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# Neurofilament light chain and oligoclonal bands are prognostic biomarkers in radiologically isolated syndrome

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The prognostic role of cerebrospinal fluid molecular biomarkers determined in early pathogenic stages of multiple sclerosis has yet to be defined. In the present study, we aimed to investigate the prognostic value of chitinase 3 like 1 (CHI3L1), neurofilament light chain, and oligoclonal bands for conversion to clinically isolated syndrome and to multiple sclerosis in 75 patients with radiologically isolated syndrome. Cerebrospinal fluid levels of CHI3L1 and neurofilament light chain were measured by enzyme-linked immunosorbent assay. Uni- and multivariable Cox regression models including as covariates age at diagnosis of radiologically isolated syndrome, number of brain lesions, sex and treatment were used to investigate associations between cerebrospinal fluid CHI3L1 and neurofilament light chain levels and time to conversion to clinically isolated syndrome and multiple sclerosis. Neurofilament light chain levels and oligoclonal bands were independent risk factors for the development of clinically isolated syndrome (hazard ratio = 1.02, $P = 0.019$, and hazard ratio = 14.7, $P = 0.012$, respectively) and multiple sclerosis (hazard ratio = 1.03, $P = 0.003$, and hazard ratio = 8.9, $P = 0.046$, respectively). The best cut-off to classify cerebrospinal fluid neurofilament light chain levels into high and low was 619 ng/l, and high neurofilament light chain levels were associated with a trend to shorter time to clinically isolated syndrome ($P = 0.079$) and significant shorter time to multiple sclerosis ($P = 0.017$). Similarly, patients with radiologically isolated syndrome presenting positive oligoclonal bands converted faster to clinically isolated syndrome and multiple sclerosis ($P = 0.005$ and $P = 0.008$, respectively). The effects of high neurofilament light chain levels shortening time to clinically isolated syndrome and multiple sclerosis were more pronounced in radiologically isolated syndrome patients with $\geqslant 37$ years compared to younger patients. Cerebrospinal fluid CHI3L1 levels did not influence conversion to clinically isolated syndrome and multiple sclerosis in radiologically isolated syndrome patients. Overall, these findings suggest that cerebrospinal neurofilament light chain levels and oligoclonal bands are independent predictors of clinical conversion in patients with radiologically isolated syndrome. The association with a faster development of multiple sclerosis reinforces the importance of cerebrospinal fluid analysis in patients with radiologically isolated syndrome.

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**Abbreviations:** CIS = clinically isolated syndrome; NfL = neurofilament light chain; RIS = radiologically isolated syndrome

# Introduction

Chitinase 3-like 1 (CHI3L1) and neurofilament light chain (NfL) are promising prognostic biomarkers in the early stages of multiple sclerosis. In particular, high CHI3L1 levels in CSF samples from patients with clinically isolated syndrome (CIS) were associated with an increased risk for conversion to multiple sclerosis (Comabella *et al.*, 2010; Canto *et al.*, 2015; Hinsinger *et al.*, 2015; Modvig *et al.*, 2015). Similar findings have been reported for high CSF levels of NfL in patients with CIS (Teunissen *et al.*, 2009; Martinez *et al.*, 2015; Arrambide *et al.*, 2016). As a more established biomarker in the CSF, the presence of IgG oligoclonal bands in the CIS population also confers an increased risk for multiple sclerosis (Tintore *et al.*, 2008, 2015). Based on these findings, here we aimed to explore an earlier pathogenic stage and investigate whether CHI3L1, NfL and oligoclonal bands could also play prognostic roles in patients with radiologically isolated syndrome (RIS).

The term RIS was first introduced by Okuda *et al.* (2009) and alludes to the incidental radiological finding of white matter abnormalities highly suggestive of multiple sclerosis in individuals who are asymptomatic or have non-specific symptoms. It is well known that individuals with RIS can evolve toward relapsing-remitting or progressive multiple sclerosis (Lebrun, 2015; Kantarci *et al.*, 2016). In this context, several studies have pursued the identification of risk factors for the development of a first clinical event in RIS patients and, for instance, age $<37$ years, male sex, and presence of spinal cord lesions were found to be independent predictors for clinical conversion (Okuda *et al.*, 2011, 2014). Whether molecular biomarkers measured in the CSF in proximity to RIS diagnosis could also play prognostic roles in predicting symptoms onset has yet to be defined and was the subject of the present study.

# Materials and methods

## Patients with radiologically isolated syndrome

Seventy-five RIS patients recruited from 13 European multiple sclerosis centres [Saint-Petersburg ($n = 18$); Lublin ($n = 13$); Barcelona - Cemcat ($n = 9$); Serbia ($n = 7$); Madrid, Puerta de Hierro ($n = 7$); Madrid, Ramón y Cajal ($n = 5$); Prague ($n = 4$); Barcelona, Hospital Clinic ($n = 3$); Madrid, Hospital Clínico ($n = 3$); Rostock ($n = 2$); Milan ($n = 2$); Girona ($n = 1$); Innsbruck ($n = 1$)] satisfying the following criteria were included in the study: (i) 2009 Okuda criteria (Okuda *et al.*, 2009); (ii) lumbar puncture performed in proximity to the RIS diagnostic MRI ($\leqslant 6$ months); and (iii) follow-up longer than 1 year after RIS diagnosis. The study was approved by the corresponding Hospital Ethics Committees, and all patients gave their informed consent.

The number of brain $T_2$ lesions (recoded into two categories: $\leqslant 9$ lesions; and 10 or more lesions), infratentorial lesions,

contrast-enhancing lesions, and spinal cord $T_2$ lesions at the time of the RIS diagnostic MRI were scored. The presence of immunoglobulin G (IgG) oligoclonal bands was determined by isoelectric focusing combined with immunoblotting of matched serum and CSF sample pairs (Sadaba *et al.*, 2004; Abraira *et al.*, 2011). IgG oligoclonal bands were considered positive when there were two or more bands present in the CSF sample but not in paired serum. Conversion to CIS after RIS diagnosis was defined as the occurrence of monophasic neurological symptoms suggestive of CNS demyelination that were not attributable to other diseases (Miller *et al.*, 2012). Diagnosis of multiple sclerosis after a CIS event was done according to the 2010 McDonald criteria (Polman *et al.*, 2011). Time of follow-up was calculated as the difference between the date of the last visit and the date of RIS diagnostic MRI. The number of patients who received immunomodulatory treatment between the RIS diagnosis and the date of conversion to CIS or multiple sclerosis for converters and during the follow-up time for non-converters was recorded.

Table 1 summarizes the main demographic and clinical characteristics of the RIS cohort. Reasons for performing MRI in RIS patients are depicted in Supplementary Table 1.

## Patients with clinically isolated syndrome and multiple sclerosis

A cohort of 65 patients [20 patients with CIS (from Hospital Ramón y Cajal, Madrid) and 45 patients with multiple sclerosis (from Hospital Ramón y Cajal and the Department of Neurology in Lublin, Poland)] was included in the study for comparison purposes. For CIS, the inclusion criteria were patients with monophasic neurological symptoms suggestive of CNS demyelination not attributable to other diseases (Miller *et al.*, 2012), lumbar puncture and brain MRI performed within 6 months of the CIS event, and with a minimum follow-up of 1 year. For multiple sclerosis, inclusion criteria were patients with a relapsing-remitting course fulfilling 2010 McDonald criteria (Polman *et al.*, 2011), and with lumbar puncture performed in proximity to the brain MRI ($\leqslant$6 months). None of these patients were receiving treatment at the time of CSF collection.

Demographic characteristics of CIS/multiple sclerosis patients are shown in Table 1.

## CSF sampling and quantification of CHI3L1 and neurofilament light chain levels

CSF samples were collected by lumbar puncture, centrifuged to remove cells and stored frozen at −80°C until used.

CSF CHI3L1 levels were determined by enzyme-linked immunosorbent assay (ELISA; MicroVue YKL-40 EIA Kit, Quidel) according to the manufacturers' recommendations. Undiluted CSF samples were measured in duplicate and blinded to clinical data. ELISA intra-assay and inter-assay coefficients of variation were 3.9% and 8.8%, respectively.

CSF NfL levels were determined by ELISA (UmanDiagnostics AB) according to the manufacturers' recommendations. Undiluted CSF samples were measured in duplicate and blinded to clinical data. The intra-assay and inter-assay coefficients of variation were 4.0% and 15.5%, respectively.

All the ELISA assays to quantify CSF CHI3L1 and NfL levels were performed in at a single centre (Cemcat).

## Statistical analysis

Data were analysed using IBM SPSS statistics 20. A Mann-Whitney U-test was used to compare CSF CHI3L1 and NfL levels between RIS patients and CIS/multiple sclerosis patients, and between RIS patients stratified according to their conversion to CIS. Univariable and multivariable Cox proportional hazard regression models including as covariates age at RIS diagnosis, number of brain lesions, sex and treatment were used to evaluate the association between CHI3L1 levels, NfL levels, IgG oligoclonal bands and time to CIS and multiple sclerosis. Receiver operating characteristic (ROC) curve analyses were used to calculate the best cut-off value to classify CSF NfL levels into 'high' and 'low'. Time to CIS and time to multiple sclerosis in RIS patients with high and low CSF CHI3L1 and NfL levels, and with positive and negative oligoclonal bands were assessed by Kaplan-Meier survival analyses with log-rank tests. Kaplan-Meier analyses were performed in the whole RIS population and following age stratification according to a cut-off of 37 years (Okuda *et al.*, 2014).

# Results

## Clinical characteristics of the radiologically isolated syndrome cohort

As presented in Table 1, mean clinical follow-up time of RIS patients was 2.8 years. A total of 23 RIS patients (30.7%) converted to CIS with a mean conversion time of 1.8 years, and 52 patients remained as RIS during follow-up. Of the 23 RIS patients who converted to CIS, 21 patients (91.3%) were later diagnosed as having multiple sclerosis according to the 2010 McDonald criteria (Polman *et al.*, 2011), with a mean conversion time from RIS to multiple sclerosis of 2.2 years. Six of these 21 patients (33.3%) were diagnosed of multiple sclerosis at the time of the CIS event. IgG oligoclonal bands were positive in 53 RIS patients (70.7%). Only six patients with RIS (8.0%) received immunomodulatory treatment during the follow-up. None of the patients included in the study developed a primary progressive course of multiple sclerosis.

## CSF CHI3L1 levels are increased in clinically isolated syndrome/multiple sclerosis patients

We first compared CSF CHI3L1 and NfL levels between the whole cohort of RIS patients and CIS/multiple sclerosis patients. As shown in Fig. 1A, CHI3L1 levels were significantly higher in CIS/multiple sclerosis patients compared to RIS patients ($P = 0.013$). However, within the RIS group CHI3L1 levels were comparable between patients who

**Table 1 Demographic and baseline clinical characteristics of the patients included in the study**

| Baseline characteristics | CIS/MS | RIS, whole group | RIS–RIS | RIS–CIS |
|---|---|---|---|---|
| *n* | 65 | 75 | 52 (69.3%) | 23 (30.7%) |
| Age (years) | 38.7 (11.3) | 36.6 (10.4) | 38.0 (10.9) | 33.4 (8.6) |
| Female/male (% female) | 47/18 (72.3) | 55/20 (73.3) | 34/18 (65.4) | 21/2 (91.3) |
| Follow-up time, years | - | 2.8 (1.8) | 2.7 (1.8) | 2.8 (1.9) |
| IgG oligoclonal bands, *n*/*N* (% positive)[a] | - | 53/75 (70.7) | 31/52 (59.6) | 22/23 (95.7) |
| Time to CIS conversion, years | - | - | - | 1.8 (1.4) |
| % Patients with $\geqslant$10 $T_2$ brain lesions, *n*/*N* (% positive)[b] | 25/45 (55.6) | 54/68 (79.4) | 36/46 (78.3) | 18/22 (81.8) |
| % Patients with infratentorial lesions[c] | - | 58.7 | 56.0 | 65.2 |
| % Patients with contrast-enhancing lesions[d] | - | 18.7 | 21.2 | 13.0 |
| % Patients with spinal cord lesions[e] | - | 48.0 | 50.0 | 42.9 |
| % Treated patients[f] | - | 8.0 | 7.7 | 8.7 |

MS = multiple sclerosis.

Data are expressed as mean (standard deviation) unless otherwise stated. CIS/MS refers to the cohort of patients with CIS and multiple sclerosis included for comparison purposes; RIS–RIS refers to patients who remain as RIS during follow-up; RIS–CIS refers to patients who convert to CIS during follow-up.

[a]*n*/*N* (% positive) = number of patients with positive IgG oligoclonal bands / total number of patients in whom oligoclonal bands were determined (percentage of patients with IgG oligoclonal bands).

[b]*n*/*N* (% positive) = number of patients with $\geqslant$10 brain $T_2$ lesions / total number of patients with available information (percentage of patients with $\geqslant$10 brain $T_2$ lesions).

[c]Information available in 71 patients (94.7%).

[d]Information only available in 25 patients (33.3%).

[e]Information only available in 43 patients (57.3%).

[f]Percentage of patients who received immunomodulatory treatment between the RIS diagnosis and the date of conversion to CIS for converters, and during follow-up time for non-converters.

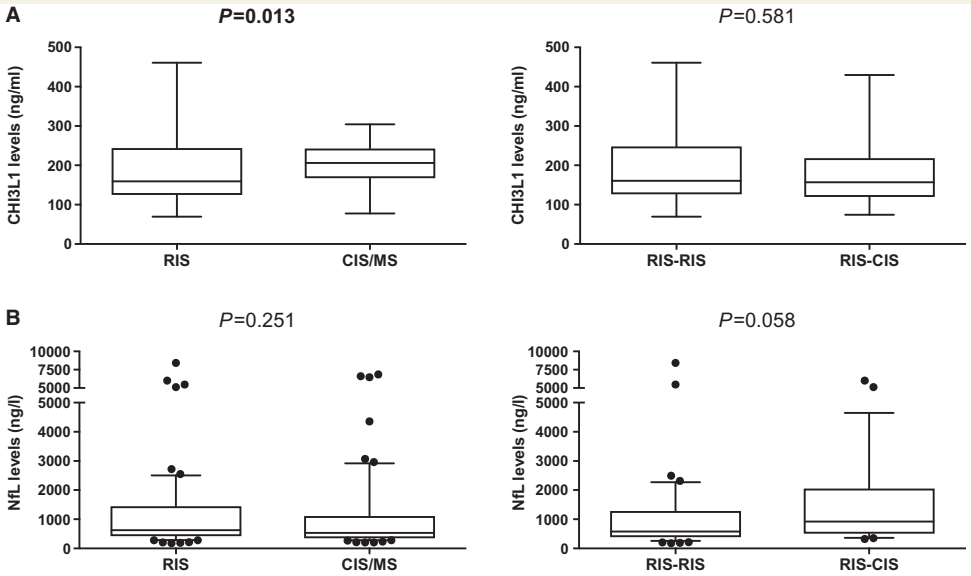


**Figure 1 Comparison of CSF CHI3L1 and NfL levels between patients with RIS and CIS/multiple sclerosis and between RIS non-converters and converters to CIS.** Boxplots showing CSF levels of CHI3L1 (**A**) and NfL (**B**) in the different groups. CSF levels were measured using commercially available ELISAs and compared among groups with a Mann-Whitney U-test, as described in the 'Materials and methods' section. CHI3L1 levels are significantly higher in CIS/multiple sclerosis patients compared to RIS patients. CIS/MS refers to the combined cohort of CIS and multiple sclerosis patients; RIS refers to the whole group of RIS patients; RIS - RIS = patients who remain as RIS during follow-up; RIS - CIS = patients who convert to CIS during follow-up.

converted to CIS and patients who remained as RIS (Fig. 1A). Levels of NfL did not differ between patients with RIS and patients with CIS/multiple sclerosis (Fig. 1B). Further stratification of the RIS group resulted in a trend towards increased NfL levels in RIS patients who converted to CIS compared to patients who continued as RIS (Fig. 1B).

CSF levels of CHI3L1 and NfL were not influenced by the time between brain MRI and lumbar puncture, insomuch as correlations between the length of this interval and biomarker levels were not statistically significant [Pearson correlation coefficient (*P*-value): −0.02 (0.875) for CHI3L1 and −0.16 (0.194) for NfL].

**Table 2 Univariable and multivariable Cox regression analysis of risk factors for the conversion to CIS and multiple sclerosis in RIS patients**

| | CIS conversion | | | Multiple sclerosis conversion | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *P*-value | HR | 95% CI | *P*-value |
| **Univariable** | | | | | | |
| CHI3L1 | 0.94 | (0.71–1.24) | 0.672 | 1.02 | (0.77–1.34) | 0.903 |
| NfL | 1.01 | (1.00–1.03) | **0.021** | 1.02 | (1.00–1.03) | **0.005** |
| OB | 10.31 | (1.37–76.61) | **0.024** | 9.29 | (1.24–69.41) | **0.030** |
| Age | 0.96 | (0.92–1.01) | 0.095 | 0.97 | (0.92–1.01) | 0.147 |
| $T_2$ lesions | 0.90 | (0.30–2.71) | 0.856 | 1.65 | (0.38–7.24) | 0.505 |
| Sex | 4.01 | (0.94–17.15) | 0.061 | – | – | – |
| Treatment | 1.20 | (0.28–5.15) | 0.808 | 1.31 | (0.30–5.67) | 0.721 |
| **Multivariable** | | | | | | |
| CHI3L1 | 0.88 | (0.61–1.27) | 0.499 | 1.03 | (0.72–1.49) | 0.869 |
| NfL | 1.02 | (1.00–1.04) | **0.019** | 1.03 | (1.01–1.05) | **0.003** |
| OB | 14.70 | (1.80–120.15) | **0.012** | 8.86 | (1.04–75.61) | **0.046** |
| Age | 1.00 | (0.95–1.05) | 0.968 | 1.00 | (0.94–1.05) | 0.780 |
| $T_2$ lesions | 0.39 | (0.11–1.43) | 0.156 | 0.36 | (0.07–1.85) | 0.220 |
| Sex | 3.15 | (0.64–15.52) | 0.158 | – | – | – |
| Treatment | 0.73 | (0.15–3.48) | 0.692 | 0.63 | (0.13–3.20) | 0.579 |

For CHI3L1, hazard ratio (HR) indicates the increase in risk for every 100 ng/ml increment. For NfL, hazard ratio indicates the increase in risk for every 50 ng/l increment. OB = presence of IgG oligoclonal bands. Age refers to age at RIS diagnosis. $T_2$ lesions = the number of brain $T_2$ lesions recoded into $\leqslant 9$ lesions (reference category) and $\geqslant 10$ lesions. Sex: females group were taken as reference category; conversion to multiple sclerosis was not calculated for this variable as none of the male patients converted to the disease. Treatment refers to whether patients received immunomodulatory treatment between the RIS diagnosis and the date of conversion to CIS or multiple sclerosis for converters, and during follow-up time for non-converters. Statistically significant values are highlighted in bold.

## Neurofilament light chain and oligoclonal bands are independent risk factors for conversion to clinically isolated syndrome and multiple sclerosis

We next investigated the prognostic value of CHI3L1 and NfL levels measured in CSF samples from RIS patients in the later conversion to CIS and multiple sclerosis. As shown in Table 2, in univariable Cox regression models NfL levels and IgG oligoclonal bands were associated with increased risk for conversion to CIS [hazard ratio (HR) = 1.01, 95% confidence interval (CI) 1.00–1.03, $P = 0.021$ for NfL; HR = 10.31, 95% CI 1.37–76.61, $P = 0.024$ for oligoclonal bands] and multiple sclerosis (HR = 1.02, 95% CI 1.00–1.03, $P = 0.005$ for NfL; HR = 9.29, 95% CI 1.24–69.41, $P = 0.030$ for oligoclonal bands). In contrast, CSF CHI3L1 levels as well as other factors such as age, number of brain $T_2$ lesions, sex and treatment were not predictive of future conversion to CIS or multiple sclerosis (Table 2).

In multivariable Cox regression models, NfL levels and oligoclonal bands were independent predictors of conversion to CIS (HR = 1.02, 95% CI 1.00–1.04, $P = 0.019$ for NfL; HR = 14.70, 95% CI 1.80–120.15, $P = 0.012$ for oligoclonal bands) and multiple sclerosis (HR = 1.03, 95% CI 1.01–1.05, $P = 0.003$ for NfL; HR = 8.86, 95% CI 1.04–75.61, $P = 0.046$ for oligoclonal bands) in RIS patients (Table 2). Similar to the univariable analysis, other factors including CHI3L1 levels were not associated with the risk of conversion to CIS or multiple sclerosis (Table 2). Likewise, as shown in Supplementary Table 2, the number of infratentorial lesions and other variables that were available in a subgroup of RIS patients such as the number of spinal cord lesions and number of contrast-enhancing lesions were not influencing the conversion to CIS and multiple sclerosis in univariable and multivariable analyses.

## High CSF neurofilament light chain levels and presence of oligoclonal bands are associated with shorter time to clinically isolated syndrome and multiple sclerosis

As a next step, we examined time to CIS and time to multiple sclerosis by Kaplan-Meier survival analysis in RIS patients with high and low CSF CHI3L1 and NfL levels, and presence and absence of oligoclonal bands. To classify CHI3L1 levels into high and low a cut-off value of 170 ng/ml was used, based on a previous publication by our group (Canto *et al.*, 2015).

Receiver operating characteristic (ROC) curve analysis was used to estimate the best cut-off value for NfL levels in RIS patients. A trend towards significance in the area under the ROC curve (AUC) was obtained for the time to CIS (AUC = 0.65; 95% CI 0.51–0.78; $P = 0.058$), which became statistically significant when the time to multiple sclerosis was analysed (AUC = 0.68; 95% CI 0.54–0.82; $P = 0.025$). The best cut-off value to classify

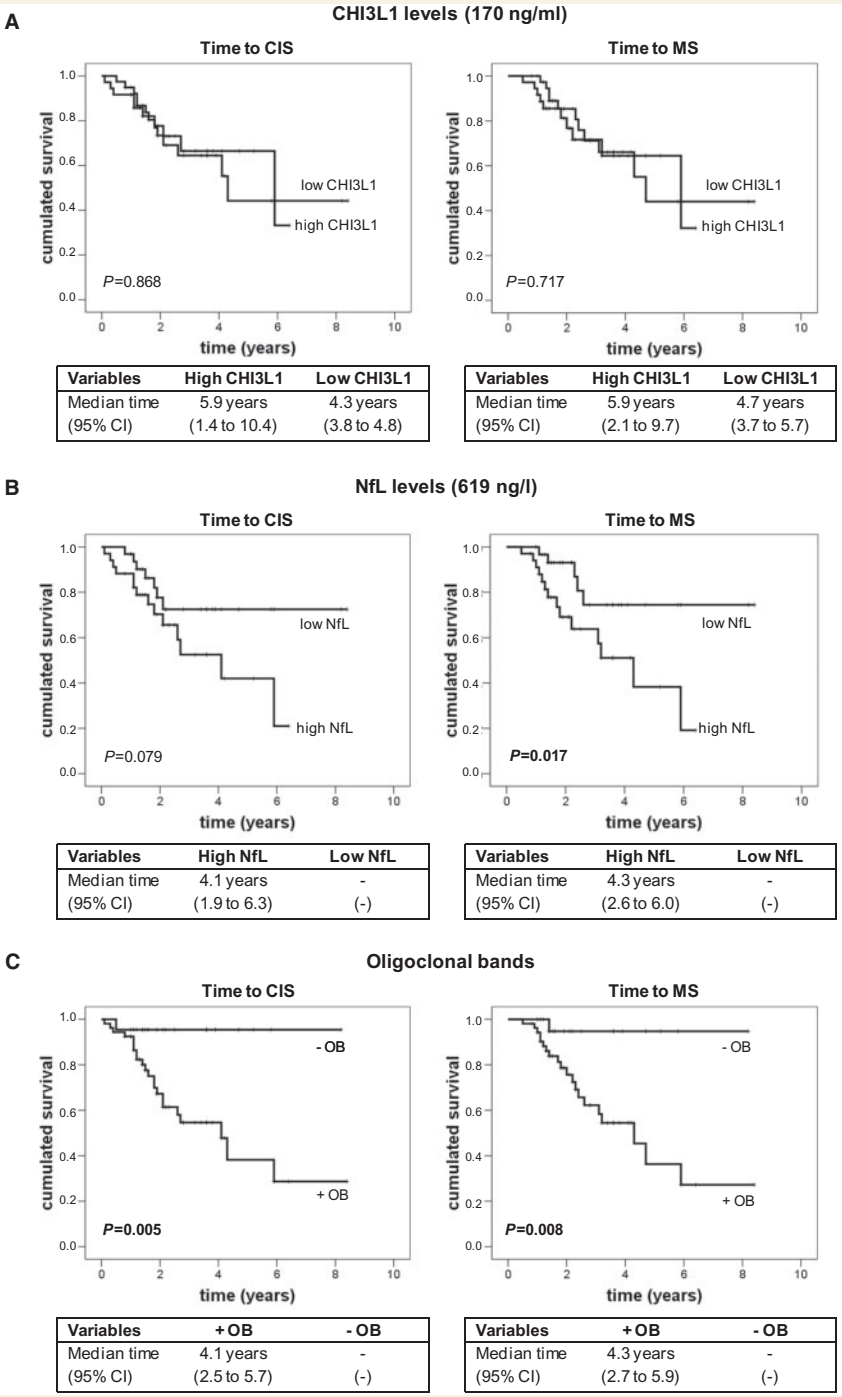


**Figure 2 Kaplan-Meier curves for time to CIS and time to multiple sclerosis in RIS patients according to high and low CSF CHI3L1 and NfL levels and positive and negative oligoclonal bands.** (**A**) CHI3L1 levels were classified into high and low based on a cut-off value of 170 ng/ml. (**B**) For NfL, a cut-off value of 619 ng/l was used to stratify protein levels into high and low. (**C**) For oligoclonal bands, patients were classified as oligoclonal bands positive (+OB) and oligoclonal bands negative (−OB). Graphs show log-rank *P*-values. Tables indicate mean times (95% CI). MS = multiple sclerosis.

CSF NfL levels into high and low in RIS patients was 619 ng/l. For this cut-off, sensitivities and specificities were 67% and 57%, respectively, for time to CIS, and 74% and 58%, respectively, for time to multiple sclerosis.

As shown in Fig. 2B, CSF NfL levels above the 619 ng/l cut-off were associated with a trend towards shorter time to CIS (log-rank *P*-value = 0.079), and with significant shorter time to multiple sclerosis ($P = 0.017$). Similarly, RIS patients with positive oligoclonal bands converted to CIS and multiple sclerosis faster than patients without oligoclonal bands ($P = 0.005$ and $P = 0.008$, respectively; Fig. 2C). In contrast, rates of conversion to CIS and

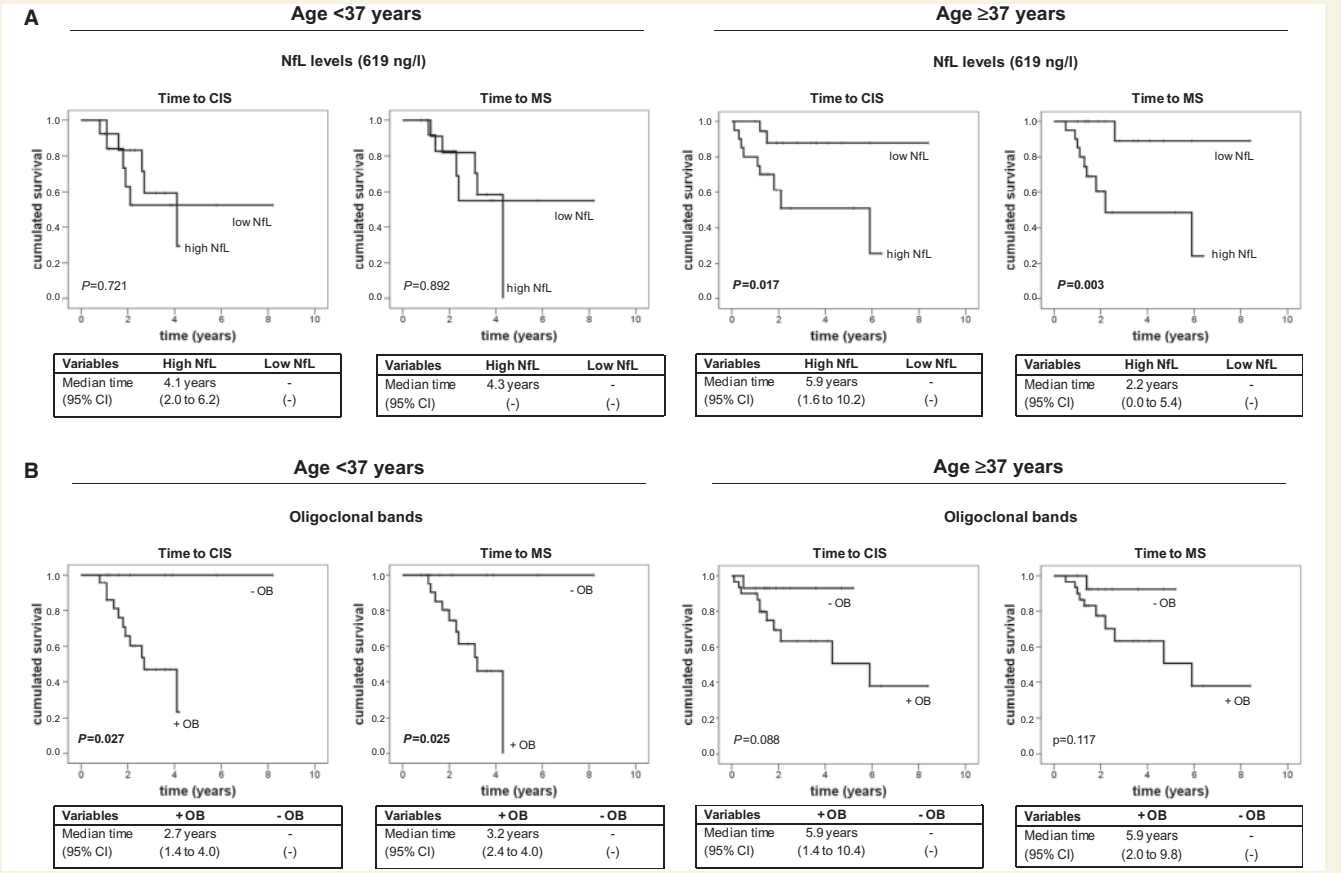


**Figure 3 Kaplan-Meier curves for time to CIS and time to multiple sclerosis based on high and low NfL levels and positive and negative oligoclonal bands in RIS patients stratified according to age.** RIS patients were classified according to age using a cut-off value of 37 years. The effects of high and low CSF NfL levels based on a cut-off value of 619 ng/l (**A**) and presence or absence of oligoclonal bands (**B**) were analysed in patients with <37 years ($n = 31$) and ≥37 years ($n = 44$). Graphs show log-rank *P*-values. Tables indicate mean times (95% CI). + OB = patients with positive oligoclonal bands. −OB = patients with negative oligoclonal bands. MS = multiple sclerosis.

multiple sclerosis were similar between RIS patients with high and low CSF CHI3L1 levels (Fig. 2A).

When RIS patients were classified according to age, a known predictor of clinical conversion (Okuda *et al.*, 2011, 2014), the prognostic value of high CSF NfL levels in the time to CIS and multiple sclerosis was restricted to patients aged ≥37 years ($P = 0.017$ and $P = 0.003$, respectively) whereas it was not observed in RIS patients with age <37 years (Fig. 3A). In contrast, as shown in Fig. 3B the presence of CSF oligoclonal bands was associated with a comparable effect on the time to CIS and time to multiple sclerosis regardless of age, although it was more pronounced in younger patients (in patients with <37 years: $P = 0.027$ and $P = 0.025$ for time to CIS and multiple sclerosis, respectively; in patients with ≥37 years: $P = 0.088$ and $P = 0.117$ for time to CIS and multiple sclerosis, respectively). A similar stratified analysis was not conducted for other predictors of clinical conversion such as sex and spinal cord lesions (Okuda *et al.*, 2011, 2014), insomuch as only two males with RIS converted to CIS and none to multiple sclerosis, and information on spinal cord lesions was missing in 66% of RIS patients (Table 1).

# Discussion

The present study, aimed to investigate the prognostic value of CHI3L1, NfL, and IgG oligoclonal bands determined in CSF samples from patients at the time of RIS diagnosis, shows that NfL levels together with the presence of IgG oligoclonal bands are independent predictors of conversion to CIS and multiple sclerosis in RIS patients, and associate with shorter times to CIS and multiple sclerosis development. In contrast, CHI3L1 does not seem to play a prognostic role in this pathogenic stage.

NfL levels have been found to be increased in CSF samples from CIS patients who later convert to multiple sclerosis compared to those patients who remain as CIS (Teunissen *et al.*, 2009; Martinez *et al.*, 2015; Arrambide *et al.*, 2016). In multivariable analyses, CSF NfL levels were also found to be independent predictors of conversion to multiple sclerosis in CIS patients (Arrambide *et al.*, 2016). In our study, CSF NfL levels were similar between RIS and CIS/multiple sclerosis patients, though within the RIS group a trend towards increased NfL levels was observed in patients who later converted to CIS. The lower $T_2$ lesion burden observed in CIS/

multiple sclerosis patients may explain that CSF NfL levels were not found to be increased in this group of patients when compared to RIS patients. Univariable and particularly multivariable analyses including as covariates age at RIS diagnosis, treatment, number of brain $T_2$ lesions, sex, and CHI3L1 levels showed that CSF NfL levels were independent risk factors for conversion to CIS in RIS patients. When the outcome analysed was time to multiple sclerosis according to the 2010 McDonald criteria (Polman *et al.*, 2011), CSF NfL levels were slightly better predictors of conversion to multiple sclerosis in RIS patients. A CSF NfL value of 619 ng/l resulted in the best cut-off to classify protein levels into high and low, and patients with high CSF NfL levels exhibited shorter time to CIS and multiple sclerosis. Altogether these findings indicate that neuroaxonal damage is already present in preclinical stages of the disease, and quantification of NfL levels in CSF samples collected at the RIS stage may help to identify those patients at higher risk for and faster conversion to CIS and multiple sclerosis.

CSF IgG oligoclonal bands are known to play prognostic roles in CIS patients, and patients with positive oligoclonal bands are at higher risk for multiple sclerosis independently of other covariates present at the time of the CIS event (Tintore *et al.*, 2008, 2015). In RIS patients, oligoclonal bands were also independent predictors of later conversion to CIS and multiple sclerosis, with strong effects as reflected by the high hazard ratios obtained for this covariate. In a previous study conducted in 451 RIS patients (Okuda *et al.*, 2014), the presence of an abnormal CSF (defined by an IgG index $>0.7$ or the presence of oligoclonal bands) was predictive of clinical symptom development in a univariable analysis, although the statistical significance was lost in the multivariable analysis. The use of different criteria for CSF abnormalities (we only considered the positivity for IgG oligoclonal bands but not the IgG index) or the inclusion of different covariates in the multivariable analysis are factors that may have probably contributed to the discrepancies observed between both studies regarding the predictive role of IgG oligoclonal bands in RIS patients.

Similar to high CSF NfL levels, in our study the presence of oligoclonal bands was associated with shorter time to CIS and multiple sclerosis compared to individuals without oligoclonal bands. These findings indicate that oligoclonal bands may help to identify those patients in whom the RIS event is part of the multiple sclerosis spectrum and not due to other neurological conditions.

Previous studies have shown that age, sex, and spinal cord abnormalities are independent predictors for clinical conversion in RIS patients (Okuda *et al.*, 2011, 2014). In our study, age *per se* was not influencing later conversion to CIS and multiple sclerosis of RIS patients in univariable or multivariable analyses. However, it is worth mentioning that the effects of high CSF NfL levels shortening time to CIS and multiple sclerosis were restricted to the older RIS population, using 37 years as cut-off for age (Okuda *et al.*, 2014). Whether this heightened predictive capacity of NfL in older patients is associated with age-related neuronal loss deserves further investigation. Male sex has also been associated with worse prognosis in RIS patients (Okuda *et al.*, 2014). Uni- and multi-variable analyses did not show significant associations of sex with conversion to CIS or multiple sclerosis. These negative findings were probably influenced by the reduced number of male patients reaching conversion outcomes in our RIS cohort. Finally, the number of spinal cord lesions did not seem to influence the conversion to CIS or multiple sclerosis, although these data should be considered with caution owing to the small number of patients with available information on this variable, being one limitation of the study.

Regarding CHI3L1, high CSF levels in CIS patients have been associated with increased risk for multiple sclerosis independent of oligoclonal bands and MRI abnormalities (Canto *et al.*, 2015). In our study, CHI3L1 levels were not predictive of symptoms onset in RIS patients. Considering that CSF CHI3L1 levels may reflect the degree of astrocyte activation secondary to inflammation, one explanation for these negative findings would be that reactive astrogliosis secondary to the inflammatory insult is a less prominent pathological feature in RIS patients compared to clinical stages such as CIS or multiple sclerosis. This hypothesis is supported by the increased CSF CHI3L1 levels observed in CIS/multiple sclerosis patients compared to RIS patients.

In summary, our findings indicate that both CSF NfL levels and IgG oligoclonal bands play prognostic roles in RIS patients in terms of predicting later development of clinical stages such as CIS and multiple sclerosis. In contrast, CSF CHI3L1 levels do not seem to be predictive in preclinical stages of the multiple sclerosis spectrum. Although treatment of RIS patients is at present controversial, CSF NfL and oligoclonal bands may be used as biomarkers to select patients at higher risk for CIS and multiple sclerosis who may benefit from early treatment to delay conversion outcomes. These findings also reinforce the importance of CSF analysis in patients with RIS.

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## Supplementary material

Supplementary material is available at *Brain* online.

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