Morphometry Confirms the Presence of Considerable Nuclear Size Overlap Between “Small Cells” and “Large Cells” in High-Grade Pulmonary Neuroendocrine Neoplasms

Alberto M. Marchevsky, MD,1 Anthony A. Gal, MD,2 Swati Shah, MD,1 and Michael N. Koss, MD3

Key Words: Small cell lung carcinoma; Large cell neuroendocrine carcinoma; Morphometry; Classification

Abstract

We morphometrically evaluated 5-µm H&E-stained sections from 28 surgically resected high-grade pulmonary neuroendocrine neoplasms, including 16 small cell lung carcinomas (SCLCs) and 12 large cell neuroendocrine carcinomas (LCNECs). For each case, 200 tumor nuclei and 20 to 100 normal lymphocytes were measured. The frequency distributions of tumor cell/lymphocyte (TC/L) size ratios were plotted in bins ranging from 1 to 6, classified into 6 histogram types with TC/L size ratio peaks ranging from 2 to 6 (A-E) and a histogram with a wide distribution (F). SCLCs fit histograms A through E; LCNECs, A through F. Morphometry demonstrated considerable nuclear size overlap in high-grade neoplasms. Approximately one third of SCLCs exhibited considerable numbers of neoplastic cells that were larger than 3 normal lymphocytes, while 4 of 12 LCNECs had a predominant number of small cells. Ten tumors exhibited a B histogram with a “borderline” peak TC/L of 3. The rule that a TC/L size ratio larger than 3 helps distinguish “large” from “small” neoplastic cells was confirmed in only 9 of 28 cases. The use of more generic terminology such as “high-grade neuroendocrine carcinoma” or “grade III neuroendocrine carcinoma” for SCLC and LCNEC is discussed.

The World Health Organization and the International Association for the Study of Lung Cancer (WHO/IASLC) recently revised the diagnostic criteria for neuroendocrine neoplasms of the lung.1 New criteria were adopted for the distinction between typical and atypical carcinoid tumors, and large cell neuroendocrine carcinoma (LCNEC) was added as a new variant of large cell carcinoma. Both small cell lung carcinoma (SCLC) and LCNEC can exhibit focal areas with squamous, glandular, or large cell differentiation; these neoplasms are now designated as SCLC or LCNEC, combined variant. Previous subtypes of SCLC such as “oat” cell and “intermediate” cell carcinomas and SCLC, mixed type are no longer included in classification schema.2,3 In addition, it has been recognized that pulmonary adenocarcinomas, squamous cell carcinomas, and large cell carcinomas can exhibit immunophenotypic evidence of neuroendocrine differentiation when studied with immunocytochemical methods and electron microscopy. They are classified by the recent WHO/IASLC scheme as non–small cell carcinoma-neuroendocrine.1 Mitotic densities and presence of necrosis are the most important morphologic features to distinguish typical carcinoid tumors, atypical carcinoid tumors, and high-grade neuroendocrine carcinomas such as SCLC and LCNEC.1-4 Typical carcinoid tumors have fewer than 2 mitoses per 10 high-power fields (HPF) and no necrosis. Atypical carcinoid tumors exhibit 2 to 10 mitoses per 10 HPF and frequently show focal areas of punctate necrosis. LCNEC and SCLC exhibit more than 11 mitoses per 10 HPF and larger areas of necrosis than atypical carcinoid tumors. Both LCNEC and SCLC are composed of round to oval cells with hyperchromatic nuclei, inconspicuous nucleoli,
and a high nuclear/cytoplasmic ratio, arranged in similar histopathologic patterns, indicative of neuroendocrine differentiation. The differential diagnosis between these carcinomas is based on nuclear size and the identification of certain cytologic features.5-10

The cell size of SCLC has been ambiguously defined in various studies as ranging from 1.5 to 4 times the size of normal lymphocytes.9 Standard textbooks have proposed the use of an arbitrary cutoff of 3 times the size of lymphocytes to distinguish “small” from “large” cells, based presumably on clinical experience.9 A morphometric study by Lee and associates11 demonstrated that the cells of SCLC are approximately twice the size of lymphocytes. This study also demonstrated that technical factors such as specimen size and degree of tissue crushing had a substantial impact on nuclear size.11 Cytologic features are valuable for the distinction of SCLC from LCNEC. Nucleoli and vesicular or fine chromatin are seen in the nuclei of LCNEC, while the cells of SCLC exhibit absent or faint nucleoli and finely granular nuclear chromatin.1,8,9 However, reliance on the identification of nucleoli or vesicular nuclear chromatin for the diagnosis of LCNEC can be misleading in difficult cases, particularly in instances of SCLC or LCNEC, combined type. Cell size is the most important diagnostic criterion to distinguish SCLC from LCNEC.9 However, size can be difficult to recognize with certainty as the cells of both neoplasms usually exhibit a spectrum of nuclear sizes. The subjective estimate by a pathologist of the relative proportions of small and large cells has an important role in the differential diagnosis of pulmonary neuroendocrine neoplasms.

A study of interobserver variability by Travis and associates6 raised questions about whether the distinction between these high-grade neuroendocrine neoplasms of the lung is truly reproducible. We report a study of SCLC and LCNEC of the lung using a simple morphometric method to test whether a cutoff of nuclear size based on the size of 3 lymphocytes can objectively distinguish SCLC from LCNEC.

**Materials and Methods**

Histologic slides from patients with surgically resected SCLC and LCNEC of the lung were retrieved from the files of the department of pathology, Cedars-Sinai Medical Center, Los Angeles, CA, and Emory University, Atlanta, GA. Sixteen SCLCs and 12 LCNECs that could be diagnosed by consensus by 3 experienced pulmonary pathologists (A.M.M., A.A.G., and M.N.K.) were studied. We evaluated 5-µm H&E-stained sections by morphometry.

**Morphometry**

The nuclear area of 200 randomly selected tumor nuclei and 20 to 100 normal lymphocytes was measured from each case using a SAMBA 4000 image analysis system (IPI, Trenton, NJ).12 The system segments the nuclei automatically from the background, and the investigator needs only to edit manually adjacent nuclei that overlap with each other.

**Tumor Cell/Lymphocyte Size Ratios**

The average nuclear size of normal resting lymphocytes was calculated for each tumor. Lymphocytes with a round nucleus with regularly distributed granular chromatin were selected. Lymphoid cells with irregular chromatin distribution, cleaved nuclei, and/or prominent nucleoli were excluded from measurements. In each neoplasm, the nuclear size of each tumor cell was divided by the average lymphocyte size for the case and a tumor cell/lymphocyte (TC/L) size ratio was calculated. The tumor TC/L size ratios for all cells of each case were plotted in a frequency distribution histogram using bins ranging from 2 to 6 in peak TC/L.

**Histogram Types**

Histograms were classified into 6 types (A-F) according to their predominant TC/L peak. Histogram type A was defined as illustrating a frequency distribution with a peak at a TC/L size ratio of 2. Histogram types B through E illustrated frequency distributions with TC/L size ratio peaks ranging from 3 to 6. Histogram type F illustrated a frequency distribution with a wide distribution of cells that exhibited no predominant peak. We theorized that if the “3-times-lymphocyte-size” rule is valid to distinguish neoplastic small cells from large cells, a majority of SCLCs should exhibit type A histograms Image 1, and LCNECs should show type C through F histograms Image 21. Histogram type B would illustrate “borderline” cases Image 31 composed predominantly of cells with a peak at a TC/L size ratio of 3 Image 31.

**Results**

Substantial overlap in the TC/L size ratio frequency distribution was encountered in both variants of high-grade neuroendocrine neoplasms. The frequency distribution of nuclear sizes of SCLCs yielded type A through E histograms (n = 4, 7, 2, 2, and 1, respectively) while LCNECs yielded type A through F histograms (n = 1, 3, 2, 4, 1, and 1, respectively). Five of 16 SCLCs exhibited a predominant number of neoplastic cells that were larger than 3 normal lymphocytes, while 4 of 12 LCNECs had a predominant number of small cells. Ten neoplasms (SCLC, 7; LCNEC, 3) exhibited borderline type B histograms.
Morphometric analysis of high-grade pulmonary neuroendocrine neoplasms confirms with an objective and graphic method the presence of considerable nuclear size overlap in SCLC and LCNEC. Our results are similar to those of older morphometric studies by Vollmer\textsuperscript{13} and Lee and associates\textsuperscript{14,15} demonstrating a continuum of cell sizes.
from small cell to large cell undifferentiated carcinoma of the lung. These studies demonstrated that the subclassification of small cell carcinomas into oat cell and intermediate cell variants was spurious and that there was no clear size cutoff between small cell and large cell carcinomas of the lung. In our study, the rule that the identification of a predominant neoplastic population composed of cells larger than 3 lymphocytes permits the distinction between SCLC and LCNEC was confirmed in only 9 of 28 neoplasms. Moreover, 10 of 28 neoplasms exhibited a borderline spectrum of nuclear sizes centered on a TC/L size ratio of 3. Our results provide an objective explanation for considerable interobserver variability in the diagnosis of high-grade pulmonary neoplasms and do not support the use of an arbitrary size cutoff for a reproducible distinction between SCLC and LCNEC.

In a study by Travis and associates, including two of the present authors (A.A.G. and M.N.K.), a group of 40 surgically resected neuroendocrine tumors of the lung, chosen from the files of the Armed Forces Institute of Pathology, were reviewed independently by 5 experienced pulmonary pathologists. Unanimous diagnostic agreement occurred in 70% of SCLCs and 40% of LCNECs. The most frequent disagreement fell between SCLC and LCNEC. The study indicated a need for more careful definition and application of diagnostic criteria for the differential diagnosis of SCLC vs LCNEC. Morphometric measurements of cell size are unlikely to be useful for the differential diagnosis of high-grade pulmonary neuroendocrine neoplasm. Indeed, if we were to use a TC/L size ratio of 2, as supported by the previous morphometric study of Lee et al, or any other TC/L cutoff, there would still be considerable cell size overlap between the cells of SCLC and LCNEC.

It is controversial whether the distinction between SCLC and LCNEC has clinical value, as patients with both neoplasms are being treated with similar therapeutic protocols. Surgery is advocated for the treatment of patients with LCNEC and limited stage SCLC. Chemotherapy has a limited value for patients with early-stage LCNEC; the neoplasms pursue a very aggressive clinical course despite therapy. In contrast, some SCLCs respond initially to chemotherapy, but frequently relapse and metastasize. To our knowledge, there are no retrospective or prospective clinicopathologic studies demonstrating statistically significant differences in survival rates for patients with LCNEC and patients with SCLC. A study of 200 patients with pulmonary neuroendocrine tumors, using current diagnostic criteria, suggested that patients with LCNEC may have a slightly better prognosis (5-year survival, 27%; 10-year survival, 9%) than people with SCLC (5-year survival, 9%; 10-year survival, 5%). However, the differences in survival rates were not statistically significant. Dresler and associates reported a 13% 5-year survival rate for patients with LCNEC in stage I; adjuvant therapy did not improve survival. It is difficult to collect at any institution a large cohort of patients with well-staged LCNEC and limited-
stage SCLC to compare their clinical courses. The fact that our data and previous observations question current diagnostic criteria for the distinction between the neoplasms would make this task more difficult.

There has been recent support for the designation of neuroendocrine carcinoma grades I through III similar to updated terminology for the classification of epithelial neuroendocrine neoplasms arising in other organs. 21,22 Wick 22 reviewed these concepts in a recent editorial and supported the use of the designation “grade III neuroendocrine carcinoma” for the classification of pulmonary neuroendocrine neoplasms, as it “avoids the potential confusion surrounding the ‘baggage’ attached to the terms small cell and large cell carcinoma of the lung.”

Studies with immunocytochemical and molecular methods support the concept that high-grade neuroendocrine lung tumors are a common group of neoplasms, distinct from carcinoid tumor and atypical carcinoid tumor 25,30-61 (Z.K. Arbiser, J.A. Arbiser, C.C. Cohen, et al. Neuroendocrine lung tumors: grade correlates with proliferation but not angiogenesis; written communication, 2001). SCLC and LCNEC have similar genetic changes, cell cycle abnormalities, development of angiogenesis factors, and other molecular abnormalities. For example, Brambilla and associates 59 described different incidences of p53 mutations and Bcl2/bax ratios between low-grade (carcinoid and atypical carcinoid tumors) and high-grade neuroendocrine carcinomas (SCLC and LCNEC). Debelenko and associates 60 reported molecular changes in the gene responsible for the multiple endocrine neoplasm 1 syndrome (MEN1) in pulmonary neuroendocrine neoplasms, supporting the hypothesis that SCLC and lung carcinoids develop via distinct molecular pathways. Helpap and Kolllermann 61 used double-labeling immunohistochemical methods to detect the expression of MIB-1 and neuroendocrine markers, such as chromogranin and synaptophysin, in high- and low-grade neuroendocrine neoplasms arising from the lung and other organs. Their findings suggest that high-grade and low-grade neuroendocrine tumors might arise from different precursor cell populations. Walch and associates 27 described similar complex cytogenetic changes in SCLC and LCNEC detected by comparative genomic hybridization. Haruki and associates 5 characterized mutations in the menin gene, which has a role in the development of MEN1, and demonstrated that loss of heterozygosity of the gene was more prevalent in LCNEC (50%) than in SCLC (22%), suggesting a possible distinction between these neoplasms.

Future studies of high-grade neuroendocrine neoplasms of the lung probably need to include objective methods for estimating the percentage of cells larger or smaller than a particular cutoff and better descriptors of nuclear features to differentiate SCLC and LCNEC with improved accuracy. A morphometric study by Jutting and associates 24 of pulmonary neuroendocrine neoplasms demonstrated that only chromatin features were independent predictors of survival. The study included carcinoid tumors, well-differentiated neuroendocrine carcinomas (which would presumably be classified as atypical carcinoid under current WHO/IASLC guidelines), and SCLC, but not cases of LCNEC. Future studies also would need to define more precisely the cytologic features that would enable a pathologist to reliably distinguish large cells from an LCNEC from those present in an SCLC, combined variant.

Our study reaffirms that there is considerable overlap in nuclear sizes between SCLC and LCNEC, which contributes to considerable difficulty in separating these variants of high-grade neuroendocrine lung tumors. Improved diagnostic criteria and future prospective clinicopathologic studies are needed to validate the concept that patients with LCNEC have a different clinical course from that of those with SCLC. Current evidence supports the use of the common terminology of “high-grade neuroendocrine carcinoma” or “grade III neuroendocrine carcinoma,” perhaps with a note indicating whether the pathologist favors a small or large cell subtype, for the diagnosis of both neoplasms in daily practice. This practice would avoid interobserver diagnostic problems and would have no substantial negative impact on clinical care.

From the Departments of Pathology, 1Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA; 2Emory University Hospital, Atlanta, GA; and 3University of Southern California/Los Angeles County Hospital.


Address reprint requests to Dr Marchevsky: Dept of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, 8700 Beverly Blvd, Los Angeles, CA 90048.

References


