Serum Biomarker gMS-Classifier2: Predicting Conversion to Clinically Definite Multiple Sclerosis

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Abstract

Background: Anti-glycan antibodies can be found in autoimmune diseases. IgM against glycan P63 was identified in clinically isolated syndromes (CIS) and included in gMS-Classifier2, an algorithm designed with the aim of identifying patients at risk of a second demyelinating attack.

Objective: To determine the value of gMS-Classifier2 as an early and independent predictor of conversion to clinically definite multiple sclerosis (CDMS).

Methods: Data were prospectively acquired from a CIS cohort. gMS-Classifier2 was determined in patients first seen between 1995 and 2007 with ≥ two 200 μL serum aliquots (N = 249). The primary endpoint was time to conversion to CDMS at two years, the factor tested was gMS-Classifier2 status (positive/negative) or units; other exploratory time points were 5 years and total time of follow-up.

Results: Seventy-five patients (30.1%) were gMS-Classifier2 positive. Conversion to CDMS occurred in 31/75 (41.3%) of positive and 45/174 (25.9%) of negative patients (p = 0.017) at two years. Median time to CDMS was 37.8 months (95% CI 10.4–65.3) for positive and 83.9 months (95% CI 57.5–110.5) for negative patients. gMS-Classifier2 status predicted conversion to CDMS within two years of follow-up (HR = 1.8, 95% CI 1.1–2.8; p = 0.014). gMS-Classifier2 units were also independent predictors when tested with either Barkhof criteria and OCB (HR = 1.2, CI 1.0–1.5, p = 0.020) or with T2 lesions and OCB (HR = 1.3, CI 1.1–1.5, p = 0.008). Similar results were obtained at 5 years of follow-up. Discrimination measures showed a significant change in the area under the curve (ΔAUC) when adding gMS-Classifier2 to a model with either Barkhof criteria (ΔAUC 0.0415, p = 0.012) or number of T2 lesions (ΔAUC 0.0467, p = 0.009), but not when OCB were added to these models.

Conclusions: gMS-Classifier2 is an independent predictor of early conversion to CDMS and could be of clinical relevance, particularly in cases in which OCB are not available.
Introduction

Evidence exists that both the number of lesions observed using baseline magnetic resonance imaging (MRI) [1,2] and the presence of IgG oligoclonal bands (OCB) in the cerebrospinal fluid (CSF) [3–5] of patients with clinically isolated syndromes (CIS) are independent predictors of conversion to clinically definite multiple sclerosis (CDMS). However, MS is a highly heterogeneous disease, and the search for other biomarkers that could improve the prediction of conversion to CDMS may still be necessary for early and appropriate therapeutic decision making [6,7].

A complex array of covalently attached glycans densely covers the surface of all cells and many proteins, and these molecules are a major component of the extracellular matrix. Thus, glycans are a prime antigen source and play a vital role in immunity. Indeed, antibodies against these molecules have been implicated in a number of autoimmune diseases [8], for example, those directed against galactose in collagen type II in rheumatoid arthritis [9], ganglioside GGQ1b in Miller-Fisher syndrome [10] and oligomannose, mannosibiose, laminaribiose, chitobiose, laminin and chitin epitopes in Crolin’s disease [11,12]. IgM antibodies directed against glycans composed of alpha-glucose disaccharides have been found in MS patients and demonstrated to distinguish relapsing-remitting MS patients from those with other neurological diseases [13–15]. One of the identified antibodies was directed against P63, a polymer of alpha-glucose molecules comprising two different carbohydrate structures [Glc(α1,6)Glc(α1) and Glc(α1,3)Glc(α1)]. Thus, a classification rule named gMS-Classifier2, which is based on the combination of polyclonal serum IgM antibody levels against P63 and age, was developed after an exploration analysis of clinical data and anti-glycan antibody levels in samples collected in the “Betaferon® in Newly Emerging multiple Sclerosis For Initial Treatment” (BENEFIT) trial. In this study, this classification rule identified CIS patients at higher risk of converting to CDMS during the first two years of disease evolution [16]. To validate these preliminary results, herein we aimed to analyze the gMS-Classifier2 predictive value for early conversion of CIS patients to CDMS and to determine whether gMS-Classifier2 is an independent predictor of conversion to CDMS.

Patients and Methods

Ethics Statement

This study received approval from the Clinical Research Ethics Committee (CREC) of Vall d’Hebron University Hospital and Research Institute (Comité Étique d’Investigación Clínica –CEIC- de l’Hospital Universitari Vall d’Hebron-Institut de Recerca). Participants provided their written informed consent to participate in this study.

The present study is based on longitudinal clinical, CSF, serum and MRI data prospectively acquired from a cohort of CIS patients which started in 1995. Patients presenting for the first time with monophasic neurologic symptoms of the type seen in MS were recruited at the Vall d’Hebron University Hospital in Barcelona, Spain. Inclusion criteria were as follows: a CIS suggestive of central nervous system (CNS) demyelination involving the optic nerve, brainstem, spinal cord or other topography that were not attributable to other diseases; age ≤50 years; and onset of symptoms within three months of both clinical and MRI examinations. Patients were seen every three to six months and if relapses occurred. IgG OCB were examined using agarose isoelectric focusing combined with immunoblotting. The remaining biological samples were stored at −80°C until testing. Brain MRIs were performed after the first demyelinating event and repeated after twelve months and five years of follow-up. From 2001 onwards, baseline cranial MRIs were performed at three months after the first demyelinating event. Further clinical, CSF and MRI assessments have been detailed elsewhere [1].

Cases were selected from the CIS cohort based on the following eligibility criteria: consecutive patients older than 18 years of age seen between 1995 and 2007, with a minimum of two available 200 μL serum aliquots that had not undergone previous thawing.

A diagnosis of conversion to CDMS was made when new symptoms occurred after an interval of at least one month and only when other diagnoses had been excluded [17]. Time of follow-up was calculated based on the difference between the date of the baseline visit and the date of the last visit. De-identified and coded serum samples obtained at the time of enrolment in the department-wide sample repository were shipped to Glycominds, Ltd (Mod’in, Israel) for analysis. Clinical data were not shared with collaborators at Glycominds until after the results of the serological analysis had been returned.

Serum samples were thawed according to the following protocol to prevent IgM precipitation: i) Samples were allowed to reach room temperature; ii) Samples were incubated at 37°C for 2 hours; iii) Samples were vortexed to homogeneity. IgM antibody measurement is stable if these conditions are met for no more than two freeze-thaw cycles.

Levels of anti-P63 IgM antibodies were measured in IgG-depleted serum samples by enzyme immunoassay (EIA) in duplicate. Briefly, microtiter 96-well plates with immobilized P63 were prepared as described elsewhere [18]. IgG was depleted from the samples using rheumatoid factor removal reagent (Chemicon, Australia, Cat. RFRR) according to the manufacturer’s instructions. Following IgG removal, serum samples (using a dilution of 1:600 instead of the 1:1200 originally described) were dispensed into microtiter wells in duplicate, incubated for 180 minutes at 4°C, and washed with wash buffer. Bound antibodies were labelled with horseradish peroxidase (HRP)-conjugated goat anti-human IgM antibody, washed, and 3, 3′, 5, 5′-tetramethylbenzidine was added for detection. After 30 minutes, the enzymatic reaction was stopped by adding 1% sulphuric acid solution to the wells, and the optical density (OD) was read at 450 nm using a Victor 1420 plate reader (Wallac, Turku, Finland). Each plate included a 5-point calibration curve. Anti-P63 serum levels were reported in arbitrary EIA units (EU). gMS-Classifier2 units were calculated according to the following algorithm: [1.171 – 0.082×age in years at the time of blood collection] × [0.015 × anti-P63 (EU)]. The gMS-Classifier2 was considered positive when the number of units was equal to or greater than 0.289 [16,19].

Statistical Analysis

Parametric and nonparametric comparative statistics were performed depending on the normality of the distributions of the continuous variables. Fisher’s exact test was performed to compare categorical variables. Kaplan-Meier analysis was used to estimate cumulative survival probabilities and to construct survival plots. To assess whether gMS-Classifier2 can independently predict time to CDMS, a multivariate analysis using Cox proportional hazard regression was performed for both gMS-Classifier2 status (positive or negative) and continuous values. Baseline MRI parameters such as number of Barkhof Criteria (BC), the number of T2 lesions (0, 1–9, >9 lesions) and OCB were considered as potentially relevant covariates. Age was already included in the gMS-Classifier2 algorithm as a covariate; the role of gender and CIS topography as
Results

Between 1995 and 2007, 723 patients were included in the CIS cohort. gMS-Classifier2 units were determined in a subgroup of 249 (34%) patients that met the present study’s selection criteria. The screened cohort was similar to the non-screened cohort in age, follow-up time, and proportion of both positive OCB and baseline number of Barkhof criteria. There were differences in the proportion of females and the topography of disease presentation (Table 1). When comparing the demographic variables between patients with positive and negative gMS-Classifier2 status, including median time of follow-up, there was a difference in the proportion of females and in the distribution of CIS topography, but since the gMS-Classifier2 hazard ratio (HR) estimate was not substantially modified when gender or topography were included in the model, it was not considered necessary to adjust the results for these clinical variables (data not shown). There was also a difference in the median time of follow-up; however, as it was of approximately 5 months between groups, it was not considered relevant when the total follow-up time was up to 14 years.

Predictive Value of gMS-Classifier2 Status for Conversion to CDMS

In a univariate analysis, gMS-Classifier2 status (positive/negative), Barkhof criteria, number of T2 lesions and presence of OCB were predictors for early conversion to CDMS at two years of follow-up (Table 3). In the multivariate analyses at two years of follow-up, gMS-Classifier2 status remained significant when tested with number of Barkhof criteria (HR = 1.8, 95%CI 1.1–2.8; p = 0.014) or number of T2 lesions (HR = 1.7, 95%CI 1.1–2.7; p = 0.020). When combining gMS-Classifier2 status and OCB, the former’s significance was lost (HR = 1.5, 95%CI 0.9–2.4; p = 0.095). When combining gMS-Classifier2 status with OCB and either Barkhof criteria or number of T2 lesions the HR were non-significant (HR = 1.5, 95%CI 0.9–2.5; p = 0.081 and HR = 1.5, 95%CI 0.9–2.4, p = 0.100, respectively) (Table 4). When adding treatment to these models, there were no statistically significant changes in the HR of gMS-Classifier2 (data not shown).

Table 1. Demographic, clinical and MRI characteristics of screened and non-screened patients: gMS-Classifier2 serum assay.

| Group characteristics (1995–2007) | Screened CIS cohort (N = 249) | Non-screened CIS cohort (N = 474) | p-value |
|----------------------------------|--------------------------------|----------------------------------|---------|
| Mean age in years ± SD           | 31.6±7.9                       | 31.6±7.9                         | 0.455   |
| Females (%)                      | 187 (75.1)                     | 315 (66.5)                       | 0.017   |
| Median follow-up in months (range)| 68.7 (0.53–177.0)             | 63.2 (0.30–171.2)               | 0.002   |
| Topography N (%):                |                                |                                  |         |
| ON                               | 106 (42.6)                     | 154 (32.5)                       |         |
| Brainstem                        | 51 (20.5)                      | 144 (30.4)                       |         |
| Spinal cord                      | 65 (26.1)                      | 122 (25.7)                       |         |
| Other                            | 27 (10.8)                      | 54 (11.4)                        | 0.014   |
| Positive OCB N (%)*              | 152 (64.4)                     | 181 (60.1)                       | 0.311   |
| Barkhof criteria on baseline MRI N (%):** | | | |
| 0                                | 88 (35.5)                      | 173 (38.6)                       |         |
| 1–2                              | 56 (22.6)                      | 104 (23.2)                       |         |
| 3–4                              | 104 (41.9)                     | 171 (38.2)                       | 0.601   |

Abbreviations: CIS = clinically isolated syndrome; SD = standard deviation; ON = optic neuritis; OCB = oligoclonal bands; MRI = magnetic resonance imaging. *The total number of patients with available cerebrospinal fluid for OCB determination was 236 for the screened CIS cohort and 301 for the non-screened CIS cohort. Percentages in the table correspond to these figures. **The total number of patients with available baseline MRI for Barkhof criteria determination was 248 for the screened CIS cohort and 448 for the total CIS cohort. Percentages in the table correspond to these figures.
Predictive Value of gMS-Classifier2 Continuous Unit Values for Conversion to CDMS

In the univariate analysis, gMS-Classifier2 continuous units, Barkhof criteria, number of T2 lesions and presence of OCB were predictors for early conversion to CDMS at two and five years of follow-up (Table 3).

In the multivariate analyses at two years of follow-up, gMS-Classifier2 continuous units remained significant when tested with number of Barkhof criteria (HR = 1.3, 95%CI 1.1–1.5, p = 0.003) or number of T2 lesions (HR = 1.3, 95%CI 1.1–1.6, p = 0.001). When combining gMS-Classifier2 continuous units and OCB, the former's significance was lost (HR = 1.1, 95%CI 0.9–1.4, p = 0.088), but when combining gMS-Classifier2 continuous units with either Barkhof criteria or number of T2 lesions the HR were once again statistically significant (HR 1.2, 95%CI 1.0–1.5, p = 0.020 and HR 1.3, 95%CI 1.1–1.5, p = 0.008, respectively) (Table 5). When adding treatment to these models, there were no statistically significant changes in the HR of gMS-Classifier2 (data not shown).

Figure 1. Time to reach CDMS based on gMS-Classifier2 status. Dotted line: median time of follow-up. HR = hazard ratio; CI = confidence interval. doi:10.1371/journal.pone.0059953.g001

Table 2. gMS-Classifier2 status and number of patients converting to CDMS at specified time points.

| Specified time points | CDMS in positive patients (75) | CDMS in negative patients (174) | p-value |
|-----------------------|---------------------------------|---------------------------------|---------|
|                       | N (%)                           | N (%)                           |         |
| Two years             | 31 (41.3)                       | 45 (25.9)                       | 0.017   |
| Five years            | 38 (50.7)                       | 67 (38.5)                       | 0.093   |
| Total time of follow-up (up to 14 years) | 40 (53.3) | 77 (44.3) | 0.214 |

Abbreviations: CDMS = clinically definite multiple sclerosis. doi:10.1371/journal.pone.0059953.t002
Similar results were obtained at five years of follow-up in the univariate and multivariate analyses for gMS-Classifier2 status and continuous units (Tables 3, 4 and 5). At total time of follow-up, gMS-Classifier2 status and continuous units were not predictive of conversion to CDMS in the univariate analysis (Table 3), but when included in the multivariate models, gMS-Classifier2 continuous units remained independent predictors except when combined with OCB, whereas gMS-Classifier2 status yielded mostly negative results (Tables 4 and 5).

### Discrimination Measures: ROC Curve Analyses

To assess the clinical utility of gMS-Classifier2, the calibration measures for number of Barkhof criteria, OCB and gMS-Classifier2 continuous units yielded a p value of 0.303; and the one performed for number of T2 lesions, OCB and gMS-Classifier2 continuous units showed a p value of 0.664. When performing the ROC analyses as discrimination measures, in the model using number of Barkhof criteria, the ROC association

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**Table 3.** Univariate Cox proportional hazard regression for conversion to CDMS.

| Univariate model | Two years of follow-up | Five years of follow-up | Total time of follow-up |
|------------------|------------------------|-------------------------|-------------------------|
|                  | N                      | HR 95%CI | p     | HR 95%CI | p     | HR 95%CI | p     |
| gMS-Classifier2 status (positive or negative) | 249 | 1.8 1.1–2.8 | 0.017 | 1.5 1.0–2.3 | 0.034 | 1.4 1.0–2.1 | 0.061 |
| gMS-Classifier2 continuous units* | 249 | 1.2 1.0–1.4 | 0.027 | 1.1 1.0–1.3 | 0.038 | 1.1 1.0–1.3 | 0.117 |
| 1–2 Barkhof criteria | 56 | 3.9 1.6–9.4 | 0.002 | 4.3 2.1–8.6 | <0.0001 | 5.3 2.7–10.6 | <0.0001 |
| 3–4 Barkhof criteria | 104 | 7.5 3.4–16.5 | <0.0001 | 6.7 3.6–12.8 | <0.0001 | 8.1 4.3–15.4 | <0.0001 |
| 1–9 T2 lesions | 76 | 5.6 1.7–18.9 | 0.005 | 10.0 3.1–32.6 | <0.0001 | 11.5 3.6–37.4 | <0.0001 |
| ≥10 T2 lesions | 107 | 12.5 3.9–39.9 | <0.0001 | 17.4 5.5–55.2 | <0.0001 | 21.4 6.7–68.1 | <0.0001 |
| Positive OCB | 152 | 3.7 1.9–7.3 | <0.0001 | 3.1 1.8–5.3 | <0.0001 | 2.9 1.8–4.8 | <0.0001 |

**Table 4.** Multivariate Cox proportional hazard regression for conversion to CDMS according to gMS-Classifier2 status (positive or negative).

| Multivariate models | Two years of follow-up | Five years of follow-up | Total time of follow-up |
|---------------------|------------------------|-------------------------|-------------------------|
|                     | HR 95%CI | p     | HR 95%CI | p     | HR 95%CI | p     |
| gMS-Classifier2 status | 1.8 1.1–2.8 | 0.014 | 1.6 1.1–2.4 | 0.019 | 1.5 1.0–2.2 | 0.031 |
| Number of Barkhof criteria, N = 1–2 vs. 0*² | 4.1 1.7–9.8 | 0.002 | 4.4 2.2–8.9 | <0.0001 | 5.5 2.8–10.9 | <0.0001 |
| Number of Barkhof criteria, N = 3–4 vs. 0.75 | 3.4–16.5 | <0.0001 | 6.9 3.6–13.1 | <0.0001 | 8.3 4.4–15.7 | <0.0001 |
| gMS-Classifier2 status | 1.7 1.1–2.7 | 0.020 | 1.5 1.0–2.3 | 0.044 | 1.4 1.0–2.1 | 0.074 |
| Number of T2 lesions, N = 1–9 vs. 0 | 5.4 1.6–18.0 | 0.007 | 9.6 3.0–31.3 | <0.0001 | 11.2 3.4–36.4 | <0.0001 |
| Number of T2 lesions, N ≥10 vs. 0 | 12.2 3.8–39.1 | <0.0001 | 17.2 5.4–54.8 | <0.0001 | 21.4 6.7–67.9 | <0.0001 |
| gMS-Classifier2 status | 1.5 0.9–2.4 | 0.095 | 1.3 0.9–2.0 | 0.217 | 1.2 0.8–1.8 | 0.307 |
| Positive OCB | 3.6 1.8–7.0 | <0.0001 | 3.1 1.8–5.2 | <0.0001 | 2.9 1.7–4.6 | <0.0001 |
| gMS-Classifier2 status | 1.5 0.9–2.5 | 0.081 | 1.4 0.9–2.1 | 0.126 | 1.3 0.9–2.0 | 0.151 |
| 1–2 Barkhof criteria | 3.1 1.2–7.6 | 0.015 | 3.5 1.7–7.3 | 0.001 | 4.4 2.2–9.0 | <0.0001 |
| 3–4 Barkhof criteria | 5.2 2.3–11.9 | <0.0001 | 5.3 2.7–10.2 | <0.0001 | 6.5 3.3–12.5 | <0.0001 |
| Positive OCB | 2.2 1.1–4.5 | 0.022 | 1.9 1.1–3.4 | 0.014 | 1.8 1.1–2.9 | 0.023 |
| gMS-Classifier2 status | 1.5 0.9–2.4 | 0.100 | 1.3 0.9–2.0 | 0.204 | 1.2 0.8–1.9 | 0.267 |
| Number of T2 lesions, N = 1–9 vs. 0 | 3.9 1.1–13.7 | 0.030 | 7.6 2.3–25.2 | 0.001 | 9.1 2.7–30.0 | <0.0001 |
| Number of T2 lesions, N ≥10 vs. 0 | 8.2 2.5–27.1 | 0.001 | 13.1 4.0–42.7 | <0.0001 | 16.5 5.1–53.3 | <0.0001 |
| Positive OCB | 2.2 1.1–4.4 | 0.024 | 1.9 1.1–3.2 | 0.021 | 1.7 1.0–2.8 | 0.030 |

Abbreviations: HR = hazard ratio, CI = confidence interval, OCB = oligoclonal bands.

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*For continuous values, HR indicates how much the hazard (for CDMS) increases per unit increase in gMS-Classifier2.

**Biomarker gMS-Classifier2 and Multiple Sclerosis**
Panel agrees that the inclusion of CSF in the criteria requires further evaluation [5,23]. Furthermore, an ideal biomarker should be non-invasive and simple to use, making potential serum prognostic markers a good option, as they would be easy to obtain [24].

Glycans are potential antigens, and indeed, antibodies against various types of glycans have been found in serum. Such antibodies were first described for the human blood group ABO antigens [25], and later findings have linked antibodies directed against glycans to several autoimmune diseases, either by association only or as etiopathogenic [8–12]. Consequently, diverse assays have been designed to identify anti-glycan antibodies in autoimmune diseases [18,26] including MS [27–29]. Because some of these glycans are found within the type IV collagen matrix of the blood-brain barrier [14], it has been hypothesized that in MS patients, an inflammatory response could lead to the release of these carbohydrate antigens with the subsequent development of a humoral response [30]. Therefore, an array of glycans was screened in RRMS patients and healthy controls, observing that IgM antibodies to various alpha-glucose are elevated in the former group. Among the alpha-glucose, GAGA4 was the most notable and gMS-Classifier Dx was developed as GAGA4 normalized to total IgM. It was further concluded that anti-glycan antibodies in autoimmune diseases [18,26] including MS [27–29]. Because some of these glycans are found within the type IV collagen matrix of the blood-brain barrier [14], it has been hypothesized that in MS patients, an inflammatory response could lead to the release of these carbohydrate antigens with the subsequent development of a humoral response [30]. Therefore, an array of glycans was screened in RRMS patients and healthy controls, observing that IgM antibodies to various alpha-glucose are elevated in the former group. Among the alpha-glucose, GAGA4 was the most notable and gMS-Classifier Dx was developed as GAGA4 normalized to total IgM. It was further concluded that anti-glycan antibodies in autoimmune diseases [18,26] including MS [27–29].

Table 5. Multivariate Cox proportional hazard regression models for CDMS conversion according to gMS-Classifier2 continuous units.

| Multivariate models | Two years of follow-up | Five years of follow-up | Total time of follow-up |
|---------------------|------------------------|-------------------------|-------------------------|
|                     | HR $^{\#}$ 95%CI p     | HR 95%CI p              | HR 95%CI p              |
| gMS-Classifier2 units** | 1.3 1.1–1.5 0.003 | 1.3 1.1–1.5 0.001 | 1.2 1.1–1.4 0.004 |
| 1–2 Barkhof criteria | 4.5 1.8–10.9 0.001 | 4.9 2.4–10.0 <0.0001 | 6.0 3.0–12.0 <0.0001 |
| 3–4 Barkhof criteria | 8.3 3.7–18.4 <0.0001 | 7.6 4.0–14.5 <0.0001 | 9.1 4.8–17.4 <0.0001 |
| gMS-Classifier2 units | 1.3 1.1–1.6 0.001 | 1.3 1.1–1.5 0.001 | 1.3 1.1–1.5 0.001 |
| Number of T2 lesions, n=1–9 vs. 0 | 6.0 1.8–20.5 0.004 | 10.7 3.3–34.8 <0.0001 | 12.4 3.8–40.3 <0.0001 |
| Number of T2 lesions, n=10 vs. 0 | 15.0 4.6–48.8 <0.0001 | 20.9 6.5–67.3 <0.0001 | 25.8 8.0–82.9 <0.0001 |
| gMS-Classifier2 units | 1.1 0.9–1.4 0.088 | 1.1 0.9–1.3 0.157 | 1.1 0.9–1.2 0.283 |
| Positive OCB | 3.4 1.9–7.2 <0.0001 | 3.1 1.8–5.2 <0.0001 | 2.9 1.8–4.7 <0.0001 |
| gMS-Classifier2 units | 1.2 1.0–1.5 0.020 | 1.2 1.0–1.4 0.015 | 1.2 1.0–1.4 0.020 |
| 1–2 Barkhof criteria | 3.4 1.3–8.4 0.010 | 3.8 1.8–7.9 <0.0001 | 4.8 2.3–9.8 <0.0001 |
| 3–4 Barkhof criteria | 5.7 2.5–13.1 <0.0001 | 5.8 2.9–11.3 <0.0001 | 7.1 3.6–13.8 <0.0001 |
| Positive OCB | 2.2 1.1–4.4 0.025 | 1.9 1.1–3.3 0.019 | 1.7 1.1–2.9 0.031 |
| gMS-Classifier2 units | 1.3 1.1–1.5 0.008 | 1.2 1.1–1.5 0.007 | 1.2 1.0–1.4 0.009 |
| Number of T2 lesions, n=1–9 vs. 0 | 4.4 1.3–15.2 0.020 | 8.3 2.5–27.6 0.001 | 9.9 3.0–32.9 <0.0001 |
| Number of T2 lesions, n=10 vs. 0 | 9.9 2.9–33.1 <0.0001 | 15.6 4.8–51.3 <0.0001 | 19.6 6.0–64.2 <0.0001 |
| Positive OCB | 2.2 1.1–4.3 0.029 | 1.8 1.1–3.1 0.031 | 1.7 1.0–2.7 0.044 |

Abbreviations: HR = hazard ratio, CI = confidence interval, OCB = oligoclonal bands.

**For continuous values, HR indicates how much the hazard (for CDMS) increases per unit increase in gMS-Classifier2.

Discussed statistics showed that when number of Barkhof criteria, OCB and gMS-Classifier2 were put together, the area under the curve (AUC) was 0.786 (95%CI 0.7169–0.8403), in comparison with an ROC AUC of 0.7552 (95%CI 0.6945–0.8160) when not including gMS-Classifier2. Thus, the AUC change (ΔAUC) was 0.0415, p = 0.020. But when the model excluded OCB, the AUC change (ΔAUC) was 0.0407, p = 0.023. When the model excluded OCB, the results were the following: AUC ROC 0.7552 (95%CI 0.6945–0.8160) when not including gMS-Classifier2. Thus, the AUC change (ΔAUC) was 0.023 (p = 0.0788). But when the model excluded OCB, the results were the following: AUC ROC 0.7552 (95%CI 0.6945–0.8160) when not including gMS-Classifier2. Thus, the AUC change (ΔAUC) was 0.023 (p = 0.0788).
The early years of the disease and decreased with long-term follow-up. When gMS-Classifier2 status was evaluated together with MRI variables in the multivariate analyses at two and five years of follow-up, it remained an independent predictor of conversion to CDMS, but not when evaluated with OCB. With MRI and OCB findings, continuous unit values of gMS-Classifier2 independently predicted the development of an early second relapse, indicating the increased risk of relapse with increased serum levels of the biomarker. When performing the ROC analyses, the model for gMS-Classifier units was statistically significant only when OCB were excluded. However, recent publications emphasize that testing for any improvement using discrimination measures such as the change in the area under the ROC curve is extremely conservative [36,37]. Thus, we consider the HRs to be sufficient to support the role of gMS-Classifier2 as an independent predictor of conversion to CDMS.

As for the added value of gMS-Classifier2 to OCB findings in predicting early CDMS conversion, the differing results obtained are probably partly due to the higher resolution of a biomarker that is measured in continuous units compared to a dichotomous biomarker [35].

We conclude that gMS-Classifier2 is an independent predictor for conversion of CIS patients to CDMS in the first years of the disease course and therefore could be of clinical relevance to determine which patients are at higher risk, particularly in cases in which OCB are not available.

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Author Contributions

Contributed in analyzing the MRI data: GA CE JY ND XM MT. Performed the experiments: EF LS. Analyzed the data: GA CE JY ND AD AR XM MT. Contributed reagents/materials/analysis tools: GA CE JY EF LS ND AD XM MT. Wrote the paper: GA CE JY AR XM MT.

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