Diagnostic value of serum and bronchoalveolar lavage neuron specific enolase levels in pulmonary malignancies*

Ülkü YILMAZ[1], Çiğdem BİBER[1], Şerif AKMAN[2], Ayşe ÖZYILDIRIM[1], Sinan ÇOPUR[1], İshan Atila KEYF[1]

[1] Atatürk Pulmonary Diseases and Surgery Center,
[2] Dept. ot Biochemistry, Gülhane Military Medical Academy, Ankara, TURKEY

The diagnostic value of serum and bronchoalveolar lavage (BAL) levels of Neuron Spesific Enolase (NSE) in pulmonary malignancies were evaluated. Thirty-one patients with primary pulmonary carcinomas (12 small cell lung cancer-SCLC and 19nonsmall cell lung cancer-NSCLC) and 11 patients with benign lung disease were included in this study.
NSE levels in serum and BAL were significantly higher in SCLC ($p<0.05$) when compared to NSCLC and benign cases. In BAL standardized by total protein, NSE levels were significantly higher only in SCLC cases, compared with benign cases ($p<0.05$). [Turk J Med Res 1994; 12(5): 206-209]

Key Words: Bronchoalveolar lavage, Neuron Specific Enolase

An acidic protein of the brain tissue; 14-3-2 is a specific protein for neurons. Nowadays, this protein is known as neuron specific enolase, cell specific isoenzyme of enolase which is a glycolytic enzyme (1,2).

Immunreactivity of NSE is present in all diffuse neuroendocrine systems, including the lungs. Usually, anaerobic glycolytic enzyme activity enhances in malignant tissues and NSE, being a glycolytic enzyme, is found elevated in neuroendocrine malignancies, including small cell lung carcinoma (3,4).

In our study, serum and bronchoalveolar lavage (BAL) levels of NSE is evaluated in order to determine the value of NSE in differentiating between pulmonary malignancies and benign pulmonary diseases.

MATERIALS AND METHODS

In Ataturk Pulmonary Diseases and Surgery Center, 42 patients were evaluated between January 1992-May 1992. Thirty-eight patients were male and four were female. Median age was 52.5 (17-76). Thirty-one patients had primary pulmonary malignancy. Eleven patients with benign pulmonary disease were taken as control group (Table 1).

Diagnosis of pulmonary neoplasm was made by histopathological or cytological evaluation of the specimens taken by bronchoscopy, computed tomography (CT) guided needle aspiration biopsy or surgery.

In control group, there were five patients with pneumonia. While an etiological agent couldn't be detected in four of them, one was serologically positive for Mycoplasma pneumonia. There were three patients with pulmonary tuberculosis (1 miliary, 1 endobronchial, 1 parenchyma tuberculosis). Diagnosis of miliary tuberculosis was made by bronchoscopic transbronchial biopsy and endobronchial tuberculosis by bronchoscopic forceps biopsy.

Olympus BF Type I T-20-D (Olympus-Hyde Park New York) fiberoptic bronchoscope was used for the study.

In 24 cases of pulmonary carcinoma, BAL was performed in the involved segment and in 5 cases with no endobronchial lesion, it was performed in the segment detected by CT or conventional radiography.

In control group, BAL was performed from the involved segment. It was performed in middle or lingular lobes in disseminated disease cases. Interventions like biopsy and brushing were not performed before BAL. Hemorrhagic lavages were excluded from the study.

After bronchoscope was introduced to the involved segment, 80 ml of saline was given in aliquoits, each containing 20 ml. Suction was applied after each instillation.

Received: July 15,1994 Accepted: Sept. 18,1994

Correspondence: Ülkü YILMAZ
Atatürk Pulmonary Diseases and
Surgery Center, Ankara, TURKEY

It has been presented XXI.International Respiratory Research Association Congres.

Table 1. Characteristics of the patients

Diagnosis	Number of Patients
Primary pulmonary malignancies	31
SCLC	12
NSCLC	19
Squamous cell carcinoma	10
Adenocarcinoma	7 •
Bronchoalveolar cell carcinoma	1
Large cell carcinoma	1
Control	11
Pneumonia	5
Pulmonary tuberculosis	3
Bronchiectasis	1
Chronic obstructive pulmonary disease	1
Chondroma	1

Every BAL sample was centrifugated at 3500 rpm for ten minutes and then stored at -20°C.

NSE was measured by ELISA method using CIS Biointernational EIA-NSE KIT. In several previous studies cut off value for serum NSE was reported as 12.3 ng/ml or 16 ng/ml (5-10). We took the cut off level as 16 ng/ml.

BAL cut off level was accepted as 12.5 ng/ml in Kosmas's study (9) and 16 ng/ml in Macchia's study (6). In our study, we accepted NSE cut off value as 30 ng/ml for standardized BAL and 20 ng/ml for non-standardized BAL.

Total protein levels in BAL were measured by "Lowry's" method. We used total protein as the reference protein denominator assuming that it is diluted to the same degree as tumor markers by the instilled liquid.

Statistical analysis was made by "Student-t" test.

Evaluating the results of serum and BAL NSE levels to predict the diagnosis of SCLC and NSCLC, we calculated sensitivity, specificity and the predictive values of these variables, expressing the fractions as percentages. The following equations were used:

$$\text{Sensitivity (S)} = \frac{\text{Number of patients with cancer who have a positive test (True positive)}}{\text{Number of patients with cancer}}$$

$$\text{Specificity (Sp)} = \frac{\text{Number of patients without cancer who have a negative test (True negative)}}{\text{Number of patients without cancer}}$$

Prevalance (P)=Incidence of disease in the population studied

$$\text{Positive predictive value (PPV)} = \frac{(S)x(P)}{(S)(P)+(1-Sp)\ (1-P)}$$

Table 2. Median±Standard error (SE) NSE values of the groups

NSE	Benign	SCLC	NSCLC
Serum*	19.81 ±0.33	36.41 ±0.44	17.21 ±0.11
BAL*	13.54±0.1	19.8±0.25	14.26±0.1
Standardised BAL**	85.99±0.79	44.63±0.49	86.89±0.58

ng/ml
ng/mg

RESULTS

In our study, serum levels of NSE were found elevated in 83.3% of SCLC group, 67.8% of NSCLC and 45.4% of benign pulmonary disease group.

Median NSE levels in serum, BAL and standardised BAL are given in Table 2.

NSE level distribution of the groups in serum, BAL and standardised BAL are shown in Figure 1.

In our study, serum NSE levels of SCLC group was found significantly higher than the NSE levels in NSCLC and benign pulmonary disease group ($p<0.05$). The difference in NSE levels of NSCLC group and benign pulmonary disease group was not statistically significant ($p>0.05$).

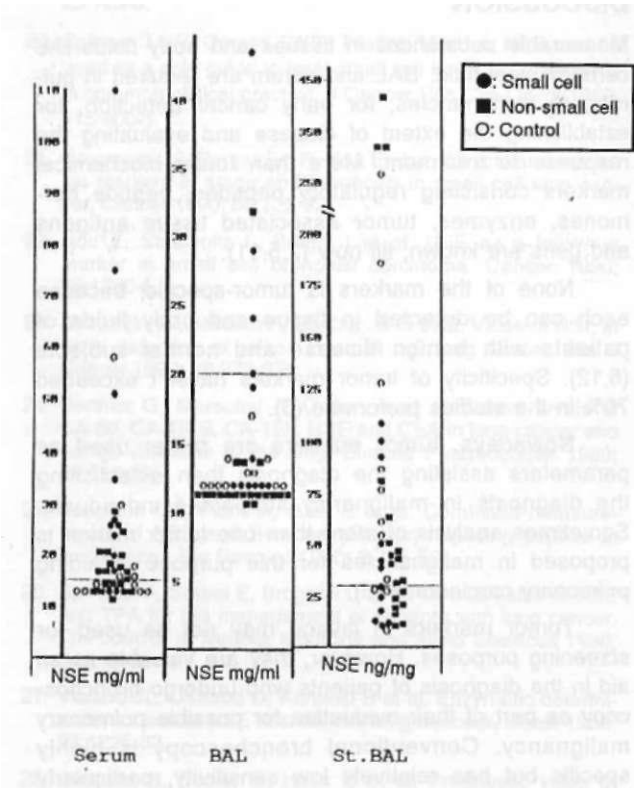

Figure 1. NSE level distribution of the groups in serum, BAL and standardised BAL

Table 3. Standardised BAL, BAL and serum NSE levels, sensitivity, specificity and positive predictive values

		Specificity (%)	Sensitivity (%)	ppv (%)
Serum	LC	36	73	81
	SCLC	36	83	66
BAL	LC	45.4	48.8	88
	SCLC	33	97	44
Standardised BAL	LC	36	58	69
	SCLC	50	13	23

Non standardised BAL NSE levels of SCLC group was significantly higher than the NSCLC and benign pulmonary disease group ($p<0.05$). The difference between benign pulmonary disease and NSCLC group was not statistically significant ($p>0.05$).

When standardised BAL levels were compared, SCLC group showed significantly higher levels than the benign pulmonary disease group ($p<0.05$). The differences between pulmonary disease group and NSCLC; SCLC and NSCLC were not statistically significant ($p>0.05$).

Standardised BAL, BAL and serum NSE levels, sensitivity, specificity and ppv results of the groups are shown in Table 3.

DISCUSSION

Measurable substances in tissues and body fluids like cerebrospinal fluid, BAL and serum are required in pulmonary malignancies, for early cancer detection, for establishing the extent of disease and evaluating the response to treatment. More than fourty biochemical markers consisting regulatory peptides, peptide hormones, enzymes, tumor associated tissue antigens and gens are known, till now (2,5,11).

None of the markers is tumor-specific, because each can be detected in tissue and body fluids of patients with benign disease and normal subjects (6,12). Specificity of tumor markers haven't exceeded 70% in the studies performed (6).

Nowadays, tumor markers are rather used as parameters assisting the diagnosis than establishing the diagnosis in malignancy suspected individuals. Sometimes analysis of more than one tumor marker is proposed in malignancies for this purpose including pulmonary carcinomas (6).

Tumor markers in lavage may not be used for screening purposes. However, they are valuable as an aid in the diagnosis of patients who undergo bronchoscopy as part of their evaluation for possible pulmonary malignancy. Conventional bronchoscopy is highly specific but has relatively low sensitivity, particularly when dealing with solitary pulmonary nodules. Therefore, in the patient with negative bronchoscopy findings, further testing is necessary. The sensitivity of the procedure increases substantially when the results of conventional bronchoscopy and tumor marker levels in lavage are combined (12).

NSE is an enoiase isoenzyme found at high levels in neurons and neuroendocrine cells. This tumor marker is found elevated in neuroendocrine malignancies like SCLC and neuroblastoma (13). High serum levels are detected in 70% of untreated SCLC patients. Moreover, elevated serum NSE levels are detected in NSCLC and malignancies derived from other organs. However, the levels in progressive NSCLC cases are four-five fold higher than the levels in other progressive malignancies (1,13).

Studies performed revealed that serum NSE levels are useful to follow up the treatment response and to detect metastases and relapse (5,7,14-20). Studies indicating the contrary of this concept are also reported (8,21 -23).

Burghuber and coll, assented the cut-off value of serum NSE as 12.3 ng/ml. They found that serum NSE levels are significantly higher in SCLC patients than NSCLC cases (7). Various studies supporting this concept has been reported (8,14,15,24-26), Sensitivity and spesificity of serum NSE levels found in various studies are 23% to 76% and 85% to 93%, respectively (24,27).

Serum NSE levels in limited and extensive stage SCLC has been evaluated in various studies and is found significantly higher in extensive stage disease (8-10,14,17,19,25-27). Sensitivity is found 100% in a study performed on extensive stage SCLC patients (27). Reports are also present in favor of the contrary (28).

Kosmas et al, found that BAL NSE levels are elevated along with the serum levels in extensive stage SCLC patients. However, they declared that values are inadequate to remark a statistical significance. In the same study serum and BAL NSE levels in NSCLC patients were also elevated. But they were not as high as the levels detected in SCLC patients (9). Sensitivity of BAL and serum NSE levels were 85.7%, 57%, respectively. Specificities were both 76% (9).

Macchia and Coll, in their study, found BAL NSE positivity in 71% of SCLC patients, while it was 30% in NSCLC group (6).

Serum NSE levels found in our study correlates with the literature, but there are a few studies investigating NSE levels of BAL and standardised BAL. As we have mentioned above, NSE levels in nonstandardised BAL is found significantly higher in SCLC group than NSCLC group and benign pulmonary disease group.

Sensitivity and specificity of serum, BAL and standardised BAL levels in our study, also shows correlation with the literature.

Table 1. Characteristics of the patients

Diagnosis	Number of Patients
Primary pulmonary malignancies	31
SCLC	12
NSCLC	19
Squamous cell carcinoma	10
Adenocarcinoma	7
Bronchoalveolar cell carcinoma	1
Large cell carcinoma	1
Control	11
Pneumonia	5
Pulmonary tuberculosis	3
Bronchiectasis	1
Chronic obstructive pulmonary disease	1
Chondroma	1

Every BAL sample was centrifugated at 3500 rpm for ten minutes and then stored at -20°C.

NSE was measured by ELISA method using CIS Biointernational EIA-NSE KIT. In several previous studies cut off value for serum NSE was reported as 12.3 ng/ml or 16 ng/ml (5-10). We took the cut off level as 16 ng/ml.

BAL cut off level was accepted as 12.5 ng/ml in Kosmas's study (9) and 16 ng/ml in Macchia's study (6). In our study, we accepted NSE cut off value as 30 ng/ml for standardized BAL and 20 ng/ml for non-standardized BAL.

Total protein levels in BAL were measured by "Lowry's" method. We used total protein as the reference protein denominator assuming that it is diluted to the same degree as tumor markers by the instilled liquid.

Statistical analysis was made by "Student-t" test.

Evaluating the results of serum and BAL NSE levels to predict the diagnosis of SCLC and NSCLC, we calculated sensitivity, specificity and the predictive values of these variables, expressing the fractions as percentages. The following equations were used:

$$\text{Sensitivity (S)} = \frac{\text{Number of patients with cancer who have a positive test (True positive)}}{\text{Number of patients with cancer}}$$

$$\text{Specificity (Sp)} = \frac{\text{Number of patients without cancer who have a negative test (True negative)}}{\text{Number of patients without cancer}}$$

Prevalance (P)=Incidence of disease in the population studied

$$\text{Positive predictive value (PPV)} = \frac{(S)x(P)}{(S)(P)+(1-Sp)\ (1-P)}$$

Table 2. Median±Standard error (SE) NSE values of the groups

NSE	Benign	SCLC	NSCLC
Serum*	19.81 ±0.33	36.41 ±0.44	17.21 ±0.11
BAL*	13.54±0.1	19.8±0.25	14.26±0.1
Standardised BAL**	85.99±0.79	44.63±0.49	86.89±0.58

ng/ml
ng/mg

RESULTS

In our study, serum levels of NSE were found elevated in 83.3% of SCLC group, 67.8% of NSCLC and 45.4% of benign pulmonary disease group.

Median NSE levels in serum, BAL and standardised BAL are given in Table 2.

NSE level distribution of the groups in serum, BAL and standardised BAL are shown in Figure 1.

In our study, serum NSE levels of SCLC group was found significantly higher than the NSE levels in NSCLC and benign pulmonary disease group ($p<0.05$). The difference in NSE levels of NSCLC group and benign pulmonary disease group was not statistically significant ($p>0.05$).

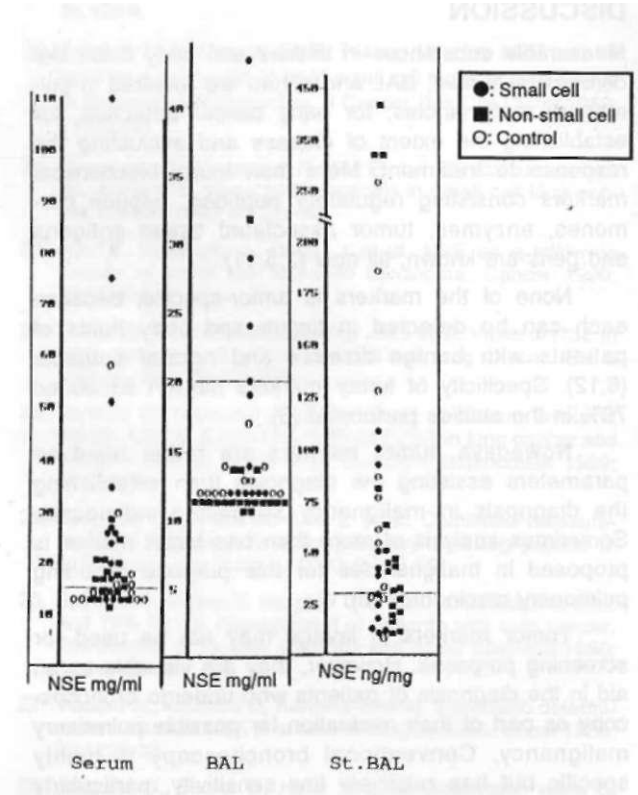

Figure 1. NSE level distribution of the groups in serum, BAL and standardised BAL

Table 3. Standardised BAL, BAL and serum NSE levels, sensitivity, specificity and positive predictive values

		Specificity (%)	Sensitivity (%)	ppv (%)
Serum	LC	36	73	81
	SCLC	36	83	66
BAL	LC	45.4	48.8	88
	SCLC	33	97	44
Standardised BAL	LC	36	58	69
	SCLC	50	13	23

Non standardised BAL NSE levels of SCLC group was significantly higher than the NSCLC and benign pulmonary disease group ($p<0.05$). The difference between benign pulmonary disease and NSCLC group was not statistically significant ($p>0.05$).

When standardised BAL levels were compared, SCLC group showed significantly higher levels than the benign pulmonary disease group ($p<0.05$). The differences between pulmonary disease group and NSCLC; SCLC and NSCLC were not statistically significant ($p>0.05$).

Standardised BAL, BAL and serum NSE levels, sensitivity, specificity and ppv results of the groups are shown in Table 3.

DISCUSSION

Measurable substances in tissues and body fluids like cerebrospinal fluid, BAL and serum are required in pulmonary malignancies, for early cancer detection, for establishing the extent of disease and evaluating the response to treatment. More than fourty biochemical markers consisting regulatory peptides, peptide hormones, enzymes, tumor associated tissue antigens and gens are known, till now (2,5,11).

None of the markers is tumor-specific, because each can be detected in tissue and body fluids of patients with benign disease and normal subjects (6,12). Specificity of tumor markers haven't exceeded 70% in the studies performed (6).

Nowadays, tumor markers are rather used as parameters assisting the diagnosis than establishing the diagnosis in malignancy suspected individuals. Sometimes analysis of more than one tumor marker is proposed in malignancies for this purpose including pulmonary carcinomas (6).

Tumor markers in lavage may not be used for screening purposes. However, they are valuable as an aid in the diagnosis of patients who undergo bronchoscopy as part of their evaluation for possible pulmonary malignancy. Conventional bronchoscopy is highly specific but has relatively low sensitivity, particularly when dealing with solitary pulmonary nodules. Therefore, in the patient with negative bronchoscopy findings, further testing is necessary. The sensitivity of the procedure increases substantially when the results of conventional bronchoscopy and tumor marker levels in lavage are combined (12).

NSE is an enolase isoenzyme found at high levels in neurons and neuroendocrine cells. This tumor marker is found elevated in neuroendocrine malignancies like SCLC and neuroblastoma (13). High serum levels are detected in 70% of untreated SCLC patients. Moreover, elevated serum NSE levels are detected in NSCLC and malignancies derived from other organs. However, the levels in progressive NSCLC cases are four-five fold higher than the levels in other progressive malignancies (1,13).

Studies performed revealed that serum NSE levels are useful to follow up the treatment response and to detect metastases and relapse (5,7,14-20). Studies indicating the contrary of this concept are also reported (8,21-23).

Burghuber and coll, assented the cut-off value of serum NSE as 12.3 ng/ml. They found that serum NSE levels are significantly higher in SCLC patients than NSCLC cases (7). Various studies supporting this concept has been reported (8,14,15,24-26), Sensitivity and spesificity of serum NSE levels found in various studies are 23% to 76% and 85% to 93%, respectively (24,27).

Serum NSE levels in limited and extensive stage SCLC has been evaluated in various studies and is found significantly higher in extensive stage disease (8-10,14,17,19,25-27). Sensitivity is found 100% in a study performed on extensive stage SCLC patients (27). Reports are also present in favor of the contrary (28).

Kosmas et al, found that BAL NSE levels are elevated along with the serum levels in extensive stage SCLC patients. However, they declared that values are inadequate to remark a statistical significance. In the same study serum and BAL NSE levels in NSCLC patients were also elevated. But they were not as high as the levels detected in SCLC patients (9). Sensitivity of BAL and serum NSE levels were 85.7%, 57%, respectively. Specificities were both 76% (9).

Macchia and Coll, in their study, found BAL NSE positivity in 71% of SCLC patients, while it was 30% in NSCLC group (6).

Serum NSE levels found in our study correlates with the literature, but there are a few studies investigating NSE levels of BAL and standardised BAL. As we have mentioned above, NSE levels in honstandardised BAL is found significantly higher in SCLC group than NSCLC group and benign pulmonary disease group.

Sensitivity and specificity of serum, BAL and standardised BAL levels in our study, also shows correlation with the literature.

As a conclusion, it can be said that serum NSE is a helpful parameter in diagnosis. However the results about NSE levels in BAL are controversial, to make a definitive decleration, studies on large populations are needed.

However, studies with larger populations are needed in order to reach a conclusion, about the value of NSE levels in BAL in patients with lung cancer.

Akciğer kanserlerinde nöron spesifik enolaz (NSE) serum ve bronkoalveoler lavaj düzeylerinin tanı değeri

Küçük hücreli akciğer kanserlerinde serum ve bronkoalveoler lavaj (BAL) NSE düzeylerinin tanısal değerinin araştırılması amaçlandı. 12 küçük hücreli, 19 küçük hücre' dışı akciğer kanserinden oluşan 31 primer akciğer kanserli ve 11 benign akciğer hastalıklı olgu çalışma kapsamına alındı.

Küçük hücreli akciğer kanseri bulunan olgularda, serum ve BAL NSE düzeyleri, küçük hücre dışı akciğer kanserli ve benign hastalıklı olgulara oranla anlamlı yüksek bulundu (p<0.05). Total protein ile standardize edilen bronkoalveoler lavajda ise NSE düzeyi, yalnızca küçük hücreli akciğer kanserli olgularda benign gruba göre anlamlı yükseklik gösterdi (p<0.05).

[Turk JMed Res 1994; 12(5): 206-209]

REFERENCES

1. Kaiser E, Kuzmitz R, Pregant P et al. Clinical biochemistry of NSE. Clinical Chimica Acta 1989; 183:13-32.
2. Bates SE. Clinical applications of serum tumor markers. Annals of Internal Medicine 1991; 115:623-38.
3. Esscher T, Steinholtz L, Bergh J et al. Neuron specific enolase: A useful diagnostic serum marker for small cell carcinoma of the lung. Thorax 1985; 40:85-90.
4. Fufita K, Haimoto H, Imaizumi M et al. Evaluation of gamma-enolase as a tumor marker for lung cancer. Cancer 1987; 60:362-9.
5. Lombardi C, Tassi GF, Pizzocolo G et al. Clinical significance of a multiple biomarker assay in patients with lung cancer. Chest 1990; 97:639-44.
6. Macchia V, Mariano A, Cavalcantii M et al. Tumor markers and lung cancer: Correlation between serum and bronchial secretion levels of CEA, TPA, Can Ag CA 50, NSE and ferritin. The International Journal of Biological Markers 1987;2:151-6.
7. Burghuber OC, Worofka B, Schernthaner G et al. Serum NSE is a useful tumor marker for small cell lung cancer. Cancer 1990; 65:1386-90.
8. Harding M, McAllister J, Hulks G et al. Neuron specific enolase in small cell lung cancer. Br J Cancer 1990; 61:60-5-7.
9. Kosmas E, Panayotou A, Parastadides S. NSE in bronchial washings: A better diagnostic marker for small cell lung cancer. Eur J Cancer 1991; 27:948.
10. Rapellino M, Pecchio F, Arasio C et al. NSE and CEA determination in serum and patients small cell lung cancer. The Journal of Nuclear Medicine and Allied Sciences 1990; 34:147-9.
11. Semenzato G, Spatafora M, Feruglio C et al. Bronchoalveolar lavage and the immunology of lung cancer. Cancer 1990 (Lung Suppl);1041-9.
12. Goldstein N, Lippmann ML, Goldberg SK et al. Usefulness of tumor markers in serum and bronchoalveolar lavage of patients undergoing fiberoptic bronchoscopy. Am Rev Respir Dis 1985; 132:60-4.
13. Duffy MJ. New Cancer markers. Ann Clin Biochem 1989; 26:379-87.
14. Bork E, Hansen M, Urdal P et al. Early detection of response in small cell bronchogenic carcinoma by changes in serum concentrations of creatine kinase and gastrin releasing peptide. Eur J Cancer Clin Oncol 1988; 24:1033-8.
15. Fischbach W, Schwarz-Wallrauch C, Jany BS. NSE and thymidine kinase as an aid to the diagnosis and treatment monitoring of small cell lung cancer. Cancer 1989; 63:1143-9.
16. Gaast A, Putten WU, Oosterom R et al. Prognostic value of serum thymidine kinase, tissue polypeptide antigen and NSE in patients with small cell lung carcinoma. Br J Cancer 1991;64:369-72.
17. Gamm SA, Keevil BG, Thatcher N et al. The value of tumour markers in lung cancer. Br J Cancer 1988; 58:797-804.
18. Jorgenson LGM, Osterlind K, Hansen HH et al. The prognostic influence of serum NSE in small cell lung cancer. Br J Cancer 1988; 58:805-7.
19. Jorgensen LGM, Hansen HH, Cooper EH. Neuron specific enolase, CEA and LDH as indicators of disease activity in small cell lung cancer. Eur J Cancer Clin Oncol 1989; 25:123-8.
20. Splinter TAW, Carney DNB, Teeling M et al. NSE can be used as a sole guide to treat small cell lung cancer patients in common clinical practice. J Cancer Res Clin Oncol 1989; 115:400-1.
21. Gronowitz JS, Bergstrom R, Nou E et al. Clinical and serologic markers of stage and prognosis in small cell lung cancer. Cancer 1990; 66:722-32.
22. Nou E, Steinholtz L, Bergh J et al. NSE as a follow-up marker in small cell bronchial carcinoma. Cancer 1990; 65:1380-5.
23. Zandwijk NV, Jassem E, Bonfrer JMG et al. Value of NSE in early detection of relaps in small cell lung cancer. Eur J Cancer 1990; 26:273-376.
24. Berthiot G, Marechal F, Cattan A et al. Serum -levels of CA-50, CA-19, 9, CA-125, NSE and CEA in lung cancer and benign diseases of the lung. Biomed Pharmocother 1989; 49:613-20.
25. Scagliotti GV, Piani M, Gatti E et al. Combined measurements of NSE and Bombesin/Gastrin releasing peptide in lung cancer. Eur Respir J 1990; 2:746-50.
26. Spinazzi A, Soresi E, Broghini U et al. Clinical value of NSE and TPA for the management of patients with lung cancer. The Journal of Nuclear Medicine and Allied Sciences 1990; 34:147-9.
27. Viallard JL, Caillaud D, Kantelip B et al. Enzymatic determination of serum NSE in small cell lung cancers. Chest 1988; 93:1225-33.
28. Jacques G, Bepler G, Holle R et al. Prognostic value of pretreatment CEA, NSE and creatine-kinase-BB lewels in sera of patients with small cell lung cancer. Cancer 1988; 62:125-34.