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Eur Respir J
1989, 2, 746–750

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ABSTRACT: Pretreatment serum neuron specific enolase (NSE) and plasma bombesin/gastrin releasing peptide (BN/GRP) were measured in 92 lung cancer patients and 17 controls. The mean level of NSE ($p<0.001$) and BN/GRP ($p<0.05$) was significantly raised in patients with small cell lung cancer (SCLC, n=62) compared to non-SCLC (n=30) and controls. The mean concentration of NSE in extensive stage SCLC was significantly greater ($p<0.005$) than in limited stage but with a substantial overlap of values. Fortyseven out of 62 SCLC patients had at least one of the two markers raised (sensitivity 76%, specificity 83%), 44 had raised NSE (sensitivity 71%, specificity 89%) but only 24 had BN/GRP raised (sensitivity 42%, specificity 91%). At restaging, 16 of 19 patients with SCLC responsive to chemotherapy showed a significant fall of NSE; on the other hand, BN/GRP fell significantly in only 3 patients, remaining unchanged in the majority of responding patients. In conclusion, the combined determination of NSE and BN/GRP in SCLC, at diagnosis and during the follow-up, was not found to be superior to NSE determination alone.

Eur Respir J., 1989, 2, 746–750

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Keywords: Biological markers; bombesin; gastrin releasing peptide; lung cancer; neuron specific enolase; small cell lung cancer.

Received: April, 1988; accepted after revision April 15, 1989.

In recent years neuron specific enolase (NSE) and bombesin/gastrin releasing peptide (BN/GRP) have been identified as biological markers of small cell lung cancer (SCLC) cell lines [1, 2]. The adult human lung contains immunoreactive NSE and BN/GRP only in small amounts, localized both in neuroendocrine cells and neuroepithelial bodies [3, 4].

NSE is the neuronal form of the glycolytic enzyme enolase (E.C. 4.2.1.11) which is considered to be a marker of APUD (amine precursor uptake and decarboxylation) cells [5]. SCLC and the more benign pulmonary carcinoid are presumed to arise from normal APUD cells in the respiratory tract [6]. In SCLC patients serum NSE levels correlate positively with tumour extent and response to chemotherapy [7, 8].

The biological activity of BN, a tetradecapeptide discovered in amphibian skin, is related to the C-terminal decapeptide sequence which differs only in one residue from the corresponding sequence of GRP (GRP-10), the mammalian equivalent of BN [9]. Furthermore, BN and GRP-10 act as growth factors for normal bronchial epithelial cells [10] and as autocrine growth factors for SCLC [11, 12].

The purpose of the present experimental study was:

a) to evaluate the pretreatment pattern of distribution of serum NSE and plasma BN/GRP in lung cancer (SCLC and non-SCLC) and controls;

b) to determine whether the combination of these two biochemical markers would improve the diagnostic accuracy;

c) to assess the change of NSE and BN/GRP levels in the patients with SCLC responsive to induction chemotherapy.

Patients and methods

Venous blood collected from 92 lung cancer patients, 62 SCLC and 30 non-SCLC, histologically or cytologically proven, was retrospectively analysed for NSE and BN/GRP pretreatment levels. Seventeen blood donors were chosen as normal controls. All lung cancer patients were staged initially with radionuclide bone scan, upper abdomen ultrasonography and computed axial tomography of the chest. SCLC patients were also routinely examined by computed axial tomography of the brain and unilateral bone marrow biopsy. Limited disease was defined as tumour confined to one hemithorax including ipsilateral, mediastinal and supraclavicular nodes; patients with disease elsewhere, including those with pleural effusion, were classified as having extensive disease.

For BN/GRP determination blood was collected in glass tubes with ethylenediaminetetra-acetic acid (EDTA) as anticoagulant and aprotinine was immediately added to

the collection tube to prevent degradation of the peptides in the specimen. Blood samples for NSE and BN/GRP determination were immediately centrifuged, divided into multiple aliquots and stored at -20°C until assayed. In our laboratory plasma BN/GRP and serum NSE concentrations were measured by a radioimmunoassay technique using, respectively, the NSE-RIA kit (Pharmacia, Uppsala, Sweden) and bombesin I-125 RIA kit (Immunonuclear, Minnesota, USA) with a second antibody suspension. The antibody to BN shows a 24% cross-reactivity with porcine GRP [13].

Mean +2 SD of the analyte in healthy subjects was considered as the upper limit of reference for the classification of results as normal or raised.

In 27 of the 62 patients with SCLC, serum NSE and plasma BN/GRP were measured again at restaging after induction chemotherapy (cyclophosphamide, 4-epidoxorubicin, vincristine alternated with high dose cisplatin and etoposide). The response status was coded according to standard criteria [14]: for complete response all known sites of disease must have disappeared for more than one month; for partial response there must have been >50% decrease in volume of all sites of disease for more than one month. When there was an increase in size >25% of measurable or evaluable lesions, the patient was classified as having progressive disease.

In the follow-up data a significant decrease in the level of NSE and BN/GRP was defined as a fall >25% of pretreatment value.

Statistical analysis of the results was performed by the Wilcoxon rank sum test for the comparison of NSE and BN/GRP levels among the groups and between limited and extensive stage. The Chi-squared and McNemar's test were used to examine the association between the disease status and raised level of markers. Discriminant analysis and other classification analysis were not used because of the relatively small sample size of patients considered in this study. Pearson's correlation was used for the correlation between markers.

Table 1. – Major clinical characteristics of patients

	SCLC	non-SCLC
Total	62	30
Sex ratio (male:female)	56:6	23:7
Age: median yrs	59	62
range	38–72	36–78
Extent of disease		
limited	32	19
extensive	30	11
Histology		
oat cell	43	
intermediate cell	19	
squamous cell		15
adenocarcinoma		10
large cell		5
Performance status (Karnofsky)		
100/80	49	24
70/50	13	6

SCLC: small cell lung cancer.

Results

Table 1 outlines the major clinical characteristics of lung cancer patients. All patients had paired samples of serum NSE and plasma BN/GRP measured at the time of clinical diagnosis of lung cancer. Tables 2 and 3 show the results obtained and proportion of raised values for serum NSE and plasma BN/GRP, respectively, in controls and lung cancer patients.

The mean serum NSE and plasma BN/GRP concentrations in SCLC were significantly higher ($p<0.001$ and $p<0.05$, respectively) than the mean values in controls or in patients with non-SCLC. Patients with SCLC with extensive disease stage had only a significantly greater mean serum NSE ($p<0.005$) than patients with limited disease stage (tables 2 and 3).

The relationship of the level of pretreatment markers to clinical stage of SCLC was analysed within four quantitative categories (table 4). Substantial overlap exists, especially for BN/GRP, but only 3 out of 32 with limited disease stage had NSE level >50 $ng \cdot ml^{-1}$ compared to 15 out of 30 (50%) of patients with extensive disease stage. At the other end of the spectrum 29 of 32 patients with limited disease had NSE level <50 $ng \cdot ml^{-1}$, while 9 of 30 with extensive disease had a similar quantitative level.

In 62 patients with SCLC the initial value of serum NSE and plasma BN/GRP were slightly correlated ($r=0.53$).

Table 2. – Serum neuron specific enolase at presentation

Group	Mean $ng \cdot ml^{-1}$	SEM	Range	Raised values (%)	Chi-squared
A) Controls	8.1	0.5	4.8–12.9	1/17 (6)	
B) Non-SCLC	8.4	0.9	1.0–28.4	4/30 (13)	
C) SCLC	47.6	7.3	5.1–200	44/62 (71)	p<0.001
1) limited	28.4	5.2	5.1–85.4	18/32 (56)	
2) extensive	68.3	6.5	6.9–200	26/30 (87)	p<0.01

A and B *vs* C: $p<0.001$ (Wilcoxon rank sum test); C1 *vs* C2: $p<0.005$ (Wilcoxon rank sum test); raised values were considered to be those >12 $ng \cdot ml^{-1}$; SCLC: small cell lung cancer.

Table 3. – Plasma bombesin/gastrin releasing peptide at presentation

Group	Mean pg·ml^{-1}	SEM	Range	Raised values (%)	Chi-squared
A) Controls	124	5.6	92–176	1/17 (6)	
B) Non-SCLC	120	6.6	75–197	3/30 (10)	
C) SCLC	176	9.1	84–308	26/62 (42)	p<0.001
1) limited	154	9.5	106–280	11/32 (34)	
2) extensive	183	10.8	84–308	15/30 (50)	p=0.32

A and B *vs* C: p<0.05 (Wilcoxon rank sum test); raised values were considered to be those >170 pg·ml^{-1}; SCLC: small cell lung cancer.

Table 4. – Distribution of NSE and BN/GRP in relationship to stage of disease in SCLC

	Quantitative level			
NSE ng·ml^{-1}	0–12	>12–50	>50–85.4	>85.4
Limited	15	14	3	0
Extensive	3	6	15	6
BN/GRP pg·ml^{-1}	0–170	>170–235	>235–280	>280
Limited	21	8	3	0
Extensive	15	7	5	3

NSE: neuron specific enolase; BN/GRP: bombesin/gastrin releasing peptide; SCLC: small cell lung cancer.

Table 5. – Classification of disease status in 62 patients with SCLC using single or combined determination of NSE and BN/GRP

		BN/GRP*		Both markers raised**		At least one marker raised***	
		C	I	C	I	C	I
NSE	C	23	21	23	21	44	0
	I	3	15	0	18	3	15

C: correct; I: incorrect; *, **, ***: McNemar test, p<0.0002, p<0.0001, p=0.083, respectively; for other abbreviations see legend to table 4.

Table 6. – Diagnostic value of NSE, BN/GRP and their combination in small cell lung cancer

	Sensitivity	Specificity	Diagnostic accuracy
NSE	71% (60–82)	89% (80–98)	79% (71–87)
BN/GRP	42% (40–54)	91% (83–99)	63% (54–72)
At least one marker raised	76% (25–49)	98% (94–100)	63% (54–72)
Both markers raised	37% (65–87)	98% (72–94)	63% (71–87)

The values in parentheses are the approximate 95% confidence limits; for abbreviations see legend to table 4.

At presentation, 76% of patients with SCLC had at least one marker raised compared to 20% of patients with non-SCLC. Only 3 of the 62 patients with SCLC had raised plasma BN/GRP associated with normal serum NSE level, compared to 44 patients with raised NSE level at presentation in 23 of whom there was also a raised BN/GRP level.

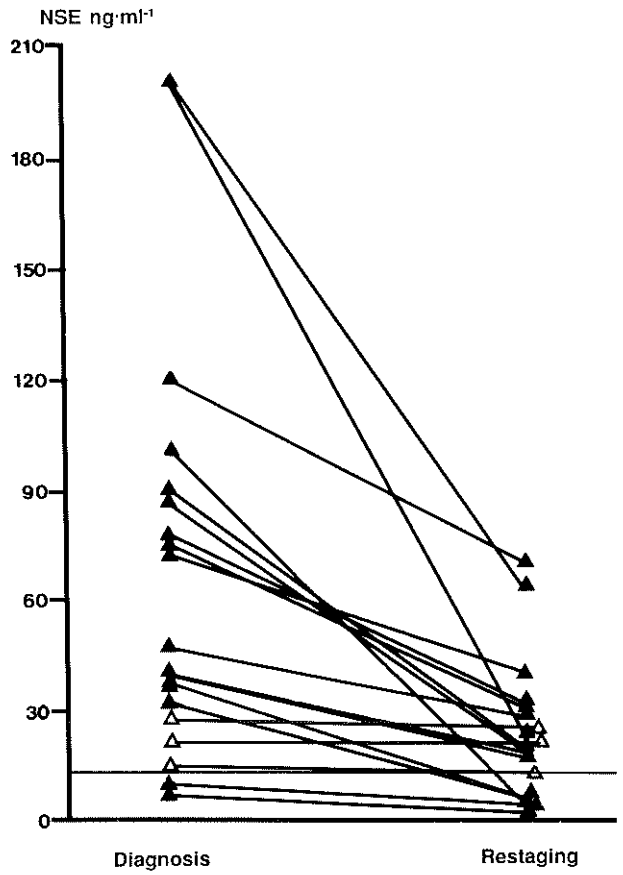

Fig. 1. – Serial neuron specific enolase (NSE) determination in 19 small cell lung cancer patients responsive to induction chemotherapy (s: significant decrease; Δ: no significant decrease). Continuous horizontal line represents the upper limit of normal range (mean±2SD).

Since NSE showed the greatest accuracy of classification it was paired with BN/GRP to determine whether a combination of these markers would improve the diagnostic accuracy (table 5). Unfortunately, the combination did not improve the accuracy of correctly classifying the patients with SCLC when we had also coded as "abnormal" the elevation of at least one of the two markers. Table 6 shows sensitivity, specificity and diagnostic accuracy of single and combined determination of NSE and BN/GRP in SCLC.

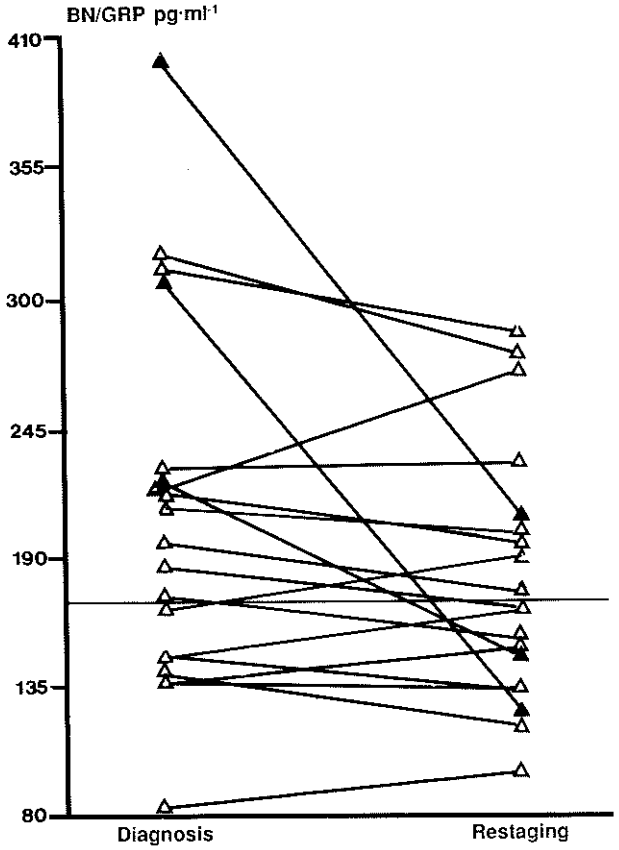

Fig. 2. – Serial bombesin/gastrin releasing peptide (BN/GRP) determination in 19 small cell lung cancer patients (s: significant decrease; Δ: no significant decrease). Continuous horizontal line represents the upper limit of normal range (mean±2SD).

At restaging, in 27 patients with SCLC re-evaluated for NSE and BN/GRP levels, the clinical response rate to induction chemotherapy was 70%. At presentation, 17 and 11 of these 19 responding patients showed a raised NSE or BN/GRP level, respectively. The change of serum NSE and plasma BN/GRP in 19 responders is shown in figures 1 and 2. At restaging, all patients who were clinically progressive showed a further increase of serum NSE while this pattern was observed for BN/GRP in only 2 out of 8 progressive patients.

Discussion

In SCLC many biological markers have been investigated and several hormonal peptides have been found to have elevated levels, and in particular calcitonin, antidiuretic and adrenocorticotrophic hormones have been studied extensively. The pretreatment frequency of elevated levels varies greatly from one clinical study to another; ranging from 24–30% (for adrenocorticotrophic hormone) to 25–75% (for calcitonin) with some correlations with disease stage and response to induction chemotherapy [15].

More recently, the expression of peptides (particularly BN, and BN-like peptides) and enzymes (L-dopa decarboxylase, NSE, isoenzyme BB of creatine kinase) in SCLC cell lines and, subsequently, in the serum of patients with SCLC were tested. BN and these enzymes are more selectively expressed, although with considerable heterogeneity, in SCLC cell lines than other peptide hormones, *i.e.* calcitonin, and are rarely found in non-SCLC cell lines.

In the last 4–5 yrs a substantial body of clinical experience of the measurement of serum NSE, at the initial diagnosis and during follow-up of SCLC, has built up with promising results [7, 8, 16, 17]. There is a general agreement about the existence of a positive correlation between the NSE level and the occurrence of SCLC and stage of SCLC. Serial determinations of serum NSE, during therapy for SCLC, has revealed a good correlation with the disease stage, declining after successful treatment and increasing before a relapse. Nevertheless, the level of NSE reached at remission does not predict the type of objective response obtained (complete or partial).

There are few reports available on plasma BN/GRP in SCLC. PERT and SCHUMACHER [18] noted three patients with extensive SCLC with a marked elevation of BN while six patients with limited disease had lower levels. By contrast, WOOD *et al.* [19] found no elevation of plasma BN in 31 patients with SCLC, ten of whom had extensive disease.

The results reported in our study show that the mean serum NSE and plasma BN/GRP levels were elevated in SCLC compared to both non-SCLC and controls. When we analysed the data obtained at diagnosis of SCLC, 44 of 62 patients had a raised serum NSE but only 23 of them had a concurrent elevation of plasma BN/GRP. On the other hand, 20% of non-SCLC patients had one or both markers raised and 24% of SCLC patients had both markers in the normal range. Consequently, our study has demonstrated that between two markers, NSE and BN/GRP, NSE is the marker with the best accuracy in correctly classifying patients according to disease status (SCLC or non-SCLC, limited or extensive disease) and that the combination of NSE and BN/GRP (both or at least one marker raised) does not enhance the accuracy of classification.

At restaging, the evaluation of 19 patients with SCLC responsive to induction chemotherapy revealed a significant fall of NSE in 14 of 17 patients with raised levels at presentation while, for BN/GRP, this pattern was

observed in only 3 of 11 responding patients with a raised level at presentation.

The different results reported for BN from cell lines and plasma from SCLC patients may be partially explained by the rapid degradation in plasma of secreted BN/GRP. Moreover, plasma might not be an ideal biological medium to measure the level of BN/GRP: in this way, considering also the pattern of secretion and the role of BN/GRP as an autocrine growth factor for SCLC, its measurement in the bronchoalveolar lavage fluid, obtained from the corresponding lobar bronchus, might be a more suitable procedure. The immunohistochemical demonstration of positive NSE and BN/GRP cells in the initial biopsy specimen of the primary tumour may help to pick out that subgroup of SCLC patients in whom the monitoring of disease activity by appropriate biological markers may be more strictly indicated.

In conclusion, the addition of plasma BN/GRP levels to serum NSE in the initial evaluation and follow-up of SCLC, seldom gives more information than measuring NSE alone. However, in the clinical-diagnostic staging of a patient with suspected SCLC the use of serum NSE seldom provides information not otherwise obtainable by physical examination or standard routine procedures while more can be obtained in the follow-up of SCLC as previously reported [16].

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Mesure combinée de la neuron specific enolase et du peptide secrétant la bombesin/gastrin dans le cancer du poumon. G.V. Scagliotti, M. Piani, E. Gatti, F. Gozzelino, C. Albera, E. Pozzi.
RÉSUMÉ: Les taux sériques de neuron specific enolase (NSE) et les taux plasmatiques du peptide libérant bombesin/gastrin (BN/GRP) ont été mesurés dans 92 cas de cancer pulmonaire et chez 17 sujets-contrôle avant tout traitement. Le niveau moyen de NSE ($p<0.001$) et de BN/GRP ($p<0.05$) est élevé de manière significative chez les patients atteints de cancer pulmonaire à petites cellules (SCLC, n=62), par comparaison avec les cancers non micro-cellulaires (n=30) et avec les contrôles. La concentration moyenne de NSE dans les stades avancés de SCLC est significativement plus élevée que dans les stades limités ($p<0.005$) mais avec un chevauchement substantiel des valeurs observées. 47 des 62 cancers micro-cellulaires avaient une augmentation d'au moins 1 des 2 marquers (sensibilité 71%, spécificité 83%); 43 d'entre eux avaient une augmentation de NSE (sensibilité 71%, spécificité 89%); 24 seulement avaient une élévation de BN/GRP (sensibilité 42%, spécificité 91%). Lors des contrôles ultérieurs, 16 des 19 patients atteints d'un cancer micro-cellulaire répondant à la chimiothérapie avaint une chute significative de NSE; par contre BN/GRP n'a diminué que chez 3 patients et est resté inchangé chez la majorité des répondeurs. En conclusion, la détermination combinée de NSE et de BN/GRP dans le cancer micro-cellulaire, que ce soit au diagnostic ou pendant la période d'observation, n'a aucune supériorité par rapport à la détermination isolée de NSE.
Eur Respir J., 1989, 2, 746–750