Evaluation of evoked potentials and lymphocyte subsets as possible markers of multiple sclerosis: one year follow up of 30 patients

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SUMMARY Evoked potentials and T-lymphocyte helper/suppressor ratio (H/S) were evaluated serially together with neurological status in 30 definite multiple sclerosis patients to evaluate their possible role in monitoring disease progression. Evoked potentials in many cases reflected the clinical status of the pathways tested, but some exceptions were observed, probably due to sub-clinical relapses or physical factors. In some instances the occurrence of subclinical relapses was suggested by increased H/S ratios. Serial H/S values increased in parallel with clinical and sub-clinical relapses, and seemed to show specific patterns in relation to the type of clinical course (relapsing, stable, chronic progressive). Our results suggest that evoked potentials and H/S ratio serial analysis can contribute to a better assessment of the progress of multiple sclerosis.

Multiple sclerosis is a neurological disease with great variability of symptoms and progress, which makes the assessment of the course of the disease difficult. There is evidence that clinical data alone insufficiently describe the real extent of central nervous system (CNS) involvement, as demonstrated by the presence, in multiple sclerosis patients, of abnormal cranial CT scans and evoked potential abnormalities without related signs.¹ Evoked potentials permit a quantifiable and objective evaluation of neurological damage of the pathways tested, even in asymptomatic patients. They could provide useful data on neurological status and its evolution but published findings appear conflicting.²⁻⁸ Several other laboratory indices of multiple sclerosis have been proposed,⁹ mainly related to immunopathological processes. Recent papers stress the presence of lymphocyte abnormalities, mainly concerning the number and the function of blood and CSF suppressor T-cells (for a review see ref 10). The helper/suppressor (H/S) ratio, defined by monoclonal antibodies has been found to be increased during clinical relapses.¹¹⁻¹³ However, the correlation between laboratory and clinical findings is not always present, thus affecting the interpretation of this laboratory finding.¹⁴⁻¹⁷

The aim of our study was to evaluate and compare evoked potentials and lymphocyte subpopulations as possible indices for monitoring the course of multiple sclerosis.

Subjects and methods

Patients

Thirty definite multiple sclerosis patients (according to the criteria of Poser *et al*¹⁸ were studied: 14 males, 16 females, mean age 31.6 years (range 17-44), mean duration of disease 7.8 years (range 2-20). Disease evolution was assessed approximately monthly, recording clinical history (a relapse was defined as the appearance of new symptoms or signs) and neurological status. The same day, lymphocyte subsets were evaluated collecting blood samples always at the same hour (9.00-10.30 am); evoked potentials were recorded quarterly, in the afternoon. The follow up lasted 1 year. All patients gave their consent to take part in the study.

Visual evoked potentials (VEPs)

VEPs were recorded in response to a LED pattern reversal (total field 5.5°, single check 55'). Further details are reported elsewhere.^{19,20} P₂ latency was considered: in our laboratory the normal upper limit (+3 SD) is 113.1 ms, the limit of inter-trial variability (assessed in 10 normal subjects) is 8.7 ms.

Somatosensory evoked potentials (SEPs)

SEPs were recorded in response to medial nerve stimulation at wrist: 500-1000 stimuli were given, with intensity sufficient to produce thumb adduction, at the frequency of 4 Hz. Responses were recorded from Erb's point (bandwidth

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Fig 1 Visual evoked potential latency changes in the course of the study. Patients with stable responses were omitted. Normal range (mean $\pm$ 3 SD) is marked by the grey strip. The mean value of P2 latency (99.6 ms) and the limits of inter-trial variability ($\pm$ 8.7 ms) are reported.

Table 1 Evoked potential results at entry and at the end of the follow up

	Abnormal response at entry	Follow up results: responses				
		Unchanged	Improved	Worsened	Temporarily improved	Temporarily worsened
VEPs	25 (11)	19 (19)	2 (1)	6 (3)	1 (0)	2 (1)
BAEPs	15 (11)	21 (18)	1 (0)	6 (3)	0	2 (1)
SEPs	17 (3)	20 (20)	0	10 (2)	0	0

() number of patients whose neurophysiological data were in agreement with the clinical evaluation of the tested pathways.

1–1000 Hz), from the 7th cervical spine (bandwidth 1–2000 Hz) and from the contralateral parietal focus (bandwidth 1–2000 Hz), 2 cm behind C3–C4, referenced to Fz. In this study, only N₉–N₁₃, N₁₃–N₁₉ and N₉–N₁₉ interpeak latency (IPL) were considered. In our laboratory the upper normal limits ($\pm$ 3 SD) are respectively 5.2, 7.4, 11.2 ms. The upper limits of inter-trial variability (in 10 normal subjects) were respectively 1.1, 2.1, 2.3 ms.

Brainstem auditory evoked potentials (BAEPs)

BAEPs were recorded from C2-ipsilateral mastoid electrodes in response to 1000–2000, 11 Hz alternating clicks, 70 dB above sensation level (bandwidth 100–3000 Hz).

Only I–III, I–V, III–V interpeak latency (IPL) was considered. In normal subjects the upper limits ($\pm$ 3 SD) are respectively 2.61, 4.47, 2.52 ms; the limits of inter-trial variability (in 10 normal subjects) were 0.25, 0.43, 0.23 ms.

Fig 2 H/S ratio profiles in the course of the study. Patients are grouped according to their clinical course. The normal range (mean $\pm$ 2 SD) is marked by the grey strip.

Criteria of evoked potentials evaluation

For each patient the limit of inter-trial variability, defined in normal controls, was recalculated proportionally to his/her own (generally delayed) latencies. For example for VEP P₂ = 128 ms the limit was 12.4 ms (= 128 × 8.7/99.6), where 8.7 and 99.6 are respectively the inter-trial variability and the mean P₂ latency of controls. According to this criterion, responses were classified as "stable", "worsened", improved".

T-lymphocyte subsets

T-lymphocyte subpopulations of the peripheral blood were analysed by direct immunofluorescence on an automated laser flow cytometry system (ORTHO SPECTRUM III).

Monoclonal antibodies of the OKT series were used (ORTHO-MUNE): OKT₃ defines mature T-cells, OKT₄ marks the helper/inducer subset, OKT₈ the suppressor/cytotoxic subset.

In this study the OKT₄/OKT₈ ratio (H/S) was considered: in our laboratory the normal value was 1.5 ± 0.2. The upper limit was 1.9 (+2 SD).

Results

Neurophysiological results at entry and evoked potentials changes in the follow up are reported in table 1 correlated with the involvement of the CNS pathway tested. Responses were classified as "stable", "worsened" or "improved" considering the whole profile during the period of observation. In a small number of cases changes were only temporary. VEP changes in the individual patients are given in fig 1; BAEP and SEP results are omitted because of their complexity (three parameters for each side respectively).

VEP P₂ latency increased steadily in six cases (nos 12, 17, 21, 22, 27, 30), without clinical correspondence in three of them. In two cases (nos 13, 28) the latency increased temporarily but only in one (no 28) was it related to an attack of optic neuritis. In one case (no 20) a temporary improvement was noticed in one eye, with return of latency within normal limits (99 ms), followed by a stable worsening (latency: 128 ms) in successive recordings. A progressive decrease of latency was observed in two patients (nos 23, 24). Responses were unchanged in the remaining 19 cases (see fig 1).

BAEPs were unchanged in 21 cases, including three patients who had a relapse involving the brainstem.

Responses worsened in eight cases (in three without corresponding clinical signs): in three cases for an increase of I-V and III-V IPL (bilateral in two), in three cases for disappearance of wave V in one side and of wave III and successive ones in the other side. In two of them BAEPs worsened temporarily. In one case responses improved progressively.

SEPs did not change in 20 cases, worsened steadily in ten (in eight without clinical correspondence) and improved in none. In 14 arms the worsening concerned the IPL N13-N19, in six the IPL N9-N13.

In six cases evoked potentials deteriorated in the absence of related neurological signs, but were associated with deterioration of neurological status.

Serial analysis of H/S ratios is illustrated Fig 2, in which patients are grouped according to their clinical course during the period of observation. Three distinct patterns were observed: patients with constantly high values (12 cases with no more than three normal determinations), patients with normal baseline but occasionally increased values (ten cases), patients with constantly normal H/S ratios (eight cases), (see also table 2).

The first group consisted of all six chronic-progressive patients plus patients 9, 10, 14 (stable) and patients 21, 22, 24 (relapsing). In the second group the temporarily raised H/S values were related with clinical relapses in four cases (nos 16, 18, 19, 20) (that is the relapse occurred in the two weeks preceding or following the blood analysis) unrelated in four cases (nos 7, 8, 12, 13) both related and not in two cases (nos 17, 23).

H/S ratio fluctuations were unrelated to clinical relapses but associated with evoked potential worsening in five instances (twice in case 21 and one respectively in cases 13, 17, 19) suggesting the occurrence of sub-clinical exacerbations. It must be noted that in patient 19 the subclinical relapse (BAEP wave V disappearance) followed a clinical relapse (lower limbs paresis) while H/S ratios were increased. In four instances (nos 11, 18, 19, 20) evoked potentials deteriorated while H/S ratio remained within normal values. It must be noted that patient 15 had a clinical relapse unrelated with any H/S fluctuation or evoked potentials worsening.

Laboratory data compared with clinical course are

Table 2 Analytical correlation of clinical course with evoked potential and H/S ratio results during the period of observation

Patients	Clinical course																													
	Stable														Relapsing										Chronic progressive					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Evoked potentials	=	=	=	=	=	=	=	=	=	=	+	+	+	+	=	=	+	+	+	+	+	+	-	-	=	=	+	+	+	+
H/S ratios	=	=	=	=	=	=	+	+	+	+	=	+	+	+	=	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

EPs: = stable + increased latencies (at least in one EP) - decreased latencies (at least in one EP).

H/S ratios: = stable + temporary fluctuations + constantly high.

Table 3 Correlation of clinical course with H/S ratio and evoked potentials results

Course	H/S ratio			Evoked potentials		
	Constantly high	Temporarily high	Normal	Stable	Improved	Worsened
Chronic progressive n = 6	6	0	0	2	0	4
Relapsing n = 10	3	6	1	2	2	6
Stable n = 14	3	4	7	10	0	4
Statistical significance	χ^2 16.5 (p < 0.005)			χ^2 3.9 (p < 0.10)		

The correlation EP results—clinical course reached a statistical significance grouping chronic progressive and relapsing patients—active MS—(χ^2 6.9, p < 0.025).

reported in table 2. In the stable group, both evoked potentials and H/S ratios were stable in six out of 14 cases.¹⁻⁶ Among the ten relapsing patients, both evoked potentials and H/S ratios fluctuated in agreement with the clinical course in eight cases (nos 17-24). In the chronic-progressive group evoked potentials worsened and H/S ratios were constantly high in four out of six cases. The H/S ratio pattern appeared significantly related to the clinical course (p < 0.005, table 3). The correlation between evoked potentials pattern and clinical course reached statistical significance (χ^2 = 6.9 p < 0.025) only grouping chronic-progressive and relapsing patients (active multiple sclerosis), indicating that stable responses were more frequently observed in stable patients (inactive multiple sclerosis).

Discussion

The long term assessment of multiple sclerosis patients presents several difficult problems, and it would be useful to have reliable indicators of disease activity in addition to clinical evaluation.⁹ Our study was undertaken to evaluate whether serial evoked potentials and lymphocyte subsets determinations could contribute to the assessment of disease progression. The role of evoked potentials in monitoring multiple sclerosis lesions has been evaluated by several investigators²⁻⁸ but an important methodological question has not been adequately answered: whether changes in successive recordings are related to actual changes of neurological status or to normal variability.⁴ A variation of $\pm 10\%$ ⁵ has been proposed as a criteria to evaluate test to test variability. Aminoff *et al*⁸ calculated the inter-trial variability in a control group but used this data only for statistical comparison of variability between multiple sclerosis patients and controls. Weerd & Jonkman³ considered the inter-trial variability of normal subjects and calculated its normal upper limit. However, we believe that this is insufficient, because multiple sclerosis patients frequently have abnormally prolonged latencies. For this reason we proportionally adjusted the limit of inter-trial variability in each patient, to fit their latencies.

Having defined this methodological question, it

seems important to discuss the role of evoked potentials in the evaluation of the clinical course. A concordance between serial neurophysiological responses and neurological status of the tested pathways was observed in 24 cases testing by VEPs, in 22 testing by BAEPs, in 22 testing by SEPs. Such a concordance appears slightly more frequent in our series than appear in published reports and probably depends on our more restrictive limits of intertrial variability.

In 19 instances evoked potentials changed in the absence of related clinical signs. BAEPs appeared unchanged in three patients in spite of a relapse involving the brainstem. It is interesting to note that in six patients evoked potentials deteriorated in the absence of clinical signs of the tested pathways, but were associated with involvement of other, untested pathways.

In a small number of recordings evoked potentials improved: temporarily in one instance, progressively in the course of successive recordings in three others. This finding has also been observed in other series⁵ and may be ascribed to different mechanisms: the reduction of oedema,²² the removal of blocking factors active on synaptic transmission,²³ the remyelination of CNS.^{13 24 25} In 16 instances (four for VEPs, four for BAEPs, eight for SEPs; see table 1) evoked potentials worsened without clinical correlation: this fact can be interpreted as the occurrence of a subclinical relapse (suggested in cases with high H/S ratio: see below). However, other factors, unrelated to pathological events, may intervene: the increased susceptibility of demyelinated fibres to chemical-physical factors such as pH, electrolyte changes and temperature,^{22 24 26} and the possible role of blocking factors.²³

In addition to serial evoked potentials recordings, a further contribution to the definition of the clinical course is derived from the analysis of blood lymphocyte subpopulations. The possible role of T-cell subset analysis as a reliable marker of disease activity has been suggested by some authors^{17 27 28} but denied by others.^{14 16} In our series a statistically significant relationship between H/S ratios and clinical course was observed in a large number of cases (table 3): constantly normal values were associated with a stable course (half of 14 cases), baseline normal levels with

temporary increases to a relapsing-remitting course (in six out of 10 cases), baseline high levels to a chronic-progressive course (all of six cases). However, some exceptions were observed: as already stated, three stable and three relapsing patients showed constantly high H/S ratios, whereas four clinically stable subjects demonstrated transitory abnormal values. Finally, an isolated patient suffered from a clinical exacerbation without any laboratory change. It must be stressed that in five instances a rise in H/S ratio over normal values was related to a subclinical relapse as defined by evoked potentials deterioration, whereas four other times a subclinical relapse was accompanied by a normal H/S ratio.

Some authors^{27,28} have pointed out that some clinical relapses are unrelated to any lymphocyte abnormality: the occurrence of transient fluctuation of neurological status (for example fatigue), misinterpreted as a disease exacerbation, has been noted. To avoid this source of error, we restricted the definition of relapse to the appearance of new symptoms or signs: in this way the occurrence of a relapse associated to a normal H/S ratio was observed only in one patient. The opposite phenomenon (transient T subset modification not followed by a clinical relapse) has also been described,^{27,28} suggesting that demyelinating lesions can involve silent CNS sites or produce only a subclinical damage. In this study the parallel analysis of T-lymphocyte subsets and evoked potentials contributed in clarifying the possible occurrence of subclinical exacerbations in patients with temporarily increased H/S ratios and apparently stable clinical course: in four patients an increased ratio was observed to coincide with the worsening of at least one evoked potential.

However, the comparison of clinical, immunological and neurophysiological results must be considered in the light of some inevitable restrictions: in our study H/S ratios were assessed monthly whereas evoked potentials were recorded only quarterly, because of the obvious limitations in time. In some instances, this led to difficulty in evaluating the exact temporal relation between neurophysiological and immunological findings. More frequent evoked potentials recordings and the association of neuroimaging techniques probably would further increase the number of cases in which increased H/S ratios could be related to clinically silent relapses. However, such an approach involves high costs and is time consuming. Comparing clinical and laboratory data, a concordance between the course and the indices of disease activity was found in many cases: in the clinically stable group, stable evoked potentials recordings and H/S ratios were observed in five patients whereas in the remaining subjects the laboratory findings showed an unsuspected activity of the

disease. It will be of interest to observe whether, in the future, stable patients with high H/S ratios and progressive worsening of evoked potentials will change their clinical course to a chronic-progressive one. In the relapsing group both an increased H/S ratio and a worsening of evoked potentials were observed in six out of ten subjects as related to the exacerbation of the pathological process. In the chronic-progressive group we observed a most peculiar pattern: baseline high H/S ratios together with a tendency to a progressive worsening of successive evoked potentials recordings in four out of six cases.

The role of laboratory tests in multiple sclerosis monitoring has been differently evaluated in previous reports (see introduction). Our results point out the significant correlation between T-lymphocyte subsets and clinical course. Furthermore the association of evoked potentials recordings clarified the phenomenon of lymphocyte subset fluctuations in stable patients, disclosing the occurrence of subclinical relapses in some instances. The correlation between evoked potentials data and clinical course was less strict, but a relationship between stable responses and stable courses has been demonstrated. In some instances, evoked potentials revealed changes unsuspected on the basis of clinical evaluation. To conclude, evoked potentials recordings and lymphocyte subset determination seem to contribute to better identification of multiple sclerosis activity and disease progression.

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