Neuroendocrine Carcinomas of the Lung
A Critical Analysis

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Abstract

Neuroendocrine carcinomas represent an important group of primary neoplasms in the lung. During the last decades, the nomenclature of these tumors has evolved and the current use of immunohistochemical and molecular biology studies have, to some extent, expanded the conventional view of these tumors. However, the primary diagnosis of most of these lesions is performed on limited biopsy specimens, which may not translate well when one is confronted with a nomenclature that is based on resected material. In addition, for some of these specific entities, some confusion and controversy apparently remain, allowing for the proliferations of different terms that, although they may be dismissed as “semantics,” may have a role in interpretation, further subclassification, and, possibly, treatment. Herein we review current concepts regarding the classification of these neoplasms and the role of this classification in our daily practice and discuss how it may impact treatment.

Neuroendocrine tumors are ubiquitous neoplasms that have been the subject of investigation for more than a century. Although the low-grade tumors were initially described as a form of neoplasms that behave better than conventional carcinoma (carcinoid tumor), in time, different studies of these neoplasms have focused on the concept of a family of neoplasms that may expand from the indolent and insignificant small lesion (so-called tumorlet), which is most often encountered by chance, to low-, intermediate-, and high-grade malignancies. Different studies, including morphologic analyses, immunohistochemical studies, and, more recently, molecular studies have attempted to conceptualize this family of neoplasms to provide a better understanding in terms of clinical course, natural behavior, and possible histogenesis; however, universal agreement is still lacking. Some reviews on the subject1 precede the most recent classification of lung tumors by the World Health Organization (WHO)2 or have separated these tumors in the conventional 3-way category system.3 Although other studies have followed the WHO classification, those studies have stated the difficulty that exists in making these diagnoses on surgical biopsy specimens.4

The problem deepens by the fact that the designation given to some of these tumors depends largely on the anatomic site in which they may appear. An example of this issue is the WHO classification for tumors of the pleura, lung, thymus, and heart,2 in which tumors in the thymus are separated into low- and high-grade malignancies, whereas tumors occurring in the lung are separated into a 4-grade system, creating serious concerns for professionals involved in the treatment of the patients.

Although the entire concept (body length) of neuroendocrine tumors requires revisitation, we cannot be that inclusive...
in this review, and we will limit our discussion to tumors occurring in the lung, not only because they are not that uncommon but also because the pulmonary nomenclature has been borrowed to classify these tumors in other anatomic areas. Currently, there are some entities in the spectrum of neuroendocrine tumors in the lung that not only are still ambiguous in their definition but also require an in-depth appraisal of their clinical significance.

**Historic Aspects**

Although the credit is given to Siegfried Oberndorfer for the initial description of carcinoid tumor, the credit should be for coining the term carcinoid. Bunting, from Johns Hopkins in 1904, described a case under the designation of “multiple primary carcinomatata” and made reference to other possible descriptions that dated back to the 18th century. Thus, it seems that this tumor may have been recognized well over a century ago. In 1914, Gossett and Masson described similar tumors in the appendix and made an analogy to the one previously described in the literature by Oberndorfer.

At the same time that knowledge of these tumors was being gained, ie, in the gastrointestinal tract, similar tumors in the respiratory tract, essentially those occurring in the bronchial wall, were being coded as bronchial adenomas. In this regard, Gmelich et al identified the presence of Kulchitsky cells in bronchioles, establishing the relationship of these cells and the occurrence of these neoplasms in the lung. It is interesting that Hausman and Weimann described a case (which seems to be a low-grade neuroendocrine carcinoma) with lymph node metastasis under the designation of pulmonary tumorlet. The authors alluded to the fact that these tumors have a low malignant potential. In their description of the case, the tumor measured 1.5 cm, and it had spindle cell morphologic features with lymph node metastasis.

On the other hand, Azzopardi in a study of 100 cases of what he called “oat cell carcinoma,” based on 16 surgical cases and 84 cases from autopsy material, stated that oat cell carcinoma has positive structural features that identify this tumor, including streams, ribbons, rosettes, and ductules. Judging by this definition and at least one of the illustrations presented in this review, it is very likely that some of the cases described in this study may not represent oat cell carcinoma as it is defined today. Thus, it is possible that some of the cases presented by Azzopardi may correspond to low- or intermediate-grade neuroendocrine carcinomas of today. Similar experience may be drawn from the 138 cases of oat cell carcinoma described by Kato et al, in which some of the tumors, although neuroendocrine in nature, may not necessarily be of the oat cell type as it is defined today.

**Histogenesis, Differentiation, Multidirectional Differentiation, and Divergent Differentiation**

Essentially, the first 2 of these terms are intrinsically embedded in surgical pathology. However, although these terms are similar, they are different and, unfortunately, have been often misused when it comes to tumor description. Histogenesis can be defined as the formation or development of tissues from the undifferentiated cells of the germ cell layers of the embryo. On the other hand, differentiation can be defined as the process of acquiring distinct or individual characters.

Gould et al introduced the term multidirectional differentiation after observing the presence of neuroendocrine, mucosubstance-producing, and squamous cells in pulmonary carcinomas. In addition, Gould et al made observations that certain tumors may share similar patterns of differentiation. It is interesting that Gould et al also described cases, which by electron microscopy showed predominant features of squamous differentiation, wherein the authors designated the tumors as neuroendocrine carcinomas with squamous differentiation. The authors also alluded to the fact that some squamous cell carcinomas and adenocarcinomas of the lung may show membrane-bound and dense core granules by electron microscopy, thus setting the platform for what later was transformed into large cell neuroendocrine carcinoma and large cell carcinoma with neuroendocrine differentiation. Nevertheless, Gould also warned about the possibility of having cell populations with similar or identical patterns of differentiation, which may not necessarily share identical or even closely related embryogenesis.

To that extent, other authors had documented earlier the presence of neoplasms of the non–small cell type, which histologically may look like squamous cell carcinomas or adenocarcinomas, but in ultrastructural studies show the presence of neurosecretory granules. Such descriptions have raised high concerns about the true significance of the many classification systems available for neuroendocrine tumors. In addition, tumor differentiation raises even higher issues, such as the fact that some small cell carcinomas may lack the presence of immunohistochemical differentiation for neuroendocrine markers and/or the presence of neurosecretory granules by ultrastructural studies, at the same time showing the presence of ultrastructural features of epithelial tumors. Are these tumors “small cell variants of squamous cell carcinomas” or truly undifferentiated carcinomas of the small cell type? Both designations have been suggested for tumors with the aforementioned characteristics.

The issue of histogenesis and differentiation can even get more confusing when it is associated with growth pattern, morphologic features, and cell size. How small does the cell have to be to fall into the category of small cell carcinoma?
Is the presence of nucleoli the sole discriminatory feature to separate small from non–small cell carcinoma? Is the presence of a single neuroendocrine marker evidence enough to categorize a tumor as neuroendocrine? Which neuroendocrine marker? Are all of these tumors part of a spectrum? A fact remains that regardless of the histologic subtype, all lung tumors are capable of showing neuroendocrine differentiation by immunohistochemical analysis or electron microscopy.

More recently, other authors,17 arguing that the current revised classification of tumors by the WHO2 clearly defines each one of the neuroendocrine tumors of the lung, introduced the term divergent differentiation (divergent: tending apart; deviating or radiating away from a common point), which describes essentially the same tumors that Gould et al14 had described under the term multidirectional differentiation. Nevertheless, the authors alluded to the presence of specific markers (chromogranin, synaptophysin, and neural cell adhesion molecule) to help in the differentiation of neuroendocrine tumors and further declared that all lung tumors derive from a common endodermal stem cell (ie, histogenesis). Furthermore, the authors stated that divergent differentiation applies to a subset of non–small cell carcinomas that are not considered morphologically neuroendocrine but that express neuroendocrine differentiation with neuroendocrine markers (so-called non–small cell carcinoma with neuroendocrine differentiation).

At this point, it is prudent to take a step back and ask one important question: Are we defining neuroendocrine tumors by morphologic features, by the presence of neuroendocrine differentiation through immunohistochemical studies, or by ultrastructure? As presented by Brambilla et al,17 it seems that the designation and grouping of certain tumors is a morphologic approach, and, if that is the case, the next question is: Why would anyone perform immunohistochemical studies on a tumor that does not show “the right neuroendocrine morphologic features”\(^\)? What if the tumor shows the right morphologic features, but the neuroendocrine markers are negative? Can we still call it neuroendocrine? Even greater questions would be: What are the right morphologic features and are they reproducible by most surgical pathologists? What would happen with tumors that have these right morphologic features but that are immunohistochemically negative for neuroendocrine markers? Once again, are we classifying tumors by immunohistochemical differentiation or by morphologic features? Currently it is both, but that negates proper classification of tumors in either side, morphologic features alone or neuroendocrine differentiation without the right morphologic features.

Also, from a practical standpoint, are we dealing with biopsy specimens or resection specimens? If the answer is biopsy specimens, then the growth pattern of the tumor may be very difficult to assess, which means that the interpretation of these tumors may be a diagnosis of resected specimens only, a subject that has been ignored by the WHO classification of lung tumors.

In addition, Brambilla et al\(^17\) alluded to the presence of the so-called tumorlet, which the authors described as a small tumor-like hyperplastic lesion. However, the same authors also acknowledge that these so-called tumorlets do not differ in the cellular components from the so-called typical carcinoid, and these tumorlets also display divergent differentiation. It is perhaps this so-called tumorlet, which arbitrarily has been assigned a size of no more than 5 mm, that may represent the true carcinoid tumor in the lung. After all, the original description of carcinoid in the gut was for tumors that behaved better than conventional carcinoma.

Tumorlets in the lung are purportedly benign lesions, as one would expect from something that looks like carcinoma but behaves much better than carcinoma. Although there are reports of metastatic tumorlets,11 those early reports are incorrect and represent the current so-called typical carcinoid. What is more puzzling in some reviews on this subject endorsing the “clear definitions of the WHO classification system,” is the description of some of these entities. For example, in the review by Brambilla et al\(^17\) on large cell neuroendocrine carcinoma, the authors state that “because this large cell phenotype can be misleading with non–small cell lung carcinoma, the use of neuroendocrine markers is necessary to assess the diagnosis.” That statement leads anyone to argue that the so-called right morphologic features have been ambiguously presented at best. Brambilla et al\(^17\) state that there is an overlap between morphologic patterns. Thus, the question arises, has it been well defined? Obviously the answer is that, although great attempts have been made, there is no universal agreement among pathologists when it comes to diagnosing large cell neuroendocrine carcinoma. As a matter of fact, as discussed later, even among chosen experts, a unanimous consensus of the diagnosis of this entity is not high enough.

Classifications

Neuroendocrine lung neoplasms have been a subject of numerous classification systems; many of them, although logical, fail to provide the practical approach, whereas others, although practical, fail to properly define this complex group of tumors. Herein we evaluate the most important past and present classification systems and, at the same time, highlight their practical use or the lack of it.

In 1977, Gould\(^18\) introduced the terms neuroendocrineomas and neuroendocrine carcinomas by drawing an analogy of these tumors with the APUD (amine precursor uptake and decarboxylation) cell system neoplasms and their aberrant secretory activities. Gould\(^18\) emphasized the numerous
neoplasms that may belong to this APUD system, which is not limited to the respiratory tract or to a particular group of tumors, ie, carcinoid tumor. Gould\textsuperscript{18} also warned against the overuse of terms like carcinoid when applied to certain tumors regardless of primary site, synthetic capabilities, or behavior. Gould\textsuperscript{18} also elaborated on abandoning traditional terms such as bronchial adenoma, a term that does not convey the true nature of these neoplasms and, at the same time, is used to encompass a diverse group of tumoral conditions. However, 3 important issues may be highlighted in his article\textsuperscript{18}: (1) the preferred term of bronchopulmonary neuroendocrine tumor as opposed to bronchopulmonary carcinoid; (2) the inference that “oat cell” carcinomas represent the malignant counterpart of carcinoid; and (3) the continued use of the term undifferentiated oat cell carcinoma.

The 3 issues, in later literature, have become a central point of controversy and discussion. Admittedly, Gould\textsuperscript{18} did not propose a new classification system at that time. It was not until 1983 that Gould et al\textsuperscript{19} presented a new classification system for neuroendocrine pulmonary neoplasms.

The Gould Classification

It is important to note that one of the goals of this classification system was to define the term carcinoid. In the view of Gould et al,\textsuperscript{19} such a term has been overused and provoked unnecessary confusion. They also noted the presence of carcinoids with atypical histologic features and capable of aggressive behavior that have been termed pleomorphic, atypical, and anaplastic. Similarly, another goal was to rid the literature of terms such as small cell bronchogenic carcinoma, undifferentiated small cell carcinoma, and anaplastic small cell carcinoma. In retrospect, the classification presented by Gould et al\textsuperscript{19} failed to eliminate such terminology and, in fact, introduced some vague terms to this conundrum. Novel to this classification system is the introduction of a 4-way classification system instead of the conventional 3-way split. The schema of Gould et al\textsuperscript{19} is as follows:

Bronchopulmonary Carcinoid

Bronchopulmonary carcinoids have typical histologic features, are locally invasive, and have the potential for recurrence and distant metastasis. Let us analyze this more carefully. Bronchopulmonary carcinoid, first of all, is separated from neuroendocrine carcinoma (see the following sections). An analysis of the cases presented reveals that all cases showed penetration of the bronchial wall and mediastinal soft tissue. In addition, 6 cases showed direct invasion into lymph nodes at the time of initial diagnosis. Although the authors argue that no vascular invasion was identified in these cases, such a finding is commonly observed and is not considered (at least for the time being) as part of the criteria for upgrading a tumor.

Well-Differentiated Neuroendocrine Carcinoma

Even though Gould et al\textsuperscript{19} state that this term may not represent an entirely satisfactory designation, this designation is for tumors that retain a clearly organoid pattern, moderate cellular pleomorphism, mitosis (the authors do not state a number), and “true” lymph node metastasis. This latter feature is rather ambiguous, leading one to assume that, in what they call bronchopulmonary carcinoid, the lymph node invasion that they described in 6 cases is not a “true invasion or metastasis.”

Neuroendocrine Carcinoma of Intermediate-Sized Cells

This term represents a variant of small cell neuroendocrine carcinoma. The cells are twice the size of “small cell” counterparts with prominent nucleoli and abundant mitosis (once again, a number is not provided). It is interesting that Gould et al\textsuperscript{19} state that 7 of 11 cases showed features of glandular or squamous differentiation.

Neuroendocrine Carcinoma of Small Cell Type

These carcinomas exhibit typical oat cell carcinoma, abundant mitoses, and inconspicuous nucleoli. Gould et al\textsuperscript{19} comment that not all tumors in this category are neuroendocrine and recommend the systematic use of immunohistochemical studies to separate the tumors that are neuroendocrine from tumors that are not neuroendocrine.

Other Classifications

In 1985, Warren et al\textsuperscript{20} reported a study of 81 cases of pulmonary neuroendocrine neoplasms, assessing the Gould classification system and determining its usefulness for the proper classification and treatment of patients with those neoplasms. During the same year, Paladugu et al\textsuperscript{21} presented a new classification system, designated Bronchopulmonary Kulchitsky Cell Carcinomas (KCCs), and reverted to the 3-way classification of neuroendocrine neoplasms. Paladugu et al\textsuperscript{21} used the designations KCC-I, KCC-II, and KCC-III for typical carcinoid, atypical carcinoid, and small cell carcinoma, respectively. It is interesting that Paladugu et al\textsuperscript{21} provided mortality rates of 1.7% for KCC-I and 27% for KCC-II. Histologically, the tumors coded under KCC-II showed a mitotic activity of 1 mitosis per high-power field (HPF; range, 0.3-6.7). In the table provided in the article, the mitotic count provided is 3 to 67 per 10 HPF (perhaps a typographical error). Nevertheless, in either case, this mitotic count is rather ambiguous at best. Paladugu et al\textsuperscript{21} concluded that their nomenclature is preferred to that of Gould et al\textsuperscript{19} because they consider the Kulchitsky cell as the origin of these tumors, and, in addition, it is simpler and less confusing.

However, it was Arrigoni et al\textsuperscript{22} who in 1972 laid the foundation for the concept of atypical carcinoid by separating those tumors based on cellular atypia and mitotic activity with an average of 1 mitotic figure per 1 or 2 HPF, thus leaving a
window of 5 to 10 mitotic figures per 10 HPF. It is of interest to note that Arrigoni et al., Gould et al., and Paladugu et al., although their most important criterion was essentially the number of mitotic figures, failed to provide a more specific number per 10 HPF, leaving a gap in this interpretation.

Given that state of confusion, in 1982, Mills et al. reported a study of 17 cases of atypical carcinoid tumors of the lung in which the mitotic count varied from 2 to 28 mitotic figures (mean, 14; median, 13). Also Valli et al. reported a study of 33 cases of atypical carcinoid tumors of the lung in which the mitotic activity varied from 4 to 80/1.52 mm². In another report, the resulting criterion is that of increased mitotic activity, greater than 1 per 1 or 2 HPF. At this point, one can only assume that the criterion to separate carcinoid from atypical carcinoid has been less than ideal when it comes to the issue of mitotic activity.

In 1995, Capella et al. revised the classification of neuroendocrine tumors of the lung, pancreas, and gut. Capella et al. reclassified these tumors as follows: (1) benign or low-grade malignant, nonfunctioning, well-differentiated tumor as the equivalent for conventional carcinoid; (2) low-grade malignant, nonfunctioning, well-differentiated carcinoma as equivalent for atypical carcinoid; and (3) high-grade malignant, functioning or nonfunctioning, poorly differentiated carcinoma for the large cell, small cell, or intermediate type. The mitotic count to separate typical from atypical carcinoid was established at no more than 3 mitotic figures per 10 HPF. The authors added that if metastasis or gross invasion is present, tumors should be called low-grade neuroendocrine carcinoma.Although the mitotic count is specific, the grouping of tumors is ambiguous, and, for the first time, the presence of lymph node invasion is used to upgrade a “tumor” to “carcinoma.” Needless to say, the groupings of benign or low-grade and the “real” low-grade are ambiguous. In addition, the large cell type of neuroendocrine malignancy lacks diagnostic criteria and, in addition, requires the use of immunohistochemical markers. Capella et al. do not mention the possibility of non–small cell carcinomas with neuroendocrine differentiation. As a result, one may assume that any large cell carcinoma with neuroendocrine differentiation belongs to the high-grade group of neuroendocrine carcinomas.

In 1991, Travis et al. reported a study of 35 cases of neuroendocrine carcinomas of the lung in which a proposed criterion was presented for large cell neuroendocrine carcinoma. In this study, previous criteria for other neuroendocrine carcinomas were followed (with all the ambiguity), and large cell carcinoma was presented as a tumor with a “neuroendocrine pattern” and high mitotic activity, an average 66 mitoses per 10 HPF, and prominent nucleoli. Although Travis et al. stated that their study is comprehensive, the fact is that they reported only 5 cases of what they called large cell neuroendocrine carcinoma. Thus, Travis et al. claimed the importance of classifying these tumors into a 4-category system. However, they suggested that the prognosis of large cell neuroendocrine carcinoma is between that of atypical carcinoid and small cell carcinoma. This latter statement goes against previous descriptions in which similar tumors were categorized as high-grade malignancies.

In 1998, Travis et al. reported a new study of neuroendocrine neoplasms in which their goal was to provide clear definitions for the 4 neuroendocrine tumors, in addition to modifying the criteria for the diagnosis of carcinoid and atypical carcinoid. In short, the new classification system correctly placed large cell neuroendocrine carcinoma in the high-grade category of tumors, in contrast with the previous study. However, close analysis of the criteria deserves a few notes. In the new system, the diagnosis conventional carcinoid tumor is restricted to no more than 2 mitoses per 10 HPF; the diagnosis atypical carcinoid is for a tumor with 2 to 10 mitotic figures per 10 HPF or necrosis (often punctate); the diagnosis large cell neuroendocrine carcinoma is for a tumor with “neuroendocrine morphology,” mitoses of more than 10 per 10 HPF, cytologic features of large cell carcinoma, and positive immunohistochemical staining for neuroendocrine markers; and the diagnosis small cell carcinoma is for a tumor with the cytologic features of small cell tumor cells (absent nucleoli), mitotic figures of more than 10 per 10 HPF, and frequent necrosis.

Although at first this seems to be a reasonable rationalization of neuroendocrine neoplasms, there is an important drawback to this classification: What about biopsy specimens? If one is to apply this classification, the diagnosis of small cell carcinoma cannot be accomplished in a great majority of the cases in which there is a simple transbronchial biopsy specimen as the sole material for evaluation. Furthermore, the possibility of overdiagnosing atypical carcinoid tumor in a small biopsy specimen is high, and the diagnosis of large cell neuroendocrine carcinoma simply cannot be accomplished with a biopsy specimen because one of the most important criteria is the presence of neuroendocrine morphologic features, whatever those might be. Therefore, this classification system, which is essentially repeated in the last version of the WHO for the classification of tumors of the lung, pleura, thymus, and heart, is a classification for resected specimens, which is far from the reality of the daily practice of surgical pathology.

On the other hand, let us examine the other goal of this classification, which was to provide clear definitions of the 4 groups of tumors. In a separate study on the reproducibility of the proposed classification of neuroendocrine lung tumors, conducted by Travis, et al., 5 experienced pulmonary pathologists were selected to participate, and 40 surgical resection specimens of neuroendocrine tumors were carefully chosen. Unanimous agreement was reached in only 55% of the cases. More puzzling is the fact that the most common disagreements were between large cell neuroendocrine carcinoma and small
cell carcinoma. Although one would not conceive agreement of 100%, a 55% agreement among 5 experienced pulmonary pathologists falls short of what one would expect and leaves deep doubts as to whether the definitions are, in fact, clear. In this study with 40 resected specimens, the fact that the majority of disagreement was between small cell and large cell carcinoma is rather worrisome.

In 2002, Huang et al. presented the last attempt to classify neuroendocrine tumors of the lung. They reported a study of 234 cases and classified them into 5 categories. This system essentially follows the criteria of Travis et al. for the separation of carcinoid and atypical carcinoid, which are called well- and moderately differentiated neuroendocrine carcinomas. Large cell neuroendocrine carcinoma and small cell carcinoma are kept with the modifier “undifferentiated” with mitotic counts of more than 30 per 10 HPF. The new category is what Huang et al. call “poorly differentiated neuroendocrine carcinoma,” which is conceptualized as an atypical carcinoma with an increased mitotic activity of more than 10 mitoses per 10 HPF. Serious drawbacks about this system include returning to the obsolete term undifferentiated and, worst of all, adding this so-called poorly differentiated neuroendocrine carcinoma between atypical carcinoid and large cell neuroendocrine carcinoma. Once again, for practical application, this system is based on complete surgical resection specimens and not on biopsy specimens. Just the same as the attempt by Travis et al., Huang et al. also failed to consider the tumors that may have the right morphologic features but failed to demonstrate neuroendocrine differentiation. Where do we group those tumors? Large cell carcinomas with neuroendocrine morphologic features?

In the interest of criteria for specific conditions and more detail of the different types of neuroendocrine pulmonary neoplasms, let us examine each individually.

Carcinoid and Atypical Carcinoid

The current designation provided by the WHO defines these tumors as having neuroendocrine morphologic features, ie, organoid, trabecular, insular, palisading, ribbon, and rosette-like features (Image 1). However, the separation of conventional carcinoid and atypical carcinoid, according to the WHO, is that the former has fewer than 2 mitotic figures for 10 HPF, whereas the latter has 2 to 10 mitotic figures per 10 HPF (Image 2). Clinically, these tumors may be associated with carcinoid syndrome and show a spectrum of cell differentiation that includes spindle cells and oncocytic and melanocytic features, among others.

However, other authors believe that the most accurate designation for these neoplasms is neuroendocrine carcinoma, which conveys the true nature of these tumors and honors the previous classification of Gould et al., in which atypical carcinoid is equivalent to well-differentiated neuroendocrine carcinoma. Important to mention about this designation of tumors is the lack of incorporation of the so-called tumorlet, which in reality may represent the true carcinoid (looks like carcinoma but is benign). In any case, immunohistochemical studies for these 2 neoplasms revealed similar attributes in terms of showing positive staining for neuroendocrine markers, ie, chromogranin, synaptophysin, and CD56. In addition, the use of thyroid transcription factor-1 has also been shown to be helpful in the evaluation of neuroendocrine neoplasms of the lung.

The use of proliferation markers such as Ki-67 in the separation of conventional and atypical carcinoids has revealed that a 4% cutoff provides significant differences for the 4-year overall survival rate. In this study, the methods followed for the classification of neuroendocrine neoplasms were those of Arrigoni et al. and Warren et al., and the study was performed on resected specimens. This study raises 2 important issues: (1) whether the current WHO classification is any better than previously presented classifications in terms of restricting the diagnosis of conventional carcinoid to 2 mitotic figures per 10 HPF; and (2) that the complete interpretation of these tumors rests on surgical resection specimens and not on biopsy specimens, in which the labeling of proliferative markers may be deceiving.

Carcinoids and atypical carcinoids have also been the subject of more modern techniques such as chromosomal studies in which 11q deletions seem to be shared by both tumors. Losses of 10q and 13q may also be responsible for the possible aggressive behavior shown by some of these tumors. Survival rates of patients with these tumors are difficult to address meaningfully owing to the lack of real comparisons between previous and current schemas for the classification of these tumors. Wilkins et al. in 1984, obviously using a different schema (most likely the criteria of Arrigoni et al.), reported a study of 111 patients who underwent surgical resection for “bronchopulmonary carcinoid.” Of the patients, 11 atypical carcinoid, 45% of them died in a period of 33 months, and patients who survived were followed up for 16 to 48 months. Unfortunately, even though Wilkins et al. provided a survival rate of 82% for a 10-year period, close examination of data provided is rather limited, and no clear-cut survival rate is provided for the conventional carcinoid.

A better-defined study of typical carcinoid is that by Schreurs et al. in a study of 93 patients for a period of 25 years. Once again, even though the histologic criteria are not clearly mentioned, it is very likely that the criteria of Arrigoni et al. were used for separating typical from atypical carcinoids. Schreurs et al. provide an important survival rate of 100% for 86 patients at 5, 10, and 15 years (according to Schreurs et al., 7 patients died of unrelated causes) for surgically treated patients. However, the selection process for this study excluded patients with distant metastasis, raising the issue that staging of the neoplasm at diagnosis also has an
Image 1 A, Low-power view of a resected well-differentiated neuroendocrine carcinoma (carcinoid) tumor. Note the nested, well-organized pattern (H&E, ×10). B, Intermediate magnification showing homogeneous cellular proliferation with absence of necrosis (H&E, ×10). C, Higher magnification showing mild nuclear atypia and lack of mitotic activity (H&E, ×20).

Image 2 A, Low-power view of a resection specimen of a moderately differentiated neuroendocrine carcinoma (atypical carcinoid) showing tumor necrosis (comedo-like) (H&E, ×4). B, Higher magnification showing nuclear atypia and mitotic activity (H&E, ×10).
important role in this group of tumors and impacts heavily on the survival rate.

**Small Cell Carcinoma**

Small cell carcinoma is defined by the WHO as a malignant tumor containing cells with scant cytoplasm and absent or inconspicuous nucleoli. The necrosis is extensive and mitotic count high. As a matter of fact, the mitotic count provided is 60 mitoses per 2 mm$^2$. This latter histologic feature could not be detached further from the reality that most surgical pathologists confront—making a diagnosis on small biopsy specimens in which one cannot possibly obtain enough high-power fields and in which the finding of mitotic figures, at least in the range specified by the WHO, makes the diagnosis of small cell carcinoma an impossible task. It is interesting that in a study of 100 cases of small cell carcinoma, Nicholson et al clearly state that in more than 90% of the cases, the diagnosis of small cell carcinoma can be established on small biopsy specimens. However, the results of this study of mitotic activity in the range of 18 to 286 per mm$^2$ became part of the criteria for small cell carcinomas in the latest WHO classification of tumors. Needless to say, an important fact that must be emphasized is that most patients with small cell carcinoma have disease beyond a stage amenable to surgical resection of the tumor, as noted by Nicholson et al and others, leaving surgical pathologists with a small biopsy specimen and diagnostic criteria that, although elegant, help mainly with autopsy cases.

The topic of small cell carcinoma has not been exempt from controversy. In 1985, Vollmer et al reported a study in which they advocated the subclassification of small cell carcinoma into oat cell type or intermediate type. However, in 1988, the Pathology Committee of the International Association for the Study of Lung Cancer recommended that the use of the important role in this group of tumors and impacts heavily on the survival rate.

![Image 3](https://academic.oup.com/ajcp/article-abstract/131/2/206/1765899)

**Image 3** A, Transbronchial biopsy specimen of small cell carcinoma. Panoramic view. Note the difficulty in examining 10 high-power fields of viable tumor (H&E, ×2). B, Intermediate magnification showing prominent crush artifact and only focal areas of viable cells (H&E, ×10). C, High-power view showing a focal area of viable tumor cells with a couple of mitotic figures (not the 10 mitotic figures required by the World Health Organization) (H&E, ×20).
small cell carcinoma designation would include tumors previously denominated as oat cell and intermediate subtypes. Even terms such as small cell neuroendocrine carcinoma may fall under scrutiny because a proportion of these tumors may not show positive reactions with antibodies against neuroendocrine markers. In a study on the immunohistochemical staining of small cell carcinoma, Guinee et al found that the neuroendocrine marker chromogranin was positive in 60% of open lung biopsy specimens but in only 47% of tranbronchial biopsy specimens, whereas synaptophysin showed 5% and 19% positivity, respectively. Nevertheless, most authors concur that small cell carcinoma represents the end of the spectrum of neuroendocrine carcinomas of the lung. Also, it is important to mention that in small poorly preserved biopsy specimens, in which the intensity of neuroendocrine markers is marked, one should include better differentiated neuroendocrine tumors in the differential diagnosis of small cell carcinoma.

It cannot be overemphasized that there are implications for treatment in the diagnosis of small cell carcinoma because patients may undergo chemotherapy, radiation therapy, or both. In addition, it has been estimated in large studies that the long-term survival of patients with small cell carcinoma is approximately 10% at 2 years.

Large Cell Neuroendocrine Carcinoma, Large Cell Carcinoma With Neuroendocrine Morphologic Features, and Large Cell Carcinoma With Neuroendocrine Differentiation

These entities are shown in Image 5 and Image 6. It is interesting that there is no specific entry for the diagnosis of large cell neuroendocrine carcinoma in the latest WHO classification of tumors. Rather, these tumors are grouped under the rubric “large cell carcinoma” and defined as undifferentiated non–small cell carcinomas that lack features of small cell carcinoma, adenocarcinoma, or squamous cell carcinoma. More important is the fact that under synonyms for large cell carcinoma, the WHO lists neuroendocrine carcinoma with intermediate differentiation and large cell carcinoma with neuroendocrine differentiation and adds that before the description of large cell neuroendocrine carcinoma, those terms were used, but now all of these tumors are designated as large cell carcinoma with neuroendocrine differentiation. One cannot help but wonder whether it means that now any tumor with neuroendocrine differentiation falls into the category of large cell neuroendocrine carcinoma. If that is the case, the analysis of the so-called neuroendocrine pattern becomes irrelevant. In addition, according to the WHO, the main distinguishing feature of large cell neuroendocrine carcinoma and small cell carcinoma is the presence of prominent nucleoli, which leads us to recall the study on reproducibility in which the distinction of these 2 tumors was the major discrepancy among 5 pulmonary pathologists.

In addition, these tumors are further described as having large zones of necrosis with a mitotic count higher than 11 mitotic figures per 2 mm² (average, 75) of viable tumor. This latter definition clearly applies only to resected tumors; thus, such a diagnosis can hardly be accomplished with small biopsy specimens in which one may not have enough viable tumor, may not be able to evaluate the so-called neuroendocrine pattern, or may not be able to evaluate the mitotic count of more than 11 mitotic figures.

Image 4 A, Resection specimen of a small cell carcinoma. It is easier to identify areas of necrosis and viable tumor cells (H&E, x4). B, In resected specimens where one can focus on viable tumor, mitoses are not difficult to find in large numbers (H&E, ×10).
In 1988, Mooi et al\textsuperscript{52} described 11 primary lung carcinomas under the rubric of non–small cell carcinoma with neuroendocrine features. These tumors had been diagnosed as large cell carcinoma or squamous cell carcinoma, but all the tumors had similarities to bronchial carcinoid and small cell carcinoma. Of 7 cases in which electron microscopy studies were available, 6 showed dense-core granules, and all cases showed positive staining for neuron-specific enolase. (Some of the antibodies available today may not have been available in 1988.) Mooi et al\textsuperscript{52} also stated that neuroendocrine features might be suspected by using light microscopy. Judging by some of the illustrations provided, one cannot help but assume that some, if not all, of the tumors are similar to the tumors today designated as large cell neuroendocrine carcinoma.

Dresler et al,\textsuperscript{53} in 1997, analyzed 40 cases of large cell neuroendocrine carcinoma and concluded that large cell neuroendocrine carcinomas, identified by histologic examination, have a poor prognosis. Dresler et al\textsuperscript{53} also suggested that lesions previously categorized as large cell carcinoma with neuroendocrine features should be regarded as “large cell carcinoma with occult neuroendocrine differentiation.” This latter suggestion specifically addresses an issue that goes to the core of these types of tumors, which is how to group tumors that have a neuroendocrine pattern but fail to show immunoreactivity for neuroendocrine markers. As the criteria are currently presented, to make a diagnosis of large cell neuroendocrine carcinoma, one must have the right morphologic features and positive staining for at least 1 neuroendocrine marker.\textsuperscript{2} This issue has become even more confusing by the use of terms such as \textit{small cell–like large cell neuroendocrine carcinoma} in some cytologic studies.\textsuperscript{54} In addition, following the presence of nucleoli to separate small from non–small cell carcinoma may prove difficult, just as using cell size for their differentiation.

Marchevsky et al\textsuperscript{55} evaluated, by morphometric means, 28 surgically resected high-grade pulmonary neuroendocrine

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{A. Low-power magnification of a resected non–small cell carcinoma with neuroendocrine features. Note the comedo-type of necrosis (H&E, ×2). B. High-power magnification to demonstrate the non–small cell nature of the neoplasm. Mitotic figures are easily identifiable (H&E, ×20). C. Immunohistochemical stain for synaptophysin confirming the neuroendocrine nature of the neoplasm (×20). Coupled with the morphologic features, this tumor would be called a large cell neuroendocrine carcinoma.}
\end{figure}
Moran et al / Neuroendocrine Carcinomas of the Lung

carcinomas (16 small cell carcinomas and 12 large cell neuroendocrine carcinomas). They concluded that there is considerable nuclear overlap between these entities, which helped to separate only 9 of 28 cases studied, and suggested the use of more generic terminology such as high-grade neuroendocrine carcinoma or grade III neuroendocrine carcinoma.

The entire topic of large cell neuroendocrine carcinoma has generated a myriad of articles to bring light to this confusing topic. For example, Iyoda et al, in a study of 2,070 cases of large cell carcinomas, divided such tumors into 4 categories: large cell neuroendocrine carcinoma, large cell carcinoma with neuroendocrine differentiation, large cell carcinoma with neuroendocrine morphologic features, and “classic” (whatever that may be) large cell carcinoma. In a multivariate analysis, Iyoda et al grouped the 3 former entities into a single entity, which they denominated as large cell carcinoma with neuroendocrine features separate from the classic large cell carcinoma, and concluded that those tumors are more aggressive. What is interesting and important in this study is the fact that tumors in which Iyoda et al did not find morphologic evidence of neuroendocrine change were grouped as large cell carcinomas with neuroendocrine morphologic features, yet Iyoda et al found in their multivariate analysis that these tumors, the same as those grouped as large cell neuroendocrine carcinoma and large cell carcinoma with neuroendocrine differentiation, behaved similarly, as opposed to the classic large cell carcinoma. We may find in this report some semantic issues in terms of what Iyoda et al call neuroendocrine morphology and neuroendocrine differentiation, which others may have used differently. Nevertheless, their point raises higher issues: whether the important issue is mainly determining neuroendocrine “differentiation” by immunohistochemical means and whether a specific diagnosis is warranted for those tumors.

Some other articles on this topic have lumped large cell neuroendocrine carcinomas and large cell carcinomas...
with neuroendocrine morphologic features under the same designation. Unfortunately, in many articles on the subject, the authors claimed that they used rigorous application of the WHO criteria, but their efforts have been in the tumors that fit the morphologic and immunohistochemical criteria, leaving unanswered the question of what to do with tumors that show the morphologic but not the immunohistochemical criteria.

In other reports, it is difficult to discern which cases accounted for large cell neuroendocrine carcinoma (the terminology used seems ambiguous) and whether there were cases that morphologically were candidates for the designation but failed to show positive staining with neuroendocrine markers. In others, the nomenclature used is not that of the WHO classification system. In some reports, these tumors have been correlated with pathologic stage, raising the issue of staging, since these tumors may manifest in different pathologic stages. More worrisome is the fact that, in some recent articles, emphasis has been made on separating large cell neuroendocrine carcinoma from small cell carcinoma by using immunohistochemical and molecular studies. If one is to apply the WHO criteria or any of the other schemes presented for either of those diagnoses, such distinction should not be a difficult task because one is small cell and the other is non–small cell with prominent nucleoli. Furthermore, the problem of separating small from non–small cell carcinomas should not require immunohistochemical or molecular studies.

On the other hand, some reports have concentrated on non–small cell carcinomas with neuroendocrine differentiation or neuroendocrine morphologic features. Howe et al studied 439 cases of non–small cell carcinoma, which were evaluated using immunohistochemical stains for neuroendocrine markers. They reported that 36% of these tumors showed positive staining for at least 1 neuroendocrine marker and concluded that the presence of neuroendocrine differentiation in non–small cell carcinoma is of no prognostic significance, as also reported by others. More recently, Ionescu et al arrived at similar conclusions after reviewing 609 cases of non–small cell carcinoma. However, important to mention is the fact that some adenocarcinomas may show neuroendocrine differentiation, which has been suggested as an important prognostic feature, but other authors have had a more circumspect approach to this particular issue.

A few issues arise from critical analysis of the literature: (1) The diagnosis of large cell neuroendocrine carcinoma, in most of the series reported, has been a diagnosis in retrospect after analysis of all large cell carcinomas. (2) The cases included in a good number of reports, despite the “rigorous” application of the WHO criteria, suggest that some of the tumors included may not exactly belong in that designation. (3) The fact that resection specimens have been evaluated speaks volumes to the issue that these tumors have already been treated as non–small cell carcinomas. (4) Although all authors believe that these tumors are of high grade, there is no unanimous agreement regarding the best method of treatment for patients diagnosed, using whatever criteria, with large cell neuroendocrine carcinomas. In this regard, some authors have advocated that large cell neuroendocrine carcinoma is potentially treatable with surgery, whereas others advocate additional medical treatment. (5) There is great confusion about the use of terms such as neuroendocrine differentiation and neuroendocrine morphologic features. (6) Staging may still represent an important independent factor in the prognosis of these tumors.

Although one would expect that the issue of large cell carcinoma may be restricted to the issue of neuroendocrine morphologic features or neuroendocrine differentiation, the WHO separated yet another tumor that may provide even more confusion to the current classification, the so-called basaloid carcinoma of the lung. This latter entity was described as a new morphologic and phenotypic entity; however, close analysis of the histopathologic description shows that 19 of the 38 cases described showed areas of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma, raising the possibility that at least half of the tumors described may be grouped into one of those more specific categories. On the other hand, the authors of the WHO classification recognized that the distinction between large cell neuroendocrine carcinoma and basaloid carcinoma is difficult and often requires immunohistochemical studies. Furthermore, the authors of the WHO classification acknowledged that in 10% of cases of basaloid carcinoma, a neuroendocrine marker may be seen as positive, raising the possibility that the entity of basaloid carcinoma may represent a growth pattern rather than a specific entity. Further studies on basaloid carcinoma have stressed the use of immunohistochemical studies, namely cytokeratins 1, 5, 10, and 14 (34ΒΕ12) to differentiate such tumors from large cell neuroendocrine carcinoma. However, Lyda and Weiss reported positive staining for 34ΒΕ12 in 1 of 6 large cell neuroendocrine carcinomas.

Carcinomas With Mixed Histologic Features

It is well known that pulmonary carcinomas in a small but well-represented number of cases may show combined histologic features, namely of the small cell/non–small cell categories. It seems that the consensus with these types
of tumors is that the behavior shown is more aggressive than that of pure small cell carcinomas, leading to shorter survival. However, an important issue that needs further investigation is the possible association of small cell carcinoma, large cell neuroendocrine carcinoma, or large cell carcinoma with neuroendocrine differentiation. Whether that distinction has practical value in the treatment of a tumor at the higher end of neuroendocrine carcinomas may be just an academic exercise.

Molecular Biology of Neuroendocrine Tumors

From the molecular point of view, genetic studies have been performed showing a gain of 3q in about 66% of small cell carcinomas, whereas deletions of 10q, 16q, and 17p were less frequent in large cell neuroendocrine carcinomas than in small cell carcinomas. Other studies have shown that gene expression profiling failed to distinguish small cell from large cell neuroendocrine carcinomas and have led some authors to suggest that both entities should be grouped under the designation high-grade neuroendocrine tumors. Other studies have placed more emphasis on the issue of the spectrum of differentiation of neuroendocrine tumors and have found, through studies of expression of gene products, that there is no evidence linking carcinoids and small cell carcinoma. A similar finding has been encountered by other authors, leading to the suggestion that small cell carcinomas are derived from epithelial cells and that bronchial carcinoids are related to neural crest–derived tumors.

Analysis of p53, k-ras-2, and c-raf-1 by some authors has led to the suggestion that typical and atypical carcinoids are genetically distinct from high-grade neuroendocrine carcinomas. Regarding atypical carcinoids, it has been documented that loss of heterozygosity at 3p14.2-p21.3 is significantly more extensive in atypical carcinoids, whereas typical carcinoids are strongly positive for the cytoplasmic Fhit protein, similar to normal lung epithelia. It is interesting that studies on the expression of bcl-2 have concluded that the expression of bcl-2 is involved in the pathogenesis of small cell carcinoma and carcinoid tumors of the lung. Also, the expression of retinoblastoma (RB) in neuroendocrine tumors has disclosed the presence of the RB in carcinoids and atypical carcinoids, whereas it is absent in small cell and large cell neuroendocrine carcinomas. More recently, it has been suggested that hASH-1 (human homologue of Mas1) is involved in the neuroendocrine differentiation of small and non–small cell carcinomas.

Practical Approach Based on Current Concepts and Approaches

A practical classification system for neuroendocrine carcinomas may be conceptualized (even with the current definitions and criteria) as follows:

Surgical Resection Specimens

- Carcinoid tumorlet: tumorlet (lesions <0.5 cm)
- Grade I neuroendocrine carcinoma: well-differentiated neuroendocrine carcinoma; low-grade neuroendocrine carcinoma (typical carcinoid): tumor with fewer than 3 mitotic figures per 10 HPF and only focal punctate necrosis
- Grade II neuroendocrine carcinoma: moderately differentiated neuroendocrine carcinoma; intermediate-grade neuroendocrine carcinoma (atypical carcinoid): tumor with more than 3 but fewer than 10 mitotic figures per 10 HPF and necrosis, usually comedo-like necrosis
- Grade III neuroendocrine carcinoma: poorly differentiated neuroendocrine carcinoma; high-grade neuroendocrine carcinoma
- Small cell carcinoma (with or without positive neuroendocrine markers, tumors with more than 10 mitotic figures per 10 HPF and necrosis)
- Large cell neuroendocrine carcinoma: large cell carcinoma with positive neuroendocrine morphologic features and positive neuroendocrine markers and large cell carcinoma with positive morphologic features and negative staining for neuroendocrine markers

Biopsy Specimens

- Neuroendocrine carcinoma, if the tumor is in the spectrum of grade I or II; well- or moderately differentiated carcinoma (carcinoid or atypical carcinoid) and the tumor radiologically is more than 5 mm in greatest diameter (specify the possibilities)
- Small cell carcinoma
- Squamous cell carcinoma
- Adenocarcinoma
- Non–small cell carcinoma

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