferation index	<5%	5–20%	40- > 90%	70- > 95%
TTF1 expression	Peripheral tumors = positive Central tumors = negative	Peripheral tumors = positive Central tumors = negative	Frequently positive	Frequently positive
p40 expression	Negative	Negative	Negative	Negative
Combined with NSCLC component	No	No	Up to 25% of resected cases	Up to 25% of resected cases

Table 2.
Histologic and immunophenotypic characteristics of low and high-grade neuroendocrine tumors of the lung.

(NETs), and the poorly differentiated neuroendocrine carcinomas (NECs) including SCLC and LCNEC. Among primary lung neuroendocrine carcinomas, SCLC is most frequent, with a prevalence of approximately 14% among lung cancers in the United States. In contrast, typical and atypical carcinoids account for less than 2% of all lung malignancies.

LCNEC and SCLC are poorly differentiated and can contain different histologic components of other non-small cell carcinomas (usually adenocarcinoma or squamous cell carcinoma) and can be further classified as combined LCNEC or combined SCLC. For typical and atypical carcinoids, it is exceedingly rare to contain these other histologic components. These tumors are classified according to the degree of necrosis and the mitotic count, and for high grade LCNEC and SCLC, the cytologic features. Ki-67 is also commonly used by pathologists to differentiate the carcinoid tumors from the high-grade tumors, although it is not among the WHO diagnostic criteria. Further research is required to determine the optimal approach for using Ki-67 to differentiate typical versus atypical carcinoid, and carcinoids from LCNEC and SCLC. Classification is based primarily on mitotic counts per mm2, presence or absence of necrosis, and small cell versus large cell cytological features. Mitoses should be counted in areas of highest mitotic activity. Ki-67 is used to aid in distinguishing carcinoid tumors (NETs) from LCNEC and SCLC (NECs). Regarding Ki-67 proliferation index, based on most experts opinion, tumors with a Ki-67 index <5% are likely TC, >5% to <20% are AC, and > 40% are most likely high grade NEC (LCNEC or SCLC).

3.1 Diffuse idiopathic pulmonary neuroendocrine hyperplasia (DIPNECH)

Diffuse idiopathic pulmonary neuroendocrine hyperplasia (DIPNECH) represents the precursor lesion in a subset of carcinoid tumors and is comprised of multifocal areas of neuroendocrine cell hyperplasia associated with tumorlets in small airways.

While the pathogenesis remains unclear, the underlying etiology is thought to be secondary to chronic pulmonary (airway) injury. The proliferating neuroendocrine cells have round to oval nuclei with salt-and-pepper chromatin, with moderate amounts of amphophilic cytoplasm. Proliferations of >5 (single cells or in clusters) measuring <5 mm that invade beyond the bronchoalveolar wall are classified as tumorlets. DIPNECH expresses neuroendocrine markers (chromogranin, synaptophysin, CD56), TTF-1, and pancytokeratins, and is negative for p40, p63, and high-molecular weight cytokeratins. All neuroendocrine hyperplastic nodules larger than 5 mm are classified as carcinoids.

3.2 Typical carcinoid (G1 NET), atypical carcinoid (G2 NET), and carcinoid tumor NOS

Well differentiated carcinoid tumors are rare and commonly seen in younger patients with no smoking history. They occur both centrally and peripherally (TC = more central; AC = more peripheral). Current terminology proposed by the International Agency for Research on Cancer (IARC) and WHO Classification of Tumors Group includes well differentiated grade 1 (G1 NET) corresponding to TC, and well differentiated grade 2 (G2 NET) corresponding to AC. Carcinoids are associated with the precursor lesion diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) in 60–75% of cases [7]. The tumors have well differentiated architecture and neuroendocrine morphology. Tumor cells are small to intermediate in size, cuboidal to polygonal and uniform, with finely granular salt-and-pepper chromatin and abundant eosinophilic cytoplasm and inconspicuous nucleoli. Trabeculae, rosettes, palisading, and organoid nesting may be seen. Carcinoid tumors are typically positive for low molecular weight cytokeratins, chromogranin A, synaptophysin, CD56, INSM1, and can express TTF-1, the latter being more common in peripheral lesions (**Figure 1**). Ki-67 is not required for diagnosis, with still no consistent threshold for Ki-67 proliferation index in distinguishing TC (G1 NET) from AC (G2 NET), with its main function in excluding NEC (LCNEC and SCLC). Typical carcinoids have a Ki-67 index of <5% with <2 mitoses/2 mm2 without necrosis. Atypical carcinoids exhibit a Ki-67 index of 5–20% with 2–10 mitoses/2 mm2 and foci of necrosis (**Table 2**). The distinction of TC versus AC on biopsy can be challenging and should be reserved for the resection specimen, with the terminology of "carcinoid tumor NOS" being recommended. If features suggestive of AC are present (higher Ki-67 index, punctate necrosis) this can be mentioned in a comment favoring classification as AC even on limited tissue. For patients with metastatic pulmonary carcinoids, the term "metastatic carcinoid tumor NOS" is preferred. 3.3 Large Cell Neuroendocrine Carcinoma (LCNEC).

Large cell neuroendocrine carcinomas (LCNEC) are primarily located in the lung periphery. Metastases are present in 40–50% of patients at presentation, with common sites including liver and bone, with brain metastases occurring in approximately 50% of patients [8]. LCNEC is highly associated with smoking and represents 3% of resected lung carcinomas [9]. On gross appearance, the cut surface appears tan-red, well circumscribed, and necrotic. The tumor is high grade, poorly differentiated, and exhibits a neuroendocrine growth pattern with organoid nesting, trabecular, peripheral palisading, and rosettes. The tumor cells are large (usually greater than 3 lymphocytes), with coarsely granular chromatin, moderate to abundant cytoplasm, and prominent nucleoli. There is abundant necrosis and high

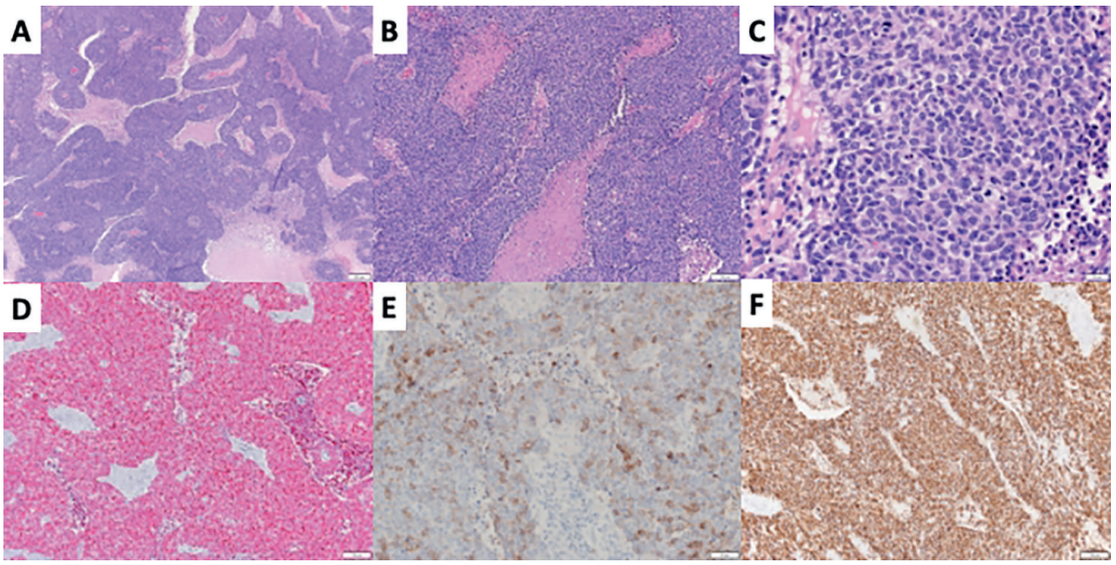


Figure 2.
Large cell neuroendocrine carcinoma (LCNEC). In LCNEC, neoplastic cells form large nests with extensive areas of central necrosis (a, 40X; B, 100X). At higher magnification the contrast to small cell carcinoma is obvious: The cytoplasm is conspicuous, there is no nuclear molding, the chromatin is condensed or clear with prominent nucleoli, crush artifact is absent or, at most, focal (C, 200X). Pan-cytokeratin is diffusely positive (D, AE1/AE3, 100X). Neuroendocrine markers may be weak or patchy, but usually are more consistently positive than in SCLC (E, synaptophysin, 200X). CD56 is very useful as a surrogate neuroendocrine marker when diffusely and strongly positive (F, CD56, 100X).

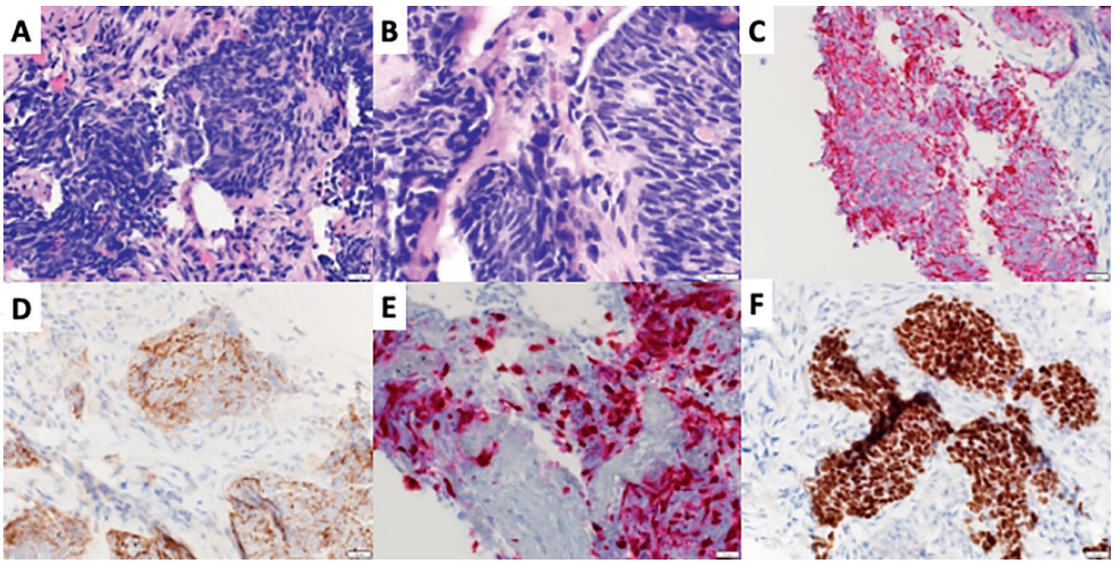


Figure 3.
Small cell lung carcinoma (SCLC). SCLC is comprised of sheets of atypical cells with extensive necrosis and crush artifact due to nuclear fragility (a, 200X). Tumor cells display high nuclear to cytoplasm ratio and there is nuclear molding due to close nuclear vicinity. Mitotic activity is brisk, nuclei are hyperchromatic or have granular (salt and pepper) appearance with inconspicuous or very small nucleoli (B, 400X). Cytokeratin is present in majority of cases, but frequently is discontinuous or punctate (C, AE1/AE3, 200X). Neuroendocrine markers, when expressed, are seen in most neoplastic cells, but occasionally may be patchy, focal or entirely absent (D, chromogranin, 200X). In cases with absent neuroendocrine differentiation, the diagnosis relies solely on morphology. Proliferation index is markedly increased, typically more than 70% (E, Ki-67, 200X). Expression of nuclear TTF-1 is not organ specific, but when present, indicates that the neoplasm is more likely to originate from lung than other organs (F, TTF-1, 200X).

mitotic counts (>10 mitoses/2 mm2, generally very high) (**Figure 2**). A majority of tumors express at least two neuroendocrine markers (synaptophysin, chromogranin A, CD56), pankeratins, and 50% also express TTF1 [10]. Tumor cells usually lack napsin A, with weak staining in a minority of cases, therefore the combination of strong TTF1 staining with negative/weak napsin A is likely to represent a NEC (LCNEC or SCLC) versus adenocarcinoma. The Ki-67 index is >40%. LCNEC can exist as a combined tumor, combined LCNEC, with components of adenocarcinoma, squamous cell carcinoma, spindle cell carcinoma, or giant cell carcinoma. 3.4 Small Cell Lung Carcinoma (SCLC).

Small cell lung carcinomas are typically centrally located in major airways, and can frequently involve mediastinal lymph nodes, with metastatic spread to other organs common at the time of presentation. Patients may present with acute symptoms due to intrathoracic tumor growth, extrapulmonary spread, or paraneoplastic syndromes, the latter being most common in SCLC and can be the patient's initial presentation. SCLC is a malignant epithelial tumor comprised of small cells with finely granular chromatin, scant cytoplasm, and inconspicuous or absent nucleoli. The tumor cells are small to mid-size (usually less than the size of 3 resting lymphocytes) and are oval to spindle shape. They have a high mitotic count (>10 mitoses/2 mm2) with frequent and vast necrosis (**Figure 3**). Most SCLCs express neuroendocrine differentiation markers (synaptophysin, chromogranin, CD56). SCLC may be negative for neuroendocrine markers in 5–10% of cases. INSM1 is consistently positive in SLCL with positive staining for pancytokeratins and TTF1, and is generally negative for CK20, Napsin A, p63, and p40 [11]. On gross examination, the tumor appears as a large perihilar mass with a tan, necrotic cut surface. SCLC can exist as a combined lesion of non-small cell carcinoma which can include LCNEC, adenocarcinoma, squamous cell carcinoma, large cell carcinoma, spindle cell carcinoma, or giant cell carcinoma. In combined SCLC, the second component should make up > = 10% of the tumor. While not part of the diagnostic criteria, Ki-67 can be useful in crushed biopsies to avoid the pitfall of misdiagnosing carcinoid tumors, which may also show significant crush artifact, with a very high Ki-67 index (>70%) excluding TC or AC.

4. Gastrointestinal tract

Overall, based on the NCI-SEER database, the gastrointestinal tract has the highest incidence of neuroendocrine tumors (NETs) in the body, comprising about 55% of all NETs. By contrast the second most common site is the bronchopulmonary system, accounting for 25% of NETs. In the gastrointestinal tract, the small intestine is by far the site with highest incidence, accounting for approximately 45% of all neuroendocrine tumors, followed by rectum (20%), appendix (17%), colon (11%) and stomach (7%).

4.1 Esophagus

Esophageal neuroendocrine neoplasms (NENs), like other organ systems, are comprised of well differentiated neuroendocrine tumors (NETs), poorly differentiated neuroendocrine carcinomas (NECs), and mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs), which includes mixed adenoneuroendocrine carcinoma (MANEC). NEN are typically small lesions most commonly occurring in the lower

esophagus, often associated with Barrett esophagus, and infiltrative at the time of diagnosis [12, 13]. Esophageal NEN share similar features of NEN of other anatomic sites. While NET of the esophagus has been described in the literature, the majority of cases are in fact NECs, and are further subclassified as small cell NEC (SNEC) and large cell NEC (LCNEC) [14]. The NEC is comprised of medium-sized to large cells (SNEC and LCENC, respectively) arranged in solid, rosette-like, or palisading architecture with elongated nuclei with fine chromatin, inconspicuous nucleoli in SNEC and large nucleoli in LCNEC, and scant basophilic cytoplasm. Mitotic activity and necrosis are substantial. The immunohistochemical profile of NEC is similar to that of other neuroendocrine carcinomas and includes variable reactivity for neuroendocrine markers (synaptophysin > chromogranin), TTF1 (majority of cases), and absence of p40 and CK5/6 expression. NET share similar histopathologic features with NEC, however NET will have less necrosis, with stronger reactivity for chromogranin (versus NEC). Additionally, many NETs express hormone immunoreactivity (glucagon, PP, gastrin, and calcitonin) [15, 16]. MiNENs of the esophagus usually consist of NEC and either squamous cell carcinoma or adenocarcinoma (if underlying Barrett mucosa or ectopic gastric mucosa is identified) [17]. Grading of esophageal NENs is performed using the same grading system as used for gastroenteropancreatic NENs.

4.2 Stomach

The classification for neuroendocrine neoplasms of the stomach is identical to that of the esophagus, as follows: neuroendocrine neoplasms (NENs) comprised of well differentiated neuroendocrine tumors (NETs), poorly differentiated neuroendocrine carcinomas (NECs), and mixed neuroendocrine–non-neuroendocrine neoplasms (MiNENs) including MANEC. NEN of the stomach occur with a site-specific anatomic distribution based on tumor subtype. Enterochromaffin-like–cell (ECL-cell) NETs arise in the corpus/fundus, D-cell and G-cell NETs in the antrum, and enterochromaffin-cell (EC-cell) NETs in both the antrum and corpus/fundus [18]. NECs and MiNENs usually occur in the antral or cardiac regions but can occur at any site. NETs are comprised of a population of monotonous cells with round nuclei and finely stippled chromatin, with NECs composed of sheets of small or large poorly differentiated cells. The immunohistochemical profile of gastric NENs is like that of other gastrointestinal neuroendocrine neoplasms at other anatomic sites. Gastric NECs are comprised of sheets of poorly differentiated small or large cells, and are further subtyped as small cell NEC (SCNEC) and large cell NEC (LCNEC) based on cell cytomorphologic size. LCNECs are comprised of large cells with vesicular nuclei with prominent nucleoli and abundant eosinophilic cytoplasm. SCNECs are comprised of small to mid-sized cells with scant cytoplasm and hyperchromatic nuclei and inconspicuous or absent nucleoli. NECs have a high mitotic count (> 20 mitoses/mm^2) and high Ki-67 proliferation index (>40–50%). NECs express positivity for neuroendocrine markers (synaptophysin > chromogranin), may be focally positive for TTF-1, and have high mitotic counts and an increased Ki-67 proliferation index. Mixed adenocarcinoma neuroendocrine carcinomas (MANECs) are MiNENs composed of adenocarcinoma associated with NEC component. The neuroendocrine component of MANEC has a high Ki-67 proliferation index (> 55%) [19]. Mixed adenocarcinoma-NETs are comprised of areas of tubular, papillary, or mucinous adenocarcinoma mixed with areas of G1 or G2 NET.

Gastric ECL-cell NETs are histamine-producing, and typically show smaller microlobular or trabecular architecture and lack necrosis. They are comprised of

monotonous well differentiated cells with abundant cytoplasm and round nuclei and have inconspicuous or absent nucleoli. Mitotic figures are rare to absent. Type 1 ECL-cell NETs usually infiltrate beyond the muscularis mucosae, with a majority of cases classified as G1 or G2. Type 2 ECL-cell NETs are confined to the mucosa and submucosa when they are G1, with Type 3 NETs (G1 to G3) invading into the gastric wall with distant and lymph node metastases common. ECL-cell NETs are immunoreactive for VMAT2, HDC, and SSTR2A, and can show scattered positivity for serotonin, ghrelin, somatostatin, and α-hCG [20, 21]. Gastric EC-cell NETs produce serotonin and have histomorphological features similar to ileal EC-cell NETs, with nests of uniform tumor cells with peripheral palisading. EC-cell NET cells are immunoreactive for serotonin, SSTR2A, and CDX2. Gastric G-cell NETs produce gastrin and usually show monotonous cells with scant cytoplasm in a trabecular or gyriform pattern, and are immunoreactive for gastrin and SSTR2A. Gastric D-cell NETs produce somatostatin and are comprised of well differentiated monomorphic cells that are immunoreactive for somatostatin, chromogranin, synaptophysin, and SSTR2A. Grading of gastric NENs is performed by using the same system used for other gastroenteropancreatic NENs. Staging of gastric NETs is performed using NET-specific Union for International Cancer Control (UICC) TNM staging system; NEC and MiNEN are staged following the system used for adenocarcinomas.

4.3 Small intestine and ampulla

Neuroendocrine neoplasms (NENs) of the small intestine and ampulla, like other NEN of the GI tract, are epithelial neoplasms with neuroendocrine differentiation of the duodenum, jejunum, and ileum, are classified as follows: well differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs). In the small intestine MiNENs have an exocrine component (commonly adenocarcinoma) and a neuroendocrine component (usually NEC), each accounting for ≥30% of the neoplasm. NETs are further subclassified based on hormone production, and include gastrinoma, somatostatin-producing neuroendocrine tumor, gangliocytic paraganglioma, NEC (further subtyped as small cell neuroendocrine carcinoma (SNEC) and large cell neuroendocrine carcinoma (LCNEC), and MiNEN. Duodenal NETs are located in part 1 or 2, with those in part 2 predominating in the ampullary region in 95% of cases. Somatostatin-producing NET, gangliocytic paraganglioma, and NEC are almost exclusively located in the ampullary region. Uniquely, jejuno-ileal NETs are often multifocal (2–100 tumors) in at least 30% of cases [22, 23], and those located in the upper jejunum tend to be large and locally infiltrative [24].

NETs of the small intestine are composed of rather bland appearing tumor cells with oval to round nuclei with finely granular chromatin. High-grade poorly differentiated NECs grow in sheets, although poorly formed trabeculae or nests may be seen. The tumor cells are moderately pleomorphic and may show large cell or small cell architecture. Some NETs may have a concurrent adenocarcinoma component (MiNEN), while some arise in association with an adenoma. Duodenal gastrin-expressing NETs (G-cell NETs) are more commonly arranged in trabeculae. Ampullary Somatostatin-expressing NETs (D-cell NETs) have tubuloglandular architecture and may contain psammoma bodies. Jejuno-ileal serotonin-expressing NETs (EC-cell NETs) are composed of nests cells with peripheral palisading, often with pseudoglandular formation. Triphasic histology is typically seen in gangliocytic paraganglioma, and includes neuroendocrine, Schwannian, and ganglion cell–like

components. NENs are comprised of round to oval shaped cells with neuroendocrine architecture with round nuclei and finely stippled chromatin. The immunohistochemical profile of NENs includes expression of neuroendocrine markers (synaptophysin and chromogranin) and keratins (cytokeratin AE1/AE3 and CAM5.2). NENs may also express peptide hormones and/or biogenic amines. A unique consideration is ileal serotonin-producing EC-cell NETs, as they and their metastases are positive for CDX2, and > 90% show positivity for SSTR2A [25]. NECs are comprised of sheets of poorly differentiated cells of either small or large size, and similar to NENs of other anatomic sites, have a high mitotic count and Ki-67 index, however, NECs are less likely to express the typical markers of neuroendocrine differentiation. In comparison with G3 NETs, NECs have loss of RB1 and aberrant p53. NENs of the small intestine and ampulla are graded using the same system used for other gastroenteropancreatic NENs. A staging system for NETs is in place; staging of NECs and MiNENs should be performed using the same staging system used for adenocarcinoma.

4.4 Appendix

Keeping consistent with other neuroendocrine neoplasms of the GI tract, appendiceal neuroendocrine neoplasms (NENs) of the appendix are epithelial neoplasms with neuroendocrine differentiation and are classified as well differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs, further subclassified as small cell neuroendocrine carcinoma (SNEC) and large cell neuroendocrine carcinoma (LCNEC)). MiNENs of the appendix are epithelial neoplasms with a neuroendocrine component combined with a non-neuroendocrine component, with each component comprising at least 30% of the neoplasm. Many small NETs are not macroscopically visible, and as such, grossing of appendiceal specimens should include the entirety of the appendiceal tip in two longitudinal sections [26, 27]. EC-cell and L-cell NETs are composed of large nests of cells; peripheral palisading and glandular formation is often seen. L-cell NETs show a distinct glandular or trabecular growth pattern. Mitotic figures are rare to absent, and necrosis is typically not seen. Examination of the stroma will show a fibrotic response in most cases. NECs of the appendix are rare and histomorphologically identical to colonic NECs, with small or large cell cytomorphology (SCNEC and LCNEC, respectively), sheets of poorly differentiated cells, and high Ki-67 proliferation indices [26, 28, 29]. Serotonin production can be determined by immunohistochemistry in EC-cell NETs, along with reactivity for typical neuroendocrine markers (chromogranin, synaptophysin). There may be S100-positive spindle cells surrounding the nests of tumor cells. Interestingly, L-cell NETs are immunoreactive for chromogranin B rather than chromogranin A. Appendiceal NENs are graded using the same system used for other gastroenteropancreatic NENs and staged using the parameters for appendiceal adenocarcinomas.

4.5 Colon and Rectum

Colorectal neuroendocrine neoplasms (NENs), like other gastrointestinal NENs, are epithelial neoplasms with neuroendocrine differentiation, and include well differentiated neuroendocrine tumors (NETs), poorly differentiated neuroendocrine carcinomas (NECs), and mixed neuroendocrine–non-neuroendocrine neoplasms (MiNENs). NETs can be further subclassified into serotonin-producing

enterochromaffin-cell neuroendocrine tumor and glicentin-PYY-producing L-cell neuroendocrine tumor, while NECs can be further subclassified as small cell neuroendocrine carcinoma (SNEC) and large cell neuroendocrine carcinoma (LCNEC).

Colorectal NETs are comprised of a bland appearing monotonous population of cells with round nuclei and finely stippled salt-and-pepper chromatin. NETs are immunoreactive for neuroendocrine markers (synaptophysin, chromogranin) and are usually G1 or G2. EC-cell NETs are immunoreactive for neuroendocrine markers (synaptophysin, chromogranin), serotonin, SSTR2A, and CDX2 (minority of cases). L-cell NETs are immunoreactive for neuroendocrine markers (synaptophysin > chromogranin) and PYY, glicentin, GLP-1, GLP-2, SSTR2A, and PAP (minority of cases) [30–32]. Colorectal NECs are comprised of sheets of poorly differentiated small cell or large cells with organoid architecture. Trabecular, rosette-like, palisading, and solid patterns can be seen. Necrosis is often present. The poorly differentiated carcinoma cells may have small cell features (small cells with scant cytoplasm) or large cell features (large cells, abundant cytoplasm). NECs have a high degree of cellular pleomorphism, a high mitotic count, and elevated Ki-67 proliferation index. NECs are immunoreactive for neuroendocrine markers (CD56, synaptophysin > chromogranin). TTF1, SSTR2A, and CDX2 may also be positive [33–35]. Colorectal MiNENs are usually comprised of both a poorly differentiated NEC component mixed with an adenocarcinoma component [36–38]. Staging of colorectal NETs follows the criteria of the Union for International Cancer Control (UICC) TNM classification and the American Joint Committee on Cancer (AJCC) cancer staging manual. Staging of NEC and MiNEN follows the criteria for adenocarcinoma.

4.6 Anal Canal

Anal canal neuroendocrine neoplasms (NENs) are epithelial neoplasms with neuroendocrine differentiation and include well differentiated neuroendocrine tumor (NET), poorly differentiated neuroendocrine carcinomas (NECs), including small cell neuroendocrine carcinoma (SNEC) and large cell neuroendocrine carcinoma (LCNEC), and mixed neuroendocrine–non-neuroendocrine neoplasms (MiNENs). Anal NENs are comprised of various architectures of cells with neuroendocrine differentiation that express immunoreactivity for neuroendocrine differentiation (synaptophysin, chromogranin). NETs are graded using the same system used for other gastroenteropancreatic NETs, and are often G1 or G2 [39]. NEC is comprised of sheets of cells with severe nuclear pleomorphism, high nuclear to cytoplasmic ratio, high mitotic activity, and increased Ki-67 proliferation index. NECs of the anal canal are often immunoreactive for TTF1. MiNENs, like in MiNENs of other gastrointestinal sites, are usually comprised of a NEC component mixed with an adenocarcinoma component [40, 41]. MiNENs consisting of SCNEC and SCC can also occur.

In the anal canal, the differentiation of NEC and MiNEN from poorly differentiated/basaloid SCC, poorly differentiated adenocarcinoma, melanoma of the anal canal, and basal cell carcinoma of the perianal skin is important. An immunohistochemical workup can be helpful in distinguishing these entities; with chromogranin and synaptophysin confirming the diagnosis of NEC or a NEC component in MiNEN, p63/p40 positivity excluding NEC (positive in SCC and basal cell carcinoma), and CK7 positivity excluding NEC. Additional melanoma markers can be useful in ruling out melanoma of the anal canal.

4.7 Liver

Hepatic neuroendocrine neoplasms (NENs) are epithelial neoplasms with morphological and immunohistochemical features of neuroendocrine differentiation and include well differentiated neuroendocrine tumors (NETs), poorly differentiated neuroendocrine carcinomas (NECs) including small cell neuroendocrine carcinoma and large cell neuroendocrine carcinoma (SCNEC and LCNEC, respectively), and mixed neuroendocrine–non-neuroendocrine neoplasms (MiNENs). Primary hepatic NENs are much rarer than metastatic lesions. Hepatic NETs are comprised of a monotonous population of cells with mild cytologic atypia and coarse chromatin and are generally graded as G1 or G2. Hepatic G3 NETs have yet to be described in the literature [42]. NETs are strongly and diffusely immunoreactive for neuroendocrine markers (synaptophysin, chromogranin), with a Ki-67 index of <20% [43]. Hepatic NECs are comprised of sheets of poorly differentiated small or large cells with frequent necrosis and mitotic figures that are immunoreactive for neuroendocrine markers (synaptophysin > chromogranin). The Ki-67 index is markedly increased (>50%). Commonly, NEC will be identified as a component of a mixed hepatocellular-neuroendocrine carcinoma. Hepatic MiNENs are generally more common than NECs. MiNENs are comprised of a NEC component mixed with a non-neuroendocrine component, either hepatocellular carcinoma or cholangiocarcinoma. Each component is required to be morphologically and immunohistochemically discrete, and account for ≥30% of the neoplasm. Most cases include a component of hepatocellular carcinoma, which may be the predominant component [44–47]. Hepatic NETs are staged using the Union for International Cancer Control (UICC) criteria for neoplasms of the intrahepatic bile ducts. There is no staging system specific for hepatic NECs or MiNENs.

4.8 Gallbladder and bile ducts

Gallbladder and bile duct neuroendocrine neoplasms (NENs) are very rare epithelial neoplasms with neuroendocrine differentiation, and include well differentiated neuroendocrine tumors (NETs), poorly differentiated neuroendocrine carcinomas (NECs) including small cell neuroendocrine carcinoma and large cell neuroendocrine carcinoma (SNEC and LCNEC, respectively), and mixed neuroendocrine–non-neuroendocrine neoplasm (MiNEN). NETs of the gallbladder and bile ducts are comprised of nests, trabeculae, or tubules of bland appearing cells with mild cytologic atypia. Tumor cells have uniform round to oval nuclei, inconspicuous nucleoli, and moderate amounts of cytoplasm. NETs are immunoreactive for neuroendocrine markers of differentiation (chromogranin, synaptophysin). A unique subtype of NET found in the gallbladder, clear cell NET, is characterized by abundant tumor cells with characteristic foamy cytoplasm. Of note, clear cell NETs of the gallbladder can be associated with VHL, and unlike sporadic cases, may stain positive for inhibin [48, 49]. NECs of the gallbladder and bile duct include small cell and large cell subtypes. SNECs are comprised of sheets of molded round cells with hyperchromatic nuclei and inconspicuous nucleoli. Tubules and rosette-like structures can be seen. LCNEC is comprised of sheets of large poorly differentiated pleomorphic cells with vesicular nuclei, prominent nucleoli, and moderate cytoplasm. NECs have a markedly increased Ki-67 proliferation index and high mitotic activity, with both single cell and confluent necrosis. NECs are variably immunoreactive for neuroendocrine markers of differentiation (synaptophysin > chromogranin), and epithelial markers (AE1/AE3), with SNECs often positive for TTF1. Gallbladder and bile duct NENs are staged in a similar manner to other gallbladder carcinomas.

4.9 Pancreas - introduction

Akin to other neuroendocrine neoplasms, pancreatic NENs (PanNENs) show neuroendocrine differentiation and express synaptophysin and usually chromogranin. PanNEN includes malignant well differentiated NENs, neuroendocrine tumors (NETs), and poorly differentiated NENs, neuroendocrine carcinomas (NECs). PanNENs are further classified into functioning and non-functioning neoplasms. PanNENs associated with a clinical syndrome secondary to abnormal hormone secretion are classified as functioning PanNETs and include insulinomas, gastrinomas, glucagonomas, and VIPomas, along with tumors that produce serotonin, ACTH, GHRH, PTHrP, and CCK. Functioning tumors the pancreas will not be discussed in this chapter. Non-functioning PanNENs may secrete hormones or biogenic substances (PP, somatostatin, chromogranin), however not to an extent to produce symptoms or cause a clinical syndrome. PanNETs are well differentiated tumors of low, intermediate, or high grade, based on their proliferative activity; G1 (<2 mitoses/2 mm^2 and a Ki-67 proliferation index <3%), G2 (2–20 mitoses/2 mm^2 or a Ki-67 proliferation index of 3–20%), and G3 (>20 mitoses/2 mm^2 or a Ki-67 proliferation index >20%). PanNECs are poorly differentiated high grade NENs comprised of markedly atypical small or large cells, and have markedly increased proliferative activity (>20 mitoses/2 mm^2 or a Ki-67 proliferation index >20%). PanNENs should be differentiated as NET or NEC based on histology and immunohistochemistry. PanNETs are graded as G1, G2, or G3, while PanNECs are high grade, by default. MiNEN is a mixed neoplasm with a neuroendocrine component mixed with a non-neuroendocrine component. Staging of PanNEN follows the Union for International Cancer Control (UICC) TNM classification and the American Joint Committee on Cancer (AJCC) cancer staging manual. Tumor site, size, and metastatic extent should be evaluated.

4.10 Pancreas - nonfunctioning neuroendocrine tumors

Pancreatic non-functioning (non-syndromic) neuroendocrine tumors (NF-PanNETs) are well differentiated epithelial neuroendocrine neoplasms (PanNENs). NF-PanNETs usually are ≥0.5 cm and lack a distinct hormonal syndrome. NF-PanNETs measuring <0.5 cm are subclassified as microadenomas, with the multifocal occurrence of microadenomas known as microadenomatosis. NF-PanNETs can be further subclassified as oncocytic NF-PanNET, pleomorphic NF-PanNET, clear cell NF-PanNET, and cystic NF-PanNET.

NF-PanNETs are comprised of well differentiated cells growing an organoid growth pattern with mild cytologic atypia and salt-and-pepper chromatin. While organoid architecture is the most common growth pattern, solid-nesting, solid-paraganglioma-like, trabecular, gyriform, and glandular growth patterns can be seen. NF-PanNETs lack necrosis and mitoses, and have a vascular, dense, collagenized stroma. The stroma may contain calcifications (including psammoma bodies). The tumor cells will contain variable amounts of membrane-bound nonspecific granules.

A subset of NF-PanNETs have unique cytological features. Oncocytic NF-PanNETs are comprised of cells with markedly abundant eosinophilic cytoplasm, and nuclei with prominent and enlarged nucleoli. Pleomorphic NF-PanNETs are comprised of cells with marked pleomorphism, however, the tumors cells have a normal N:C ratio and a low Ki-67 proliferation index. Clear cell NF-PanNETs are comprised of tumor cells with markedly increased cytoplasmic lipid vacuoles and nuclear scalloping. Like NENs of the gallbladder and bile duct, clear cell NF-PanNETs

are often identified in patients with VHL. NF-PanNETs associated with VHL are usually immunoreactive for HIF1A and CAIX [50, 51]. NF-PanNETs are immunoreactive for synaptophysin (diffuse, strong) and chromogranin (focal), and NSE, CD56, and CD57. Expression of CEA and CA19–9 may be seen. NF-PanNETs also express ISL1, which can be helpful in determining pancreatic primary versus metastasis.

Despite being clinically nonfunctional, a large proportion of NF-PanNETs express positivity for peptide hormones, with 40% of tumors being multihormonal [52]. The hormones typically expressed include glucagon, PP, and somatostatin, with hormone expression linked with unique histomorphology. Glucagon-positive NF-PanNETs may show cystic change with a trabecular or reticular pattern [53]. Somatostatin-positive NF-PanNETs may show paraganglioma-like pattern with glandular structures and psammoma bodies [54]. Serotonin-positive NF-PanNETs may show small cell nests and tubules within dense stromal sclerosis arising adjacent to the main duct, causing duct obstruction and dilatation [55, 56]. Staging of NF-PanNETs follows the (UICC) TNM classification and the American Joint Committee on Cancer (AJCC) cancer staging.

4.11 Pancreas: neuroendocrine carcinoma

Pancreatic neuroendocrine carcinoma (PanNEC) is a high-grade malignant epithelial neoplasm with neuroendocrine differentiation, and includes small cell neuroendocrine carcinoma (SNEC) and large cell neuroendocrine carcinoma (LCNEC). PanNECs are comprised of sheets of small or large poorly differentiated tumor cells. The tumor cells of NECs of small cell type have round nuclei with finely granular chromatin and scant cytoplasm. Molding is often seen. The tumor cells of NECs of large cell type often have a nesting or trabecular pattern, with round large nuclei with vesicular chromatin, prominent nucleoli, and moderate amphophilic cytoplasm. Necrosis is often seen in addition to increased mitoses (>20 mitoses/2 mm^2) and a markedly elevated Ki-67 proliferation index (typically >60–80%). It is important to identify the immunohistochemical profile of NEC, as many markers of neuroendocrine differentiation can be variably or focally expressed. The carcinoma cells will often be synaptophysin positive, with variable expression of chromogranin. Small cell carcinomas lack abundant cytoplasmic granules and are more commonly negative for chromogranin [57]. CD56 has low specificity and may or may not be expressed. Additionally, focal expression of neuroendocrine markers is not uncommon in some non-neuroendocrine neoplasms of the pancreas [58].

If neuroendocrine architecture and expression of neuroendocrine markers is identified in a tumor with a low Ki-67 index, other possibilities should be considered. Acinar cell carcinomas are often confused for NECs due to their similar appearance, high-grade nature, and common expression of neuroendocrine markers (at least focally). NECs can be associated with other non-neuroendocrine carcinoma types, including ductal adenocarcinoma or acinar cell carcinoma. If each component accounts for ≥30% of the tumor, the term "mixed neuroendocrine–non-neuroendocrine carcinoma (MiNEC)" is appropriate. Staging of PanNECs is based on the Union for International Cancer Control (UICC) TNM classification for carcinomas of the exocrine pancreas, rather than the staging for more well differentiated NETs (PanNETs).

4.12 Pancreas: MiNENs

Pancreatic mixed neuroendocrine–non-neuroendocrine neoplasms (MiNENs) are mixed neuroendocrine and non-neuroendocrine neoplasms with each component

constituting ≥30% of tumor volume, with the neuroendocrine component being substantiated by immunohistochemistry (expression of synaptophysin or chromogranin). While the majority of the neuroendocrine component of MiNENs is composed of carcinomas, MiNENs with a component of a well differentiated neuroendocrine tumor also exist. If a MiNEN is identified with a well differentiated neuroendocrine component, exclusion of collision tumor is appropriate. MiNEN itself is a conceptual category and not a discrete entity, and as such, diagnoses indicating the specific cellular components should be applied.

5. Conclusion

While the general classification of neuroendocrine neoplasms follows a similar overall structure for most organs, there is now a concerted effort to bridge the gap that exists in the lung regarding classification of neuroendocrine tumors with high-grade morphology that maintain general morphologic features of neuroendocrine tumors, but demonstrate a higher mitotic activity than what is accepted for atypical carcinoid tumors. Although the behavior of these neoplasms falls between atypical carcinoid group and high-grade neuroendocrine carcinomas (SCLC and LCNEC) these tumors are still classified as either small or large cell neuroendocrine carcinomas. It is the general expectation that this category will be better defined and accepted as separate group of neoplasms and will render the classification of lung tumors more consistent and closer to general classification of neuroendocrine neoplasms in other organs.

Conflict of interest

The authors declare no conflict of interest.

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