Neuron Specific Enolase Concentration Is Increased in Serum and Decreased in Platelets of Patients with Active Systemic Sclerosis

PAULO S. MASSABKI, NEUSA P. SILVA, DAYSE M. LOURENÇO, and LUIS E.C. ANDRADE

ABSTRACT. ***Objective.*** To determine frequency, origin, and clinical associations of elevated serum neuron specific enolase (NSE) in systemic sclerosis (SSc).

Methods. Serum was obtained from 75 patients with SSc, 20 systemic lupus erythematosus, 8 polymyositis, 10 idiopathic interstitial lung disease, and 10 healthy volunteers. NSE status was determined in serum (in all individuals) and in platelet lysate (in volunteers and 30 patients with SSc).

Results. Elevated serum NSE (mean 22.6 ng/ml, range 12.1–68.2 ng/ml) was observed in 26 patients with SSc (34.6%). Those with diffuse SSc had higher serum NSE than those with limited disease (16.5 ± 13.4 vs 9.6 ± 5.0 ng/ml, p = 0.006). No association was found between serum NSE and lung or esophagus involvement. Patients with long-standing disease had lower serum NSE than those with early disease (10.8 ± 7.3 vs 16.1 ± 13.6 ng/ml, p = 0.05). Serum NSE was 19.4 ± 13.0 ng/ml in patients with total skin score (TSS) > 20, 8.3 ± 2.1 ng/ml in patients with TSS < 5, and 6.0 ± 3.1 ng/ml in volunteers (p = 0.01). NSE platelet lysate concentration was 3.6 ± 2.9 ng/ml in patients with TSS > 20, 12.4 ± 4.1 ng/ml in those with TSS < 5, and 14.1 ± 6.5 ng/ml in healthy individuals (p < 0.001). Volunteers and SSc patients with low TSS had comparable S/PL–NSE index (serum/platelet lysate NSE concentration) (0.42 ± 0.16 and 0.75 ± 0.33, respectively), both lower than SSc patients with high TSS (7.45 ± 5.57) (p < 0.001).

Conclusion. Elevated serum NSE was observed in one-third of SSc patients but not in other autoimmune rheumatic diseases. The inverse relationship between serum and platelet lysate NSE concentration suggests platelet activation as the origin of high serum NSE in SSc. NSE S/PL was the best discriminatory variable between healthy volunteers and SSc patients as well as between patients with high and low TSS. High serum NSE and high NSE-S/PL index seemed to be associated with SSc disease activity. Further work is warranted to investigate a possible role for this marker in assessing disease activity and therapy response. (J Rheumatol 2003;30:2606–12)

Key Indexing Terms:
SYSTEMIC SCLEROSIS   NEURON SPECIFIC ENOLASE
TUMOR MARKERS   PLATELET ACTIVATION

Tumor markers are molecules that have their serum concentrations abnormally elevated in certain kinds of malignant neoplasias[1-5]. Most known tumor markers are proteins that are usually present in very low serum concentration or are undetectable in the serum. Despite the designation, tumor markers are not restricted to cancer. In fact, elevated serum levels of tumor markers can be found in a variety of non-neoplastic states, such as some chronic inflammatory diseases, tabagism, alcoholism, and pregnancy[4-6]. Even autoimmune rheumatic diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis have been reported to be associated with modest increases in serum concentration of some tumor markers[7-9].

Neuron specific enolase (NSE) was originally described by Moore and McGregor in 1965 as an enzyme enriched in neurons in general and in peripheral neuroendocrine cells[10]. Later it was found that NSE is also present in high levels in platelets and erythrocytes[11,12]. NSE is considered to be a useful tumor marker for small cell lung carcinoma[13-17] and neuroblastoma[18,19]. It has also been associated with non-specific central nervous system injury, such as cerebral vascular accident[20,21].

In a previous survey on tumor marker serum levels in autoimmune rheumatic diseases, we observed a high frequency of patients with systemic sclerosis (SSc) presenting with high NSE serum levels[22]. In the present study we explored this finding by determining the frequency

of elevated NSE serum levels in a larger sample of SSc patients and by investigating the possible clinical associations and the origin of elevated serum NSE in these patients.

MATERIAL AND METHODS

Seventy-five patients with SSc, 20 with SLE, and 8 with polymyositis were sequentially recruited from the Rheumatology Outpatient Clinic at the Medical School Hospital of the Universidade Federal de São Paulo. An additional 10 patients with idiopathic interstitial lung fibrosis were obtained from the Interstitial Disease Outpatient Clinic at the same institution. Ten healthy non-smoking volunteers were recruited among the medical staff. Diagnosis was established according to standard American College of Rheumatology classification criteria and the American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias[23-26]. All patients and volunteers signed an informed consent form approved by the University Ethics Committee for this study.

Clinical characterization of patients included determination of disease duration, disease subtype, presence of Raynaud's phenomenon, interstitial lung disease (ILD), and esophageal dysmotility. For the determination of disease duration the onset of disease was considered as the date of appearance of characteristic skin involvement. Limited SSc was defined as skin involvement of face and limbs distal to the elbows and/or knees. Diffuse SSc was considered when there was also involvement of the skin of proximal regions of the limbs and/or abdomen and chest. The severity of skin involvement was registered by the modified Rodnan's total skin score (TSS) in 30 patients with SSc[27-29]. Raynaud's phenomenon was considered as the report of consistent episodes of paroxysmal and transient bluish or pale discoloration of fingers induced either spontaneously or by cold exposure. ILD was investigated in all SSc patients and established by thorax high resolution computerized tomography showing characteristic ground-glass appearance or fibrotic changes or spirometry indicating diffusing capacity for carbon monoxide (DLCO) below 80% predicted, forced vital capacity below 80% predicted, or 10% loss in forced vital capacity between 2 consecutive tests. Esophageal dysmotility was determined by standard barium esophagogram in all SSc patients.

Blood for NSE determination was obtained from superficial cubital veins without tourniquet from all patients and controls. For 20 SSc patients, additional blood from the radial artery was obtained. After coagulation serum was separated and stored at –85° C until used. For the 10 healthy volunteers and 30 of the 75 SSc patients (those who had the TSS determined), an additional 9 ml volume was collected into a tube containing 1 ml of anticoagulant solution (38 mM citric acid, 60 mM sodium citrate, 14 mM dextrose) for platelet separation and platelet lysate NSE determination. All steps were performed carefully in order to avoid hemolysis and platelet activation. No macroscopic evidence of hemolysis was observed in any of the samples. Platelet count on whole blood was performed on automated analyzer (Cell-Dyn 3700). Anticoagulated blood was gently homogenized and centrifuged at 2000 rpm for 10 min. From the resultant platelet-rich plasma 1 ml was further centrifuged at 3,000 rpm for 15 min. The platelet-rich pellet was washed 3 times in modified Tyrode's buffer (0.2 M EDTA, 0.1M $MgCl_2$, 137 mM NaCl, 2.7 mM KCl, 0.4 mM NaH_2PO_4, 12 mM $NaHCO_3$, 5 mM glucose, 0.25 mg/dl gelatin, pH 6.5) and resuspended in 1 ml modified Tyrode's buffer with 1.4 mM $MgCl_2$ instead. The platelet suspension was then sonicated at setting 2 (Branson 250, Danbury, CT, USA) twice for 30 s on ice with 1 min interval. The resultant platelet lysate was stored at –85ºC for future NSE determination.

NSE concentration was determined in the serum and platelet lysate by immunochemoluminescence (LIA-mat® NSE, Sangtec Medical, Dietzenbach, Germany) according to the manufacturer's instructions. Briefly, 200 µl of serum or platelet lysate was added to polystyrene tubes internally coated with monoclonal anti-NSE. After incubation at 37ºC for 30 min, tubes were washed twice with washing buffer and incubated with a second monoclonal anti-NSE antibody conjugated to isoluminol. Antibody binding was determined by recording reactive light units (RLU) at 425 nm and interpolation onto a standard curve for estimation of NSE concentration. Reference range for normality in serum is < 12 ng/ml[30]. Lactic dehydrogenase (LDH) was determined by enzymatic ultraviolet method (Wiener, Rosario, Argentina) in an automated analyzer (Cobas Miraplus, Roche, Mannheim, Germany)[31]. Platelet lysate NSE concentration was normalized to 100,000 platelets/mm^3 count in whole blood. NSE-S/PL (serum/platelet lysate) index was obtained by dividing serum NSE by the normalized platelet lysate NSE concentration.

Comparisons of NSE concentration among 3 or more groups were analyzed by Kruskall-Wallis' test. Comparisons of NSE concentration between 2 independent groups were analyzed by Student's t test. NSE concentrations obtained in different samples from the same individual were compared by means of paired Student's t test. Serum NSE and LDH concentrations as well as serum and platelet lysate NSE concentrations were compared by Spearman's correlation test. Statistical significance was set at 5%.

RESULTS

Sixty-four of the 75 SSc patients were female. Mean age was 45 years (range 22 to 68). Among healthy volunteers, there were 8 women and 2 men, mean age 33 years (range 25 to 50). All SLE patients were women, with a mean age of 34.3 years (range 18 to 49). There were 3 men and 5 women with polymyositis, with mean age of 37.2 years (range 27 to 49). In the idiopathic lung fibrosis group, there were 6 men and 4 women, mean age 55 years (range 42 to 74).

All patients with SSc had Raynaud's phenomenon. Forty-two SSc patients (56%) had diffuse and 33 (44%) had limited SSc. Disease duration was less than 3 years in 37 patients (49.3%) and greater than 5 years in 33 patients (44%). Five patients (6.7%) had disease duration between 3 and 5 years. Esophageal dysmotility was identified in 38 (51%) and ILD was diagnosed in 26 (35%) of the patients. TSS was determined in 30 patients: TSS was > 20 in 20 (67%) and < 5 in 10 (33%). NSE serum concentration was significantly higher in SSc patients (13.4 ± 11.1 ng/ml) versus patients with SLE (5.1 ± 2.5 ng/ml), polymyositis (3.4 ± 1.3 ng/ml), idiopathic lung fibrosis (7.4 ± 4.5 ng/ml), and healthy volunteers (6.0 ± 3.1 ng/ml) (p < 0.001). NSE serum levels > 12 ng/ml upper normal limit were observed in 26 (34.6%) of the 75 SSc patients (mean 24.0 ng/ml, range 12.1 to 68.2 ng/ml), in only one patient with idiopathic interstitial lung fibrosis, and in no patient with SLE or polymyositis. SSc patients with diffuse disease presented significantly higher NSE serum levels than patients with limited SSc (Table 1). Patients with early disease (< 3 yrs) presented higher serum NSE as compared to those with disease duration above 5 years (16.1 ± 13.6 vs 10.8 ± 7.3 ng/ml, p = 0.05). Accordingly, there was a significant inverse correlation between disease duration and NSE serum levels (Spearman's correlation –0.243, p = 0.03). On the other hand, no association was observed between higher NSE serum levels and the presence of esophageal dysmotility or ILD (Table 1). Patients with TSS > 20 presented significantly higher NSE serum concentration as

Table 1. NSE concentration data in patients with systemic sclerosis, according to disease and therapy characteristics.

	Serum NSE (ng/ml)		p	Platelet Lysate NSE (ng/ml)		p	S/PL-NSE Index		p
Disease subtype	Diffuse (n = 42)	Limited (n = 33)		Diffuse (n = 20)	Limited (n = 10)		Diffuse (n = 20)	Limited (n = 10)	
	16.5 ± 13.4$	9.6 ± 5.0	0.006	5.8 ± 4.9	8.1 ± 6.2	0.27	6.17 ± 5.87	3.31 ± 4.48	< 0.001
Disease duration	≥ 5 years (n = 33)	≤ 3 years (n = 37)		≥ 5 years (n = 11)	≤ 3 years (n = 15)		≥ 5 years (n = 11)	≤ 3 years (n = 15)	
	10.8 ± 7.3	16.1 ± 13.6	0.05	7.2 ± 6.7	5.7 ± 4.8	0.51	3.65 ± 3.58	6.78 ± 6.75	0.01
Total skin score	> 20 (n = 20)	< 5 (n = 10)		> 20 (n = 20)	< 5 (n = 10)		> 20 (n = 20)	< 5 (n = 10)	
	19.4 ± 13.0	8.3 ± 2.1	0.01	3.6 ± 2.9	12.4 ± 4.1	< 0.001	7.45 ± 5.57	0.75 ± 0.73	< 0.001
				Clinical Feature					
	Present (n = 38)	Absent (n = 37)		Present (n = 12)	Absent (n = 18)		Present (n = 12)	Absent (n = 18)	
Esophageal dysmotility	12.1 ± 11.0	14.8 ± 11.1	0.29	6.9 ± 6.1	6.3 ± 5.0	0.76	4.60 ± 5.07	5.60 ± 5.90	0.62
Interstitial lung disease	(n = 26)	(n = 49)		(n = 11)	(n = 19)		(n = 11)	(n = 19)	
	12.8 ± 7.4	13.8 ± 12.7	0.71	5.2 ± 4.6	6.9 ± 5.7	0.40	5.64 ± 5.95	4.97 ± 5.44	0.75
				Drug Therapy					
	Yes (n = 53)	No (n = 22)		Yes (n = 18)	No (n = 12)		Yes (n = 18)	No (n = 12)	
Oral prednisone#	13.6 ± 11.4	11.8 ± 10.0	0.53	5.6 ± 5.0	7.8 ± 5.8	0.28	5.43 ± 4.90	4.90 ± 6.60	0.80
IV cyclophosphamide	(n = 19)	(n = 56)		(n = 8)	(n = 22)		(n = 8)	(n = 22)	
	17.0 ± 11.1	12.2 ± 11.0	0.10	4.9 ± 5.3	7.1 ± 5.4	0.34	8.24 ± 5.81	4.12 ± 5.13	0.07
D-penicillamine	(n = 25)	(n = 50)		(n = 11)	(n = 19)		(n = 11)	(n = 19)	
	12.1 ± 8.9	14.2 ± 12.0	0.42	5.5 ± 5.1	7.1 ± 5.5	0.45	6.02 ± 6.16	4.75 ± 5.27	0.55
IV xylocaine	(n = 51)	(n = 24)		(n = 25)	(n = 5)		(n = 25)	(n = 5)	
	15.1 ± 12.7	10.0 ± 5.0	0.06	6.0 ± 5.3	9.1 ± 5.4	0.25	5.94 ± 5.80	1.61 ± 1.15	0.11

$ Mean ± standard deviation. # Prednisone dose < 30 mg/day. IV: intravenous.

compared to those with TSS < 5 (19.4 ± 13.0 ng/ml vs 8.3 ± 2.1 ng/ml, p = 0.01; Table 1 and Figure 1).

The therapeutic regimen routinely used in our patients includes low dose prednisone, low dose D-penicillamine, intravenous cyclophosphamide, and intravenous xylocaine. Although not statistically significant, there was a trend for increased NSE serum levels in patients receiving cyclophosphamide or xylocaine (Table 1).

Among 10 volunteers NSE concentration in the platelet lysate was higher than serum NSE (14.1 ± 6.5 ng/ml vs 6.0 ± 3.1 ng/ml; p < 0.001). The opposite was observed for SSc patients, in whom platelet lysate NSE concentration was lower than that observed in the serum (6.5 ± 5.4 ng/ml versus 15.7 ± 11.9 ng/ml, p = 0.002). SSc patients presented significantly lower platelet lysate NSE concentration than normal volunteers (6.5 ± 5.4 ng/ml vs 14.1 ± 6.5 ng/ml, p < 0.001). Patients with TSS > 20 had lower platelet lysate NSE concentration as compared to those with low skin score (3.6 ± 2.9 vs 12.4 ± 4.1 ng/ml, p < 0.001) (Figure 1). Platelet lysate NSE concentration showed no significant association with disease subtype, disease duration, esophageal dysmotility, or ILD (Table 1).

In order to investigate other possible sources for the elevated serum NSE concentration in SSc patients we compared arterial- and venous-derived serum from 20 SSc patients and showed that the NSE concentration was similar (9.9 ± 5.9 ng/ml vs 9.7 ± 6.1 ng/ml). Since erythrocytes are rich in NSE, hemolysis should be considered as a possible source of elevated NSE serum concentration. However, there was no significant correlation between NSE and LDH serum levels in 13 SSc patients and in 10 volunteers (Spearman's correlation test: r = –0.1; p = 0.5).

In comparison with the isolated variables serum NSE concentration and normalized platelet lysate NSE concentration, the NSE-S/PL index better discriminated volunteers and SSc patients, and SSc patients with high versus low TSS (Figure 2). The NSE-S/PL index was significantly higher in patients with high TSS (7.45 ± 5.57) as compared to those with low TSS and volunteers (0.75 ± 0.33 and 0.42 ± 0.16, respectively; p < 0.001). Patients with diffuse disease and early disease showed higher NSE-S/PL index than those with limited disease and late disease, respectively, but no association was observed with esophageal dysmotility or ILD (Table 1). There was a trend for higher NSE-S/PL index in patients receiving intravenous cyclophosphamide or xylocaine.

Patients and volunteers with NSE serum concentration within the normal range (below 12 ng/ml) presented higher platelet lysate NSE concentration than SSc patients with elevated serum NSE concentration (10.2 ± 6.6 vs 3.7 ± 3.0, p = 0.004). The same was true when only SSc patients were considered (8.15 ± 5.84 vs 3.73 ± 3.00, p = 0.02). Figure 3
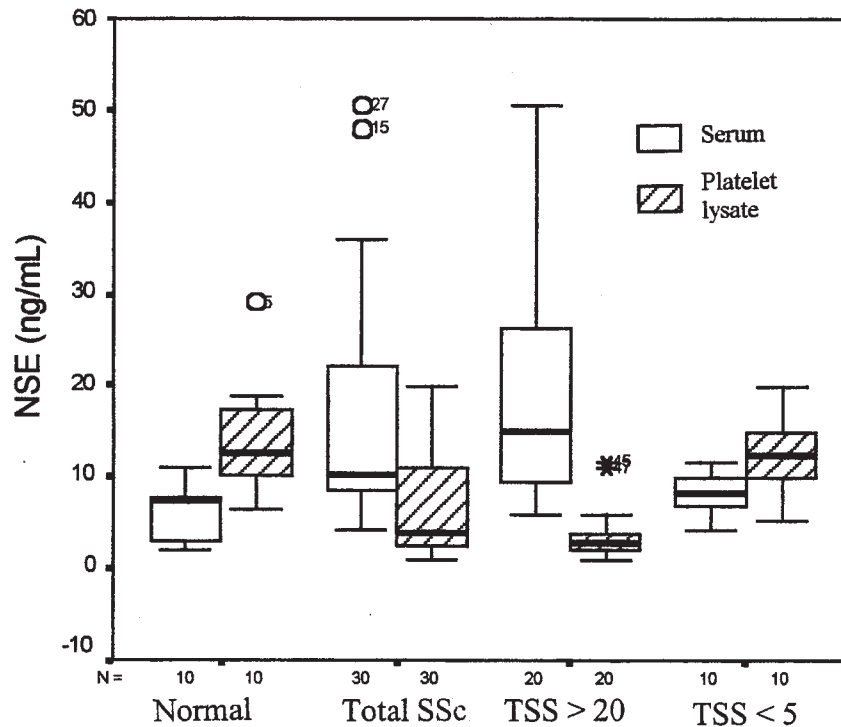
Figure 1. Distribution of serum and normalized platelet lysate NSE concentration in healthy volunteers and in patients with SSc as a whole and grouped according to degree of skin involvement. Rectangles depict 50% of the sample; thick horizontal bar corresponds to median; upper and lower horizontal bars represent highest and lowest figures, respectively. Symbols above the upper horizontal bars represent outliers.
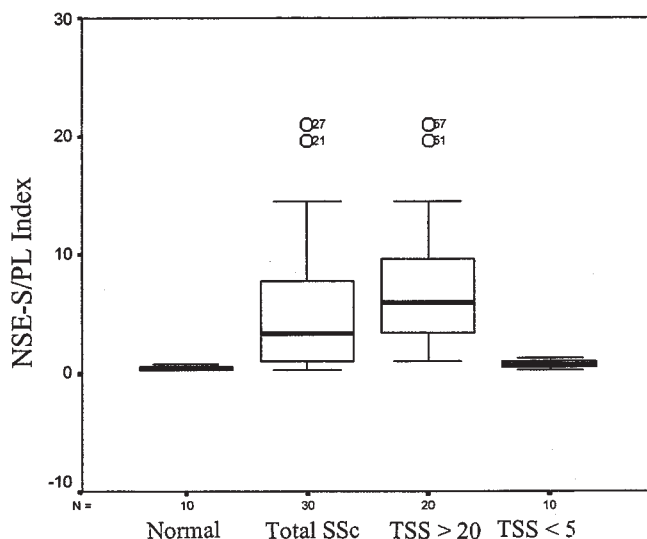

Figure 2. Distribution of the S/PL-NSE index in healthy volunteers and in patients with SSc as a whole and grouped according to degree of skin involvement. Rectangles depict 50% of the sample; thick horizontal bar corresponds to median; upper and lower horizontal bars represent highest and lowest figures. Symbols above the upper horizontal bars represent outliers.
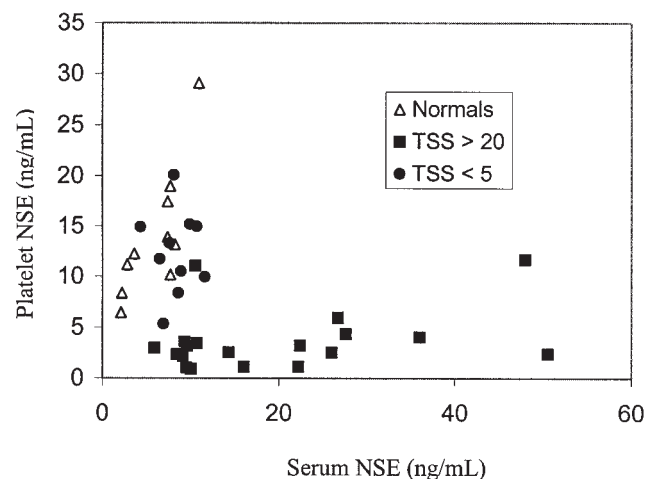
Spearman's correlation: r=-0.400; p<0.01.

Figure 3. Dispersion graph for platelet lysate and serum NSE concentration in 30 patients with SSc and 10 healthy volunteers.

depicts an inverse correlation between NSE concentration in the serum and platelet lysate obtained in volunteers and SSc patients (Spearman's test: r = –0.400; p = 0.01).

DISCUSSION

We were able to demonstrate that a significant fraction of SSc patients presented high NSE serum levels. In many of the patients the magnitude of NSE levels was comparable to that found in cancer patients[13]. This abnormality was restricted to SSc, since all patients with SLE and polymyositis, and all but one patient with idiopathic ILD, presented normal NSE serum concentration. Additionally, high NSE serum concentration appeared to be associated with disease activity as taken by its association with high Rodnan's TSS determination and with early disease.

Although the number of patients in each control group was small, the non-SSc controls comprised 48 individuals, with only one of them (one patient with ILD) with elevated serum NSE concentration, which is strikingly different from the 35% frequency of elevated serum NSE concentration in SSc. There was some variation in the ages of individuals in the various groups. However, this variation was within the young-to-middle-age range and was according to the expected age distribution of each of the diseases involved. This age heterogeneity was not felt to be relevant since there are no reports in the literature of age-interference in NSE serum levels. In addition the analysis of the present series showed no influence of age on NSE serum concentration in non-SSc patients and controls (r = 0.137; p = 0.35). The same is true for ethnic background and smoking habit. Although no drug showed statistically significant association with increased NSE serum levels, there was a trend for higher NSE serum concentration and NSE-S/PL index among patients receiving cyclophosphamide or xylocaine. We believe this was due to the fact that these 2 therapeutic agents are usually used in patients with severe forms of disease and high TSS.

Although there are scant data about NSE in rheumatic diseases, this tumor marker has been reported to be elevated in the serum of patients with non-malignant illnesses such as tuberculosis, severe asthma, cerebral vascular accident, and overt diabetes mellitus[32-36]. Nyberg, et al[9] and Scagliotti, et al[37] have reported on the occurrence of high NSE concentrations in the pleural fluid from patients with non-malignant lung diseases, rheumatoid arthritis, and SLE. Scagliotti, et al have found that NSE serum levels were lower than NSE pleural fluid concentrations[37]. Nyberg, et al confirmed a high pleural fluid/serum NSE ratio in patients with rheumatoid arthritis and suggested local production of NSE[9].

Since high NSE serum levels are usually observed in patients with small cell lung carcinoma, we reasoned that also in SSc the observed high NSE serum concentration might be derived from affected lung tissue. We found no

evidence to support this hypothesis. If that were the case, arterial blood NSE concentration would likely be higher than venous blood NSE levels. In 20 SSc patients, arterial-derived serum showed NSE concentrations similar to those observed in venous-derived serum. Additionally, as pointed out above, patients with idiopathic ILD had serum NSE levels in the normal range. Finally, SSc patients with and without ILD did not differ in NSE serum concentration.

Another possible source of NSE is the erythrocyte and thus the abnormally elevated NSE serum levels in SSc could be related to red cell fragility and subclinical *in vivo* or *in vitro* hemolysis. Since even mild hemolysis is associated with elevated LDH serum concentration, we sought a possible correlation between NSE and LDH serum levels in 13 SSc patients and in 10 healthy volunteers. Spearman's correlation test showed lack of significant correlation between these 2 variables, which indicates that the elevated NSE serum levels observed in SSc are not due to hemolysis.

According to Day and Thompson, NSE is present in platelets at concentrations similar to those observed in neuron cells, while erythrocytes have 10 times less NSE[12]. The present finding of an inverse relationship between serum and platelet lysate NSE concentrations strongly suggests that the elevated NSE serum levels in SSc patients are derived from circulating platelets. This observation is consistent with the well-known demonstration of platelet activation in SSc. In fact SSc patients have been shown to present elevated serum levels of ß-thromboglobulin, platelet factor 4, and thromboxane, as well as circulating platelet aggregates[38-42]. It is believed that platelet activation is triggered by the injured endothelium in the SSc microcirculation and that activated platelets themselves play an important role in the development of SSc microangiopathy[43-47].

A very interesting point in our study was the finding that NSE serum levels were higher in patients with high skin score than in those with low TSS. Although Rodnan's skin score is a subjective index that addresses a single aspect of SSc, it is considered an important variable for disease activity and severity. Apart from rare exceptions, patients with skin score < 5 have less disease activity than patients with skin score > 20. Therefore the isolated classification of patients with high and low TSS may suffice for a crude separation of 2 groups with active and quiescent disease, respectively. With this assumption in mind, our findings suggest that high NSE serum concentration and low NSE platelet lysate levels are associated with disease activity. This interpretation is further supported by the fact that patients with recent disease presented more conspicuous NSE abnormalities than those with long-standing disease. Given the platelet origin for the observed elevated serum NSE levels in SSc, these findings are consistent with previous observations that platelet activation is associated with disease activity in SSc[39]. However, it must be kept in

mind that a single TSS estimation conveys combined information about disease activity and disease severity. To establish the association between NSE abnormalities and disease activity a longitudinal assessment of TSS and serum/platelet lysate NSE concentration is required. The strategy of combining serum and platelet lysate NSE concentrations by means of the NSE-S/PL index proved helpful in emphasizing the differences between SSc patients and volunteers, as well as between SSc patients with high skin score and those with low skin score.

Our study has shown abnormal NSE concentration in the serum and platelets of SSc patients. These abnormalities were associated with the extent of skin involvement and with early disease, suggesting an association with disease activity and platelet activation. This finding may be useful for monitoring disease activity in SSc patients. An appealing feature of NSE as a marker of platelet activation in SSc is that other methods of evaluating platelet activation are rather cumbersome and mostly restricted to investigation laboratories, while NSE determination is part of the menu of most clinical laboratories. Further studies are warranted in order to evaluate a possible application of NSE-S/PL index as an ancillary variable for monitoring disease activity and therapeutic response in systemic sclerosis.

REFERENCES

1. Farghali SA. Tumor markers in gynecologic cancer. Gynecol Obstet Invest 1992;34:65-72.
2. Cooner WH. Definition of the ideal tumor marker. Urol Clin North Am 1993;20:575-9.
3. Pandha HS, Waxman K, Sikora K. Tumour markers. Br J Hosp Med 1994;51:297-303.
4. Azis JK. Tumor markers: Current status and future applications. Scand J Clin Lab Invest 1995;55:153-5.
5. Azis JK. Tumor markers: Reclassification and new approaches to evaluation. Adv Clin Chem 1999;33:169-99.
6. Bates SE. Clinical applications of serum markers. Ann Intern Med 1991;115:623-38.
7. Shimomura C, Eguchi K, Kawakami A, et al. Elevation of a tumor associated antigen CA 19-9 levels in patients with rheumatic diseases. J Rheumatol 1989;16:1410-5.
8. Moncayo R, Moncayo H. Serum levels of CA 125 are elevated in patients with active systemic lupus erythematosus. Obstet Gynecol 1991;77:932-4.
9. Nyberg P, Söderblom T, Petterson T, Riska H, Klockars M, Linko L. Neuron-specific enolase levels in pleural effusions in patients with rheumatoid arthritis. Thorax 1996;51:92-4.
10. Moore BW, McGregor T. Chromatographic and electrophoretic fractionation of soluble proteins of brain and liver. J Biol Chem 1965;240:1647-53.
11. Marangos PJ, Campbell IC, Schmechel DE, Murphy DL, Goodwin FK. Blood platelets contain a neurone-specific enolase subunit. J Neurochem 1980;34:1254-8.
12. Day INM, Thompson RJ. Levels of immunoreactive aldolase C, creatine kinase-BB, neuronal and non-neuronal enolase, and 14-3-3 protein in circulating human blood cells. Clin Chim Acta 1984;136:219-28.
13. Cooper EH. Neuron-specific enolase. Int J Biol Markers 1994;4:205-10.
14. Carney DN. Serum neuron-specific enolase: a marker for disease extent and response to therapy of small-cell lung cancer. Lancet 1982;1:583-5.
15. Cooper EH, Splinter TAW. Neuron-specific enolase (NSE): a useful marker in small-cell lung cancer. Lung Cancer 1987;3:61-6.
16. Jörgensen L, Österlin K, Hansen HH, Cooper EH. The prognostic influence of neuron-specific enolase in small-cell lung cancer. Br J Cancer 1988;58:805-7.
17. Gomm SA, Keevil BG, Thatcher N, Hasleton PS, Swindell RS. The value of tumour markers in lung cancer. Br J Cancer 1998;58:797-804.
18. Cooper EH, Prichard J, Bailey CC, Ninane J. Serum neuron-specific enolase in children's cancer. Br J Cancer 1987;56:65-7.
19. Villard JL, Tiget F, Hartmann O. Serum neuron-specific enolase/non-neuronal enolase ratio in diagnosis of neuroblastomas. Cancer 1988;62:2546-53.
20. Karkela J, Bock E, Kaukinen S. CSF and serum brain-specific creatine kinase isoenzyme (CK-BB), neuron-specific enolase (NSE) and neural cell adhesion molecule (NCAM) as prognostic markers for hypoxic brain injury after cardiac arrest in man. Neuro Sci 1993;116:100-9.
21. Beelen MA, Twijnstra A, van der Pol M, Menheere PPCA. Neuron-specific enolase in cerebrospinal fluid of patients with metastatic and non-metastatic neurological disease. Eur J Cancer 1993;29A:193-5.
22. Massabki PS, Oliveira RM, Oda EE, Andrade LEC. Tumor markers in systemic lupus erythematosus and systemic sclerosis. Rev Bras Reumatol 1998;38:215-20.
23. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee: Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581-90.
24. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus (SLE). Arthritis Rheum 1982;25:1271-7.
25. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975;292:344-7.
26. American Thoracic Society. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. Am J Respir Crit Care Med 2002;165:277-304.
27. Rodnan GP, Lipinski E, Luksick J. Skin thickness and collagen content in progressive systemic sclerosis (scleroderma) and localized scleroderma. Arthritis Rheum 1979;22:130-40.
28. Clements PJ, Lachenbruch PA, Seibold JR, Zee B, Steen VD, Brennan P. Skin thickness score in systemic sclerosis: An assessment of interobserver variability in 3 independent studies. J Rheumatol 1993;20:1892-6.
29. Clements PJ, Lachenbruch PA, Seibold JR, et al. Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. J Rheumatol 1995;22:1281-5.
30. Pahlman S, Esscher T, Bergvall P, Odelstad L. Purification and characterization of human neuron-specific enolase: radioimmunoassay development. Tumor Biol 1984;5:127-39.
31. Mathieu M, Aubry C, Arthur Y, et al. Societé Francais de Biologie Clinique (SFBC). Commission Enzymologie. Ann Biol Clin 1982;40:163-4.
32. Collazos J, Esteban C, Fernández A, Genollá J. Measurement of the serum tumor marker neuron-specific enolase in patients with benign pulmonary diseases. Am J Respir Crit Care Med 1994;150:143-5.
33. Prados C, Alvarez-Sala R. Clinical evaluation of tumor markers in bronchoalveolar lavage fluid [Spanish]. Med Clin Barc 1994;102:174-7.
34. Sellman M, Ivert T, Ronquist G, Persson L, Semb BKH. Central nervous system damage during cardiac surgery assessed by 3 different biochemical markers in cerebrospinal fluid. Scand J Thor

Cardio Surg 1992;26:39-45.

35. Ruibal A, Encabo G, Genollá J, Mañé S, Miralles EM. La enolasa
    específica neuronal sérica en pacientes afectos de patología no
    tumorales. Rev Esp Oncol 1985;32:185-6.

36. Bishop AE, Polak JM, Facer P, Ferri GL, Marangos PJ, Pearse AGE.
    Neuron specific enolase: A common marker for the endocrinal cells
    and innervations of the gut and pancreas. Gastroenterology
    1982;83:902-15.

37. Scagliotti GV, Gatti E, Michelotto F, Pozzi E. Neuron specific
    enolase levels in exudative pleural effusions. J Tumor Marker Oncol
    1990;5:373-7.

38. Kahaleh MB, Osborn I, LeRoy EC. Elevated levels of circulating
    platelet aggregates and beta-thromboglobulin in scleroderma. Ann
    Intern Med 1982;96:610-3.

39. Lee P, Norman CS, Sukenik S, Alderdice CA. The clinical
    significance of coagulation abnormalities in systemic sclerosis
    (scleroderma). J Rheumatol 1985;12:514-7.

40. Lima J, Fonollosa V, Fernández-Cortijo J, et al. Platelet activation
    and endothelial cell dysfunction in the absence of anticardiolipin
    antibodies in systemic sclerosis. J Rheumatol 1991;18:1833-6.

41. Lau CS, McLaren M, Saniabadi A, Belch JJF. Increased whole
    blood platelet aggregation in patients with Raynaud's phenomenon
    with or without systemic sclerosis. Scand J Rheumatol
    1993;22:97-101.

42. Herrick AL, Illingworth K, Blann AD, Hay CRM, Hollis S, Jayson
    MIV. Von Willebrand factor, thrombomodulin, thromboxane,
    beta-thromboglobulin and markers of fibrinolysis in primary
    Raynaud's phenomenon and systemic sclerosis. Ann Rheum Dis
    1996;55:122-7.

43. Blann AD, Illingworth K, Jayson MIV. Mechanisms of endothelial
    cell damage in systemic sclerosis and Raynaud's phenomenon.
    J Rheumatol 1993;20:1325-30.

44. Kahaleh MB, LeRoy EC. Autoimmunity and vascular involvement
    in systemic sclerosis (SSc). Autoimmunity 1999;31:195-214.

45. Massberg S, Enders G, Leiderer R, et al. Platelet-endothelial cell
    interactions during ischemia/reperfusion: the role of P-selectin.
    Blood 1998;92:507-15.

46. Goodfield MJD, Orchard MA, Rowell NR. Whole blood platelet
    aggregation and coagulation factors in patients with systemic
    sclerosis. Br J Haematol 1993;84:675-80.

47. Kahaleh MB, Scharstein KK, LeRoy EC. Enhanced platelet
    adhesion to collagen in scleroderma. Effect of scleroderma plasma
    and scleroderma platelets. J Rheumatol 1985;12:468-71.