Stability and covalent modification of P-glycoprotein in multidrug resistant KB cells. *Biochemistry* 1988, 27, 7607–7613.
19. Levine AJ. The p53 tumour supressor gene and product. In Levine AJ, ed. *Cancer Surveys* 12: *Tumour Supressor Genes, the Cell Cycle and Cancer*. New York, Cold Spring Harbour Press, 1992, 59–78.
20. Maltzman W, Czyzyk L. UV irradiation stimulates levels of p53 tumor antigen in nontransformed mouse cells. *Molec Cell Biol* 1984, 4, 1689–1694.
21. Kastan MB, Onyekwere O, Sidaransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res* 1991, 51, 6304–6311.
22. Oren M, Maltzman W, Levine AJ. Post-translational regulation of the 54k cellular tumour antigen in normal and transformed cells. *Molec Cell Biol* 1981, 1, 101–110.

Acknowledgements—We thank Victor Ling for helpful discussions regarding immunoprecipitation procedures and are grateful to Mary Wallace for her skilled secretarial assistance.

Eur J Cancer, Vol. 29A, No. 16, pp. 2248–2250, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00

Inability of Serum Neuron-specific Enolase to Predict Disease Extent in Small Cell Lung Cancer

Elisabeth Quoix, Anne Charloux, Elisabeth Popin and Gabrielle Pauli

Serum neuron-specific enolase (NSE) levels were measured before treatment in 112 patients diagnosed as having small cell lung cancer in our department. All these patients underwent exhaustive staging procedures: 53 had limited disease (LD) and 59 extensive disease (ED). Serum NSE was elevated in 83% of the patients (i.e. 71% of the patients with LD and 93% of the patients with ED). Mean values of NSE differed significantly according to disease extent. A receiver-operating characteristic curve was constructed with different cut-off levels of serum NSE in order to determine the accuracy of NSE for identifying ED. There was no level of NSE capable of predicting with sufficient accuracy the presence of ED. The best compromise was given by a threshold of 35 μg/l: 60% of the ED patients had a serum NSE above 35 μg/l but 30% of the LD patients also had a serum NSE above 35 μg/l.
Eur J Cancer, Vol. 29A, No. 16, pp. 2248–2250, 1993.

INTRODUCTION

PROGNOSIS IN small cell lung cancer (SCLC) with respect to response rate and survival is strongly related to the extent of disease [1].

The current two-category staging system (limited disease and extensive disease) requires complex initial staging procedures which are time consuming and unpleasant for these patients. Although some authors [2, 3] have found that the disease stage could be replaced by a more simple prognostic index based on laboratory parameters and performance status, Rawson and Peto [1] could not find a model omitting disease stage that could act as a useful substitute. Moreover, mediastinal irradiation combined with chemotherapy improves local control and overall survival outcome in limited stage disease [4]. Therefore, selection of appropriate therapy requires staging of the patients.

Serum neuron-specific enolase (NSE) is an established useful diagnostic marker for all tumours originating from neuroendocrine cells such as SCLC. Elevated serum levels of NSE have been found in SCLC [5–9] with a sensitivity between 65 and 79%. Specificity is high, since 82–86% of non-small cell lung cancer (NSCLC) have serum NSE levels within normal limits [5, 6].

Consistently higher levels of serum NSE have been found in patients with extensive disease (ED) compared to those with limited disease (LD) [5–10]. As could be expected, survival also correlated with the initial level of NSE [6, 11].

The aim of our study was to determine if the initial serum NSE level could predict the disease stage (LD versus ED) with sufficient accuracy.

PATIENTS AND METHODS

During a 4.5-year period (15 October 1987–15 April 1992), 157 patients were diagnosed as SCLC in our department. Among these, a pretherapeutic measurement of serum NSE was performed in 112 (100 males, 12 females). The average age was 58 years (range 37–84).

Initial staging procedures performed in all patients were physical examination, chest X-ray, computerised tomographic (CT) scan of the thorax, bronchoscopy, abdominal ultrasound and/or CT scan, radionuclide imaging study of the bones and/or unilateral bone marrow biopsy, and CT scan of the brain.

All patients were staged according to the standardised classification. LD stage was defined as a disease limited to one hemithorax, including the mediastinal nodes, ipsi- and/or contralateral supraclavicular nodes, and ipsilateral pleural effusion. ED stage was defined as disease beyond that described above. In addition to this classification, tumour size (T) and mediastinal node involvement (N) were assessed.

Determination of serum NSE was done by radioimmunoassay (Pharmacia, Uppsala, Sweden). According to the manufacturer, normal values are below 12.5 μg/l in 96% of the controls.

Serum samples were obtained prior to any treatment.

Correspondence to E. Quoix.
The authors are at the Pavillon Laennec, Hôpitaux Universitaires de Strasbourg, 1 place de l'Hôpital, 67091 Strasbourg, Cedex, France.
Revised 21 Apr. 1993; accepted 11 June 1993.

Statistics

To evaluate the ability of NSE to predict ED stage, indices of sensitivity (Se) and specificity (Sp) were calculated [12] for different levels of serum NSE. We also plotted a receiver-operating characteristic (ROC) curve [13]. For different cut-offs of NSE levels, the ROC curve shows the proportion of patients with a true-positive (TP) test (i.e. patients with ED and a serum NSE above the given level) as compared with the proportion with a false-positive (FP) test (i.e. patients with LD and a serum NSE above the given level).

If a test is of no use, then both proportions are roughly equal for all values and the ROC curve is a straight line with a slope of one. A useful test has a ROC curve that rises rapidly then reaches a plateau; the point of inflection represents the value of the test giving the best compromise between TP and FP.

RESULTS

Among the 112 patients, 83% had a serum NSE above the normal limit (71% of the 53 LD patients and 93% of the 59 ED patients). Mean values of NSE differed significantly with the degree of disease extent, as shown in Table 1.

The ROC curve (Fig. 1) constructed with different cut-off levels of NSE, illustrated the limited accuracy of serum NSE to predict ED stage.

The best compromise between TP (Se) and FP (1-Sp) was given by the threshold 35 μg/l: 60% of the ED patients had a serum NSE above 35 μg/l but 30% of the LD patients also had serum NSE levels above this threshold.

DISCUSSION

Using the conventionally accepted upper limit of normal serum NSE (i.e. 12.5 ng/ml), 83% of our SCLC patients had increased levels, which is a proportion very similar to that reported by others using the same method of analysis (Pharmacia NSE RIA test) [5, 9, 11,14]. The percentages of increased serum NSE levels are slightly lower in the other studies ranging from 65 to 73%, but the measurement methods [6–8] or cut-off level differed [9].

Table 1. Serum neuron-specific enolase concentrations in small cell lung cancer according to disease extent

Extent of disease	Number of patients	Serum NSE concentrations (ng/ml) Mean (m) [range] Standard deviation (S)
LD (Stage I to IIIB)	53	m = 30 [4–153] S = 24.9
Stage I, II, IIIA	20	m = 17.5 [4–54] S = 12.8
Stage IIIB	33	m = 27.5 [8–153] S = 27.5
ED	59	m = 68.53 [10–503] S = 82.4
One metastatic site	35	m = 56.2 [10–276] S = 50.65
⩾ two metastatic sites	24	m = 86.5 [11–503] S = 113.0
Liver involvement	27	m = 105.2 [15–503] S = 108.3
Bone involvement	34	m = 77.4 [11–503] S = 96.5
CNS involvement	12	m = 31.6 [10–76] S = 19.5

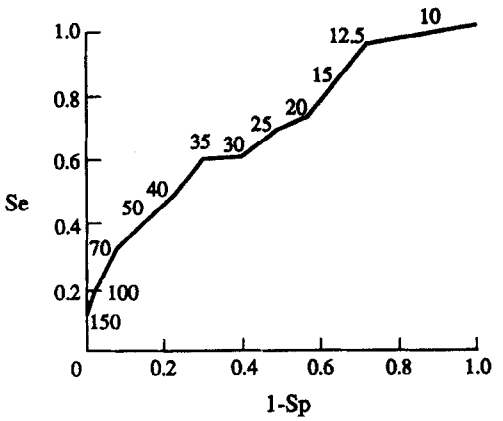

Fig. 1. ROC curve analysis of discrimination between limited and extensive disease stage at various cut-off levels of serum NSE.

One possible explanation for some patients with SCLC having a normal serum NSE could be the absence of correlation between the NSE content of the tumour cells and the serum NSE [15].

We confirmed, like others [5–10], that the percentage of increased levels of NSE is significantly higher in ED patients and that there is a significant difference between the levels of serum NSE in ED patients and in LD patients. Nevertheless, there is a somewhat important overlap of the values between the two stages, as illustrated by the ROC curve.

Several explanations may account for the very high levels of NSE in LD stage.

Firstly, staging procedures may differ from one study to another. Improved and up-staging procedures can shift patients from the LD to ED category; if only a few staging procedures are performed, some patients may be falsely classified as having LD. A distribution of NSE levels in LD stage ranging from 7 to 141 ng/ml, with 21% of the patients above 25 ng/ml was found by Harding *et al.* [11]. In this latter study, bone scans and brain CT scans were performed only if clinically indicated, liver ultrasound being the only systematic investigation. Even in our study, in which patients had exhaustive staging procedures, out of the 53 LD patients, 25 had serum NSE values above 25 ng/ml and 7 above 50 ng/ml. On the other hand, when exhaustive staging procedures are performed, the suboptimal sensitivity of some of them may account for the apparently unexplained elevation of serum NSE.

Secondly, haemolysis is known to elevate serum NSE concentration [8], which may also account for some high levels of NSE observed in LD stage. Although sera with visible haemolysis were excluded from our series, one cannot be sure that there was absolutely no haemolysis.

Thirdly, serum NSE level has been shown to be correlated with the total tumour mass [14] which may be very important even in a LD stage. In our series there was a significant difference between the serum NSE levels in patients with stage I to IIIA and patients with stage IIIB disease; all LD patients with NSE levels above 50 ng/ml had bulky mediastinal disease.

There were 4 ED patients with NSE values below 12.5 ng/ml and 15 between 12.5 and 25 ng/ml, compared with values ranging from 12 to 372 ng/ml found by Esscher *et al.* [8] with 1 out of 55 patients with a serum NSE level ⩽ 12.5 ng/ml and 4 in the 13–25 range. In the study by Akoun *et al.* [6], 6 patients out

of 26 had serum NSE below the upper limit. Some metastatic sites such as the brain are not correlated with high levels of serum NSE [9, 10], whereas liver and bone metastases are generally associated with very high levels of serum NSE [10, 16]. In our patient population, the highest levels of NSE were observed for patients with liver and bone metastases, and the lowest for cutaneous, peripheral lymph node, choroid and brain metastases as the only extrathoracic disease.

In conclusion, we have shown, in accordance with other authors, that serum NSE reflects to a certain degree the extent of disease in SCLC. Nevertheless, the importance of the overlap between the values for limited and extensive disease prevents its use for determining disease stage with sufficient accuracy.

1. Rawson NSB, Peto J. An overview of prognostic factors in small cell lung cancer. *Br J Cancer* 1990, **61**, 597–604.
2. Souhami RL, Bradbury J, Geddes DM, Spiro SG, Harper PG, Tobias JS. Prognostic significance of laboratory parameters measured at diagnosis in small cell carcinoma of the lung. *Cancer Res* 1985, **45**, 2878–2882.
3. Vincent MD, Ashley SE, Smith IE. Prognostic factors in small cell lung cancer: a simple prognostic index is better than conventional staging. *Eur J Cancer Clin Oncol* 1987, **23**, 1589–1599.
4. Arriagada R, Ihde DC, Johnson DH, Perry MC, Pignon JP, Souhami RL and the SCLC Meta-analysis Study Group. Meta-analysis of randomized trials evaluating the role of thoracic radiotherapy in limited small cell lung carcinoma. *Lung Cancer* 1991, 7 (suppl.), A 359.
5. Burghuber OC, Worofka B, Schernthaner G, *et al.* Serum neuron-specific enolase is a useful tumour marker for small cell lung cancer. *Cancer* 1990, **65**, 1386–1390.
6. Akoun GM, Scarna HM, Milleron BJ, Benichou MP, Herman DP. Serum neuron-specific enolase. A marker for disease extent and response to therapy for small-cell lung cancer. *Chest* 1985, **87**, 39–43.
7. Carney DN, Ihde DC, Cohen MH *et al.* Serum neuron-specific enolase: a marker for disease extent and response to therapy of small-cell lung cancer. *The Lancet* 1982, 583–585.
8. Esscher T, Steinholtz L, Bergh J, Nöu E, Nilsson K, Pahlman S. Neuron specific enolase: a useful diagnostic serum marker for small cell carcinoma of the lung. *Thorax* 1985, **40**, 85–90.
9. Cooper EH, Splinter TAW, Brown DA, Muers MF, Peake MD, Pearson SL. Evaluation of a radioimmunoassay for neuron specific enolase in small cell lung carcinoma. *Br J Cancer* 1985, **52**, 333–338.
10. Johnson DH, Marangos PJ, Forbes JT, *et al.* Potential utility of serum neuron specific enolase levels in small cell carcinoma of the lung. *Cancer Res* 1984, **44**, 5409–5414.
11. Harding M, McAllister J, Hulks G, *et al.* Neuron specific enolase in small cell lung cancer: a tumour marker of prognostic significance? *Br J Cancer* 1990, **61**, 605–607.
12. Feinstein AR. Clinical biostatistics XXXI. On the sensitivity, specificity and discrimination of diagnostic tests. *Clin Pharmacol Ther* 1975, **17**, 104–116.
13. Robertson EA, Zweig HH. Use of receiver operating characteristic curves to evaluate the clinical performance of analytical systems. *Clin Chem* 1981, **27**, 1569–1574.
14. Muller LC, Gasser R, Huber H, Klinger A, Salzer GM. Neuron-specific enolase (NSE) in small-cell lung cancer: longitudinal tumour marker evaluation. *Lung Cancer* 1992, **8**, 29–36.
15. Jorgensen LGM, Hirsch FR, Skov BG, Osterlind K, Cooper EH, Larsson LI. Occurrence of neuron specific enolase in tumour tissue and serum in small cell lung cancer. *Br J Cancer* 1991, **63**, 151–153.
16. Jacques G, Bepler G, Holle R, *et al.* Prognostic value of pretreatment carcinoembryonic antigen, neuron-specific enolase and creatine kinase-BB levels in sera of patients with small cell lung cancer. *Cancer* 1988, **62**, 125–134.

Eur J Cancer, Vol. 29A, No. 16, pp. 2250–2255, 1993.
Printed in Great Britain
0959–8049/93 $6.00 + 0.00

Differential Expression of the Intercellular Adhesion Molecule-1 (ICAM-1) in Lung Cancer Cell Lines of Various Histological Types

Cordula Schardt, Jochen Heymanns, Christof Schardt, Martin Rotsch and Klaus Havemann

Ten small cell lung carcinoma and 12 non-small cell lung carcinoma cell lines of various histological types were studied for constitutive expression of the intercellular adhesion molecule-1 (ICAM-1). ICAM-1 was present in all squamous and large cell carcinoma cell lines whereas two out of five adenocarcinoma and all small cell lung cancer (SCLC) cell lines showed no basal ICAM-1 expression. ICAM-1 expression was upregulated by tumour necrosis factor-α (TNF-α) in a time- and dose-dependent manner in cell lines with basal ICAM-1 expression. Western blot analysis revealed a molecular size of 85 kDa for ICAM-1 in all but one cell line. The TNF-α-induced upregulation of ICAM-1 occurs on the transcriptional level. Adhesion of peripheral blood mononuclear cells to lung tumour cell lines could be inhibited by monoclonal antibodies (MAb) (CD11a;CD18) against the receptor of ICAM-1, the leukocyte function-associated antigen-1 (LFA-1), but not by a MAb (CD54) against ICAM-1 itself.
Eur J Cancer, Vol. 29A, No. 16, pp. 2250–2255, 1993.

INTRODUCTION

CELL ADHESION molecules (CAM) of the immunoglobulin supergene family are relevant for tumorigenesis and the development of metastatic sites [1, 2]. Important molecules of the immunoglobulin supergene family are the intercellular adhesion molecules 1 and 2 (ICAM-1, ICAM-2). ICAM-1 is a transmembrane glycoprotein with five immunoglobulin-like domains [3]. The molecular weight ranges from 74 to 114 kDa due to the extent of glycosylation [4]. ICAM-1 is constitutively expressed on a variety of haematopoietic and non-haematopoietic cells and tissues, whereas ICAM-2 [5] is restricted to haematopoietic cells and to vascular endothelium. ICAM-1 expression can be upregulated