Lung tumours with neuroendocrine differentiation

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Introduction

Neuroendocrine (NE) tumours of the lung include the low grade typical carcinoid (TC), intermediate grade atypical carcinoid (AC) and the high grade large cell NE carcinoma (LCNEC) and small cell carcinoma (SCLC) (Table 1). NE tumours of the lung comprise approximately 20–25% of all invasive lung malignancies. The most common NE lung tumour is SCLC, which accounts for 15–20% [1], followed by LCNEC, which is about 3% in surgical series, and carcinoid, which accounts for 1–2% of lung cancers. The rarest NE lung tumour is atypical carcinoid which comprises approximately 10% of all lung carcinoids, accounting for approximately 0.1–0.2% of invasive lung cancers [2]. These tumours have certain morphologic, ultrastructural, immunohistochemical and molecular characteristics in common [3–7], but there are important differences in incidence, clinical, epidemiologic, histological, survival, and molecular characteristics [8]. Diffuse idiopathic NE cell hyperplasia (DIPNECH) is a pre-invasive lesion for carcinoid tumors, but it is very rare.

There are major clinical, epidemiologic and genetic differences between carcinoid tumours and the high grade SCLC and LCNEC although they share the neuroendocrine phenotype [3–7]. LCNEC and SCLC are associated with older age, more smoking and male gender compared to TC and AC. Multiple endocrine neoplasia (MEN) type I or DIPNECH can occur in patients with TC and AC but is not recognised in LCNEC and SCLC patients [9]. Also, histological heterogeneity with other major histological types of lung carcinoma, such as adenocarcinoma or squamous cell carcinoma, can occur with both LCNEC and SCLC, but it is not characteristic of TC or AC [9–11].

NE lung tumours are problematic for a variety of reasons. First, histological classification has also evolved over the past few decades with recognition of AC [12] and LCNEC [9]. However, widely varied terminology, diagnostic criteria and concepts including well-differentiated NE carcinoma, NE carcinoma (grade 1–3), intermediate cell NE carcinoma, malignant carcinoid and peripheral small cell carcinoma resembling carcinoid [13,14] have led to a great deal of confusion in the literature often making it difficult to understand what tumour types are included in some papers. AC and LCNEC are rare so few pathologists have much experience diagnosing them. In small biopsies, the morphologic appearance of these tumours can overlap. Surgical specimens rather than small biopsies or cytology specimens are usually required to separate TC from AC and to diagnose LCNEC. Light microscopy is sufficient to diagnose SCLC, TC and AC in most cases without the need for special tests, but the diagnosis of LCNEC still requires demonstration of NE differentiation by immunohistochemistry or electron microscopy. In some cases, immunostains are helpful in sorting out the differential diagnosis, especially in small biopsies. In addition, the high grade SCLC and LCNEC show many genetic changes, but in TC there are relatively few and in AC the changes are intermediate [15–19].

There are no specific immunohistochemical or molecular markers that allow for separation of these tumours. In addition, the optimal therapy for AC and LCNEC is not established, so once a diagnosis is established, clinicians are often unsure how to treat patients [2].

This review will focus on describing the histological spectrum of pulmonary NE lesions and diagnostic criteria. The spectrum of NE proliferations and neoplasms in the lung are summarised in Table 1. Each of these topics will be covered in this review. The diagnostic criteria for the main NE lung tumours are summarised in Table 2.

Classification

SCLC and carcinoid were the first NE tumours recognised. In 1972, Arrigoni and colleagues described AC as a more aggressive form of pulmonary carcinoid [12] and in 1991, Travis and colleagues described LCNEC as a high-grade NE non-small cell

Table 1
The spectrum of neuroendocrine (NE) proliferations and neoplasms[†]

(I)	NE cell hyperplasia and tumourlets
	(A) NE cell hyperplasia
	1. NE cell hyperplasia associated with fibrosis and/or inflammation
	2. NE cell hyperplasia adjacent to carcinoid tumours
	3. Diffuse idiopathic NE cell hyperplasia with or without airway fibrosis/obstruction
	(B) Tumourlets (less than 0.5 cm)
(II)	Tumours with NE Morphology
	(A) Typical carcinoid (0.5 cm or larger)
	(B) Atypical carcinoid
	(C) Large cell NE carcinoma
	Combined large cell NE carcinoma[‡]
	(D) Small cell carcinoma
	Combined small cell carcinoma[‡]
(II)	Non-small cell carcinomas with NE differentiation (NED)
(IV)	Other tumours with NE properties
	(A) Pulmonary blastoma
	(B) Primitive neuroectodermal tumour
	(C) Desmoplastic round cell tumour
	(D) Carcinomas with rhabdoid phenotype
	(E) Paraganglioma

[†] Modified from reference [8].
[‡]The histological type of the other component of non-small cell carcinoma should be specified.

Table 2
Criteria for diagnosis of neuroendocrine tumours [8]

Typical carcinoid

A tumour with carcinoid morphology and less than 2 mitoses per 2 mm^2 (10 high-power field (HPF)*), lacking necrosis and 0.5 cm or larger

Atypical carcinoid

A tumour with carcinoid morphology with 2–10 mitoses per 2 mm^2 (10 HPF*) OR necrosis (often punctate)

Large cell neuroendocrine carcinoma

A tumour with a neuroendocrine morphology (organoid nesting, palisading, rosettes, trabeculae)

High mitotic rate: 11 or greater per 2 mm^2 (10 HPF*), median of 70 per 2 mm^2 (10 HPF*)

Necrosis (often large zones)

Cytologic features of a non-small cell carcinoma (NSCLC): large cell size, low nuclear to cytoplasmic ratio, vesicular or fine chromatin, and/or frequent nucleoli. Some tumours have fine nuclear chromatin and lack nucleoli, but qualify as NSCLC because of large cell size and abundant cytoplasm.

Positive immunohistochemical staining for one or more neuroendocrine markers (other than neuron specific enolase) and/or neuroendocrine granules by electron microscopy.

Small cell carcinoma

Small size (generally less than the diameter of three small resting lymphocytes)

Scant cytoplasm

Nuclei: finely granular nuclear chromatin, absent or faint nucleoli

High mitotic rate (11 or greater per 2 mm^2, median of 80 per 2 mm^2)*

Frequent necrosis often in large zones

*10 HPF in a microscope with field of view of 0.2 mm^2; however, the number of HPF to reach 2 mm^2 vary depending on the field of view, see reference [20].

carcinoma [9]. A major development that allowed the recognition of the diagnosis of LCNEC was the introduction of NE immunohistochemical markers such as chromogranin and synaptophysin into routine practice of surgical pathology. Pulmonary NE tumours have been classified with many different terms and criteria such as well, moderately and poorly differentiated NE carcinoma, NE carcinoma (grade 1–3), intermediate cell NE carcinoma, malignant carcinoid and peripheral small cell carcinoma resembling carcinoid [13,14,21]. Some have argued that the terminology should be changed with the idea it may help solve some of the problems of classification of pulmonary NE tumours. However, the problems are the same whatever terms are used. Additional terms and classification systems only cause further confusion, often making it difficult to understand what tumour types are included in some papers. Another problem is that the diagnosis of AC and LCNEC is difficult in small biopsies or cytology and a definitive diagnosis usually requires a surgical specimen. One of the greatest challenges with pulmonary NE tumours is that there is no optimal therapy established for AC and LCNEC [2].

Neuroendocrine cell hyperplasia and tumourlets

NE cells are normally situated at the base of the bronchial and bronchiolar respiratory mucosa as non-ciliated, round to oval shaped cells. Cytologically, these cells demonstrate a moderate amount of cytoplasm, round to oval nuclei with finely granular chromatin [22]. There are cytoplasmic processes extending from the cell surface that rarely reach the airway lumen [23]. When clusters of four to ten NE cells are present, they are called a NE body [22]. NE cell hyperplasia may manifest as either linear arrays of NE cells or clusters of NE cells along the base of respiratory epithelium [24–26]. NE cells play an important role in lung development through regulation of airway branching [27–31]. They also are involved in repair of lung injury in conditions such as cystic fibrosis and bronchopulmonary dysplasia [31, 32]. It is thought that NE bodies function as oxygen sensors and thus, chronic hypoxia can contribute to the development of NE cell hyperplasia [32–36]. NE bodies have been shown to be innervated with channels responsive to mechanical stretching forces that occur in lung growth and development [37].

Tumourlets are composed of nodular aggregates of NE cells in association with airways that demonstrate morphology similar to those of carcinoid tumours. They should measure less than 0.5 cm in greatest diameter. More often they are incidental histological findings of no clinical significance, although they are often found in the setting of bronchiectasis [38], interstitial fibrosis [39], chronic abscesses [40], or tuberculosis [41].

The rare exception is in the setting of diffuse idiopathic pulmonary NE cell hyperplasia.

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

"Diffuse idiopathic pulmonary NE cell hyperplasia" (DIPNECH) is a very rare condition consisting of widespread peripheral airway NE cell hyperplasia and/or multiple tumourlets, with less than 40 cases reported [24,42–44].. Patients may present with the clinical picture of interstitial lung disease characterised by airway obstruction due to small airway narrowing by the NE cell proliferation and/or bronchiolar fibrosis. DIPNECH is thought to represent a pre-invasive lesion for carcinoid tumours since a subset of these patients has one or more carcinoid tumours [44].

The paper by Aguayo and colleagues described six patients with widespread pulmonary NE cell hyperplasia, tumourlets, multiple carcinoids and airway fibrosis. In this paper, they proposed that the NE cell proliferation was the primary lesion and that airway fibrosis was promoted substances such as bombesin produced by the NE cells [24]. Largely based on this paper, the World Health Organisation (WHO) committee for the 1999 classification of tumours of the lung and pleura decided to include DIPNECH as a pre-invasive lesion for carcinoid tumours for the first time, realising this affects only a very small subset of carcinoid patients [44]. More recently, Davies and colleagues reported 19 cases with 15 females and four males and 16 non-smokers [45]. In this study, patients presented in two major ways: nine patients presented with mild interstitial lung disease, such as symptomatic cough and/or dyspnea, averaging 8.6 years prior to diagnosis, while the other ten patients presented as a consequence of an incidental radiographic finding during routine radiologic evaluation for another disorder, usually cancer. Nine patients had TC and tumourlets. AC was found in three patients and one had MEN1 syndrome. The clinical course was stable in most patients, but a few progressed to severe airflow obstruction [45].

The pathologic finding in lung biopsies from patients with DIPNECH consists of extensive NE cell hyperplasia and tumourlets that may be associated with airway narrowing and/or obliteration. TC or

AC may be present and they can be multiple. The surrounding lung parenchyma is generally normal.

Peripheral carcinoid tumours can be associated with NE cell hyperplasia in the mucosa of adjacent bronchioles as demonstrated by Miller and colleagues in 75% of 25 peripheral carcinoid tumours with obliterative bronchiolar fibrosis in 32% of cases [26]. The distinction of DIPNECH from incidental NE cell hyperplasia and tumourlets can be difficult, but DIPNECH is favoured if the histological changes are diffuse throughout representatively sampled available lung tissue and/or if there is clinical evidence of airflow obstruction or computed tomography (CT) evidence of air trapping or multiple nodules consistent with tumourlets and/or carcinoid tumours.

Carcinoid tumours

Clinical features

The mean age for TC and AC patients is 45–55 years but they can occur at any age and there is no sex predilection [46–50]. Up to 50% of patients with TC and AC can be asymptomatic at initial diagnosis. The most common presenting manifestations are dyspnea, haemoptysis, a cough and post-obstructive pneumonia [48,51]. Peripheral carcinoids are more likely to present as an incidental radiologic finding in an asymptomatic patient [52].

A variety of paraneoplastic syndromes can occur such as the carcinoid syndrome [48], Cushing's syndrome [53,54], and acromegaly [55]. Bronchial carcinoids occur in approximately 5% of patients with MEN, type I [56].

Pathological features

The location of pulmonary carcinoid tumours can be central or peripheral with up to 40% presenting as peripheral tumours. It is common to have an endobronchial component in central carcinoids. The tumours are typically circumscribed with a tan, yellow cut surface with an average size of 2–3 cm.

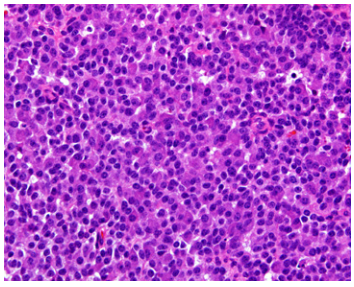

Fig. 1. Typical carcinoid. This tumour shows an organoid nesting pattern of uniform cells with a moderate amount of eosinophilic cytoplasm and finely granular nuclear chromatin.

The histological features of both TC and AC consist of an organoid and nesting growth pattern with uniform cytological features consisting of a moderate amount of eosinophilic cytoplasm (Fig. 1). The nuclear chromatin is finely granular.

A variety of histological patterns can be seen in both AC and TC including spindle cell [57], trabecular [9], palisading [9], glandular, follicular [9], rosette-like [9],, sclerosing, clear cell and papillary patterns [57]. Rarely, carcinoid tumours can show oncocytic or melanocytic features [9,58,59]. Also, stromal ossification or calcification is rarely so extensive that there is mineralisation on radiographs [60, 61].

The criteria for AC are a carcinoid tumour with mitoses between 2 and 10 per $2mm^2$ area of viable tumour and/or the presence of necrosis (Figs. 2A, B and C) [20]. Other histological features, such as pleomorphism, vascular invasion and increased cellularity, are not as helpful in separating TC from AC [9,12]. In AC, the necrosis usually consists of small punctate foci.

Frozen sections in pulmonary carcinoid tumours can be problematic. A recent study showed that the most common misdiagnosis was squamous cell carcinoma (7/66 cases, 11%) while lymphoma (12/40, 30%) and

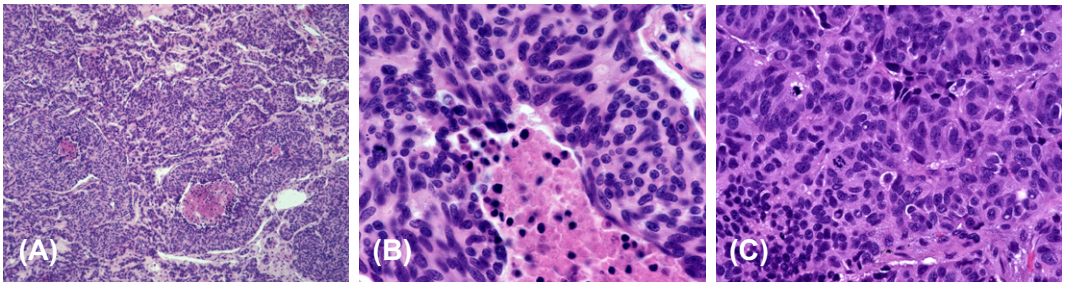

Fig. 2. Atypical carcinoid shows A) punctate foci of necrosis within sheets and nests of carcinoid tumour cells. B) Closer view shows punctate necrosis and tumour cells with finely granular nuclear chromatin. C) There are apoptotic cells and a single mitosis (center, left) in one tumour cell.

metastatic breast cancer (4/38, 13%) were frequently mistaken for carcinoid tumours [62]. The most helpful pathological features in recognition of carcinoid versus lymphoma, squamous cell carcinoma (SCC) or metastatic breast carcinoma were central location (favouring carcinoid or SCC), stromal hyalinisation (favouring carcinoid), salt and pepper chromatin (favouring carcinoid), nuclear pleomorphism (favouring breast cancer and SCC), irregular nuclear membrane (favouring breast cancer, SCC or lymphoma), and mitoses greater than 5 per 10 high-power field (HPF) (favouring SCC or breast cancer) [62].

Immunohistochemistry and electron microscopy

The most useful NE immunohistochemical markers are chromogranin, CD56 and synaptophysin. The expression of TTF-1 in TC and AC has varied widely with some claiming all negative [63] and others frequent positive staining [64,65]. One study demonstrated that TTF-1 was expressed predominantly in peripheral carcinoids rather than central ones [64]. Up to 20–25% may be keratin negative, although most are keratin positive. The proliferation rate by Ki-67 staining is usually low in TC, less than 5%, while in AC it is higher, usually between 5 and 20% [66–68]. Several studies have shown that the proliferation rate can be most helpful in small crushed biopsies to separate TC or AC from LCNEC or SCLC [66–68]. Although electron microscopy is rarely performed anymore because of the reliability of immunohistochemistry, dense core granules by electron microscopy are characteristic of pulmonary carcinoids and they tend to be fewer in AC compared to TC.

Differential diagnosis

The differential diagnosis for carcinoid tumours includes the separation of TC from AC as well as from the high grade SCLC and LCNEC. The criteria for separation of TC and AC are summarised in Table 2. TC cannot be reliably distinguished in small biopsies and one can usually only make a diagnosis of carcinoid tumour. In small biopsies or cytology specimens, a diagnosis of AC may be suspected, if necrosis and/or mitoses are seen, but the distinction between TC and AC usually requires a surgical lung biopsy because it is difficult to determine the level of mitotic activity and to identify the characteristic punctate necrosis. The same crush artifact often seen in SCLC or LCNEC can be seen in carcinoid tumours in small biopsies or cytology specimens and the identification of mitotic activity can be difficult. Immunohistochemistry for Ki-67 can be helpful in such cases because it typically shows a high proliferation rate compared to TC or AC. The high-grade tumours can be distinguished from TC and AC more easily in resected specimens by their high mitotic rate.

Carcinoid tumours can be confused with many other tumours including smooth muscle tumours, sclerosing haemangioma, mucoepidermoid carcinoma, solitary fibrous tumour, metastatic breast carcinoma and metastatic melanoma. However, because carcinoid tumours can show distinctive morphologic features and they have distinctive immunohistochemical properties, such as positive staining for NE markers, the differential diagnosis can usually be sorted out with immunohistochemistry.

Treatment and prognosis

The main therapy for pulmonary carcinoids is surgical resection [48,69–72] and there is an excellent prognosis for TC patients who rarely die from their tumours [48,73]. The treatment of choice is lobectomy in most cases. In some cases it may be possible to perform lung sparing procedures such as segmentectomy, wedge excision, bronchial sleeve resection or endoscopic resection with or without laser removal. However, in such cases, lymph node sampling should be performed if possible for staging purposes. In a study of 23 TC and 11 AC that presented with lymph node metastases, Thomas and colleagues reported that most patients with TC and lymph node metastases had a favourable prognosis [74].

The 5-year survival for AC is significantly reduced (61–88%, Fig. 3) compared to that for TC (92–100%) [46,51,75–79]. Lymph node metastases can be found in 4–14% of TC and 35–64% of AC at presentation.

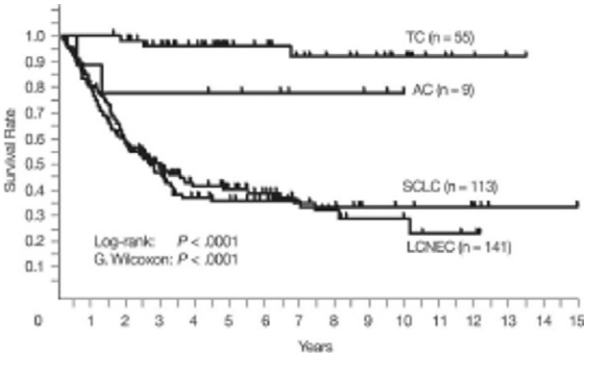

Fig. 3. Significant survival differences for pulmonary neuroendocrine tumours in overall survival from Japanese NE Registry ($P < 0.0001$, $n = 318$ total), with TC ($n = 55$), AC ($n = 9$), LCNEC ($n = 141$), and SCLC ($n = 113$). From reference [46].

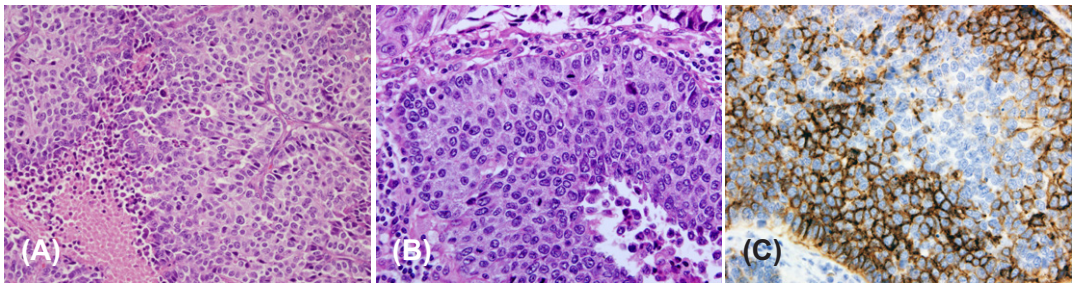

Fig. 4. Large cell neuroendocrine carcinoma. A) The tumour shows necrosis and grows in organoid nests with peripheral palisading, rosette-like structures and prominent mitoses. B) The tumour cells show peripheral palisading, rosette-like structures, abundant cytoplasm, vesicular chromatin, prominent nucleoli and several mitoses. C) CD56 stains many of the tumour cells with a membranous pattern.

For the first time, TNM staging is recommended for pulmonary carcinoids in the seventh edition UICC/AJCC TNM staging system [80]. This is a change from the sixth edition of the AJCC and UICC which did not formally accept that TNM staging applies to carcinoid tumours [81–84].

TC and AC tumours are relatively resistant to chemotherapy and radiation therapy; therefore, when possible, metastatic disease can sometimes best be managed surgically. Unfortunately, no proven optimal therapy has been demonstrated for metastatic or unresectable TC or AC.

Large cell neuroendocrine carcinoma

LCNEC is a high grade non-small cell NE carcinoma that was first recognised by the WHO classification in 1999 and maintained in the 2004 WHO classification [8,44,85]. Historically, this tumour has been included with other tumours such as AC, large cell carcinoma, non-small cell carcinoma with NE differentiation or combined small cell carcinoma/large cell carcinoma [6,86–88].

LCNEC is classified as a variant of large cell carcinoma [8,44]. There are four major ways that large cell carcinomas can express NE differentiation: 1) LCNECs with NE features, as shown by light microscopy as well as by immunohistochemistry and/or electron microscopy, 2) large cell carcinomas with NE morphology (LCC-NEM) but no NE differentiation, as shown by electron microscopy or immunohistochemistry, 3) large cell carcinomas with NE differentiation (LCC-NED) but no NE morphology, as shown by immunohistochemistry or electron microscopy, and 4) classic large cell carcinomas (LCCs) that lack both NE morphology and NE differentiation, as shown by special studies [9,44].

Clinical features

LCNEC is found in approximately 3% of surgically resected lung cancers (range 1–5%) [10,85,89]. A history of cigarette smoking is found in virtually all patients and most are heavy smokers with greater than a 50 pack/year history of smoking [9]. The strong male predominance probably reflects a predominance of smoking in males compared to females.

The median age is 62 years (range 33–87 years) [10]. Ectopic hormone production and paraneoplastic syndromes are uncommon [10,90,91].

The most common manifestations are chest pain, followed by haemoptysis, dyspnea, cough, fever and weight loss with up to 24% of patients being asymptomatic [88]. By CT, most tumours are in the lung periphery (84%) and the upper lobes (63%) [92]. Less common findings include endobronchial growth (5%), obstructive pneumonia (8%) and pleural effusion (24%) [92].

Pathological features

Most LCNEC are large peripheral tumours, with an average size of 3–4.0 cm (range from 0.9 up to 12 cm) [20,72,92–94]. Grossly, the tumours are typically circumscribed with a necrotic, tan-red cut surface. The diagnosis of LCNEC requires 1) NE morphology with organoid nesting, palisading or rosette-like structures (Fig. 4A), ,2) high mitotic rate greater than 10 mitoses per 2 mm^2 (average 60–80 mitoses per 2 mm^2), 3) non-small cell cytological features including large cell size, low nuclear/cytoplasmic ratio, nucleoli, or vesicular chromatin, and (Fig. 4B), 4) NE differentiation by immunohistochemistry with antibodies such as chromogranin, CD56 (Fig. 4C) or synaptophysin or electron microscopy [8,20]. Necrosis is frequent (Fig. 4A).

The diagnosis of combined LCNEC is made when there is a LCNEC with components of adenocarcinoma, squamous cell carcinoma, giant cell carcinoma and/or spindle cell carcinoma [8,20]. The most common histologic component is adenocarcinoma, but squamous cell, giant cell or spindle cell carcinoma can

be present. If SCLC is present, the tumour becomes a combined SCLC and LCNEC.

The diagnosis of LCNEC is very difficult based on small biopsies or cytology, due to the problems in identifying the NE morphologic pattern and demonstrating NE differentiation by immunohistochemistry in small tissue samples. For this reason, a definite diagnosis of LCNEC will require a surgical lung biopsy in most cases.

The diagnosis of LCNEC based on cytology specimens is also difficult; however, several papers have addressed this subject [95–101]. The difficulty in making this diagnosis is reflected by the study of Kakinuma and colleagues where the diagnosis of LCNEC was not made based on any of the 20 cases evaluated by cytology prior to the diagnosis which was rendered on a surgical specimen [97]. The most common diagnosis made in these cases was carcinoma, histological type undetermined, or, less often, squamous cell carcinoma, AC, small cell carcinoma, and large cell carcinoma [97].

Immunohistochemistry/electron microscopy

The diagnosis of LCNEC requires documentation of NE differentiation by immunohistochemistry or electron microscopy [8,20]. LCNEC express cytokeratin antibodies such as AE1/AE3 and CAM5.2 [94,102]. Positive staining is found for NE markers including chromogranin (55–82%), CD56/NCAM (73–100%), and synaptophysin (40–91%) [9,91,103,104]. A panel of these markers is recommended. TTF-1 is positive in 41–75% of cases [63,102,105]. The proliferation rate is high with Ki-67 staining in 50–100% of tumour cells.

Differential diagnosis

Separation of LCNEC from the intermediate grade AC is important because the latter has a much better prognosis and different biologic behaviour. This is best done based on mitotic activity, where AC should only have up to 10 mitoses per 2 mm^2 [8,9]. However, LCNEC should have a mitotic count of 11 or more per 2 mm^2 but mitotic counts typically range between 50–100 mitoses per 10 HPF [8]. The extent of necrosis in most LCNEC is greater than in AC where it usually consists of punctate foci. The nuclear chromatin of AC is usually finely granular in contrast to LCNEC where it is usually vesicular or coarse.

In addition, the differential diagnosis of LCNEC includes SCLC, large cell carcinoma, large cell carcinoma with NE differentiation (LCC-NED), large cell carcinoma with NE morphology (LCC-NEM) and classical large cell carcinoma (LCC) without NE features. The distinction of LCNEC from SCLC requires consideration of multiple histological features such as cell size, nucleoli, chromatin pattern, and nuclear/cytoplasmic ratio, rather than a single criterion. Fixation or frozen section artifacts can complicate this distinction. The most important stain is a good quality haematoxylin and eosin-stained section. If histological sections are too thick or over stained, it can be difficult to see the cellular and nuclear detail required to make an accurate diagnosis. The difficulty in making this distinction is reflected in the retrospective review of a group of previously diagnosed SCLC where up to 44% of cases were reclassified as LCNEC [91,106].

In a reproducibility study among five pathologists, 18% of LCNEC were called SCLC and 4% AC [106]. The fewest disagreements were for SCLC, where 4% were called AC and 4% LCNEC. However, overall, kappa values were mostly between 0.70–0.77 ('substantial'), with a few kappa values at 0.60 which is at the upper limit of 'moderate' [106].

Large cell carcinoma with neuroendocrine morphology

This tumour was first mentioned in the 1999 WHO classification where it was recognised that some large cell carcinomas have NE morphology (LCC-NEM), but lack positive staining with NE markers [44]. Since these cases are very rare, there is little information available regarding the clinical characteristics of these patients [88,93,107]. However, the existing data suggests that clinical features such as age, gender predilection, smoking history, stage distribution and survival are very similar to LCNEC [88,93,107]. For example, Iyoda and colleagues found LCC-NEM had similar clinical properties to LCNEC with the exception that LCC-NEM had a more significantly elevated serum tissue polypeptide antigen (TPA) compared to LCNEC and a higher lactate dehydrogenase (LDH) than LCCs [93].

Non-small cell carcinomas with neuroendocrine differentiation

Non-small cell carcinomas (NSCLC) with NE differentiation (NSCLC-NED) (Table 1) consist of lung carcinomas which do not show NE morphology under light microscopy but do exhibit NE differentiation when using immunohistochemistry and/or ultrastructure. Immunohistochemistry will show NE differentiation in 10–20% of squamous cell carcinomas, adenocarcinomas, and large cell carcinomas [108]. It is seen most often in adenocarcinomas. The data do not show any consistent clinical significance

either with regard to prognosis or responsiveness to chemotherapy [89,109,109–125].

Treatment and prognosis

LCNEC patients have an aggressive clinical course with overall 5-year survivals ranging from 15–57%. The reported variation in survival is probably due to differences in distribution of lower versus higher stage and the thoroughness of the approach to operative staging. It is likely that the favourable survival data stage for stage observed in some series can be attributed to the careful approach to surgical staging such as systematic nodal dissection [88].

Survival for LCNEC is significantly worse than that for other non-small cell carcinomas. According to Jiang and colleagues, LCNEC patients had a 58.8% and 44.8% 1- and 5-year survival, respectively, which was significantly worse than for patients with other non-small cell carcinomas ($P = 0.046$) [103]. Iyoda also found that the survival for LCNEC was significantly worse than that for LCCs [93]. Takei and colleagues showed that survival in Stage I patients with LCNEC, poorly differentiated NSCLC, and LCC were 67%, 88% and 92%, respectively, and found significant differences between LCNEC and NSCLC ($P = 0.003$), LCNEC and LCC ($P = 0.03$) but not between LCNEC and SCLC [91].

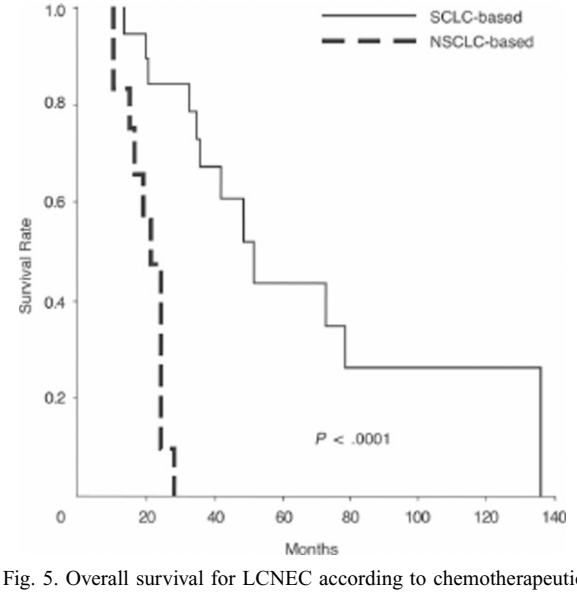

Fig. 5. Overall survival for LCNEC according to chemotherapeutic regimens in the metastatic setting. Median overall survival for patients who received platinum plus etoposide versus gemcitabine and taxanes was 51 and 21 months, respectively. NSCLC = non-small cell lung cancer; SCLC = small cell lung cancer. From reference [126].

There is little data about the efficacy of chemotherapy for LCNEC. The major question is whether LCNEC should be treated in a similar way to SCLC because of the NE features. While the aggressive clinical course and tendency to metastasise is similar to SCLC, it remains to be proven whether LCNEC is also chemotherapy-sensitive. In recent years, several studies have demonstrated clinical response to cisplatin-based chemotherapeutic regimens similar to those used for SCLC [126–128]. However, these are retrospective studies of small numbers of patients who received adjuvant therapy following surgery. The study by Rossi and colleagues showed that patients who received platinum-etoposide-based chemotherapy had the best survival in both the adjuvant and metastatic setting (Fig. 5) [126]. A small series reported by Filosso and colleagues suggested that octreotide may be effective alone or in combination with radiation therapy when given as adjunctive treatment [129].

There is too little data regarding radiation to know whether it is effective in LCNEC or not [86,91,93, 130,131].

Further investigation is needed regarding the spectrum of clinical and pathological features of LCNEC and optimal therapeutic approach. Hopefully, future work will further define the differences in survival and response to therapy for LCNEC compared to AC, LCC-NEM, LCC-NED, LCC and SCLC.

Small cell carcinoma

Clinical features

Small cell lung cancer (SCLC) is the most common pulmonary NE tumour accounting for an estimated 28,000 of the 215,020 lung cancer cases diagnosed in the US in 2008 [132]. According to the US National Cancer Institute's Surveillance, Epidemiologic, and End Results (SEER) database, the proportion of SCLC cases among all lung cancers in the US has decreased from 17% to 13% in the last 30 years [133].

Virtually all SCLC patients are cigarette smokers [134]. In fact, if the diagnosis of SCLC is suggested in a non-smoker, the pathology diagnosis should be carefully re-evaluated. Clinically, SCLC is a tumour that grows rapidly and metastasises early [134]. The presenting manifestations of SCLC can be divided into four categories: constitutional, pulmonary, the result of extrathoracic spread, or due to paraneoplastic disorders [134]. Typical symptoms include fatigue, a cough, dyspnea, decreased appetite, weight loss, pain and haemoptysis. The typical radiological finding is a large central mass invading or compressing the

mediastinum with hilar or mediastinal adenopathy. In 10% of patients, superior vena cava obstruction is found at presentation [134]. Rarely, SCLC presents as a solitary pulmonary nodule [135]. Most patients with SCLC have metastases at diagnosis involving sites such as bone, brain, liver and adrenals [134]. The paraneoplastic syndromes associated with SCLC include the syndrome of inappropriate anti-diuretic hormone (SIADH), Cushing's syndrome, or neurologic paraneoplastic syndromes such as autoimmune neuropathies and encephalomyelitis that probably have autoimmune mechanisms [134].

Because of its tendency to metastasise early, a two stage system of limited versus extensive stage disease, apart from TNM staging, has been recommended by the Veterans' Administration Lung Study Group (VALSG) for SCLC [136]. Approximately two-thirds of patients with SCLC have extensive disease at diagnosis, and one-third have limited-stage disease [133]. According to the VALSG system, limited stage consists of tumour confined to one hemithorax that can be "encompassed" in a "tolerable" radiation field. Less than 10% of patients, who have a tumour only involving the lung, are surgical candidates. Recently, analysis of a large database of over 8000 patients demonstrated that TNM staging is effective for SCLC and use of TNM staging will be recommended in the upcoming 7th edition [137].

Pathology

Because most patients are unresectable at presentation, the majority of specimens obtained for the diagnosis of SCLC are small specimens such as bronchoscopic biopsies, fine needle aspirates, core biopsies, and cytology. The diagnosis can readily be established based on these specimens in the vast majority of cases. While some of the description in the 2004 WHO classification deals with issues involving surgical resection specimens [8], most of the criteria are applicable to small biopsies as well.

When surgically resected, the diagnosis of SCLC has not usually been established prior to surgery and the tumour consists of a peripheral nodule measuring 2–4 cm in size. The tumour is usually circumscribed with a tan, necrotic cut surface.

The diagnosis of SCLC is based primarily on light microscopy (Fig. 6A). Necrosis is common, frequently with large areas. Although there is no absolute criterion for cell size, in general the tumour cells measure less than the diameter of three small resting lymphocytes. Tumour cells are usually round to fusiform with scant cytoplasm. Nuclear chromatin

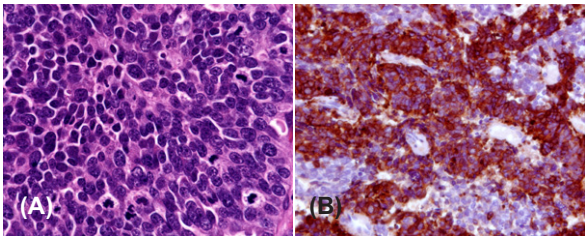

Fig. 6. Small cell carcinoma. A) This tumour consists of dense sheets of small cells with scant cytoplasm, finely granular nuclear chromatin, frequent mitoses and nucleoli are inconspicuous or absent. B) Chromogranin staining is positive.

is finely granular and nucleoli are inconspicuous or absent [8,138]. There is usually a high mitotic rate, averaging 60–80 per 2 mm^2. Although this may be more difficult to appreciate in small biopsy specimens, frequent mitoses can usually be detected. The crush artifact and tumour streaming often seen in small transbronchial or mediastinal biopsy specimens can complicate pathological interpretation. These artifacts can also occur with NSCLC, lymphoma, carcinoid and chronic inflammation. The cells of SCLC appear larger than in small biopsies in surgically resected specimens where the tumour is better fixed [8,138, 139]. SCLC are called combined SCLC when there is also a component of NSCLC, such as adenocarcinoma, squamous cell carcinoma, large cell carcinoma, spindle cell carcinoma or giant cell carcinoma. In this setting the specific histology of the non-small cell component should also be mentioned [8,138]. In resected specimens, combined SCLC may occur in up to 28% of cases [8,138]. Although there is no percentage requirement for the components of adenocarcinoma, squamous cell, spindle cell or giant cell carcinoma when these result in a combined SCLC, in order to diagnose combined SCLC and large cell carcinoma, the large cell carcinoma component must comprise at least 10% of the overall tumour [8,138].

The most important special stain for a SCLC diagnosis is a good quality haematoxylin and eosin stain that is not too thick or over stained. Immunohistochemistry may be helpful but if the histological features are classic, it may not be needed. Since virtually all SCLC stain for keratin, a pancytokeratin antibody such as AE1/AE3 is useful to confirm that the tumour is a carcinoma. If keratin is negative, stains for other tumours such as lymphoma, including CD45 and CD20, or stains for primitive neuroectodermal tumours (PNET), such as CD99, may be helpful. The most useful NE markers include CD56, chromogranin (Fig. 6B) and synaptophysin, which are best used as a panel. TTF-1 expression is found in 70–80% of SCLC [63,102,105].

SCLC must be separated from other NSCLC, carcinoid tumours, malignant lymphoma, and sarcomas such as PNET. This differential diagnosis can be very difficult in small, crushed biopsy specimens. Since cytology is often obtained at the time of bronchoscopic biopsy and SCLC shows very characteristic cytological features, comparison of biopsy material with the cytology specimen can be very helpful. In particular, LCNEC and the basaloid variant of large cell carcinoma can be difficult to distinguish from SCLC. SCLC can be distinguished from carcinoids by a high proliferation rate of 80–100% with Ki-67.

In approximately 5% of cases, SCLC can be difficult even for expert lung cancer pathologists to separate from NSCLC [106,140,141]. These cases may require special scrutiny using a consensus approach among other pathology colleagues. If a consensus diagnosis cannot be reached, it may be appropriate to refer the case for extramural consultation. Immunohistochemical markers can be of assistance in crushed specimens, as SCLC may demonstrate positive staining for cytokeratin, chromogranin, CD56, synaptophysin, TTF-1 and a high proliferation index with Ki-67 [11]. However, some preserved tumour cells with characteristic morphology should be seen on light microscopy to confirm the diagnosis. Up to 10% of SCLC may be negative for all NE markers if a panel of antibodies including CD56 is utilised, so if all other features are present the diagnosis of SCLC should not be avoided [142]. Since TTF-1 can be positive in extrapulmonary small cell carcinomas, it should not be used to determine the primary site of small cell carcinomas [143].

Differential diagnosis

The differential diagnosis for SCLC has been addressed with regard to LCNEC, other NSCLC and the carcinoids. In addition, the use of immunohistochemistry to distinguish SCLC from lymphoma and PNET has been mentioned. Problems in small crushed biopsies have also been discussed. While most cases can be diagnosed with routine haematoxylin and eosin-stained sections, ensuring a good quality haematoxylin and eosin stain that is not too thick or over stained is one of the most important steps to a correct diagnosis.

Treatment and prognosis

Survival for SCLC is poor with the SEER programme database reporting overall survival at 2, 3, and 5 years of 12%, 7%, and 5%, respectively [144]. Poor prognostic factors include performance status, Cushing's syndrome, continued smoking and metastases to sites such as the liver, brain, bone marrow and bone [134]. Female gender has been associated with improved survival and response to therapy [134].

The mainstay of treatment for SCLC is combination chemotherapy, typically with etoposide plus either cisplatin or carboplatin [134]. Patients with limited stage disease are usually given chemotherapy concurrently with radiation.

Molecular changes in pulmonary neuroendocrine tumours

Genetic studies reveal important molecular differences among the spectrum of NE lung tumours. In general, LCNEC and SCLC show frequent genetic changes with fewer seen in the carcinoids. A limited number of genetic markers demonstrate significant differences between TC and AC or LCNEC and SCLC, but these findings support the concept that these tumours should be classified separately.

Onuki and colleagues demonstrated that the high-grade LCNEC and SCLC had a significantly higher frequency of loss of heterozygosity (LOH) for 3p, RB, 5q21, 9p, and p53 than in the carcinoids [17]. Significantly more frequent 5q21 LOH was found in SCLC compared to LCNEC as well as in the high-grade carcinomas compared to the carcinoids. In addition, there were increasing percentages of p53 abnormalities by immunohistochemistry, LOH and mutation analysis from TC to AC and the high-grade SCLC and LCNEC [17]. No p53 mutations were found in TC with 25% in AC, 59% in LCNEC and 71% in SCLC. These results are similar to data reported by others in high-grade NE carcinomas with p53 expression ranging between 40 and 86% and p53 mutations from 27–59% [7,18,67,145–147]. Interestingly Onuki and colleagues found that 58% of the point mutations found in high-grade NE tumours were G:C to T:A or other transversions [17]. The G:C to T:A transversions are associated with carcinogens found in cigarette smoke, consistent with the high frequency of heavy cigarette smoking in LCNEC and SCLC patients [148]. However, these transversions were not present in the mutations found in AC, corresponding with the fact that AC patients have significantly less smoking histories compared to LCNEC and SCLC [17]. A single K-RAS mutation was found in one LCNEC [17].

The $P16^{INK4}$/cyclin D1/Rb pathway is involved with regulation of G1 arrest in the cell cycle and it is frequently affected in NE tumours [149,150]. Rb loss

is found in a high percentage of SCLC and LCNEC but not in TC and in only 21% of AC. There is an inverse relationship between Rb and P16 in the high-grade tumours and a direct relationship between cyclin D1 and Rb in all tumours indicating that p16 and cyclin D1 act exclusively on the Rb pathway for cell cycle regulation [149]. Igarashi also demonstrated overexpression of cyclin B1 in a high percentage of LCNEC and SCLC. These data demonstrate that loss of Rb is the most frequent mechanism of Rb cell cycle pathway deregulation in LCNEC and SCLC [149,150]. The frequent expression of cyclin B1 in LCNEC and SCLC is consistent with the concept that regulation of cyclin B1 expression and G2/M arrest are consistently compromised in the high-grade NE carcinomas [150].

Study of apoptosis shows that LCNECs have a high index (1.3–6.8%) of apoptosis compared to carcinoids that have a variable apoptotic index and SCLCs that have almost no apoptosis [7]. In contrast to TC and AC that have predominant Bax expression, LCNEC and SCLC have a high Bcl2/bax ratio [7,151]. These findings are consistent with the concept that LCNECs have a high rate of cell division that could be worsened by abrogation of cell death which is favoured by high Bcl2 and low Bax levels; this could result in a short doubling time and tumour aggressiveness [7].

Several studies have evaluated C-kit protein expression in pulmonary NE tumours. Pelosi and colleagues found frequent expression in the high-grade tumours and distinguished membranous from cytoplasmic patterns of staining with membranous/cytoplasmic staining in 77/44% of LCNEC, 67/70% of SCLC and much less staining with 7/5% in carcinoids [152] C-kit staining was demonstrated in 55% and 61% of LCNEC by Araki and Casali and colleagues [153,154]. A significantly worse prognosis ($P = 0.046$), as well as a higher rate of recurrence ($P = 0.037$), was found by Casali and colleagues for patients with C-kit positive LCNEC [154]. However, with LCNEC and SCLC, neither Pelosi and colleagues nor Araki and colleagues found any prognostic significance to C-kit expression [152,153].

LOH at chromosome 11q13, the site of the MEN1 gene, is found in lung carcinoids from familial MEN1 patients [155]. In addition, LOH at this locus and MEN1 gene mutations can be demonstrated in up to 36% of sporadic carcinoids, particularly AC [156]. However, MEN mutations are very rare in LCNEC and they are not found in SCLC [106,157,158]. In one of 13 LCNECs, Debelenko and colleagues found a somatic frameshift in the MEN1 gene (1226delC) that represented the first mutation observed in a tumour not typically associated with MEN1 [158]. On the other allele, neither a deletion nor mutation were detected and wild-type mRNA sequence was expressed. This suggested that the typical two-hit mechanism of MEN1 gene inactivation had not taken place [158].

As more study is made of molecular alterations in NE tumours, it is hoped that it may lead to novel therapeutic approaches. As we do not have effective therapies for LCNEC, AC, or TC with metastases, an understanding of the molecular changes in the entire spectrum of pulmonary NE tumours is important. Since these tumours are so uncommon, there are few molecular studies that have examined large numbers of these rare variants. Also, since only a few institutions have frozen tissue banks, most molecular studies of these tumours have been retrospective studies performed on formalin-fixed and paraffin-embedded tissue samples, limiting the type of molecular studies that can be performed.

International registry of pulmonary neuroendocrine tumours

Because of the great need for collaboration to gather sufficient numbers of the rare subtypes of pulmonary NE tumours including TC with metastases, AC, LCNEC and surgically resected SCLC, the International Association for the Study of Lung Cancer has developed an International Registry of Pulmonary NE Tumours [159]. This will provide a network of collaborations to foster research of these tumours with the hope it will lead to development of novel molecular targeted therapies for these tumours.

Conflict of interest statement

None declared.

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