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Cerebrospinal fluid biomarkers as predictors for disease outcome and functional disability in multiple sclerosis

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Abstract

In multiple sclerosis (MS) research, cerebrospinal fluid (CSF) is preferred over other body fluids because of its close relation to the pathological alterations of MS, and CSF biomarkers can shed light into different aspects of the disease's pathophysiology.

The aim of this research was to evaluate the predictive value of immune-inflammatory and metabolic CSF biomarkers for disability in multiple sclerosis and whether these biomarkers assessed at diagnosis add prognostic value using a population-based MS cohort.

After screening 340 suspected MS patients, a dataset of 83 eligible patients with secondary progressive MS (SPMS) and relapsing-remitting MS (RRMS) treated with interferon beta-1a was established. A 2-year longitudinal cohort study employing an integrated approach was conducted, combining quantitative and qualitative methods to analyze CSF patterns.

Results indicated a significant association between CSF lactate, IgG, and IgA levels with Expanded Disability Status Scale (EDSS) scores, suggesting their potential for predicting disease outcome. Notably, CSF lactate emerged as a significant early predictor, indicating its utility in prognosticating disease course and functional disability. Furthermore, a multiple regression model identified admission CSF lactate levels and CSF IgG as significant early predictive risk factors for functional disability in MS patients. These findings highlight the importance of CSF biomarkers in enhancing prognostic assessments and treatment planning for MS patients, providing valuable insights into disease progression and functional outcomes.

Key words: cerebrospinal fluid, immune-inflammatory, metabolic biomarkers, multiple sclerosis, EDSS, disability

Introduction

Conventionally viewed as an immune-mediated disorder, multiple sclerosis (MS) is characterized by chronic inflammatory demyelination in the central nervous system, often associated with blood-brain barrier breakdown and immune cell infiltration, resulting in acute tissue damage and neurological deficits (Frischer et al., 2009).

Exploring various biomarkers including CSF glucose, total protein, and immunoglobulin levels provides valuable insights into the pathology of MS (Deisenhammer et al., 2019). Despite challenges in standardization, these biomarkers offer potential for early prognostication and therapeutic optimization in managing MS.

The 2017 revision of the McDonald criteria (Thompson et al., 2017) facilitates MS diagnosis, requiring a clinical neurological exam, the presence of oligoclonal bands (OCBs) in cerebrospinal fluid (CSF), magnetic

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resonance imaging (MRI), and exclusion of relevant differential diagnoses. MS diagnosis is based on the coexistence of clinical or biomarkers indicating both dissemination in space (DIS; involvement of different central nervous system (CNS) locations) and dissemination in time (DIT; e.g., 2 relapses), essential for confirming the chronic nature of the disease.

Recent studies explore novel CSF and blood serum indicators for MS diagnosis (Porter et al., 2020; Probert et al., 2021). Proton- magnetic resonance spectroscopy (MRS) studies demonstrate elevated CSF lactate levels in MS patients, potentially indicating a link to disease activity (Regenold et al., 2008). Elevated CSF lactate levels in MS patients, attributed to anaerobic metabolism in damaged tissue, suggest a link between lactate and disease activity. Several studies (Albanese et al., 2016; Amorini et al., 2014) have reported elevated CSF lactate levels in MS patients compared to healthy controls, suggesting a potential association between lactate and disease activity. Despite this association, the precise role of CSF lactate as a biomarker for MS diagnosis or disease progression remains unclear.

Immunoglobulins (Ig) are typically absent in normal cerebrospinal fluid (CSF), but the most characteristic abnormality in patients with multiple sclerosis is certainly the intrathecal synthesis of IgG (Adam et al., 2001), which can be demonstrated with different sensitivity, by various methods (qualitative and quantitative). While IgG mainly originates from peripheral sources crossing the blood-brain barrier (BBB), its presence in CSF doesn't always indicate inflammation (Falip et al., 2001; Lunding et al., 2000). The golden standard for the demonstration of intrathecal synthesis of IgG is the detection of CSF OCGBs by IEF and silver staining. For instance, the proportion of patients with definite multiple sclerosis that demonstrate oligoclonal bands was reported in the range of 80% (Farina et al., 2017; Pryce and Baker, 2018; Rojas et al., 2012)., and these bands were demonstrable even in cases where CSF γ-globulin levels were not elevated (Berner et al., 1972), showing superiority of the IEF method and, lower sensitivity of the quantitative detection of IgG by laser immunonephelometry. Immunologically based measure, IgG index, offer diagnostic and prognostic insights (Link and Huang, 2006; Senel et al., 2019).

The IgG Index analysis is a quantitative measurement of intrathecal IgG synthesis in which a positive result for the CSF IgG index indicates an inflammatory CNS condition (Link and Tibbling, 1997). However, other Ig isotypes (IgM, IgA) are also differentially increased in MS CSF relative to blood. Elevated IgM levels in CSF correlate with more severe MS progression and MRI lesion severity (Huss et al., 2018).

Total protein concentration in CSF may indicate the integrity of the BBB. Markedly increased total protein (>100 mg/dL) or increased white blood cell count beyond 50 cells/μL should be considered a red flag and is more suggestive of an infectious or other inflammatory disease rather than MS (Hegen et al., 2016). BBB integrity may be assessed by calculation of the albumin quotient (Tibbling et al., 1977) and despite the fact that the test is not sensitive or specific for the diagnosis of MS, the finding of high albumin quotient is associated with systemic inflammation.

Three investigations evaluated glucose levels in individuals with multiple sclerosis (MS), two scrutinizing serum glucose (Pitt et al., 2000; Yelamanchi et al., 2016) and one concentrating on cerebrospinal fluid (CSF) glucose (Kim et al., 2017), all revealing decreased glucose levels in MS subjects compared to controls, with Dickens et al. (2014) specifically highlighting lower glucose levels in secondary progressive MS (SPMS) versus relapsing-remitting MS (RRMS).

This study aims to investigate the prognostic value of routine CSF biochemical variables, specifically the IgG index and presence of OCGBs, in predicting disability at the time of MS diagnosis using a population-based MS cohort. The research aims to assess the capacity of biochemical and protein markers to predict disease outcome and functional disability in MS patients, providing clinicians with early prognostic information to tailor treatment strategies effectively.

Material and methods

Study subjects

The study initially screened a total of 340 patients suspected of MS, of whom 293 met the criteria for clinical definite MS and were enrolled in the study. Inclusion criteria were strictly applied, ensuring that patients did not have any other neurological, psychiatric, or neurodegenerative disorders, immunological disorders, or severe comorbidities, and had not received specific medications for at least two months prior to admission. Additionally, exclusion criteria included specific patterns detected through DISC-PAGE densitometry of CSF immunological patterns and contraindications for lumbar puncture. After applying these criteria, 163 patients were deemed eligible for the cohort study. Ultimately, the final dataset consisted of 83 patients with secondary progressive MS (SPMS) and relapsing-remitting MS (RRMS) who were treated with interferon beta-1a and exhibited observable changes in CSF immunological patterns at admission yet did not experience severe disability during the 2-year treatment period following diagnosis. The flow process of the clinical trial is illustrated in Fig. 1. Ethical approval was obtained from the Human Research Ethics Committee at the Ss. Cyril and Methodius University, Medical Faculty, Skopje, and all participants provided informed consent prior to participation in the study.

Biological samples and biochemical analysis

Venous blood samples were collected in the morning, after overnight fasting and left undisturbed at room

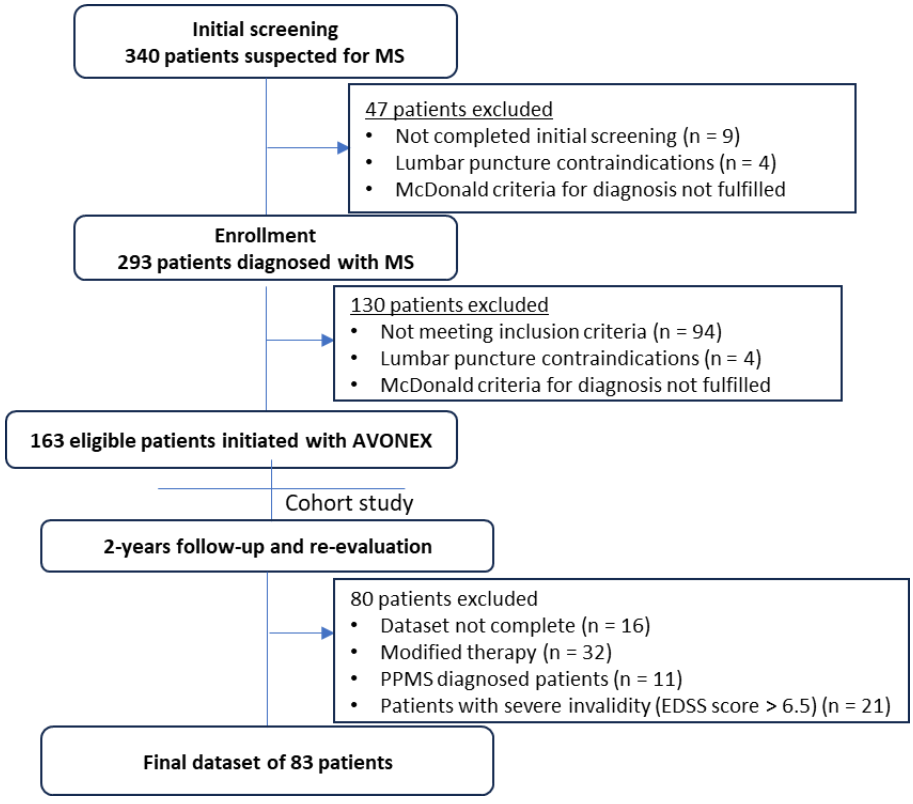

Fig. 1. Flow diagram for the clinical cohort study.

temperature for 30 min to clot, then centrifuged at 3,000×g, 15 min, at 4 °C. Further, the serum aliquots were stored at –80 °C until use. Simultaneously, diagnostic lumbar puncture and CSF sampling was also performed at admission, CSF was cooled on an ice-bath during sampling, centrifuged at 200×g for 15 min (at 4 °C), and the cell-free CSF was aliquoted and stored at –80°C until further analysis.

The quantitative detection of total CSF proteins, total serum proteins, CSF lactate levels, CSF glucose, CSF urea levels and CSF creatinine were performed on Mindray BS-200 Chemistry Analyzer. The quantitative detection of CSF IgG, serum IgG, CSF IgA, CSF IgM, serum albumins and CSF albumins was performed with laser immunonephelometry and particle-enhanced immunonephelometry. A parallel sample of serum and CSF were run for the albumin and IgG levels, in order to calculate the corresponding quotients. The following equations were used for this purpose, in compliance to Tibbling et al. (1977):

$$\text{Albumin index} = Q_{\text{Alb}} \times 1000 = \frac{\text{CSF albumin (mg}\cdot\text{L}^{-1})}{\text{Serum albumin (mg}\cdot\text{L}^{-1})} \times 1000$$

$$\text{IgG index} = \frac{Q_{\text{IgG}}}{Q_{\text{Alb}}} = \frac{\frac{\text{CSF IgG}}{\text{Serum IgG}}}{\frac{\text{CSF Alb}}{\text{Serum Alb}}}$$

The IgG synthesis rate was calculated in compliance with the Tourtellotte's formula - TOURT (Tourtellotte, 1975):

$$\text{IgG synthesis rate mg/24 h} = \left[\left(\text{IgG}_{\text{CSF}} - \frac{\text{IgG}_{\text{serum}}}{369}\right) - \left(\text{Alb}_{\text{CSF}} - \frac{\text{Alb}_{\text{serum}}}{230}\right) \times \left(\frac{\text{IgG}_{\text{serum}}}{\text{Alb}_{\text{serum}}}\right) \times 0.43\right] \times 5$$

The CSF Discontinuous Polyacrylamide Gel Electrophoresis (DISC-PAGE) of CSF was performed following the procedure of Ornstein (1964) and Davis (1964). The separation was made by using 5% stacking (0.125 mol/L Tris-HCL, pH = 6.8) and 7.5% separating (0.375 mol/L Tris-HCL, pH = 8.8) gels, in TRIS-glycine running buffer with ionizable tracking dye (0.005% bromophenol blue). The electrophoresis was run at 5 mA per sample. After the separation, the gels were stained with Amido black 10B (1 g in 200 mL 7% acetic acid). Interpretation and densitometric analysis of CSF fractions were performed and each protein zone was identified and quantified according to the peak area and then compared to a standard. The obtained electrophoretic patterns were assigned to one of the CSF immunological patterns in the sense of the neuro-immunological classification: *inflammatory CSF pattern* (INF, reflecting local synthesis) is characterized by clearly increased G zone (25-30%

increase in peak density), clear subdomination of the G band in the total band composition (usually around 3 times larger band percentage, when compared to normal CSF, band % usually exceeding 28%) and albumin zone usually slightly decreased (around 30-38% of the albumin band in the total band composition); *non-inflammatory transudate CSF pattern (TU*, reflecting abnormal barrier function) is characterized by a significant reduction in the prealbumin zone peak density (approximately 40%) and peak area (10-15%), an increased albumin zone, with the albumin band dominating the total band composition (band percentage surpassing 48% and peak area exceeding 5000), a notable increase in the A zone by 20-40% in peak density and around 30% in peak area and a clear decrease in the B2 zone by 30% in peak density. Additionally, α2-macroglobulin and haptoglobin 1-1 bands typically appear between the B2 and G zone, termed as "post-transferrin" fractions. A distinctive trait of the TU pattern is the presence of haptoglobin 2-1 and 2-2 polymers, absent in normal CSF; *inflammatory plus transudate CSF pattern* (INF+TU, reflecting local synthesis plus abnormal barrier) having the characteristics of the both previously described patterns. The pattern resembles to a larger extent to the TU type, with the only difference of the largely increased G zone, as in the INF type.

An integrated approach has been applied by combination of different available methods, including quantitative measurements (determination of total CSF proteins, CSF glucose levels, CSF lactate levels, CSF urea and CSF creatinine levels by spectroscopy; determination of CSF albumins and CSF IgG by laser immunonephelometry, determination of IgA and IgM by particle-enhanced immunonephelometry), semiquantitative methods (DISC-PAGE with densitometry tracing) and qualitative methods (IEF with immunofixation and silver staining) to significantly increase the sensitivity of our findings.

Statistical analysis

Categorical variables were presented as percentages via segmented bar graphs, and the $\chi 2$ test was employed to evaluate significant differences in frequencies among groups. Given that most quantitative variables (excluding age, disease onset, and CSF urea levels) significantly deviated from normality (Shapiro-Wilk test $p < 0.05$), results were reported as median and interquartile range (IQR), illustrated using boxplots. Central tendency comparisons among groups were assessed using the Kruskal−Wallis H test, with subsequent Mann−Whitney U tests for between-group multiple comparisons.

Effect size for the nonparametric Mann−Whitney test was determined using the correlation coefficient *r*, following Cohen's proposal, computed as $Z/\sqrt{N}$. Additionally, the square of the correlation coefficient (r2¬) was reported to gauge the proportion of variance in ranks of the dependent variable accounted for by the categorical variable (calculated as $r2 = \eta 2 = Z2/N$). Interpretation of r and η2 adhered to Cohen's suggested guidelines (for *r:* 0.1 – small effect, 0.3 – moderate effect, 0.5 – large effect; for η2: 0.01 − small effect, 0.06 − moderate effect, 0.14 − large effect; Cohen, 1988). Conversely, epsilon squared estimate (ε2) served as an effect size statistic for the Kruskal–Wallis test (calculated as $\varepsilon 2 = H/[(N2 – 1)/(N + 1)]$). Categorical data effect sizes were expressed using the extension of the ϕ statistic (Cramér's V, calculated as $V = \sqrt{\chi 2}/\sqrt{(N – k)}$)

The bivariate statistical analyses were performed by using non-parametric correlation with the Spearman ρ correlation coefficient. The prediction of the functional disability (EDSS scores) was performed by building multiple regression models, with a stepwise-backward selection, using the standard least squares method. In all cases, the level of statistical significance was defined as $p < 0.050$ (marked as *), i.e. $p < 0.001$ for highly significant (***).

All data were analyzed using IBM SPSS Statistics® 21, with additional modeling performed in JMP® 14 (Copyright © 2018 SAS Institute Inc.).

Results and discussion

Basic characteristics of the patients

Eighty-three patients have completed the study. Their basic demographic, clinical and biochemical characteristics are summarized in Table 1. As it can be seen, the study has included predominantly female patients with MS (67.5%), with a median age of 36 years and a median disease onset of 30 years. Most of the patients at admission were characterized by INF electrophoretic pattern (63.9%; electrophoretic patterns shown on Fig. 2a); IgG oligoclonal bands were also detected in more than half of the studied patients (60.2%). The median values for the examined admission CSF biomarkers can be also seen in Table 1.

Two years later, on re-evaluation, most of the patients were diagnosed with RRMS (81.9%). The median EDSS score in the cohort group after 2-years follow-up and interferon beta-1a treatment was 2.5 (which corresponds to mild disability in one functional system, or minimal disability in two systems).

Relations between the presence of OCGBs and the examined CSF biochemical markers

Most of the CSF variables (total proteins, albumins, IgM levels, glucose levels, albumin index, urea levels, creatinine levels) were not associated with the presence of OCGBs. Nevertheless, some of the immunological markers have shown strong positive relations (Fig. 2); for instance, as expected, all IgG markers were positively associated with the presence of the oligoclonal patterns, with the variable IgG synthesis rate (mg/24 h) showing the strongest positive relation ($r = 0.638$, $\eta^2 = 0.407$).

Table 1. Demographic, clinical and biochemical characteristics of the patients

Variables	Frequency[a] / Median (IQR)[b]
Admission characteristics	
Sex	
Male participants	32.5%
Female participants	67.5%
Age	36 (13)
Disease onset	30 (12)
Admission CSF electrophoretic signatures assigned to the defined CSF types	
Inflammatory CSF type (INF)	63.9%
Non-inflamatory transudate CSF type (TU)	19.2%
Inflammatory plus transudate CSF type (INF+TU)	16.9%
Qualitative detection of CSF oligoclonal IgG bands	
Presence of OCGBs	60.2%
Admission CSF biomarkers levels	
Total CSF proteins (g/L)	0.37 (0.20)
CSF albumins (mg/L)	193 (117)
CSF IgG (mg/L)	41.50 (28.90)
CSF IgA (mg/L)	3.170 (3.670)
CSF IgM (mg/L)	0.794 (1.610)
Albumin index (Q_{Alb} × 1000)	4.20 (2.60)
IgG index	0.780 (0.360)
IgG *de novo* synthesis rate (mg/24 h)	4.6 (9.6)
CSF glucose levels (mmol/L)	3.70 (0.70)
CSF lactate levels (mmol/L)	1.41 (1.06)
CSF urea levels (mmol/L)	3.70 (0.94)
CSF creatinine levels (mmol/L)	46.0 (22.0)
CSF immunological patterns according to cut-off values of CSF IgG and CSF Albumin (in compliance to Schuller and Sagar, 1981	
Inflammatory CSF type (INF; CSF IgG > 40 mg/L)	73.5%
Non-inflamatory transudate CSF type (TU; CSF albumin > 334mg/L)	2.4%
Inflammatory plus transudate CSF type (INF+TU)	7.2%
Pattern assessed as normal	16.9%
Characteristics on re-evaluation (after 2 years follow-up and interferon beta-1a treatment)	
MS type (according to the disease progression)	
SPMS	18.1%
RRMS	81.9%
Functional disability quantified by the EDSS score	2.5 (2)

[a]The categorical variables are expressed as total frequency (% of individuals) for each group
[b]The continuous variables are expressed as median (IQR)

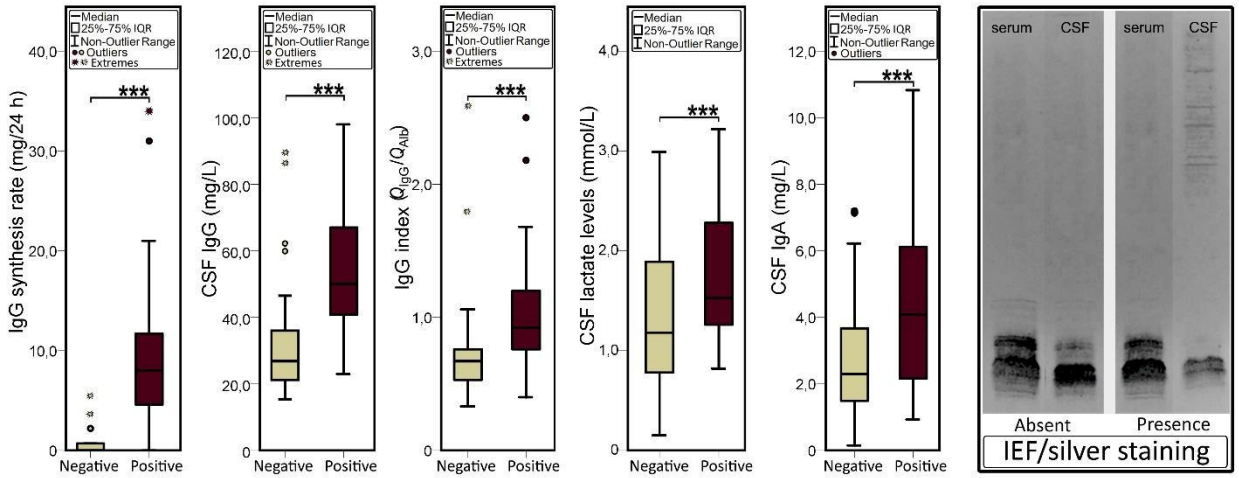

Fig. 2. Detection of OCGBs (variables ordered according to the effect size of the association with the presence of IgG oligoclonal pattern). *$p < 0.05$; ***$p < 0.001$.

As it can be seen from Fig. 2, when compared to patients with absence of OCGBs, the patients with IgG oligoclonal pattern were characterized with almost double higher median values of the CSF IgG (50.1 VS 26.9 mg/L); the differences were found statistically significant, with a relatively large effect size ($U = 240.5$, $p = 5.35 \cdot 10^{-9}$, $r = 0.597$), suggesting that 35.6% of the variability of IgG levels can be attributed to the presence of OCGBs. The IgG index ($U = 352.0$, $p = 0.00001$) and CSF lactate levels ($U = 513.5$, $p = 0.0037$), were also significantly higher in patients with IgG oligoclonal pattern, with a moderate effect size ($r = 0.483$ and $r = 0.318$, respectively). CSF IgA levels were also found significantly higher in patients with OCGBs, although the effect size of the relation was small (only 8.8% of the variability of IgA levels can be explained by the presence of oligoclonal bands; $U = 534.5$, $p = 0.0069$, $r = 0.297$). As mentioned, the variable IgG synthesis rate has shown the largest effect size ($U = 208.0$, $p = 6.17 \cdot 10^{-9}$, $r = 0.638$), suggesting that 40.7% of the variability in IgG synthesis rate can be accounted by the presence of oligoclonal bands; according to the follow-up ROC analyses, the value of only ≥ 0.75 can fairly detect patients that will show OCGBs (96% sensitivity, 75.8% specificity; ROC AUC = 0.874, $p = 9.41 \cdot 10^{-9}$).

Concerning the OCGBs, all IgG related variables have shown some relation with the presence of OCGBs detected by IEF and silver staining. Nevertheless, the variable IgG synthesis rate have shown the highest association, suggesting the utility of the TOURT formula for estimation of the *de novo*, locally synthetized IgG in the CNS (Spence, 1983; Tourtellotte, 1975). On the basis of our findings, we have proposed the value of ≥ 0.75 mg/24 h for the IgG synthesis rate as optimal for detection of patients with local (intrathecal) IgG synthesis, which was characterized by high sensitivity and specificity (96% sensitivity, 75.8% specificity; ROC AUC = 0.874, $p = 9.41 \cdot 10^{-9}$).

Factors affecting the functional disability in MS patients

Most of the CSF biomarkers have shown positive and significant correlations with the EDSS scores (Table 2). Bivariate fitting models were built in order to assess these relationships further. Despite the relatively weak correlation between age and EDSS scores (Fig. 3a), a discernible positive trend was evident. Notably, CSF lactate, IgG, and IgA exhibited robust correlations with EDSS scores, accompanied by narrower 95% confidence intervals, suggesting their potential utility in predicting EDSS scores (Fig 3b, c). Bivariate regression analyses indicated that the relationship between EDSS and total CSF proteins and albumin index was more pronounced at lower values, with density contours primarily clustered between values of 2 – 6 for the albumin index and between 20 and 60 g/L for total CSF proteins (Fig. 3d). Additionally, density contours for the relationship between EDSS and the 24-hour IgG synthesis rate were concentrated between values of 0 and 15 mg/24 hours, indicating potential limitations in estimating EDSS scores at higher synthesis rates (Fig. 3e).

Concerning the categorical variables, the MS type has also shown significant and moderate-to-large effect on the EDSS scores; the EDSS scores were significantly higher in SPMS, when compared to the RRMS patients (median EDDS 4.0 VS 2.0, respectively; $U = 161.0$; $p = 0.00003$, $r = 0.456$; Fig. 3f). The EDSS scores were also significantly affected by the presence of IgG oligoclonal bands, with a moderate effect size (median EDSS 1.5 for absent OCGBs VS 3.0 for patients with detected OCGBs; $U = 462.0$, $p = 0.0007$, $r = 0.373$; Fig. 3g). Finally, the presence of specific CSF immunological patterns has also shown significant and large effect size on the EDSS scores ($H = 27.634$, $p = 9.99\ 10^{-7}$, $\varepsilon 2 = 0.337$; Fig. 3h); patients with combined INF+TU pattern were characterized by significantly higher

EDSS scores, when compared both to the patents with INF pattern (U = 231.0, p = 0.030, r = 0.239), and the patients with TU pattern (U = 17.5, p = 0.00007, r = 0.436).

Concerning the prognostication of functional disability, most of the CSF biomarkers have shown positive and significant correlations with the EDSS scores. In compliance with the performed bivariate fitting models (Fig. 3), the relations of CSF lactate, IgG, and IgA with the EDSS scores were quite strong, suggesting that they might be particularly useful for prediction of the EDSS scores; the bivariate regression analyses for total CSF proteins and albumin index have shown strong relationship only for their lower values (see Fig. 3d), and the density contours for the relationship between EDSS and the 24 h IgG synthesis rate were concentrated only between the values of 0 and 15 mg/24 h, suggesting that higher values for these markers might not be suitable for estimation of the EDSS scores. The IgG index, although assessed as useful marker for reflection of the local synthesis, did not correlate significantly with the EDSS scores. These results are in good congruence with several previous studies which have confirmed absence of relationship between the severity or the activity of the disease and the intrathecal production of IgG detected by the IgG index (Lamers and Wevers, 1995; Sobek et al., 2000; Valenzuela et al., 1982), despite the fact that an increased IgG index is found in more than 80% of patients with multiple sclerosis (Adam et al., 2001).

Multiple regression models for prediction of the MS functional disability

Following the initial bivariate analyses, it was determined that the linearity assumption was not met for several variables: disease onset, CSF IgM, IgG index, CSF glucose, CSF creatinine, and CSF urea levels (with correlation coefficients less than 0.2, as shown in Table 2). Consequently, these variables were initially excluded from the model, so these variables were not initially included in the model. The multiple regression modeling for prediction of the EDSS scores (as a quantitative measure of the MS functional disability) was performed with a stepwise-backward selection, using the standard least squares method. For building up the model, initially, all 8 variables which have shown significant correlations (and corr. coefficients higher than 0.2) were included in the analyses (Table 2); moreover, the categorical variables – presence of OCGBs and presence of combined INF+TU pattern – were also added (individually, and as a crossed variable). Four cycles were performed, with step-by step backward-elimination of the variable characterized by the highest p value as non-significant predictor in each cycle; the final, optimal multiple regression model characterized with the highest R^2_{Adj} and the highest overall predictive capacity was generated in the fifth cycle, consisting of five independent variables (model effects), 2 of them estimated as significant predictors (Table 3).

Table 2. Non-parametric correlations for age, disease onset and admission CSF biomarker levels with the EDSS scores, 2-years later (N = 83)

Variable	Spearman Cor. Coeff. (ρ)	Sig. (2-tailed)
Admission CSF lactate levels (mmol/L)	0.659	$1.29 \cdot 10^{-11}$ (***)
Admission CSF IgG (mg/L)	0.548	$8.07 \cdot 10^{-08}$ (***)
Admission CSF IgA (mg/L)	0.420	0.00008 (***)
Admission total CSF proteins (g/L)	0.416	0.00009 (***)
Admission IgG synthesis rate (mg/24 h)	0.315	0.004 (*)
Admission CSF albumins (mg/L)	0.295	0.007 (*)
Admission albumin index ($Q_{Alb} \times 1000$)	0.274	0.012 (*)
Age (years)	0.261	0.017 (*)
Admission CSF IgM (mg/L)	0.193	0.080 (ns)
Disease onset	0.115	0.302 (ns)
Admission IgG index	0.081	0.468 (ns)
Admission CSF glucose levels (mmol/L)	−0.009	0.935 (ns)
Admission CSF creatinine levels (mmol/L)	−0.017	0.880 (ns)
Admission CSF urea levels (mmol/L)	−0.142	0.199 (ns)

The variables are sorted according to the value of the corr. coeff. ρ.
***$p < 0.001$; *$p < 0.05$.

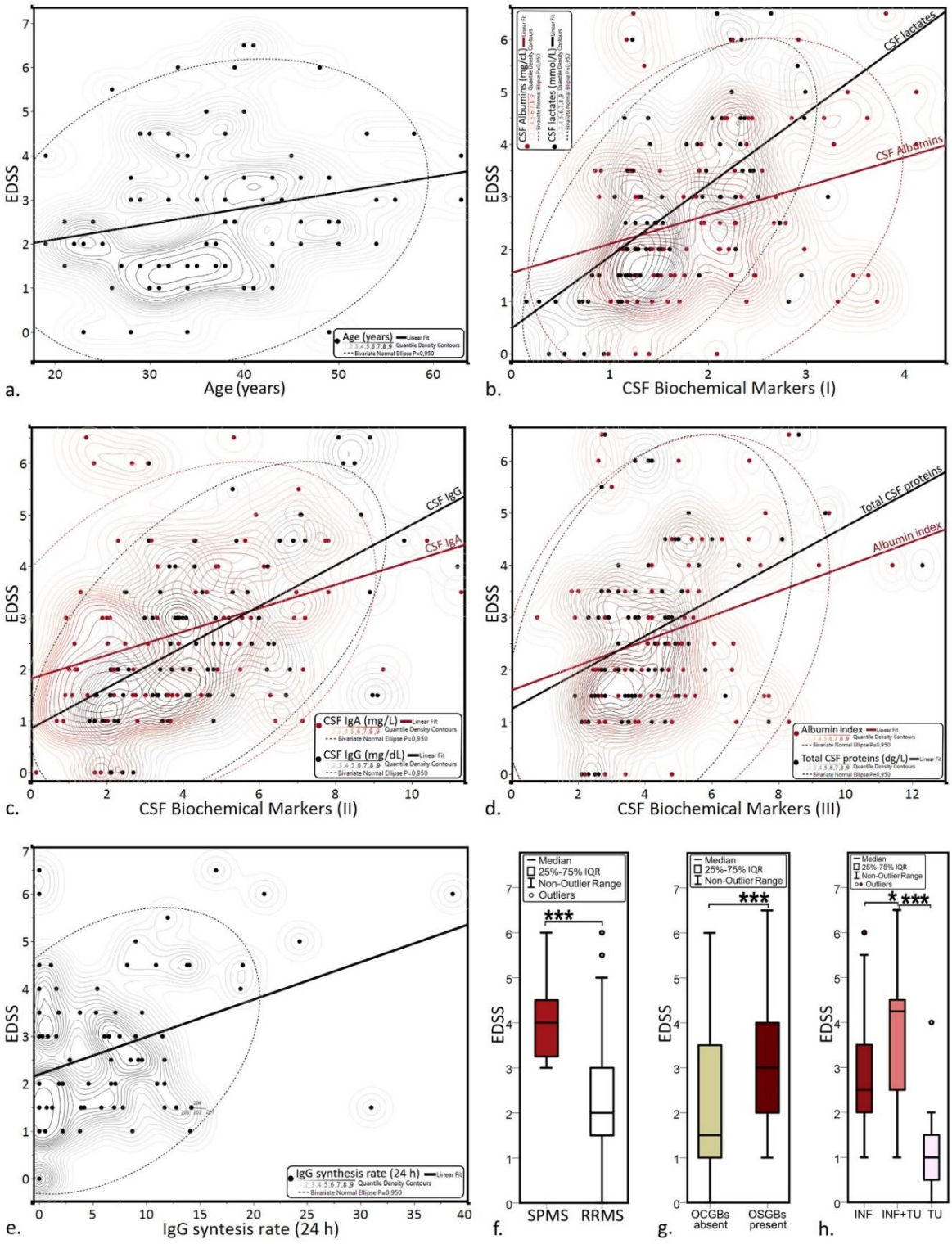

Fig. 3. Factors affecting functional disability (expressed as EDSS scores) in interferon beta-1a treated MS patients.

In the model, the variables admission CSF lactate levels and admission CSF IgG were estimated as the only significant predictive risk factors for functional disability (high EDSS scores); among them, the CSF lactate levels has shown the largest effect size (detected both by the LogWorth of the effects and the standardized β coefficient); 1 unit increase of CSF lactates was associated with 1 unit increase of the EDSS score.

It is important to stress that the highest R^2_{Ad} value was generated when the effects of the two categorical variables (presence of OCGBs and presence of combined INF+TU pattern) were crossed; since the effect of the generated crossed variable had higher effect in the overall model, when compared to the effects of the two variables alone, an effect of interaction between the presence of OCGBs and combined INF+TU pattern can be speculated.

The following formula for prediction of the MS functional disability (expressed as EDSS scores) can be defined, based on the model:

$$EDSS = 1.0176 \text{ x (CSF lactate levels/mmol·L}^{-1}) + 0.0250 \text{ x (CSF IgG/mg·L}^{-1}) + 0.0732 \text{ x (CSF IgA/mg·L}^{-1}) + 0.0141 \text{ x (Age/years)} - 0.8751 + a,$$

where *a* summarizes the effects of the presence of OCGBs and the presence of combined INF+TU pattern.

Several statistical tests have proven that the constructed model for prediction of the EDSS scores has good overall characteristics and fit. The overall predictive capacity of the model was estimated as moderate-to-high (48.9% of the variation in the EDSS scores was accounted by the defined five model effects; $R^2 = 0.52075$, $R^2_{Adj} = 0.48922$, Root Mean Square Error = 1.11178), and the model was assessed as highly significant (One-way ANOVA: $F = 16.51635$, $p = 5.2 \cdot 10^{-11}$). The relation between the actual and predicted EDSS scores was high and highly significant ($\rho = 0.7400$; $R^2_{adj} = 0.51476$, $F = 86.92816$, $p = 2.0 \cdot 10^{-14}$), suggesting good overall fit of the model (Fig. 4a). Moreover, the homoscedasticity assumption was checked by plotting the standardized residuals versus the predicted EDSS scores and the scatterplot has shown equal distribution across all values of the independent variable (Fig. 4b; evident absence of any correlation between the residuals and the predicted EDSS scores: $\rho = 0.11205$; $R^2 = 0.00$, $F = 0.00$, $p = 1.00$). Finally, no multicollinearity issues were associated with the developed model (VIF values less than 1.5, Tab. 3) and the residuals followed approximately normal distribution, as proven by the Shapiro-Wilk test of normality ($W = 0.97902$, $p = 0.2076$, Fig. 4c, d).

Our developed multiple regression model characterized by the highest R^2_{Ad} value have shown that admission CSF lactate levels and admission CSF IgG can be considered as the only significant early predictive risk factors for functional disability in MS patients; among them, the CSF lactate levels has shown the largest effect size, with 1 unit increase of CSF lactates associated with 1 unit increase of the EDSS score. The backward-stepwise elimination has excluded the total CSF proteins, 24 h IgG synthesis rate and the albumin index from the final model as non-significant effects. The final model, however, has included the effects of the presence of OCGBs, the presence of combined INF+TU CSF type, CSF IgA levels and age as non-significant predictors, which can largely contribute to the final predictive capacity of the model by their strong direct and indirect effects; although not estimated as significant prognostic risk factors, these variables must be therefore considered as fundamental part of the developed model.

It is interesting to note that CSF lactate levels were assessed as significant early predictor with the highest effect size both in terms of prognostication of the disease course (and prediction od SPMS) and the functional disability. CSF lactate levels were recently proposed as a useful parameter of cerebral metabolism (Adam et al., 2001), since their levels are not influenced by the plasmatic concentration. Lactate is produced only in small amounts in the CSF in the case of anaerobic glycolysis, and therefore high lactate levels can be considered as a reflection of ischemia, and (consequently) anaerobic metabolism. Several publications hitherto have associated the high lactate concentration with adult meningitis and have proposed the value of >2.1 mmol/L as sensitive marker for CNS tissue destruction (Genton and Berger, 1990; Leib et al., Leen et al., 2012). The predictive value of admission CSF lactates in MS can be therefore explained by the fact that, patients with CNS tissue ischemia and destruction at admission are more likely to develop disease progression and functional disability over the years.

Conclusion

In conclusion, our study undertook a two-year longitudinal cohort investigation among interferon beta-1a-treated MS patients, employing a multifaceted methodology integrating various quantitative, semiquantitative, and qualitative techniques to enhance sensitivity of our findings.

The IgG synthesis rate, particularly estimated using the TOURT formula, exhibited a robust association with the presence of OCGBs, suggesting its potential utility in estimating locally synthesized IgG within the CNS with high sensitivity and specificity.

The primary focus of the study centered on evaluating the prognostic capability of CSF immunological patterns and admission CSF biomarkers in predicting disease outcome and functional disability in MS patients. Notably, admission CSF lactate levels and CSF IgG emerged as significant early predictors of functional disability, with CSF lactate levels demonstrating the largest effect size, highlighting its potential as a robust prognostic marker for both disease course and functional impairment.

The findings underscore the importance of CSF biomarkers, particularly lactate levels, in early prognostication of disease progression and disability in MS patients, potentially informing clinical decision-making and therapeutic interventions to mitigate long-term neurological sequelae.

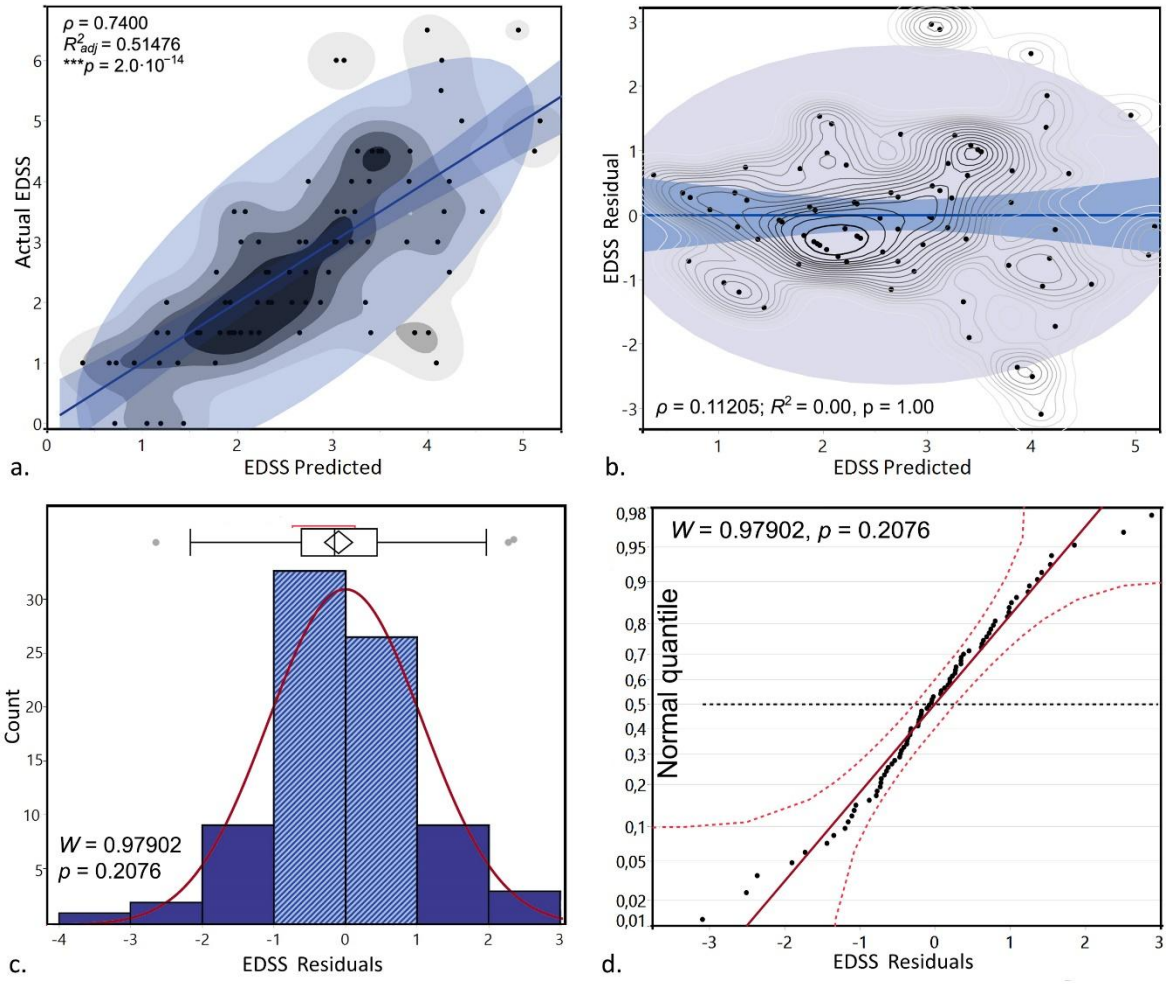

Fig. 4. Overall characteristics and fit of the multiple regression model for prediction of the MS functional disability.

References

Adam, P., Táborský, L., Sobek, O., Hildebrand, T., Kelbich, P., Průcha, M., Hyánek, J., 2001. Cerebrospinal fluid. Adv. Clin. Chem. 36, 1-62. https://doi.org/10.1016/s0065-2423(01)36024-9

Albanese, M., Zagaglia, S., Landi, D. Boffa, L., Nikoletti, C.G., Marciani, M.G.,Mandolesi, G., Marfia, G.A., Buttari, F., Mori, F., Centonze, D., 2016. Cerebrospinal fluid lactate is associated with multiple sclerosis disease progression. J. Neuroinflammation 13, 36. https://doi.org/10.1186/s12974-016-0502-1

Amorini, A.M., Nociti, V., Petzold, A., Gasperini, C., Quartuccio, E., Lazzarino, G., Di Pietro, V., Belli, A., Signoretti, S., Vagnozzi, R., Lazzarino, G., Tavazzi, B., 2014. Serum lactate as a novel potential biomarker in multiple sclerosis. Biochim. Biophys. Acta 1842(7), 1137-1143. doi: 10.1016/j.bbadis.2014.04.005

Berner, J.J., Ciemins, V.A. and Schroeder, E.F., 1972. Radial immunodiffusion of spinal fluid: Diagnostic value in multiple sclerosis. AJCP 58(2), 145-152. https://doi.org/10.1093/ajcp/58.2.145

Davis, B.J., 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121, 404-427. Available at: https://doi.org/10.1111/j.1749-6632.1964.tb14213.x

Deisenhammer, F., Zetterberg, H., Fitzner, B., Zettl, U.K., 2019. The Cerebrospinal Fluid in Multiple Sclerosis. Front. Immunol. 10, 726. doi: 10.3389/fimmu.2019.00726

Dickens, A.M., Larkin, J.R., Griffin, J.L., Cavey, A., Matthews, L., Turner, M.R., Wilcock, G.K., Davis, B.G., Claridge, T.D.W., Palace, J., Anthony, D.C., Sibson, N.R., 2014. A type 2 biomarker separates relapsing-remitting from secondary progressive multiple sclerosis. Neurology 83, 1492–1499. doi: 10.1212/wnl.0000000000000905

Falip, M., Tintoré, M., Jardí, R., Duran, I., Link, H., Montalban, X., 2001. Clinical usefulness of oligoclonal bands. Rev. Neurol. 32(12), 1120–1124. PMID: 11562840.

Farina, G., Magliozzi, R., Pitteri, M., Reynolds, R., Rossi, S., Gajofatto, A., Benedetti, M.D., Facchiano, F., Monaco, S., Calabrese, M., 2017. Increased cortical lesion load and intrathecal inflammation is associated with oligoclonal bands in multiple sclerosis patients: a combined CSF and MRI study. J. Neuroinflammation 14(40). doi: 10.1186/s12974-017-0812-y

Frischer, J.M., Bramow, S., Dal-Bianco, A., Lucchinetti, C.F., Rauschka, H., Schmidbauer, M., Laursen, H., Sorensen, PS., Lassmann, H., 2009. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain 132(5), 1175–1189. https://doi.org/10.1093/brain/awp070

Genton, B., Berger, J.P., 1990. Cerebrospinal fluid lactate in 78 cases of adult meningitis. Intensive Care Med. 16(3), 196-200. doi: 10.1007/BF01724802.

Hegen, H., Auer, M., Zeileis, A. and Deisenhammer, F., 2016. Upper reference limits for cerebrospinal fluid total protein and albumin quotient based on a large cohort of control patients: implications for increased clinical specificity. Clin. Chem. Lab Med. 54, 285–292. doi: 10.1515/cclm-2015-0253

Huss, A., Abdelhak, A., Halbgebauer, S., Mayer, B., Senel, M., Otto, M., Tumani, H., 2018. Intrathecal immunoglobulin M production: A promising high-risk marker in clinically isolated syndrome patients. Ann. Neurol. 83(5), 1032–1036. https://doi.org/10.1002/ana.25237

Kim, H.H., Jeong, I.H., Hyun, J., Kong, B.S., Kim, H.J., Park, S.J., 2017. Metabolomic profiling of CSF in multiple sclerosis and neuromyelitis optica spectrum disorder by nuclear magnetic resonance. PLoS One 12, e0181758. doi: 10.1371/journal.pone.0181758

Lamers, K.J.B. and Wevers, R.A., 1995. Cerebrospinal fluid diagnostics: Biochemical and clinical aspects. Clin. Biochem. Metab. 3(24), 63-75. https://hdl.handle.net/2066/223321

Leib, S.L., Boscacci, R., Gratzl, O. and Zimmerli, W., 1999. Predictive value of cerebrospinal fluid (CSF) lactate level versus CSF/blood glucose ratio for the diagnosis of bacterial meningitis following neurosurgery. Clini. Inject. Dis. 29, 69-74. doi: 10.1086/520184.

Link, H. and Tibbling, G., 1997. Principles of albumin and IgG analyses in neurological disorders. III. Evaluation of IgG synthesis within the central nervous system in multiple sclerosis. Scand. J. Clin. Lab. Invest. 37(5), 397–401. doi: 10.1080/00365517709091496.

Link, H. and Huang, Y.M., 2006. Oligoclonal bands in multiple sclerosis cerebrospinal fluid: An update on methodology and clinical usefulness. J. Neuroimmunol. 180(1–2), 17–28. doi: 10.1016/j.jneuroim.2006.07.006.

Lunding, J., Midgard, R. and Vedeler, C.A., 2000. Oligoclonal bands in cerebrospinal fluid: A comparative study of isoelectric focusing, agarose gel electrophoresis and IgG index. Acta Neurol Scand. 102(5), 322–325. https://doi.org/10.1034/j.1600-0404.2000.102005322.x

Ornstein, L., 1964. Disc electrophoresis. I. Background and theory. Ann. N.Y. Acad. Sci. 121, 321-349. doi: 10.1111/j.1749-6632.1964.tb14207.x.

Pitt, D., Werner, P. and Raine, C.S., 2000. Glutamate excitotoxicity in a model of multiple sclerosis. Nat. Med. 6, 67–70. doi: 10.1038/71555

Porter, L., Shoushtarizadeh, A., Jelinek, G.A., Brown, C.R., Lim, C.K., de Livera, A.M., Jacobs, K.R., Weiland, T.J., 2020. Metabolomic Biomarkers of Multiple Sclerosis: A Systematic Review. Front Mol. Biosci. 7, 574133. doi: 10.3389/fmolb.2020.574133.

Probert, F., Yeo, T., Zhou, Y., Sealey, M., Arora, S., Palace, J., Claridge D.W.T., Hillenbrand, R., Oechtering, J., Leppert, D., Kuhle, J., Anthony D.C., 2021. Integrative biochemical, proteomics and metabolomics cerebrospinal fluid biomarkers predict clinical conversion to multiple sclerosis, Brain Comms 3(2), fcab084. https://doi.org/10.1093/braincomms/fcab084

Pryce, G. and Baker, D., 2018. Oligoclonal bands in multiple sclerosis; functional significance and therapeutic implications. Does the specificity matter?. Multiple Sclerosis and Related Disorders 25, 131-137. doi: 10.1016/j.msard.2018.07.030

Regenold, W.T., Phatak, P., Makley, M.J., Stone, R.D., Kling, M.A., 2008. Cerebrospinal fluid evidence of increased extra-mitochondrial glucose metabolism implicates mitochondrial dysfunction in multiple sclerosis disease progression. J. Neurol. Sci. 275(1-2), 106-112. doi: 10.1016/j.jns.2008.07.032.

Rojas, J.I., Tizio, S., Patrucco, L., Cristiano, E., 2012. Oligoclonal bands in multiple sclerosis patients: worse prognosis? Neurol. Res. 34, 889-892. doi: 10.1179/1743132812Y.0000000088

Schuller, E. and Sagar H.J., 1981. Local synthesis of CSF immunoglobulins. A neuroimmunological classification. J. Neurol. Sci. 51(3), 361-70. https://doi.org/10.1016/0022-510X(81)90114-3

Senel, M., Mojib-Yezdani. F., Braisch, U., Bachhuber, F., Lewerenz, J., Ludolph, A.C., Otto, M., Tumani, H., 2019. CSF Free Light Chains as a Marker of Intrathecal Immunoglobulin Synthesis in Multiple Sclerosis: A Blood-CSF Barrier Related Evaluation in a Large Cohort. Front Immunol. 10, 641. doi: 10.3389/fimmu.2019.00641.

Sobek, O., Adam, E., Tfiborsk, L., Zeman, D., Kelbich, E., Hildebrand, T., 2000. Protein fractions and other parameters of cerebrospinal fluid in patients with multiple sclerosis. Clin. Biochem. Metab. 8(29), 209-212.

Spence, A.M., 1983. Immunoglobulins in cerebrospinal fluid in multiple sclerosis. Hum. Pathol. 14, 99-103. https://doi.org/10.1016/S0046-8177(83)80237-8

Tibbling, G., Link, H. and Ohman, S., 1977. Principles of albumin and IgG analyses in neurological disorders. 1. Establishment of reference values. Scand. J. Clin. Lab. Invest. 37, 385-390. doi: 10.1080/00365517709091496.

Thompson, A.J., Banwell, B.L., Barkhof, F., Carroll, W.M., Comi, C.G., Correale, J., Fazekas, F., Filippi, M., Freedman, M.S., Fujihara, K., Galetta, S.L., Hartung, H.P., Kappos, L., Lublin, F.D., Marrie, R.A., Miller, A.E., Montalban, X., Mowry, E.M., Sorensen, P.S., Tintore, M., Traboulsee, A.L., Trojano, M., Uitdehaag, B.M.J., Vukusic, S., Waubant, E., Weinshenker, B.G., Reingold, S.C., Cohen, J.A., 2018. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 17(2), 162–173. doi: 10.1016/S1474-4422(17)30470-2

Tourtellotte, W.W., 1975. What is multiple sclerosis? Laboratory criteria for diagnosis. In: Davison, A.N., Humpfrey, J.H., Liversledge, A.L., McDonald W.I. and Porterfield, J.S. (Eds.), Multiple Sclerosis Research, Elsevier, New York, pp. 9-26.

Valenzuela, R., Mandler, R. and Goren, H., 1982. Immunonephelometric quantitation of central nervous system IgG daily synthesis in multiple sclerosis. AJCP 78(1), 22-28. doi: 10.1093/ajcp/78.1.22.

Yelamanchi, S.D., Jayaram, S., Thomas, J.K., Gundimeda, S., Khan, A.A., Singhal, A., Prasad, T.S.K., Pandey, A., Somani, B.L., Gowda, H., 2016. A pathway map of glutamate metabolism. J. Cell Commun. Sign. 10, 69–75. doi: 10.1007/s12079-015-0315-5

Резиме

Биомаркери на цереброспинална течност како предвидувачи за исходот на болеста и функционална попреченост кај мултиплекс склероза

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Клучни зборови: цереброспиналната течност, имуно-воспалителни, метаболички биомаркери, мултиплекс склероза, EDSS, попреченост

Во истражувањето за мултиплекс склероза (МС), цереброспиналната течност (CSF) има предност во однос на другите телесни течности поради нејзината блиска поврзаност со патолошките промени на при МС, пришто биомаркерите на цереброспиналната течност можат да откријат различни аспекти на патофизиологијата на болеста.

Целта на ова истражување беше да се процени предиктивната вредност на имуно-воспалителните и метаболичките CSF биомаркери за попреченост кај мултиплекс склерозата и дали овие биомаркери, оценети при дијагнозата, додаваат прогностичка вредност користејќи популациска група на МС пациенти.

По скрининг на 340 сомнителни пациенти со МС, беше добиена база на податоци од 83 подобни пациенти со секундарна прогресивна МС (SPMS) и релапс-ремитирачка МС (RRMS) третирани со интерферон бета-1а врз кои беше спроведена 2-годишна лонгитудинална кохортна студија со интегриран пристап, комбинирајќи квантитативни и квалитативни методи за да се анализираат CSF обрасците.

Резултатите укажаа на значајна поврзаност помеѓу нивоата на CSF лактат, IgG и IgA со резултатите од проширената скала за статусот на попреченост (EDSS), што укажува на нивниот потенцијал за предвидување на исходот на болеста. Имено, CSF лактатот се покажа како значаен ран предиктор, што укажува на неговата корисност во прогнозирањето на текот на болеста и функционалната попреченост. Понатаму, моделот на мултипла регресија ги идентификуваше нивоата на CSF лактат и CSF IgG како значајни рани предвидувачки фактори на ризик за функционална попреченост кај пациенти со МС. Овие наоди ја нагласуваат важноста на биомаркерите на цереброспиналната течност за подобрување на прогностичките проценки и планирањето на третманот за пациентите со МС, обезбедувајќи вредни увиди за прогресијата на болеста и функционалните исходи.