Clinical Characterization of Pulmonary Large Cell Neuroendocrine Carcinoma and Large Cell Carcinoma with Neuroendocrine Morphology

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BACKGROUND. Large cell carcinoma has been classified as four potential types based on its neuroendocrine morphology and evidence of neuroendocrine differentiation discernible by immunohistochemistry or electron microscopy. However, the clinical relation among these four categories has not been clearly defined. In 1999, the World Health Organization (WHO) categorized large cell neuroendocrine carcinoma as a variant of large cell carcinoma.

MATERIAL AND METHODS. The authors analyzed 119 cases of large cell carcinoma from a total of 2070 primary lung carcinoma cases resected surgically between 1969–1999. Using light microscopy, electron microscopy, and immunohistochemical staining, the authors reclassified these cases into large cell neuroendocrine carcinoma (LCNEC), large cell carcinoma with neuroendocrine differentiation (LCCND), large cell carcinoma with neuroendocrine morphology (LCCNM), and classic large cell carcinoma (CLCC).

RESULTS. In multivariate analyses, the authors found that large cell carcinoma with neuroendocrine features, which combined LCNEC, LCCND, and LCCNM, impacted both the overall survival and disease-free survival of patients. The clinical behavior of LCCNM was similar to that of LCNEC.

CONCLUSIONS. Large cell carcinomas with neuroendocrine features appear to be more clinically aggressive than CLCCs. The authors’ findings suggest that the histologic identification of neuroendocrine features in tumor tissue from patients diagnosed with large cell carcinoma of the lung may have clinical relevance.

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KEYWORDS: pulmonary, large cell neuroendocrine carcinoma, neuroendocrine morphology, neuroendocrine differentiation, neuroendocrine feature.

In the 1970s, pulmonary neuroendocrine tumors were classified into three histologically defined categories, typical carcinoid (TC), atypical carcinoid (AC),1 and small cell lung carcinoma (SCLC). ACs have shown more evident cytologic atypia, cellularity, and focal necrosis than TC tumors, and AC clinical aggressiveness has been less than that of SCLC. More recently, Travis et al.2 and others3–5 recognized a fourth high-grade neuroendocrine tumor of the lung, large cell neuroendocrine carcinoma (LCNEC) or intermediate cell neuroendocrine carcinoma. LCNEC has been categorically placed between AC and SCLC in terms of clinical aggressiveness.6–8 Recently the World Health Organization (WHO) classified LCNEC as a variant of large cell carcinoma.9 In this lung tumor classification schema, large cell carcinomas are classified into four types based on neuroendocrine morphology as determined by light microscopy and neuroendocrine differentiation demonstrable by immunohistochemistry and/or electron microscopy.
microscopy as follows: 1) LCNEC has both neuroendocrine morphology and evidence of neuroendocrine differentiation by immunohistochemistry and/or electron microscopy; 2) Large cell carcinoma with neuroendocrine differentiation (LCCND) lacks neuroendocrine morphology but has neuroendocrine markers by immunohistochemistry and/or electron microscopy; 3) Large cell carcinoma with neuroendocrine morphology (LCCNM) has neuroendocrine morphologic features but lacks neuroendocrine markers by immunohistochemistry and/or electron microscopy; 4) Classic large cell carcinoma (CLCC) lacks neuroendocrine morphology or differentiation. However, the clinical relation among these four histologic categories has not been defined. In this study, we sought to examine the clinical and biologic behavior of these different tumor types.

MATERIAL AND METHODS

Of 2070 primary lung carcinoma cases resected surgically at Chiba University Hospital from 1969–1999, we analyzed 119 cases (5.7%) diagnosed as large cell carcinoma. We excluded so-called combined carcinomas analyzed 119 cases (5.7%) diagnosed as large cell carcinoma, i.e., large cell-size, low nuclear to cytoplasmic ratio, vesicular or fine chromatin, and/or frequent nucleoli. Large cell carcinomas without neuroendocrine morphology were classified as LCCND or CLCC.

To detect neuroendocrine differentiation, immunohistochemical staining and electron microscopy were performed. Immunohistochemical staining was performed on 118 cases using both a polyclonal anti-chromogranin A antibody (Nichirei Corporation, Tokyo, Japan) and a monoclonal antisynaptophysin antibody (DAKO, Glostrup, Denmark). Formalin-fixed, paraffin-embedded, 4 μm sections were treated with 3% hydrogen peroxide in phosphate-buffered saline (0.01 moles per liter [M] of sodium phosphate buffer, pH 7.2, 0.15 M of NaCl) to block endogenous peroxidase and normal goat serum (chromogranin A) and normal rabbit serum (synaptophysin) to block nonspecific binding sites and were incubated overnight at 4 °C with the primary antibody. They were incubated with biotinylated goat antirabbit immunoglobulin G (IgG [chromogranin A]) and rabbit antimouse IgG+IgA+IgM (synaptophysin) for 10 minutes followed by peroxidated streptavidin for 5 minutes. The peroxidase activity was observed with 3,3′-diaminobenzidine. The primary antibody was omitted in negative controls. Sections were counterstained with hematoxylin.

Electron microscopy was performed on 11 cases because epoxy–resin-embedded blocks were available in these cases. Resected tumor was cut into pieces and fixed in 2.5% glutaraldehyde with 0.1 M cacodylate buffer at 4 °C, postfixed in 1% osmium tetroxide for 1 hour, dehydrated through an ascending series of alcohols, and embedded in EPON 812 (Electron Microscopy Sciences, Fort Washington, PA). Semithin sections were cut from the resin-embedded blocks from cases, stained with toluidine blue, and used for light microscopy orientation. Ultrathin sections were cut from selected areas, mounted on coated copper grids, and stained with uranyl acetate and lead citrate. They were examined under a JEM1200EXS electron microscope (JEOL Ltd., Tokyo, Japan) at 80 kV. Three or more sections on a grid were examined in each case.

We detected neuroendocrine differentiation by positive immunohistochemical staining for antichromogranin A, or antisynaptophysin, or neuroendocrine granules by electron microscopy.

Clinical information was culled from the medical records as follows: gender, age, smoking index, preoperative serum tumor-marker levels (lactate dehydrogenase [LDH], carcinoembryonic antigen [CEA], α-fetoprotein [AFP], ferritin, carbohydrate antigen 19–9 [CA19-9], tissue polypeptide antigen [TPA], squamous cell carcinoma antigen [SCC], neuron specific enolase [NSE], and carbohydrate antigen 125 [CA125]), surgical procedure, chemotherapy, radiation therapy, and patient outcome.

We compared clinicopathologic features of LCNEC, LCCND, LCCNM, and CLCC, and we examined differences between CLCC and large cell carcinoma with neuroendocrine features (morphology or differentiation), which combined LCNEC, LCCND, and LCCNM.

Statistical Analysis

The Fisher exact test was used to compare binomial proportions. The chi-square test was used to assess differences in patient’s serum tumor-marker levels among the different tumor types. The unpaired t test was used to detect significant differences in patients’ age, tumor size, smoking index, and tumor mitotic rates. Patient survival time was calculated from the date of surgery until the time of first local or distant recurrence (disease-free survival) or death (overall survival) and was evaluated using the Kaplan–Meier method.10 The curves obtained were compared with
the log rank test. The Cox proportional hazards multivariable regression model was used to investigate the prognostic impact of pretreatment variables as follows: gender, age, smoking index, histologic type (neuroendocrine features [LCNEC, LCCND, and LCCNM] vs. CLCC), tumor size, mitotic rate, tumor T (T1, T2 vs. T3, T4) and N classifications (N0 vs. N1, N2, and N3). A $P$ value of $< 0.05$ was considered statistically significant.

RESULTS
We examined 119 cases of pulmonary large cell carcinoma. Fifty-nine cases had evidence of neuroendocrine differentiation by immunohistochemical staining or electron microscopy. Neuroendocrine differentiation was detected by immunohistochemical staining in 58 cases and by electron microscopy in 1 case whose paraffin-embedded materials were not available. Sixty-three cases demonstrated neuroendocrine...
morphology by light microscopy (Figures 1–4). Fifty cases (42.0%) were classified as LCNEC, 9 (7.6%) as LCCND, 13 (10.9%) as LCCNM, and 47 (39.5%) as CLCC.

Patient Characteristics (Table 1)
The mean age of LCCNM patients was significantly higher than those of CLCC ($P = 0.0439$) and LCCND ($P = 0.0498$) patients. Patients were predominantly male (LCNEC, 84.0%; LCCND, 88.9%; LCCNM, 92.3%; CLCC, 89.4%). On smoking indices, there was no significant difference among categories. The tumor size of LCCND cases was significantly larger than those of LCNEC cases ($P = 0.0033$) and CLCC cases ($P = 0.0259$). The mitotic rate of LCCNM cases was significantly higher than those of LCNEC cases ($P = 0.0071$), LCCND cases ($P = 0.0488$), and CLCC cases ($P < 0.0001$). The mitotic rate of LCNEC cases was significantly higher than that of CLCC cases ($P = 0.0108$). There were no significant differences on surgical

**FIGURE 3.** Histologic features of large cell carcinoma with neuroendocrine morphology (LCCNM). By light microscopy (left; H&E stain, original magnification $\times 40$) the tumor cells look similar to LCNEC cells; however, (right; original magnification $\times 64$), they do not stain with the antichromogranin A antibody.

**FIGURE 4.** Histologic features of classic large cell carcinoma (CLCC). Light microscopy (left; H&E stain, original magnification $\times 40$). The tumor cells do not have a neuroendocrine morphology. There is no evidence suggestive of small cell carcinoma, glandular or squamous differentiation. Immunohistochemistry (right; original magnification $\times 64$). The tumor cells do not stain with the antichromogranin A antibody.
methods and chemotherapy among these four categories. Radiation therapy was not performed in any case of LCCNM.

The extent of lymph node metastases among the four categories was not significantly different. Although it was more frequent in LCCND and in LCCNM, lymph node metastases were found in only 5 cases of LCCND and in only 9 cases of LCCNM (Table 2).

We compared the proportion of cases with elevated, preoperative, serum tumor-markers among the four histologic types (Table 3). The proportion of cases with an elevated LDH was significantly higher in LCNEC and LCCNM cases than in CLCC cases (LCNEC vs. CLCC, \( P = 0.02 \); LCCNM vs. CLCC, \( P = 0.0004 \)). The proportion of cases with an elevated TPA was significantly higher in LCCNM cases than in LCNEC cases (\( P = 0.0123 \)), and the proportion of cases with an elevated CEA was significantly higher in LCNEC cases than in CLCC cases (\( P = 0.029 \)). The proportion of cases with an elevated NSE was not significantly different among the four categories.

### TABLE 1
Characteristics of Patients with Classic Large Cell Carcinoma or Large Cell Carcinoma with Neuroendocrine Features

|                | CLCC          | Large cell carcinomas with neuroendocrine features | LCNEC          | LCCND          | LCCNM          |
|----------------|---------------|---------------------------------------------------|----------------|----------------|----------------|
| No. of cases (%) | 47 (39.5)     | 72 (60.5)                                         | 50 (42.0)      | 9 (7.6)        | 13 (10.9)      |
| Years of age (mean range) | 62 (36-90)   | 64 (35-82)                                       | 64 (38-82)     | 58 (35-78)     | 69 (50-80)     |
| Gender          |               |                                                   |                |                |                |
| Male (%)        | 42 (89.4)     | 62 (86.1)                                         | 42 (84.0)      | 8 (88.9)       | 12 (92.3)      |
| Female (%)      | 5 (10.6)      | 10 (13.9)                                         | 8 (16.0)       | 1 (11.1)       | 1 (7.7)        |
| Smoking index (mean range) | 921.5 (0–3500) | 947.3 (0–2600)                                     | 906.8 (0–2600) | 823.9 (460–1350) | 1188.5 (600–2550) |
| Tumor size (cm) (mean range) | 4.6 (0.7–14.0) | 4.5 (1.3–10.0)                                    | 4.1 (1.3–10.0) | 6.7 (2.2–10.0) | 4.6 (2.0–10.0) |
| Mitotic rate (mean range) | 37.9 (5-244) | 67.2 (9-251)                                      | 60.6 (12-212)  | 51.0 (9-156)  | 103.7 (42-251) |
| Stage           |               |                                                   |                |                |                |
| IA              | 10            | 13                                                | 11             | 2              |
| IB              | 10            | 14                                                | 12             | 1              |
| IIA             | 2             | 2                                                 | 1              | 1              |
| IIIB            | 7             | 5                                                 | 3              | 2              |
| IIIA            | 8             | 23                                                | 15             | 2              |
| IIIB            | 6             | 14                                                | 8              | 3              |
| IV              | 4             | 1                                                 | 1              | 1              |
| Surgery         |               |                                                   |                |                |                |
| Partial resection | 1             | 3                                                 | 2              | 1              |
| Segmentectomy   | 1             | 1                                                 |                |                |
| Lobectomy       | 41            | 51                                                | 37             | 6              |
| Bilobectomy     | 1             | 8                                                 | 5              | 2              |
| Pneumonectomy   | 4             | 9                                                 | 5              | 1              |
| Chemotherapy    | 19            | 34                                                | 22             | 6              |
| Induction       | 15            | 11                                                | 6              | 4              |
| Adjuvant        | 15            | 32                                                | 21             | 5              |
| Radiation       | 6             | 11                                                | 7              | 4              |
| Preoperative    | 1             | 1                                                 | 1              |                |
| Postoperative   | 5             | 10                                                | 6              | 4              |

\( ^{a} \) LCCNM vs. CLCC (\( P = 0.0439 \)); LCCNM vs. LCCND (\( P = 0.0498 \)).

\( ^{b} \) LCCND vs. LCNEC (\( P = 0.0013 \)), LCCND vs. CLCC (\( P = 0.0259 \)).

\( ^{c} \) Large cell carcinoma with neuroendocrine features vs. CLCC (\( P = 0.0013 \)).

\( ^{d} \) LCNEC vs. CLCC (\( P = 0.0108 \)).

\( ^{e} \) LCCNM vs. LCNEC (\( P = 0.0071 \)), LCCNM vs. LCCND (\( P = 0.0488 \)), LCCNM vs. CLCC (\( P < 0.0001 \)).

### TABLE 2
The Extent of Lymph Node Metastases Observed for each Histologic Type of Tumor

| N0 cases (%) | N1 cases (%) | N2 cases (%) | N3 cases (%) |
|--------------|--------------|--------------|--------------|
| CLCC         | 28 (59.6)    | 5 (10.6)     | 10 (21.3)    | 4 (8.5)      |
| Large cell carcinomas with NE features | 34 (47.2)    | 10 (13.9)    | 24 (33.3)    | 4 (5.6)      |
| LCNEC        | 26 (52.0)    | 6 (12.0)     | 17 (34.0)    | 1 (2.0)      |
| LCCND        | 4 (44.4)     | 1 (11.1)     | 2 (22.2)     | 2 (22.2)     |
| LCCNM        | 4 (30.8)     | 3 (23.1)     | 5 (38.5)     | 1 (7.7)      |

N0–N3: World Health Organization staging and classification system, 1999; NE: neuroendocrine.
The overall survival for patients with LCCND was significantly lower than that for those with CLCC (P = 0.0092) (Figure 5 and Table 4). The disease-free survivals for patients with LCNEC, LCCND, and LCCNM were significantly lower than those for patients with CLCC (LCNEC vs. CLCC, P = 0.031; LCCND vs. CLCC, P = 0.04; LCCNM vs. CLCC, P = 0.0351) (Figure 6 and Table 4).

When we compared CLCC clinicopathologic findings with large cell carcinoma with neuroendocrine features, which combined LCNEC, LCCND, and LCCNM, we found that the mitotic rate of large cell carcinoma with neuroendocrine features was significantly higher than that of CLCC (P = 0.0013). On age, gender, smoking indices, and tumor size, there was no significant difference between CLCC and large cell carcinoma with neuroendocrine features. In CLCC, induction or adjuvant chemotherapy was performed in 19 of 47 patients, and radiation therapy was done in 6 patients. In large cell carcinoma with neuroendocrine features, induction or adjuvant chemotherapy was performed in 34 of 72 patients, and radiation therapy was done in 11 patients (Table 1). Patients with large cell carcinoma with neuroendocrine features had lymph node metastases more frequently than those with CLCC, although there was no statistically significant difference (Table 2). The proportions of cases with elevated LDH and CEA were significantly higher in large cell carcinoma with neuroendocrine features than in CLCC (LDH, P = 0.0054; CEA, P = 0.0467) (Table 3). The overall and disease-free survivals for patients who had large cell carcinoma with neuroendocrine features were significantly lower than those for patients who had CLCC (overall survival, P = 0.0196; disease-free survival, P = 0.0066) (Table 4).

Multivariate analyses for patient survival were performed to look for differences in overall and disease-free survivals (Table 5). The presence of tumors with neuroendocrine features impacted both overall survival and disease-free survival in all of these analyses. Univariate analysis showed that neuroendocrine features, large tumor size, advanced T stage, and lymph node metastasis predicted poorer overall survival and poorer disease-free survival. Multivariate analysis showed that neuroendocrine features, older age, large tumor size, increased tumor mitotic rate, and lymph node metastasis predicted poorer overall and disease-free survivals.

**DISCUSSION**

The current study was designed to examine the clinicopathologic features of pulmonary large cell carcinoma with neuroendocrine features. Previous studies have compared LCNEC with typical carcinoid, atypical...
carcinoid, and small cell carcinoma; however, there have been few reports that have examined the relation between LCNEC and CLCC or that have examined LCCNM and LCCND in detail and compared the prognosis of these tumors with that of LCNEC.

LCNEC is an aggressive tumor. Travis et al. found that the patient survival for LCNEC was worse than that for atypical carcinoid and was no different than that for small cell lung carcinoma. Dresler et al. showed that the 5-year survival for Stage I LCNEC patients was 18%, that the 5-year survival for all stages was 13%, and that LCNEC had a remarkably poor prognosis even in very early stage disease. Jiang et al. reported 22 cases of LCNEC and indicated that the 5-year survival for patients with LCNEC was 44.8%.

The 5-year survival for LCNEC patients in our study was intermediate, between Dresler’s results and Jiang’s results. Przygodzki et al. performed analysis of p53, K-ras, and C-raf-1 in pulmonary neuroendocrine tumors and revealed that LCNEC was more akin to large cell carcinoma with neuroendocrine features.

![FIGURE 6. The disease-free survival curves for each tumor by histologic type.](image)

| TABLE 4 |
|---|
| Overall and Disease-Free Survivals for each Histologic Type of Tumor |
|  |
| **Overall survival** |  |
| **MST** | **5-year survival rate** | **95% CI** | **10-year survival rate** | **95% CI** |
| CLCC | 25 | 0.484 | 0.319–0.649 | 0.484 | 0.319–0.649 |
| Large cell carcinomas with NE features | 16.5 | 0.323 | 0.205–0.441 | 0.298 | 0.180–0.416 |
| LCNEC | 19 | 0.353 | 0.208–0.498 | 0.317 | 0.172–0.462 |
| LCCND | 8 | 0.222 | 0.0–0.494 | 0.222 | 0–0.494 |
| LCCNM | 14 | 0.273 | 0.010–0.545 |  |

| **Disease-free survival** |  |
| **MST** | **5-year survival rate** | **95% CI** | **10-year survival rate** | **95% CI** |
| CLCC | 24 | 0.433 | 0.278–0.588 | 0.433 | 0.278–0.588 |
| Large cell carcinomas with NE features | 11.5 | 0.254 | 0.148–0.360 | 0.185 | 0.083–0.287 |
| LCNEC | 12.5 | 0.274 | 0.143–0.405 | 0.205 | 0.076–0.332 |
| LCCND | 6 | 0.222 | 0–0.494 | 0.222 | 0–0.494 |
| LCCNM | 11 | 0.182 | 0–0.489 | 0 | 0

Cl: confidence interval; MST: median survival time; NE: neuroendocrine.

| TABLE 5 |
|---|
| Multivariate Analyses for Survival |
|  |
| **Overall survival** |  |
| Univariate | Multivariate |
| Histology | 0.0234 | 0.0192 |
| T classification | 0.0057 | 0.3478 |
| N classification | < 0.0001 | < 0.0001 |
| Mitosis | 0.0882 | 0.0016 |
| Tumor size | < 0.0001 | < 0.0001 |
| Age | 0.0537 | 0.0021 |
| Gender | 0.7000 | 0.0607 |
| Smoking index | 0.8365 | 0.3469 |

| **Disease-free survival** |  |
| Univariate | Multivariate |
| Histology | 0.0084 | 0.0079 |
| T classification | 0.0066 | 0.3247 |
| N classification | < 0.0001 | < 0.0001 |
| Mitosis | 0.2110 | 0.0051 |
| Tumor size | < 0.0001 | < 0.0001 |
| Age | 0.0537 | 0.0021 |
| Gender | 0.7000 | 0.0607 |
| Smoking index | 0.8365 | 0.3469 |

a Histology (large cell carcinoma with neuroendocrine features vs. classic large cell carcinoma).
b Tumor T classification (T1, T2 vs. T3, T4), World Health Organization (WHO) classification system, 1999.
c Tumor N classification (N0 vs. N1, N2 and N3), WHO system, 1999.

FIGURE 6. The disease-free survival curves for each tumor by histologic type. The disease-free survivals for patients with LCNEC, LCCND, and LCCNM were significantly lower than that for those with CLCC (LCNEC vs. CLCC, P = 0.031; LCCND vs. CLCC, P = 0.04; LCCNM vs. CLCC, P = 0.0351).
genetically and immunohistochemically to small cell lung carcinoma, although it was categorized as nonsmall cell carcinoma.

Although LCNEC cannot be distinguished from LCCNM by routine light microscopy, neuroendocrine differentiation is observed only in the former tumor (by immunohistochemistry or electron microscopy). We found that patient characteristics, clinical behavior, and the pathology of LCCNM cases were similar to those of LCNEC, e.g., the proportion of males to females, mean age of diagnosis, smoking index, tumor size, and serum levels of LDH. However, the proportion of cases with elevated serum TPA and tumor mitotic rates were significantly higher in patients with LCCNM tumors than in those with LCNEC. Lymph node metastases were observed more frequently in LCCNM than in LCNEC. These findings would suggest that LCCNM is more clinically aggressive than LCNEC. Therefore, it may be clinically relevant to differentiate LCCNM from LCNEC by immunohistochemistry or electron microscopy.

We indicated in this study that the patients with LCND had larger tumors than those with CLCC and that the overall and disease-free survivals for LCND patients were significantly poorer than those for CLCC patients. Accordingly, it is clinically significant to differentiate LCND from CLCC by immunohistochemistry or electron microscopy.

A total of nine tumor markers were analyzed in the current study to determine whether they may be useful in distinguishing neuroendocrine tumors from CLCC. Our findings indicated that there were significant differences in serum LDH and CEA. Although NSE is used clinically as a neuroendocrine tumor marker, it does not appear to be very specific, which we confirmed in this study.

Because too few cases existed in each of the four subcategories for statistically significant conclusions, we tried to compare CLCC with the large cell carcinomas that had neuroendocrine features, which included LCNEC, LCCND, and LCCNM. The mitotic rate of the large cell carcinomas that had neuroendocrine features was significantly higher than that of CLCC, and lymph node metastases were more frequently observed in the large cell carcinomas that had neuroendocrine features than in CLCC. There were significant differences in serum LDH and CEA levels between the large cell carcinomas that had neuroendocrine features and CLCC. Multivariate analyses showed that the presence of neuroendocrine features in these tumors was an important negative prognostic factor for both overall and disease-free patient survival.

Berendsen et al. reported a negative prognosis for biopsies that contained > 50% positive-staining nonsmall cell carcinoma cells with the neuroendocrine marker MOC1. Jiang et al. reported that there was a significant difference in overall survival for LCNEC patients versus nonsmall cell lung carcinoma patients. Sunadesan et al. observed statistically significant correlations between nodal status and neuroendocrine differentiation and between disease stage and neuroendocrine differentiation, although there was no correlation between neuroendocrine differentiation and survival. On the contrary, Linnoila et al. showed that neuroendocrine differentiation was not predictive of recurrence in patients with resected nonsmall cell lung carcinoma. The results of our multivariate analyses strongly suggest that it is important to determine by histology the presence or absence of neuroendocrine features in tumors from patients diagnosed with large cell carcinoma.

CONCLUSION

Pulmonary large cell carcinoma with neuroendocrine features has a poorer prognosis than classic large cell carcinoma. The identification of neuroendocrine, histologic features in tumor tissue from patients diagnosed with large cell carcinoma of the lung may have clinical relevance.

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