Adjuvant chemotherapy, extent of resection, and immunoistochemical neuroendocrine markers as prognostic factors of early-stage large-cell neuroendocrine carcinoma

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Abstract
Background: We investigated whether adjuvant chemotherapy, extent of resection, and immunoistochemical neuroendocrine markers affected survival of patients with the early stage of large-cell neuroendocrine cancer.

Methods: This was a retrospective multicenter study including consecutive patients undergoing resection of node negative large-cell neuroendocrine carcinoma. Five-year survival and disease-free survival rate were evaluated by the Kaplan–Meier method and the log-rank test in relation to adjuvant chemotherapy, extent of resection, and immunoistochemical neuroendocrine markers (synaptophysin, chromogranin A, and neuron-specific enolase).

Results: Our study population included 117 patients; 47 (40%) of these received adjuvant chemotherapy. Patients treated with adjuvant chemotherapy had better survival (74% vs. 45%, \(p = 0.002\)) and disease-free survival (79% vs. 40%, \(p = 0.001\)) in all cases except patients with tumor <20 mm (79.5% vs. 57.4%, \(p = 0.43\)). Lobectomy compared to sublobar resection was associated with better survival (67% vs. 0.1%, \(p < 0.0001\)) and disease-free survival (65% vs. 0.1%, \(p < 0.0001\)) also in patients with tumor <20 mm (79% vs. 28%, \(p = 0.001\)). Patients with triple-positive neuroendocrine markers had better survival (79% vs. 35%, \(p = 0.001\)) and disease-free survival (69% vs. 42%, \(p = 0.0008\)). Regression analysis showed that tumor size <20 mm, lobectomy, adjuvant chemotherapy, and triple-positive immunoistochemical neuroendocrine markers were significant favorable prognostic factors for survival outcomes.

Conclusions: Lobectomy seems to be the management of choice in patients with large-cell neuroendocrine cancer <20 mm while adjuvant chemotherapy should be administered only in patients with tumor >20 mm.

KEYWORDS
adjuvant chemotherapy, immunoistochemical neuroendocrine markers, large-cell neuroendocrine carcinoma, lobectomy, surgery

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INTRODUCTION

Large-cell neuroendocrine carcinoma (LCNEC) of the lung is a rare malignant tumor and accounts for only 2–3% of all primary lung cancers. Although previously classified as a subgroup of large-cell carcinoma, in 2015 LCNEC was reclassified as a high-grade neuroendocrine tumor, including the subgroups small-cell lung cancer (SCLC), typical carcinoid, and atypical carcinoid.

Primary surgery remains the main treatment for patients with limited LCNEC, but the prognosis is poor even in patients with pathologic stage I because of its aggressive course and high potential for metastasis of LCNEC. This led many physicians to consider LCNEC together with SCLC and to routinely do adjuvant chemotherapy regardless of pathologic stage, while others did not show any benefits associated with adjuvant chemotherapy even in early stage of LCNEC. The different clinico-pathological features of LCNEC among these studies likely explain the contrasting results. Thus, the optimal treatment remains to be established in these subsets of patients.

In this study, we investigated clinico-pathologic features and survival outcomes in patients with early-stage LCNEC to evaluate whether adjuvant chemotherapy, extent of resection, and immunohistochemical neuroendocrine markers affect survival outcomes.

MATERIALS AND METHODS

Study design

This was a retrospective multicenter study including the clinical data of consecutive patients undergoing curative surgery and receiving a diagnosis of LCNEC in three different thoracic surgery centers from January 2010 to January 2020. We excluded (i) patients with incomplete data sets and follow-up, (ii) patients with lymph node involvement (>N0) and/or metastatic disease (M1), (iii) patients with tumor larger than 50 mm, (iv) patients with margin-positive resection (R1, R2, or unknown), (v) patients with a diagnosis of mixed LCNEC combined with elements of SCLC, (vi) patients who died within 30 days of surgery; and (vii) patients without immunohistochemical investigation of the following neuroendocrine markers: synaptophysin, chromogranin A, and neuron-specific enolase (NSE).

The end points of the paper were to evaluate the impact of adjuvant chemotherapy, type of resection, and immunohistochemical neuroendocrine markers on survival outcomes (overall survival and disease-free survival) in order to stratify the best treatment for each subgroups of patients.

The study protocol was approved by the local ethics committees of each participating center; no specific code approval was needed because it was a retrospective study that did not change the standard clinical practice. All patients gave written informed consent for the treatment and the data were anonymously used.

Patients’ data

The following parameters were investigated from the medical records: patient gender, age, smoking index, pathologic tumor size, surgical procedure, regimen of adjuvant chemotherapy, time and site of recurrence, and time and cause of death. Before operation all patients underwent standard cardio-respiratory evaluation and oncological staging through imaging of the chest and abdomen, and positron emission tomography (PET). If indicated, histopathology evaluation of mediastinal nodes was performed via cervical mediastinoscopy, endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration (TBNA), thoracoscopy, or chamberlain incision. Resections included lobectomies and sublobar resections (segmentectomy or wedge resection) as indicated. Systematic lymph node dissection was carried out in all cases. The diagnosis of LCNEC was confirmed based on the WHO criteria, including the presence of neuroendocrine morphology and positive staining for synaptophysin, chromogranin A, and NSE. Tumors were categorized as pure LCNEC or combined LCNEC based on the presence of mixed histologic components such as adenocarcinoma, squamous cell carcinoma, or giant cell carcinoma. Based on tumor size, patients were divided into three groups: tumors <20 mm, tumors between 20 and 30 mm, and tumor between 30 and 50 mm.

Postoperative treatment and follow-up

Adjuvant chemotherapy was defined as given after the surgical resection while treatment given for disease progression or recurrence was excluded. Chemotherapeutic regimen, time of administration from surgery, and duration of treatment were at the discretion of the treating centers. Five-year survival rate (5-YSR) was calculated from the day of operation to the date of death from any cause or of the last follow-up. Five-year disease-free survival rate (5-YDFS) was calculated from the day of operation to the time of the first recurrence. Tumor recurrence and cause of death were determined for each patient. Loco-regional recurrence was defined as that occurring within the ipsilateral hemithorax while distant recurrence was defined as that developing within the contralateral hemithorax or a distant solid organ.

Statistical analysis

The summary statistics of patient characteristics were tabulated either as mean ± standard deviation (SD) for continuous variables or as number of patients and percentages for categorical variables. Student’s t test and the chi-square test were used to compare different variables, as appropriate.
| Variable | All | Adjuvant chemotherapy | No adjuvant chemotherapy | p value |
|----------|-----|-----------------------|--------------------------|---------|
| Number of patients | 117 | 47 (40%) | 70 (60%) | – |
| Age (years) | 67 ± 3.9 | 67 ± 9.8 | 67 ± 7.9 | 0.83 |
| Sex (male) | 87 (74%) | 37 (79%) | 50 (71%) | 0.37 |
| Smokers | 110 (94%) | 42 (89%) | 68 (97%) | 0.08 |
| Previous comorbidity (total) | 91 (78%) | 35 (78%) | 56 (80%) | 0.48 |
| Diabetes | 15 (65%) | 7 (20%) | 8 (14%) | |
| Hypertension | 15 (65%) | 5 (14%) | 10 (18%) | |
| Cardiac | 21 (31%) | 8 (23%) | 13 (23%) | |
| Cerebral | 1 (1%) | 0 | 1 (2%) | |
| COPD | 30 (33%) | 11 (31%) | 19 (34%) | |
| Neoplastic | 9 (10%) | 4 (12%) | 5 (9%) | |
| Symptoms | | | | |
| None | 50 (27%) | 21 (47%) | 29 (41%) | 0.72 |
| Cough | 25 (21%) | 17 (36%) | 18 (26%) | |
| Thoracalgia | 5 (4%) | 2 (4%) | 3 (4%) | |
| Expectoration | 6 (5%) | 2 (4%) | 4 (6%) | |
| Hemoptysis | 9 (8%) | 4 (8%) | 5 (7%) | |
| Pyrexia | 7 (6%) | 3 (6%) | 4 (6%) | |
| Weight loss | 15 (13%) | 6 (13%) | 9 (13%) | |
| Respiratory function | | | | |
| FEV1% | 78 ± 21 | 78 ± 15 | 77 ± 32 | 0.45 |
| DLCO % | 73 ± 18 | 73 ± 22 | 72 ± 21 | 0.21 |
| 6-MWT (metres) | 365 ± 59 | 366 ± 63 | 364 ± 49 | 0.39 |
| Tumor site | | | | |
| Peripheral | 79 (67%) | 30 (64%) | 49 (70%) | 0.38 |
| Central | 38 (23%) | 17 (36%) | 21 (30%) | |
| PET | | | | |
| Mean value SUV value | 6.9 ± 2.9 | 6.7 ± 2.9 | 6.9 ± 4.9 | 0.29 |
| Patients with SUV > 2.5 | 113 (%) | 45 (96%) | 68 (97%) | 0.68 |
| Preoperative biopsy (total) | 85 (73%) | 35 (74%) | 50 (71%) | 0.71 |
| Diagnostic | 8 (9%) | 3 (6%) | 5 (7%) | |
| Inconclusive | 5 (6%) | 2 (4%) | 3 (4%) | |
| Positive for malignancy | 72 (85%) | 32 (68%) | 40 (57%) | |
| Type of resection | | | | |
| Lobectomy | 97 (83%) | 40 (85%) | 57 (81%) | 0.60 |
| Segmentectomy | 17 (14%) | 6 (12%) | 11 (16%) | |
| Wedge resection | 3 (3%) | 1 (3%) | 2 (3%) | |
| Complications (total) | 21 (4%) | 4 (8%) | 17 (24%) | 0.03 |
| Prolonged air leak | 11 (52%) | 1 (2%) | 10 (14%) | |
| Atelectasis | 3 (14%) | 3 (6%) | 0 | |
| Atrial fibrillation | 6 (28%) | 0 | 6 (8%) | |
| ARDS | 1 (6%) | 0 | 1 (1%) | |
| Histology | | | | |
| Pure | 90 (77%) | 35 (74%) | 55 (78%) | 0.62 |
| Mixed | 27 (23%) | 12 (26%) | 15 (22%) | |
| pTumor size | | | | |
| <20 mm | 3.9 ± 2.5 | 3.8 ± 1.1 | 3.9 ± 1.3 | 0.49 |
| 20 to 30 mm | 29 (25%) | 12 (25%) | 17 (24%) | 0.87 |
| 30 to 50 mm | 46 (40%) | 19 (40%) | 27 (38%) | 0.84 |

(Continues)
TABLE 1 (Continued)

| Variable                        | All       | Adjuvant chemotherapy | No adjuvant chemotherapy | p value |
|---------------------------------|-----------|-----------------------|--------------------------|---------|
| Immunoistochemical neuroendocrine markers |           |                       |                          |         |
| Triple positive                 | 67 (57%)  | 13 (32%)              | 54 (77%)                 | <0.0001 |
| Nontriple-positive              | 50 (43%)  | 34 (68%)              | 16 (23%)                 | <0.0001 |
| Recurrence (total)              | 45 (38%)  | 10 (21%)              | 35 (50%)                 | <0.0001 |
| Loco-regional                   | 30 (67%)  | 7 (15%)               | 23 (33%)                 |         |
| Distant                         | 5 (11%)   | 2 (4%)                | 3 (4%)                   |         |
| Loco-regional + distal          | 10 (22%)  | 1 (2%)                | 9 (13%)                  |         |

Abbreviations: 6-MWT, 6-minute walking test; ARDS, acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disease; DLCO, diffusing capacity of the lungs for carbon monoxide; FEV1%, forced expiratory volume in the first second; SUV, standard uptake value.

The 5-YSR and the 5-YDFSR were evaluated by the Kaplan–Meier method and the log-rank test was used to calculate the difference between subgroups. The Cox multivariate proportional hazards regression model was used to identify independent risk factors for death and recurrence. A p value of less than 0.05 was considered statistically significant. MedCalc statistical software (version 12.3) was used.

RESULTS

In the study period, 153 patients underwent surgical resection for LCNEC. Among these, 36 patients were excluded because of missing clinical data and incomplete follow-up (n = 13), of N1-N2 disease (n = 7), of tumor larger than 50 mm (n = 10), of diagnosis of mixed LCNEC combined with elements of SCLC (n = 3), or of lack of investigation of immunoistochemical neuroendocrine markers (n = 3). Thus, our study population included the 117 patients summarized in Table 1.

The mean age was 67 ± 3.9 years old. All patients but seven (94%) were current smokers or had a history of intense tobacco consumption. At the time of presentation, 50 (27%) patients were asymptomatic and the tumor was found incidentally on a chest computed tomography (CT) scan. There were 79 (67%) peripheral and 38 (23%) central tumors shown by CT imaging and in all cases CT did not show specific features that were meaningful for the differential diagnosis of other types of lung cancer. A preoperative biopsy was made in 85 patients (73%). Only a small fraction of these patients were diagnosed with LCNEC (n = 8, 9%), while most were diagnosed with nonspecific cell types, including NSCLC (n = 72, 85%). In the remaining 32 (27%) patients intraoperative biopsy was performed with diagnosis of NSCLC in 30 patients and of LCNEC in two. Operative procedures performed included 97 lobectomies (84.1%) and 20 sublobar resections (17 segmentectomies and three wedge resections). LCNEC was categorized as pure (n = 90, 77%) or mixed (n = 27, 23%) with features of both LCNEC and NSCLC, mainly adenocarcinoma (73%). The mean tumor size was 3.9 ± 2.5 cm; 29 (25%) patients had tumor less than 20 mm, 46 (40%) tumor >2 to 30 mm, and 42 (35%) tumor >30 to 50 mm. Twenty-one (4%) patients had postoperative complications including prolonged air-leaks (n = 11, 52%), atelectasis (n = 3, 14%), atrial fibrillation (n = 6, 28%), and acute respiratory distress syndrome (ARDS) (n = 1, 6%).

Recurrence and survival

Forty-five (38%) patients had recurrence, 30 (67%) local recurrence, five (11%) distant recurrence, and 10 (22%) developed both local and distant recurrences. A total of 38 out of 45 (84%) patients were treated for recurrence with chemotherapy (n = 25, 65%), radiotherapy (n = 5, 13%), and combined radio-chemotherapy (n = 8, 22%). The median follow-up was 41 months (range 10–130 months). At the end of follow-up, there were 75 surviving patients (64%); 11 (15%) of whom had progressive disease. Forty-two (36%) patients died from disease progression (n = 34, 81%), cardiac disease (n = 5, 12%), and respiratory failure (n = 3, 7%). The 5-YSR and 5-YDFSR were 53% and 52%, respectively.

Survival in relation to adjuvant chemotherapy

The results are summarized in Tables 1 and 2. Seventy (60%) patients underwent surgical resection alone and 47 (40%) received adjuvant chemotherapy. These patients were treated with an SCLC-based regimen (etoposide/cisplatin, n = 20, 42%) or an NSCLC-based regimen (n = 27, 58%) including gemcitabine (n = 7, 26%), vinorelbine (n = 7, 26%), pemetrexed (n = 7, 26%), and taxol (n = 6, 22%). Patients with less postoperative complications (p = 0.03) and no triple-positive immunoistochemical neuroendocrine markers (p < 0.0001) were more likely to receive chemotherapy after surgery. Patients treated with adjuvant chemotherapy compared to those who did not receive adjuvant chemotherapy had better 5-YSRT (74% vs. 45%, p = 0.002; Figure 1(a)) and 5-YDFSR (79% vs. 40%, p = 0.001; Figure 1(b)). When adjuvant chemotherapy was stratified in relation to tumor size, no significant differences
were found in patients with tumor <20 mm (79.5% vs. 57.4%, p = 0.43, Figure 1(c); 81% vs. 72%, p = 0.51, Figure 1(d)) while adjuvant chemotherapy was associated with better 5-YSR rates and 5-YDFSR in patients with tumor >20 to 30 mm (72% vs. 36.2%, p = 0.01, Figure 1(e); 73% vs. 45%, p = 0.02, Figure 1(f)) and in those with tumor >30 to 50 mm (68.8% vs. 27%, p = 0.01, Figure 1(g); 61% vs. 13.8%, p = 0.002, Figure 1(h)).

### Survival in relation to extent of resection

The results are summarized in Table 2. Patients treated with lobectomy had better outcomes than those treated with sublobar resection, with higher 5-YSR (67% vs. 0%, p < 0.0001; Figure 2(a)) and 5-YDFSR (65% vs. 0%, p < 0.0001; Figure 2(b)). When the extent of resection in relation to tumor size was evaluated, lobectomy was associated with a better 5-YSRT and 5-YDFSR in patients with tumor <20 mm (79% vs. 28%, p = 0.001, Figure 2(c), 89% vs. 38%, p = 0.001, Figure 2(d)), in patients with tumor between 20 and 30 mm (62% vs. 14.8%, p = 0.0004, Figure 2(e); 71% vs. 0%, p < 0.0001; Figure 2(f)) and in those with tumor between 30 and 50 mm (51% vs. 0%, p < 0.0001, Figure 2(g); 30% vs. 0%, p = 0.0001, Figure 2(h)).

### Survival in relation to immunoistochemical neuroendocrine markers

The results are summarized in Table 2. Immunoistochemical staining was positive for synaptophysin in 79 (67%) patients, for chromogranin A in 59 (50%) patients, and for NSE in 99 (84%). Sixty-seven tumors (57%) were positive for all three neuroendocrine markers (triple-positive group), while 50 (43%) were negative for one or two markers (nontriple-positive group).

5-YSRT and 5-DFSRT were better in the triple-positive group compared to the nontriple-positive group (79% vs. 35%, p = 0.0001, Figure 3(a); 69% vs. 42%, p = 0.0008

### Abbreviations

- 5-YDFSR, 5-year disease free survival rate
- 5-YSR, 5-year survival rate
Figure 3(b)). These results were confirmed also in patients with tumor <20 mm (92.3% vs. 40%, \( p = 0.001 \), Figure 3(c); 84% vs. 62%, \( p = 0.02 \), Figure 3(d)), in patients with tumor between 20 and 30 mm (74.9% vs. 28.1%, \( p = 0.01 \), Figure 3(e); 79% vs. 28%, \( p = 0.03 \), Figure 3(f)) and in those with tumor between 30 and 50 mm (60.4% vs. 29%, \( p = 0.0005 \), Figure 3(g); 38% vs. 23%, \( p = 0.003 \), Figure 3(h)). When patients were stratified for the administration of adjuvant chemotherapy, in patients who received chemotherapy triple-positive compared to no triple-positive group had
worse 5-YSR (61% vs. 90%, \( p = 0.043 \), Figure 3(i); 5-YDFS (68% vs. 91%, \( p = 0.031 \), Figure 3(j)) while patients who did not receive chemotherapy triple-positive compared to no triple-positive group had better 5-YSR (72% vs. 16%, \( p = 0.0003 \); Figure 3(k)) and 5-YDFS (66% vs. 20%, \( p = 0.0008 \); Figure 3(l)).
Prognostic factors

The results are summarized in Table 3. Cox regression analysis showed that tumor size <20 mm, lobectomy, adjuvant chemotherapy, and triple-positive immunohistochemical neuroendocrine markers were significant favorable prognostic factors for overall survival and disease-free survival while age, sex, comorbidity and histology did not affect overall survival and disease-free survival.

DISCUSSION

LCNEC has poor prognosis even in resected patients with early stage, and it is still debated whether this tumor should be treated in the same manner as NSCLC or SCLC. The topic of sublobar resection versus lobectomy for stage I tumors smaller than 20 mm is controversial in NSCLC, while lobectomy seems to be superior to sublobar resection even for early-stage SCLC. Furthermore, adjuvant chemotherapy is indicated for stage II or III NSCLC while retrospective studies support the routine administration of adjuvant therapy even for stage I SCLC. Previous papers, summarized in Table 4, evaluated several prognostic factors as the extent of resection, the administration of adjuvant chemotherapy, and the expression of immunohistochemical neuroendocrine markers to define the best treatment of LCNEC in relation to its clinical and pathological characteristics. However, the results were contrast as the existing studies were heterogenous. Some studies evaluated all stages of LCNEC while others included only patients with early stage. Previous papers included patients undergoing different perioperative treatment as radiotherapy and chemotherapy administered before and after operation while others evaluated only patients who received adjuvant chemotherapy alone or associated with radiotherapy. Yet, only a few studies evaluated the extent of resections, but none of these correlated the type of resection with the expression of immunohistochemical neuroendocrine markers, as in the present. To overcome these limitations, the present study included only patients with early-stage LCNEC who received adjuvant chemotherapy alone or associated with radiotherapy. The clinico-pathologic features of our study population were similar to other studies. LCNECs generally affected
males (74%) and almost exclusively smokers (94%). CT scan did not present specific features that allowed LCNEC to be differentiated from other NSCLCs and preoperative diagnosis of LCNEC was obtained in only 9% of cases while in most cases (91%) the diagnosis of LCNEC was obtained by careful identification of cell morphology, mitotic phase and immunohistochemical markers of surgical specimens.

First, in line with other previous papers, adjuvant chemotherapy was associated with better survival outcomes compared to surgery alone. This survival association was found for patients with tumors >20 mm, and was strongest for those with tumors >30 mm, but no significant differences were found for patients with tumor <20 mm. Our results were in contrast with those of Kujtan et al., who found that adjuvant chemotherapy was associated with a better survival in both stage IA and IB patients. The benefit remained significant after multivariable adjustment and was further supported by propensity score-matched analyses. In this analysis, the tumor stage was classified based on the International Union Against Cancer/American Joint Committee on Cancer (UICC/AJCC) TNM staging system sixth and seventh edition staging classification, which limited the comparison with our data. Furthermore, other authors did not find any advantages associated with the administration of adjuvant chemotherapy for stage I patients. Kim et al. found significant survival benefit from adjuvant treatment only for stage II or higher, but not for stage I. However, 30% of patients with stage I LCNEC presented distant recurrence independently whether they received adjuvant therapy or not. Yet, multivariate analysis showed that adjuvant therapy was a significant survival prognostic factor. All these factors may likely demonstrate the benefit of adjuvant therapy for LCNEC also in early stage. Veronesi et al. and Tanaka et al. found a significant survival benefit for adjuvant chemotherapy in the whole population, but it was not significant for stage I disease. However, in both papers there was a trend to better outcome with chemotherapy in stage I disease, and probably the small number of subjects did not allow a statistically significant difference to be obtained.

Second, lobectomy was associated with better survival not only for patients with large tumors (>30 mm) but also in those with small lesions (<30 and <20 mm). Yet, lobectomy was a favorable prognostic factor for survival in multivariate analysis, in line with previous studies. In a large retrospective study including 1530 patients with all-stage LCNEC, Cao et al. found that surgery was a positive independent prognostic factor for survival, and lobectomy was associated with better outcomes compared to other types of resections, such as sublobar or pneumonectomy. Similarly, Wakeam et al. reported that sublobar resection for stage I LCNEC was correlated with worse survival than lobectomy. Iyoda et al. found that patients with limited resection of primary LCNEC tumors experienced more recurrence than those undergoing lobectomy.

Third, patients with triple-positive markers had better survival outcomes than the control group and these results were also observed when patients were stratified according to tumor size. Neuroendocrine markers are often negative in poorly differentiated neuroendocrine cancers. Thus, LCNEC with nontriple-positive markers tended to be similar to SCLC and thus associated with a poor prognosis. By contrast, in patients receiving adjuvant chemotherapy, triple-positive patients had worse survival compared to nontriple-positive patients. As observed by Tanaka et al., the LCNEC might become resistant to chemotherapy through coexistence and mutual interaction of all three proteins while the lack of any of these proteins may reduce the resistance of tumor to chemotherapy. Similarly, SCLCs that show a poor prognosis may likely demonstrate the benefit of adjuvant therapy for LCNEC also in early stage. Veronesi et al. and Tanaka et al. found a significant survival benefit for adjuvant chemotherapy in the whole population, but it was not significant for stage I disease. However, in both papers there was a trend to better outcome with chemotherapy in stage I disease, and probably the small number of subjects did not allow a statistically significant difference to be obtained.

Fourth, from a clinical point view the results of this study suggest different treatments in relation to characteristics of LCNEC, as summarized in Figure 4. Patients with LCNEC scheduled for surgical resection should be treated in a similar way as for SCLC, and lobectomy routinely performed also even for small tumors (<20 mm). By contrast, adjuvant chemotherapy should be not routinely administered in patients with LCNEC as performed for SCLC, but only in patients with tumor >20 mm. This is in line with the current National Comprehensive Cancer Network (NCCN) guidelines that recommend adjuvant therapy in patients with “high-risk” features, including poorly differentiated neuroendocrine histology and pathologic stage IB NSCLC.

| Table 3 | Prognostic factors for overall survival and disease-free survival |
|---------|---------------------------------------------------------------|
| **Factors** | **Overall survival** | | **Disease-free survival** | |
| | HR | 95% CI | p value | HR | 95% CI | p value |
| Age (≤70 vs. >70) | 0.89 | 0.78–2.78 | 0.56 | 0.78 | 0.68–1.98 | 0.46 |
| Sex (male vs. female) | 1.07 | 0.97–1.87 | 0.76 | 1.17 | 0.87–1.37 | 0.66 |
| Comorbidity (yes vs. no) | 0.76 | 0.56–2.21 | 0.58 | 0.86 | 0.46–2.61 | 0.68 |
| Tumor size (<20 vs. >20 mm) | 2.98 | 1.45–2.98 | 0.001 | 2.38 | 1.25–2.65 | 0.002 |
| Resection (lobar vs. sublobar) | 4.19 | 2.21–3.34 | 0.002 | 4.45 | 2.31–4.54 | 0.001 |
| Histology (pure vs. mixed) | 1.56 | 1.98–4.91 | 0.49 | 1.34 | 1.54–3.87 | 0.51 |
| Adjuvant chemotherapy | 2.17 | 1.56–3.65 | 0.001 | 2.30 | 1.76–4.10 | 0.002 |
| Triple positive markers | 3.91 | 1.34–2.98 | 0.003 | 3.91 | 1.58–3.16 | 0.005 |

*Abbreviations: CI, confidence interval; HR, hazard ratio.*
| Authors          | Population               | Variables                          | Results                                                                 | Conclusions                                                                 |
|------------------|--------------------------|------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Wakeam et al.13  | 1770 resected pts        |                                    | 59% vs. 45%, \( p < 0.0001 \), all pts                                  | ACT was associated with significantly longer survival for tumors larger than 3 cm and possibly for tumors between 2 and 3 cm |
|                  | Surgery alone 1,307 pts   | 5-YSR (ACT vs. NACT)               | 59.8% vs. 42%, \( p < 0.0001 \), >3 cm                                  |                                                                            |
|                  | Surgery + ACT 463 pts     | Prognostic Factors                 | 60% vs. 42%, \( p = 0.002 \), 2–3 cm                                    |                                                                            |
|                  |                          |                                    | 54% vs. 51%, \( p = 0.27 \), <2 cm                                      |                                                                            |
|                  |                          |                                    | ACT, \( p < 0.0001 \)                                                   |                                                                            |
|                  |                          |                                    | T stage \( p = 0.006 \)                                                 |                                                                            |
|                  |                          |                                    | R1 \( p = 0.008 \)                                                      |                                                                            |
|                  |                          |                                    | Sublobar resection \( p < 0.0001 \) CT within                           |                                                                            |
|                  |                          |                                    | 3 months \( p < 0.0001 \), within 3–6 months                            |                                                                            |
|                  |                          |                                    | \( p = 0.005 \)                                                         |                                                                            |
| Kujtan et al.14  | 1232 pts                 |                                    | 64% vs. 48%, \( p < 0.001 \), all pts                                  | ACT improved survival in patients with stage I A and stage IB              |
|                  | Surgery alone 957 (77.7%) | 5-YSR (ACT vs. NACT)               | 59% vs. 50%, \( p = 0.006 \), stage IA                                  |                                                                            |
|                  | Surgery + ACT 275 (22.3%) | Prognostic factors                 | 68% vs. 44%, \( p < 0.001 \), stage IB                                  |                                                                            |
|                  |                          |                                    | Age < 70 y, \( p < 0.0001 \)                                            |                                                                            |
|                  |                          |                                    | Non white, \( p = 0.002 \) Lobectomy,                                  |                                                                            |
|                  |                          |                                    | \( p = 0.003 \)                                                         |                                                                            |
|                  |                          |                                    | ACT, \( p < 0.0001 \)                                                   |                                                                            |
| Kim et al.15     | 139 pts                  |                                    | 62% vs. 48%, \( p = 0.212 \), all pts                                  | AT improved survival in patients with stage II or higher                  |
|                  | Surgery alone 50 pts     | 5-YSR (AT vs. NAT)                 | 100% vs. 61%, \( p = 0.2 \), stage I                                    |                                                                            |
|                  | Surgery + AT (CT and/or  | 5-YDFS (AT vs. NAT)                | 52% vs. 31%, \( p = 0.02 \), stage II                                   |                                                                            |
|                  | CT + RT) 89 pts          | Prognostic factors 5-YSR           | 46% vs. 35%, \( p = 0.308 \), all pts                                   |                                                                            |
|                  |                          | 5-YDFS (5-YSF)                     | 80% vs. 50%, \( p = 0.3 \), stage I                                    |                                                                            |
|                  |                          |                                    | 39% vs. 18%, \( p = 0.03 \), stage II                                   |                                                                            |
|                  |                          |                                    | \( pN (p < 0.001) \)                                                     |                                                                            |
|                  |                          |                                    | R0 resection \( p = 0.02 \)                                             |                                                                            |
|                  |                          |                                    | AT \( p = 0.003 \)                                                       |                                                                            |
|                  |                          |                                    | \( pN (p < 0.001) \)                                                     |                                                                            |
|                  |                          |                                    | Pneumonectomy \( p = 0.04 \)                                           |                                                                            |
|                  |                          |                                    | R0 resection \( p = 0.009 \)                                            |                                                                            |
|                  |                          |                                    | AT \( p < 0.001 \)                                                       |                                                                            |
| Veronesi et al.16| 144 resected pts         |                                    | 43% all pts, 52% stage I, 59% stage II, 20% stage III                   | There is a trend to better outcome with chemotherapy in stage I disease   |
|                  | 21 had induction therapy | 5-YSR (ACT vs. NAT)                | 100% vs. 58%, \( p = 0.077 \)                                           |                                                                            |
|                  | and 24 ACT               | Prognostic factors                 | Pneumonectomy, \( p = 0.02 \)                                           |                                                                            |
|                  |                          |                                    | Stage III, \( p = 0.004 \)                                              |                                                                            |
| Tanaka et al.17  | 63 resected pts          |                                    | 74.4% vs. 32.3%, \( p = 0.042 \)                                        | There is a trend to better outcome with chemotherapy in stage I disease   |
|                  | Surgery alone 40 pts     | 5-YSRT (ACT vs. NACT)              |                                                                            |                                                                            |
|                  | Surgery + ACT 23 pts     | Prognostic factors                 |                                                                            |                                                                            |
| Raman et al.18   | 2642 pts                 |                                    | 53%, all pts, 56% vs. 54%, \( p = 0.1 \), stage IA                      | ACT improved survival in stage IB but not in stage IA                      |
|                  | Surgery alone 2.161 pts  | 5-YSR (ACT vs. NAT)                | 62% vs. 43%, \( p < 0.0001 \), stage IB                                 |                                                                            |
|                  | Surgery + ACT 481 pts    | Prognostic factors IA, IB          | Lobectomy, \( p < 0.001 \)                                              |                                                                            |
|                  |                          |                                    | Lobectomy, \( p = 0.02 \)                                               |                                                                            |
|                  |                          |                                    | ACT, \( p = 0.007 \)                                                     |                                                                            |

(Continues)
| Authors          | Population                              | Variables                          | Results                                                                 | Conclusions                                      |
|------------------|-----------------------------------------|------------------------------------|-------------------------------------------------------------------------|--------------------------------------------------|
| Roesel et al.19  | 251 pts                                  | 5-YSR (ACT vs. NACT)               | 60.9% stage I, 31% stage II, 22% stage III                              | ACT may improve survival in stage Ib and higher   |
|                  | Surgery alone 150 pts                    |                                    | 34.6% vs. 37.8%, \( p = 0.02 \) for all pts, \( p = 0.005 \) for stage I |                                                  |
|                  | Surgery + ACT 101 pts (19 had induction therapy) |                                    | 34.6% vs. 37.8%, \( p = 0.02 \) for all pts, \( p = 0.005 \) for stage I |                                                  |
|                  |                                          |                                    | \( p = 0.001 \) for stage II                                          |                                                  |
| Sarkaria et al.20| 100 resected pts                         | 5-YSR (ACT vs. NACT)               | 50% vs. 45%, \( p = 0.1 \), all pts                                    | ACT may improve survival in advanced-stage patients|
|                  | Surgery alone 42                         | Prognostic factors                 | 37% vs. 51%, \( p = 0.052 \), stage IB-IIIA                             |                                                  |
|                  | Surgery + ACT 30 pts                     |                                    | Gender (\( p = 0.007 \))                                              |                                                  |
|                  |                                          |                                    | Co-morbidity (\( p = 0.012 \))                                        |                                                  |
|                  |                                          |                                    | Stage (\( p = 0.011 \))                                               |                                                  |
| Iyoda et al.21   | 72 resected pts                          | Recurrence (ACT vs. NACT)          | 10 (33%) vs. 26 (61.9%) (\( p = 0.017 \))                              | ACT is useful to prevent recurrence               |
|                  | Surgery alone 42                         | 5-YDFSRT (ACT vs. NACT)            | 58.9% vs. 33%, \( p = 0.044 \)                                        |                                                  |
|                  | Surgery + ACT 30 pts                     | Prognostic factors for 5-YDFSRT    | ACT, \( p = 0.005 \)                                                  |                                                  |
|                  |                                          |                                    | Stage, \( p = 0.025 \)                                               |                                                  |
|                  |                                          |                                    | Second cancer, \( p = 0.008 \)                                        |                                                  |
| Iyoda et al.22   | 38 resected pts                          | 2-YSR (ACT vs. NACT)               | 88.9% vs. 65.2%, \( p = 0.025 \)                                      | ACT was associated with significantly longer survival |
|                  | Surgery alone 23 pts                     | 5-YSR (ACT vs. NACT)               | 88.9% vs. 47.4%,                                                      |                                                  |
|                  | Surgery + ACT 15 pts                     | 2-YDFSRT (ACT vs. NACT)            | 86.7% vs. 47.8%, \( p = 0.013 \)                                      |                                                  |
|                  |                                          | 5-YDFSRT (ACT vs. NACT)            | 86.7% vs. 34.8%                                                       |                                                  |
| Saji et al.23    | 45 pts                                   | 5-YSR (ACT vs. NACT)               | 87.5% vs. 58.5%, \( p = 0.04 \)                                       | Adjuvant chemotherapy improved the survival even in stage I disease |
|                  | Surgery alone 22 pts                     | Prognostic factors for 5-YSR       | ACT, \( p = 0.045 \)                                                 |                                                  |
|                  | Surgery + ACT 23 pts                     |                                    |                                                                        |                                                  |

*Abbreviations: 5-YSR, 5-year survival rate; 5-YDFSRT, 5-year disease free survival rate; ACT, adjuvant chemotherapy; AT, adjuvant therapy; pts, patients.*
but do not explicitly recommend routine adjuvant therapy for stage IA and IB LCNEC. The lack of triple-positive markers seems to be associated with poor prognosis, but a better response to chemotherapy. In theory, it may influence the decision of adjuvant chemotherapy in selected patients with tumor size <20 mm (i.e. nontriple-positive markers). However, our data are not strong enough to support that different neuroendocrine marker profiles may influence therapeutic strategy. Future studies, including molecular studies, may improve the treatment stratification of these subsets of patients. Rossi et al.26 analyzed the molecular profile of 83 LCNEC patients. They found that patients with mesenchymal epithelial transition factor (MET)-positive samples had better median overall survival than the control group with MET-negative samples (24 vs. 18 months). Other authors27,28 supported the use of epidermal growth factor receptor (EGFR)-targeted therapy due to the presence of EGFR-activating mutations in mixed LCNECs with an adenocarcinoma component, while Mairinger et al.29 hypothesized the use of anti-angiogenic-targeted drugs in association with chemotherapy as the angiogenesis could be involved in LCNEC metastasisization. Furthermore, other innovative therapeutic targets could be represented by tropomyosin-related kinase B and brain-derived neurotrophic factor, which are highly expressed in LCNEC.30

**STUDY LIMITATIONS**

This study had some limitations that should be considered before drawing definitive conclusions. First, because of the retrospective and multicenter nature of the study, the choice of type of resection (lobectomy or sublobar), multimodality treatment (surgery plus chemotherapy or surgery alone), adjuvant chemotherapy regimen (SCLC-based regimen or NSCLC-based regimens), dosages and timing of administration of chemotherapy, and the strategy for management of recurrence (CT, RT, combined CT and RT) was based on the decision of each participating center rather than on structured protocol. Second, patients who received adjuvant chemotherapy may have been selected among those with better functional status, thus the effect attributed to treatment could be due to patients’ more favorable status. Third, the sublobar group included patients undergoing segmentectomy and wedge resection. However, anatomic segmentectomy has traditionally been considered superior to wedge resection and this could affect the results. Due to the relative rarity of LCNEC, the study population was rather small, precluding the ability to obtain more powerful results.

**CONCLUSIONS**

LCNEC represents a rare entity of neuroendocrine pulmonary malignancies that is associated with poor prognosis and high recurrence rate, also in patients with early stage cancer undergoing surgical resection. Lobectomy should be routinely performed for management of limited LCNEC while adjuvant chemotherapy is indicated in patients with tumor >20 mm. The presence of multiple immunostochemical neuroendocrine markers is also associated with a poor prognosis in early-stage LCNEC. Because of the small sample size in this paper, a multicenter, prospective, randomized control trial is necessary to define the role of adjuvant chemotherapy in early-stage LCNEC in relation to immunostochemical neuroendocrine expressions.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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**FIGURE 4** Therapeutic strategy in patients with early-stage large cell neuroendocrine carcinoma

[Diagram of therapeutic strategy]

- Clinically resectable early stage LCNEC
- Lobectomy is the first choice in all cases
- Adjuvant chemotherapy
  - Tumor < 20 mm
  - Tumor > 20 mm

[Box highlighting different treatment options]
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