Large Cell Neuroendocrine Carcinoma of the Lung: A 10-Year Clinicopathologic Retrospective Study

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Background. **Large cell neuroendocrine carcinoma is a recently recognized histologic entity whose clinical features and optimal treatment have not yet been well defined and are still being assessed. We report our retrospective assessment of cases of large cell neuroendocrine carcinoma observed from 1989 to 1999 in terms of survival.**

Methods. **Cases of large cell neuroendocrine carcinoma diagnosed between 1989 and 1999 were reassessed retrospectively according to the World Health Organization classification. The clinical outcome and pathologic features of all cases are described. Survival rates of patients with large cell neuroendocrine carcinoma are compared with those patients with small cell lung cancer treated in the same period.**

Results. **Patients were 41 men and 7 women with an average age of 63.7 years. Twenty-nine patients (60.4%) had pathologic stage I disease, 11 patients (22.9%) had pathologic stage II disease, and 7 patients (14.6%) had pathologic stage IIIA disease. One patient (2.1%) had pathologic stage IIIB disease. No patient underwent induction chemotherapy. Two patients underwent adjuvant chemotherapy and 2 underwent mediastinal radiotherapy for N2. No death was reported in the perioperative period. The median follow-up was 5 years. The actuarial survival for the entire group was 60.4% at 1 year, 27.5% at 3 years, and 21.2% at 5 years. The actuarial survival of accurately staged, stage I patients at 5 years was 27%.**

Conclusions. **The findings suggest that treating large cell neuroendocrine carcinoma by means of applying treatment for nonsmall cell lung cancer leads to a prognosis that is worse than that for nonsmall cell lung cancer, even in terms of low pathologic stages.**

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Neuroendocrine (NE) lung tumors represent a broad clinical-pathologic spectrum with varying morphologic features and biological behavior. There is still much debate concerning classification. In 1998 Travis [1] proposed a scheme that included tumors with low-grade malignancy such as typical carcinoid, medium-grade malignancy such as atypical carcinoid (a type introduced by Arrigoni [2] in 1972) and high-grade malignancy such as large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC).

Large cell neuroendocrine carcinoma of the lung has the immunohistological and morphologic appearance of high-grade malignant NE tumors and presents nonsmall cell nuclear features. In 1999 The World Health Organization (WHO) proposed a classification with rigorous histologic criteria for each subtype of LCNEC [3]: (1) NE morphologic features (organoid nesting, palisading, rosettes, trabeculae); (2) high mitotic rate ($>$ 10 per 10 high-power field); (3) necrosis (often large zones); (4) cytologic features of a nonsmall cell carcinoma (large cell size, low nuclear/cytoplasmatic ratio, vecicular or fine chromatin and prominent nucleoli); (5) positive immunohistochemical staining for one or more neuroendocrine markers including chromogranin A, synaptophysin and neural cell adhesion molecule (NCAM/CD56).

Large cell neuroendocrine carcinoma represents 1% to 2% of malignant pulmonary neoplasms [4]; the prognosis is poor and it is not yet clear what the correct therapeutic approach might be. Currently, LCNEC is considered a nonsmall cell lung cancer (NSCLC) and is thus treated according to the criteria for the latter but whether adjuvant or induction chemotherapy is useful remains to be proven.

Some studies found in the literature report the morphologic aspects of LCNEC and the clinical outcome in limited series of patients [4–8]. Our study, conducted from 1989 to 1999, is based on a single series of 48 patients treated in the same center. The aim of the study was to outline the morphologic features and describe the clinical outcome of LCNEC.

Material and Methods

In the period from 1989 to 1999, 1,530 patients with primitive pulmonary neoplasm underwent surgical intervention in our unit. Routine hematoxylin and eosin-stained sections of these patients were reviewed by a single pathologist. In 113 (7.3%) of the tumors that presented NE morphology immunohistochemistry was performed to confirm the NE phenotype. Primary anti-

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bodies against chromogranin A (BIOGENEX, monoclonal 1:500; BIOGENEX Laboratories, Inc, San Ramon, CA) and synaptophysin (BIOGENEX, monoclonal 1:100) were used. The sections with NE morphology that expressed one immunohistochemical marker in at least 10% of tumor cells were considered positive. The following histologic diagnoses were made: typical carcinoid (n = 23), atypical carcinoid (n = 2), LCNEC (n = 53), SCLC (n = 22), large cell carcinomas with NE morphology (n = 13).

A diagnosis of LCNEC was made when all WHO classification criteria were present in the section.

All patients underwent a preoperative computed tomography scan of the chest, adrenal gland, liver, and brain, bone scanning, fibrobronchoscopy and biopsy (endo/transbronchial, transthoracic) when possible. The stage of disease was based on the TNM classification using the International Union Against Cancer (UICC) staging system [9]. All patients underwent surgery and had no local macroscopic or microscopic neoplastic residue.

Statistical analysis was carried out using an SPSS package (SPSS, Chicago, IL). The overall survival of the patients was calculated by means of the Kaplan-Meier methods [10]. Comparison survival curves between patients with LCNEC and patients with SCLC was performed by log-rank tests.

Results

Of the 66 patients with large cell carcinomas of the lung with neuroendocrine morphology, 53 had positive results for at least one immunohistochemical neuroendocrine marker. Therefore, LCNEC represented 3.5% of all resected lung tumors. Of these 53 patients, 5 did not go through follow-up and were thus dropped from the study. Of the 48 patients studied, 41 were men and 7 were women. The average age of patients was 63.7 years (range, 39 to 81). Forty-one patients had a history of cigarette smoking. No patient presented clinical signs of paraneoplastic syndromes. Only 1 patient had a central tumor with positive bronchoscopy. In 27 patients was made a preoperative histologic diagnosis of NSCLC. A preoperative diagnosis of LCNEC was not made for any of the cases owing to the difficulty in assensing a definitive diagnosis with immunoistochemical study on small sample. The clinical stages of LCNEC were IA, 7 patients; IB, 35 patients; IIA, no patient; IIB, 6 patients; IIIA, no patient. The pathologic stages were IA, 5 patients; IB, 24 patients; IIA, no patient; IIB, 11 patients; IIIA; 7 patients; and IIIB, 1 patient. The following radical surgical procedures were performed: 46 lobectomies, 1 bilobectomy, and 1 pneumonectomy. No patient underwent minor pulmonary resection. A mediastinal lymph node dissection was performed for all patients: 9 patients were positive for N1 and 6 patients were positive for N2. Of the 7 patients in clinical stage IA, 2 were IIIA (T1N2) in the pathologic stage. Of the 35 patients in clinical stage IB, 4 were IIIA (T2N2) and 7 patients were IIB (T2N1). Of the 6 IIB patients, 1 were IIIA (T3N1) and 1 IIIB (T4N1). No patient underwent induction chemotherapy. Of the 6 N2 positive patients, 2 underwent cisplatin-based adjuvant chemotherapy and 2 underwent mediastinum radiotherapy.

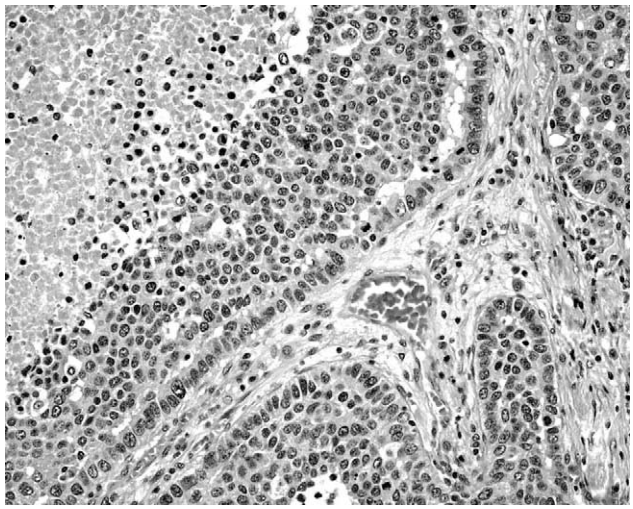

Fig 1. The tumor is composed of nodules with a peripheral palisading and large areas of central necrosis. Neoplastic cells have an abundant eosinophilic cytoplasm, with open nuclear chromatin and evident nucleoli. Mitoses are numerous (hematoxylin & eosin, ×200).

In all cases, the neoplasm presented an NE appearance characterized by organoid nesting, palisading, or trabecular growth. The cancer cells were medium-to-large size, with large cytoplasm, atypical nucleus, and often evident nucleoli. There were numerous mitoses (> 10 per 10 high-power field, often > 100), and tumoral necrosis was abundant in all cases (Fig 1).

The NE phenotype was confirmed in all cases by immunohistochemical positivity for chromogranin A (Fig 2) or synaptophysin in at least 10% of neoplastic cells.

Clinical follow-up lasted 12 years with a median fol-

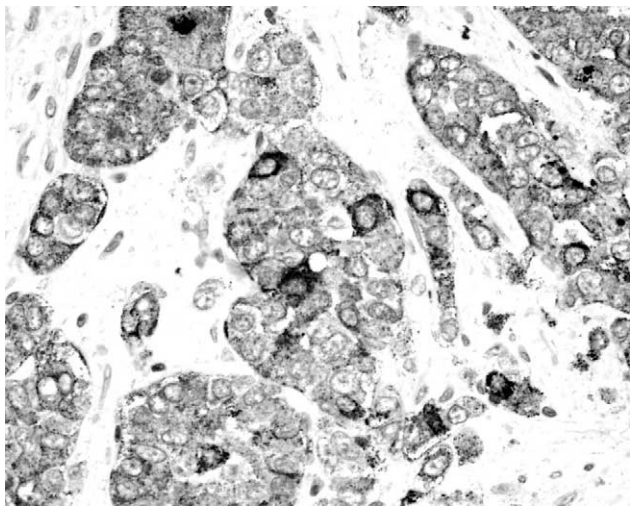

Fig 2. Immunohistochemically, tumor cells are positive for chromogranin A (ABC, ×400).

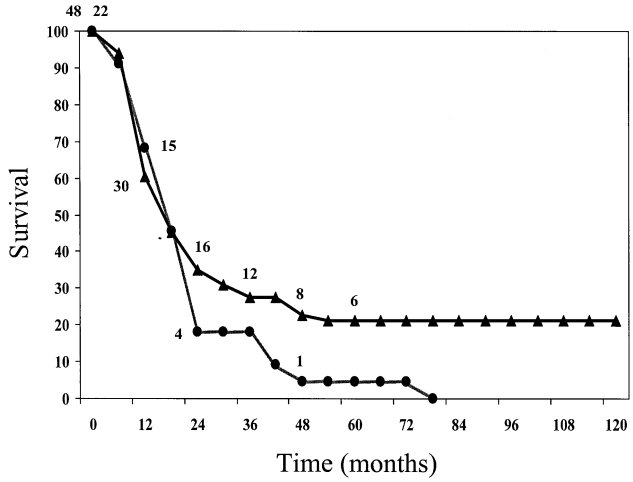

Fig 3. Kaplan-Meier survival estimates for all patients comparing survival between small cell lung cancer (circles, *22 patients) and large cell neuroendocrine carcinoma* (triangles, *48 patients). (Log rank test;* p = *0.088, not significant).*

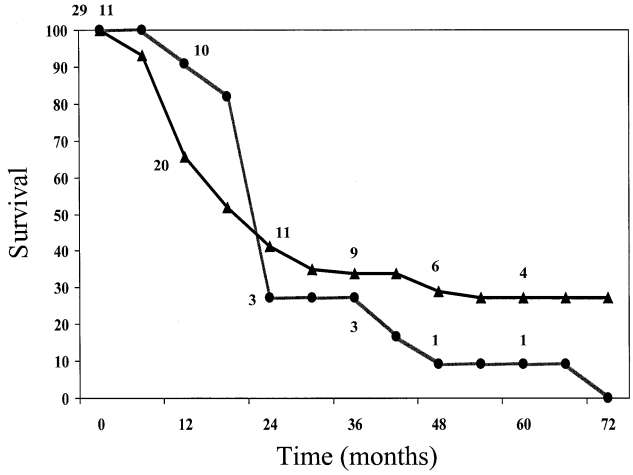

Fig 4. Kaplan-Meier survival estimates for stage I patients comparing survival between small cell lung cancer (circles, *11 patients) and large cell neuroendocrine carcinoma* (triangles, *29 patients). (Log rank test;* p = *0.394, not significant).*

low-up of 5 years. No patient dropped out of the follow-up during the study period. Four patients died at 13, 23, 41, and 47 months of causes other than neoplastic disease, and 34 patients died of metastatic spread. No locoregional recurrence was noted. Two N2 positive patients treated with adjuvant chemotherapy died at 11 and 13 months after surgery, and the 2 patients treated with mediastinum radiotherapy died at 10 and 24 months. The interval of time the patients were free of disease varied from 2 to 51 months, with an average of 16.9 months. Eight patients survived to 5 years after surgery, among them 2 IA patients, 4 IB patients, and 2 IIB patients. The actuarial survival of accurately staged, stage IA patients at 5 years was 66.6% (2 of 3 patients: of the 5 patients in pathologic stage IA, 2 were added, in 1998 and in 1999 respectively. Having a follow-up of 37 and 49 months respectively by the end of the study these patients were not included in the calculation for 5-year survival). The 5-year actuarial survival of patients in stage IB was 9.5% (2 of 21: of the 24 patients in the pathologic stage IB, 3 died at 23, 41, and 47 months of causes other than neoplastic disease), in stage IIB was 18.1% (2 of 11, all T2N1). The actuarial survival of patients in stage IIIA and IIIB was 0% at 5 years. Overall survival was 60.4% for 1 year, 27.5% for 3 years, and 21.2% for 5 years (Fig 3). The actuarial survival of accurately staged, stage I patients at 5 years was 27% (Fig 4). The 5-year actuarial survival of patients in stage II and III was 18.1% and 0% respectively (Figs 5 and 6). All patients alive are free of disease.

Of the 22 patients with SCLC identified, 19 were men and 3 were women. The average age of patients was 62.6 years (range, 47 to 75). No patient had a central tumor. A preoperative diagnosis of SCLC was not made for any of the case. All patients underwent lobectomy with mediastinal lymph node dissection. The pathologic stages of SCLC were IA, 8 patients; IB, 3 patients; IIA, 2 patients; IIB, 4 patients; IIIA, 5 patients. All patients underwent cisplatin-based adjuvant chemotherapy. Eight patients underwent mediastinum radiotherapy. No patient underwent prophylactic cranial irradiation. Clinical follow-up lasted 12 years. No patient was lost to follow-up. All patients died of metastatic disease. The interval of time the patients were free of disease varied from 4 to 68 months with an average of 16.7 months. Overall survival was 68.1% for 1 year, 18.1% for 3 years, and 4.5% for 5 years (Fig 3). The actuarial survival of accurately staged, stage I patients at 5 years was 9% (Fig 4). The actuarial survival of patients in stage II and III was 0% at 5 years (Figs 5, 6).

The actuarial survival of patients with LCNEC and SCLC was compared for all stages. Not significant differ-

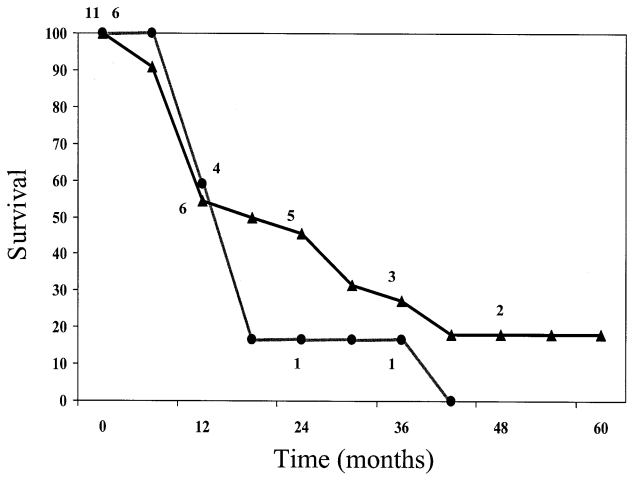

Fig 5. Kaplan-Meier survival estimates for stage II patients comparing survival between small cell lung cancer (circles, *6 patients) and large cell neuroendocrine carcinoma* (triangles, *11 patients). (Log rank test;* p = *0.263, not significant).*

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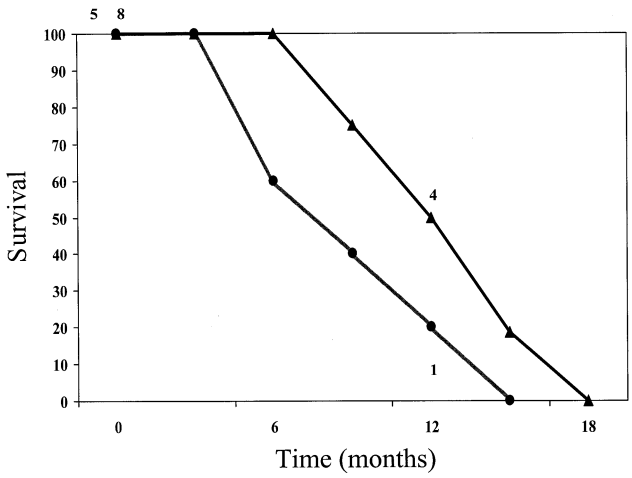

*Fig 6. Kaplan-Meier survival estimates for stage III patients comparing survival between small cell lung cancer (*circles*, 5 patients) and large cell neuroendocrine carcinoma (*triangles*, 8 patients). (Log rank test;* p = *0.107, not significant).*

ence was found in survival between patients with LCNEC and SCLC (overall, $p = 0.088$; stage I, $p = 0.394$; stage II, $p = 0.263$; stage III, $p = 0.107$) (Figs 3 to 6).

Comment

The classification of NE tumors has undergone numerous changes over the years, thereby causing diagnostic difficulties for pathologists. To these difficulties can be added the still not clearly defined therapeutic course to be taken. Large cell neuroendocrine carcinoma differs from low grade and medium grade NE forms in terms of its greater quantity of mitosis (> 10 per 10 high-power field) and abundant necrosis, and from SCLC exclusively in terms of its cytologic characteristics. There are four types of large cell tumors, each determined by its morphology and neuroendocrine differentiation: (1) LCNEC with a morphologic appearance and NE differentiation under electron microscope or immunohistochemistry; (2) large cell carcinoma with NE differentiation that does not have an NE carcinoma morphology but tests positive immunohistochemicaly or under electron microscope for its NE differentiation; (3) large cell carcinomas with NE morphology that have an NE morphology but not the neuroendocrine phenotype as seen under an electron microscope or immunohistochemically; (4) classic large cell carcinomas that have neither NE morphology nor differentiation. The distinction therefore from large cell carcinoma depends essentially on the absence in this last of an NE morphologic appearance under light microscopy and on the absence of a NE differentiation under electron microscope or immunohistochemically. Large cell neuroendocrine carcinoma differs from NSCLC tumors with NE immunophenotype (10% to 20% of NSCLC), known as NSCLC with NE differentiation (NSCLC-ND) due to the absence in the latter of a NE morphology under light microscopy. The term "combined LCNEC" should be used for those LCNEC that have components of adenocarcinoma, squamous cell carcinoma, or large cell carcinoma.

Diagnosing LCNEC can be particularly difficult for pathologists due to the overlapping of the above-described entities, and a differential diagnosis with SCLC is particularly difficult [12]. Other morphometric studies have shown a significant overlapping between the nuclear dimensions of LCNEC and SCLC [13].

A preoperative diagnosis of LCNEC was not made for any patient. It is difficult to perform transbronchial biopsy on these usually peripheral tumors and cytology with immunohistochemistry on fine needle biopsy is not sufficient to accurately diagnose these tumors [7]. In our series, the patients with LCNEC represented 3.5% of all patients undergoing surgery for pulmonary neoplasm. These results are slightly higher than those reported in the literature [5, 14]. Overall survival of patients with LCNEC at 1 year (60.4%), at 3 years (27.5%), and at 5 years (21.2%) was lower than usually seen for NSCLC, and more similar to that for SCLC (in our series overall survival was 68.1% for 1 year, 18.1% for 3 years, and 4.5% for 5 years). The comparison of actuarial survival between patients with LCNEC and SCLC was not significant for all stages (overall, $p = 0.088$; stage I, $p = 0.394$; stage II, $p = 0.263$; stage III, $p = 0.107$). These findings are in line with what has been described in the literature [1, 4, 5, 7, 14].

The 5-years actuarial survival of patients with LCNEC in stage I (27%) was particularly low when compared with the literature [8]. Affecting this is predominantly the poor survival rate recorded for stage IB (9.5%). The interval patients were free of disease was 16.9 months on average, with recurrence in 70.8% of cases. The recurrence rate in the literature is 57% [15]. In a series of patients described by Mazières [7], the response to cisplatin-based chemotherapy was 20% and partial. This pathology is therefore to be considered as having a high grade of malignancy. Induction chemotherapy was not carried out in our series of patients. Given the extreme histologic, immunohistochemical, and molecular similarity [16] between LCNEC and SCLC and the fact that LCNEC's clinical outcome is more similar to that of SCLC than to that of NSCLC, we wonder whether pathologists' interpretive difficulty has a real clinical effect and whether it might not be better to define a new therapeutic course that differs from that for NSCLC, for example one that foresees a combined modality treatment for each clinical case even when chemosensitivity seems lower than that in SCLC [7], with resistance attributed to the high frequency of MDR 1 gene expression [17]. In NSCLC patients NE differentiation appears to be an independent prognostic factor in terms of chemotherapy response [18]. Few studies have been done on adjuvant chemotherapy and these do not demonstrate improved survival rates [15]. The presence of lymph node involvement, mitotic rate, or the presence of molecular anomalies such as p53 and Bcl-2 do not seem to be correlated to clinical outcome [7]. Adjuvant radiotherapy seems to be effective in controlling the disease locally [7]. Some

authors [14] have suggested classifying LCNEC and SCLC in one group called "high-grade malignant neuroendocrine tumors" and using this biological information to define the treatment program.

In conclusion, LCNEC represents a subtype of NE tumor that is highly malignant and whose poor prognosis is more similar to that for SCLC than to that for NSCLC. While optimal treatment remains to be defined, the criteria applied to NSCLC are currently used. In our opinion a combined modality treatment approach that uses new chemotherapy regimens should be explored.

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