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THE SIGNIFICANCE OF NEUROENDOCRINE DIFFERENTIATION IN PATIENTS WITH RESECTED NON-SMALL CELL LUNG CARCINOMA

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ABSTRACT:

Purpose: Neuroendocrine differentiation (NED) can be detected in 10-30% of non-small cell lung carcinomas (NSCLCs), with the highest frequency in adenocarcinomas (ACs) and large cell carcinomas (LCCs), by immunohistochemical or electron microscopic techniques. These tumors are collectively referred to as non-small cell lung carcinomas with neuroendocrine differentiation (NSCLC-NED). However, the clinical significance of this feature is not fully elucidated in NSCLC-NED. The purpose of this study was to evaluate the prevalence and the clinical significance of NED in patients with stage I-IV NSCLC by using immunohistochemistry for neuroendocrine markers.

Materials and Methods: The relationship between NED and prognosis was investigated by immunohistochemistry using neuron specific enolase (NSE), chromogranin A (Chr A), and neurofilament (NF) in 71 patients with surgically resected stage I-IV NSCLC. The immunostaining results of NE markers were compared with survival data.

Results: While NSE expression was detected most often in LCCs (66.7%), that of Chr A was seen mostly in ACs (25.8%) but none of the NSCLC cases showed immunoreactivity with NF. Multivariate analysis showed that pneumonectomy versus lobectomy carried a poorer prognosis while NSE expression predicted a better prognosis in NSCLC cases.

Conclusion: NED may be of prognostic significance in patients with resected NSCLC but the clinical significance of NED in this subpopulation needs further analysis with specific antibodies in larger series.

Key words: Non-Small Cell Lung Carcinoma, Neuroendocrine Differentiation, Prognosis, Immunohistochemistry.

REZEKE KÜÇÜK HÜCRELİ DISI AKCİĞER KARSİNOMLU HASTALARDA NÖROENDOKRİN DİFERANSİYASYONUN ÖNEMİ

ÖZ:

Amaç: Nöroendokrin diferansiyasyon (NED) en sık adenokarsinomlar (AD'ler) ve büyük hücreli karsinomlar (BHK'ler) olmak üzere küçük hücreli dısı akciğer karsinomlarının (KHDAK'lerin) %10-30'unda immünhistokimyasal veya elektron mikroskopik yöntemler ile saptanabilir. Bu tümörler kollektif olarak "nöroendokrin diferansiyasyon gösteren küçük hücreli dısı akciğer karsinomları (KHDAK-NED)" olarak isimlendirilirler. Ancak bu özelliğin klinik olarak önemi KHDAK-NED'de tam olarak aydınlatılamamıstır. Bu çalısmada evre I-IV KHDAK'li hastalarda nöroendokrin belirleyiciler için immünhistokimyasal yöntem kullanılarak NED'nin sıklığı ve klinik öneminin değerlendirilmesi amaçlanmıstır.

Gereç ve Yöntem: Cerrahi olarak rezeke edilmis evre I-IV KHDAK'li 71 hastada immünhistokimyasal olarak nöron spesifik enolaz (NSE), kromogranin A (Kr A) ve nörofilament (NF) kullanılmasıyla NED ve prognoz arasındaki iliski arastırılmıstır. Nöroendokrin belirleyicilerin immünboyanma sonuçları sağkalım verileri ile karsılastırılmıstır.

Bulgular: NSE ekspresyonu en sık BHK'lerde (%66.7) saptanırken, Kr A ekspresyonu ise en sık AD'lerde (%25.8) gözlenmistir ancak NF ile hiçbir KHDAK olgusunda immünreaktiviteye rastlanmamıstır. Multivaryans analiz KHDAK olgularında; lobektomiye karsılık pnömomektomi uygulanmasının olumsuz, NSE ekspresyonunun ise olumlu prognostik faktör olduğunu ortaya koymustur.

Sonuç: Rezeke KHDAK hastalarında NED'nin prognostik bir önemi olabilir ancak daha genis olgu serileri üzerinde spesifik antikorlarla çalısılarak, bu hasta popülasyonunda NED klinik açıdan daha ayrıntılı arastırılmayı gerektirmektedir.

Anahtar Kelimeler: Küçük Hücreli Dısı Akciğer Karsinomu, Nöroendokrin Diferansiyasyon, Prognoz, İmmünhistokimya.

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INTRODUCTION

In the past 30 years, it has been shown that some of the primary lung tumors reflecting the "gray area" between small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC) demonstrated immunohistochemical and/or ultrastructural evidence of neuroendocrine differentiation (NED) without showing neuroendocrine (NE) morphology by light microscopy. NED shown by the investigations including clinical trials based on immunohistochemical or electron microscopic techniques has been detected in 10%-30% of NSCLCs and it is observed with the highest frequency in adenocarcinomas (ACs) and large cell carcinomas (LCCs).[1,2] These tumors are collectively referred to as non-small cell lung carcinomas with neuroendocrine differentiation (NSCLC-NED).

It has been hypothesized that NSCLC-NED like SCLCs would carry a worse prognosis and moreover these tumors would be more responsive to chemotherapy. While this issue has attracted much interest, there is controversy over whether these tumors are associated with worse or better survival rates and whether they are more or less responsive to chemotherapy than NSCLCs lacking NED.

The purpose of this study was to investigate the relationship between NED and prognosis by evaluating the biological significance of NE markers using immunohistochemistry by correlating staining results with the recurrence and survival of NSCLC cases following a surgical resection.

MATERIALS AND METHODS

Tumor Specimens and Clinicopathological Analysis

A total of 71 bronchial margin negative retrospective cases diagnosed as NSCLC, composed of 31 squamous cell carcinomas (SCCs) (18 well/moderately, 13 poorly differentiated), 31 ACs (20 well/moderately, 11 poorly differentiated), and 9 LCCs in which pneumonectomy, lobectomy/bilobectomy, and segmentectomy were performed, were chosen from the archives of the Pathology Department of the Medical Faculty of Ankara University between 1995 and 1999. Selection of cases was based on the availability of well-fixed paraffin-embedded material with sufficient tumor material for serial sections. Clinical data with available follow-up information (follow-up ranging from 8 to 50 months) were obtained from the records of the Department of Thoracic Surgery of the Medical Faculty of Ankara University. The tumors were staged according to TNM 1986[3] and TNM 1996[4] staging systems.

Histopathological Examination

The tumors were classified according to the World Health Organization (WHO) 2004 criteria.[1] The histological features considered in subtyping and grading of the tumors were summarized as follows: **well differentiated SCC:** extensive keratinization, intercellular bridges or pearl formation; **moderately differentiated SCC:** keratinization and/or pearl formation easily seen but not extensive; **poorly differentiated SCC:** focal morphological features of squamous differentiation with prominent cytological atypia; **well differentiated AC:** prominent glandular differentiation and/or mucin production; **moderately differentiated AC** (Fig. 1): morphologically in between well differentiated and poorly differentiated ACs; **poorly differentiated AC:** solid areas containing five or more cells staining positively for mucin (mucicarmine and periodic acid-Schiff with diastase) in at least two high-power fields; and **LCC:** tumors lacking the cytological features of SCLC and glandular or squamous differentiation.

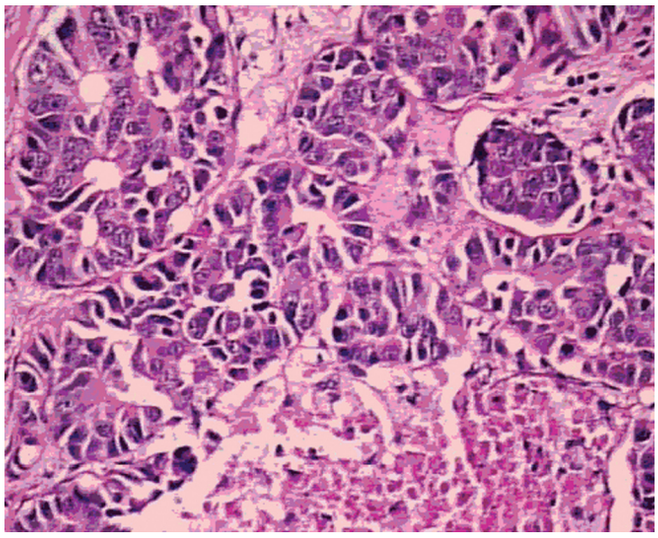

Figure 1: Moderately differentiated adenocarcinoma showing glandular structures with narrow lumens, morphologically reminiscent of neuroendocrine differentiation (Hematoxylin and eosin, × 400).

Immunohistochemistry

Four-micron sections were cut from the most representative tumor subtype and grade of 10% formalin-fixed, paraffin-embedded blocks and were stained using the streptavidin-peroxidase method. Antibodies against neuron-specific enolase (NSE) (Zymed, 1:50), chromogranin A (Chr A) (Novocastra, 1:50), and neurofilament (NF) (Immunon, 1:40) were used as NE markers. While diffuse cytoplasmic reddish-brown staining in intrapulmonary nerve fibers and ganglion cells was regarded as a positive control for NSE and NF, fine granular cytoplasmic reddish-brown staining in mucosal NE cells within the small intestine was considered a positive control for Chr A.

Staining results were scored for both the staining intensity (SI) and percentage of positive tumor cells [distribution score (DS)]. SI was categorized as negative=no staining; +=weak staining; ++=moderate staining; and +++=strong staining. DS was defined as negative: <20% and positive: ≥20% of the tumor cells were stained with one of the NE markers. Positive staining was accepted as DS ≥20% or SI ≥++.

Statistical Analysis

For statistical analysis, SPSS 9.0 for Windows was used. Well and moderately differentiated tumors were combined where there were limited cases in groups that did not permit us to draw precise conclusions. Correlation between categorical variables was assessed using Student's t test/Mann-Whitney U test, chi-square test, Fisher's exact test, Spearman rank correlation, Cochran Q test, and Kruskal-Wallis variance analysis. Life probability calculations were performed using the Kaplan-Meier method. Long rank test, Breslow test, and Cox's regression model were used for statistical evaluation of the prognostic effects of different variables on survival. For multivariate analysis, categorical variables were broken into dichotomous groups. $p<0.05$ was considered significant and $0.15>p>0.05$ was interpreted as "may have an effect on prognosis".[5]

RESULTS

Histopathological/Clinicopathological and Immunohistochemical Results and Their Relationships with Prognostic Factors

The study population in the current investigation consisted of 71 NSCLC retrospective cases with stages I-IV. While 91.5% of the patients were men, 8.5% of them were women. The median age of the patients was 58.9 (59.0 in men and 57.0 in women). No statistically significant correlation was detected between histological subtypes and grades of the tumors and the median age of the patients and gender distribution. The proportions of the patients who underwent lobectomy, pneumonectomy, and segmentectomy were 77.5%, 21.1%, and 1.4%, respectively. No statistically significant correlation existed between the histological subtypes and grades of the tumors and the distribution of resection types. The features of the study cases are summarized in Table 1.

Table 1. Patient/specimen characteristics (n=71)

Age (year)	
Mean±SD	58.9±8.7
Range	42-76
Gender [n (%)]	
Female	6 (8.5)
Male	65 (91.5)
Resection type [n (%)]	
Lobectomy/Bilobectomy	55 (77.5)
Pneumonectomy	15 (21.1)
Segmentectomy	1 (1.4)

Table 2 shows the immunohistochemical expressions of NE markers in NSCLC cases. It can be seen that 57.7% of NSCLC cases stained for NSE and 12.7% of them showed immunoreactivity for Chr A. However, none of the cases showed positivity for NF. A statistical significance was detected in terms of NE marker expression between NSE and Chr A, NSE and NF ($p<0.0001$), and Chr A and NF ($p<0.005$). The NE marker expression rates according to the histological subtypes of the tumors were as follows: NSE positivity was detected in 66.7% of LCCs, 61.3% of SCCs, and 51.6% of ACs, and Chr A immunoreactivity was observed in 25.8% of ACs and 3.2% of SCCs. However, none of the LCCs expressed Chr A and none of the NSCLCs showed positivity for NF. When the NE marker expression rates were evaluated according to the grades of the tumors, the highest incidence of NSE expression was seen in poorly differentiated SCCs and the highest incidence of Chr A expression was observed in well/moderately differentiated ACs. None of the well/moderately differentiated SCCs expressed Chr A.

Table 2. Expression of neuroendocrine markers in NSCLCs

Tumors		**Positive cases [n (%)]***	
Histology and grade	**n**	**NSE**	**Chr A**	**NF**
Adenocarcinoma	31	16 (51.6)	8 (25.8)a
Well/moderate	20	12 (60.0)	6 (30.0)b
Poor	11	4 (36.4)	2 (18.2)
Squamous cell carcinoma	31	19 (61.3)	1 (3.2)
Well/moderate	18	9 (50.0)	
Poor	13	10 (76.9)	1 (7.7)
Large cell carcinoma	9	6 (66.7)	
Total NSCLC	71	41 (57.7)	9 (12.7)

*: DS ≥20% or SI ≥++
a: $p<0.05$ when compared with squamous cell carcinoma
b: $p<0.05$ when compared with well/moderately differentiated squamous cell carcinoma

When the cases were evaluated according to the staining criteria scored as DS≥20% or SI≥++, staining positivity was obtained for each NE marker according to the histological subtypes and grades of the tumors. Statistical analysis was performed to investigate if a significant correlation existed between the histological subtypes and grades of the tumors and the staining positivities of these NE markers. According to this investigation, no statistically significant correlation was found between the histological subtypes and grades of the tumors and NSE positivity. However, a significant correlation was detected between ACs and SCCs and well/moderately differentiated ACs and well/moderately differentiated SCCs with respect to Chr A positivity ($p<0.05$).

The association of clinicopathological/prognostic parameters with expression of NE markers in NSCLC cases is depicted in Table 3. When the NE marker staining was evaluated with respect to T-stage of NSCLCs, the highest proportion of NSE positive cases was staged as T3, whereas the highest incidence of Chr A expression was observed with T2 tumors. The NE marker expressions according to nodal status of NSCLCs were as follows: while 66.7% of N2, 57.1% of N1, and 56.3% of N0 showed immunoreactivity for NSE,

Parameter	**n**	**NSE**	**Chr ANF**
T-stage	-	-	-
T1	7	4 (57.1)	-
T2	42	25 (59.5)	7 (16.7)
T3	20	12 (60.0)	2 (10.0)
T4	2	-	-
N-stage			
N0	48	27 (56.3)	6 (12.5)
N1	14	8 (57.1)	1 (7.1)
N2	9	6 (66.7)	2 (22.2)
TNM 1986			
Stage I	32	18 (56.3)	4 (12.5)
Stage II	10	5 (50.0)	1 (10.0)
Stage III	27	16 (59.3)	4 (14.8)
Stage IV	2	2 (100.0)	-
TNM 1996			
Stage I	32	18 (56.3)	4 (12.5)
Stage II	24	13 (54.2)	3 (12.5)
Stage III	13	8 (61.5)	2 (15.4)
Stage IV	2	2 (100.0)	-
Recurrence			
Absent	27	16 (59.3)	6 (22.2)
Local	3	2 (66.7)	1 (33.3)
Mediastinal	3	3 (100.0)	-
Distant metastasis	19	10 (52.6)	-

Table 3. Association of clinicopathological/prognostic parameters with expression of neuroendocrine markers in NSCLCs [n (%)]

22.2% of N2, 12.5% of N0, and 7.1% of N1 showed positive staining for Chr A. No significant correlations were detected between T- and N-stages of any histological subtypes or grades of NSCLCs and expressions of NE markers. Although the cases were re-evaluated according to the new versions of T- and N-staging systems obtained by union of T1 and T2 versus union of T3 and T4 and union of N1 and N2 versus N0, no significant correlations were observed between these new versions of T- and N-stages of any histological subtypes or grades of NSCLCs and NE marker positivities. No significant correlations were observed between TNM 1986 and the new version of TNM 1986 (obtained by union of stages I and II versus union of stages III and IV) stages of any histological subtypes or grades of NSCLCs and expressions of NE markers. However, a statistically significant finding was detected between stage II ACs showing 27.3% NSE expression and stage III ACs showing 100.0% NSE expression with respect to TNM 1996 staging system ($p<0.05$). When the cases were re-evaluated according to the new version of the TNM 1996 staging system obtained by union of stage I and II versus union of stage III and IV, a statistically significant finding was detected between stage I and II well/moderately differentiated ACs and stage III and IV well/moderately differentiated ACs with respect to NSE positivity ($p<0.05$). No significant correlations were detected between tumor recurrence or distant metastasis and NSE expression. However, a statistically significant finding was observed between NSCLC cases that did not have recurrence and had distant metastasis with respect to Chr A expression ($p<0.05$).

Immunohistochemical Staining Patterns

Non-neoplastic Lung Tissue

Strong diffuse cytoplasmic NSE staining was observed in bronchial epithelial cells, bronchial glands, alveolar epithelial cells, and walls of vascular structures. Chr A showed strong fine granular cytoplasmic staining in solitary NE cells, hyperplastic cells, and dysplastic epithelial cells. Strong cytoplasmic NSE, Chr A, and NF staining was seen in intrapulmonary nerve fibers or ganglion cells.

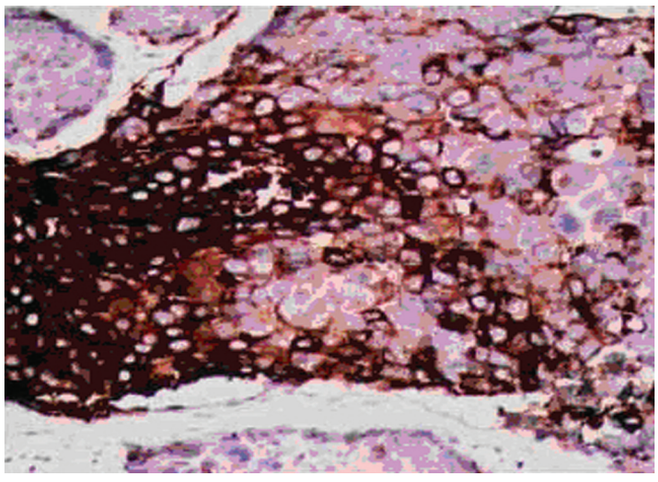

Figure 2: Moderately differentiated adenocarcinoma showing strong granular cytoplasmic immunoreactivity for Chr A (Diaminobenzidine, × 400)

Tumors

Strong diffuse cytoplasmic NSE staining was detected in the majority of LCCs and in some of the well/moderately and poorly differentiated ACs. Strongly stained cells among weakly stained cells were noted in some ACs. Generally weak-moderate staining and focal strong staining were observed in the majority of SCCs. Chr A showed strong fine granular cytoplasmic staining in generally well/moderately differentiated ACs (Fig. 2). NF displayed weak cytoplasmic staining in only one poorly differentiated AC.

Survival Data

Survival data for 57 patients was obtained. According to these data, the mean survival duration for NSCLC cases was 40 months; the longest duration was detected in SCCs, with 43 months, and the shortest duration was observed in LCCs, with 27 months (Table 4). Univariate analysis was carried out for the possible dichotomous factors that could influence prognosis. Table 5 shows the influences of these dichotomous factors on the survival of NSCLCs. Based on the results of univariate analyses, it was concluded that pneumonectomy carried a worse prognosis in NSCLC cases ($p<0.005$). Although the number of cases was limited to allow precise conclusions about age and NSE expression, patients over 65 years could have a worse prognosis ($0.15>p>0.05$), whereas NSE expression could have a better prognosis ($0.15>p>0.05$) in NSCLC cases. According to the histological subtypes of NSCLCs, univariate analyses showed that age over 65 years and pneumonectomy had worse prognostic effects on ACs ($p<0.05$), while age over 65 years, NSE expression, and ≥1 NE marker expression could have better prognostic effects on SCCs ($0.15>p>0.05$), pneumonectomy and the presence of recurrence could have worse prognostic influences on SCCs ($0.15>p>0.05$), and age over 65 years had a worse prognostic effect on LCCs ($p<0.05$).

Table 4. Summary of survival data according to the histological subtypes and grades of the tumors (months)

Histology and grade	n	Mean	Standard error	95% CI
Adenocarcinoma	24	38	4	30-45
Well/moderate	17	34	5	24-43
Poor	7	-	-	-
Squamous cell carcinoma	28	43	3	38-49
Well/moderate	17	45	3	40-50
Poor	11	39	5	29-49
Large cell carcinoma	5	27	7	13-41

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Squamous cell carcinoma	28	43	3	38-49
Well/moderate	17	45	3	40-50
Poor	11	39	5	29-49
Large cell carcinoma	5	27	7	13-41

Table 5. Influences of dichotomous factors on the survival of NSCLCs

Variable	Groups	p-value	Effect on survival
	1 2	Logrank Breslow	
Age (1: ≤65, 2: >65)	42±2 (37-47) 34±5 (24-45)	0.2089 0.1202	0, ? (↓)
Gender (1: Male, 2: Female)	39±3 (34-44) -	0.2411 0.2538	0
Resection (1: Lobectomy, 2: Pneumonectomy)	43±2 (39-48) 24±5 (15-33)	0.0025 0.012	* (↓)
Recurrence (1: Absent, 2: Present)	43±3 (36-49) 39±3 (33-45)	0.4925 0.802	0 0
T stage (1: T1+T2, 2: T3+T4)	41±3 (36-47) 38±4 (29-46)	0.4881 0.5715	0 0
N stage (1: N0, 2: N1+N2)	39±3 (33-45) 42±3 (35-48)	0.4942 0.478	0
TNM 1986 (1: Stage I+II, 2: Stage III+IV)	41±3 (35-47) 39±3 (33-46)	0.8622 0.9372	
TNM 1996 (1: Stage I+II, 2: Stage III+IV)	40±3 (35-45) 40±5 (30-49)	0.9213 0.9878	
NSE (1: -, 2: +)	36±4 (28-43) 43±3 (38-48)	0.2116 0.1241	0, ? (↑)
Chr A (1: -, 2: +)	40±2 (35-45) 32±4 (23-40)	0.9944 0.8539	0
NF (1: -, 2: +)	40±2 (36-45)		
1: -, 2: ≥1 NE marker	37±4 (29-44) 42±3 (37-47)	0.4139 0.3092	0

*(↓): p<0.05, the second group has a negative effect on prognosis.

? (↓): 0.15>p>0.05, the number of samples is inadequate, but the second group may have a negative effect on prognosis.

? (↑):0.15>p>0.05, the number of samples is inadequate, but the second group may have a positive effect on prognosis.

0: No statistical significance on prognosis.

-: Kaplan-Meier survival analysis could not be performed due to no exitus in the group.

.: Statistical analysis could not be performed due to the absence of cases in one of the groups.

Multivariate analysis using Cox's regression model showed that the patients who underwent lobectomy had 5.62 times longer survival compared with those who underwent pneumonectomy and the patients that showed NSE expression had 33.3 times longer survival compared with those that did not show NSE positivity. These data showed that pneumonectomy versus lobectomy carried a poorer prognosis while NSE expression predicted a better prognosis in NSCLCs ($p<0.05$) (Table 6).

Table 6. Summary of multivariate analysis data based on Cox's regression model in NSCLCs

Covariable	Regression coefficient (β±SE)	Relative risk eβ (95% CI)	p-value (two-sided)
Age (≤65/>65)	0.61±0.73	1.84 (0.44-7.73)	0.4042
Resection (Lobectomy/Pneumonectomy)	1.73±0.76	5.62 (1.27-24.85)	0.0229
NSE (+/-)	-3.47±1.74	0.03 (0.001-0.94)	0.0460

DISCUSSION

Tumors morphologically similar to SCLCs that may occur especially in the gastrointestinal tract, the genitourinary system, and in the head and neck, collectively referred to as extrapulmonary small cell carcinomas, often have a more indolent course than SCLCs and may respond to cytotoxic therapy. The occurrence of NE differentiation in NSCLCs is significant not only because it suggests that the major forms of lung cancer represent a continuum of differentiation within a common cell lineage but also because the response of NSCLC-NED to chemotherapy has been shown to be similar to that of SCLC by in vitro chemosensitivity profiles and in vitro drug sensitivity testing. It has been demonstrated that NSCLC-NE lines are similar to SCLC lines and their IC50 values are significantly lower than the values for NSCLCs and carcinoids.[6] In vitro drug sensitivity testing of SCLC, NSCLC, and NSCLC-NED cell lines has shown that SCLC and NSCLC-NED cell lines are more responsive to cytotoxic drug treatment than non-NSCLC-NED.[7] Similarly, several studies have demonstrated that patients with NSCLC-NED show increased response to chemotherapy compared to those with non-NSCLC-NED.[8-14]

As SCLC is the prototype of highly malignant and therapy-sensitive NE tumor of the lung, a theoretical assumption has arisen from these findings that tumors showing NED may, like SCLCs, be associated with an adverse prognosis. Perhaps more importantly, it has been proposed that expression of NE features in NSCLCs may identify a subset that is more respon-

sive to chemotherapy. Thus there may be clinical benefits for identifying this NSCLC subgroup and treating it accordingly.[6] To further evaluate the clinical significance of NED in NSCLCs and to see if NED represents an aggressive nature in these tumors as it is characterized in SCLCs, the current study examined the relationship between the expression of NE markers and survival in surgically operable NSCLC patients.

Recent immunohistochemical studies have shown the presence of multiple NE markers in 10%-20% of NSCLCs.[1,15,16] In previous reports, while NSE immunoreactivity varied from 10% to 70%[17,18] that of Chr A varied from 0% to 17%.[19,20] The current study showed that 57.7% and 12.7% of NSCLC cases stained positively for NSE and Chr A, respectively. Our results regarding the incidence of NSE immunoreactivity are in accordance with the studies by Linnoila et al.,[15] Said et al.,[19] and Sundaresan et al.[21] Our finding that 12.7% of NSCLC cases were Chr A positive was within the 10%-20% threshold reported by the WHO classification for establishing NED in conventional NSCLCs[1] and is in keeping with the studies carried out by Gazdar et al.,[16] Graziano et al.,[18] and Loy et al.[20] Studies have shown that while NSE immunoreactivity is seen most often in LCCs[17,19-22] that of Chr A is detected mostly in ACs.[8,18,22] We also found that LCCs were associated with the highest incidence of NSE expression and Chr A positivity was seen mostly in ACs.

The clinical implications of NED in patients with NSCLC remain largely unclear. The initial investigations conducted on the potential clinical significance of NED in NSCLCs and the development of a spectrum of NE markers prompted systematic efforts by multiple groups to further elucidate the clinicopathological impact of NED. However, studies comparing clinical outcomes with NED in NSCLC cases have shown conflicting results. Several studies[23] have correlated NSCLC-NED with shorter survival, more advanced disease stage, and/or increased chemosensitivity.[8,10,12,21,24-30] Other investigators, however, have failed to show any correlation between NED and prognosis or susceptibility to the therapy[14,18,28,31-37] or even have reported better survival rates for patients with NSCLC-NED.[8,12,14,36,38] Differences in tissue processing, techniques or markers used for highlighting NED, definitions of positive results, and study population selection may account for these contradictory results reported previously on the prognosis and survival of patients with NSCLC-NED.

We were able to obtain survival data for 57 patients. Unfortunately, the follow-up duration, which ranged between 8 and 50 months, was not uniform in the groups with respect to the histological subtypes and grades of the tumors. Therefore, we were unable to perform Kaplan-Meier survival analysis in the groups where all the patients were still alive. In this study, multivariate analysis using Cox's regression model demonstrated that the patients who underwent lobectomy had 5.62 times longer survival compared with those who underwent pneumonectomy and the patients that showed NSE expression had 33.3 times longer survival compared with those that did not show NSE positivity. These results were interpreted as showing that pneumonectomy versus lobectomy carried a poorer prognosis while NSE expression predicted a better prognosis in NSCLC cases.

Our current results are in accordance with the studies by Graziano et al.,[8] Carles et al.,[12] and Schleusener et al.[14] on NSCLCs that showed an effect of NED on survival. Our results are particularly in agreement with the findings concerning NSE expression that correlated with longer survival in univariate and multivariate analyses in the study by Carles et al.[12]

There are problems both technical and conceptual in interpretation of the data about the results concerning the incidences of NED in different studies since there is no "gold standard" for defining and detecting NED. The problem is compounded by differences in sensitivity and specificity for identical antibodies in different centers and in interpretation. However, on the basis of many studies, the use of a panel of antibodies has been recommended, with the suggestion that reactivity with two or more should be required before NED is accepted.[39]Although a broad spectrum of immunohistochemical markers can highlight NED in lung tumors, Chr and/or synaptophysin are accepted as the most reliable markers in the detection at the present time due to their close relation with the ultrastructural evidence of neurosecretory granules and small clear vesicles, respectively.[39-44] However, NSE is no longer considered a specific NE marker since it stains up to two-thirds of NSCLCs.[1,2] Therefore, our finding that NSE was a positive prognostic factor should be interpreted with caution.

Although which genetic program induces NED in NSCLCs has not been elucidated, some results indicate that the clustered NE cell component of NSCLC-NED is not a mere expression of intratumoral heterogeneity due to pluripotential capabilities of transformed stem cells, but it likely reflects genetic changes within individual neoplasms and represents an indicator of the potential for new, biologically more aggressive clones to emerge in tumor development.[30]

There are still important questions to be answered regarding NSCLC-NED, namely whether these tumors behave aggressively like SCLCs and therefore respond to chemotherapy or if NED indicates a more benign behavior as in typical carcinoids. Alternatively, is NED of no clinical significance, merely reflecting the general heterogeneity of lung tumors?

In conclusion, in the current study NSE expression was detected most often in LCCs, whereas Chr A expression was seen mostly in ACs. However, none of NSCLC cases showed immunoreactivity for NF. Multivariate analysis showed that pneumonectomy versus lobectomy carried a poorer prognosis while NSE expression predicted a better prognosis in NSCLC cases. This study suggests that additional clinicopathological studies with larger series using reliable NE markers and a con-

sistent classification will be needed to clarify whether NED indicates an independent prognostic factor in NSCLC cases until NSCLC-NED could be accepted as a separate category in a histological classification.

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REFERENCES

1. Travis WD, Brambilla E, Müller-Hermelink HK, Harris CC, eds. Pathology and genetics of tumours of the lung, pleura, thymus and heart. Lyon: International Agency for Research on Cancer Press; 2004.

2. Brambilla E, Lantuejoul S, Sturm N. Divergent differentiation in neuroendocrine lung tumors. Semin Diagn Pathol 2000; 17: 138-148.

3. Naruke T, Tsuchiya R, Kondo H, et al. Implications of staging in lung cancer. Chest 1997; 112: 242S-248S.

4. Mountain CF. Revisions in the international system for staging lung cancer. Chest 1997; 111: 1710-1717.

5. Kardaun OJWF. Kidney-survival analysis of IgA nephropathy patients: A case study. In: Rao CR, Chakraborty R, eds. Handbook of statistics 8. Amsterdam: Elsevier Science Publishers BV.; 1991. p. 407-459.

6. Gazdar AF. Advances in the biology of lung cancer. Clinical significance of neuroendocrine differentiation. Chest 1989; 96: 39S-41S.

7. Gazdar AF. Advances in the biology of non-small cell lung cancer. Chest 1986; 89: 277-283.

8. Graziano SL, Mazid R, Newman N, et al. The use of neuroendocrine immunoperoxidase markers to predict chemotherapy in patients with non-small cell lung cancer. J Clin Oncol 1989; 7: 1398-1406.

9. Linnoila RI, Jensen S, Steinberg S, et al. Neuroendocrine differentiation in non-small lung cancer correlates with favorable response to chemotherapy. Proc Am Soc Clin Oncol 1989; 8: 248.

10. Berendsen HH, Leij L, Poppema S, et al. Clinical characterization of non-small cell lung cancer tumors showing neuroendocrine differentiation features. J Clin Oncol 1989; 7: 1614-1620.

11. Gazdar AF, Kadoyama C, Venzon D, et al. Association between histological type and neuroendocrine differentiation on drug sensitivity of lung cancer cell lines. J Natl Cancer Inst Monogr 1992; 13: 191-196.

12. Carles J, Rosell R, Ariza A, et al. Neuroendocrine differentiation as a prognostic factor in non-small cell lung cancer. Lung Cancer 1993; 10: 209-219.

13. Shaw GL, Gazdar AF, Phelps R, et al. Correlation of in vitro drug sensitivity testing results with response to chemotherapy and survival: comparison of non-small cell lung cancer and small cell lung cancer. J Cell Biochem Suppl 1996; 24: 173-185.

14. Schleusener JT, Tazelaar HD, Jung S, et al. Neuroendocrine differentiation is an independent prognostic factor in chemotherapy-treated non-small cell lung carcinoma. Cancer 1996; 77: 1284-1291.

15. Linnoila RI, Mulshine JL, Steinberg SM, et al. Neuroendocrine differentiation in endocrine and nonendocrine lung carcinomas. Am J Clin Pathol 1988; 90: 641-652.

16. Gazdar AF, Helman LJ, Israel MA, et al. Expression of neuroendocrine cell markers L-dopa decarboxylase, chromogranin A, and dense core granules in human tumors of endocrine and nonendocrine origin. Cancer Res 1988; 48: 4078-4082.

17. Kayser K, Schmid W, Ebert W, et al. Expression of neuroendocrine markers (neuronspecific enolase, synaptophysin and bombesin) in carcinoma of the lung. Path Res Pract 1988; 183: 412-417.

18. Graziano SL, Tatum AH, Newman NB, et al. The prognostic significance of neuroendocrine markers and carcinoembryonic antigen in patients with resected stage I and II non-small cell lung cancer. Cancer Res 1994; 54: 2908-2913.

19. Said JW, Vimadalal S, Nash G, et al. Immunoreactive neuron-specific enolase, bombesin, and chromogranin as markers for neuroendocrine lung tumors. Hum Pathol 1985; 16: 236-240.

20. Loy TS, Darkow GVD, Quesenberry JT. Immunostaining in the diagnosis of pulmonary neuroendocrine carcinomas. An immunohistochemical study with ultrastructural correlations. Am J Surg Pathol 1995; 19: 173-182.

21. Sundaresan V, Reeve JG, Stenning S, et al. Neuroendocrine differentiation and clinical behaviour in non-small cell lung tumours. Br J Cancer 1991; 64: 333-338.

22. Brambilla E, Veale D, Moro D, et al. Neuroendocrine phenotype in lung cancers. Comparison of immunohistochemistry with biochemical determination of enolase isoenzymes. Am J Clin Pathol 1992; 98: 88-97.

23. Carnaghi C, Rimassa L, Garassino I, et al. Clinical significance of neuroendocrine phenotype in non-small cell lung cancer. Ann Oncol 2001; 12: S119-S123.

24. Pujol JL, Boher JM, Grenier J, et al. Cyfra 21-1, neuron specific enolase and prognosis of non-small cell lung cancer: prospective study in 621 patients. Lung Cancer 2001; 31: 221-231.

25. Hiroshima K, Iyoda A, Shibuya K, et al. Prognostic significance of neuroendocrine differentiation in adenocarcinoma of the lung. Ann Thorac Surg 2002; 73: 1732-1735.

26. Iyoda A, Hiroshima K, Baba M, et al. Pulmonary large cell carcinomas with neuroendocrine features are high-grade neuroendocrine tumors. Ann Thorac Surg 2002; 73: 1049-1054.

27. Pujol JL, Simony J, Demoly P, et al. Neural cell adhesion molecule and prognosis of surgically resected lung cancer. Am Rev Respir Dis 1993; 148: 1071-1075.

28. Skov BG, Sorensen JB, Hirsch FR, et al. Prognostic impact of histologic demonstration of chromogranin A and neuron specific enolase in pulmonary adenocarcinoma. Ann Oncol 1991; 2: 355-360.

29. Kibbelaar RE, Moolenaar KEC, Michalides RJAM, et al. Neural cell adhesion molecule expression, neuroendocrine differentiation and prognosis in lung carcinoma. Eur J Cancer 1991; 27: 431-435.

30. Pelosi G, Pasini F, Sonzogni A, et al. Prognostic implications of neuroendocrine differentiation and hormone production in patients with Stage I non-small cell lung carcinoma. Cancer 2003; 97: 2487-2497.

31. Gajra A, Tatum AH, Newman N, et al. The predictive value of neuroendocrine markers and p53 for response to chemotherapy and survival in patients with advanced non-small cell lung cancer. Lung Cancer 2002; 36: 159-165.

32. Graziano SL, Tatum A, Herndon JE II, et al. Use of neuroendocrine markers, p53, and HER2 to predict response to chemotherapy in patients with Stage III non-small cell lung cancer: a Cancer and Leukemia Group B study. Lung Cancer 2001; 33: 115-123.

33. Graziano SL, Kern JA, Herndon JE, et al. Analysis of neuroendocrine markers, HER2 and CEA before and after chemotherapy in patients with Stage IIIA non-small cell lung cancer: a Cancer and Leukemia Group B study. Lung Cancer 1998; 21: 203-211.

34. Abbona G, Papotti M, Viberti L, et al. Chromogranin A gene expression in non-small cell lung carcinomas. J Pathol 1998; 186: 151-156.

35. Hage R, Elbers HR, Brutel de la Riviere A, et al. Neural cell adhesion molecule expression: prognosis in 889 patients with resected non-small cell lung cancer. Chest 1998; 114: 1316-1320.

36. Wertzel H, Grahmann PR, Bansbach S, et al. Results after surgery in undifferentiated large cell carcinoma of the lung: the role of neuroendocrine expression. Eur J Cardiothorac Surg 1997; 12: 698-702.

37. Linnoila RI, Piantadosi S, Ruckdeschel JC. Impact of neuroendocrine differentiation in non-small cell lung cancer. Chest 1994; 106: 367S-371S.

38. Harada M, Yokose T, Yoshida J, et al. Immunohistochemical neuroendocrine differentiation is an independent prognostic factor in surgically resected large cell carcinoma of the lung. Lung Cancer 2002; 38: 177-184.

39. Addis BJ. Neuroendocrine differentiation in lung carcinoma. Thorax 1995; 50: 113-115.

40. Kiriakogiani-Psaropoulou P, Malamou-Mitsi V, Martinopoulou U, et al. The value of neuroendocrine markers in non-small cell lung cancer: a comparative immunohistopathologic study. Lung Cancer 1994; 11: 353-364.

41. Capella C, Heitz PU, Hofler H, et al. Revised classification of neuroendocrine tumours of the lung, pancreas and gut. Virchows Arch 1995; 425: 547-560.

42. O'Connor DT, Frigon RP. Chromogranin A, the major catecholamine storage vesicle soluble protein: multiple size forms, subcellular storage, and regional distribution in chromaffin and nervous tissue elucidated by radioimmunoassay. J Biol Chem 1984; 259: 3237-3247.

43. Varndell IM, Llyod RV, Wilson BS, et al. Ultrastructural localization of chromogranin: a potential marker for the electron microscopical recognition of endocrine cell secretory granules. Histochem J 1985; 17: 981-992.

44. Wiedenmann B, Huttner WB. Synaptophysin and chromogranins/secretogranins-widespread constituents of distinct types of neuroendocrine vesicles and new tools in tumor diagnosis. Virchows Archiv B Cell Pathol 1989; 58: 95-121.