

Pulmonary Atypical Carcinoid: Predictors of Survival in 106 Cases

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Pulmonary neuroendocrine tumors (NE) include a spectrum of tumors from typical carcinoid (TC) to atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC), and small cell carcinoma (SCLC). Little is known about prognostic predictors for AC because of its rarity. Survival analysis was performed on 106 ACs with clinical follow-up from the AFIP and the Pathology Panel of the International Association for the Study of Lung Cancer (IASLC). The tumors fulfilled the 1999 WHO/IASLC criteria for AC of a NE tumor with a mitotic rate of 2 to 10 per 2 mm² of viable tumor or coagulative necrosis. Multiple clinical and histologic features were analyzed by Kaplan-Meier and Cox regression analysis. Of the clinical features, higher stage ($P = .003$) and a tumor size of 3.5 cm or greater ($P = .003$) were associated with a worse prognosis. Features that were histologically unfavorable by univariate analysis were mitotic rate ($P = .002$), pleomorphism ($P = .018$), and aerogenous spread ($P = .007$). Histologically favorable features by univariate analysis were the presence of palisading ($P = .008$), papillary ($P = .039$), pseudoglandular ($P = .026$), and rosette ($P = .022$) patterns. Female gender showed a trend toward a poorer prognosis ($P = .085$) and was included in the multivariate model. Multivariate analysis stratified for stage showed mitoses ($P < .001$), a tumor size of 3.5 cm or greater ($P = .017$), and female gender ($P = .012$) to be the only negative independent predictors of prognosis and the presence of rosettes ($P = .016$) to be the only independent positive predictor. We further divided the AC into subgroups of low (2 to 5 mitoses/2 mm²) and

The major primary lung tumors with neuroendocrine (NE) morphology include typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC), and small cell carcinoma (SCLC). Before the relatively recent development of this 4-tiered

high (6 to 10 mitoses/2 mm²) mitotic rate and compared the survival with TC and with LCNEC. Within the category of AC, the patients with a higher mitotic rate had a significantly worse survival than those with a lower mitotic rate ($P < .001$) stratified for stage. Five- and 10-year survival rates for AC (61% and 35%, respectively) stratified for stage were significantly worse than for TC and better than that for LCNEC and SCLC. Chemotherapy or radiation therapy was given in 12 of 52 and 14 of 52 cases, respectively, but the data were insufficient to evaluate tumor response. We conclude that AC is an aggressive neuroendocrine neoplasm with survival intermediate between TC and LCNEC and SCLC. Higher mitotic rate, tumor size of 3.5 cm or greater, female gender, and presence of rosettes are the only independent predictors of survival. Surgical resection remains the treatment of choice, and the role of chemotherapy and radiation therapy remains to be proven. HUM PATHOL 31:1255-1265. This is a US Government work. There are no restrictions on its use.

Key words: carcinoid, atypical carcinoid, lung, pulmonary, neuroendocrine, mitoses, bronchus, large cell neuroendocrine carcinoma.

Abbreviations: TC, typical carcinoid; AC, atypical carcinoid; LCNEC, large cell neuroendocrine carcinoma; SCLC, small cell carcinoma; HPF, high-power field; WHO, World Health Organization; AFIP, Armed Forces Institute of Pathology; IASLC, International Association for the Study of Lung Cancer.

classification schema, neuroendocrine tumors were generally classified as carcinoid tumor or SCLC. Pulmonary carcinoid tumors were described by Muller in 1882¹ and established as a pathologic entity by Kramer² in 1930, using the term *bronchial adenoma*. In 1968, Bensch et al³ first described a relationship between carcinoid tumor and SCLC based on electron microscopy studies,³ and this finding was further substantiated by subsequent studies.^{4,5}

In the earlier literature, carcinoids were regarded as essentially benign tumors or, at worst, as low-grade malignancies. The concept of aggressive or malignant pulmonary carcinoids emerged in the literature beginning in 1944.⁶⁻⁹ These reports described varying histologic features of irregular cell size and shape, prominent nucleoli, hyperchromasia and mitoses. However, it was not until Arrigoni et al¹⁰ defined AC in 1972 that more precise criteria were established. Arrigoni et al originally defined AC as a carcinoid tumor with (1) 1 mitotic figure per 1 to 2 high-power fields (HPF) or 5 to 10/10 HPF; (2) necrosis; (3) pleomorphism, hyper-

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0046-8177/00/3110-0014\$00.00/0
doi:10.1053/hupa.2000.19294

chromatism, and an abnormal nuclear cytoplasmic ratio; and (4) areas of increased cellularity with disorganization.¹⁰ It was not specified whether 1 or all of these criteria needed to be met, and the subjectivity of the latter criteria led to inconsistencies in application of the criteria.

Since Arrigoni et al's description, additional reports have appeared in the literature that describe tumors with intermediate histology between TC and SCLC along with several proposed classification schemes.¹¹⁻¹⁴ Some of the criteria used to define the tumors included in these studies varied greatly from those originally described by Arrigoni et al. Mills et al¹¹ included tumors with an average of 14 mitoses per single HPF and Warren et al¹⁴ included tumors with up to 40 mitoses per 10 HPF. Therefore, one can presume that at least some of the tumors included in these studies would be better classified as LCNEC by current criteria.¹⁵

The appreciation of high-grade neuroendocrine malignancies that were not SCLC led to the separation of these tumors into the category of LCNEC by Travis et al, in 1991.¹⁵ With the establishment of diagnostic criteria for LCNEC, AC was then restricted to the tumors with low mitotic rates as originally proposed by Arrigoni et al. A more precise definition of AC was still needed, however, to eliminate the subjectivity of some aspects of the original criteria and to provide criteria that would improve diagnostic accuracy. A modified set of criteria for AC was recently proposed by Travis et al,¹⁵ after critical evaluation and statistical analysis of 200 neuroendocrine tumors. Based on this analysis, the criteria for AC were re-defined as a carcinoid tumor with a mitotic count of 2 to 10 per 2 mm² of viable tumor or coagulative necrosis.¹⁶ These criteria sharpened the definition of AC as a neuroendocrine tumor with a prognosis intermediate between TC and the high-grade LCNEC and SCLC, even when stratified for stage.¹⁶ These criteria were subsequently adopted into the current World Health Organization (WHO) International Association for the Study of Lung Cancer (IASLC) Histologic Classification of Lung and Pleural Tumors, and are the currently accepted criteria for AC.¹⁷

In addition to the clinical and pathologic studies that have established AC as a defined entity, molecular studies have further shown multiple genetic differences between AC and the other neuroendocrine tumors, especially the high-grade LCNEC and SCLC. Most of these molecular studies show an intermediate level of abnormalities in p53, 3p, 9p, FHIT, and 5q compared with those seen in TC and the high-grade neuroendocrine tumors.¹⁸⁻²³ MEN1 mutations appear to be seen mostly in AC rather than TC, and they are typically absent in LCNEC or SCLC.^{24,25}

Because AC is an established entity, the purpose of this article was not to evaluate the criteria for AC or to compare AC with other neuroendocrine or non-neuroendocrine pulmonary malignancies. The aim of the study is rather to examine prognostic indicators within the category of AC alone, as defined by the recent 1999 WHO criteria, in a sufficiently large group of tumors to

yield statistically significant results. Our goal was to identify possible predictors of prognosis within the category of AC and to evaluate the effectiveness of adjuvant therapy.

METHODS

Neuroendocrine tumors originally diagnosed as TC, AC, and "malignant carcinoid" were evaluated. The tumors were obtained from the files of consultation cases at the Armed Forces Institute of Pathology (AFIP) and from cases submitted by the Pathology Panel of the IASLC. The cases were examined by using the histologic criteria proposed by Travis et al²⁶ that were later adopted by the recent WHO/IASLC Histologic Classification of Lung and Pleural Tumors.¹⁷ These diagnostic criteria are based purely on light microscopy without the need for immunohistochemistry or electron microscopy. Cases were excluded if they did not meet the current criteria for AC or if clinical follow-up was not available. All of the cases were reviewed by the first author and senior author (M.B.B., W.D.T.), and a large number of the cases were reviewed by the panel members of the IASLC. Discrepancies were resolved by consensus evaluation of the slides. A total of 106 tumors were included in the study. To make some comparisons in survival between this group of AC and the other types of neuroendocrine lung tumors, survival information for TC, LCNEC, and SCLC was used from a previously published series of neuroendocrine lung tumors.¹⁶

Clinical Features

Clinical features were examined that were thought to be possible predictors of aggressive behavior. They included age, sex, smoking history, race, tumor size, tumor location, stage, surgical procedure, and history of adjuvant therapy. Clinical information and follow-up were obtained from patient records and from referring pathologists and clinicians. Complete information on each of the clinical features evaluated was not available on all cases.

Pathologic Features

Pathologic features assessed included histologic features, as well as gross and immunohistochemical findings, when available. Histologic features were assessed by examination of hematoxylin and eosin-stained sections. Features evaluated were aerogenous spread, amyloid-like stroma, follicular pattern, interstitial growth, necrosis, nucleoli, organoid pattern, papillary formation, palisading pattern, pleomorphism, presence of rosettes, solid growth, spindle cell pattern, trabecular pattern, and vascular invasion. The presence of clear or oxyphilic cytoplasm also was evaluated. The percentage of each growth pattern and cytoplasmic features present in a tumor were estimated, but for statistical analysis a pattern was regarded as present or absent. The presence of nucleoli was graded as absent, inconspicuous, clearly present, or prominent. Nucleoli that were regarded as absent or "inconspicuous" were considered absent for statistical purposes, and nucleoli regarded as "clearly present" or "prominent" were evaluated as present. Pleomorphism, defined as variation in cell size and shape, was graded as minimal, moderate, or marked, with those regarded as moderate or marked being grouped together as "present" for analysis purposes. Necrosis, presence of amyloid-like stroma, and vascular invasion were regarded as present or absent.

Mitoses were counted on an Olympus BH2 microscope at an HPF magnification of 40× and a standard field of view number of 20 (0.2 mm²); therefore, 10 HPF using this microscope equals 2 mm². It is important to note that 2 mm² does not equal 10 HPF on all microscopes, and adjustments must be made in the number of fields counted to accurately apply these criteria.²⁶ Mitoses were counted in the most mitotically active areas, as recommended by other authors.²⁷⁻²⁹ Such areas were identified by scanning the tumor at a medium magnification, and mitoses were counted in areas where viable tumor cells constituted the entire field diameter. Areas of necrosis and prominent stroma were avoided when possible. Three sets of 10 HPF were counted and the results averaged.

The gross features of the tumor were obtained from pathology reports accompanying the submitted material.

Although not required to make a diagnosis of AC by the current criteria, immunohistochemical studies were performed at the AFIP by using a standard avidin-biotin peroxidase technique with antigen retrieval, using formalin-fixed, paraffin-embedded material, in cases with material available. Primary markers evaluated were pan-cytokeratin (AE1/3/CK1/LP34, 200:40, Boehringer Mannheim, Indianapolis, IN/Dako, Carpinteria, CA), CAM 5.2 (Cam5.2, 1:50, Becton-Dickinson, San Jose, CA), chromogranin (Chromogranin AB, 1:100; Ventana, Tucson, AZ), synaptophysin (synaptophysin, 1:1, Ventana), and Leu-7 (HNK-1, 1:20, Becton-Dickinson). When positive staining was present, the intensity and percentage of tumor cells staining was evaluated. Intensity was regarded as absent (0), weak (1+), moderate (2+), or strong (3+). Distribution was evaluated by quartiles with scores given for 1% to 25%, 26% to 50%, 51% to 75% and 76% to 100% staining.

Statistics and Survival Analysis

Statistical analysis was performed by using SPSS 9.0 for Windows. Chi-square (χ²) tests, Kaplan-Meier, and Cox multivariate were used regarding a P-value of .05 or less as significant. Only death related to tumor was regarded as a censored event.

Each feature was analyzed by using univariate analysis. Features that proved to be significant by univariate analysis were then examined by multivariate analysis after stratification for stage.

RESULTS

Clinical Features

The clinical findings are summarized in Table 1. The mean follow-up interval was 4.74 years, with a range of 0.3 to 23 years. Fifty-one cases occurred in women and 54 in men (gender was unknown in 1 patient), with an average patient age of 54 years (range, 20 to 85). Race was known in 44 of the cases and consisted of 35 whites, 6 blacks, 2 Asians, and 1 Hispanic. Of 63 patients with information available, 44 reported a positive smoking history. Smoking pack-years ranged from 0 to 120, with a mean of 28 and a median of 7.

Forty-seven patients were dead of disease at the time of the study, and 59 were alive or dead of unrelated causes. In 62 patients with known clinical information regarding disease presentation, 28 were asymptomatic, with tumor discovered incidentally on chest

TABLE 1. Clinical Features

	n*	
Sex	106	Male = 54 Female = 51 Not available = 1
Age	106	Range = 20-85 Mean = 54
Race	44	White = 35 Black = 6 Asian = 2 Hispanic = 1
Smoking history	63	Yes = 44 No = 19
Pack years	37	Range = 1-120 Mean = 28 Median = 7
Tumor size	89	Range = 0.7-12.0 cm Mean = 3.1
Tumor location: central v peripheral	90	Central = 44 Peripheral = 40 Mid-portion = 6
Tumor distribution	93	Right upper lobe = 16 Right middle lobe = 11 Right lower lobe = 16 Right upper and middle lobed = 3 Right middle and lower lobes = 5 "Right lung," not further specified = 5 Left upper lobe = 14 Left lower lobe = 16 "Left lung," not further specified = 7
Stage	100	Stage I = 57 Stage II = 21 Stage III = 14 Stage IV = 8
Chemotherapy	52	Yes = 12 No = 40
Radiation	48	Yes = 13 No = 35
Surgical procedure	97	Lobectomy = 56 Pneumonectomy = 16 Wedge resection = 12 Bilobectomy = 7 Bronchial biopsy/biopsy of metastasis = 5 Sleeve resection = 1
Follow-up interval	106	Range = 0.30-23 years Mean = 4.74
Survival	106	Dead of disease = 47 Alive/dead of other cause = 59 Overall survival 61% (5 yr), 35% (10 yr), 28% (15 yr)
5-year survival by stage		Stage I: 71% Stage II: 46% Stages III & IV: 37%

* n = number of patients with information available.

radiograph. Twenty-two patients reported cough or dyspnea, 11 hemoptysis, and 8 chest pain. Several patients presented with multiple symptoms. Three patients had carcinoid syndrome, and 3 had features of Cushing's syndrome. Two of the 3 patients with carcinoid syndrome had liver metastases at presentation, whereas none of the patients with Cushing's syndrome had metastatic disease at presentation. One patient was reported to have multiple endocrine neoplasia syndrome 1, and a second reported a family history of multiple endocrine neoplasia syndrome 1.

TABLE 2. Summary of Histologic Features and Prognostic Significance for Survival

Growth Pattern	% of tumors present	Prognostic Significance (P)*
Organoid	89	.964
Trabecular	71	.843
Solid	58	.072
Spindle	54	.995
Pseudoglandular	52	.026†
Rosettes	44	.022†
Palisading	44	.008†
Aerogenous	27	.007†
Interstitial	23	.632
Follicular	23	.132
Papillary	9	.039†
Mitotic rate‡		.002†
Nuclear Pleomorphism	52	.018†
Nucleoli	42	.081
Vascular invasion	62	.07
Necrosis	67	.501
Oxyphilic cytoplasm	26	.71
Clear cytoplasm	26	.305
Amyloid-like stroma	20	.228

* All values analyzed by Kaplan-Meier analysis except for mitoses, which were analyzed by the Cox regression method.

† Significant P value in univariate analysis (P < .05).

‡ See Text for mean and range.

Forty-nine percent of tumors were central, 44% were peripheral, and 7% were described as mid-lung. Fifty-six tumors presented in the right lung and 37 in the left with roughly equal lobe distribution. Average tumor size was 3.1 cm (range, 0.7 to 12.0). Staging information was known on 100 patients, with 57 patients presenting with stage I disease, 21 with stage II, 14 with stage III, and 8 with stage IV. In cases with lymph node involvement, the peribronchiolar and perihilar lymph nodes were most commonly positive. Distant metastases, either at initial presentation or as a recurrence, most commonly occurred in the liver, followed by bone, brain, adrenal, and ovary. Metastatic disease to the breast, abdominal wall, spleen, pancreas, skin and retro-orbital region were also reported.

Gross Features

Gross descriptions were available for 45 of the tumors. Twenty-five tumors were described as white to gray, 19 tumors were reported as tan, pink to yellow, and 4 as brown to red. Several tumors were described as more than 1 color. Gross necrosis was noted in only 4 tumors, and 3 were described as “friable.” Six tumors were specifically described as circumscribed, and 1 was reported as “irregular.” Consistency was reported equally as “soft or rubbery” and “firm or hard.” Three tumors were described as hemorrhagic.

Histologic Features

The histologic findings are summarized in Table 2. Of the growth patterns examined, the organoid pattern was most common (89%) followed by the trabecular

(71%), solid (58%), spindled (54%), pseudoglandular (52%) (Fig 1), rosette (44%) (Fig 2), and peripheral palisading (44%) (Fig 3) patterns. Less common were the follicular (23%) (Fig 4), interstitial (23%) (Fig 5), and papillary (9%) (Fig 6) patterns. In most cases, more than 1 histologic pattern was present, and individual patterns were sometimes present only focally in a given tumor. Necrosis was present in 67% of cases and nucleoli in 42%. Necrosis was usually present in the form of coagulative necrosis in the central portion of tumor nests and was often focal and punctate (Fig 7). In 8 cases, larger areas of necrosis were present. Large zones of necrosis were not necessarily found in tumors of larger size or those having a higher mitotic rate. Large zones of necrosis were noted in tumors as small as 1.2 cm and having as few as 1 to 2 mitoses/2 mm². Nine cases contained areas of necrosis but had fewer than 2 mitoses per 2 mm². The average number of mitoses per 2 mm² was 4.9, with a range of 0.33 to 10 (Fig 8). Vascular invasion was identified in 62% and aerogenous spread in 27% (Fig 9) of cases. Nuclear pleomorphism (Fig 10) was present in 52% of cases. All of the cases were pure forms of AC, and we did not encounter AC combined with other major histologic types lung carcinoma, such as adenocarcinoma, squamous cell carcinoma, or SCLC.

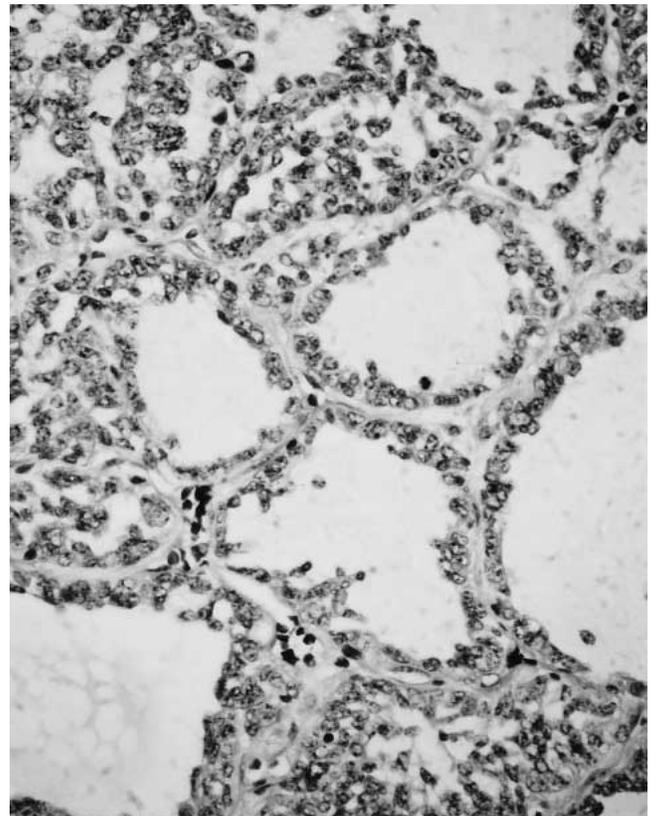


FIGURE 1. The pseudoglandular pattern is characterized by growth in gland-like structures.

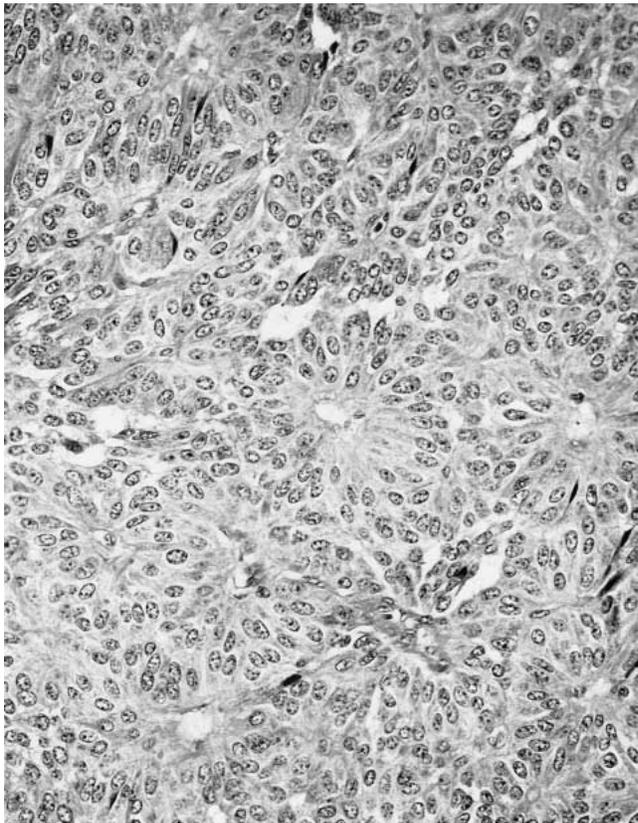


FIGURE 2. Tumor cells showing formation of rosettes.

Immunohistochemistry

Immunohistochemical studies were available on 38 cases, although they are not required for the diagnosis of AC by the WHO criteria. Cytokeratin “cocktail” stains were available on 31 cases. Twenty-six of the cases (84%) showed at least some degree of positive staining. Eleven of 13 (85%) cases stained with CAM 5.2 were positive, 31 of 33 cases (94%) were positive for chromogranin, 30 of 33 cases (91%) were positive for synaptophysin, and 21 of 28 cases (75%) were positive for Leu-7. All of the cases with immunohistochemical studies available showed some degree of staining for at least 1 of the neuroendocrine markers. Analysis of each marker was made with regard to positive versus negative staining, and no statistically significant results were found. When the percentage of cells staining with a particular marker was analyzed, a trend toward a better prognosis was identified in tumors with 75% of cells or greater staining with chromogranin compared with tumors with less than 75% of cells staining ($P = .082$). No other significant results were identified with regard to percentage of cells staining or intensity of staining. A summary of the distribution and intensity of staining with these markers appears in Table 3.

Survival Analysis

The overall 5-, 10-, and 15-year survival rates for AC were 61%, 35%, and 28%, respectively (Fig 11). Five-

year survival was significantly better for stage I (71%) than for stage II (46%) and for stages III and IV combined (37%, $P = .022$).

Of the clinical parameters, higher stage ($P = .003$) and larger tumor size were significantly correlated with a poor prognosis. Size of the tumor was significant when analyzed as both a continuous variable ($P = .003$) and when divided into groups of smaller than 3.5 cm and larger than 3.5 cm and analyzed by the Kaplan-Meier method ($P = .041$). Sex of the patient showed an interesting trend, with female patients having a worse 5-year survival than males (50% and 67%, respectively, $P = .085$).

Histologic parameters that were negative predictors of prognosis by univariate analysis were mitotic rate ($P = .002$), pleomorphism ($P = .018$), and aerogenous spread ($P = .007$). Features that were shown to be positive predictors of prognosis were palisading ($P = .008$), papillary formation (.039), rosettes ($P = .022$), and the pseudoglandular pattern ($P = .026$).

Given the worse survival in females, each of the histologic features examined was also evaluated with regard to patient gender. Necrosis was the only histologic parameter that had a significant gender difference, and it was found more frequently in women ($P = .004$). Evaluation by the Cox method, however, failed to show that necrosis accounted for the reduced survival in females ($P = .825$), even when stratified by gender or stage.

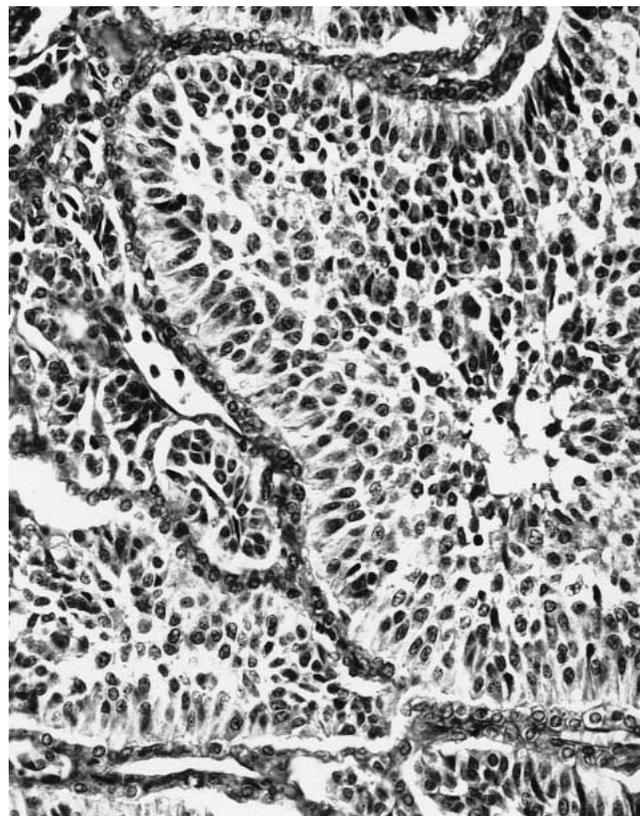


FIGURE 3. Palisading refers to a row of cells arranged perpendicular to the primary cellular arrangement.

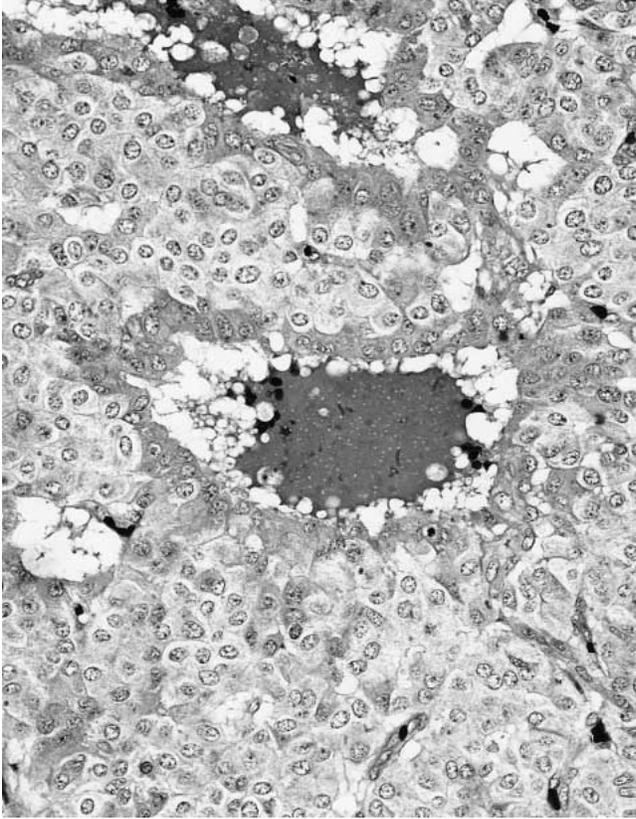


FIGURE 4. The follicular growth pattern is characterized by the formation of gland-like structures, somewhat similar to the pseudoglandular pattern, but the lumen is filled with eosinophilic "colloid-like" material showing peripheral scalloping reminiscent of thyroid follicles.

Multivariate analysis stratified for stage was then performed on the variables found to be significant by univariate analysis. Gender of the patient also was included in the multivariate model because of the suggestion of a survival difference in the univariate analysis. The multivariate analysis revealed that mitotic rate, size 3.5 cm or greater, and female gender were the only negative predictors of prognosis ($P < .001$, $P = .017$, and $P = .012$, respectively), and the presence of rosettes was the single positive predictor of prognosis ($P = .016$).

Given the strong significance of mitotic rate, we compared survival for patients with ACs having 2 to 5 mitoses/ 2 mm^2 and 6 to 10 mitoses/ 2 mm^2 as well as with that of TC and LCNEC to determine whether there were differences in survival. Five- and 10-year survival rates were 60% and 40% for AC with a mitotic range of 2 to 5 mitoses/ 2 mm^2 compared with 50% and 10% for AC with a mitotic rate of 6 to 10 mitoses/ 2 mm^2 ($P = .0019$) (Fig 12). Stratified for stage, patients with AC having low mitotic counts (2 to 5 mitoses/ 2 mm^2) had a significantly worse survival than those with TC ($P < .001$) and patients with AC having high mitotic counts (6 to 10 mitoses/ 2 mm^2) had a significantly better prognosis than those with LCNEC ($P = .006$).

Therapeutic Findings

Surgical resection was performed in 95% of cases. The procedures performed were lobectomy in 56 cases, pneumonectomy in 16, wedge resection in 12, bi-lobectomy in 7, and sleeve resection in 1. Bronchial biopsy or biopsy of a metastasis were performed in 5 of the cases, and at the time of follow-up, these patients had not received additional surgery. In cases with information on adjuvant therapy available, chemotherapy or radiation therapy was given in 12 of 52 and 13 of 48 cases, respectively. In the patients in whom a response was assessed, 8 of 9 tumors showed no response to chemotherapy, and 3 of 3 tumors showed no response to radiation therapy. The patients who received adjuvant therapy all presented with advanced disease, and the exact agents and dosages are largely unknown. Administration of adjuvant therapy did not appear to improve overall survival. The small number of patients and lack of uniformity in approach to adjuvant treatment precluded meaningful statistical analysis.

DISCUSSION

Our study provides a comprehensive survival analysis of multiple clinical and histologic factors in a large series of AC. Because AC is the rarest of the major pulmonary neuroendocrine tumors, it was difficult to

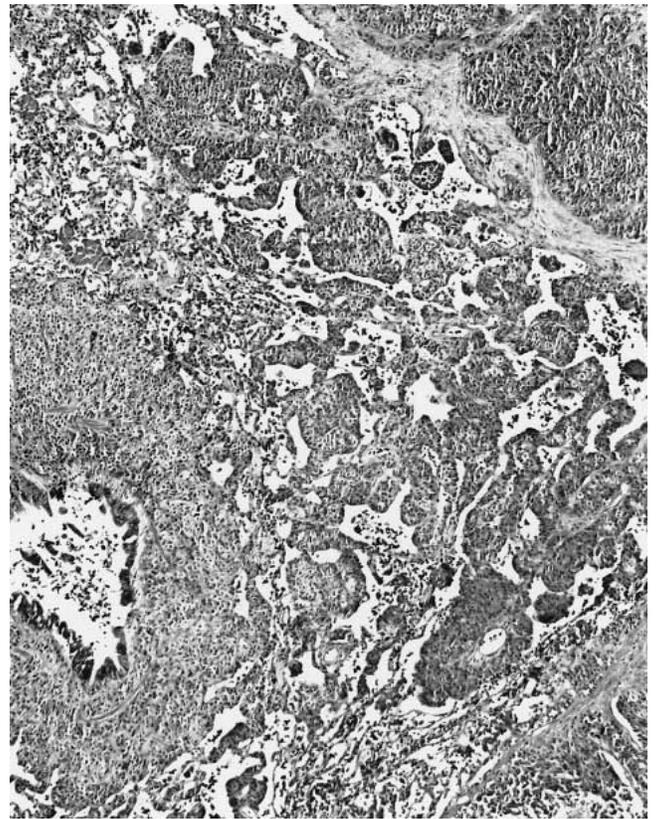


FIGURE 5. The interstitial pattern is characterized by the growth of tumor within existing alveolar septa.

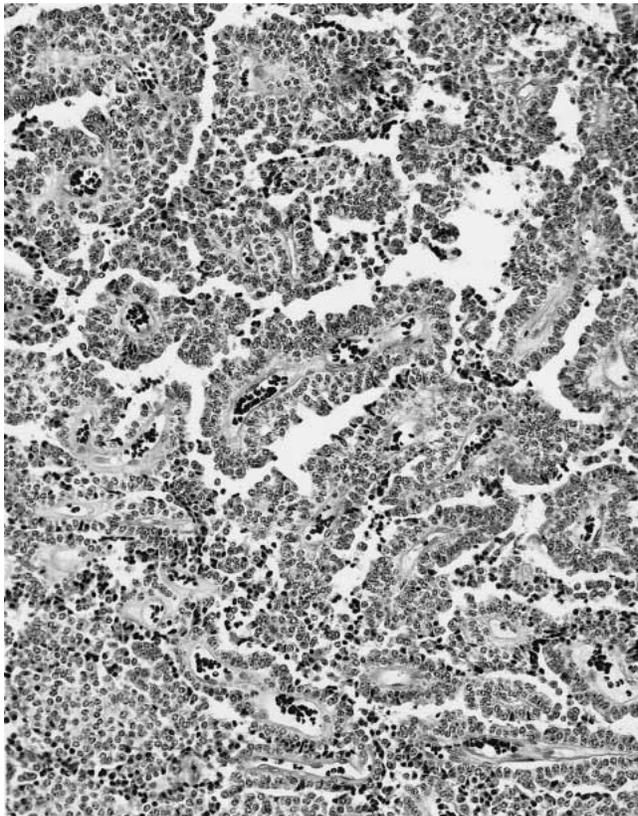


FIGURE 6. The papillary pattern is characterized by tumor cells surrounding a fibrovascular core.

collect this series of 106 cases, requiring not only cases from the files of the AFIP but also contributions from pathologists in multiple countries throughout the world representing the pathology panel of the IASLC. Multiple clinical and microscopic parameters were found to be predictors of survival. Histologic patterns that correlated with a favorable prognosis by univariate analysis included palisading, pseudoglandular, papillary, and rosettes, whereas unfavorable factors included aerenogenous spread and nuclear pleomorphism. The variables that were found to be of importance in a multivariate analysis stratified for stage included the unfavorable factors of mitoses, female gender, and tumor size greater than 3.5 cm, as well as the favorable presence of rosettes. The growth patterns that correlated with a favorable prognosis may be reflective of a higher degree of differentiation. Patient gender also proved to be a negative predictor of survival in the multivariate model stratified for stage, with female patients having a worse prognosis. The reason for this is not clear. In an attempt to explain this finding, the histologic patterns were analyzed with regard to gender, and the only significant difference was the higher frequency of necrosis in women. However, by the Cox method, necrosis did not account for the difference in survival, even when stratified for gender or stage. This finding leads us to believe that factors other than histologic features account for the worse survival in fe-

males. Higher stage and greater tumor size were poor prognostic variables. These findings are not particularly surprising, because these are poor prognostic indicators in lung carcinomas in general.³⁰ Additionally, tumor size of greater than 3.0 cm was shown to be of significance in carcinoid tumors in general in a paper by McCaughan et al,³¹ so it is not surprising that it should be of predictive value in AC alone.

We found a variety of histologic patterns in our AC, including the more common organoid and trabecular patterns as well as rosette formation, aerenogenous spread, interstitial, solid, papillary, pseudoglandular, and palisading patterns. These patterns often appear only focally, and more than 1 pattern is often present within a single tumor. Tumor heterogeneity in the form of AC combined with other major histologic types of lung carcinoma, such as squamous cell carcinoma or adenocarcinoma, was not observed. Also of interest is the spindle cell pattern that was observed in slightly over half of the tumors in this study and was found to be equally distributed between tumors of central and peripheral location. The presence of a spindled pattern was not found to be prognostically significant in this study.

Additionally, the presence of necrosis was not prognostically significant. The necrosis in our AC was mostly punctate, but in a small percentage, larger zones of infarct-like necrosis were present. However, because

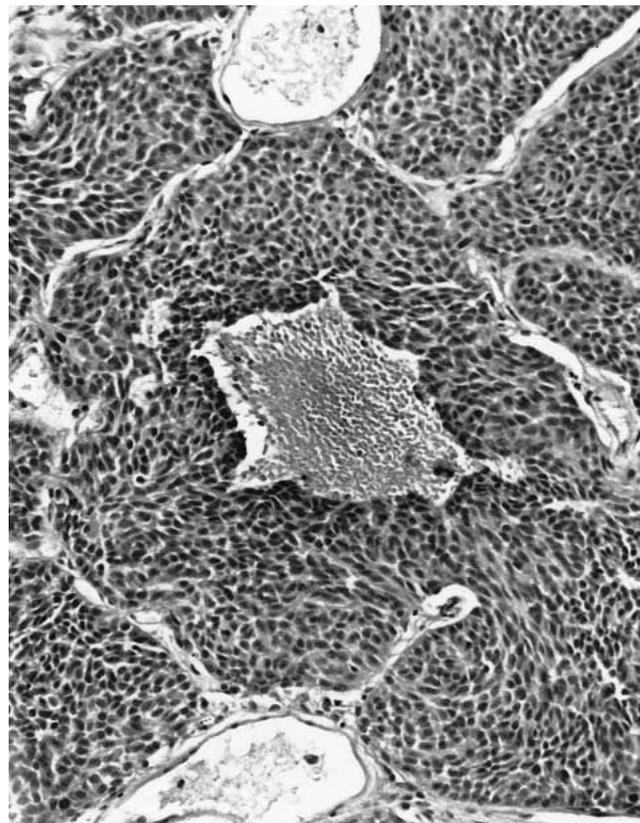


FIGURE 7. Atypical carcinoid with organoid nests showing central punctate foci of necrosis.

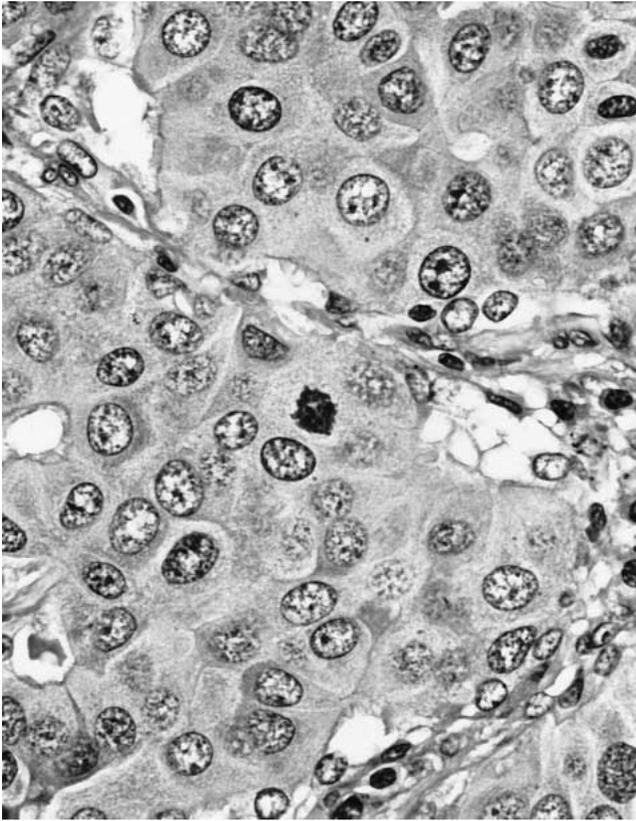


FIGURE 8. The tumor cells have moderate amount of cytoplasm and nuclei with finely granular nuclear chromatin. A single mitosis is present.

necrosis is one of the defining features of AC, it is not surprising that we did not find prognostic significance for this variable within the category of AC itself.

The recognition of AC is important because of the prognostic significance when compared with the lower-grade TC and the high-grade LCNEC and SCLC.²⁶ The main differential diagnosis that arises with AC is the separation of this tumor from the other neuroendocrine neoplasms. When distinguishing AC from TC, it is important to closely evaluate the tumor for the presence of mitoses or necrosis, because these may be focal. The necrosis may be focal and punctate and is not always readily apparent at low power. Similarly, areas of mitotic activity may vary from section to section, and it is important to count the mitoses in the most active areas. Separating AC from TC on a transbronchial or needle biopsy may be difficult, because mitoses and necrosis may occur only focally in AC and may not be represented on a small biopsy. The diagnosis of AC was made in 2 cases in this study on transbronchial biopsy; however, these biopsy specimens were rather large and contained diagnostic features of necrosis or mitotic figures. Similarly, crush artifact, frozen section artifact, and thick or overstained sections may compound the difficulty of separating AC from TC, and other NE tumors in general. All of these factors can obscure nuclear detail and impair accurate counting of mitoses.

Well-prepared histologic sections are therefore critical for accurate classification of this neoplasm.

In differentiating AC from LCNEC or SCLC, mitotic count is the most critical discriminator. Necrosis is usually present in the high-grade tumors, and the amount of necrosis in AC may be extensive, so this is not a discriminating feature. In most of the high-grade tumors, mitotic figures are readily apparent, so the distinction is not often difficult. Problems may occur when an LCNEC or SCLC has a well-organized neuroendocrine pattern and minimal necrosis. Even in the presence of a well-organized neuroendocrine pattern, if the mitotic count is 11 per 2 mm² or greater, the tumor should be classified as either LCNEC or SCLC, depending on the cellular features of the tumor. The current WHO criteria stipulate that both LCNEC and SCLC should have a mitotic rate of greater than 10 mitoses per 2 mm², and AC should have a mitotic rate of 2 to 10 mitoses per 2 mm².¹⁷ These criteria are largely based on statistical analysis of the spectrum of NE tumors by Travis et al,¹⁶ with an optimum mitotic range for AC of 2 to 10 mitoses/2 mm² being supported by these analyses. In this study, the median mitotic rate for LCNEC was 70 mitoses/2 mm², and there were only 6 cases with a mitotic rate between 11 and 30 mitoses/2 mm².¹⁶ Although mitotic rate is the major discriminator between AC and the high-grade

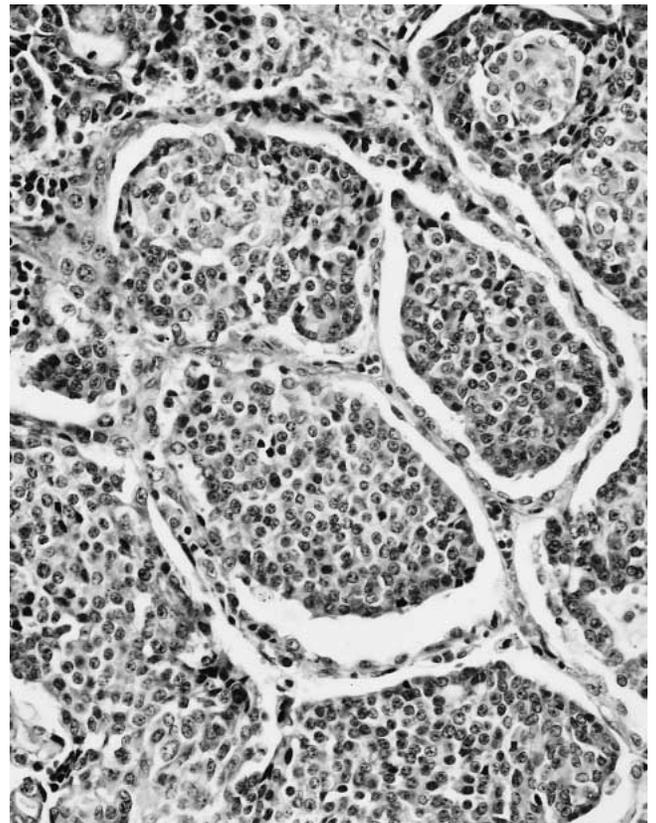


FIGURE 9. Aerogenous spread is characterized by tumor growth, which appears to spread into the alveolar spaces without accompanying destruction of the septa.

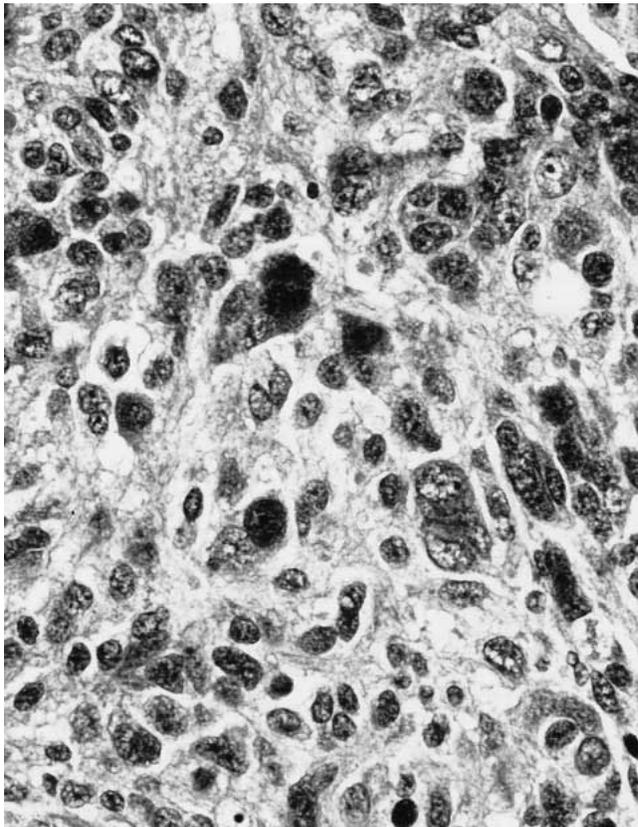


FIGURE 10. The enlarged nuclei with angulated shapes and dense chromatin with hyperchromasia reflect nuclear pleomorphism.

tumors, we acknowledge that there is probably a continuum of mitotic activity, particularly between AC and LCNEC. However, current data support that even in tumors with strong carcinoid morphology, if the mitotic rate per 2 mm² is 11 or greater, it should be classified as a high-grade tumor, because it is likely to behave in a more aggressive fashion.¹⁷ In a recent study of reproducibility in neuroendocrine lung tumor classification, we found that there was excellent agreement in the separation of carcinoids, including AC from the high-grade neuroendocrine tumors LCNEC and SCLC.³²

The immunohistochemical results indicate that AC occasionally can be negative for cytokeratins. Chromogranin proved to be the most sensitive neuroendocrine

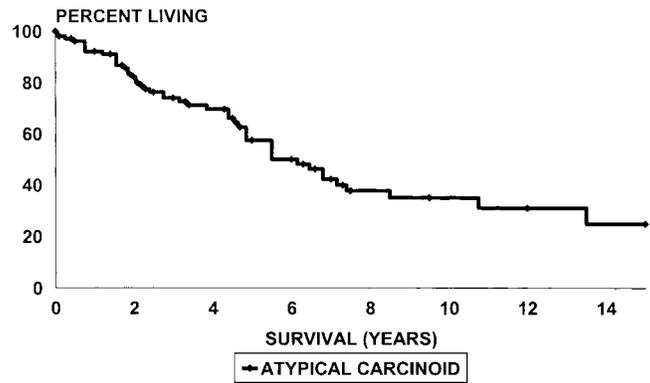


FIGURE 11. Overall survival for patients with atypical carcinoid.

marker, followed by synaptophysin and Leu-7. Rare cases were negative for individual neuroendocrine markers, but neuroendocrine differentiation was shown by at least 1 of the markers in all of the cases in which immunostained material was available. This suggests that a panel of neuroendocrine markers may be the best approach for demonstrating NE differentiation. Although not required for the diagnosis of AC by the WHO criteria, immunohistochemical studies are often used to support the histologic findings, so it is important to recognize that not all cases of AC will stain for every neuroendocrine marker, and that AC may be negative for keratin in some cases. We had thought that tumors negative for neuroendocrine markers might behave more aggressively. Although we did observe a slight trend toward a better prognosis in tumors with greater than 75% of cells staining with chromogranin, we found no additional statistically significant results regarding immunohistochemical staining and survival.

Despite our hopes and efforts to assess the role of adjunctive therapy, the optimal treatment for AC remains unclear. Traditionally, the treatment of choice for AC has been surgical resection. There is disagreement in the literature regarding the extent of resection required for AC, with some authors recommending parenchymal sparing procedures^{33,34} and others endorsing complete lobectomy.^{35,36} Regardless of the surgical technique employed, regional lymph nodes should be assessed in all cases of AC for staging.^{31,36} Given the difficulty of distinguishing TC from AC on a

TABLE 3. Immunohistochemistry Results

Stain	Percentage of Cells Staining					Total Positive	Intensity of Positive Staining		
	Negative 0%	<25% Positive	26-50% Positive	51-75% Positive	76-100% Positive		Weak (1+) Staining	Moderate (2+) Staining	Strong (3+) Staining
Cytokeratin "cocktail"	166	19	3	16	45	84	12	36	52
CAM 5.2	15	31	8	0	46	85	18	45	36
Chromogranin	6	0	9	12	73	94	3	7	90
Synaptophysin	9	9	15	6	61	91	10	31	59
Leu-7	25	29	7	10	29	75	5	50	45

NOTE. The total number of cases evaluated by each antibody is listed in the Results section.

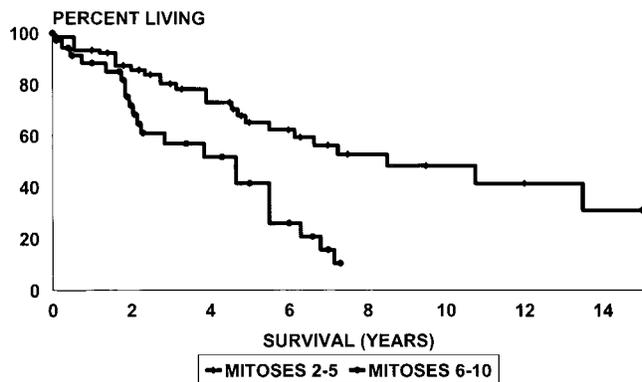


FIGURE 12. Survival for atypical carcinoid patients with 2 to 5 mitoses per 2 mm² compared with 6 to 10 mitoses per 2 mm² ($P = .0019$).

small biopsy or frozen section specimen, and the potential for up to 15% of TC to metastasize, lymph nodes should be assessed on all cases of carcinoid tumor for staging purposes.^{31,37}

Our study supports surgical resection as the optimal therapy for AC. We were unable to evaluate the effectiveness of adjuvant chemotherapy or radiation therapy, because of the small number of patients who received adjuvant therapy. Because the information was obtained retrospectively, the exact agents used and dosages given were largely unknown. Furthermore, most of the patients who received adjuvant therapy presented with advanced stage disease, and what constituted a "response" on a clinical level was not defined. Several previous studies have included patients treated with chemotherapy or radiation therapy. Although a minority of patients are reported to have shown a response in a few reports, no study conclusively proves that adjuvant therapy offers a significant survival advantage.^{13,38-41} Evaluation of prior studies is difficult, however, because most of these studies either do not provide specific histologic detail³⁸ or have included tumors of much higher grade that were probably LCNEC.^{13,40,41} The rarity of AC precludes the existence of a randomized therapeutic trial and necessitated the retrospective and multi-institutional nature of this study. However, the fact that these patients were not treated in a uniform fashion is not a major detriment to the validity of the survival data, because after surgical resection it has not been proved that chemotherapy or radiation therapy are effective. What is needed to address this issue is a prospective randomized clinical trial to evaluate the effectiveness of adjuvant therapy in these patients. Because of the rarity of these tumors, such a study would require collaboration among major oncology institutions or working groups.

We were surprised to find that mitoses were such a strong predictor of survival within such a narrow range between 2 and 10 per 2 mm². The fact that we found a worse prognosis in patients with tumors showing a higher mitotic rate of 6 to 10 mitoses/2 mm² compared with those with 2 to 5 mitoses/2 mm² underscores the importance of mitosis counting in predicting prognosis

in pulmonary carcinoids. It also suggests, along with the other variables found to be significant in the multivariate analysis, that it may be possible to identify AC that are at risk for poor survival based on the number of mitoses and absence of rosettes. Because of the complexity of pulmonary neuroendocrine tumor classification and the lack of data regarding effectiveness of adjuvant therapy, we are unwilling to split AC into distinct subsets at this time and prefer to maintain this as a single entity. However, based on our data, we recognize that there may be a spectrum of survival for these patients and certain features that are helpful in predicting prognosis. Ultimately these histologic features, especially the mitotic counts, should be taken into consideration when evaluating effectiveness of adjuvant therapy.

In summary, AC is an intermediate-grade pulmonary neuroendocrine carcinoma that has a significantly worse prognosis than TC but a significantly better prognosis than the high-grade LCNEC and SCLC. Within the category of AC, the presence of increased numbers of mitoses, size of 3.5 cm or greater, and female gender are predictive of a poor outcome, whereas the presence of rosettes is favorable, as shown by multivariate analysis stratified for stage. Surgical resection remains the treatment of choice, but the role of adjuvant therapy awaits a prospective clinical trial because this issue is very difficult to address in a retrospective fashion.

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