Letters to the Editor

Serum neurone-specific enolase in the primary antiphospholipid syndrome: A new biochemical marker for cerebral vascular involvement?

Sirs,

Neurone-specific enolase (NSE) is a peripheral marker of brain neurons affected by ischemia: the subunit gamma (in particular the specific gamma-gamma homodimer) is present in neurons, peripheral nerve tissue and neuroendocrinal tissues (1, 2). NSE is an enzyme of the glycolytic pathway supporting the energy needs of neurons; therefore it is considered to be a sensitive, specific marker of neuroendocrine tumours and of other, non-tumoural diseases such as brain vascular disorders, epilepsy, Guillain-Barrè syndrome, Creutzfeldt-Jacob disease, etc. (1, 2).

In cerebral vascular disorders, serum and spinal NSE levels correlate with the extent of the damage and have a prognostic significance (3). In primary antiphospholipid syndrome (pAPS),the clinical features consist of thrombosis, fetal loss and thrombocytopenia, occurring in association with autoantibodies cross-reacting with cardiolipin and a variety of other negatively charged phospholipids. Stroke, dementia, and multiple small cerebral infarcts are the more common neurologic manifestations of pAPS (4).

In the present study we evaluated the presence and specificity of serum NSE in pAPS, especially for a laboratory screening of those pAPS patients with possible asymptomatic involvement of the brain. We examined 20 pAPS patients, diagnosed after having carefully excluded other disorders which can cause venous and/or thrombosis. Eighteen patients had no signs or symptoms of brain disease, while one 34-year-old female was affected by chronic migraine, and a 45-year-old male had history of ischemic encephalopathy. The 20 pAPS patients were compared with 20 control subjects, and with 10 patients with acute cerebral ischaemia (ACI),10 patients with previous history of cerebral ischaemia (PCI), 10 patients with acute haemorrhagic cerebral lesion (AHCL), and 10 patients with a previous history of haemorrhagic cerebral lesion (PHCL).

Serum samples from patients and controls were tested for NSE (immunoradiometric assay, CIS Diagnostics SpA, Italy), antiphospholipid antibodies (aPL IgG-IgM Screen, immunometric assay, ORGenTec, Germany), and anti-cardiolipin antibodies (Coliza-aCL IgG-IgM, immunometric assay, Chromogenix, Sweden). The pAPS patients and those in the ACI,PCI,AHCL and PHCL groups underwent magnetic resonance imaging (MRI) of the brain; controls did not undergo this examination for obvious ethical reasons.

The patients' and controls' characteristics and the % of detected serum abnormalities are shown in Table I. All 20 (100%) pAPS patients had high aPL titres and 16 (80%) also had elevated aCL titres:this latter finding was due to the heterogeneity of the aPL in their epitopic specificity (5). On the contrary, aPL and aCL were in the normal range in controls and in the other 4 groups. The mean (± SD) serum levels of the different parameters are also shown in Table I. NSE titres were within normal limits in the controls and in the patients with a history of ischemic or haemorrhagic disorder of the brain (Table I). In contrast, a significant increase in serum NSE levels was seen in 10 (50%) pAPS patients, 7 ACI patients (70%) and 9 (90%) AHCL patients (Table I).

Table II shows the serum NSE values for each group. In particular, among the 10 pAPS patients with increase of NSE, 8 (80%) subjects did not present cerebral vascular disorders, while 2 (20%) had acute ischaemic or haemorrhagic cerebral lesion.

To clarifythesignificance of the serum NSE increase in the pAPS patients, they underwent brain MRI. The male patient with a history of ischaemic encephalopathy presented signs of a previous ischaemic lesion at the level of the cerebral frontal lobe. The female patient with chronic migraine and

Table I. Characteristics and serum abnormalities of pAPS patients (n = 20), ACI patients (n = 10), CCI patients (n = 10),AHCL patients (n = 10), CHCL patients (n = 10) and controls (n = 20).

	pAPS n = 20	ACI n = 10	CCI n = 10	AHCL n = 10	CHCL n = 10	Controls n = 20
Male	7	5	6	5	7	7
Female	13	5	4	5	3	13
Mean age (m ± ds)	41 ± 6.4 yrs.	53 ± 7.2 yrs.	55 ± 5.9 yrs.	48 ± 4.9 yrs.	51 ± 6.8 yrs.	40 ± 5.3 yrs.
Mean age of disease (m ± ds)	5.2 2.1 yrs.	2 0.2 days	4.9 2.3 yrs.	1 1.2 days	4.4 3.7 yrs.	
NSE	10 (50%)*	7 (70)*	2 (20%)*	9 (90%)*	1 (10%)*	0
aCL IgG-IgM	16 (80%)*	0	0	0	0	0
aPL IgG-IgM	20 (100)*	0	0	0	0	0

pAPS:Primary antiphospholipid syndrome; ACI:acute cerebral ischaemia; CCI: chronic cerebral ischaemia; AHCL:acute haemorrhagic cerebral lesion; CHCL: chronic haemorrhagic cerebral lesion; aCL: anticardiolipin antibodies; aPL: antiphospholipid antibodies.
*Percentage refers to the total number of patients included in each group.

Serum levels (mean ± SD) of aCL, aPL and NSE in the patients from each group.

aCL IgG (nv < 15 GPD/ml)	32.2 ± 4.1[a]	9.4 ± 1.3[a]	8.9 ± 2.2[a]	9.5 ± 3.0[a]	7.2 ± 1.1[a]	8.1 ± 1.9[a]
aCL IgM (nv < 11 MPL/ml)	24.4 ± 3.8[a]	7.2 ± 0.9[a]	6.6 ± 0.1[a]	7.8 ± 1.1[a]	6.6 ± 0.8[a]	6.9 ± 1.2[a]
aPL IgG (nv < 5 GPL/ml)	18.8 ± 4.4[a]	4.3 ± 0.4[a]	3.8 ± 1.1[a]	3.9 ± 0.7[a]	4.1 ± 1.0[a]	3.4 ± 0.8[a]
aPL IgM (nv < 5 MPL/ml)	16.5 ± 2.4[b]	3.6 ± 1.1[b]	2.9 ± 0.4[b]	3.2 ± 0.9[b]	4.1 ± 1.1[b]	3.9 ± 0.4[b]
NSE (nv: 4.7-14.7 ng/ml)	18.3 ± 4.9[b]	16.2 ± 3.6[b]	9.4 ± 2.1[b]	17.1 ± 4.0[b]	8.3 ± 1.3[b]	7.9 ± 1.4[b]

[a] $p < 0.001$ (Student's t-test); [b] $p < 0.05$.

the remaining 8 pAPS patients with increased serum NSE levels (representing 45% of the pAPS patients) showed multiple small ischemic lesions in the cerebral white substance, a sign of cerebral vasculopathy. Moreover, among the 10 (50%) pAPS patients with normal serum NSE values, only one (10%) showed signs of cerebral vasculopathy; the remainder (90%) had a completely normal MRI.

First of all, our study shows that cerebral vasculitis is particularly frequent in pAPS, as the MRI images demonstrated, even in the absence of clinical neurological manifestations. This evidence emphasises the importance of monitoring pAPS patients, including those who appear asymptomatic, for possible vascular involvement of the brain. Furthermore, our study shows that the serum NSE level could represent a useful screening parameter for identifying pAPS patients at risk of vascular brain disorders, thus possibly avoiding more invasive and expensive examinations. In conclusion, a rise in serum NSE levels seems to be an early peripheral marker of cerebral vasculopathy and could be very useful in the assessment of pAPS patients.

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References

1. MARANGOS PJ, SCHMECHEL DE: Neuron-specific enolase, a clinically useful marker for neurons and neuroendocrine cells. *Ann Rev Biochem* 1987; 10: 269-95.
2. STEVEN H, JAAKOBS C,DE JAGER AE, *et al*.: Neuron-specific enolase and N-acetyl-aspartase and potential makers of ischaemic stroke. *Eur J Clin Invest* 1999; 29: 6-11.
3. CUNNINGHAM RT, MARROW JI, JOHNSTON CF, BUCHANAN KD: Serum neurone-specific enolase concentrations in patients with neurological disorders. *Clin Chim Acta* 1994; 320: 117-24.
4. LEVINE SR, BREY RL: Neurological aspects of antiphospholipid antibody syndrome. *Lupus* 1996; 5: 347-53.
5. GHRAVI AE, HARRIS AN, ASHERSON RA, HUGHES GRV: Anticardiolipin antibodies: Isotype distribution and phospholipid specificity, *Ann Rheum Dis* 1987; 46: 415-7.

Long-term efficacy and toxicity of a combination therapy in rheumatoid arthritis patients

Sir,

We read with interest the paper by Pascalis *et al.* published in a recent issue of this journal (1), in which they treated refractory rheumatoid arthritis (RA) patients with a combination therapy consisting of cyclosporine A (CsA), methotrexate (MTX) and steroids. They concluded that this treatment regimen demonstrated extremely good results in terms of efficacy, tolerability and safety after 96 months of treatment. We would like to make some comments concerning the above study.

Firstly, the concept of combination therapy usually means the use of 2 or more disease modifying antirheumatic drugs (DMARDs) at the same time together with other antirheumatic drugs. In the paper reported by Pascalis such a combination was not used for the following reasons: (a) the main drug given by the authors during the whole period of treatment was CsA, even when it was given on alternative days; (b) at the beginning MTX was administered every week for the first 1 or 2 months, then every 2 weeks, and thereafter every month, for a total period of 12-18 months, after which it was discontinued. For the remaining 6 or 7 years the patients continued to receive CsA.

Secondly, it would be somewhat difficult for a general practitioner or a rheumatologist in private practice to follow the authors' protocol because it is complicated: (a) at entry CsA was administered at a dose of 5 mg/kg/day and then was stopped for 2 or 3 days per week; (b) the MTX dose usually ranged between 0.1 - 0.3 mg/kg/week, administered per os or intramuscularly. In the present study MTX was given intravenously in relatively high doses (15 mg/week). However, this treatment schedule was followed only for a few weeks, after which MTX was administered every 2 weeks and then monthly; (c) steroids were given in a dose ranging between 80 - 130 mg/week/patient, which reflects a daily dose of 10 - 20 mg per patient.

Thirdly, the duration of treatment, which was 96 months or 8 years, should also be taken into consideration. It is amazing that the authors captured the idea of using CsA in combination therapy with MTX and steroids at the beginning of 1989, when CsA was administered as monotherapy in refractory RA patients (2). Nevertheless, their impressive results, presented in Tables II and III, which showed that almost all of the treated patients demonstrated beneficial effects after 2 or 4 years of therapy, have not been published in the meantime. To date, except for the present study, there are only a few published reports concerning the efficacy and safety of CsA alone or in combination therapy for a period of less than 4 years (3-7).

Finally, RA patients are usually treated with one DMARD until complete remission is reached. Unfortunately, complete remission occurs only in a very small number of patients. Furthermore patients are seldom treated for long periods with the same drug or with a combination therapy. The most common reasons for treatment withdrawal are insufficient suppression of disease activity (inefficacy) and/or adverse drug reactions. In the study by Pascalis, drug inefficacy, disease flare and/or severe side effects were not reported, and the combination treatment proposed by the authors has probably solved the problem of treating RA patients.

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References

1. PASCALIS L,ARESU G, PIA G: Long-term efficacy and toxicity of cyclosporin A + fluocortolone + methotrexate in the treatment of rheumatoid arthritis. *Clin Exp Rheumatol* 1999; 17: 679-88.
2. DOUGADOS M, AWADA H, AMOR B: Cyclosporine in rheumatoid arthritis; A double blind placebo controlled study in 52 patients. *Ann Rheum Dis* 1988; 47: 127-33.
3. ZEIDLER HK,KVIEN TK, HANNONEN P *et al*.: Progression of joint damage in early active severe rheumatoid arthritis during 18 months of treatment: Comparison of lowdose cyciosporin and parenteral gold. *Br J Rheumatol* 1998; 37: 874-82.
4. DROSOS AA, VOULGARI PV, PAPADOPOULOS IA, POLITI EN, GEORGIOU PE, ZIKOU AK: Cyclosporine A in the treatment of early rheumatoid arthritis. A prospective, randomized 24-month study. *Clin Exp Rheumatol* 1998; 18: 695-701.
5. DROSOS AA, VOULGARI PV, KATSARAKI A, ZIKOU AK: Influence of cyclosporine A on radiological progression in early rheumatoid arthritis patients. A 42-month prospective study. *Rheumatol Int* 2000; 19: 113-8..
6. TUGWELL P, PINCUS T, YOCUM D: Combination treatment of severe rheumatoid arthritis with cyciosporine and methotrexate in severe rheumatoid arthritis. *N Engl J Med* 1995; 333: 137-41.
7. JOHNS K,LITTLEJOHN G: Clinical experience with combination disease-modifying antirheumatic drug therapy with cyclosporine. *Clin Exp Rheumatol* 1999; 17 (Suppl. 18): S91-4.