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Mult Scler 2011 17: 327 originally published online 1 December 2010
DOI: 10.1177/1352458510388543

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Intrathecal IgM synthesis in pediatric MS is not a negative prognostic marker of disease progression: quantitative versus qualitative IgM analysis

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Abstract

Background: Intrathecal IgM synthesis is reported to be associated with a worse prognosis in adults with multiple sclerosis (MS).

Objective: To study the predictive value of intrathecal IgM synthesis for the clinical course of pediatric MS.

Methods: Seventy children with onset of MS before the age of 16 years and followed for a median period of 10.4 years (range: 0.4–22.8 years) were studied. The two subgroups with ($n=44$) or without ($n=26$) intrathecal IgM synthesis were distinguished by a new, very sensitive, evaluation of quantitative analysis in cerebrospinal fluid (CSF)/serum quotient diagrams (Reibergrams). The clinical course and EDSS (Expanded Disability Status Scale) scores at five and ten years were compared with IgM frequencies between both groups with a new statistics program for CSF data.

Results: The cohort of children without intrathecal IgM production had higher numbers of attacks in the first two years and shorter time intervals between first and second attack, although this was not statistically significant ($p=0.04$, $p=0.15$ respectively). In addition there was also a trend for girls without intrathecal IgM synthesis to have a higher EDSS score after 10 years compared with the group with IgM synthesis.

Conclusion: Intrathecal IgM synthesis is not associated with a more rapid progression of disability in pediatric MS. Reevaluation of data from previous reports about the negative predictive value of intrathecal IgM synthesis in adult MS with a CSF statistics tool show that the apparent contradiction is due to a methodological bias in the qualitative detection of 'oligoclonal' IgM or linear IgM index.

Keywords

cerebrospinal fluid, children, IgM, multiple sclerosis

Date received: 10th May 2010; revised: 13th August 2010; 28th September 2010; accepted: 30th September 2010

Introduction

In multiple sclerosis (MS) intrathecal IgM synthesis occurs in 41% of adult patients and in 55% of pediatric patients with an onset of disease before 16 years.¹ These data were evaluated with a new sensitive statistics concept, which corrects for age-related dynamics of blood-derived proteins in cerebrospinal fluid (CSF), and indicate that frequency and patterns of the humoral immune response is similar in adult and pediatric MS patients.^{1–3} The only difference between both MS groups was the quantity of intrathecal IgG and IgM synthesis.¹

Intrathecal IgM synthesis in neurological disease is part of the disease-related IgG/IgA/IgM patterns, but,

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unlike the isotype switch observed in the blood of patients with an infection, it is not an indication of an acute phase of disease.^{4,5}

Intrathecal IgM synthesis in adult MS patients has been thought to represent a prognostic marker for a more rapid disease progression,⁶ based on interpretations of intrathecal IgM by the linear IgM index.⁷ But, as shown recently, the linear evaluation of an index is unsuitable for the statistical comparison of groups¹ and does not allow an unbiased discrimination between blood-derived and brain-derived IgG or IgM.⁵ A particular confusion in the field comes from interpretation of the qualitative detection of 'oligoclonal' IgM, mainly propagated by a single group.^{8,9} In contrast with the established sensitivity of oligoclonal IgG, the existence of 'oligoclonal' IgM, as detected after isoelectric focusing, is questioned by other groups.⁴

In order to evaluate intrathecal IgM in CSF as a prognostic marker in pediatric MS, we compared groups using an established, nonlinear, evaluation program^{4,5} which now allows a more sensitive detection of intrathecal IgM for statistical purposes^{2,3} and accurately evaluates the frequencies and mean quantities of intrathecal IgM synthesis.¹ In addition, we used this program to reevaluate the quantitative data for MS patients reported in the literature combined with the qualitative analysis of 'oligoclonal' IgM.⁶ This report provides, for the first time, an explanation of the false negative and false positive interpretations due to oligoclonal IgM analysis.

Methods and patients

Pediatric patients

The pediatric MS group contained 70 patients (46 girls, 24 boys, ratio 2:1) diagnosed as definitely having MS according to the Poser or McDonald criteria^{10,11} and a disease manifestation before the age of 16 years. All patients were part of a larger, referral-based cohort ($n=121$) with suspected MS who were admitted to the Department of Pediatric Neurology, Georg-August University Göttingen, Germany, between 1987 and 2007. Median age at MS onset was 13.0 (range: 4–16 years). Only children with a set of data including oligoclonal IgG, albumin, IgG and IgM values in serum and CSF, were included. The group of MS patients overlaps with the group of pediatric patients in reference [1].

Baseline data collection included gender, age at disease onset, clinical presentation, and neuroradiological and CSF findings (Table 1). A relapse was defined as a worsening of preexisting neurological symptoms or the appearance of new symptoms lasting more than

24 hours. In general, children were seen every six months until the age of 18 years with the EDSS determined at each visit.

Patients who were older than 18 years or were not seen within the previous 6 months ($n=58$) were evaluated via a standardized telephone interview¹² to assess the EDSS score, recent relapses and changes in treatment modalities. Families and adult patients gave written consent before the telephone interview. Children older than 16 years of age at the time of MS diagnosis were not included in the study. The study was approved by the Ethics committee of the Medical Faculty of the University of Göttingen, Germany.

CSF studies

Data were obtained from CSF routine analyses in the Neurochemistry Laboratory, Department of Neurology, Georg-August University, Göttingen, Germany.

The spinal tap was performed in the majority of children ($n=60$) after the first attack. In 10 children the spinal tap was performed after the second attack. Children were not treated with steroids or disease modifying drugs at the time of the spinal tap. Albumin, IgG, and IgM were analyzed by standard immunochemical nephelometry (Dade-Behring nephelometer, Marburg, Germany).

Data were evaluated numerically and graphically in CSF/serum quotient diagrams.^{1,5,13} The intrathecal, locally synthesized contribution of IgX (G, M) in CSF was calculated as intrathecal fraction IgX_{LOC} (mg/l).^{4,5} For statistical purposes the intrathecal IgG and IgM synthesis was interpreted with reference to the $Q_{\text{mean}} + 2\text{SD}$ (standard deviations) as upper limit of the reference range for blood-derived proteins in CSF, instead of the diagnostically more specific border $Q_{\text{lim}} = Q_{\text{mean}} + 3\text{SD}$.^{2,3} This improved the sensitivity for detection of intrathecal IgM synthesis. The figures of $\text{IgM}_{\text{LOC}} > 0$ and $\text{IgM}_{\text{LOC}}(\text{mean}) > 0$ for the different reference lines are compared in a previously published study.⁵ The new CSF statistics tool which counts frequencies of increased values and also calculates mean intensities for comparison of groups compensates for the age-related difference of the barrier function.^{1–3}

The CSF data including cell count, total protein and oligoclonal bands for most patients have been reported previously.^{1,14}

Treatment

Treatment for relapses consisted of intravenous methylprednisolone 20 mg/kg/day for three consecutive days. Although until recently not approved for children with MS, immunomodulatory treatment options were offered after the second attack in the majority of

Table 1. Clinical and demographic data of 70 children with MS, divided into two groups depending on the presence (Group 1) or absence (Group 2) of intrathecal IgM synthesis

Clinical and demographic data	All children	Group 1 IgM positive	Group 2 IgM negative	p-value
Number	<i>n</i> = 70 ^{a,b}	<i>n</i> = 44	<i>n</i> = 26	
Gender (♀/♂), ratio	46/24 (1.9:1)	30/14 (2.1:1)	16/10 (1.6:1)	
Gender, age at disease onset (♀/♂)				
4–10 years, <i>n</i> (ratio)	8/8 (1:1)	7/4 (1.8:1)	1/4 (0.25:1)	
>10–16 years, <i>n</i> (ratio)	37/17 (2.2:1)	22/11 (2:1)	15/6 (2.5:1)	
Age at disease onset, median (range)	13.0 (7.0–16.0)	13.2 (7.0–16.0)	13.0 (7.0–16.0)	0.419
Disease duration ^c in years, median (range)	10.4 (0.4–21.6)	10.4 (0.4–21.6)	10.5 (3.9–18.3)	0.261
Disease course, <i>n</i> (%)				
RRMS, EDSS 0	32 (45.7)	24 (55.0)	8 (30.7)	
RRMS, EDSS ≥ 1 and ≤ 4	28 (40.0)	14 (31.8)	14 (53.8)	
SPMS, EDSS ≥ 4	10 (14.2)	6 (13.6)	4 (15.4)	
Total number of attacks, median (range)	4.5 (1–47)	4.0 (1–47)	5.5 (1–26)	0.325
Number of attacks in year 1 + 2, median (range)	2.0 (1–11)	2.0 (1–11)	3.0 (1–10)	0.043
Interval between 1st and 2nd attack (months), median (range)	7.0 (1.0–72.0)	7.1 (1.0–72.0)	6.5 (1.0–42.6)	0.155
Poly- versus monosymptomatic presentation at initial attack	51/19	31/13	20/6	0.584
Ratio	2.7:1	2.4:1	3.3:1	
Disease modifying therapies				
Current therapy unknown, <i>n</i> (%)	8 (11.4)	5 (11.4)	3 (11.5)	
Patients without therapy, <i>n</i> (%)	20 (28.6)	13 (29.5)	7 (26.9)	
Patients with therapy, <i>n</i> (%)	42 (60.0)	26 (59.0)	16 (61.5)	
Interferons	28 (66.6)	17 (65.3)	11 (68.8)	
Glatiramer acetate	8 (19.0)	4 (15.4)	4 (25.0)	
Intravenous immunoglobulins	2 (4.7)	1 (3.8)	1 (6.3)	
Azathioprine	1 (2.3)	1 (3.8)	0	
Mitoxantron	1 (2.3)	1 (3.8)	0	
Others (e.g. monoclonal antibodies)	2 (4.7)	2 (7.7)	0	

^a all children were from Caucasian descent, apart from one african.

^b 67/70 children had oligoclonal bands.

^c disease duration: from initial clinical symptom onset.

children seen after 1998. Of the 70 children, 61 had been treated in the past with immunomodulatory drugs including interferons, glatiramer acetate, intravenous immunoglobulins or azathioprine. At the time of evaluation 43 subjects were still on immunomodulatory treatment (Table 1).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences for Windows (SPSS Inc., Chicago, IL, USA, Version 15.0) and the CSF statistics tool.^{2,3} Normal distribution of data was tested using the Kolmogorow–Smirnov test. Due to significant departure from normality at an alpha level of 0.05 for several variables investigated, non-parametric tests were used

throughout the analyses. Between-group comparisons of metric data were performed using a two-tailed Mann–Whitney U test, and for categorical data the Chi-square and Fishers Exact test were used. Spearman's rank correlation was applied to analyze associations among intrathecal IgM synthesis, number of relapses during the first two years, interval between first and second attack, mono- or polysymptomatic first attack, gender and EDSS score.

Multiple linear regression analysis was applied to evaluate the influence of sex, age, IgM status, cell count, protein and oligoclonal bands on the number of attacks. To control for the rate of false positive test results due to multiple comparisons, Bonferroni correction was applied, with a more conservative level of significance set at 0.001.

Results

Seventy patients with pediatric MS and a median disease duration of 10.4 years (range 0.4–22.8 years) were included in this study (Table 1). All children had a relapsing–remitting disease course of which 10 developed a secondary–progressive course during follow-up. Seventeen of the 70 children (24%) were younger than 10 and 55/70 children (76%) were between 10 and 15 years of age at initial presentation. In the total group, girls were more likely to be affected than boys (Table 1).

The majority of all children (51/70) had a polysymptomatic manifestation at the first attack including visual ($n=31$), cerebellar ($n=24$), brainstem ($n=23$), sensory ($n=18$) and pyramidal symptoms ($n=15$) (Table 1). The median time interval between the first and second attack was seven months (range: 1–72 months). In the first two years after disease onset the median number of attacks was 2.0 (range: 1–11).

The median EDSS score after one year was 0.0 (range 0.0–4.0; $n=70$) and after two years 0.0 (range 0.0–7.0; $n=62$). At 5 and 10 years of disease duration the median EDSS score increased to 0.5 (range 0.0–6.5; $n=60$) and 1.0 (range 0.0–5.5; $n=26$) respectively (Table 2).

Factors which were correlated with a higher EDSS score at 5 and 10 years of disease duration were the number of attacks in year one and two ($r=0.381$, $p=0.002$ and $r=0.434$, $p=0.024$, respectively) and the total number of relapses ($r=0.356$, $p=0.005$ and $r=0.686$, $p\leq 0.001$ respectively). In addition a shorter time interval between the first and second attack was weakly correlated with a higher EDSS score after one and two years of disease duration ($r=-0.266$, $p=0.032$ and $r=-0.289$, $p=0.025$ respectively).

Female as compared to male gender was linked with a significantly higher number of relapses in the first two

years (3.3 ± 2.0 vs 2.3 ± 1.5 ; $p=0.018$) and a shorter time interval between first and second attack (10.9 ± 13.4 vs 19.8 ± 19.2 ; $p=0.047$). Female gender was linked with a higher EDSS score at 10 years ($EDSS\ 2.4\pm 2.3$ vs 0.7 ± 1.0), although not statistically significant ($p=0.054$).

The interval between first and second attack, a polysymptomatic initial presentation and age at disease onset were not associated with a higher EDSS score at 5 and 10 years of disease duration.

Sensitive detection of intrathecal IgM synthesis

Intrathecal IgM synthesis was determined with reference to the $Q_{mean} + 2SD$ as upper border of the reference range for blood-derived proteins in CSF. This increased the frequency of IgM synthesis in the first available lumbar puncture in our cohort of pediatric MS patients compared with reference to Q_{lim} ($Q_{mean} + 3SD$) from 50% to 61%.¹ As shown in Figure 1 the cohort was divided into two groups according to the presence ($n=44$ patients, filled squares) or absence ($n=26$ patients, open circles) of intrathecal IgM synthesis. Baseline characteristics such as gender, age at presentation, time of follow-up, EDSS score at the beginning of the disease, presence of oligoclonal bands and the number of children treated with immunomodulatory drugs were not different between the two groups (Table 1).

Clinical correlations

The main results of this part of the study were as follows: 1) children without intrathecal IgM synthesis had a higher number of attacks in the first two years (median: 3.0 (range 1–10) vs median: 2.0 (range 1–11); $p=0.043$) (Table 1); 2) girls without intrathecal IgM

Table 2. Median (range) Expanded Disability Status Scale (EDSS) score for all children with pediatric MS from our cohort ($n=70$) after 1, 2, 5 and 10 years of disease duration and depending on the presence or absence of an intrathecal IgM synthesis

	EDSS score, median (range)			
	All children	Group 1 IgM positive	Group 2 IgM negative	<i>p</i> -value
1 st year	0.0 (0.0–4.0) ($n=70$)	0.0 (0.0–3.5) ($n=44$)	0.0 (0.0–4.0) ($n=26$)	0.939
2 nd year	0.00 (0.0–7.0) ($n=62$)	0.0 (0.0–6.0) ($n=36$)	0.3 (0.0–7.0) ($n=26$)	0.695
5 th year	0.5 (0.0–6.5) ($n=60$)	0.0 (0.0–6.0) ($n=34$)	2.3 (0.0–6.5) ($n=26$)	0.242
10 th year	1.0 (0.0–5.5) ($n=26$)	1.0 (0.0–3.5) ($n=13$)	3.0 (0.0–5.5) ($n=13$)	0.258

Figure 1. Comparison of CSF data from 70 children with MS with (squares) and without (open circles) intrathecal IgM synthesis. The reference range (bold line for discrimination between blood- and brain-derived fractions in CSF) is based on data of 4100 patients. The hyperbolic function to fit the data is theoretically founded. The diagram in this figure was created with a new CSF Statistics Tool (www.COMED-com.de).

synthesis had a three-fold higher EDSS score (median: 3.5, range 0.0–7.5) after ten years of disease duration compared with girls with intrathecal IgM synthesis (median: 1.0, range 0.0–3.5; $p=0.094$) (Table 3); 3) girls without intrathecal IgM synthesis had more attacks in the first two years after disease onset than girls with intrathecal IgM synthesis (median: 4.0 (range 2.0–10.0) vs median: 2.0 (range 1–11); $p=0.048$) (Table 3).

Further results which underscore that intrathecal IgM synthesis is not associated with a worse prognosis were that: 4) children with intrathecal IgM synthesis experienced their second attack after 7.1 months (range 1.0–72.0) as compared with children without intrathecal IgM synthesis after 6.5 months (range 1.0–42.6); $p=0.16$) (Table 1); 5) children without IgM synthesis had a higher number of attacks as compared with children without intrathecal IgM synthesis (median: 5.5 (range 1–26) vs median: 4.0 (range 1–26); $p=0.33$) (Table 1); 6) children with intrathecal IgM synthesis had a lower EDSS score after 5 ($p=0.24$) and 10 years ($p=0.26$) (Table 2); 7) the total number of attacks in girls without intrathecal IgM synthesis was higher compared with girls with intrathecal IgM synthesis (median: 6.0 (range 2–26) vs median: 4.0 (range 1–26); $p=0.28$) (Table 3); 8) the intrathecal IgM synthesis was not associated with the presence or absence of a secondary progressive course in our cohort ($p=0.84$), which correlated predominately with duration of the disease ($p=0.002$).

No significant association was found between a monosymptomatic or polysymptomatic manifestation of the initial attack and presence/absence of intrathecal IgM synthesis ($p=0.78$).

In multiple linear regression analysis, neither presence/absence of intrathecal IgM synthesis ($p=0.87$), gender ($p=0.23$), nor age at onset, as metric variable ($p=0.79$) was significantly associated with the number of attacks. Including age as a categorized variable in our regression models ($</>10$ years) did not change our findings. Similarly, the above mentioned variables were not significantly associated with the time interval between the first and second attack (all $p \geq 0.20$).

In addition, intrathecal IgM synthesis did not significantly correlate with the number of inflammatory cells ($r=0.27$, $p=0.079$), protein level ($r=0.077$, $p=0.53$) or age at onset ($r=0.14$, $p=0.24$).

Individual time course of intrathecal IgM synthesis

Sixteen children with intrathecal IgM synthesis in the initial spinal tap had more than one lumbar puncture for diagnostic reasons. In 10/16 patients the IgM_{LOC} (mean) remained constant (investigated over up to 7 years), in two the intrathecal amount decreased and in four the amount increased (see also reference [1]).

Table 3. (a) Comparison of girls with multiple sclerosis and an intrathecal IgM synthesis ($n = 30$) and girls without intrathecal IgM synthesis ($n = 16$) concerning the median EDSS score (range) after 5 and 10 years of disease duration, the total number of attacks and the number of attacks in the first two years after MS manifestation. (b) Comparison of boys with multiple sclerosis and an intrathecal IgM synthesis ($n = 14$) and boys without intrathecal IgM synthesis ($n = 10$) concerning the median EDSS score (range) after 5 and 10 years of disease duration, the total number of attacks and the number of attacks in the first two years after MS manifestation

(a)			
	Girls IgM positive	Girls IgM negative	<i>p</i> -value
EDSS 5 th year; median (range)	0.3 (0.0–6.0) ($n = 22$)	1.0 (0.0–6.5) ($n = 15$)	0.521
EDSS 10 th year; median (range)	1.0 (0.0–3.5) ($n = 8$)	3.5 (0.0–5.5) ($n = 7$)	0.094
Total number of attacks; median (range)	4.0 (1.0–26.0) ($n = 30$)	6.0 (2.0–26.0) ($n = 16$)	0.227
Number of attacks in year one and two; median (range)	2.0 (1.0–11.0) ($n = 30$)	4.0 (2.0–10.0) ($n = 16$)	0.048
(b)			
	Boys IgM positive	Boys IgM negative	<i>p</i> -value
EDSS 5 th year; median (range)	0.0 (0.0–4.0) ($n = 12$)	2.3 (0.0–3.5) ($n = 10$)	0.349
EDSS 10 th year; median (range)	0.5 (0.0–1.5) ($n = 4$)	0.0 (0.0–3.0) ($n = 5$)	0.893
Total number of attacks; median (range)	5.0 (1.0–47.0) ($n = 14$)	5.0 (1.0–25.0) ($n = 10$)	0.976
Number of attacks in year one and two; median (range)	2.0 (1.0–7.0) ($n = 14$)	3.0 (1.0–40) ($n = 10$)	0.169

Discussion

As reported earlier for this group of children with MS,¹ the complete, neuroimmunological pattern in CSF of a chronic inflammatory CNS disease is already present in pediatric MS at first clinical manifestation, that is, inflammatory signs are not gradually evolving.^{1,14} Regarding the time related dynamics of the immunoglobulin concentrations and intrathecal antibody synthesis, we do not observe a change of immunological patterns. This is confirmed by the individual time course of intrathecal IgM synthesis in 16 patients in this report where the IgM response remains constant in the individual patient over a long time. These observations are consistent with the absence of an IgM/IgG isotype switch in CNS.^{4,5} The IgM class synthesizing B cells migrate in a transcapillary fashion into the CNS and are not transformed there, as they are in the primary lymphatic organs.^{15,16} However, it remains interesting to consider whether intrathecal IgM synthesis is associated with a more severe course of the disease as stated in earlier reports.^{6,8,9}

Several studies in adult MS patients report that intrathecally synthesized IgM is associated with

relapses and an earlier onset of secondary progressive disease. Villar et al. showed that 'oligoclonal' IgM in the CSF detected by isoelectric focusing is associated with a poor long term outcome.^{6,8,9} Only one study so far reported that intrathecal synthesis is not associated with an increased risk of a relapse after a first clinically isolated syndrome.¹⁷ This study also showed that there was no correlation between the presence of IgM-OCB, the number of such bands and the IgM index.

Our results are also in contradiction to the reports for adults with MS: the presence of intrathecal IgM synthesis in children with MS correlates with a lower relapse rate in the first two years.^{18–20} We also show that intrathecal IgM synthesis is not linked with other negative prognostic markers such as female gender or a polysymptomatic first attack.^{18,19} Furthermore, girls with MS and an intrathecal IgM synthesis had a lower EDSS score at 5 and 10 years of follow-up, which is also in contrast to findings in adults with MS.^{6,8,9}

These discrepancies led us to question the method used in the earlier reports and the reliability of their interpretations. We reevaluated the quantitative IgM data reported in one publication⁶ in parallel with the interpretation of 'oligoclonal IgM'. The evaluation with

Figure 2. Data of MS patients from Villar et al.⁶ transferred in a CSF/serum quotient diagram (Reibergram with hyperbolic reference line for blood-derived versus brain-derived IgM in CSF). Squares are reported by Villar et al. as positive for CSF restricted oligoclonal IgM bands ($n = 10$). Open circles are reported by Villar et al. as negative for oligoclonal IgM ($n = 12$). From the diagram only 5 cases are above the diagnostically relevant upper limit ($Q_{lim} = Q_{mean} + 3SD$), shown as bold line of the reference range, and definitely above the statistical discrimination line (not shown) with $Q_{mean} + 2SD$. This means that one patient got a *false negative* interpretation applying the oligoclonal IgM method. With reference to the statistically relevant range ($Q_{mean} + 2SD$, including 96% of the normal controls) only a total of 7 cases (filled squares) were detected as intrathecal IgM synthesis in the statistically founded diagram. This means that 4 cases in the group with oligoclonal IgM synthesis according to Villar are with high probability *false positive* interpretations by the qualitative method. High CSF IgM values associated with high albumin quotients (the two squares most to the right) are false interpretations, indicating that the qualitative method does not clearly discriminate between blood- and brain-derived IgM. The resulting consequences for the data correlation with clinical EDSS score in the newly arranged groups of patients according to the quantitative evaluation are shown in Table 4.

the statistics tool³ using CSF/serum quotients results in a different interpretation of patients as shown in Figure 2: the detection of IgM synthesis by oligoclonal IgM can result in false negative (1/12) and false positive (4/10) interpretations. If we associate the EDSS scores from the study by Villar et al.⁶ for the newly arranged groups according to our interpretation (Table 4), the mean EDSS scores for adult MS patients are the same in the groups with and without IgM synthesis, i.e. the interpretations become similar to our results for children, and at least the contradiction disappears. The reason for the discrepancies is the bias in the method for detection of 'oligoclonal' IgM. The dissociation of IgM pentamers into monomers before F leads

Table 4. Comparison of IgM data interpretation from qualitative 'oligoclonal IgM' analysis as performed by Villar et al.⁶ and quantitative intrathecal IgM (IgM_{loc}) of the same patients. The quantitative evaluation of intrathecal synthesis with IgM_{loc} results in different numbers (n) of cases with intrathecal IgM in CSF which leads to a similar mean EDSS score in both groups, different from interpretation of the qualitative analysis, with false negative and false positive interpretations as shown in Figure 2

	'Oligoclonal' IgM ^a		IgM _{loc} ^b		
	n	EDSS score	n	EDSS score	median
		mean		mean	
IgMpos	10	1.7	7	1.21	1.5
IgMneg	12	0.79	15	1.2	1.5

^a original data of Villar et al.⁶ with IgM positive if CSF restricted oligoclonal IgM bands in IEF.

^b original data of Villar et al.⁶ reevaluated in Quotient diagrams with IgM positive if $Q_{IgM} > Q_{mean} (IgM) + 2SD$ (CSF Statistics Tool).

to a few broad beams. This is different from the numerous, sharp bands in cases of classical oligoclonal IgG analysis⁴ and may be the consequence of an arbitrary reassociation of the separated monomers. This is the reason why many researcher in the field raise the question whether these beams can be called 'oligoclonal'. In any case from our Figure 2 it is obvious that higher protein concentrations (increased Q_{alb}) which include higher blood-derived IgM fractions in CSF lead to the obviously false positive interpretations in the qualitative method.

Another bias in the interpretation of IgM data derives from the application of the linear IgM Index which must be replaced by the nonlinear, hyperbolic function²¹ for the relation between Q_{IgM} and Q_{alb} , as shown in earlier publications.^{1,4}

The main limitations of the study are the small number of children with a follow-up of 10 years and the telephone-based EDSS interview, which was chosen because patients older than 18 years were not followed at our institution. The telephone-based EDSS interview has been used in other studies and has been shown to correlate well with the actual neurological examination in older adults.¹² One important reported weakness of the telephone-based EDSS interview is that the interclass correlation coefficient for those with EDSS below 4.5 is only moderately good¹² which might have influenced our findings because most of the children prospectively followed reached EDSS levels below 4.5.

In summary, according to our data, intrathecal IgM synthesis in children with MS is associated with a better long term prognosis, in particular in girls, and does not correlate with a higher relapse rate and other negative prognostic markers. There seems to be no difference between pediatric and adult MS.

Again as shown earlier¹ pediatric and adult MS differ only quantitatively in neuroimmunological patterns but this does not allow for discrimination between 'early' and 'late' onset MS.

Acknowledgements

We wish to thank the children, adolescents and their parents who kindly agreed to take part in this study. Further, we would like to thank Dr Uli Wurster, Hannover, for his helpful discussions on the base of his expertise in isoelectric focusing of immunoglobulins. Statistical analysis was carried out by M Rauchenzauner and A Strasak.

Funding

This work was supported by a grant of the Hertiestiftung (GHS 191/00).

Conflict of interest statement

None declared.

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