Neuroendocrine Tumors of the Lung

An Update

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• ***Context.*—The 2004 World Health Organization (WHO) classification recognizes 4 major types of lung neuroendocrine tumors: typical carcinoid, atypical carcinoid, small cell lung cancer, and large cell neuroendocrine carcinoma. Markedly different prognostic implications and treatment paradigms for these tumors underscore the importance of accurate pathologic diagnosis.**

***Objective.*—To detail the clinical and pathologic features of lung neuroendocrine tumors, with emphasis on diagnostic criteria, differential diagnoses, and application of immunohistochemistry. The emerging evidence for the utility of Ki-67 (MIB1) in the diagnosis of lung neuroendocrine tumors, particularly in small biopsy and cytology, is emphasized.**

***Data Sources.*—The 2004 WHO classification, other published literature, and primary material from the author's institution.**

***Conclusions.*—The current WHO classification of neuroendocrine tumors is based on morphologic features in combination with precisely defined mitotic rate and absence or presence of necrosis. Ki-67 (MIB1) is emerging as a useful ancillary tool in the diagnosis of these tumors. Continued research efforts are needed to identify additional immunohistochemical and molecular biomarkers that can serve as ancillary diagnostic tools and as potential therapeutic targets for these diseases.**

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OVERVIEW OF CLASSIFICATION

The 2004 World Health Organization (WHO) classification recognizes 4 major types of lung neuroendocrine tumors (NETs): typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC), and small cell lung cancer (SCLC).[1] These tumors are further grouped in a 3-tiered grading system as low grade (TC), intermediate grade (AC), and high grade (LCNEC and SCLC) NETs (Table 1).

INCIDENCE OF PULMONARY VERSUS EXTRAPULMONARY NEUROENDOCRINE TUMORS

Neuroendocrine tumors represent 25% of primary lung neoplasms, with the remaining 75% composed of non–small cell carcinoma (NSCLC) and a few rare tumors. The most common lung NET is SCLC (20%), followed by LCNEC (3%), TC (2%), and AC (0.2%). Interestingly, the incidence of carcinoid tumors in the United States had an unexplained but substantial increase in the last 30 years,[2] possibly related to increased utilization of imaging techniques.

Lung is overwhelmingly the most common site of origin of small cell carcinoma in the body: more than 95% of small cell carcinomas arise in the lung, whereas extrapulmonary small cell carcinomas (such as of bladder, prostate, esophagus, and cervix) are extremely rare.[3] Therefore, the differential diagnosis of metastatic small cell carcinoma of unknown primary site should include lung origin as the top consideration.

Lung is also a common site of carcinoid tumors, which account for 30% of well-differentiated NETs in the body. Lung is second only to tubular gastrointestinal tract and is more common than pancreas in incidence of well-differentiated NETs.[4] Therefore, similar to SCLC, the differential diagnosis for metastatic well-differentiated NETs, particularly at extrahepatic sites such as bone, should include the possibility of a lung primary (whereas metastasis from gastroenteropancreatic NETs predominate in the liver, lung is a more common site of origin for skeletal metastases).[5]

CLINICAL PRESENTATION OF LUNG NEUROENDOCRINE TUMORS

There are striking differences in the clinical settings of carcinoid tumors versus high-grade neuroendocrine (NE) carcinomas: the former occur in younger patients (mean age, 45–50 years), with no predilection for sex or smoking history, whereas the latter occur predominantly in older patients (mean age, 65 years), who are more frequently male and almost invariably heavy smokers. The association of SCLC with smoking is so strong that this diagnosis in a nonsmoker is considered exceptional and should be carefully reevaluated.

In contrast to gastroenteropancreatic NETs, lung carcinoids are only rarely associated with hypersecretion: for example, carcinoid syndrome occurs in less than 2% of patients with lung carcinoids, as compared to 10% of patients with gastrointestinal carcinoids.[6] On the other hand, ectopic hormone production and paraneoplastic syndromes are frequent in SCLC, including the syndrome

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Table 1. Summary of Diagnostic Criteria and Grading of Lung Neuroendocrine Tumors Based on the 2004 World Health Organization Classification

	Typical Carcinoid	Atypical Carcinoid	Large Cell Neuroendocrine Carcinoma	Small Cell Lung Carcinoma
Grade	Low	Intermediate	High	High
Morphology	Well-differentiated NET	Well-differentiated NET	Poorly differentiated NET	Poorly differentiated NET
Mitoses per 10 HPFs[a]	<2	2–10	>10 (median, 70)	>10 (median, 80)
Necrosis	None	Present (focal punctate)	Present (extensive)	Present (extensive)

Abbreviations: HPFs, high-power fields; NET, neuroendocrine tumor.

[a] Field diameter with a ×40 objective (0.2 mm^2).

of inappropriate antidiuretic hormone secretion and Cushing syndrome. Lung carcinoids, but not high-grade NE carcinomas, arise in 5% of patients with multiple neuroendocrine neoplasia 1 (MEN1).[7]

PROGNOSIS OF PATIENTS WITH LUNG NEUROENDOCRINE TUMORS

Neuroendocrine tumors of the lung represent a spectrum of clinical behavior from indolent (TC) to rapidly fatal (SCLC). Patients with TC have excellent survival (>87%).[8,9] Nevertheless, it is recommended that the term *benign* not be used in reference to TC because these tumors are capable of regional lymph node metastasis in 10% to 15% of cases, and distant metastases (most commonly to liver and bone) in an additional 3% to 5%.[10,11] Therefore, TC is defined as a low-grade malignancy. Atypical carcinoid is significantly more aggressive than TC, with higher frequency of nodal (~50%) and distant (~20%) metastases,[10,11] and a 5-year survival of 60%.[12] Late metastases are known to occur in patients with carcinoid tumors, and a minimum of a 10-year follow-up is recommended.[13]

At the other end of the clinical spectrum is SCLC, for which typical survival is still measured in months, and long-term survival is highly unusual (5-year survival is <5%).[14] Most patients have hilar nodal metastases, frequently massive, and two-thirds of patients present with distant metastases (typical sites are brain, liver, adrenal, bone, and bone marrow).[15] Small cell lung cancer presenting as a solitary mass without evidence of metastases is a rare occurrence (<5% of SCLCs).[16]

The prognosis associated with LCNEC is less well defined. Large cell neuroendocrine carcinoma is considered a highly aggressive disease, but a wide range of survival (15%–57%) is reported,[17] with a weighted mean of 34%. In some series, prognosis of LCNEC is as poor as that of SCLC.[18] The wide range of reported clinical behavior of LCNEC probably reflects the different inclusion criteria and treatment approaches in various studies.

UPDATE ON CLINICAL MANAGEMENT

The treatment approaches to each of the lung NETs are markedly different, which underscores the importance of accurate pathologic diagnosis.

Carcinoid tumors are primarily a surgical disease; metastatic tumors are generally not sensitive to chemotherapy or radiation therapy, and little can be offered to patients with metastatic disease. Somatostatin analogues, in addition to having a role in imaging for carcinoid tumors (somatostatin receptor scintigraphy or octreotide scan), are also used therapeutically. The primary utility is to control hypersecretion in advanced tumors (which is rare in lung carcinoids), but these compounds do not significantly inhibit tumor growth.[19] Emerging therapies with radiolabeled somatostatin analogues hold a promise for controlling tumor growth, but this remains investigational.[19,20]

A unique feature of SCLC is that it is generally considered a nonsurgical disease. These tumors are exquisitely sensitive to chemotherapy (cisplatin plus etoposide is a standard regimen) and radiation therapy, but these patients derive no further benefit from surgical resection.[20,21] This dogma has been recently challenged, as evidence is emerging that a resection may indeed extend the survival of rare patients in whom SCLC presents as a solitary mass.[21–23]

Another unique clinical feature of SCLC is its propensity for brain metastases, which develop in 50% to 80% of patients.[21] Therefore, prophylactic cranial irradiation is indicated for patients with SCLC, but not other NETs or NSCLC. The unique therapeutic approaches and dismal prognosis underscore the importance of accurate diagnosis of SCLC.

There is no consensus on the clinical management of LCNEC. The efforts to establish treatment guidelines for LCNEC are hampered by the relative rarity of this tumor (~3% of lung NETs) and challenges in diagnostic reproducibility. The efforts to determine optimal treatment strategies for LCNEC are ongoing at Memorial Sloan-Kettering Cancer Center (New York, New York) and other institutions; these clinical efforts are closely tied to our growing understanding of the biology and diagnostic criteria for this disease.

NEUROENDOCRINE CELL HYPERPLASIA AND CARCINOID TUMORLETS

The normal neuroendocrine (NE) cells in the lung (known as Kulchitsky cells) are present as rare single cells or small cell clusters of 4 to 10 cells (known as neuroepithelial bodies) within bronchial and bronchiolar epithelium.[24]

Neuroendocrine cell hyperplasia refers to the proliferation of single, clustered, or linear arrays of NE cells confined within the basement membrane. Neuroendocrine cell hyperplasia is usually inconspicuous by hematoxylin-eosin staining; the clues are groups of cells with pale cytoplasm, which are frequently associated with retraction of the underlying stroma, or intraluminal fingerlike projections of the overlying respiratory epithelium (Figure 1, A and B).

Carcinoid tumorlets are distinguished from NE hyperplasia by extension beyond the basement membrane of the respiratory epithelia. They are morphologically identical to TC, but are defined by a size of 5 mm or less. Tumorlets are peribronchiolar and are typically associated with stromal fibrosis (Figure 1, C), which may lead to obliteration of the adjacent bronchiole. As an isolated lesion, carcinoid tumorlet is a fairly common incidental finding in the lung. In contrast,

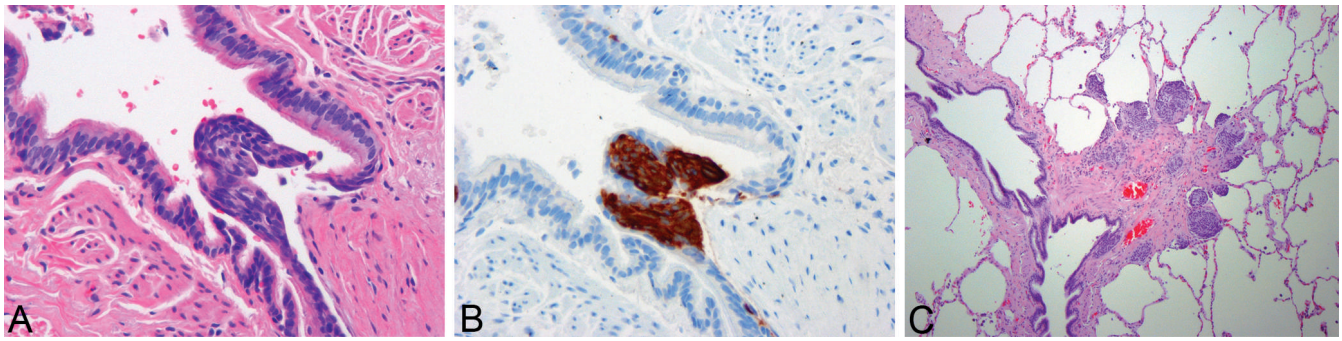

Figure 1. *Neuroendocrine cell hyperplasia and carcinoid tumorlet. A, Neuroendocrine cell hyperplasia is usually inconspicuous with hematoxylin-eosin; histologic clues are retraction from the underlying stroma and fingerlike intraluminal projections. B, Immunohistochemistry for synaptophysin (shown) or other neuroendocrine markers highlights hyperplastic neuroendocrine cells. C, Typical low-power appearance of carcinoid tumorlet: peribronchiolar location and associated stromal fibrosis (hematoxylin-eosin, original magnifications ×200 [A] and ×100 [C]; original magnification ×200 [B]).*

multiple tumorlets and diffuse NE cell hyperplasia are uncommon[25,26] and may be seen in the following 3 settings.

First, and the most common setting for multiple tumorlets and NE hyperplasia, is that of chronic lung injury, such as bronchiectasis or fibrosis. In this setting, NE proliferations are considered to be reactive in nature, and progression to carcinoid tumors is typically not seen.[1]

Second, and a much less common setting for diffuse NE proliferations, is that of an otherwise normal lung. This rare condition has been referred to as diffuse idiopathic NE cell hyperplasia (DIPNECH).[1,27,28] In this setting, progression to carcinoid tumors (both typical and atypical) has been documented,[27] and therefore, DIPNECH has been included in the 2004 WHO classification as a preneoplastic condition. This situation is curiously reminiscent of gastric carcinoids arising in a background of NE cell hyperplasia in a setting of hypergastrinemia. However, the inciting stimulus for NE proliferation in the lung is not known. Also unknown is why DIPNECH develops predominantly in women. Small airway obliteration (due to bronchiolar fibrosis, presumably triggered by hyperplastic NE cells and tumorlets) and associated respiratory symptoms due to airflow limitation were initially described as a defining feature of DIPNECH, with rare cases progressing to respiratory failure.[29] Because respiratory compromise develops only in a small subset of patients with NE hyperplasia and tumorlets, the 2004 WHO classification does not require this clinical association in the definition of DIPNECH.[1]

Lastly, NE cell hyperplasia and tumorlets are frequently but not invariably seen in lungs resected for carcinoid tumors (46%–76%), both in the vicinity and away from the tumor.[26,30] Whether NE proliferations in this setting can be considered a type of DIPNECH is uncertain. Neuroendocrine proliferations in the above 3 settings (chronic lung disease, DIPNECH, carcinoid tumor) are morphologically identical.

What are the practical issues with the diagnosis of DIPNECH? In a classic clinical setting of a patient presenting with respiratory symptoms and pathologic findings of diffuse NE hyperplasia and tumorlets in the absence of other pathologic findings, the diagnosis of DIPNECH is straightforward. However, when NE proliferations are found incidentally in the lung resected for localized lesions (such as NSCLC or carcinoid tumor), it may be unclear whether the diagnosis of DIPNECH is appropriate. In such situations, the possibility of diffuse NE proliferations in unresected lung could be raised in a note. The clinical implications are that (1) progression to carcinoid tumors may occur, (2) rarely, patients may develop respiratory symptoms, and (3) tumorlets may be detected by computed tomography scans as multiple nodules in unresected lung.

This last issue is particularly important for pathologists to be aware of because radiologically, multiple tumorlets may mimic a miliary pattern of metastasis.[25,31,32] This issue was highlighted in a study by Darvishian et al,[25] in which a group of patients with breast cancer (who were treated at Memorial Sloan-Kettering Cancer Center) was suspected of having multiple pulmonary metastases by CT scans, but subsequent pathologic examination revealed multiple carcinoid tumorlets.[25] Because of potential morphologic similarity between well-differentiated breast carcinoma and NE proliferations, this may present an important diagnostic pitfall, particularly in frozen sections.

Although usually not a diagnostic challenge, tumorlets should be distinguished from minute meningothelioid nodules (the so-called chemodectoma-like bodies)—a common lesion of no clinical significance with similarly bland cytologic features and frequent multiplicity. The distinguishing features of minute meningothelioid nodules are perivenular location with stellatelike extension into alveolar walls, whorls and intranuclear inclusions similar to meningioma, and the lack of associated fibrosis. Unlike tumorlets, minute meningothelioid nodules do not label for NE markers or cytokeratins.

CARCINOID TUMOR: PATHOLOGIC FEATURES AND DIFFERENTIAL DIAGNOSIS

It is estimated that 75% of lung carcinoids are central (bronchial) and 25% are peripheral. The typical morphologic features of carcinoid tumors are well known and are similar to well-differentiated NETs of other sites. The defining features include NE cytology (coarsely granular ''salt and pepper'' chromatin, lack of prominent nucleoli, overall uniformity) and NE architectural patterns (organoid nests, trabeculae, rosettes) (Figure 2, A). Also typical is prominent vascularity. Only 1 mitosis per 10 high-power fields (HPFs) and no necrosis are allowed for the diagnosis of TC (Table 1).

Although well-differentiated NETs of all sites share similar basic morphologic features, red granulations, characteristic of serotonin-producing ileal carcinoids, are not typically seen in the lung. Also, true glandular lumina are much less common in pulmonary than gastrointestinal carcinoids.

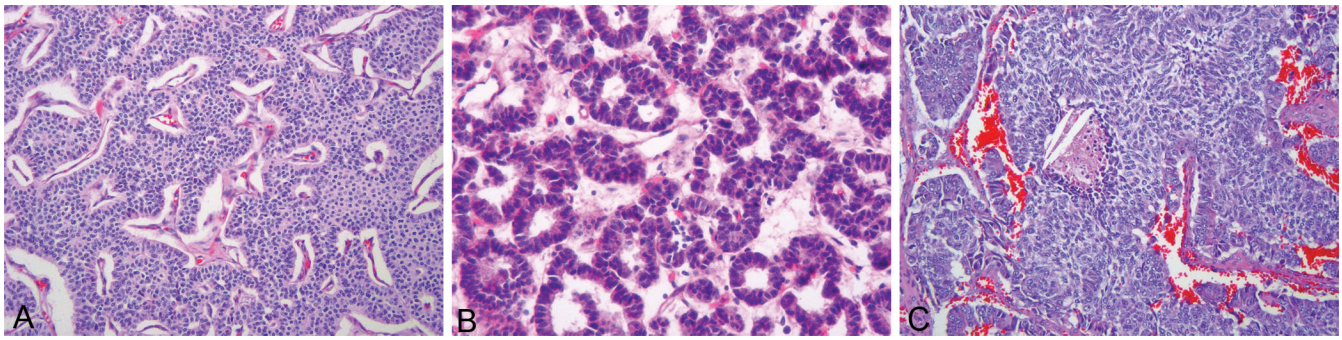

Figure 2. *Carcinoid tumor. A, Typical carcinoid with characteristic trabecular growth pattern and highly vascularized stroma. B, Typical carcinoid with a striking glandular pattern, which can mimic adenocarcinoma. C, Characteristic punctate (comedo-like) necrosis in atypical carcinoid (hematoxylin-eosin, original magnifications ×100 [A and C]; synaptophysin immunohistochemistry, original magnification ×100 [B]).*

Similar to other sites, carcinoid tumors of the lung have highly variable growth patterns, each evoking a different set of differential diagnoses.

- Carcinoid with glandular pattern should be distinguished from adenocarcinoma (Figure 2, B).
- Spindle cell carcinoid (usually peripheral) should be distinguished from bland mesenchymal tumors of the lung and pleura, particularly solitary fibrous tumor, synovial sarcoma, and smooth muscle tumors.
- Carcinoid with nested growth pattern should be distinguished from paraganglioma (although primary pulmonary paraganglioma is exceedingly rare).
- Carcinoids with prominent rosettes and cribriform growth pattern should be differentiated from metastatic adenocarcinoma with carcinoid-like appearance and bland nuclear features, which most notably include well-differentiated ductal breast carcinoma and prostate cancer.
- Carcinoid with prominent plasmacytoid features should be distinguished from a plasma cell neoplasm and melanoma.

Immunohistochemistry can effectively resolve these differential diagnoses (discussed in section on immunohistochemistry).

ATYPICAL CARCINOID

Atypical carcinoid is defined in the 2004 WHO classification as a well-differentiated NET with (1) mitotic rate of 2 to 10 per 10 HPFs, or (2) necrosis (Table 1). Neither is allowed in TC, and either mitotic rate or necrosis alone is sufficient to qualify a tumor for AC, although both are usually present. The upper limit of mitoses in AC is defined as 10 mitoses per 10 HPFs; tumors exceeding that cutoff are classified as LCNEC.

Necrosis in AC has a characteristic punctate appearance located in the centers of tumor nodules resembling comedonecrosis (Figure 2, C). Large areas of geographic necrosis typical of high-grade NE carcinomas are not seen. It is common for AC to show greater nuclear pleomorphism than seen in TC, including nucleoli and nuclear membrane irregularities, but this feature is not sensitive or specific, and it is not part of the diagnostic criteria for AC.

SMALL CELL LUNG CARCINOMA: WELL-KNOWN AND UNUSUAL MORPHOLOGIC FEATURES

The defining features of small cell carcinoma at any site are well known. The key defining feature is nuclear appearance, which includes finely granular chromatin (which is distinct from coarsely granular chromatin typical of carcinoid tumors and clumpy vesicular chromatin typical of NSCLC); lack of prominent nucleoli; and marked nuclear fragility and malleability manifesting as nuclear molding, spindling (fusiform cells), and crush artifact with nuclear streaming and incrustation of vessels (Azzopardi phenomenon). Other defining features are scant cytoplasm and indistinct cell borders. High mitotic rate, apoptotic bodies, and large areas of geographic necrosis are typical[33] (Figure 3, A).

While the typical nuclear size of SCLC is less than 3 lymphocytes, a significant size range can be seen, with a proportion of cells reaching the size of 6 to 7 lymphocytes[34,35]; even giant cells may occasionally be seen.[36] In fact, detailed morphometric studies[34,35] show that 30% of SCLCs have a predominant cell size larger than 3 lymphocytes. In our clinical practice, we accept significant size variability in SCLC as long as the defining cytologic features of SCLC (finely granular chromatin, lack of prominent nucleoli, lack of prominent cell borders) are present (Figure 3, B). This size variability of SCLC is important to bear in mind when considering the differential diagnosis with LCNEC.

The 2004 WHO classification recognizes 2 types of SCLCs: pure SCLC and combined SCLC. Previously recognized subtypes of SCLC (oat cell/lymphocyte-like, and intermediate) were found to lack diagnostic reproducibility and prognostic significance,[37–39] and these subtypes of SCLC are not distinguished in the current WHO classification (Table 2). Nevertheless, it is important to be aware of the ''intermediate'' cell appearance of SCLC. This type of SCLC is characterized by larger nuclear size and occasionally more evident cytoplasm, and it is therefore the type of SCLC that enters in the differential diagnosis of LCNEC. The classic nuclear morphology and the lack of prominent cell membranes are the most reliable distinguishing features.[40]

An underrecognized feature of SCLC is that, rather than growing as destructive sheets of cells resembling lymphoma, SCLC may have a remarkably nested (organoid) growth pattern (Figure 3, C and D). Occasional peripheral palisading and desmoplastic stromal reaction may be seen, thus resembling, at low-power magnification, basaloid squamous cell carcinoma.[38] This striking architecture of SCLC is best appreciated in resected specimens and may appear surprising because of the rarity of resected SCLC (most SCLCs are seen in small biopsy or cytology specimens).

SMALL CELL LUNG CARCINOMA: DIFFERENTIAL DIAGNOSIS

Distinction of SCLC from basaloid squamous cell carcinoma is essential clinically, but can be extremely

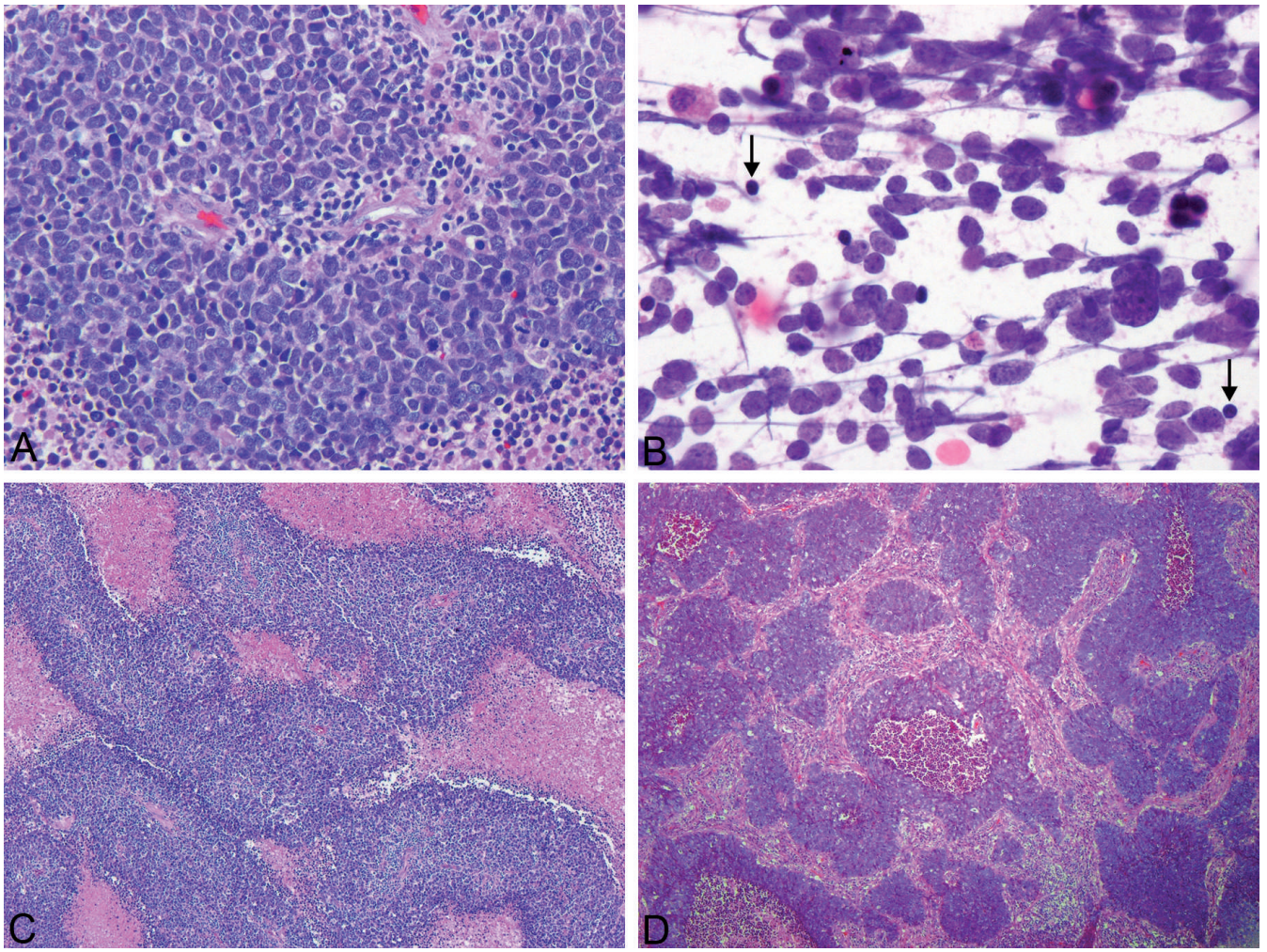

Figure 3. *Small cell lung carcinoma (SCLC). A, Typical appearance of SCLC: crowded cells with fine chromatin, inconspicuous nucleoli, and inapparent cell membranes; cells are predominantly small (<3 lymphocytes). B, Cytology preparation highlights a range of cell sizes, some far exceeding 3 lymphocytes (arrows) in otherwise typical SCLC. C, Low-power appearance of typical SCLC growing as destructive sheets with geographic necrosis. D, Low-power appearance of SCLC with a nested growth pattern and desmoplastic stroma—a growth pattern best seen in resected specimens (hematoxylin-eosin, original magnifications ×200 [A] and ×20 [C and D]; Papanicolaou stain, original magnification ×400 [B]).*

difficult morphologically, particularly in a poorly preserved specimen. Immunohistochemistry (IHC) can in general effectively resolve this differential diagnosis: expression of NE markers and thyroid transcription factor 1 (TTF-1) supports SCLC, whereas expression of p63 (4A4) and high-molecular-weight cytokeratins (CK5/6 or 34βE12) supports basaloid squamous cell carcinoma.

Crush artifact typically associated with SCLC can also be seen in some NSCLCs and carcinoid tumors. In small biopsy specimens, when NE nature of the tumor is confirmed, the presence of crush artifact may lead to overinterpretation of a carcinoid tumor as SCLC.[41] In this setting, IHC for Ki-67 (MIB1) can serve as an important ancillary tool (discussed further below).

Table 2. Summary of Classification Systems for Small Cell Lung Carcinoma[a]

WHO 1967	WHO 1981	IASLC 1998	WHO/IASLC 1999, 2004
Lymphocyte-like	Oat cell	Pure SCLC	SCLC
Polygonal Fusiform	Intermediate	Mixed SC/LC	Combined SCLC
Other (containing squamous or glandular foci)	Combined	Combined	

Abbreviations: IASLC, International Association for the Study of Lung Cancer; SCLC, small cell lung cancer; SC/LC, small cell/large cell carcinoma; WHO, World Health Organization.

[a] Modified with permission from Hirsch et al.[37]

COMBINED SMALL CELL LUNG CARCINOMA

It is estimated that 70% of resected SCLCs are pure, and 30% combined.[38] The combined SCLC is defined as SCLC in which there is a component of non–small cell carcinoma, including adenocarcinoma, squamous cell carcinoma, large cell carcinoma, or uncommonly, sarcomatoid carcinoma. These components may be present as multiple scattered foci or as discrete (collision-like) areas.

Also included in the combined category of the current WHO classification is a tumor formerly designated as mixed small cell/large cell (SC/LC) carcinoma (Table 2), which is generally defined as a tumor in which large cells

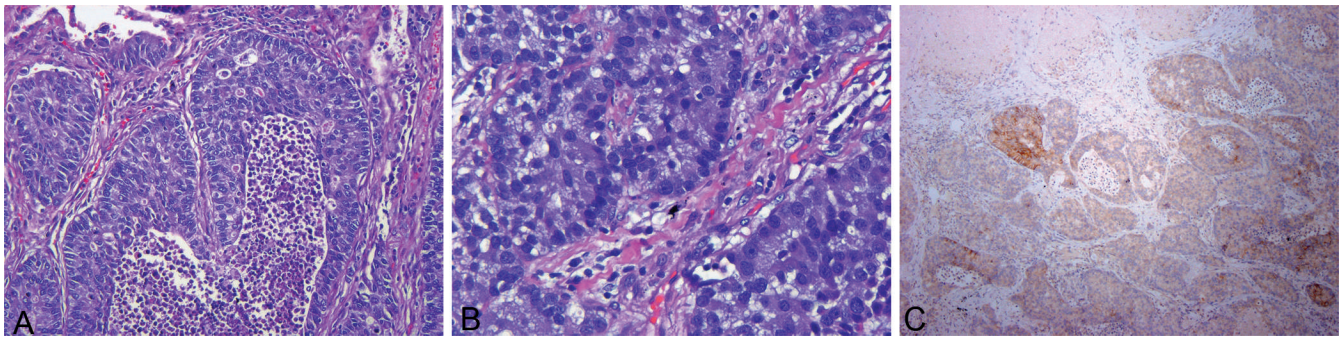

Figure 4. *Large cell neuroendocrine carcinoma (LCNEC). A, Typical low-power appearance of LCNEC showing nested/nodular growth pattern with peripheral palisading and extensive necrosis. B, Non–small cell cytologic appearance at higher power is evidenced by prominent nucleoli, vesicular chromatin, and abundant cytoplasm. C, The tumor cells label for CD56 by immunohistochemistry (hematoxylin-eosin, original magnifications ×100 [A] and ×200 [B]; original magnification ×20 [C]).*

(defined by prominent nucleoli and cell borders rather than cell size alone), rather than forming discrete clusters, are admixed with small cell carcinoma, forming a continuous spectrum from typical small cell carcinoma to large cell carcinoma.[37,38,42]

In the 2004 WHO classification, it is suggested that tumors with less than 10% large cell component be still classified as pure SCLC, whereas only tumors with more than 10% large cell component be classified as combined SCLC. This criterion was introduced to create a reproducible cutoff for SCLC with minimal (or morphologically equivocal) non–small cell component.[1,37,38] Note that the 10% criterion does not apply to the conventional type of combined SCLC; in those cases, presence of any amount of unequivocal adenocarcinoma or squamous cell carcinoma would qualify for a combined SCLC diagnosis.

The prognostic significance and clinical management of combined SCLC is controversial, although the initial treatment, as in other organs, is driven by the small cell carcinoma component. There are some suggestions that these tumors are less chemosensitive than pure SCLC,[43,44] but other studies[45] find no difference in prognosis of combined and pure SCLC. It is suggested that in contrast to pure SCLC, surgical resection should be considered after neoadjuvant therapy for combined SCLC.[23] The significance of different types of combined SCLC (eg, containing adenocarcinoma versus squamous cell carcinoma versus SC/LC carcinoma) is not known.

A notable observation in autopsy studies is that nearly 50% of patients who are initially diagnosed with pure SCLC are found to have NSCLC either exclusively or in combination with SCLC after treatment.[15,46] A possible explanation is that a minor NSCLC component (which may not be represented in small biopsy or cytology specimens) is selected because of greater chemoresistance than SCLC. Pathologists should be aware of this phenomenon when dealing with a posttreatment specimen in a patient with initial diagnosis of SCLC.

LARGE CELL NEUROENDOCRINE CARCINOMA: THE 2004 WHO DEFINITION

The category of LCNEC as currently defined in WHO classification was introduced by Travis et al in 1991.[47] The definition of LCNEC includes 3 components, as follows[1]:

1. High grade

- High mitotic rate: greater than 10 mitoses per 10 HPFs.
- Necrosis (often large zones).

2. Morphology

- Neuroendocrine architecture at low-power magnification: organoid nests, palisading, rosettes, trabeculae.
- Cytology of NSCLC: non–small cell nuclear features (prominent nucleoli, vesicular clumpy chromatin) *and/or* large cell size and abundant cytoplasm.

3. Positive IHC

- At least 1 NE marker (other than neuron-specific enolase).

To summarize, LCNEC is defined as a high-grade tumor (by virtue of high mitotic rate and necrosis), which has NE (carcinoid-like) architecture at low-power magnification, but discordant non–NE cytology at high power (prominent nucleoli, vesicular rather than finely granular chromatin, abundant cytoplasm, and evident cell membranes) (Figure 4, A through C). Cells are usually larger than those of SCLC, but similar to SCLC, there is significant size variability,[34,35] and a subset of LCNECs shows a predominant number of cells that are smaller than 3 lymphocytes.[35]

Similar to SCLC, LCNEC may be pure or combined with NSCLC. The most common combination is with adenocarcinoma.[1]

LARGE CELL NEUROENDOCRINE CARCINOMA: DIAGNOSTIC CHALLENGES

Diagnosis of LCNEC can be difficult. Highlighting the challenges in the diagnosis of this tumor is a classic reproducibility study by Travis et al,[48] which showed that among 5 expert thoracic pathologists, a unanimous agreement in the diagnosis of LCNEC was achieved in only 40% of cases. Most disagreement occurred between the diagnosis of LCNEC and SCLC. Of note, this study included only NE tumors but not NSCLC, which potentially could have made a consensus even more difficult.

The difficulty in the diagnosis of LCNEC stems from the fact that this entity has overlapping features with (1) SCLC, (2) atypical carcinoid, and (3) NSCLC, as follows.

1. LCNEC versus SCLC: While LCNEC with cytologic features that are clearly non–small cell (large nucleoli, vesicular chromatin, abundant cytoplasm, distinct cell borders) can be readily distinguished from SCLC, the greatest difficulty is with tumors that have

borderline features, such as focal and inconspicuous nucleoli, scant but apparent cytoplasm and equivocal cell borders. Particularly difficult is the diagnosis of tumors with nuclear features compatible with SCLC (fine chromatin, no nucleoli) but more abundant cytoplasm and visible cytoplasmic membranes, where the differential diagnosis is between SCLC (former intermediate-cell variant) and LCNEC. Fortunately, this situation is uncommon, and most LCNEC have both nuclear *and* cytoplasmic features that are distinct from SCLC. As discussed above, cell size alone (in the absence of other cytologic features) should not be used as a sole criterion for separation of LCNEC from SCLC because of significant overlap between these tumors.[34,35] It is recommended that even if only a minor component in an NSCLC has features that are diagnostic of SCLC, the tumor should be classified as combined SCLC rather than LCNEC.[1]

2. LCNEC versus AC: Although both tumors may have vaguely similar low-power appearance, in most cases LCNEC can be readily distinguished from AC by the obvious high-grade features, including high mitotic rate (typically much higher than the cutoff of 10 mitoses per 10 HPFs), and extensive rather than focal/punctate necrosis. In addition, the cytologic features of LCNEC and AC are distinct: LCNEC typically has prominent nucleoli (although small nucleoli may be present in AC), vesicular rather than granular chromatin, and more significant cytologic pleomorphism.
   The difficulty arises in rare tumors that show mitotic activity just slightly or focally exceeding 10 mitoses per 10 HPFs, but which otherwise would qualify as AC. Such tumors are currently classified as LCNEC, but their biologic and clinical identity awaits further investigation.
3. LCNEC versus NSCLC: While the distinction of LCNEC from other NE tumors (SCLC and AC) has received the most attention in reproducibility studies,[48] the distinction of LCNEC from NSCLC, most notably poorly differentiated adenocarcinoma, can be equally challenging.[49] Non–small cell carcinoma has cytologic features that overlap with LCNEC (prominent nucleoli, vesicular chromatin), and poorly differentiated NSCLC may show extensive necrosis with high mitotic rate. While most lung adenocarcinomas have acinar/papillary/bronchioloalveolar growth patterns, which are readily recognized as non-NE, some poorly differentiated adenocarcinomas grow as solid or cribriform nests, where focal peripheral palisading may be seen. In such cases, diagnosis of poorly differentiated adenocarcinoma versus LCNEC depends on reactivity for NE markers by IHC. However, because 10% to 20% of NSCLCs label for NE markers irrespective of morphology (referred to as NSCLC with NE differentiation [NSCLC-NED]; see next section), the classification of a tumor as LCNEC rather than poorly differentiated adenocarcinoma may hinge entirely on a subjective impression of whether a tumor has a low-power ''NE'' appearance.

Although sought after as a ''holy grail'' of the NE tumor field, currently no immunohistochemical or molecular marker can reliably aid in the differentiation of LCNEC from SCLC, AC, and NSCLC-NED in the overlapping gray-zone areas described above.

Table 3. Classification of Non–Small Cell Lung Carcinoma (NSCLC) With Neuroendocrine Features by Morphology and Immunohistochemistry

	(+) NE Markers by IHC	(−) NE Markers by IHC
(+) NE morphology[a]	**LCNEC**	**NSCLC-NEM**[b]
(−) NE morphology[a]	**NSCLC-NED**[c]	**NSCLC, NOS**[d]

Abbreviations: IHC, immunohistochemistry; LCNEC, large cell neuroendocrine carcinoma; NE, neuroendocrine; NED, neuroendocrine differentiation; NEM, neuroendocrine morphology; NOS, not otherwise specified; NSCLC, non–small cell carcinoma.

[a] NE morphology refers to the presence of organoid nests, rosettes, palisading.
[b] NSCLC-NEM refers to a tumor otherwise identical to LCNEC but without evidence of NE markers by IHC.
[c] NSCLC-NED refers to conventional NSCLC with positive NE marker(s) by IHC.
[d] NSCLC, NOS includes conventional adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.

NON–SMALL CELL LUNG CARCINOMA WITH NEUROENDOCRINE FEATURES

A highly controversial group of tumors is ''NSCLC with NE features.'' [50] These tumors are defined by discordant NE morphology and IHC (Table 3).

In 1 category are tumors that are morphologically conventional NSCLC (adenocarcinoma, squamous cell carcinoma, or large cell carcinoma), with no suggestion of NE morphology, but which are found to have NE marker expression by IHC. This group of tumors is designated ''NSCLC with NE differentiation'' (NSCLC-NED). It is estimated that 10% to 20% of otherwise typical NSCLCs have at least focal reactivity for 1 or several NE markers, with reactivity seen more commonly in adenocarcinoma than squamous cell carcinoma.[51] Over time, the data regarding the impact on prognosis and chemosensitivity of NE differentiation in NSCLC have been conflicting.[1,20] However, several recent studies[51–53] suggest an adverse prognosis when the analysis is limited to low-stage (IA–IB) tumors and NSE is excluded as a sole NE marker. Nevertheless, until a firm consensus on the clinical implications is reached, it is not recommended that NE markers be used in clinical workup of NSCLC without morphologic evidence of NE morphology.

The other category includes tumors that fit the definition of LCNEC, except that expression of NE markers cannot be confirmed by IHC. These tumors are designated as ''NSCLC with NE morphology'' (NSCLC-NEM). These cases are very rare, and their clinicopathologic characteristics are not well established. Some studies[50] suggest that these tumors carry a poor prognosis similar to that associated with LCNEC.

In summary, NSCLC-NEM, NSCLC-NED, and LCNEC may represent entities in a histologic, biologic, and clinical continuum, and future molecular and detailed clinicopathologic studies will be needed to determine their most accurate classification and clinical management.

IMMUNOHISTOCHEMISTRY HIGHLIGHTS: CARCINOID TUMORS

Carcinoids are strongly and diffusely positive for NE markers (commonly used markers include synaptophysin, chromogranin A, and CD56/NCAM). In a small minority of carcinoid tumors, particularly in AC, not all

NE markers may be expressed,[12] and therefore a panel approach is recommended.

It is important to be aware that while most carcinoid tumors are positive for cytokeratins, nearly 20% of tumors are cytokeratin negative. This can present a pitfall in differentiating a spindle cell carcinoid from mesenchymal tumors and a nested carcinoid from paraganglioma (NE markers are expressed in both, but S100-positive sustentacular cells are typical of paraganglioma but not carcinoid tumors).

Approximately 50% of lung carcinoids are reactive for TTF-1, although unlike the strong and diffuse TTF-1 expression typical of lung adenocarcinoma or SCLC, the labeling in carcinoid tumors is weak and focal. Peripheral carcinoids are more commonly TTF-1 positive than central carcinoids.[54,55] It is reported that TTF-1 is not detected in gastroenteropancreatic NETs, while most intestinal, but not pulmonary carcinoids, label for CDX2.[56] Therefore, it is suggested that analogous to adenocarcinoma of unknown primary site, TTF-1 and CDX2 could be used to suggest the site of origin of a metastatic, well-differentiated NET of unknown primary origin. Note that this is different from SCLC and LCNEC, in which TTF-1 reactivity is not lung specific.

It was recently reported that focal to diffuse immunoreactivity for estrogen receptor is found in more than 50% of pulmonary carcinoids (both typical and atypical).[57] This may present an important pitfall in the differentiation from metastatic breast cancer, particularly because of the potential carcinoid-like morphology of well-differentiated ductal breast carcinoma.

IMMUNOHISTOCHEMISTRY HIGHLIGHTS: SMALL CELL LUNG CARCINOMA

Reactivity for synaptophysin and chromogranin A is typically weak in SCLC, which is distinct from the robust reactivity typical of carcinoid tumors. While CD56 is considered to be the least specific NE marker at other sites, it is the most sensitive NE marker for SCLC: approximately 25% of SCLCs are negative for both synaptophysin and chromogranin A, but most of these tumors are positive for CD56.[34] Still, 10% of SCLCs are negative for all 3 commonly used NE markers.[1,34,58] The frequency of NE marker–negative SCLC is even higher in small biopsy and cytology specimens, in which focal reactivity may not be represented.

Weak punctate (''dotlike'') labeling for cytokeratins (such as AE1/AE3 or CAM 5.2) is typical of SCLC and is distinct from strong circumferential labeling characteristic of NSCLC. However, this feature may be seen in other high-grade carcinomas, and it is therefore not specific for SCLC.[59,60] While all resected SCLCs are cytokeratin positive, some tumors show minimal reactivity,[61] and a subset of small biopsy and cytology specimens may appear completely cytokeratin negative.

Diffuse reactivity for 34βE12 (CK903) has been suggested as an exclusion criterion for high-grade NE carcinomas at several sites, including the lung.[60,62,63] Note that presence of rare 34βE12-positive cells or focal dotlike reactivity may be seen in a subset of SCLCs and does not exclude this diagnosis.[62] In contrast, the diagnosis of basaloid squamous cell carcinoma should be considered in a tumor resembling SCLC morphologically but with diffuse 34βE12 labeling.

Thyroid transcription factor 1 is expressed in approximately 90% of SCLCs. In contrast to adenocarcinoma, TTF-1 in a small cell carcinoma setting cannot be used to distinguish pulmonary from extrapulmonary origin because expression of TTF-1 in 20% to 80% of small cell carcinomas of various sites (including prostate, bladder, cervix, and gastrointestinal tract) is well documented.[64–66]

To summarize, a small percentage of SCLCs, particularly in small biopsy or cytology specimens, may be negative for all typical markers of SCLC, including NE markers, TTF-1, and even cytokeratins. The traditional teaching remains: if morphology is classic for SCLC, the lack of supporting immunohistochemical markers should not serve as evidence against this diagnosis.[67] In the absence of confirmatory markers, it is important to exclude the possibility of lymphoma, basaloid squamous cell carcinoma, and other small round blue cell tumors.

IMMUNOHISTOCHEMISTRY HIGHLIGHTS: LARGE CELL NEUROENDOCRINE CARCINOMA

By the WHO 2004 criteria, immunohistochemical demonstration of at least 1 NE marker is required for the diagnosis of LCNEC. However, there is no minimal qualitative or quantitative requirement except for exclusion of NSE. Both synaptophysin and chromogranin A are coexpressed in most (70%) LCNECs.[68,69]

Thyroid transcription factor 1 is expressed in approximately 50% of LCNECs. Although the data are scant, several studies report that TTF-1 is expressed in extrapulmonary LCNECs including in the bladder[70] and prostate.[66] Therefore as with SCLC, TTF-1 cannot be used to assign the site of origin of metastatic LCNEC.

Expression of TTF-1 in extrapulmonary high-grade NE carcinomas (small cell and large cell) may appear puzzling. However, it is known that during embryogenesis TTF-1 is expressed not only in lung and thyroid, but also in the developing brain.[71] It is possible that TTF-1 expression in high-grade NE carcinomas of various sites is a marker of aberrant reactivation of primitive neural pathways.

As with SCLC, IHC is effective in distinguishing LCNEC (NE markers and TTF-1 positive) from basaloid squamous cell carcinoma (p63 and high-molecular-weight cytokeratin positive).

IMMUNOHISTOCHEMISTRY HIGHLIGHTS: UTILITY OF KI-67 (MIB1)

Immunohistochemistry for Ki-67 (MIB1) is not part of the 2004 WHO criteria for classifying lung NETs, but several recent studies suggest a utility for this marker, particularly in small biopsy and cytology specimens. Based on the compilation of several recent studies (Table 4), Ki-67 proliferation rate of TC is less than 2%, AC is less than 20% (typical rate ~10%), while SCLC and LCNEC have Ki-67 proliferation rates significantly higher than 20% (typical rate for SCLC is 60%–100%). It is suggested that Ki-67 rate of less than 25% excludes the diagnosis of SCLC.[72] Of note, Ki-67 proliferation rates that closely parallel the above ranges are being proposed as part of the diagnostic and prognostic criteria for gastroenteropancreatic NETs.[73]

While the utility of Ki-67 in resected specimens, in which mitotic count can be accurately performed, awaits further evaluation, this marker has been shown to be of great value in small biopsy[41,72] and cytology specimens,[74] for which distinction of carcinoid tumors, particularly

Table 4. Compilation of Reported Ki-67 (MIB1) Proliferation Rates in Lung Neuroendocrine Tumors						
			Percentage of Ki-67 (MIB1)–Positive Cells, Mean (Range)			
Source, y	Specimen Type	N	TC	AC	LCNEC	SCLC
Kobayashi et al,[81] 2004	Resection	57	0.5	5	41	76
Arbiser et al,[82] 2001	Resection	20	1	9	25	
Igarashi et al,[83] 2004	Resection	111	1.3 (0.3–2.3)	8.6 (0.2–17)	52 (36–68)	55 (44–66)
Pelosi et al,[41] 2005	Resection	220	2.3	9	47	64
Iyoda et al,[84] 2004	Resection	27			42 (28–56)	
Pelosi et al,[52] 2003	Resection	11			32 (20–61)	
Costes et al,[85] 1995	Resection	47	0.45	2.4 (0–6)		
Pelosi et al,[41] 2005	Biopsy	15	0.5 (0–1)	7.2 (1–17)		82 (60–96)
Aslan et al,[72] 2005	Biopsy and resection	22	1 (0–10)			60 (25–90)
Lin et al,[74] 2003	Cytology	63	<25			>50
Weighted Mean (Range) for the above studies			*1.5 (0–2.3)*	*7.7 (0–17)*	*46 (20–90)*	*64 (25–96)*

Abbreviations: AC, atypical carcinoid; LCNEC, large cell neuroendocrine carcinoma; SCLC, small cell lung carcinoma; TC, typical carcinoid.

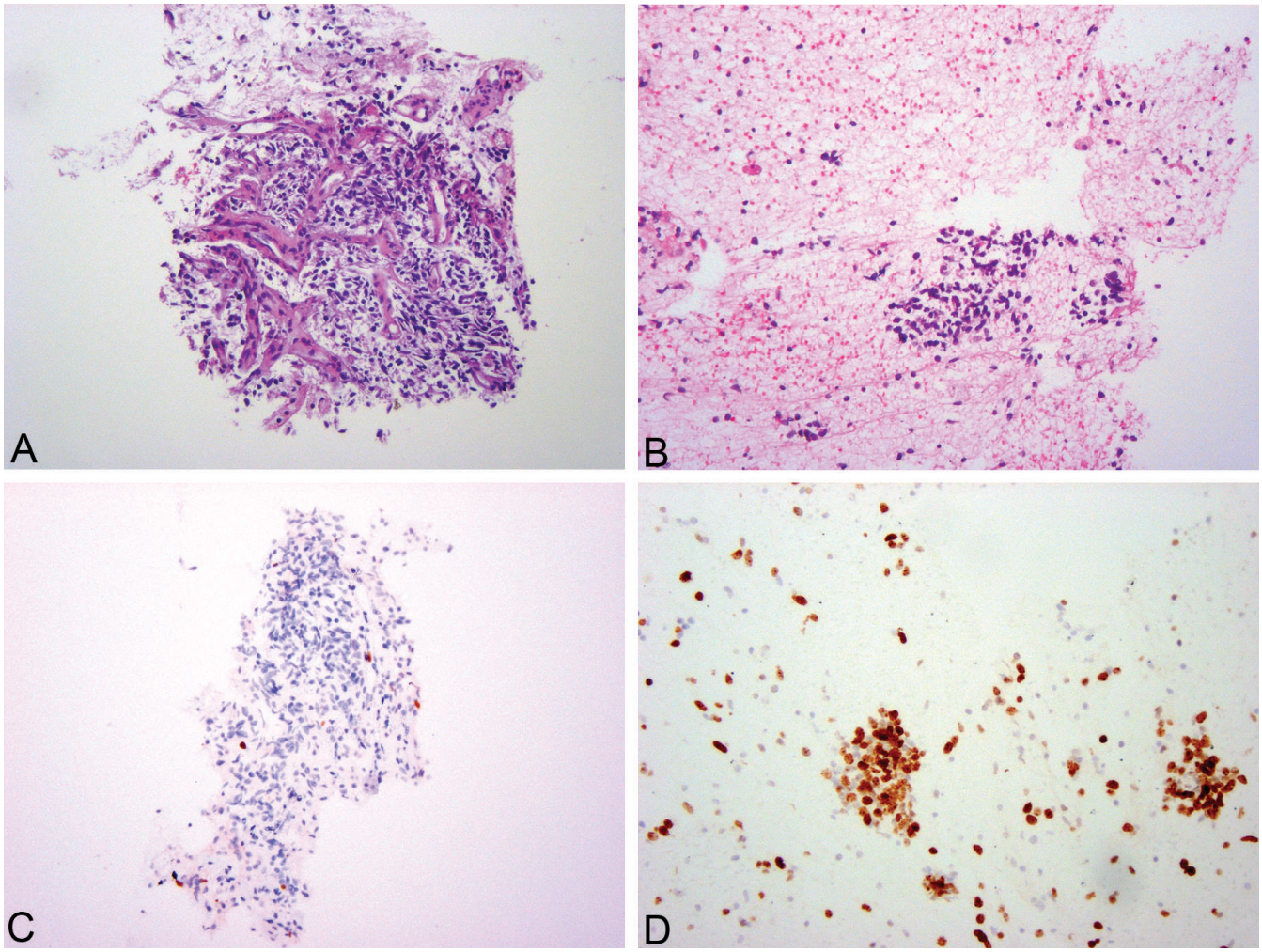

Figure 5. *Utility of Ki-67 (MIB1) in cytologic diagnosis of lung neuroendocrine tumors. Shown are hematoxylin-eosin (A and B) cell blocks and Ki-67 immunohistochemistry (C and D) on cell blocks for case 1 (A and C) and case 2 (B and D). Both cases showed neuroendocrine cytologic features and were immunoreactive for neuroendocrine markers (not shown). Less than 1% Ki-67 reactivity supports the diagnosis of case 1 as carcinoid tumor, whereas ~90% Ki-67 reactivity supports the diagnosis of case 2 as SCLC (original magnifications ×200 [A through D]).*

atypical carcinoid, from high-grade NE carcinomas may be difficult. In particular, crush artifact can be seen in small biopsies of carcinoid tumors (typical and atypical), which can lead to overdiagnosis as SCLC. In this setting, evaluation of the Ki-67 proliferation index is invaluable.[41,72] An example of Ki-67 application in cytology is shown in Figure 5, A through D.

UPDATE ON STAGING

Carcinoid tumors have in the past been excluded from the American Joint Committee on Cancer tumor, node, metastasis (TNM) staging guidelines. However, recent large-scale analysis showed that TNM parameters do have a prognostic value for carcinoid tumors of the lung.[75] Therefore, the new (7th) edition of the TNM staging system includes carcinoid tumors, which should now be staged by the same criteria as applied to NSCLC.[75]

Small cell lung cancer has been included in TMN staging, but clinicians traditionally have not used the TNM terminology in reference to SCLC. Instead, clinically used categories of SCLC are *limited disease* (defined as tumor within a single radiation portal, ie, hemithorax with mediastinal or supraclavicular node metastasis) versus *extensive disease* (contralateral or distant metastasis). As with carcinoid tumors, recent large-scale analysis showed that TNM staging is effective for SCLC,[16] and it is recommended in the 7th TNM edition that SCLC continue to be staged by the same criteria as applied to NSCLC.[76] Large cell neuroendocrine carcinomas are also staged by the same criteria as those for NSCLC.

SMALL BIOPSY AND CYTOLOGY

It is widely recognized that cytology is superior to small biopsy in the diagnosis of SCLC, since the crush artifact limiting small biopsy interpretation is minimized in cytology specimens.[77] Similarly, carcinoid tumors can be accurately diagnosed in cytology and small biopsy specimens; however the distinction of typical from atypical carcinoid is generally deferred to a resected specimen. Although the cytologic features of LCNEC have been described in retrospective analyses,[69,78] preoperatively, LCNEC is most frequently recognized in cytology as NSCLC, not otherwise specified or as adenocarcinoma.[69,79,80] As discussed above, Ki-67 (MIB1) is an extremely useful ancillary tool for the distinction of carcinoid tumors from high-grade NE carcinomas in scant morphologically equivocal cases.[41,72]

CONCLUSION

The current WHO 2004 classification system recognizes 4 major types of lung NETs—TC, AC, LCNEC, and SCLC—by morphologic features in combination with precisely defined criteria for mitotic rate and necrosis. Evidence is accumulating that Ki-67 (MIB1) can serve as a useful ancillary tool in the diagnosis of lung NETs, particularly in small biopsy and cytology specimens. Continued research efforts are needed to identify additional immunohistochemical and molecular biomarkers that can service as ancillary diagnostic tools and as potential therapeutic targets for these diseases.

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