Cut-off evaluation of intrathecal oligoclonal bands of IgM in relapsing-remitting multiple sclerosis; a retrospective study

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ABSTRACT

Background: Multiple sclerosis (MS) is the most common demyelinating disease and characterized by immunological changes. Oligoclonal bands of IgG in CSF not seen in corresponding serum have been used for many years as part of the diagnostic criteria. However, considerably less is known about the role of IgM, despite several studies showing marked changes to IgM metabolism in MS. Bands of oligoclonal IgM (o-IgM) are more difficult to determine than oligoclonal IgG, thus limiting their study, and there is no agreement as to whether o-IgM in CSF should be part of the clinical work-up of MS. Nevertheless, there is a possibility that such bands might provide a prognostic marker if a cut-off could be established.
Materials and methods: In this pilot study, paired samples of CSF and serum from 37 patients with relapsing-remitting MS (RRMS) and 57 controls with no subsequent signs of neurological disease were analysed for total IgM, and bands of o-IgM were visualised by isoelectric focusing and western blot. Patient records were used to compare mean changes in Expanded Disability Status Scale (EDSS) over a maximum of 17 years.
Results: None of the controls displayed extra o-IgM in CSF compared to corresponding serum, whereas additional o-IgM band(s) were seen in CSF in most patient samples (70%). After five years of disease, there was a significant difference in the EDSS between patients with no extra o-IgM compared to patients with at least one extra o-IgM band. This difference increased over time. If a cut-off of two or more extra bands of o-IgM in CSF was applied, this difference was not found.
Conclusion: These exploratory data suggest that o-IgM support the prognostic potential for RRMS, and though tentative, the occurrence of any bands of o-IgM restricted to CSF seems to result in poorer prognosis. Despite the small size of the groups, the data infer that the absence of CSF-restricted o-IgM is good news for the patient. The results need to be reproduced in a more comprehensive study.

List of abbreviations

MS	multiple sclerosis
IgM	immunoglobulin M
o-IgM	oligoclonal IgM
CSF	cerebrospinal fluid
RRMS	relapsing-remitting MS
EDSS	expanded disability status scale
IgG	immunoglobulin G
o-IgG	oligoclonal IgG
SPMS	secondary progressive MS
CIS	clinical isolated syndrome
CNS	central nervous system
ELISA	enzyme-linked immunosorbent assay
IgM-HRP	IgM-horseradish peroxidase
TMB	tetramethylbenzidine
HCl	hydrochloric acid
UIC	university of Illinois at Chicago
TCEP	Tris(2-carboxyethyl)-phosphine
IEF	isoelectric focusing

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SDS   sodium dodecyl sulfate
PVDF   polyvinylidene fluoride
PBS   phosphate buffered saline
ECL   electrochemiluminescence
Ln   natural logarithm
NTNU   Norges teknisk-naturvitenskapelige universitet
LP   lumbar puncture
ANOVA analysis of variance

1. Background

The diagnostic criteria for multiple sclerosis (MS) make substantial use of the measurement of IgG: in its concentration in CSF compared to serum, and calculation of the IgG index. Another qualitative use of IgG is oligoclonal IgG (o-IgG) detection performed routinely to assist the diagnosis of clinically definite MS (Thompson et al., 2018). The number of extra bands of o-IgG required in CSF that is not detected in corresponding serum has been normalized to a minimum of two (Davies et al., 2003; Link and Huang, 2006).

Although IgM is not used in MS diagnostics, it plays an important role in immunity and is a potent activator of the complement cascade in the MS brain (Sellebjerg et al., 1998). Elevated CSF levels of IgM are associated with poorer prognosis in terms of more rapid progress of disease (Magliozzi and Cross, 2020; Gastaldi et al., 2017). Several studies have measured values of IgM in CSF and the IgM index (Sindic et al., 1982; Blennow et al., 1996; Sharief et al., 1990; Sharief and Thompson, 1991) in connection with controls and MS, but to date there is no consensus for their reference values due to the low IgM concentration in normal CSF. Bands of oligoclonal IgM (o-IgM) in CSF that are not mirrored in corresponding serum are also a common feature of MS, and associated with worse prognosis and more rapid conversion to secondary progressive MS (SPMS) (Thangarajh et al., 2008; Villar et al., 2003). Oligoclonal IgM positive patients with clinically isolated syndrome (CIS) have been found to convert more frequently to MS (Tejeda-Velarde et al., 2018; Ferraro et al., 2013), though not in all studies (Ferraro et al., 2015; Schneider et al., 2007).

Whereas o-IgG can be demonstrated in the CSF of 95% of the Caucasian MS population (Fredrikson, 2010), oligoclonal bands for IgM are found less frequently and reported with considerable variation, from around 20 to 75% of patients across the entire MS spectrum, including CIS, SPMS and primary progressive MS (Ferraro et al., 2013; Beltrán et al., 2012; Villar et al., 2002). Although two extra CSF bands of IgG is the accepted cut-off for MS diagnosis (Hegen et al., 2019), there is no validated cut-off for IgM in connection with either diagnosis or prognosis. Research articles therefore vary as to whether any cut-off is mentioned at all to establish whether a patient is 'o-IgM positive'. Where a cut-off is given, it can vary from one extra o-IgM band (Sharief and Thompson, 1991, 1989), to at least two or more (Schneider et al., 2007; Villar et al., 2002; Sharief and Thompson, 1991), and for diagnostic purposes has been suggested to be at least 4 extra (Zeman et al., 2020). Villar and co-workers established a method for the detection of o-IgM two decades ago (Villar et al., 2001) which has since been widely adopted. The group applied a cut-off at two or more extra bands (Villar et al., 2002), and may explain why this is the cut-off most often adopted in similar studies. However, a requirement for the presence of two or more unique bands of o-IgM in CSF, or any other cut-off level, remains arbitrary in the absence of increased standardization.

Given the reliability of o-IgG in the diagnosis of MS, it is unlikely that o-IgM will be routinely used in addition. Despite the popularity of Villar's method, technical challenges continue to restrict general application, and therefore the potential of o-IgM for MS prognosis. Although the detection technique employed is similar in principle to that used for IgG, it must be adapted for the detection of IgM pentamers, and there are marked variations in practical detail. Ongoing work towards automation of the methodology may soon enable routine analysis of o-IgM (Cabrera and Gosis, 2020), so standardization and validation ought to be

priorities.

Nevertheless, the use of o-IgM in prognosis could provide the physician with useful extra information. In the present study, we have carried out a small retrospective study of patients with clinically definite RRMS, the majority of whom were treatment naïve. Historical records have been examined over the maximum number of years available (up to 17 years), and included a cohort of control patients with no demonstrable neurological disease. The occurrence (or not) of o-IgM bands in CSF not found in serum at baseline in patients with RRMS, was explored with respect to progression of disease as assessed by an increase in EDSS over time.

2. Materials and methods

2.1. Patients and study materials

The study employed paired CSF and serum samples obtained from patients referred by general practitioners to the Department of Neurology and Clinical Neurophysiology, University Hospital of Trondheim (St. Olav's Hospital), all of whom were Caucasian Norwegians between the ages of 18 and 60. Samples surplus to the diagnostic work-up may be stored in the Neurological Research Biobank and used for approved research projects if patients provide appropriate written, informed consent. The biobank has been licensed by the Norwegian Directorate for Health Affairs. The present study was approved by the Regional Committee for Medical Research Ethics for central Norway (2014/2275) and was in compliance with the ethical regulations of the University of Illinois at Chicago (UIC). It was carried out according to the Helsinki Declaration.

Patients with a diagnosis of RRMS ($n = 37$) according to the McDonald criteria at baseline (McDonald et al., 2001; Polman et al., 2005, 2011) were followed up to the extent possible, in five cases to a maximum of 17 years. Progression to SPMS was only recorded for six patients. Four patients died during the study: one after the first year (reason unknown), one with pancreatic cancer after nine years, and two patients with severe SPMS after 10.5 and 13 years, respectively. All patients showed brain lesions by MRI, performed at the University Hospital according to the prevailing technical criteria at the time. None had any other serious neurological diagnosis. The duration of disease was found to span a very long period, mainly due to three patients with a mild relapsing-remitting course of disease who presented at the hospital more than two decades after disease start. These patients had been aware of intermittent symptoms but did not think them severe enough to consult a physician. For this reason age at onset could not reliably be determined for all patients. Prior to baseline only one patient was taking anti-inflammatory treatment, and none received a monoclonal antibody. Subsequently, most patients received some form of anti-inflammatory treatment (steroids and/or β-inteferons), but only three received a monoclonal antibody. There was no standardization as to type or amount of medication which was tailored to the individual patient according to disease state when visiting the hospital. There were also varying levels of compliance.

CSF from all study participants was obtained by lumbar puncture at the level L3/L4 or L4/L5 with the patient lying on their side, and immediately placed in ice-water. Samples for further analysis were frozen within 30 min of sampling. Blood-contaminated samples were discarded. Serum was obtained concomitantly from venous blood and centrifuged at room temperature for 10 min, 1500 *g* within one hour of sampling. CSF cells, glucose and protein were measured by the routine procedures of the University Hospital, as were also serum and CSF albumin and IgG. Stored samples were maintained at −80 °C until analysis.

The patients in the control group ($n = 57$) had been given a spinal tap for general neurological symptoms (headaches, dizziness, paraesthesia, asthenia, fasciculations, instability, visual symptoms, pain and anxiety). None were diagnosed with a serious neurological disease, and none

subsequently developed MS. All had a CSF cell count (except for one patient with 6 cells/μL), glucose (except for one other patient with 6.6 mmol/L), protein, albumin and serum albumin within the physiological range as referenced by diagnostic standards (Fischbach and Dunning, 2009), and showed neither lesions by MRI nor extra CSF bands of o-IgG. The demographic data are shown in Table 1.

Patients with RRMS ($n = 37$) were lumbar punctured during the initial assessment (baseline, year of diagnosis, normalized to year 0), and analysis of CSF typically demonstrated over 10 extra bands of o-IgG. Some patients had moderate signs of CNS inflammation, with elevated CSF protein above the normal range up to 0.5 g/L (Regeniter et al., 2009), and sometimes a cell count above 5 cells/μL (Freedman et al., 2005).

The albumin quotient for all patients and controls was calculated according to the ratio of the concentration in CSF relative to serum. The IgG index was calculated from the concentrations of IgG relative to albumin in CSF and serum according to the ratio ((CSF IgG / serum IgG) / (CSF albumin / serum albumin)), values over 0.7 being considered pathological (Fredrikson, 2010).

2.2. Quantitation of IgM in CSF and serum

CSF IgM was quantified by capture ELISA as described by Hvaring et al. (2013). Briefly, 96-well plates (Nunc) were coated overnight at 4 °C in basic carbonate buffer with a capture antibody to human IgM (BD Pharmingen, San Jose, CA). Plates were blocked for 3 h at room temperature by direct addition of blocking buffer. After 5 rinses, each plate was incubated overnight at 4 °C with CSF samples diluted in blocking buffer from 1:5 to 1:500 (triplicate), and a standard curve prepared with commercial human IgM (MP Biomedicals, Irvine, CA). After rinsing, plates were incubated with antiserum to human IgM-HRP (Dako) for 1 h at 37 °C. After rinsing, a tetramethylbenzidine (TMB) solution was incubated for 30 min at 37 °C, stopped by HCl, and the absorbance read at 450 nm against a 620 nm reference. OD values were converted into IgM concentration using a standard curve. In this assay, OD was linear against concentration up to an OD value of 3 (Hvaring et al., 2013). All CSF IgM concentrations were measured 2–4 times. Because the physiological range of IgM concentration in CSF varies greatly in the literature, we adopted a conservative approach to define its normalcy. The upper level of 0.4 mg/L IgM was based on a large study of over 100 healthy subjects whose age (18–88 years) spans adulthood (Blennow et al., 1996). The IgM index was calculated in a similar way to the IgG index, and considered normal up to 0.07 (Sharief and Thompson, 1991). Serum IgM was quantified by immunoturbidity (Quest Diagnostics,

Schaumburg, IL).

2.3. Oligoclonal bands of IgM (o-IgM)

Oligoclonal IgM was detected in CSF by isoelectric focusing coupled to western blot, at the relevant core facility of the University of Illinois at Chicago. The facility ran numerous samples from patients with various neurological conditions and healthy control individuals, but was unaware of diagnosis at all times. Serum diluted 1:1000 in PBS (not distilled water) and undiluted CSF were reduced with 50 mM Tris(2-carboxyethyl)-phosphine (TCEP) in 0.1 M Tris–HCl, pH 9, for 30 min at room temperature. Pairs of CSF and serum were loaded side-by-side in pre-cast Novex® IEF gels pH 3–10, composed of 5% polyacrylamide (Invitrogen, Life Technologies, Grand Island, NY) in IEF Novex® sample buffer (20 mM lysine, 20 mM arginine, 15% glycerol). Samples were never heated before loading on gels and there was no SDS in the IEF Novex® system. Migration was carried out at constant voltage applied in stepwise fashion at 100 V for 1 h, 200 V for 1 h, and 500 V for 30 min. Samples were blotted on PVDF membranes using a wet transfer under constant voltage for 1 h, and incubated overnight at 4 °C with anti-human IgM-HRP antibody (Biosource Invitrogen) diluted 1:2000 in PBS, 0.1% Tween. Bands were revealed by ECL (Pierce, Rockford, IL) and pictures generated by scanner (BioRad, Hercules, CA). As a quality control, some of the samples (12%) were re-run and the pattern of o-IgM always found to be the same.

Gels were not identifiable as displaying patient or control samples when bands were counted. The number of oligoclonal bands in CSF not observed in parallel serum samples were counted independently, including samples from patients with other neurological conditions (not mentioned further in this work), by three co-authors (CH, LRW, AIB). Four MS patient samples had to be re-assessed by all three co-authors: two due to a misunderstanding, and two where there was disagreement as to the total number of extra bands of o-IgM. However, there was agreement that these two latter patients had at least two extra bands.

2.4. Statistical analyses

Statistical analyses were carried out using IBM SPSS version 27 in cooperation with a statistician (ØS), and values of $p < 0.05$ were considered significant. When comparing the RRMS and control groups (Tables 1 and 2), some data were distributed parametrically, whereas other data were non-parametric (Kolmogorov-Smirnov test), so results in the Tables are given as the median and range. Comparisons of gender were made with the chi-square test. A significant difference in age between the groups was found, so age was therefore tested as a covariate

Table 1
Demographic and laboratory data at lumbar puncture (baseline) from patients with relapsing-remitting multiple sclerosis (RRMS) and mild neurological controls, and years of follow-up.

	RRMS ($n = 37$)	Controls ($n = 57$)
Gender ratio (F:M)	26:11	44:13
Age (years)	42 (26 – 57)	36 (19 – 58) [a]
Duration of disease (months)	65 (1 – 400)	n.a.
EDSS	1.3 (0.0 – 4.0)	n.a.
CSF free cells (10^6/L)	3 (0 – 17)	1 (0 – 6) [a]
CSF glucose (mM)	3.3 (2.8 – 6.3)	3.3 (2.7 – 6.6)
CSF total protein (g/L)	0.40 (0.21 – 0.94)	0.30 (0.15 – 0.45) [a]
Follow-up (years)	10.25 (1 – 17)	n.a.

CSF: cerebrospinal fluid, EDSS: Expanded Disability Status Scale. Data are presented as the median (range). Gender was analysed using the chi-square test, age was significantly different between the groups (Mann-Whitney-U test) and subsequently used as a covariate in other comparisons. Where applicable, data that were non-parametrically distributed were transformed to the natural logarithm to approximate to a normal distribution, and all comparisons made with Student's *t*-test
[a] $p < 0.05$.

Table 2
Laboratory and immunological data at lumbar puncture (baseline) from patients with relapsing-remitting multiple sclerosis (RRMS) and mild neurological controls.

	RRMS ($n = 37$)	Control ($n = 57$)
Serum albumin (g/L)	43 (35 – 49)	44 (34 – 50)
CSF albumin (g/L)	0.27 (0.11 – 0.64)	0.19 (0.09 – 0.31) [a]
Albumin quotient · 10^2	0.61 (0.28 – 1.43)	0.47 (0.20 – 0.70) [a]
Serum IgG (g/L)	10.4 (4.7 – 14.3)	10.5 (6.4 – 18.2)
CSF IgG (mg/L)	51 (22 – 121)	23 (10 – 39) [a]
IgG index	0.77 (0.46 – 2.76)	0.48 (0.40 – 0.60) [a]
Serum IgM (g/L)	1.21 (0.28 – 2.22)	1.15 (0.42 – 2.93)
CSF IgM (mg/L)	0.49 (0.06 – 5.19)	0.16 (0.04 – 0.54) [a]
IgM index	0.08 (0.01 – 1.92)	0.03 (0.03 – 0.09) [a]

CSF: cerebrospinal fluid. Not all data were normally distributed and are therefore presented as the median (range). Where applicable, data that were non-parametrically distributed were transformed to the natural logarithm to approximate to a normal distribution, and using age as a covariate in a general linear model, all comparisons made with Student's *t*-test.
[a] $p < 0.05$.

for all parameters in a general linear model. Data were log-transformed to the natural logarithm (ln) to approximate to a normal distribution, and comparisons between the two groups made with Student's t-test.

When the patient group was split according to the number of bands of o-IgM (Table 3), comparisons of gender were again made with the chi-square test. Age, duration of disease and years of historical records were analyzed by the Kruskal-Wallis test. Other variables were log-transformed (ln) (except EDSS) and analyzed with ANOVA (Welch) (Welch, 1947) and post-hoc t-test.

We chose worsening of EDSS as a measure of disease progression. Other factors, such as new MRI lesions or self-reported relapses were also considered but found to be too unreliable in this retrospective study. EDSS values were taken from patient records at the University Hospital and confirmed by a neurologist specializing in MS (HH). To evaluate the change in EDSS over time, we assessed mean EDSS values over the years from the time of diagnosis to the last available recorded entry. Analysis over time was based on the following time groupings: group 0 (baseline, year of diagnosis), 1 (years 1–4), 2 (years 5–9) and 3 (years 10–17). Only five patients could be followed to 17 years, not primarily because patients died, but because there were no patient data further back in time when the study was terminated in 2019. Patients came to the hospital after the initial diagnosis usually only when they had to be assessed for response to treatment, or when their disease worsened. The number of visits for a given patient varied from a minimum of 2 (the patient who died after the first year as previously mentioned), to a maximum of 12 visits, mean 7. Whenever ANOVA (Welch) was significant we proceeded

Table 3

Data concerning oligoclonal bands of IgM (o-IgM) in patients with RRMS ($n = 37$) at lumbar puncture (baseline).

	o-IgM 0 extra bands ($n = 11$)	o-IgM 1 extra band ($n = 9$)	o-IgM ≥2 extra bands ($n = 17$)
Gender ratio (F:M)	10:1	4:5	12:5[a]
Age (year)	42.0 (27 – 57)	49.0 (26 – 57)	42.0 (29 – 56)
Duration of disease (months)	65 (19 – 116)	112 (1 – 333)	33 (5 – 400)
Years of historical records	10.0 (1.0 – 15.0)	10.5 (9.0 – 17.0)	11.0 (4.0 – 17.0)
EDSS	2.0 (0.0 – 2.5)	1.0 (0.0 – 3.5)	1.5 (0.0 – 4.0)
CSF free cells (10^6/L)	3 (0 – 17)	3 (1 – 8)	4 (1 – 11)
CSF glucose (mM)	3.3 (3.0 – 5.0)	3.3 (3.0 – 3.4)	3.3 (2.8 – 6.3)
CSF protein (g/L)	0.30 (0.22 – 0.73)	0.40 (0.23 – 0.65)	0.43 (0.21 – 0.94)
Serum albumin (g/L)	42 (40 – 49)	43 (37 - 48)	45 (35 - 49)
CSF albumin (g/L)	0.21 (0.16 – 0.60)	0.28 (0.12 – 0.47)	0.28 (0.11 – 0.64)
Albumin quotient · 10^3	5.00 (3.55 – 14.28)	6.34 (2.79 – 12.05)	6.22 (2.97 – 13.62)
Serum IgG (g/L)	9.9 (8.7 – 11.7)	9.6 (4.7 – 11.9)	11.4 (8.0 – 14.3)
CSF IgG (mg/L)	44 (22 – 121)	49 (26 – 89)	62 (22 – 113)
IgG index	0.65 (0.46 – 1.85)	0.79 (0.56 – 2.76)	0.77 (0.48 – 1.46)
Serum IgM (g/L)	0.95 (0.57 – 1.90)	1.28 (0.28 – 1.81)	1.35 (0.41 – 2.22)
CSF IgM (mg/L)	0.26 (0.06 – 1.09)	0.36 (0.12 – 1.73)	1.24 (0.27 – 5.19)[b]
IgM index	0.03 (0.01 – 0.23)	0.08 (0.04 – 0.12)	0.18 (0.03 – 1.92)[b]

CSF: cerebrospinal fluid, EDSS: Expanded Disability Status Scale. Not all data were normally distributed and are therefore presented as the median (range). Gender was analyzed using the chi-square test. Where applicable, metabolic data that were non-parametrically distributed were transformed to the natural logarithm to approximate to a normal distribution. Comparisons were made with ANOVA (Welch), and remaining data were analyzed with the Kruskal-Wallis-test. [a]$p < 0.05$.

[b] $p < 0.001$. Post-hoc t-test for o-IgM CSF concentration and IgM index: o-IgM group 0 extra bands vs ≥2 extra bands, both $p < 0.001$; o-IgM group 0 extra bands vs 1 extra band, $p = 0.079$ and $p = 0.053$, respectively; o-IgM group 1 extra band vs ≥2 extra bands, $p = 0.006$ and $p = 0.012$, respectively.

with post-hoc testing at the 5% significance level by the closed testing principle (Marcus et al., 1976).

3. Results

Values of IgG for patients and controls are given in Table 2, and were within expected reference limits (Tibbling et al., 1977). The CSF IgM values in the control group corresponded to the range established by Blennow et al. (1996). CSF values for IgM among patients with RRMS ranged from normal levels to considerably elevated concentrations, also as expected (Sindic et al., 1982).

The pattern of bands of o-IgM was heterogeneous in patients with RRMS, ranging from no extra bands to multiple extra bands being detected in CSF compared to corresponding serum, as reviewed by Mailand and Frederiksen (2020). Conversely, extra bands of o-IgM were not observed in any of the 57 control patient samples.

Since we did not find extra o-IgM bands in CSF in any control sample, it was natural to use zero bands as a point of reference for exploring a prognostic cut-off in this work. When the RRMS cohort was split into groups according to the number of extra bands of o-IgM in CSF compared to serum (Table 3), all oligoclonal bands seen in CSF from 11 patients could also be detected in serum. The remaining 26 (70%) demonstrated from one ($n = 9$) to multiple bands ($n = 17$) of o-IgM not visible in serum. Although no significant differences were found for most parameters in serum or CSF, patients displaying at least two extra CSF bands of o-IgM had highly significant increases in CSF IgM, also reflected in the IgM index, compared to patients where no extra bands were seen in CSF. Patients with a single extra o-IgM band did not reach significance when compared to no extra bands.

The change in EDSS over the years is shown in Fig. 1a,b. When progression of disease was analysed in time periods (Fig. 1a), there were
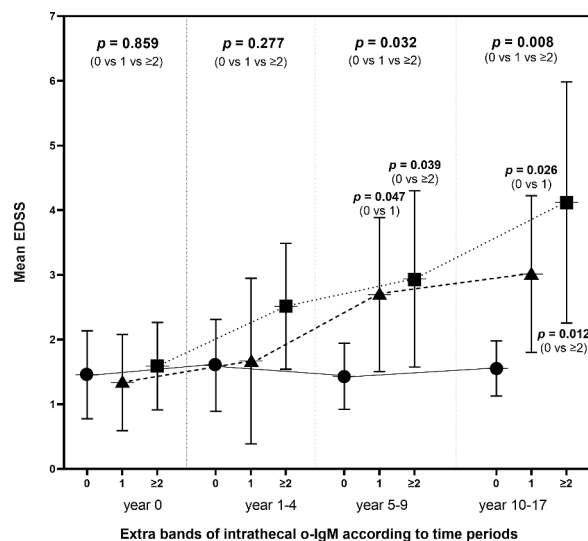
Fig. 1a. Changes of EDSS over time periods according to number of unique oligoclonal bands of IgM (o-IgM) in CSF.

When splitting the RRMS patients ($n = 37$) into groups according to o-IgM band status (group 0, 1 or ≥2 extra bands of o-IgM in CSF compared to serum) and comparing these groups to mean progression in EDSS over 4 time periods (bars represent 95% confidence intervals), analysis with ANOVA (Welsh) showed ΔEDSS at year 0 (baseline): ns; year 1–4: ns; year 5–9: $p = 0.032$; year 10–17: $p = 0.008$. (Year 0: group 0: no extra o-IgM bands, $n = 11$; group 1: one extra o-IgM band, $n = 9$; group 2: two or more extra o-IgM bands, $n = 17$. Year 1–4: group 0: $n = 10$; group 1: $n = 7$; group 2: $n = 14$. Year 5–9: group 0: $n = 9$; group 1: $n = 9$; group 2: $n = 15$. Year 10–17: group 0: $n = 8$; group 1: $n = 7$; group 2: $n = 12$.).

Post hoc analysis t-test: year 5–9; group 0 vs. 1: $p = 0.047$; group 0 vs. ≥2: $p = 0.039$. Year 10–17; group 0 vs. 1: $p = 0.026$; group 0 vs. ≥2: $p = 0.012$; group 0 vs. ≥1: $p = 0.002$. All comparisons of group 0 and 1 merged vs. ≥2: ns.
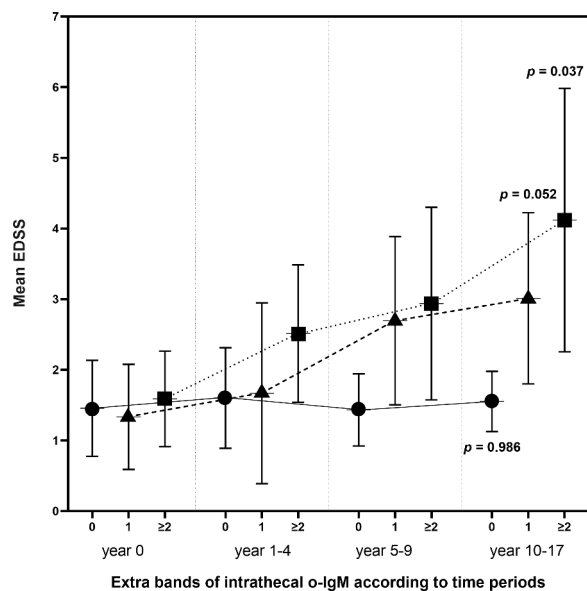
Fig. 1b. Separate comparisons for progression of mean EDSS over time groups for oligoclonal bands of IgM (o-IgM) unique to CSF.

ns: not significant. ANOVA (Welsh) showed ΔEDSS for 0 bands over time: ns, 1 band extra over time: borderline significant $p = 0.052$, ≥ 2 bands over time: $p = 0.037$.

no significant differences between the groups at year 0, the time of diagnosis. However, differences developed over time, and after five years the presence of even one extra band of o-IgM was associated with a statistically significant increase in EDSS compared to samples with no evident extra bands. This effect was more pronounced after 10 years. No significance was found between groups with 1 band and ≥ 2 bands.

When separately comparing the change in EDSS over time per group (Fig. 1b), patients with no evident extra intrathecal o-IgM band had no significant change in EDSS across the overall time period, whereas the presence of a single band was on the border of significance, and in patients with ≥ 2 extra intrathecal bands of o-IgM the change in EDSS over time was significant.

4. Discussion

Most studies (Mailand and Frederiksen, 2020) have found intrathecal IgM in MS to be a negative prognostic predictor of disability in the long term (though not all Stauch et al. 2011), and already in Thorne et al. (2004) remarked on the desirability for a good long-term prognostic marker. The present pilot study indicates that any bands of o-IgM unique to CSF may be such a marker. Interestingly, despite the small size of the groups, the occurrence of even a single extra band of o-IgM was associated with a worsening of EDSS over time. Although the groups were almost identical at baseline for EDSS and most laboratory data, there were significant differences in EDSS values after 5 years. It may be argued that a single band is potentially unreliable, but no extra bands were observed in any of our 57 control samples. The control patients had only mild neurological symptoms and none subsequently developed a neurological disease. In such patients extra bands of o-IgM in CSF seem not to occur, as reported in several other studies (Thangarajh et al., 2008; Schneider et al., 2007; Villar et al., 2002; Sharief and Thompson, 1992; Kaiser and Lücking, 1993). In MS, even when there is a relatively benign course of disease, these bands can be absent, so the occurrence of even a single extra band in CSF is useful clinical information. The physician may wish to follow up such patients more often, and consider disease modifying treatment at an earlier stage to limit the accumulation of disability.

The occurrence of any extra bands in CSF not found in serum seems

to indicate a more aggressive progression in the long term. If we had set a cut-off of at least two extra bands in CSF there would have been a loss of potentially relevant data, since nine of the patients (27%) would have been considered negative. This may also explain the discrepancy between studies regarding an association between IgM in CSF and disease progression (Mailand and Frederiksen, 2020). These preliminary data also suggest that the minimum follow-up period for MS prognosis should be at least 5 years for heterogeneous clinical patients.

There has been some debate in the literature regarding the validity of o-IgM due to the possibility that the bands represent artifacts (Stauch et al., 2011; Reiber et al., 2009), neuronal surface antigens (Beltrán et al., 2012), or that they might reflect antigen-driven maturation with hypermutations specific to CNS IgM clones (Beltrán et al., 2014). It therefore remains unclear what 'bands of o-IgM' actually represent. In their excellent review, Gastaldi et al. (2017) have pointed out that since serum is substantially diluted during preparation for the demonstration of o-IgM (1:1000 in the present work), whereas CSF is applied to gels undiluted, any extra bands appearing in CSF invisible in serum may simply be due to the dilution of serum. They may also be the result of barrier leakage, which can be more pronounced than the impression given by the albumin quotient that only provides evidence of leakage at the blood-CSF barrier. However, our group has often seen examples where bands of o-IgM that are marked in serum are not visible, or only barely visible in CSF. It follows that if such o-IgM species have not leaked significantly to CSF, then other strong bands appearing in CSF but not seen in serum are likely to have originated intrathecally. In our material, bands of o-IgM unique to the CSF were found exclusively in material from patients with MS, and in no control samples. Gel patterns were maintained with repeated testing. These preliminary data therefore support the view that the occurrence of such bands of o-IgM in CSF is of intrathecal origin due to MS-related pathophysiology. From a clinical point of view the actual nature of the bands is of less importance.

This study was retrospective, from data available in records of patients who were followed by neurologists at the University Hospital. There was considerable variation between patient check-ups, disease progression, treatments, compliance with treatments, duration of treatments, and time to diagnosis. The data are therefore not a standardized group of patients enrolled for research, though they are representative of patients in a clinical setting 10 years ago. The cohort differs from similar cohorts today in that none received a monoclonal antibody from time of diagnosis, and only three patients received such treatment later during the disease course. Although there were differences in the number of datapoints available to us, the immunological data from the patient group conforms to the expected values of an MS cohort, and in this respect can be considered representative. It is reasonable to expect that the differences would be even clearer in a larger cohort.

5. Conclusions

We tentatively conclude that bands of o-IgM have prognostic potential for RRMS and that according to our preliminary data, the occurrence of any extra band of o-IgM compared to serum results in poorer prognosis. However, it seems that a minimum follow-up period for MS prognosis should be at least 5 years. Band presence could be an indicator for earlier disease-modifying treatment. A lack of extra o-IgM in CSF appears to be good news for the patient.

Declarations

Ethics approval and consent to participate

The study was carried out in accordance with the Helsinki Declaration and was approved by the Regional Committee for Medical Research Ethics for central Norway. All participants gave written, informed consent.

Consent for publication

All participants gave written, informed consent.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

Formulated the concept for the paper: CH.

Writing main manuscript, prepared the tables and figure: CH (mainly), to a lesser extent LRW.

Training/guidance: LRW, AIB.

Practical aspects: CH, AIB, NA.

Statistics: ØS, CH, LRW.

Band interpretation: CH, AIB, LRW.

Clinical data collection: CH.

Quality control clinical aspects: HH, CH.

All authors reviewed the manuscript.

Declaration of Competing Interest

No conflicts of interest.

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References

Beltrán, E., Hernández, A., Lafuente, E.M., Coret, F., Simó-Castelló, M., Boscá, I., et al., 2012. Neuronal antigens recognized by cerebrospinal fluid IgM in multiple sclerosis. J. Neuroimmunol. 247, 63–69. https://doi.org/10.1016/j.jneuroim.2012.03.013.

Beltrán, E., Obermeier, B., Moser, M., Coret, F., Simó-Castelló, M., Boscá, I., et al., 2014. Intrathecal somatic hypermutation of IgM in multiple sclerosis and neuroinflammation. Brain 137 (10), 2703–2714. https://doi.org/10.1093/brain/awu205. Pt.

Blennow, K., Skoog, I., Wallin, A., Wikkelsö, C., Fredman, P., 1996. Immunoglobulin M in cerebrospinal fluid: reference values derived from 111 healthy individuals 18-88 years of age. Eur. Neurol. 36, 201–205. https://doi.org/10.1159/000117248.

Cabrera, C.M., Gosis, A., 2020. Semi-automated methodology for detection of IgM oligoclonal bands in cerebrospinal fluid and serum samples. J. Immunol. Methods 487, 112898. https://doi.org/10.1016/j.jim.2020.112898.

Davies, G., Keir, G., Thompson, E.J., Giovannoni, G., 2003. The clinical significance of an intrathecal monoclonal immunoglobulin band: a follow-up study. Neurology 60, 1163–1166. https://doi.org/10.1212/01.wnl.0000055864.08740.cb.

Ferraro, D., Simone, A.M., Bedin, R., Galli, V., Vitetta, F., Federzoni, L., et al., 2013. Cerebrospinal fluid oligoclonal IgM bands predict early conversion to clinically definite multiple sclerosis in patients with clinically isolated syndrome. J. Neuroimmunol. 257, 76–81. https://doi.org/10.1016/j.jneuroim.2013.01.011.

Ferraro, D., Galli, V., Vitetta, F., Simone, A.M., Bedin, R., Del Giovane, C., et al., 2015. Cerebrospinal fluid CXCL13 in clinically isolated syndrome patients: association with oligoclonal IgM bands and prediction of multiple sclerosis diagnosis. J. Neuroimmunol. 283, 64–69. https://doi.org/10.1016/j.jneuroim.2015.04.011.

Fischbach, F.T., Dunning, M.B., 2009. A Manual of Laboratory and Diagnostic Tests, 8th ed. Lippincott Williams & Wilkins.

Fredrikson, S., 2010. Clinical usefulness of cerebrospinal fluid evaluation. Int. MS J. 17, 24–27. http://www.ncbi.nlm.nih.gov/pubmed/20663418.

Freedman, M.S., Thompson, E.J., Deisenhammer, F., Giovannoni, G., Grimsley, G., Keir, G., et al., 2005. Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement. Arch. Neurol. 62, 865–870. https://doi.org/10.1001/archneur.62.6.865.

Gastaldi, M., Zardini, E., Franciotta, D., 2017. An update on the use of cerebrospinal fluid analysis as a diagnostic tool in multiple sclerosis. Expert Rev. Mol. Diagn. 17, 31–46. https://doi.org/10.1080/14737159.2017.1262260.

Hegen, H., Zinganell, A., Auer, M., Deisenhammer, F., 2019. The clinical significance of single or double bands in cerebrospinal fluid isoelectric focusing. A retrospective study and systematic review. PLoS One 14, e0215410. https://doi.org/10.1371/journal.pone.0215410.

Hvaring, C., Vujicic, S., Aasly, J.O., Feinstein, D.L., White, L.R., Boullerne, A.I., 2013. IgM to S-nitrosylated protein is found intrathecally in relapsing-remitting multiple sclerosis. J. Neuroimmunol. 256, 77–83. https://doi.org/10.1016/j.jneuroim.2012.12.011.

Kaiser, R., Lücking, C.H., 1993. Intrathecal synthesis of IgM and IgA in neurological diseases: comparison of two formulae with isoelectric focusing. Clin. Chim. Acta 216, 39–51. https://doi.org/10.1016/0009-8981(93)90137-S.

Link, H., Huang, Y.M., 2006. Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness. J. Neuroimmunol. 180, 17–28. https://doi.org/10.1016/j.jneuroim.2006.07.006.

Magliozzi, R., Cross, A.H., 2020. Can CSF biomarkers predict future MS disease activity and severity? Mult. Scler. 26, 582–590. https://doi.org/10.1177/1352458519871818.

Mailand, M.T., Frederiksen, J.L., 2020. Intrathecal IgM as a prognostic marker in multiple sclerosis. Mol. Diagn. Ther. 24, 263–277. https://doi.org/10.1007/s40291-020-00455-w.

Marcus R., Peritz E., Gabriel K.R. On closed testing procedures with special reference to ordered analysis of variance. 1976. https://about.jstor.org/terms.

McDonald, W.I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., et al., 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. Ann. Neurol. 50, 121–127. https://doi.org/10.1002/ana.1032.

Polman, C.H., Reingold, S.C., Edan, G., Filippi, M., Hartung, H.P., Kappos, L., et al., 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria. Ann. Neurol. 58, 840–846. https://doi.org/10.1002/ana.20703.

Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J.A., Filippi, M., et al., 2011. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann. Neurol. 69, 292–302. https://doi.org/10.1002/ana.22366.

Regeniter, A., Kuhle, J., Mehling, M., Möller, H., Wurster, U., Freidank, H., et al., 2009. A modern approach to CSF analysis: pathophysiology, clinical application, proof of concept and laboratory reporting. Clin. Neurol. Neurosurg. 111, 313–318. https://doi.org/10.1016/j.clineuro.2008.12.004.

Reiber, H., Teut, M., Pohl, D., Rostasy, K.M., Hanefeld, F., 2009. Paediatric and adult multiple sclerosis: age-related differences and time course of the neuroimmunological response in cerebrospinal fluid. Mult. Scler. 15, 1466–1480. https://doi.org/10.1177/1352458509348418.

Schneider, R., Euler, B., Rauer, S., 2007. Intrathecal IgM-synthesis does not correlate with the risk of relapse in patients with a primary demyelinating event. Eur. J. Neurol. 14, 907–911. https://doi.org/10.1111/j.1468-1331.2007.01871.x.

Sellebjerg, F., Christiansen, M., Garred, P., 1998. MBP, anti-MBP and anti-PLP antibodies, and intrathecal complement activation in multiple sclerosis. Mult. Scler. 4, 127–131. https://doi.org/10.1177/135245859800400307.

Sharief, M.K., Thompson, E.J., 1989. Immunoglobulin M in the cerebrospinal fluid: an indicator of recent immunological stimulation. J. Neurol. Neurosurg. Psychiatry 52, 949–953. https://doi.org/10.1136/jnnp.52.8.949.

Sharief, M.K., Thompson, E.J., 1991a. Intrathecal immunoglobulin M synthesis in multiple sclerosis. Relationship with clinical and cerebrospinal fluid parameters. Brain 114, 181–195. Pt 1 March. http://www.ncbi.nlm.nih.gov/pubmed/1998881.

Sharief, M.K., Thompson, E.J., 1991b. The predictive value of intrathecal immunoglobulin synthesis and magnetic resonance imaging in acute isolated syndromes for subsequent development of multiple sclerosis. Ann. Neurol. 29, 147–151. https://doi.org/10.1002/ana.410290206.

Sharief, M.K., Thompson, E.J., 1992. Distribution of cerebrospinal fluid oligoclonal IgM bands in neurological diseases: a comparison between agarose electrophoresis and isoelectric focusing. J. Neurol. Sci. 109, 83–87. https://doi.org/10.1016/0022-510x(92)90098-6.

Sharief, M.K., Keir, G., Thompson, E.J., 1990. Intrathecal synthesis of IgM in neurological diseases: a comparison between detection of oligoclonal bands and quantitative estimation. J. Neurol. Sci. 96, 131–142. https://doi.org/10.1016/0022-510x(90)90126-8.

Sindic, C.J., Cambiaso, C.L., Depré, A., Laterre, E.C., Masson, P.L., 1982. The concentration of IgM in the cerebrospinal fluid of neurological patients. J. Neurol. Sci. 55, 339–350. https://doi.org/10.1016/0022-510x(82)90131-9.

Stauch, C., Reiber, H., Rauchenzauner, M., Strasak, A., Pohl, D., Hanefeld, F., et al., 2011. Intrathecal IgM synthesis in pediatric MS is not a negative prognostic marker of disease progression: quantitative versus qualitative IgM analysis. Mult. Scler. 17, 327–334. https://doi.org/10.1177/1352458510388543.

Tejeda-Velarde, A., Costa-Frossard, L., Sainz de la Maza, S., Carrasco, Espiño, M., Picón, C., et al., 2018. Clinical usefulness of prognostic biomarkers in optic neuritis. Eur. J. Neurol. 25 (4), 614–618.

Thangarajh, M., Gomez-Rial, J., Hedström, A.K., Hillert, J., Alvarez-Cermeño, J.C., Masterman, T., et al., 2008. Lipid-specific immunoglobulin M in CSF predicts adverse long-term outcome in multiple sclerosis. Mult. Scler. 14, 1208–1213. https://doi.org/10.1177/1352458508095729.

Thompson, A.J., Banwell, B.L., Barkhof, F., Carroll, W.M., Coetzee, T., Comi, G., et al., 2018. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 17, 162–173. https://doi.org/10.1016/S1474-4422(17)30470-2.

Thorne, S., Con, A., McGuinness, L., McPherson, G., Harris, S.R., 2004. Health care communication issues in multiple sclerosis: an interpretive description. Qual. Health Res. 14, 5–22. https://doi.org/10.1177/1049732303259618.

Tibbling, G., Link, H., Ohman, S., 1977. Principles of albumin and IgG analyses in neurological disorders. I. Establishment of reference values. Scand. J. Clin. Lab. Investig. 37, 385–390. https://doi.org/10.1080/00365517709091496.

Villar, L.M., González-Porqué, P., Masjuán, J., Alvarez-Cermeño, J.C., Bootello, A., Keir, G., 2001. A sensitive and reproducible method for the detection of oligoclonal IgM bands. J. Immunol. Methods 258, 151–155. https://doi.org/10.1016/s0022-1759(01)00492-6.

Villar, L.M., Masjuan, J., González-Porqué, P., Plaza, J., Sádaba, M.C., Roldán, E., et al., 2002. Intrathecal IgM synthesis in neurologic diseases: relationship with disability in MS. Neurology 58, 824–826. https://doi.org/10.1212/wnl.58.5.824.

Villar, L.M., Masjuan, J., González-Porqué, P., Plaza, J., Sádaba, M.C., Roldán, E., et al., 2003. Intrathecal IgM synthesis is a prognostic factor in multiple sclerosis. Ann. Neurol. 53, 222–226. https://doi.org/10.1002/ana.10441.

Welch, B.L., 1947. The generalisation of student's problems when several different population variances are involved. Biometrika 34, 28–35. https://doi.org/10.1093/biomet/34.1-2.28.

Zeman, D., Kušnierová, P., Všianský, F., Reguliová, K., Škutová, M., Woznicová, I., et al., 2020. Cerebrospinal fluid oligoclonal IgM test in routine practice: comparison with quantitative assessment of intrathecal IgM synthesis. Clin. Chim. Acta 508, 137–145. https://doi.org/10.1016/j.cca.2020.05.021.