Contribution of blood biomarkers to multiple sclerosis diagnosis

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Background and Objectives

The constant evolution of ancillary tests has increased the diagnostic accuracy in multiple sclerosis (MS). However, invasive procedures may delay this process. We explored the added value of serum neurofilament light chain (sNfL), glial fibrillary acidic protein (sGFAP), chitinase-3-like 1 (sCHI3L1), and the humoral immune responses to the Epstein-Barr virus (EBV)-encoded nuclear antigen 1 (EBNA-1) to current MS diagnostic criteria.

Methods

- We retrospectively selected patients presenting a clinically isolated syndrome (CIS) between October 2004 and December 2022 from two prospective cohorts: Cemcat and Ramon y Cajal (RyC) Hospital, Spain.
- Baseline assessments were performed within 3 months from CIS onset. Baseline and follow-up brain and spinal MRIs were conducted using 1.5 or 3.0 Tesla (T) magnets. All baseline T1-weighted sequences were repeated after the administration of gadolinium contrast agent.
- Analyses of sNfL and sGFAP levels were performed on Simoa™ HD-1 Analyzer. Levels of sCHI3L1 were quantified by an *in-house* Simoa™-based assay. Serum IgG antibodies to EBV-EBNA-1 were quantified using ELISA.
- Patients were classified at baseline as presenting: (i) not dissemination in space (DIS) nor dissemination in time (DIT) (noDIS & noDIT); (ii) DIS, without DIT (DIS & noDIT); and (iii) both (DIS & DIT), which were used as a reference.
- Patients were clinically and radiologically followed up until disease activity allowed for MS conversion according to McDonald 2017 criteria.

Statistical analysis

- The comparisons of baseline serum biomarker levels between groups were assessed by linear mixed models. The comparisons of biomarker levels between patients with negative and positive OBs and between patients with and without disease activity during follow-up were performed with a Mann-Whitney U test. The relationship between the different biomarker levels was assessed by partial correlations. Receiver operating characteristic (ROC) curve analyses and Youden Index were used to determine the best cut-off values associated with disease activity during follow-up.

Results

Characteristics	Whole cohort	Cemcat	RyC
N	181	89	92
Age, y (SD)	35.0 (9.7)	35.4 (8.1)	34.6 (11.1)
Female / male, n (% women)	120 / 61 (66.3)	57 / 32 (64.0)	63 / 29 (68.5)
IgG oligoclonal bands, n (% positive)	143 (79.0)	69 (77.5)	74 (80.4)
Patient classification at CIS, n (%)			
noDIS & noDIT	25 (13.8)	16 (18.0)	9 (9.8)
DIS & noDIT	62 (34.3)	25 (28.1)	37 (40.2)
DIS & DIT	94 (51.9)	48 (53.9)	46 (50.0)
Time blood sample-CIS, m (IQR)	1.5 (0.6 - 2.3)	1.6 (0.9 - 2.1)	1.1 (0.3 - 2.7)
Time blood sample-diagnostic MRI, m (IQR)	1.2 (0.1 - 2.5)	1.9 (1.1 - 2.7)	0.2 (0.0 - 1.9)

Table 1. Demographic, clinical and radiological characteristics of CIS patients stratified according to participating center.

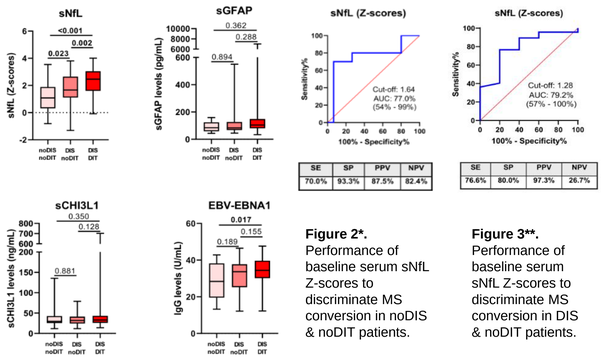

Figure 1. Distribution of baseline biomarker levels according to DIS & DIT status.

Figure 2*. Performance of baseline serum sNfL Z-scores to discriminate MS conversion in noDIS & noDIT patients.

Figure 3.** Performance of baseline serum sNfL Z-scores to discriminate MS conversion in DIS & noDIT patients.

Combination of biomarkers	SE	SP	PPV	NPV
H. sNfL Z-scores & H. sGFAP (N=30)	63.9%	**100%**	**100%**	22.7%
H. sNfL Z-scores & L. sGFAP (N=7)	15.8%	92.9%	85.7%	28.9%
L. sNfL Z-scores & H. sGFAP (N=11)	19.1%	81.8%	81.8%	7.3%
L. sNfL Z-scores & L. sGFAP (N=4)	40%	95.7%	50%	93.8%

Table 2.** Combination of levels of sNfL Z-scores and sGFAP to predict MS conversion in DIS & noDIT group. High sNfL Z-scores refer to values ≥1.28. High sGFAP levels refer to values ≥66.42 pg/mL.

Abbreviations:
H: High, L: Low, SE: sensitivity, SP: specificity, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve.

*Median (IQR) follow-up, 8.1 (5.0 - 11.7) years
Median (IQR) time to MS, 2.0 (0.9 - 5.0) years

**Median (IQR) follow-up, 6.8 (4.0 - 9.1) years
Median (IQR) time to MS, 1.1 (0.4 - 2.9) years

Key Points

1. Near CIS, only sNfL accurately discriminated the diagnostic groups.
2. Amongst noDIS & noDIT patients that converted to MS (40% of the cohort), sNfL outperformed the remainder.
3. Amongst DIS & noDIT patients converted to MS (90% of the cohort), sNfL outperformed the remainder.
4. Combined high sNfL Z-scores and high sGFAP increased diagnostic performance in DIS & noDIT patients.

Lay Summary

In this project, we explored the added value of serum body fluid biomarkers obtained from peripheral blood present in people who are newly diagnosed with a CIS. Our goal is to find an alternative to other diagnostic procedures that are usually needed. Our results show that such molecules are reliable and may be a promising alternative to diagnose MS in people experiencing a first symptom of the disease.