Routine CSF parameters as predictors of disease course in multiple sclerosis : an MSBase cohort study

Routine CSF parameters as predictors of disease course in Multiple Sclerosis: An MSBase cohort study

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Abstract

Background: It remains unclear whether routine cerebrospinal fluid (CSF) parameters can serve as predictors of multiple sclerosis (MS) disease course.

Methods: This large-scale cohort study included persons with MS with CSF data documented in the MSBase registry. CSF parameters to predict time to reach confirmed expanded disability status scale score (EDSS) 4, 6 and 7 and annualized relapse rate in the first 2 years after diagnosis (ARR2) were assessed using (cox) regression analysis.

Results: In total, 11 245 participants were included of which 93.7% (n=10 533) were persons with relapsing remitting MS (RRMS). In RRMS, presence of CSF oligoclonal bands (OCB) was associated with shorter time to disability milestones EDSS 4 (adjusted hazard ratio (HR) (95% confidence interval (CI)) =1.272 (1.089-1.485), p=0.002), EDSS 6 (HR (95% CI)=1.314 (1.062-1.626), p=0.012) and EDSS 7 (HR (95% CI)=1.686 (1.111-2.558), p=0.014). On the other hand, presence of CSF pleocytosis (≥5 cells/µl) increased time to moderate disability (EDSS 4) in RRMS (HR (95% CI)=0.774 (0.632-0.948), p=0.013). None of the CSF variables were associated with time to disability milestones in persons with primary progressive MS (PPMS). Presence of CSF pleocytosis increased ARR2 in RRMS (adjusted R^2 =0.036, p=0.015).

Conclusions: In RRMS, presence of CSF OCB predicts shorter time to disability milestones whereas CSF pleocytosis could be protective. This could however not be found in PPMS. CSF pleocytosis is associated with short-term inflammatory disease activity in RRMS. CSF analysis provides prognostic information which could aid in clinical and therapeutic decision-making.

Key message

WHAT IS ALREADY KNOWN ON THIS TOPIC

In many countries, cerebrospinal fluid (CSF) analysis is a standard procedure in the diagnostic work-up of a person with suspected multiple sclerosis (MS), but conflicting results about its prognostic value have been reported.

WHAT THIS STUDY ADDS

In this large-scale MSBase cohort study including 11 245 persons with MS, we demonstrated that presence of CSF oligoclonal bands seems an unfavorable prognostic factor as it is associated with future disability accumulation in relapsing remitting multiple sclerosis (RRMS). Additionally, we demonstrated that CSF pleocytosis (≥5 cells/µl) is associated with short-term inflammatory disease activity and appears to be a protective factor to reach moderate disability

in persons with RRMS. Interestingly, this could not be demonstrated in persons with primary progressive MS.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

Routine CSF analysis provides clinicians with useful prognostic information early in the disease course which could aid in patient counselling, clinical decision-making and guidance of treatment decisions.

1. Introduction

Multiple sclerosis (MS) is characterized by a highly variable and unpredictable disease course. The growing availability of disease modifying therapies (DMTs) with different efficacy and risk profiles, comes along with the need for reliable biomarkers that can properly identify those persons at high risk of an aggressive disease course.

Since cerebrospinal fluid (CSF) analysis is often performed in the diagnostic work-up of a person with suspected MS, identifying prognostic CSF biomarkers would be highly valuable. Indeed, CSF analysis regained attention in the latest revisions of the McDonald criteria as presence of oligoclonal bands (OCB) unique to the CSF currently substitutes for "dissemination in time", enabling faster diagnosis of MS[1]. Despite the unquestioned diagnostic value of routine CSF analysis in MS, its prognostic significance remains undetermined. Although presence of CSF OCB is independently associated with the conversion from clinically isolated syndrome to clinical definite MS[2,3], it remains unclear whether presence or absence of CSF OCB confers a better outcome regarding disease activity and disability accumulation. Several authors reported an association between presence of OCB and an unfavorable prognosis[2,4,5] while others refuted this association[6-15]. Moreover, there is some contradictory[4,10,11,16] evidence that the IgG index[4,12,17-19] and CSF pleocytosis[11,20] may have prognostic implications regarding future MS disease course. Furthermore, conflicting results have been published concerning the CSF profiles of various MS subtypes[6,21] and in particular, data about the CSF composition and its prognostic value in primary progressive MS (PPMS) remain limited.

Here, we present a large-scale, longitudinal cohort study of the association between routinely available CSF markers and future MS disease course. We hypothesize that the diagnostic CSF analysis contains prognostic information regarding future MS disease course.

2. Materials and Methods

2.1 Study population

Data were obtained from MSBase, an international MS registry approved by the Melbourne Health Human Research Ethics Committee (registered with WHO CTRN12605000455662). This database consists of prospectively collected information during routine clinical care, primarily from tertiary MS centres currently encompassing data of more than 97 000 individuals from 175 different centres and 43 countries worldwide. Data were extracted on November 2nd 2022. All participants provided informed consent as per local regulations. All participants aged ≥18 years, diagnosed with relapsing remitting (RRMS) or PPMS according to the McDonald criteria in whom CSF analysis was performed before or within a year after diagnosis and who met the minimum data requirements (documented diagnosis date, birth date, disease onset date, sex, MS course, ≥3 Expanded Disability Status Scale (EDSS) scores after diagnosis and at least one documented CSF measure of interest (OCB status and/or IgG index and/or white blood cell (WBC) count)) were eligible to participate. If multiple lumbar punctures (LPs) met these criteria, the one closest to the diagnosis date was selected and used for all analyses (="Diagnostic LP").

2.2 Outcomes

Primary outcomes were time to 6 month confirmed EDSS 4, 6 and 7 after diagnosis in RRMS and PPMS. For the respective outcome event to occur, 2 EDSS scores needed to be documented separated by a minimum time interval of 6 months. The "time to" the outcome event was determined by interval censoring. If the EDSS score dropped below the previously confirmed event during follow-up, the initial confirmation was invalidated. Participants with EDSS scores surpassing one of the outcome EDSS scores at dataset entry were not considered "at risk" for the respective event. However, they were considered "at risk" if the EDSS score dropped below the respective EDSS milestone during follow-up.

Secondary outcomes were annualized relapse rate (ARR) in the first 2 (ARR2) years after diagnosis and difference in CSF composition at diagnosis between RRMS and PPMS.

2.3 Collected variables

The following clinical variables were retained: age at diagnosis, sex, MS onset date, diagnosis date, relapses, EDSS scores and MS course. In line with De Brouwer *et al.*[22], DMTs were arbitrarily categorized into low-,moderate- and high-efficacy:

- Low-efficacy: interferons, Teriflunomide, Glatiramer acetate, Azathioprine, Methotrexate

- Moderate-efficacy: Fingolimod, Dimethyl-Fumarate, Cladribine, Siponimod, Daclizumab, Ozanimod, Ponesimod
- High-efficacy: Alemtuzumab, Rituximab, Ocrelizumab, Natalizumab, Mitoxantrone, Cyclophosphamide, Ofatumumab

Assignment to a specific treatment category occurred if participants were on treatment with a specific DMT for at least six months.

Collected CSF variables of interest were OCB status (presence or absence), IgG index and WBC count. Following CSF variables were further categorized:

- CSF leukocyte count ≥5/µl was considered elevated ("CSF pleocytosis")
- IgG index values >0.7 were considered elevated. IgG index was further categorized into 3 groups: normal (≤0.7), elevated (0.71-1.03) and highly elevated (>1.03). Median IgG index value of those participants with an elevated IgG index was used as the cut-off value to differentiate between an elevated and highly elevated IgG index, which is in line with an earlier published study[18].
- Participants with both positive CSF OCB and an elevated IgG index (>0.7) are referred to as "double positives" whereas participants with absent CSF OCB and a normal IgG index (≤0.7) are referred to as "double negatives". "Double negatives" therefore represent those participants with no signs of intrathecal IgG synthesis.

2.4 Statistical analysis

Time-to-event outcomes were analysed using Cox proportional hazards models. The multivariable model was adjusted for age at diagnosis, sex, EDSS at LP, treatment category throughout the disease course (time varying), disease duration and number of relapses until the event or censoring. The proportional hazard assumption was verified using log minus log plots. If the outcome event did not occur, the participant was censored at the last documented visit date. Analysis was performed in the "at risk population" only. CSF predictors for ARR2 were analysed using multivariable linear regression (generalised linear model) and were adjusted for age at diagnosis, sex, EDSS at LP, number of relapses between disease onset and diagnosis and highest treatment category until the first 2 years after diagnosis (DMT2y). Key assumptions of multivariable linear regression were evaluated. Differences in CSF profile between RRMS and PPMS were assessed with the Mann–Whitney U-test and Chi-square test where appropriate. Only participants with documented information on the respective CSF measure of interest were used for analysis. All analyses were performed using IBM SPSS statistics software version 29.0. P values <0.05 were considered statistically significant.

3. Results

3.1 Demographic and disease characteristics

A flowchart of participant inclusion is shown in Figure 1. At the extraction date (November 2nd 2022), data of 84 571 participants were available of which 11 245 met our inclusion criteria (10 533 RRMS and 712 PPMS). Demographic and disease characteristics are summarized in table 1. All OCB-negative participants fulfilled the McDonald criteria based on clinical and MRI findings.

3.2 Time to 6 month confirmed EDSS 4, 6 and 7

Median survival times are represented in table 2. We refer to supplementary materials for Kaplan-Meier curves graphically representing our main findings.

Univariable cox regression analysis demonstrated that none of the CSF variables was associated with confirmed disability worsening in PPMS (table 3). Due to a low number of events, multivariable analysis was not performed in PPMS, as this analysis requires a minimum of 10-15 events per predictor variable[23].

In RRMS, multivariable analysis (table 3) showed that OCB-positive participants had an increased risk to reach confirmed EDSS 4 [HR (95% CI)=1.272 (1.089-1.485); p=0.002], 6 [HR (95% CI)=1.314 (1.062-1.626); p=0.012] and 7 [HR (95% CI)=1.686 (1.111-2.558); p=0.014]. In other words, OCB-positive RRMS participants were 27.2%, 31.4% and 68.6% more likely to reach confirmed EDSS 4, 6 and 7 respectively. In addition, the hazard to reach confirmed EDSS 4 increased with a factor 1.23 [HR (95% CI)=1.228 (1.033-1.459); p=0.020] if an elevated IgG index was present (>0.7). Further analysis revealed that participants with a highly elevated IgG index (>1.03) were 24.1% more likely to reach confirmed EDSS 4 compared to those with a normal IgG index (≤0.7) [HR (95% CI)=1.241 (1.019-1.512); p=0.032]. Compared to “double negatives”, “double positive” RRMS participants were more likely to reach confirmed EDSS 4 [HR (95% CI)=1.635 (1.186-2.252); p=0.003] but not 6 and 7. Additionally, RRMS participants with CSF pleocytosis were 22.6% less likely to reach moderate disability (EDSS 4) [HR (95% CI)=0.774 (0.632-0.948); p=0.013]). Recategorization of the DMTs into low-moderate versus high-efficacy therapy did not alter the main findings (table 1 supplementary materials).

A post hoc analysis where the observation period ended 10 years after diagnosis confirmed that in RRMS, presence of CSF OCB was associated with increased risk of reaching confirmed EDSS 4 [HR (95% CI)=1.455 (1.192-1.752); p<0.001] and 6 [HR (95% CI)=1.361 (1.035-1.788); p=0.027] (table 4 and table 2 supplementary materials). Additionally, the protective role of CSF pleocytosis was confirmed, as RRMS participants with CSF pleocytosis were 29.4%

and 33.4% less likely to reach confirmed EDSS 4 [HR (95% CI)=0.706 (0.557-0.984); p=0.004] and 6 [HR (95% CI)=0.666 (0.471-0.943); p=0.022] respectively.

All multivariable analysis were adjusted for age at diagnosis, sex, EDSS at LP, treatment category throughout the disease course (time varying), disease duration and number of relapses until the event or censoring.

3.3 Annualized relapse rate in the first two years after diagnosis

Multivariable linear regression analysis adjusted for age at diagnosis, sex, EDSS at LP, number of relapses between disease onset and diagnosis and DMT2y revealed that CSF pleocytosis was associated with ARR2 in RRMS (adjusted R^2 RRMS=0.036, p=0.015, β (95%CI)=0.052 (0.010-0.094) (table 5). In other words, the designed model explained only 3.6% of the total variance. Other CSF variables could not be identified as being significantly associated with ARR2 in RRMS or PPMS (table 5).

3.4 CSF profile in RRMS versus PPMS

Differences in diagnostic CSF profile between RRMS and PPMS are summarized in table 1. In the 14 days prior to LP, 1863/10533 RRMS and 41/712 PPMS participants received high-dose corticosteroids.

The proportion of OCB-positive participants was higher in PPMS compared to RRMS (88.8% versus 84.4% respectively, p=0.002). To explore if the association between OCB-positivity and PPMS was influenced by the time interval between disease onset and LP performance, participants were divided into “very early”, “early”, “late” and “very late” LP. The quartiles of the interval between disease onset and LP were used as cut-off value. In RRMS, the proportion of participants with CSF OCB increased with increasing time intervals (very early: 82.8%, early: 84.1%, late: 85.1% and very late: 85.5%) whereas this trend was less obvious in PPMS with an almost equal proportion of OCB-positivity in the “very early” and “very late” group (very early: 85.8%, early: 92.9%, late: 90.1%, very late: 86.2%).

Repeated LPs were conducted in 421 participants with 2, 3 and 4 LPs respectively performed in 377, 36 and 5 participants and 6, 9 and 11 LPs performed in 1 participant each. In 273/421 participants, the “Diagnostic LP” was the first performed one. Median time between first and last LP was 1197 days (interquartile range (IQR) 277-2546). Most participants remained either OCB-positive (219/421) or OCB-negative (61/421). Notably, 80 participants demonstrated a change in OCB status (negative to positive or vice versa), with 19 converting to OCB-negativity. Among these, the change in OCB status occurred either between an LP performed before (30/80) or after (41/80) the “Diagnostic LP” (=LP used for analysis) or was observed on an LP performed after the disability milestones were already reached (9/80). For the remainder, OCB

status was either never determined (13/421), was initially not determined but was later either positive (27/421) or negative (5/421), or was initially positive (16/421) but never redetermined.

Regarding the IgG index, no differences between RRMS and PPMS were observed.

A higher proportion of RRMS participants demonstrated CSF pleocytosis (RRMS 44.2% versus PPMS 29.4%, <0.001). However, this seemed an age-related effect, as the proportions of participants presenting CSF pleocytosis did not differ between PPMS and RRMS when they were divided in different, arbitrarily defined age categories (table 1). The proportion of participants demonstrating CSF pleocytosis decreased with age.

Information on both OCB status and IgG index was available for 4441 RRMS and 312 PPMS participants. In RRMS, 12.3% were “double negatives”, 56.7% were “double positives”, 26.5% had CSF OCB but no elevated IgG index and 4.5% had only an elevated IgG index. In PPMS, this was 9.3%, 58.7%, 28.5% and 3.5% respectively.

For a total of 3000 RRMS and 240 PPMS participants, both WBC count and OCB status were documented. In OCB-positive RRMS participants, CSF WBC count was significantly higher compared to OCB-negative participants (RRMS: OCB-positive 4/µl (IQR 2-10), OCB-negative 2/µl (IQR 1-5), p<0.001; PPMS OCB-positive 2/µl (IQR 1-5), OCB-negative 3/µl (IQR 1;75-5), p=0.152).

4. Discussion

MS is a heterogeneous disorder characterized by a wide spectrum of disability outcomes as a consequence of a complex interplay between inflammation and neurodegeneration. To date, reliable biomarkers for prognostication of inflammatory disease activity and accumulation of disability are lacking. In many countries, CSF analysis is a standard procedure in the diagnostic work-up of a person with suspected MS. Still, conflicting results about its prognostic value have been reported. Previous studies regarding this topic were often limited by their cross-sectional design, relatively small sample sizes, inability to include confounders and heterogeneous patient populations. Moreover, often, no distinction was made between RRMS and PPMS. Here, we presented a large-scale, longitudinal cohort study of the association between routinely available CSF markers and future MS disease course.

In our cohort, presence of CSF OCB was associated with an increased risk of disability accumulation in RRMS. This is in line with a meta-analysis including 16 studies demonstrating that OCB-positive persons with MS (PwMS) were more likely to reach the disability outcome measure associated with the included studies (odds ratio=1.65 (95% CI= 1.27-2.13);

p=0.0002). However, in this meta-analysis, the disability outcomes were heterogeneous, confounding factors were not accounted for and not all studies on OCB were included. Another large-scale study (n=7322) further demonstrated that OCB-positive PwMS had an increased risk of reaching sustained EDSS 3 (HR (95% CI) =1.29 (1.12-1.48), p<0.001) and 4 (HR (95% CI)=1.38 (1.17-1.63), p<0.001)[24]. Of note, in both studies[2,24], no distinction was made between RRMS and PPMS.

The worse outcome in OCB-positive RRMS may suggest a direct link with the mechanism underlying disability accumulation. Presence of CSF OCB has been associated with increased CSF neurofilament light chain levels next to inflammatory markers linked to B-cell activity[25]. Moreover, lower numbers of plasma cells were found in brain lesions of OCB-negative PwMS[26]. Accumulation of disability in OCB-positive RRMS could therefore be a direct result of B-cell responses and their associated proinflammatory CSF profile. Earlier studies consistently linked the HLA-DRB1*15:01 allele to presence and the HLA-DRB1*04 allele to absence of CSF OCB[27]. Absence of OCB may thus signify a distinct immunogenetic phenotype leading to less aggressive immune responses.

One could argue that our results suggest the prompt initiation of high-efficacy therapy in OCB-positive RRMS. However, it must be kept in mind that OCB are part of the diagnostic McDonald criteria[1] and that advocating for such strategy would necessitate the initiation of high-efficacy therapy in about 90% of individuals with RRMS[2], including those with a benign disease course. This strategy, together with the longitudinal effect of DMTs on OCB status and the prognostic significance of changes in OCB status could be a subject of future research.

Although we confirmed the negative prognostic role of CSF OCB in RRMS[2,5,24], OCB might not represent the ultimate prognostic biomarker. OCB status is a qualitative measure yielding either a positive or negative result as assessed by visual inspection. In MS disease prognostication, a sensitive, quantitative biomarker such as the kappa free light chain index (κFLC index), might show clear advantages. The κFLC index recently emerged as a promising diagnostic biomarker with comparable diagnostic sensitivities to and clear methodological advantages over CSF OCB. In recent years, its prognostic value has also been increasingly recognized[28].

Although little researched, elevated CSF WBC were inconsistently[4,10] associated with increased relapse rates, EDSS worsening[11,20] and gadolinium enhancing lesions[11]. We demonstrated that CSF pleocytosis was associated with ARR2 and was a protective factor for developing moderate disability in RRMS. CSF leukocytes may indeed primarily correlate with

inflammatory disease activity rather than disability accumulation. Current DMTs mainly target inflammation, and some DMTs have shown to reduce CSF WBC in PwMS[11,29,30]. Persons with RRMS with CSF pleocytosis might therefore benefit more from DMT initiation which potentially accounts for the observed protective effects. This observation was not valid for PPMS, possibly due to its lower inflammatory nature. However, precision and sensitivity of CSF WBC quantification depend upon the method used (automated versus manual)[31] and on pre-analytical factors such as traumatic punctures and the time interval between LP and CSF analysis[32]. These factors combined with our very low adjusted R^2, warrant a cautious interpretation of our results on CSF WBC.

None of the CSF variables contributed to future disability accumulation in PPMS. These findings suggest that mechanisms apart from neuroinflammation might contribute to disability accrual in PPMS. For instance, tertiary meningeal follicles, which are organized structures consisting of CD8+ T-cells, CD20+ B-cells and a variable number of plasma cells[33], were shown to be associated with accelerated disability accumulation in secondary progressive MS[34], whereas their absence in PPMS was demonstrated in another study[35]. Talbot *et al.* further found few and weak associations between intrathecal inflammation and the extent of neuroaxonal damage in PPMS[36]. Finally, CSF IgM OCB have been associated with a worse disease course in RRMS but not PPMS[37]. All these findings suggest that heterogeneous pathogenic and immunological mechanisms may be involved in the different MS subtypes.

Due to limited published results on CSF in PPMS, it is unclear whether MS subtypes can be distinguished based on CSF profile. Studies addressing CSF compositions of MS subtypes suffer from obvious limitations, such as small numbers of PPMS participants as well as limited CSF parameter datasets[6,21]. In our cohort, the proportion of OCB-positive RRMS participants was somehow lower than expected (84.4%)[2], which might relate to ethnicity, genetics, patient characteristics, the detection assay used or latitude. Inconsistencies in OCB status according to latitude were indeed demonstrated in several studies[2,5]. The highest proportions of OCB-positive PwMS were typically reported in Northern European countries whereas lower proportions have been demonstrated in Southern Europe, Southern American and Asian countries[6]. As the MSBase database encompasses data of 43 countries worldwide, this might at least partially explain this lower percentage. Another potential explanation might relate to the method to detect OCB. It is possible that not all participating centres used the gold standard, i.e. isoelectric focussing, potentially reducing the sensitivity to detect OCB.

The observed association of OCB-positivity with PPMS (PPMS=88.8%, RRMS=84.4%) was consistent with earlier published studies[6], but contrasts findings of others[7,9,38]. We could not demonstrate that the association between positive CSF OCB and PPMS resulted from the

longer time interval between the disease onset and LP. However, in contrast to RRMS, CSF analysis has long been a key component in PPMS diagnostic criteria. It is therefore possible that neurologists have been more cautious in establishing a PPMS diagnosis in absence of CSF OCB. Although all our OCB-negative RRMS participants fulfilled the McDonald criteria, we cannot fully exclude that a proportion of them was misdiagnosed due to for instance misinterpretation of MRI findings.

Earlier studies reported that CSF pleocytosis is seen in about 50% of PwMS[39]. We demonstrated that this is only valid for RRMS (44.2%) but not PPMS (29.4%). However, this seemed an age-related effect, as the proportions of participants presenting CSF pleocytosis did not differ between PPMS and RRMS when they were divided in different age categories. The proportion of PwMS demonstrating CSF pleocytosis decreased with age, which can probably be explained by the decline in inflammatory immune response with increasing age[40].

This study has limitations. Using data from an international registry that collects information from routine clinical practice outside a specific study protocol is associated with some risks, including the possibility of data-entry errors and insertion of incomplete patient information. Also, some essential information such as the method used for OCB detection was not available, as the sensitivity to detect OCB depends on the technique used. We included all participants who met predefined minimum data requirements and had at least one CSF measure of interest. Consequently, CSF data were incomplete in the majority of participants. Most PwMS were OCB-positive, reducing the power to detect associations with EDSS worsening in OCB-negative participants and due to a low number of events, multivariable cox regression analysis was not feasible in PPMS. However, if CSF parameters significantly contributed to disability accrual in PPMS, this would be picked up with our univariable analysis. Finally, due to high missingness and incompleteness, information on MRI lesion load could not be included in our multivariable model. However, to the best of our knowledge, this is the largest reported CSF cohort so far, which is, next to the multicentric aspect of the study, a major advantage.

5. Conclusion

This study illustrates that routine CSF analysis offers prognostic in addition to diagnostic information. We demonstrated that presence of CSF OCB seems a biological unfavorable predictive factor regarding future disability accumulation in RRMS but not PPMS. CSF pleocytosis further seems to predict short-term inflammatory disease activity and appears to be a protective factor to reach moderate disability in RRMS. CSF analysis therefore provides

clinicians with useful prognostic information early in the disease course which could aid in patient counselling, clinical decision-making and treatment decisions.

6. Competing interests

Dekeyser, Cathérine: received travel compensation from Sanofi-Genzyme, Merck and Biogen.

Hautekeete, Matthias: None declared.

Cambron, Melissa: received travel and/or consultancy compensation from Sanofi-Genzyme, Roche, Teva, Merck, Novartis, Celgene, Biogen, Sandoz and Janssen.

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Lechner Scott, Jeanette: received travel compensation from Novartis, Biogen, Roche and Merck. Her institution receives the honoraria for talks and advisory board commitment as well as research grants from Biogen, Merck, Roche, TEVA and Novartis.

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Grammond, Pierre: has served in advisory boards for Novartis, EMD Serono, Roche, Biogen idec, Sanofi Genzyme, Pendopharm and has received grant support from Genzyme and Roche, has received research grants for his institution from Biogen idec, Sanofi Genzyme and EMD Serono.

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Gray, Orla: received honoraria as consultant on scientific advisory boards for Genzyme, Biogen, Merck, Roche and Novartis; has received travel grants from Biogen, Merck, Roche, and Novartis; has participated in clinical trials by Biogen and Merck. Her institution has received research grant support from Biogen.

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Buzzard, Katherine: received speaker honoraria and/or education support from Biogen, Teva, Novartis, Genzyme-Sanofi, Roche, Merck and Alexion; has been a member of advisory boards for Merck and Biogen.

Laureys, Guy: received travel and/or consultancy compensation from Sanofi-Genzyme, Roche, Teva, Merck, Novartis, Celgene and Biogen.

7. Contributorship statement

Dekeyser, Cathérine: Conceptualization, Methodology, Data collection, Formal analysis and investigation, Writing - original draft preparation

Hautekeete, Matthias: Conceptualization, Methodology, Data collection, Formal analysis and investigation, Writing - review and editing

Cambron , Melissa: Conceptualization, Methodology, Data collection, Writing - review and editing

Van Pesch, Vincent: Conceptualization, Methodology, Data collection, Writing - review and editing

Patti, Francesco: Data collection, Writing - review and editing

Kuhle, Jens: Data collection, Writing - review and editing

Khoury, Samia: Data collection, Writing - review and editing

Lechner Scott, Jeanette: Data collection, Writing - review and editing

Gerlach, Oliver: Data collection, Writing - review and editing

Lugaresi, Alessandra: Data collection, Writing - review and editing

Maimone, Davide: Data collection, Writing - review and editing

Surcinelli, Andrea: Data collection, Writing - review and editing

Grammond, Pierre: Data collection, Writing - review and editing

Kalincik, Tomas: Data collection, Writing - review and editing

Habek, Mario: Data collection, Writing - review and editing

Willekens, Barbara: Data collection, Writing - review and editing

Macdonell, Richard: Data collection, Writing - review and editing

Lalive, Patrice: Data collection, Writing - review and editing

Csepany, Tunde: Data collection, Writing - review and editing

Butzkueven, Helmut: Data collection, Writing - review and editing

Boz, Cavit: Data collection, Writing - review and editing

Tomassini, Valentina: Data collection, Writing - review and editing

Foschi, Matteo: Data collection, Writing - review and editing

Sánchez-Menoyo, José Luis: Data collection, Writing - review and editing

Altintas, Ayse: Data collection, Writing - review and editing

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Cartechini, Elisabetta: Data collection, Writing - review and editing

Spitaleri, Daniele: Data collection, Writing - review and editing

Gouider, Riadh: Data collection, Writing - review and editing

Soysal, Aysun: Data collection, Writing - review and editing

Van Hijfte, Liesbeth: Data collection, Writing - review and editing

Slee, Mark: Data collection, Writing - review and editing

Amato, Maria Pia: Data collection, Writing - review and editing

Buzzard, Katherine: Data collection, Writing - review and editing

Laureys, Guy: Conceptualization, Methodology, Data collection, Writing - review and editing , Supervision

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Table 1. Demographic, disease and CSF characteristics

	RRMS (n=10 533)	PPMS (n=712)	P value	Relative risk (95% CI)
Demographic characteristics				
Follow-up time (y)	10.78 (6.00-16.74)	12.74 (8.27-18.80)		
Male (%)	29.5% (n=3110)	47.5% (n=338)		
Female (%)	70.5% (n=7423)	52.5% (n=374)		
Age at diagnosis	33 (27-41)	48 (40-54)		
Interval symptom onset - Diagnosis (m)	12 (3-44)	46.5 (25-78)		
Interval date diagnostic LP - Diagnosis (d)	4 (-4-77)	3 (-1-80)		
EDSS at the moment of diagnostic LP	2 (1-3)	4 (3-5.5)		
CSF characteristics				
Documented data on CSF OCB status (n)	10 022	677		
Presence of CSF OCB (%)	84.4% (n=8457)	88.8% (n=601)	**0.002**	RRMS 0.979 (0.967-0.991)
Absence of CSF OCB (%)	15.6% (n=1565)	11.2% (n=76)		PPMS 1.433 (1.135-1.808)
Documented data on serum OCB status in OCB positive participants	4990	350		
Pattern II (CSF restricted OCB)	87.1% (n=4344)	87.1% (n=305)	1.00	
Pattern III (additional OCB in serum)	12.9% (n=646)	12.9% (n=45)		
Documented data on IgG index (n)	4790	336		
Elevated IgG index (>0.7) (%)	61.3% (n=2935)	60.7% (n=204)	0.862	RRMS 1.002 (0.987-1.017)
No elevated IgG index (≤0.7) (%)	38.7% (1855)	39.3% (n=132)		PPMS 0.978 (0.792-1.209)
IgG index (absolute value)	0.80 (0.61-1.16)	0.81 (0.62-1.19)	0.698	
Documented data on CSF WBC (n)	3246	255		
CSF pleocytosis (≥5/µl) (%)	44.2% (n=1436)	29.4% (n=75)	**<0.001**	RRMS 1.045 (1.026-1.064)
No CSF pleocytosis (<5/µl)	55.8% (n=1810)	70.6% (n=180)		PPMS 0.549 (0.423-0.712)
CSF WBC (absolute value)	4 (1.4-9)	2 (1-5)	**<0.001**	
CSF Pleocytosis (≥5/µl) ≤30 years (%)	51.2% (n=620/1212)	62.5% (n=5/8)	0.726	RRMS 0.997 (0.988-1.006) PPMS 1.587 (0.381-6.610)
CSF Pleocytosis (≥5/µl) >30 ≤40 years (%)	44.2% (n=477/1079)	41.3% (n=19/46)	0.763	RRMS 1.005 (0.981-1.029) PPMS 0.892 (0.502-1.586)
CSF Pleocytosis (≥5/µl) >40 ≤50 years (%)	37.4% (n=256/685)	27.4% (n=23/84)	0.092	RRMS 1.048 (0.998-1.10) PPMS 0.662 (0.420-1.045)
CSF Pleocytosis (≥5/µl) >50 ≤60 years (%)	31.4% (n=71/226)	23.2% (n=19/82)	0.202	RRMS 1.11 (0.968-1.272) PPMS 0.731 (0.466-1.146)
CSF Pleocytosis (≥5/µl) >60 years (%)	27.3% (n=12/44)	25.7% (n=9/35)	1.000	RRMS 1.036 (0.669-1.603) PPMS 0.956 (0.540-1.691)
Outcomes				
Confirmed EDSS 4	14.6% (n=1539)	30.3% (n=216)	<0.001	
Confirmed EDSS 6	8.3% (n=873)	36% (n=256)	<0.001	
Confirmed EDSS 7	2.8% (n=296)	18.8% (n=134)	<0.001	
ARR2	0.50 (0-0.50)	0 (0-0.0)	<0.001	

Table 1. Demographic, disease and CSF characteristics of our cohort. Continuous variables were summarized using medians and interquartile ranges (IQR, p25-p75). Abbreviations: RRMS: relapsing remitting multiple sclerosis; PPMS: primary progressive multiple sclerosis; CI: confidence interval; y: years; m: months; LP: lumbar puncture; d: days; OCB: oligoclonal bands; IgG: immunoglobulin G; CSF: cerebrospinal fluid; WBC: white blood cells; EDSS: Expanded Disability Status Scale; ARR2: annualized relapse rate in the first 2 years after diagnosis; IQR: interquartile range.

Table 2. Median survival time for time to reach confirmed EDSS 4, 6 and 7

	Median survial time (y) (95% CI)	
	RRMS	**PPMS**
EDSS 4		
Presence of CSF OCB	24.63 (23.05-26.21)	5.53 (4.74-6.32)
Absence of CSF OCB	29.59 (20.63-38.54)	5.25 (4.39-6.11)
No elevated IgG index	29.59 (20.36-38.81)	6.32 (2.70-9.95)
Elevated IgG index	25.80 (23.99-27.61)	5.78 (5.06-6.50)
Highly elevated IgG index	25.80 (23.34-28.26)	5.78 (4.68-6.88)
Double positives	26.95 (24.71-29.19)	5.79 (4.73-6.84)
No CSF pleocytosis	24.85 (22.00-27.71)	5.92 (4.68-7.17))
CSF pleocytosis	25.76 (21.62-29.90)	3.72 (1.55-5.89)
EDSS 6		
Presence of CSF OCB	34.50 (30.19-38.81)	9.09 (8.04-10.15)
Absence of CSF OCB	27.59 (26.28-28.91)*	8.33 (6.19-10.48)
No elevated IgG index	27.66 (26.94-28.38)	10.03 (7.84-12.22)
Elevated IgG index	34.78 (25.99-43.54)	9.49 (7.55-11.42)
Highly elevated IgG index	34.77 (SE 0.000)	9.75 (6.99-12.55)
Double positives	34.77 (22.90-46.63)	9.49 (7.41-11.56)
No CSF pleocytosis	32.25 (SE 0.000)	9.40 (7.73-11.078)
CSF pleocytosis	28.62 (26.83-30.41)*	8.97 (5.31-12.63)
EDSS 7		
Presence of CSF OCB	37.16 (36.23-38.10)*	19.46 (17.13-21.80)
Absence of CSF OCB	34.66 (32.72-36.60)*	15.21 (12.42-18.00)
No elevated IgG index	31.96 (29.86-34.05)*	19.85 (17.31-22.38)*
Elevated IgG index	33.74 (32.63-34.84)*	20.80 (18.30-23.30)*
Highly elevated IgG index	33.90 (32.39-35.41)*	22.33 (18.47-26.19)*
Double positives	33.73 (32.52-34.93)*	20.95 (18.28-23.62)*
No CSF pleocytosis	29.76 (28.59-30.92)*	21.24 (17.42-25.06)*
CSF pleocytosis	38.01 (36.21-39.80)*	17.51 (15.46-19.57)*

Table 2. Median time to reach confirmed EDSS 4, 6 and 7 for each CSF measure of interest. If the median survival time could not be estimated due to an insufficient amount of events, the mean survival time is presented, which is indicated with an asterixis (*). Abbreviations: Abbreviations: RRMS: relapsing remitting multiple sclerosis; PPMS: primary progressive multiple sclerosis; CI: confidence interval; y: years; CSF: cerebrospinal fluid; OCB: oligoclonal bands; IgG: immunoglobulin G; EDSS: Expanded Disability Status Scale.

Table 3. CSF predictors for time to reach confirmed EDSS 4, 6 and 7

	RRMS			PPMS		
EDSS 4	**Events (n)**	**HR (95% CI)**	**P Value**	**Events (n)**	**HR (95% CI)**	**P Value**
Presence of CSF OCB	1369	1.272 (1.089-1.485)	**0.002**	206	1.151 (0.665-1.99)	0.616
IgG index absolute value	587	1.097 (0.993-1.213)	0.069	100	1.060 (0.720-1.560)	0.769
Elevated IgG index	587	1.228 (1.033-1.459)	**0.020**	100	1.081 (0.720-1.624)	0.707
Highly elevated IgG index	587	1.241 (1.019-1.512)	**0.032**	100	1.099 (0.684-1.766)	0.697
"Double positives" vs "Negatives"	524	1.635 (1.186-2.252)	**0.003**	95	1.090 (0.512-2.321)	0.822
"Double positives" vs "OCB positives"	524	1.076 (0.878-1.32)	0.480	95	1.042 (0.657-1.653)	0.860
"Double positives" vs elevated IgG index	524	1.074 (0.728-1.586)	0.719	95	0.567 (0.203-1.58)	0.278
CSF WBC count	407	0.982 (0.970-0.994)	**0.004**	80	1.025 (0.997-1.053)	0.079
CSF pleocytosis	407	0.774 (0.632-0.948)	**0.013**	80	1.440 (0.906-2.288)	0.123
EDSS 6	**Events (n)**	**HR (95% CI)**	**P Value**	**Events (n)**	**HR (95% CI)**	**P Value**
Presence of CSF OCB	755	1.314 (1.062-1.626)	**0.012**	244	0.960 (0.630-1.464)	0.850
IgG index absolute value	331	1.047 (0.905-1.21)	0.538	120	0.876 (0.601-1.276)	0.490
Elevated IgG index	331	0.991 (0.788-1.247)	0.940	120	1.163 (0.802-1.686)	0.426
Highly elevated IgG index	331	0.961 (0.735-1.256)	0.770	120	1.089 (0.702-1.688)	0.703
"Double positives" vs "Negatives"	287	1.36 (0.874-2.115)	0.173	114	1.035 (0.533-2.008)	0.920
"Double positives" vs "OCB positives"	287	0.875 (0.665-1.15)	0.339	114	1.168 (0.762-1.791)	0.476
"Double positives" vs elevated IgG index	287	1.084 (0.626-1.878)	0.774	114	1.498 (0.471-4.763)	0.493
CSF WBC count	209	0.985 (0.969-1.001))	0.058	85	0.999 (0.970-1.028)	0.929
CSF pleocytosis	209	0.814 (0.615-1.077)	0.150	85	1.205 (0.759-1.913)	0.429
EDSS 7	**Events (n)**	**HR (95% CI)**	**P Value**	**Events (n)**	**HR (95% CI)**	**P Value**
Presence of CSF OCB	251	1.686 (1.111-2.558)	**0.014**	126	0.846 (0.485-1.476)	0.555
IgG index absolute value	114	1.043 (0.828-1.314)	0.722	59	0.685 (0.366-1.284)	0.238
Elevated IgG index	114	1.297 (0.864-1.947)	0.210	59	1.329 (0.770-2.297)	0.307
Highly elevated IgG index	114	1.18 (0.735-1.895)	0.493	59	1.039 (0.534-2.021)	0.911
"Double positives" vs "Negatives"	95	2.089 (0.894-4.879)	0.089	55	1.209 (0.43-3.404)	0.719
"Double positives" vs "OCB positives"	95	1.378 (0.827-2.298)	0.218	55	1.27 (0.673-2.395)	0.461
"Double positives" vs elevated IgG index	95	2.819 (0.686-11.577)	0.150	55	0.998 (0.239-4.164)	0.998
CSF WBC count	64	0.972 (0.937-1.008)	0.127	33	0.979 (0.914-1.049)	0.553
CSF pleocytosis	64	0.639 (0.377-1.082)	0.096	33	1.235 (0.584-2.612)	0.581

Table 3. Results of the cox regression analysis for time to reach confirmed EDDS 4, 6 and 7. For the RRMS cohort, results of the multivariable analysis are shown. Due to a low number of events in the PPMS cohort, multivariable analysis was infeasible, therefore, results of the univariable analysis are shown for PPMS. All multivariable analyses were corrected for age at diagnosis, sex, EDSS at the moment of LP, treatment category (time varying), disease duration and number of relapses until the event or censoring. For each CSF measure of interest, the number of events represents the total amount of occurred events within the at risk population. Abbreviations: RRMS: relapsing remitting multiple sclerosis; PPMS: primary progressive multiple sclerosis; EDSS: Expanded Disability Status Scale; HR: hazard ratio; CI: confidence interval; CSF: cerebrospinal fluid; OCB: oligoclonal bands; IgG: immunoglobulin G; WBC: white blood cells; vs: versus; U: univariable.

Table 4. CSF predictors for time to reach confirmed EDSS 4, 6 and 7: Post hoc analysis

	RRMS			PPMS		
EDSS 4	**Events (n)**	**HR (95% CI)**	**P Value**	**Events (n)**	**HR (95% CI)**	**P Value**
Presence of CSF OCB	965	1.455 (1.192-1.752)	**<0.001**	187	1.114 (0.633-1.959)	0.709
IgG index absolute value	436	1.093 (0.975-1.226)	0.129	88	1.032 (0.682-1.562)	0.880
Elevated IgG index	436	1.207 (0.990-1.473)	0.063	88	1.046 (0.682-1.606)	0.836
Highly elevated IgG index	436	1.239 (0.987-1.556)	0.065	88	1.056 (0.641-1.739)	0.832
"Double positives" vs "Negatives"	393	1.742 (1.195-2.538)	**0.004**	84	1.006 (0.454-2.227)	0.988
"Double positives" vs "OCB positives"	393	1.052 (0.834-1.328)	0.668	84	0.988 (0.608-1.607)	0.962
"Double positives" vs elevated IgG index	393	1.159 (0.725-1.854)	0.537	84	0.554 (0.198-1.548)	0.260
CSF WBC count	304	0.979 (0.964-0.993)	**0.004**	73	1.024 (0.996-1.053)	0.099
CSF pleocytosis	304	0.706 (0.557-0.894)	**0.004**	73	1.356 (0.842-2.183)	0.210
EDSS 6	**Events (n)**	**HR (95% CI)**	**P Value**	**Events (n)**	**HR (95% CI)**	**P Value**
Presence of CSF OCB	481	1.361 (1.035-1.788)	**0.027**	209	0.927 (0.596-1.443)	0.738
IgG index absolute value	224	1.077 (0.914-1.27)	0.376	101	0.852 (0.566-1.283)	0.444
Elevated IgG index	224	0.988 (0.749-1.304)	0.933	101	1.277 (0.847-1.923)	0.243
Highly elevated IgG index	224	0.941 (0.679-1.304)	0.713	101	1.131 (0.697-1.833)	0.619
"Double positives" vs "Negatives"	203	1.479 (0.873-2.507)	0.146	98	1.137 (0.544-2.375)	0.733
"Double positives" vs "OCB positives"	203	0.921 (0.666-1.273)	0.617	98	1.236 (0.777-1.966)	0.372
"Double positives" vs elevated IgG index	203	1.569 (0.731-3.368)	0.247	98	1.279 (0.401-4.077)	0.678
CSF WBC count	143	0.981 (0.961-1.001)	0.069	78	1.00 (0.970-1.030)	0.974
CSF pleocytosis	143	0.666 (0.471-0.943)	**0.022**	78	1.124 (0.691-1.830)	0.638
EDSS 7	**Events (n)**	**HR (95% CI)**	**P Value**	**Events (n)**	**HR (95% CI)**	**P Value**
Presence of CSF OCB	110	1.753 (0.913-3.366)	0.092	87	0.973 (0.488-1.939)	0.937
IgG index absolute value	61	0.989 (0.686-1.427)) (U)	0.955	41	0.783 (0.386-1.587)	0.497
Elevated IgG index	61	1.414 (0.816-2.452) (U)	0.217	41	1.496 (0.763-2.932)	0.241
Highly elevated IgG index	61	0.966 (0.487-1.916) (U)	0.920	41	1.193 (0.536-2.656)	0.665
CSF WBC count	39	0.973 (0.929-1.019 (U)	0.246	28	0.986 (0.922-1.054)	0.675
CSF pleocytosis	39	0.564 (0.286-1.113) (U)	0.099	28	1.495 (0.689-3.243)	0.309

Table 4. Post hoc analysis where the observation period ended 10 years after diagnosis. Results of the cox regression analysis for time to reach confirmed EDDS 4, 6 and 7. For the RRMS cohort, results of the multivariable analysis are shown. If, due to a low number of events, only univariable analysis could be performed in RRMS, this is indicated with"(U)". Due to a low number of events in the PPMS cohort, multivariable analysis was infeasible, therefore, results of the univariable analysis are shown for PPMS. All multivariable analyses were corrected for age at diagnosis, sex, EDSS at the moment of LP, treatment category (time varying), disease duration and number of relapses until the event or censoring. For each CSF measure of interest, the number of events represents the total amount of occurred events within the at risk population. Abbreviations: RRMS: relapsing remitting multiple sclerosis; PPMS: primary progressive multiple sclerosis; EDSS: Expanded Disability Status Scale; HR: hazard ratio; CI: confidence interval; CSF: cerebrospinal fluid; OCB: oligoclonal bands; IgG: immunoglobulin G; WBC: white blood cells; vs: versus; U: univariable.

Table 5. CSF predictors for ARR2

	RRMS			PPMS		
	Adjusted R^2	**β (95%CI)**	**P Value**	**Adjusted R^2**	**β (95%CI)**	**P Value**
Presence of CSF OCB	0.034	0.010 (-0.21-0.041)	0.527	0.037	-0.006 (-0.95-0.082)	0.891
IgG index absolute value	0.034	0.006 (-0.017-0.030)	0.587	0.114	0.021 (-0.029-0.071)	0.407
Elevated IgG index	0.034	0.019 (-0.014-0.052)	0.260	0.113	-0.029 (-0.106-0.048)	0.458
"Double positives"	0.033	0.032 (-0.019-0.084)	0.220	0.130	-0.085 (-0.220-0.051)	0.221
CSF WBC count	0.034	0.001 (-0.001-0.003)	0.434	0.042	-0.002 (-0.007-0.003)	0.342
CSF pleocytosis	0.036	0.052 (0.010-0.094)	**0.015**	0.053	-0.067 (-0.148-0.014)	0.103

Table 5: Results of the multivariable linear regression analysis (generalised linear model) to identify CSF variables associated with ARR2. All analyses were adjusted for age at diagnosis, sex, EDSS at the moment of LP, highest treatment category until the first 2 years after diagnosis and number of relapses between disease onset and diagnosis. Abbreviations: ARR2: annualized relapse rate in the first 2 years after diagnosis; RRMS: relapsing remitting multiple sclerosis; PPMS: primary progressive multiple sclerosis; CSF: cerebrospinal fluid; OCB: oligoclonal bands; IgG: immunoglobulin G; WBC: white blood cells.

Figure 1:

Title: Flowchart of participant selection

Caption figure 1: Flowchart of participant selection. Abbreviations: ID: identifier; MS: multiple sclerosis; CIS: clinically isolated syndrome; PPMS: primary progressive multiple sclerosis; CSF: cerebrospinal fluid; Expanded Disability Status Scale; OCB: oligoclonal bands; IgG: immunoglobulin G; WBC: white blood cells.

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