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Intrathecal IgG synthesis: marker of progression in multiple sclerosis patients

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Objectives – We study the power of IgG synthesis value as a marker of disease activity in multiple sclerosis (MS). *Material and methods* – Link index was calculated in 202 MS patients. Time between first, second and third attack and progression index (PI) were compared in patient with normal (NLI) high (HL) or very high Link index (VHLI). *Results* – Secondary progressive (SP) patients had a higher LI than relapsing–remitting (RR) and primary progressive (PP) courses (1.10 ± 0.5 for SP vs 0.86 ± 0.5 for RR and 0.81 ± 0.5 for PP, $P = 0.01$ and 0.03, respectively). Having a HLI in MS RR and SP patients has no time effect in the development of the second and third attack. PI was higher in patients with VHIL (0.67 ± 0.7) vs patients with NLI (0.42 ± 0.4, $P = 0.008$) and with HLI (0.39 ± 0.3, $P = 0.001$). *Conclusions* – This study confirmed that LI is a good marker of subsequent progression of MS.

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Though the global course of multiple sclerosis (MS) is well known when considering large cohorts of patients, it is rather difficult to make a prognosis for a given patient. It is essential however, to predict the course of the disease as early as possible in order to be able to give the patient the right treatment at the right time.

The potential of magnetic resonance imaging (MRI) to serve as a surrogate marker of disease on patients with MS is increasingly recognized. In contrast, the use of cerebrospinal fluid (CSF) analysis to evaluate the activity has received less attention and even considered of no value in diagnostic pathology (1). The most constant findings in the CSF of MS patients are the increased levels of immunoglobulin G (IgG) and oligoclonal bands. Although these abnormalities are not specific, they have been found in 90% of patients with MS (2).

Some authors reported that the negativity of intrathecal synthesis of oligoclonal IgG bands is related to short lasting (3) benign (4) clinical courses of the disease. The amount of IgG synthesis has appeared to be less dependent on clinical variables (5) but some authors found some relation with increase in gammaglobulins in spinal fluid and higher progression of disease in Spanish patients (6). In patients with isolated monosymptomatic optic neuritis, Jacobs et al. (7) found that abnormal IgG levels in the CSF correlated more strongly than abnormal MRIs with the subsequent development of clinically definite MS.

Treatments with steroids cause a significant reduction of the CSF IgG synthesis rate as measured by quantitative tests (8) while other immunocompetent treatment as azathioprine (9, 10) do not.

In this retrospective study we used IgG intrathecal shyntesis measured by Link index (LI) as a possible marker of disease activity for further evolution.

Material and methods

Patients

A total of 202 patients (115 women and 87 men) were included in this study. The patients were followed from the diagnosis (the CSF sample was obtained at the same time), until the end of the study (31-12-99). At the end of the study the

patients was classified as relapsing–remitting (RR), secondary progressive (SP) and primary progressive (PP) courses. A total of 142 patients were classified as RR courses, 23 as PP and 37 as SP courses. Mean age at onset was 29.27 ± 9.4 years and time of evolution until last examination was 10.26 ± 6.5 years. The delay at diagnosis was 3.5 ± 5 years. Onset symptoms were classified as motor, sensitive, ocular, brainstem, cerebelo-vestibular, sphincterian and plurisymptomatic.

CSF samples were obtained by lumbar puncture from the 202 patients with clinically definite MS. The CSF and a simultaneously blood sample were immediately centrifuged at 50 ×*g* for 10 min. Serum and CSF samples were stored at −80°C. A routine measurement included a CSF leukocyte count and IgG synthesis. IgG and albumin in CSF and serum were measured by immuno-nephelometry and LI calculated by each patient. We have previously published the control values of IgG synthesis calculations made in CSF of patients without neurological disease (11). The CSF was obtained after the first consultation for diagnosis purposes.

In RR and SP courses, time between first and second attack (b1–b2), and between second and third (b2–b3) were calculated and measured in days. Data of first attack was often retrospective, and delay between first attack and first consultation (data of lumbar puncture) was 3.5 ± 5 years. Progression index (PI) was defined dividing the EDSS score of last examination by the evolution time of the patients. Patients were grouped by LI as normal link index (NLI) if the LI was below our normal referential values or high link index (HLI) if the LI was above this value. We sub-classified HLI in only high link index (OHLI) and in very high link index (VHLI) for some comparisons. The cutoff was established in 1.0 (mean of LI values of abnormal group, 1.0 ± 0.5).

We also divided our patients into two groups taking into account the fact of whether they had been treated (TP) or not treated (NTP) with interferon beta in any of their presentations, routes of administration and dose.

Statistical methods: Data analysis was performed using analysis of variance (ANOVA), Bonferroni test, chi-squared, two-dimensional regression analysis and Kaplan–Meier survival curves (Log-rank test).

Results

The mean age at onset was 29.3 ± 9, age at the end of the follow-up was 39.3 ± 11 years, and mean EDSS score was 3.5 (range 0–9.5) with a typical bimodal distribution.

The percentage of HLI in our patients was 71.3. Patients with RR courses had 70% positive results for LI, in PP it was 60.9% and in SP cases 83.8%. These differences were not statistically significant. Thirteen patients underwent a second or third lumbar puncture without relevant changes in the LI, these data were not included in the analysis of results.

When we calculated the mean LI in each type of evolution SP had a higher LI than the other two (1.10 ± 0.5 for SP vs 0.86 ± 0.5 for RR and 0.81 ± 0.5 for PP, $P = 0.01$ and 0.03, respectively). Women had a higher LI (0.97 ± 0.5) than men (0.84 ± 0.4), $P = 0.03$. In our series onset symptoms neither predicted the appearance of second and third attack, nor progression of the disease. In all cases patients with VHL index tend to progress faster than others and patients with motor, sensitive and plurisymptomatic symptoms at onset, progress faster that others patients but without statistical significant differences. Sensitive onset patients with VHL index progressed even faster (Fig. 1).

The fact having a HLI in MS RR and SP courses has no time effect in the development of the second attack (time to conversion to definite MS) (b1–b2 in NLI B of 837.1 ± 897 days vs 1026 ± 1276 days in patients with HLI). The fact of having a HLI in MS RR and SP courses has no time effect in the development of a third attack after the second (b2–b3 in NLI B of 572.4 ± 556 days vs 713.4 ± 987 days in patients with HLI). Survival analysis Kaplan–Meyer curves show that the evolution of patients with HLI and NLI are identical for both b1–b2 and b2–b3 delay (Fig. 2

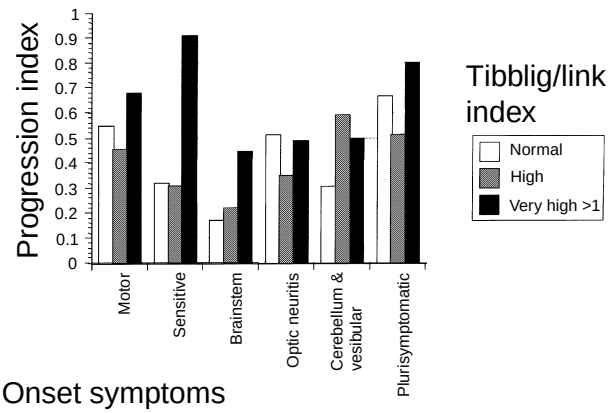

Figure 1. Onset symptoms and progression index of disease. In all cases patients with VHL index tend to progress faster than the other two groups (Normal and HLI). Patients with motor, sensitive and plurisymptomatic symptoms at onset, progress faster than other patients but without statistical significant differences. Sensitive onset patients with VHL index progressed even faster (Fig. 1).

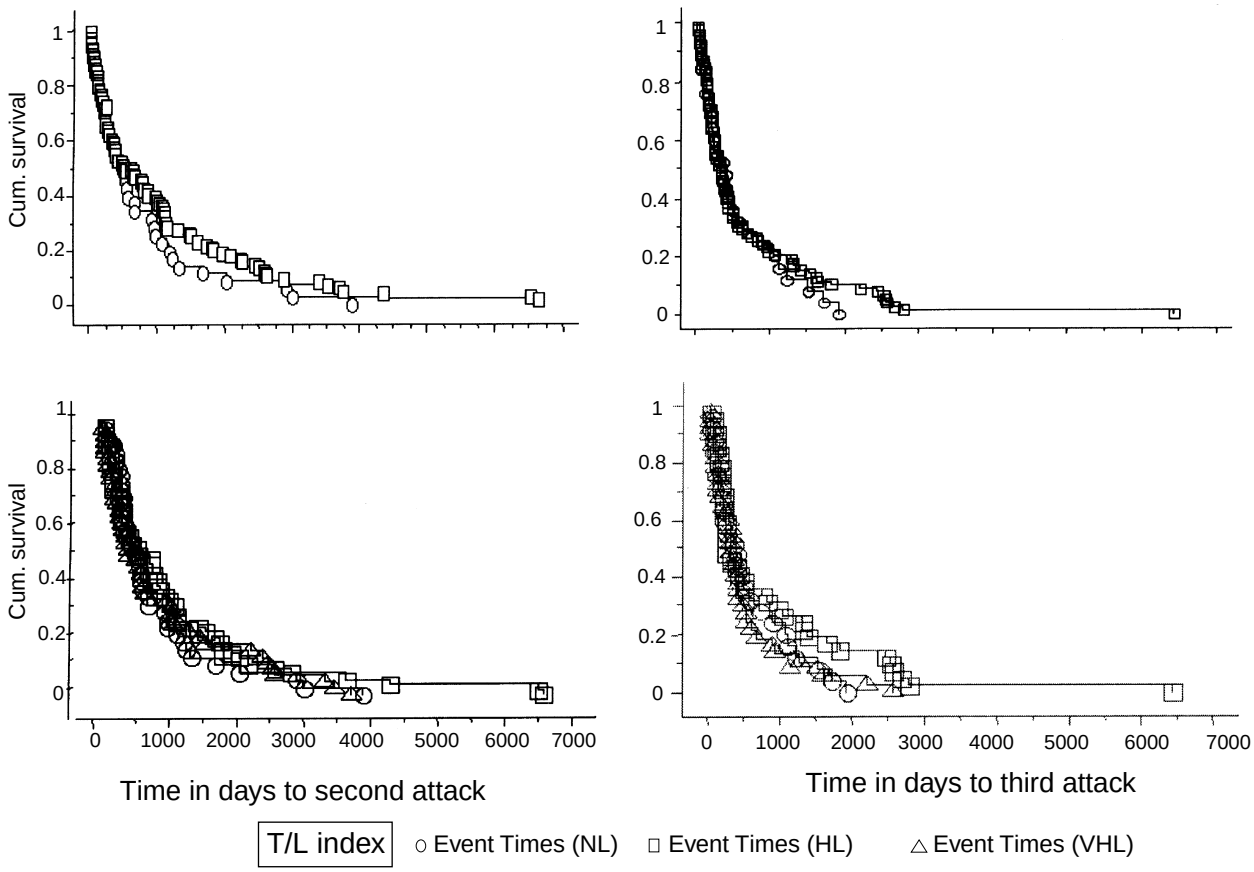

Figure 2. (A and B) Kaplan–Meier survival curves showing that the evolution of patients with HLI and NLI are identical for predicting second and third attack delay. (C and D). Kaplan–Meier survival curves showing that the evolution of patients with VHLI, HLI and NLI are identical for predicting second and third attack delay.

A, B). The differences were not significant when we considered three groups of LI (NLI, HLI and VHLI), neither for ANOVA comparisons nor for survival analysis Kaplan–Meyer curves (Fig. 2C, D). We did not observe any difference in the delay of a second attack occurrence after the first for patients with normal or HLI. In the treated group patients had a similar delay in the occurrence of a third attack in both groups (Normal and HLI groups) (Fig. 3A). In the non-treated group Kaplan–Meyer survival curves showed a faster occurrence of a third attack after the second in patients with HLI (Fig. 3b).

The PI was higher in patients with VHIL (0.67 ± 0.7 EDSS points/year, $n = 64$) in comparison with the two other groups (0.42 ± 0.4 points/year, $n = 56$, in NLI, $P = 0.008$ and 0.39 ± 0.3 points/year, $n = 78$, $P = 0.001$ in OHLI).

The 70 patients who received treatment with interferon beta had previously a higher LI that the 132 who would not receive it along the evolution of the disease (1.00 ± 0.5 vs 0.85 ± 0.5, $P = 0.04$). The patients with VHLI had more probabilities of being treated in the following years (29 out of 36, 44% than patients with HLI, 28 of 79, 35% than patients with normal LI, 13 of 45, 22%, $P = 0.03$).

Discussion

The mean age and duration of disease, gender course and EDSS distribution score of our patients are representative of the total of the 445 MS patients in our series (data not shown).

In our series mean IgG index is 0.9, lower than in Rudick et al. study (1.25), but similar to other series that used immuno-nephelometry method to measure IgG and albumin proteins. In Rudick et al. series the technique used was not standardized because the study was multicentric and in addition patients were very active in the number of relapses prior to the inclusion in the clinical trial. The percentage of positive LI was 83.8 in cases with SP courses in our study (70% in RR without conversion to SP and 60.9% in PP courses of disease). In addition SP courses had statistically significant higher mean LI than the other two groups. These differences indicate that patients who will develop progressive courses after a RR

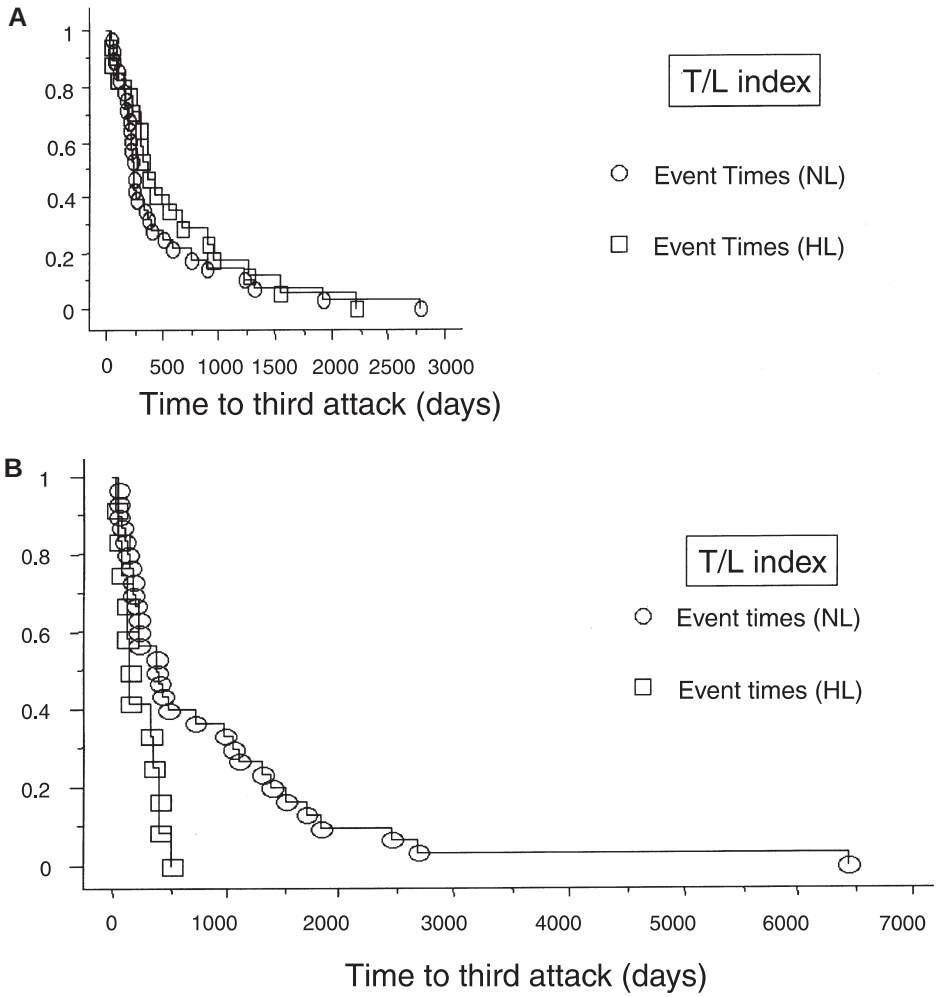

Figure 3. (A) Treated patients. In this group patients had a similar delay for presenting a third attack in both groups (Normal and HLI). (B) Non-treated patients. In this group Kaplan–Meier survival curves showed a faster occurrence of a third attack after the second in patients with HL index.

phase had a higher LI index in comparison with patients who did not change from RR to SP and also with patients who have PP courses of MS. The intrathecal IgG production tends to be stable once it is established and we think that the increase of IgG at the moment of diagnosis in MS patients, could be in relation with the subsequent development of progression.

In our patients the increase of IgG LI did not show a good correlation with the time of presentation of the second attack or with the time of appearance of the third. Some authors found that the increase of IgG index in the CSF, as well as the presence of oligoclonal bands, increase the risk of occurrence of a second relapse (12, 13).

As with Rudick et al. we found higher levels of IgG LI in females compared with males. We found a statistically significant difference between the two groups. Rudick et al. found a correlation of LI with a higher frequency of oligoclonal bands in the female group (92 vs 84% in males). We think that this data must be kept in mind although we cannot offer a good interpretation.

PI is the EDSS progression rate of disability and until now the best-known marker of clinical activity. In our patients PI is related to the presence of high LI. When we established a cut-off for normal or abnormal IgG index using our own reference values, the differences were not statistically significant, but they were when we established a cut-off in 1.0. The differences were statistically significant between patients with VHL index and the rest of the patients. The patients with VHL index at the moment of diagnosis progressed almost twice as much as the others. Other authors found also that the prognosis in MS is related to the presence of an abnormal humoral immune response within the central nervous system, and a more benign course of the disease is also more often accompanied by a normal CSF IgG index (14). This data is supported

by the recent study of Sellebjerg et al. who showed that in patients with a first attack suggestive of MS, intrathecal IgG synthesis was significantly associated with the presence of abnormalities on T2-weighted cerebral MRI (15), although the analysis of this study was only qualitative.

Other data of interest in this study is the fact that the patients who would be considered active for treatment purposes had a significant higher LI than other patients. We interpret that patients with treatment have generally a more active form of the disease and would require treatment.

Treatments with steroids cause a significant reduction of the CSF IgG synthesis rate as measured by quantitative tests while other immunocompetent treatments such as azathioprine do not. Other treatments as interferon beta 1a (AVONEX) produced a significant reduction of white blood cells (WBC) in the CSF of 62 treated patients in comparison with the placebo. In the same study the WBC was a good predictor in the placebo group of subsequent disease activity. The patients with abnormal WBC had more relapses and the EDSS increases more(0.9 vs 0.33, $P = 0.04$) after the 2-year follow-up. In the same study baseline WBC count showed a statistically moderately strong correlation with IgG index among other parameters, $r = 0.335$, $P < 0.0001$. More interestingly, IgG index and WBC at baseline showed a weak but statistically positive correlation with Gd-enhancing lesion volume and T2 lesion volume (16). With all this data it is difficult to understand why the authors of this study concluded that IgG index did not predict study disease activity in the placebo recipients.

OCB negative clinically definite MS cases seems to have a relatively benign prognosis (17), but IgG synthesis could be a better marker of disease activity measured by MRI in patients who start with optic neuritis (18).

As Iivanainen pointed out (19), more longitudinal studies are needed to clarify the sensitivity of the prognostic immunological tests and etiological significance of these abnormalities in MS.

This study confirmed that IgG levels do not correlate with the presence of more attacks in the following years, but our data show that IgG index can be a good marker of further progression of the disease. Patients with IgG index above 1.0, could have an increased risk of progression and must be considered as candidates to be treated as soon as possible with immunomodulator agents. We think that IgG index must be included as a surrogate marker of disease activity in prospective studies of MS with other clinical and paraclinical predictors and must be considered as marker of disease activity.

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