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RESEARCH ARTICLE

Neuroendocrine differentiation as an indicator of chemosensitivity and prognosis in nonsmall cell lung cancer

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Abstract

Context: Nonsmall cell lung cancers with neuroendocrine differentiation (NSCLC-ND) may demonstrate biologic behavior intermediate between NSCLC and small cell lung cancer (SCLC) with impact on prognosis.

Methods: We analyzed 116 consecutive patients with Stage III and IV NSCLC who were diagnosed and treated between 2001 and 2006. Using immuno-histochemical staining for neuron-specific enolase (NSE), chromogranin A (ChrA), and synaptophysin (Syn), 29 (25%) NSCLC-ND were identified.

Results: Expression of NSE was present in 22.4%, ChrA in 15.5% and Syn in 14.8% of patients with NSCLC. Therapeutic response was significantly better in the NSCLC-ND group and specimens with > 30% neuroendocrine (NE)-differentiated tumor cells showed favourable therapeutic response ($P < 0.05$). Multivariate binary logistic regression showed that percentage of NE positive tumor cells was a significant independent prognostic factor associated with a favourable outcome. Receiver operating characteristic (ROC) curves and areas under ROC curves confirmed that percentage of NE-differentiated tumor cells could be useful prediction factor of therapeutic response. Moreover, according to percentage of NE-differentiated tumor cells, optimal cutoffs and related sensitivities and specificities were determined for each markers.

Conclusion: Advanced-stage NSCLC with NE tumor cells are clinically less aggressive tumors. Percentage of NE-differentiated tumor cells identifies patients with favourable therapy response to paclitaxel-cisplatin

Keywords: Nonmicrocellular; lung cancer; neuroendocrine markers; chemotherapy; therapeutic response; survival

Introduction

Nonsmall cell lung carcinoma (NSCLC) makes 70–75% of all malignant lung diseases. In respect to small cell lung carcinoma (SCLC), NSCLC has a relatively low therapeutic response. *NSCLC* is defined as a group of heterogeneous clinical entities of molecular and cellular origin, diverse clinical behavior and different prognoses (Brundage et al., 2002). High resistance to radiation and chemotherapy results in low rate of 5-year survival (14%) (Spira and Ettinger, 2004). Variety of survival rates within the same stage of disease points to presence of different factors that impact on therapy and prognosis (Hiroshima et al., 2002; Jeanmart et al., 2003; Zhu et al., 2006).

Approximately, 20–25% of all lung tumors are neuroendocrine (NE) and range from relatively low-grade (carcinoids) to highly malignant neoplasms (SCLS; Travis et al., 1999, 2009; Corrin, 2000). It has become well recognized that NE properties also may be exhibited by 10–30% of carcinomas with conventional nonsmall cell morphology, traditionally considered of non-NE nature (Travis et al., 1991, 1999, 2009; Gazdar et al., 1988; Kiriakogiani-Psaropoulou et al., 1994; Senden et al., 1997; Baldi et al., 2000; Lyda and Weiss, 2000; Axiotis, 2002). These tumors are collectively referred as NSCLC with NE differentiation (NSCLC-ND; Travis et al., 1991, 1999; World Health Organisation [WHO], 1999; Brambilla et al., 2001). NSCLC-ND has not been formally

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Abbreviations:

NSCLC, nonsmall cell lung cancer;
NSE, neuron-specific enolase;
ChrA, chromogranin A;
Syn, synaptophysin;
CR, complete response;
PR, partial response;
PD, progression of disease;
SD, stable disease;
ROC, receiver operating characteristic

recognized in the WHO Classification as its clinical and therapeutic significance has not been firmly established (WHO, 1999; Brambilla et al., 2001). The identification of NE markers in NSCLC group has initiated the debate on their importance for therapy and prognosis. Previous studies in which these questions have been addressed have provided inconsistent and sometimes conflicting results. Some authors found that NSCLC-ND has better response to chemotherapy and/or better survival rates in comparison to NSCLC without NE differentiation (Graziano et al., 1989; Skov et al., 1991; Carles et al., 1993; Schleusener et al., 1996; Wertzel et al., 1997; Harada et al., 2002; Petrović et al., 2007). Other investigators, however, have failed to show any correlation between NE differentiation and prognosis or susceptibility to the therapy (Graziano et al., 1994, 1998, 2001; Linnoila et al., 1994; Kwa et al., 1996a; Abbona et al., 1998; Hage et al., 1998; Gajra et al., 2002), or even have reported shorter survival, more aggressive or advanced disease stage (Berendsen et al., 1989; Kibbelaar et al., 1991; Sundaresan et al., 1991; Pujol et al., 1993; Kwa et al., 1996b; Petersen, 1999; Travis et al., 1999; Bhattacharjee et al., 2001; Pelosi et al., 2003). The variations in results may be partly explained by study population selection (number of cases studied in different series, the stage of disease, treatment received), differences in techniques or markers used for NE differentiation detection, as well as, definitions of the IHC intensity and positivity criteria.

The purpose of this study was to evaluate the prevalence and the clinical implications of NE differentiation in a series of patients with Stage III and IV NSCLC by using immunohistochemical assays for chromogranin A (ChrA), synaptophysin (Syn) and neuron-specific enolase (NSE).

Materials and methods

Patients

The study included 116 consecutive, unselected patients (79% males, 21% females) with advanced NSCLC, Stage III and IV, who were diagnosed and treated at Military Medical Academy in Belgrade and Clinical Centre Kragujevac during the period 2001–2006. The local ethics committee approved the study, and prior to initiation, written informed consent was obtained from all subjects according to the Declaration of Helsinki.

The disease was confirmed by light microscopy using tissue sections prepared by routine paraffin technique and standard hematoxylin-eozine method. Tumor histology was classified according to the WHO classification system for lung carcinoma (Travis et al., 1999; WHO, 1999; Brambilla et al., 2001). The patients were classified into stages using TNM classification according to the revised International System for Staging Lung Cancer (Mountain, 1997), by which the therapeutic procedure was determined. The patients in Stages IIIa and IIIb without effusion (T1–T3 any N M0) were administered chemotherapy and radiation, whereas those in Stages IIIb with effusion (T4 any N M0) and IV (any T, any N, Ml) received chemoptherapy only. When the progression of disease was diagnozed in patients with Stages IIIa and IIIb without effusion, the treatment with radiation proceded, whereas in patients with more advanced stages (IIIb with effusion and IV), docetaxel, as second-line therapy, was administered. Chemotherapy consisted of paclitaxel 175 mg/m^2 followed by cisplatin 80 mg/m^2 given on first day. Chemotherapy was repeated every 3 weeks for 4–6 cycles. Six cycles were administered to the patients without progression of disease. The radiation treatment consisted of 55–60 Gy tumor dose Split Course.

Immunohistochemistry and evaluation of the results

Pathological material from all cases was obtained via endo/transbronchial biopsy. Parafin sections were deparaffinized and rehydrated through graded alcohols to a buffer solution. Endogenous peroxidase activity was blocked by incubation in 5% hydrogen peroxide for 15 min at 37°C. Immunohistochemistry was performed using the avidin-biotin-peroxidase complex technique.

The following antibodies were used according to the manufacturer's instructions: NSE (clone BBS/NC/VI14, dilution 1:50) ChrA (clone DAK-A3, dilution 1:100) and Syn (clone SY38, dilution 1:10), all from Dako, Glostrup, Denmark. After incubation with a high-sensitivity detection kit according to the manufacturer's instructions (Dako EnVision Plus-HRP, Dako), peroxidase activity was developed with chromogen (DAB liquid, Dako) to obtain a brown-colored end product. Positive controls (carcinoid tumour and small cell carcinoma) and negative controls (incubation with nonrelated, isotypic mouse immunoglobulin at a comparable dilution or omitting the specific antibody) were used in all cases. The immunohistochemical sections were then evaluated by two pathologists independently and blindly, without knowledge of each patient's identity or clinical outcome, by counting at least 1000 tumor cells in representative fields of immunostaining.

As semiquantitative assessments of staining intensity may result in a mistaken information and different ways to formulate intensity-distribution scores or cutoff points, as the criterion for evidence of NE differentiation, have been described in the literature (Berendsen et al., 1989; Sundaresan et al., 1991; Linnoila et al., 1994; Travis et al., 1999; Graziano et al., 2001; Howe et al., 2005; González-Aragoneses et al., 2007; Ionescu et al., 2007), we tried to find most rational criteria to create the overall staining index in our study. According to above literature data, semiquantitative assessments of staining intensity (none = 0, weak(+) = 1, moderate(++) = 2, strong(+++) = 3) and percentage of positive-labeled tumor cells (none = 0, 1–10% = 1, 10–49% = 2, 50–100% = 3) were made. In addition, the overall intensity-distribution (ID) score from 0 to 6 was obtained by adding the results of staining intensity to the results of the percentage of positive cells. Using this criterion, an ID score of 2 and more was considered positive. If a different evaluation of expression by the two pathologists was made, the slides were reevaluated until the assessment coincided.

Therapy and survival evaluation

The effect of therapy was determined according to Response Evaluation Criteria in Solid Tumors (RECIST; Therasse et al., 2000) as (1) *Complete response (CR)*—the disappearance of all target lesions, (2) *Partial response (PR)*—at least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD. To be assigned a status of CR or PR, changes in tumor measurements were confirmed by repeated assessments that were performed no less than 4 weeks after the criteria for response are first met. CR and PR was considered a favourable therapeutic response. (3) *Stable disease (SD)*—neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started. In the case of SD, follow-up measurements were performed at least once after study entry at a minimum interval no less than 6–8 weeks. (4) *Progression of disease (PD)*—at least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions or reccurence of the previously vanished. Patients in response categories 4–9 were considered as failing to respond to treatment (disease progression).

The major end point of this study was to compare 3-year survival in patients with NSCLC who have positive NE expression and NE marker negative NSCLC patients. Patient survival was calculated as the time between diagnostic biopsy and death or the date of the most recent contact for censored cases. Patients who were still alive at the time of data collection were censored in the statistical analysis. Survival of the investigated patients was followed up within 3-year period.

Statistical analysis

Pearson χ^2 test was used to check the independence of categorical variables. The influence of variables on therapeutic response was examined by multivariate binary logistic regression. Odds ratio was also calculated. Receiver operating characteristic (ROC) curves were constructed and the areas under the ROC curves (AUROC) were calculated for the markers. Cutoff point was determined as a value which gave the maximal score when sensitivity was multiplied by specificity. Kaplan–Meier curves were constructed and the differences in 3-year survival between patient groups were tested by the log-rank test. The influence of variables on survival was investigated by multivariate Cox's regression analysis. Hazard ratio was also calculated.

Results

The characteristics of the 116 patients are shown in Table 1. The investigated group included 92 males and 24 females, with the median age being 63 years (range 39–76 years). Sixty-two patients (53.4%) received chemotherapy plus radiation therapy, whereas 54 patients (46.6%) received chemotherapy only. The examined patients comprise 82 patients (70.7%) in Stage III and 34 patients (29.3%) in Stage IV, with squamous cell carcinoma being predominant, followed by adenocarcinoma, detected in 57 (49.1%) and 36 (31%) cases, respectively. No large cell NE carcinoma (LCNEC) were observed in this study (Table 2).

The frequency of NE markers expression (ID score of two and more) is reported in Table 3. Overall, NE differentiation was found in 29 of 116 ordinary NSCLC tumors (25%, referred to here as the NSCLC-ND group). In respect to all examined patients, NSE was identified in 26 (22.4%), ChrA in 18 (15.5%) and Syn in 16 patients (13.8%) with conventional nonsmall cell morphology tumors. Simultaneous expression of two or more markers of NE differentiation was present in 25 patients (21.5%).

Table 1. Characteristics of patients.

Characteristics of patients	Number of patients
Total	116
Average age	62.56 ± 10.92
Age rank	39–76
Age (years)	
< 60	52 (44.8%)
> 60	64 (55.2%)
Sex	
Males	92 (79.3%)
Females	24 (20.7%)
ECOG PS	
0	32 (27.6%)
1	39 (33.6%)
2	45 (38.8%)
Treatment	
Chemotherapy + Radiation therapy	62 (53.4%)
Chemotherapy	54 (46.6%)

ECOG PS, Eastern Cooperative Oncology Group scale of performance status.

The analysis of therapeutic response in relation to NE marker expression showed statistically significant difference in therapeutic response between NSCLC-ND group and those without NE marker expression. Therapeutic response was significantly more favourable (CR + PR) in the NSCLC-ND group (Table 3). Moreover, similar results regarding therapeutic response were obtained when the data were reanalyzed using more strict criterion for NE differentiation, with only the neoplasms that are positive for two markers being considered as having NE features. Out of 116 patients, 25 cases who had two or more positive NE markers included 17 (68.0%) patients with favorable therapeutic response (partial or complete), whereas the remaining 91 cases included 34 patients (37.4%) who responded to therapy (P = 0.042; Table 3).

As there is no gold standard in defining NE differentiation and overall labeling indexes, as shown by different proportion of NE positive NSCLC described in literature, we tried to find if percentage of NE positive tumor cells, solely, regardless of staining intensity, is better predictor of therapeutic response. High positive association was noted between therapeutic response and percentage of NE positive tumor cells (r = 0.623; P < 0.0001; Figure 1A). The percentage of positive tumor cells was considerably higher in the group of patients with partial or complete response (P < 0.0001). In group of patients who had complete therapeutic response, the percentage of positive tumor cells was almost 50%, in patients with partial therapeutic response, the percentage of positive tumor cells was higher than 30%, whereas in the group with progression or stable disease, this percentage was significantly lower (Figure 1B).

According to observed association between percentage of NE positive tumor cells and favorable therapeutic response, we also examined influence of some established prognostic factors and NE expression on therapeutic response in patients with NSCLC. Namely, binary logistic regression, that included different clinicopathologic variables (patient age, histological type of tumor, degree of differentiation, tumor stage, performance status, NE differentiation, etc.), showed that NE differentiation, as percentage of NE positive tumor cells, stage of the disease and smoking history were independent predictors of therapeutic response. The NE differentiation was predictor of favorable therapeutic response (P = 0.027, odds ratio = 1.080, CI = 1.009–1.115), whereas stage of disease and smoking history were negatively correlated with therapeutic response (P = 0.013, odds ratio = 0.625, CI = 0.432–0.906; P = 0.001, odds ratio = 0.404, CI = 0.193–0.883, respectively; Table 4).

As we found, the percentage of NE positive tumor cells could be useful prediction factor of therapeutic response. ROC curves and AUROC curves confirm this fact and sensitivity and specificity for individual NE markers were determined. For NSE, optimum cutoff is 29.5% of positive tumor cells (AUROC = 0.984; P < 0.0001) and this value provides sensitivity of 94% and specificity of 100%, meaning that tumors with more than 29.5% of positive tumor cells have favorable therapeutic response. Optimum cutoff for ChrA is 14.5% (AUROC = 0.732; P = 0.015) with sensitivity being 100% and specifity of 50%. For Syn, optimum cutoff is 25.0% (AUROC = 0.833; P = 0.030) with 80% of test sensitivity and 83% of test specifity (Figure 2).

Finally, we compared 3-year survival between NSCLC-ND group and NE marker negative NSCLC patients. Statistically significant difference (P < 0.0001) was found in 3-year survival of NSCLC patients in relation to the presence of NE expression. Median of survival time for the NSCLC-ND patients, shown as Kaplan-Meier curves in Figure 3, was 15 (range 4–31) months, in comparison to 8 months (range 3–19) for the patients with NE negative NSCLC. Moreover, the prognostic value of NE differentiation was confirmed by multivariate Cox's regression analysis showing that NE differentiation, as percentage of NE positive tumor cells, and stage of disease were independent predictors of overall survival. The NE differentiation was predictor of improved survival (P = 0.047, hazard ratio = 0.974, CI = 0.958–1.000), whereas stage of disease was negatively correlated with survival in NSCLC (P = 0.001, hazard ratio = 1.351, CI = 1.130–1.615; Table 5). Survival analysis showed that NE differentiation is strongly

Table 2. Pathological and histological data.

	N (%)
Stage of disease	
IIIA	24 (20.7%)
IIIB without effusion	38 (32.8%)
IIIB with effusion	20 (17.2%)
IV	34 (29.3%)
Histological form	
Squamous cell	57 (49.1%)
Adenocarcinoma	36 (31.0%)
Adenosquamous	14 (12.1%)
Large-cell cancer	9 (7.8%)

N, number of patients.

Table 3. Marker expression and response to chemotherapy.

	Marker expression	Response to chemotherapy with marker expression		
NE markers	Positive (%)	Positive (%)	Negative (%)	P value
NSE	26/116 (22.4%)	16/26 (61.5%)	21/90 (23.3%)	0.009
SYN	16/116 (13.8%)	10/16 (62.5%)	27/100 (27.0%)	0.037
ChrA	18/116 (15.5%)	14/18 (77.7%)	23/98 (23.5%)	0.004
≥2 markers	25/116 (21.5%)	17/25 (68.0%)	34/91 (37.4%)	0.042

NE, neuroendocrine; NSE, neuron-specific enolase; SYN, synaptophysin; ChrA, chromogranin A.

Table 4. Predictors of therapeutic response (multivariate binary logistic regression).

Parameter	*P* value	Odds ratio
Gender	0.146	2.047 (0.779–5.384)
Age	0.390	1.025 (0.968–1.086)
Hystological type of tumor	0.275	1.344 (0.790–2.286)
Degree of differentiation	0.623	0.869 (0.497–1.519)
NSE exspression	0.593	1.299 (0.498–3.390)
ChrA expression	0.381	0.625 (0.219–1.789)
SYN expression	0.807	0.906 (0.412–1.994)
Stage of disease	0.013	0.625 (0.432–0.906)
ECOG Performance status	0.228	1.042 (0.975–1.113)
LDH	0.187	0.999 (0.997–1.001)
Therapeutic treatment (HT ± RT)	0.126	0.455 (0.166–1.248)
Percentage of positive tumor cells	0.027	1.080 (1.009–1.155)
Smoking	0.001	0.404 (0.193–0.883)
Constant	0.401	0.058

ChrA, chromogranin A; ECOG, Eastern Cooperative Oncology Group; NSE, neuron-specific enolase; SYN, synaptophysin.

associated with improved survival in patients with advanced stage NSCLC, meaning that NE negative NSCLC have a more aggressive clinical course.

Discussion

Lung tumours are classified into two major histological subtypes that have different morphological, biological and clinical properties: (i) NE carcinomas, such as carcinoids, SCLC or LCNEC and (ii) NSCLC, which form a group of histologically heterogeneous and clinically variable tumours, including squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma and large cell carcinoma (WHO, 1999).

The biological differences between SCLC and NSCLC are well-known. Although NSCLC have been considered chemo-resistant so far, the discovery of NE differentiation in these tumors brings them closer to SCLC, on the theoretical grounds that NSCLC tumours showing NE differentiation may be associated with an adverse prognosis and greater chemosensitivity.

Over the last two decades, a number of studies have been undertaken to evaluate the therapeutic and prognostic significance of the NE differentiation in NSCLCs. However, the data are conflicting. A fundamental problem in the study of NSCLC-ND is the definition of NE differentiation (Carnaghi et al., 2001). The original definition requires the ultrastructural demonstration of membrane-bound dense core granules (Carey and Save, 1997). However, very few studies have employed this technique and, instead, majority have used immunohistochemistry. Large scope and diversity of positive findings are usually interpreted by technical and conceptual problems: different techniques and different markers are

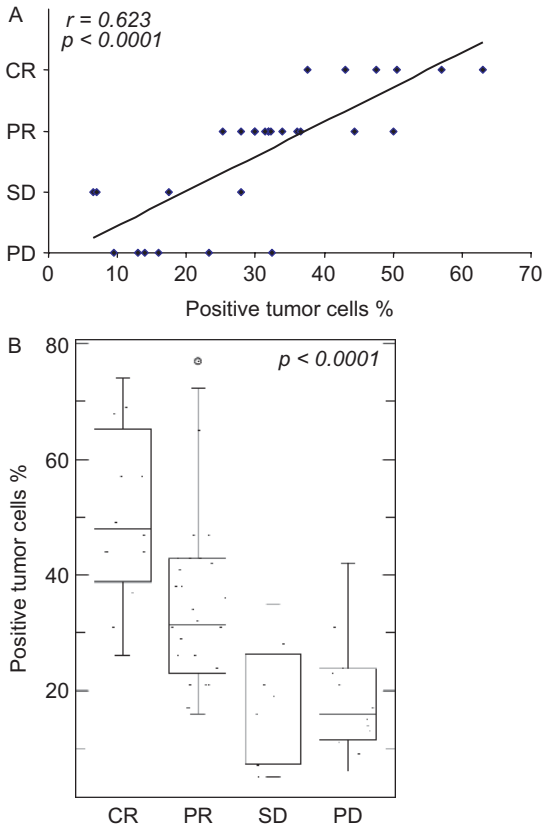

Figure 1. Percentage of neuroendocrine (NE) marker positive tumor cell in patients with different therapeutic response. Percentage of NE positive tumor cells were determined using immunohistochemical analysis. The ID score of 2 and more for any marker was used as a criterion for positive NE differentiation. (A) Data are individual percentages of positive tumor cells in respect to therapeutic response for each of NE positive NSCLC patient. Percentages of positive tumor cells showed high positive correlation with therapeutic response (Spearmen coefficients of correlation, $r = 0.623$ and $P < 0.0001$). (B) Data are presented as boxes with medians and interquartile range, and whiskers with minimum and maximum values. Statistical analysis showed a significant difference between groups ($P < 0.001$). CR, complete response, PR, partial response; SD, stable disease; PD, progression of disease.

utilized in the determination of NE differentiation, there is no gold standard in defining NE differentiation and, finally, different criteria are utilized for study population selection. The most important observation was that the insufficient correlation between the expression of the different NE markers and the percentage of tumours expressing NE features greatly depends on the marker used, suggesting that the expression of an individual NE marker is not an absolute criterion for identifying NE differentiation (Carnaghi et al., 2001). Therefore, some authors propose different cutoff points for percentage of stained neoplastic cells, > 1% (Ionescu et al., 2007); > 5% (Sundaresan et al., 1991); > 10% (Linnoila et al., 1994; Travis et al., 1999; González-Aragoneses et al., 2007); > 50% (Berendsen et al., 1989), whereas others have required diverse ID scores, ID > 1 (Howe et al., 2005), ID >

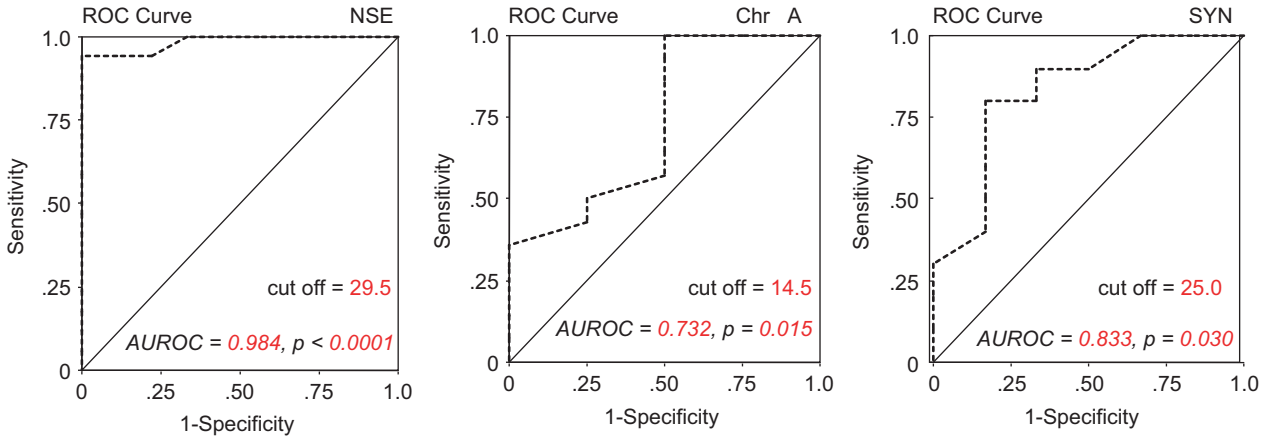

Figure 2. Cutoff points, sensitivity and specificity of NE markers. Using immunohistochemical analysis, the percentage of positive tumor cells were determined for each marker. Cutoff points as well as sensitivity and specificity for each marker were presented in the form of ROC curves for each tumor marker.

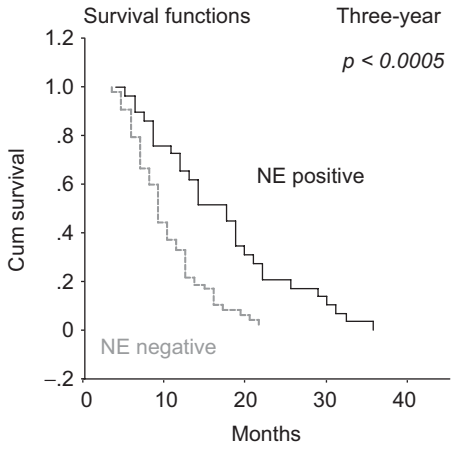

Figure 3. Three-year survival curve for patients with Stages III and IV in relation to NE marker expression. Survival of the NSCLC patients was followed up within 3-year period. Data are presented as 3-year survival Kaplan-Meier curves for NE markers postive and negative patients. Statistical analysis showed a significant difference in 3-year ($P<0.001$) survival in NE markers postive versus negative patients.

2 (Graziano et al., 2001), for any individual antibody used as the criterion for evidence of NE differentiation.

It has been proved that a broad spectrum of immunohistochemical markers can highlight NE differentiation in lung tumors. Among the most widely used NE markers, ChrA (Gosney et al., 1995) and Syn (Leung et al., 1999) still remain the most suitable markers due to their close correlation with ultrastructural evidence of NE differentiation (Travis et al., 1999, 2009; Corrin, 2000). Contrarily, a number of authors find NSE, which was used in earlier studies, insufficiently specific (Carnaghi et al., 2001; Howe et al., 2005). However, a growing body of evidence, suggesting that serum level of NSE have significant predictive and prognostic role in NSCLC (Maeda et al., 2000; Jacot et al., 2001; Pujol et al., 2001; Satoh et al., 2002; Ferrigno et al., 2003; Ishikawa et al., 2004; Tiseo et al., 2008) compel us to reexamine values of NSE as marker of NE differentiation in NSCLC.

In our study, NE differentiation (defined as an ID score of two and more) was found in 25% of the NSCLC. As might be expected for staining of NSCLC, the more specific NE markers, ChrA and Syn, were positive in fewer cases (15.5% and 13.8%, respectively), similar to that seen in previous studies (Hage et al., 1998; Petersen, 1999; Harada et al., 2002; Pelosi et al., 2003). However, contradicting the vast majority of previous studies using NSE, where higher percentage of NSE positive tumors was identified (Graziano et al., 1989, 1994, 1998, 2001; Carles et al., 1993; Linnoila et al., 1994; Sundaresan et al., 1991; Abbona et al., 1998; Gajra et al., 2002), we found that only 22.4% of the NSCLC was NSE positive, comparable to studies where the antibody panel utilized is restricted to the more specific NE markers and positivity is typically within the range 10–30% (Sundaresan et al., 1991; Kwa et al., 1996b; Schleusener et al., 1996; Hage et al., 1998; Travis et al., 1999; Howe et al., 2005; Ionescu et al., 2007; Segawa et al., 2009). The discrepancy between our results and literature data could be simply explained, as majority of previous studies utilized polyclonal NSE antibodies (Sundaresan et al., 1991; Graziano et al., 1994; Linnoila et al., 1994; Graziano et al., 1998; Graziano et al., 2001; Gajra et al., 2002), whereas we used monoclonal NSE antibodies (clon BBS/NC/VI14). Simultaneous expression of two or more markers was found in 21.5% cases and this finding is consistent with the majority of earlier studies (Graziano et al., 1989, 1994; Sundaresan et al., 1991; Abbona et al., 1998; Gajra et al., 2002), although some authors found lesser extent of simultaneous expression of NE markers (Travis et al., 1999; Graziano et al., 2001).

In clinical oncology, proper assessment of subgroup of patients who are expected to have good therapeutic response has important implications. In the present study, we found that favorable therapeutic response (PR + CR) significantly correlates with higher percentage of positive tumor cells (30–50%) in comparison to the

Table 5. Predictors of survival (multivariate Cox's regression).

Parameter	*P*	Hazard ratio
Gender	0.863	0.959 (0.599–1.536)
Age	0.864	0.998 (0.974–1.023)
Hystological type of tumor	0.754	1.037 (0.827–1.229)
Degree of differentiation	0.154	1.208 (0.932–1.565)
NSE expression	0.052	0.699 (0.487–1.003)
ChrA expression	0.876	0.969 (0.655–1.433)
SYN expression	0.872	0.974 (0.710–1.336)
Stage of disease	0.001	1.351 (1.130–1.615)
ECOG Performance status	0.104	0.974 (0.943–1.005)
LDH	0.198	1.001 (1.000–1.001)
Therapeutic treatment (HT ± RT)	0.064	1.600 (0.972–2.633)
Percentage of positive tumor cells	0.047	0.979 (0.958–1.000)

ChrA, chromogranin A; ECOG, Eastern Cooperative Oncology Group; NSE, neuron-specific enolase; SYN, synaptophysin.

group of patients with poor therapeutic response (PD + SD) where the number of positive tumor cells is considerably smaller (< 20%). Analyzing parameters that may impact therapeutic response, multivariate binary logistic regression showed that NE differentiation, as percentage of NE positive tumor cells, was predictor of favorable therapeutic response. ROC curve method confirmed that percentage of NE positive tumor cells could be a useful prediction factor of therapeutic response and obtained cutoffs gave high sensitivities and specificities. To the authors' knowledge, these findings show for the first time that usage of novel-established cutoffs for quantitative determination of NE marker expression in NSCLC specifically identified patients with enhanced probability of successful treatment. Finally, analyzing 3-year survival of NSCLC patients, we found that NE differentiation provides a survival advantage, meaning that NSCLC-ND cases have a less aggressive clinical course.

The effect of NE differentiation on therapeutic response and survival has been widely investigated. However, previous studies have offered variable and contradictory results: no association with survival and chemotherapy response, (Graziano et al., 1998; Gajra et al., 2002; Howe et al., 2005), no association with survival (Sundaresan et al., 1991; Graziano et al., 1994; Linnoila et al., 1994; Kwa et al., 1996a, 1996b; Abbona et al., 1998; Hage et al., 1998; Howe et al., 2005; Ionescu et al., 2007; Segawa et al., 2009), decreased survival (Berendsen et al., 1989; Kibbelaar et al., 1991; Travis et al., 1999; Pelosi et al., 2003; González-Aragoneses et al., 2007), no association with survival but increased response to chemotherapy, (Skov et al., 1991; Graziano et al., 2001), improved survival but no association with response to chemotherapy (Carles et al., 1993; Schleusener et al., 1996), improved survival and increased response to chemotherapy (Graziano et al., 1989).

The existing confusion regarding prognostic significance of the presence of NE differentiation, beside technical problems (different techniques and different markers, lack of gold standard and different criteria for defining NE differentiation), can also be attributed to conceptual problems, mainly to the inclusion criteria for study population, for example, stage of the disease. Our literature review (Table 6) showed that majority of similar studies included NSCLC cases in early stages, whereas there are few studies with advanced-stage NSCLC tumors. Taken together, these studies represent a collection of 4707 NSCLCs, of which 1130 (26%) demonstrated NE differentiation with variable clinical and prognostic significance. In early-stage tumors (3849 cases), there are series that have shown either no predictive/prognostic significance (Sundaresan et al., 1991; Graziano et al., 1994; Linnoila et al., 1994; Kwa et al., 1996a, 1996b; Abbona et al., 1998; Hage et al., 1998; Howe et al., 2005; Ionescu et al., 2007) or shorter survival in NSCLC-ND group of patients (Berendsen et al., 1989; Kibbelaar et al., 1991; Travis et al., 1999; Pelosi et al., 2003; González-Aragoneses et al., 2007). In the studies that included more advanced NSCLCs (858 cases), responsiveness to chemotherapy (Graziano et al., 1989, 2001; Skov et al., 1991). and association with better prognosis (Graziano et al., 1989, 2001; Carles et al., 1993; Schleusener et al., 1996; Gajra et al., 2002; Segawa et al., 2009) were noted. Few authors, however, have failed to show any correlation between NE differentiation and prognosis or susceptibility to the therapy (Graziano et al., 1998; Howe et al., 2005). Our results, in advanced NSCLCs, compare well with those reported previously and supports the argument that the clinical behavior of NSCLC-ND tumors is different in early-stage versus advanced-stage NSCLC.

NSCLC in advanced stages that show NE differentiation likely represents a clinically distinct subset of lung tumors characterized by a less aggressive clinical course and better prognosis compared with NSCLC-ND in early stages. Therefore, we believe that determination of NE differentiation in advanced NSCLC is effective and biologically rational for separating NSCLC-ND tumors into groups with different biologic and clinical properties, more akin to SCLC with regard to responsiveness to chemotherapy and carcinoids in respect to survival characteristics.

Large-scale and variety of different findings indisputably point out to the necessity for caution when interpreting the various studies that have questioned the clinical and prognostic impact of NE features in NSCLC. Obviously, clinical significance of NE differentiation remains a frustrating problem, still waiting for resolution.

Conclusion

Our article strongly suggests that advanced NSCLC with *positive expression* of NE markers have *increased*

Table 6. Summary of studies examining the clinical significance of neuroendocrine differentiation in nonsmall cell lung carcinoma.

				Percent of positive								
Author	Stage	Treatment	No.	NSE	ChrA	Syn	NCAM	NED	> 2	Criteria	CTR	Survival
Early-stage studies												
González, 2007	I	S	318			27		27		> 10%		▼
Pelosi, 2003	I	S	220		8	13		13		at least 1		▼
Hiroshima, 2002	I–II	S	90		2	5	4	8	3	> 10%		▼
Kibbelaar, 1991	I–III	S	231				19	19				▼
Berendsen, 1989	I–III	S	141				31	31		> 50%		▼
Ionescu, 2007	NA	S	588		0.4	8	9	14	0.2	> 1%		—
Kwa, 1996b	I–III	S	39		8	28	23			> 10%		—
Graziano, 1994	I–II	S	260	70	14	11			24	ID+ > 2		—
Linnoila, 1994	I–II	S	237	46	2				12	> 10%		—
Sundaresan, 1991	I–III	S	359	51	4	23		56	30	> 5%		—
Howe, 2005	I–III	S	341		6	17	28	36		ID > 1		—
Hage, 1998	I–III	S	889				14	14		Distinct		—
Kwa, 1996a	I–III	S	96				11	11		Distinct		—
Abbona, 1998	I–IV	S	40	53	13	10			15			—
Advanced-stage studies												
Howe, 2005	III–IV	CT	98		1	18	29	36		ID > 1	—	—
Gajra, 2002	III–IV	S + CT	90	47	1	17			16	Intensity > 2	—	▲[1]
Graziano, 2001	III	CT	132	38	0	5			3	ID+ > 2	▲[2]	▲[3]
Graziano, 1998	III	CT + S	38	47	3	8			11		—	—
Segawa, 2009	I–IV	CT + S	130		4	7	9	16		at least 1		▲[4]
Schleusener, 1996	III–IV	CT	107		5	24		35	11		—	▲
Carles, 1993	II–IV	CT	97	46	2	23			12		—	▲
Skov, 1991	III–IV	CT	114	16	19					> 10%	▲	—
Graziano, 1989	III–IV	CT	52	40	6				19		▲	▲

[1]Normal expression of p53 along with NED favorable factor for survival ($P = 0.05$); [2]Six of 6 (100%) synaptophysin positive tumors responded to therapy ($P = 0.04$); [3]NSE was marginally significant in multivariate analysis ($P = 0.08$); [4]Overall survival favorable but differences not signifficant.

ChrA, chromogranin A; CT, chemotherapy; CTR, chemotherapy response; ID, intensity-distribution score; NCAM, neural cell adhesion molecule; NED, neuroendocrine differentiation; NSE, neuron-specific enolase; S, surgery; Syn, synaptophysin; ▲, positive CTR or better survival; ▼, worse survival; —, no correlation.

chemosensitivity and *better survival* in comparison to NSCLC without NE expression. We have also demonstrated an interesting *novel* observation regarding the potential use of *percentage of NE positive tumor cells* as a tool for *identification* of patients with insufficient/sufficient *therapy response* to *paclitaxel-cisplatin*.

Declaration of interest

The authors declared no conflict of interest.

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