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Corresponding Author: Dr. Diana Ferraro,

Corresponding Author's Institution: University of Modena and Reggio Emilia

First Author: Diana Ferraro

Order of Authors: Diana Ferraro; Veronica Galli; Francesca Vitetta; Anna M Simone; Roberta Bedin; Cinzia Del Giovane; Franca Morselli; Maria M Filippini; Paolo F Nichelli; Patrizia Sola

Abstract: Cerebrospinal fluid (CSF) CXCL13 was shown to correlate with markers of intrathecal inflammation and CSF oligoclonal IgM bands (IgMOB) have been associated with a more severe Multiple Sclerosis (MS) course.

We correlated CSF CXCL13 levels with clinical, MRI and CSF parameters, including CSF IgMOB, in 110 Clinically Isolated Syndrome (CIS) patients.

CSF CXCL13 levels correlated with CSF cell count, total protein, IgG Index and with the presence of CSF IgGOB and IgMOB.

CSF CXCL13 levels ≥ 15.4 pg/ml showed a good positive predictive value and specificity for a MS diagnosis and for a clinical relapse within one year from onset.

Modena, February 17th 2015

I hereby declare that the manuscript entitled "Cerebrospinal fluid CXCL13 in Clinically Isolated Syndrome patients: association with oligoclonal IgM bands and prediction of Multiple Sclerosis diagnosis" contains original work and that it has not been published or submitted for publication elsewhere.

In faith,

Diana Ferraro

Ref.: Ms. No. JNI-D-15-00061

Cerebrospinal fluid CXCL13 in Clinically Isolated Syndrome patients: association with oligoclonal IgM bands and prediction of Multiple Sclerosis diagnosis

Reviewers' comments:

Reviewer #1: Review of JNI-D-15-00061

In this manuscript, the authors present compelling evidence that there is a potential association between CXCL13, IgM and MS diagnosis in a fairly large cohort (n=120) CIS patients. I have some concerns that should be addressed:

1. In section 3.1, the authors note that 86% of the patients were diagnosed with MS according to the McDonald criteria, while 45% of the patients were diagnosed with MS by the end of the follow-up period. These two percentages should be combined with the percentage that did not convert so that all three percentages add up to 100%. It is very confusing in this context.

Response

The paragraph in section 3.1 has been modified as follows:

"By the end of the follow-up period (mean: 41 ± 18 months, median: 40 months; range: 4-72), 94 (86%) patients were diagnosed with MS. Of these, 49 (45%) patients had experienced a relapse (diagnosis of CDMS), while in 45 patients (41%) patients, MS diagnosis was established based on MRI criteria of DIS and DIT, in accordance with the 2010 McDonald's criteria (Polman et al., 2011)."

To avoid confusion, table 1 has also been modified: we eliminated data on patients in whom the initial MS diagnosis was based solely on MRI criteria, since some of those patients went on to have a relapse, leading to an overlap in the percentages of patients with a diagnosis based on MRI criteria and of patients diagnosed with CDMS.

2. In relation to point 1, Table 1 should also include the denominator for each percentage calculation rather than just the "n".

Response

The denominator has been added for each percentage calculation.

3. I am not convinced that the specificity/sensitivity calculations presented in Section 3.3 are all that impressive as they do not represent an improvement from current measurements. This section should be taken out with the understanding that the paper continues to have strong biological implications without it.

Response

We eliminated the paragraph on the specificity/sensitivity calculations for the different CXCL13 cut-off values and the corresponding Table 2.

4. The authors should generate a scatter bar plot showing the concentrations of CXCL13 in the CSF of CIS patients who convert vs those who do not. Presumably, the CXCL13 levels in the CSF of CIS patients who convert should be statistically higher than in those patients who did not—or in those patients who converted later in the study.

Response

We have added a dotplot (Figure 1) showing CSF CXCL13 values (and median values) in those patients who converted to MS versus those who did not, and indicating p-value for comparison between the medians of the two groups.

5. CXCL13 is important for attracting B and T cells, rather than ALL lymphocytes (as is the case with CXCL12). Did the authors perform regressions of CXCL13 levels with B cell numbers or T cell numbers in the CSF? This would be more informative. At the very least, the authors should discuss how total CSF lymphocyte numbers (if that is what was actually measured rather than ALL CSF cells as there are others besides lymphocytes) may not be an accurate reflection of the impact of CXCL13 on CSF lymphocyte migration. The authors might also demonstrate that a more generalized chemokine (such as CXCL12) does not correlate with CSF lymphocyte numbers or total CSF cells.

Response

Our laboratory measures the number of leukocytes/ μ l and not the number of lymphocytes (although these were the prevalent type of cell at visual inspection). We could, therefore, unfortunately, not specifically discuss the impact on CXCL13 on CSF lymphocyte migration, nor could we regress CXCL13 levels with B or T cell numbers.

6. A detailed method of how cell count was calculated must be included as this set of data is central to the overall theme of the paper.

Response

We have added the following paragraph in section 2.2:

"CSF cell count was carried out within one hour from sampling as follows: test tube containing CSF was gently agitated in order to resuspend cells; CSF was incubated with Turk's solution (mixture of acetic acid and gentian violet) (1:2) for ten minutes to lyse erythrocytes and to stain leukocytes; cells were counted using Nageottes's or Fuchs-Rosenthal chamber."

7. Minor point: the use of "have been shown/has been shown/shown to be" is excessive. These should be removed. Example: In MS patients, elevated CSF CXCL13 levels have been shown to correlate with CSF cell count..." should be "In MS patients, elevated CSF CXCL13 levels correlated with CSF cell count..."

Response

We have followed your advice and corrected the manuscript accordingly.

8. Minor point: highlights point 3 regarding predictive value of CXCL13 levels in CSF should be removed.

Response

It has been removed.

Reviewer #2: This interesting manuscript confirms i) the presence of higher levels of the chemokine CXCL13 in the CSF of patients with MS, and ii) a correlation between the higher levels of CSF CXCL13 and the presence of IgM oligoclonal bands. Furthermore, the results show that CSF CXCL13 levels of >15.4pg/ml show a high positive predictive value for conversion to MS in patients presenting with a clinically isolated syndrome, and for a relapse within 1 year of onset. These findings suggest that CSF CXCL13 levels could be used as an additional tool for predicting patients who might have a more severe clinical course.

There are just a couple of minor points that could be addressed:

1. Page 5, last line of first paragraph - "used" rather than "uses"

Response

It has been corrected.

2. Page 5, last line in Methods section - "Data were analyzed" rather than "Data was analyzed"

Response

It has been corrected.

3. Section 3.2 CXCL13 values - it would be useful to have a graph showing these results, just so that the reader can get more of a feel for the distribution of the data, as it's not clear whether all of the values are reported as means, or whether median values are used in some cases.

Response

Figure 1 has been added to show distribution of CSF CXCL13 values in those CIS patients who have converted to MS and in those who have not. In section 3.2 we have specified whether numbers refer to mean or to median values.

4. Figure 1 - The X-axis labels showing "1-specificity" are awkward; it would be better to have the X axis labels showing the specificity (i.e. starting at 1 at the left side of the axis and going down to 0 on the right hand side).

Response

The figure (which has now become Figure 2) has been modified according to suggestions.

Reviewer #3: The authors have analyzed CSF CXCL13 levels in samples from 110 patients with clinically isolated syndrome (CIS). Their results confirm previous studies demonstrating a correlation of CSF CXCL13 levels with CSF cell count, total protein, IgG Index and with the presence of CSF IgG and IgM oligoclonal bands. CSF CXCL13 levels are higher in patients who had a clinical relapse within one year from onset thus confirming a predictive role of CSF CXL13 levels for the conversion to MS after a CIS. The study is well performed and is in good agreement with previously published data.

1) I have, however, one major concern and recommendation for improvement: the ROC curves shown in figure 2 are not really convincing due to the low area under the curve and the detailed statistical analysis (p-values) is missing and must be included.

Response

If I have understood your comment correctly, the p-value (that tests the null hypothesis that the area under the curve=0.50) is equal to 0.0004 for a MS diagnosis within one year and to 0.017 for a CDMS diagnosis within one year (Wilcoxon rank-sum test). This information has been added to the legend of Figure 2. We have also added the 95% confidence intervals for the area under the curves (AUC) in section 3.3: AUC for a MS diagnosis within one year=71% (CI95%:60-82); AUC for a CDMS diagnosis within one year=64% (CI95%:54-75).

2) In addition I recommend that the authors show a table with the number of patients who converted to MS according to revised the McDonald's criteria. This table should contain all demographic, MRI and CSF data (CXL13 levels and other CSF data) and a statistical analysis using binary logistic

regression analysis, which will inform the reader on the best predictor.

Response

We have added the requested information to Table 3.

3) Similarly, the cox regression analysis could not only be calculated using CXCL13 cut-off, but also with the CSF CXCL13 raw values.

Response

Cox analysis was carried out on raw CXCL13 values which significantly increased the risk of an MS (but not of a CDMS) diagnosis throughout follow-up, although the statistical significance was not confirmed at multivariate analysis. This information has been added in section 3.5.

4) Minor points: - Figure 2 (Correlations between CSF CXCL13 levels and CSF cell count Legend: R= 0,6; p<0,0001) is not necessary and should be omitted.

Response

It has been omitted.

5)- How high were the inter- and intrassay variations for the CSF CXCL13 ELISA?

Response

The following information was provided by the manufacturer:

	<i>Intra-Assay Precision</i>			<i>Inter-Assay Precision</i>		
<i>Sample</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>1</i>	<i>2</i>	<i>3</i>
<i>n</i>	20	20	20	40	40	40
<i>Mean (pg/ml)</i>	50.2	136	294	54	138	285
<i>Standard Deviation</i>	2.2	4.5	8	5.1	13.2	24.8
<i>Coefficient of Variability %</i>	4.3	3.3	2.7	9.4	9.6	8.7

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

6)- More details on the ethical approval would be appropriate (which authority was responsible, number of approval).

Response

The Ethics Committee of the province of Modena, Italy, approved the study (protocol nr. 116/09). This information has been added in section 2.2.

7)- Did the authors also measure serum CXL13 levels?

Response

No, we did not measure serum CXCL13 levels.

*List of 5 Suggested Reviewers

Potential reviewers

Johannes Brettschneider

E-mail: johannes.brettschneider@uni-ulm.de

Mohsen Khademi

Email: mohsen.khademi@ki.se

Hayrettin Tumani

E-mail: hayrettin.tumani@uni-ulm.de

Cerebrospinal fluid (CSF) CXCL13 is associated with intrathecal inflammation.

CSF CXCL13 is associated with the presence of CSF oligoclonal IgM bands.

~~Elevated CSF CXCL13 levels predict an early relapse in Clinically Isolated Syndrome.~~

Cerebrospinal fluid CXCL13 in Clinically Isolated Syndrome patients: association with oligoclonal IgM bands and prediction of Multiple Sclerosis diagnosis

Abstract

Cerebrospinal fluid (CSF) CXCL13 was shown to correlate with markers of intrathecal inflammation and CSF oligoclonal IgM bands (IgMOB) have been associated with a more severe Multiple Sclerosis (MS) course.

We correlated CSF CXCL13 levels with clinical, MRI and CSF parameters, including CSF IgMOB, in 110 Clinically Isolated Syndrome (CIS) patients.

CSF CXCL13 levels correlated with CSF cell count, total protein, IgG Index and with the presence of CSF IgGOB and IgMOB.

CSF CXCL13 levels ≥ 15.4 pg/ml showed a good positive predictive value and specificity for a MS diagnosis and for a clinical relapse within one year from onset.

1. Introduction

Increasing evidence on the importance of B lymphocytes in the immunopathogenesis of Multiple Sclerosis (MS) has encouraged the evaluation of B cell-associated biomarkers in the cerebrospinal fluid (CSF). An important chemokine that directs the migration of B cells before they differentiate to antibody-secreting cells is CXCL13 (Meinl et al., 2006). Virtually all B cells express the receptor to this chemokine, CXCR5, (Kim et al., 2001), which is also transiently induced on T cells upon activation (Langenkamp et al., 2003).

Abbreviations: Multiple Sclerosis (MS); cerebrospinal fluid (CSF); oligoclonal IgG bands (IgGOB); Clinically Isolated Syndrome (CIS); oligoclonal IgM bands (IgMOB); Clinically Definite MS (CDMS); isoelectric focusing (IEF); area under the curve (AUC); positive predictive value (PPV); negative predictive value (NPV); central nervous system (CNS); Experimental Autoimmune Encephalitis (EAE); Relapsing-Remitting MS (RRMS)

In MS patients, elevated CSF CXCL13 levels ~~have been shown to correlate~~correlated with CSF cell count, IgG index, presence of oligoclonal IgG bands (IgGOB), number of MRI brain lesions and presence of MRI gadolinium-enhancing lesions (Festa et al., 2009; Khademi et al., 2011; Krumbholz et al., 2006; Kuenz et al., 2008; Ragheb et al., 2011; Sellebjerg et al., 2009; Senel et al., 2014). Furthermore, they were ~~shown to be~~ associated with a higher relapse rate and to a higher risk of conversion to MS in Clinically Isolated Syndrome (CIS) patients (Brettschneider et al., 2010; Khademi et al., 2011).

In addition, CSF CXCL13 levels ~~were shown to correlate~~correlated with the number of CSF CD5+ B cells and with intrathecal IgM production (Villar et al., 2010). Intrathecal IgM synthesis (Mandrioli et al., 2008; Sola et al., 2011; Villar et al., 2003, 2002), and intrathecal lipid-specific IgM synthesis (Thangarajh et al., 2008; Villar et al., 2005) have been associated with a more aggressive disease course and with an earlier conversion to Clinically Definite MS (CDMS) in CIS patients (Boscá et al., 2010; Ferraro et al., 2013). Our aim was to correlate CSF CXCL13 levels with clinical, MRI and CSF parameters, including CSF oligoclonal IgM bands (IgMOB), in a cohort of CIS patients who were followed up for at least two years or until a relapse occurred, and to explore the possible role of CXCL13 in predicting a MS/CDMS diagnosis and an “early” MS/CDMS diagnosis, defined as a diagnosis or a relapse occurring within one year from onset.

2. Methods and Materials

2.1 Patient Data collection

Of all consecutive patients seen at the MS Center of the Nuovo Ospedale Civile Sant'Agostino Estense (Modena, Italy) between January 2007 and November 2011, we included patients with the following inclusion criteria: patients at their first demyelinating

event who performed a spinal tap and a brain MRI scan, and whose CSF and serum samples were stored at -80°C in our laboratory within two hours of collection. Patients were followed-up for at least two years or until a clinical relapse occurred. Of these, we recorded demographical (sex, age at onset), clinical (symptoms at onset, EDSS at onset, clinical recovery), MRI (presence and number of brain and spinal cord MRI lesions, presence and number of gadolinium-enhancing lesions on MRI, presence of infratentorial lesions) and CSF variables (CSF proteins, cell count, Link's IgG Index, presence of IgGOB), as well as follow-up data including the occurrence of relapses and dissemination in space (DIS) and time (DIT) of demyelinating lesions on MRI.

2.2 Laboratory procedures

CSF cell count was carried out within one hour from sampling as follows: test tube containing CSF was gently agitated in order to resuspend cells; CSF was incubated with Turk's solution (mixture of acetic acid and gentian violet) (1:2) for ten minutes to lyse erythrocytes and to stain leukocytes; cells were counted using Nageottes's or Fuchs-Rosenthal chamber.

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Laboratory CXCL13 determination and IgMOB detection was procedures were carried out on CSF (and serum samples, in case of IgMOB detection) which, at the time of the diagnostic spinal tap, had been centrifuged at 3000 rpm for 10 minutes and stored in cryovial tubes at - 80°C within two hours from collection.

2.2.1 CXCL13 determination

CSF CXCL13 levels were measured using the Human CXCL13/BLC/BCA-1 Quantikine ELISA Kit (R&D Systems, Minneapolis, Minnesota, USA), according to the instructions as supplied by the manufacturer. The minimal detectable dose (MDD), as reported by the manufacturer, is 1.64pg/ml. We used a standard curve ranging from 0 to 500 pg/ml. Measurements were performed in duplicates using 50 µl undiluted CSF. The optical density was determined using a microplate reader (Beckman Coulter DTX 800/880) set for dual wavelength analysis to 450/620 nm.

2.2.2 IgM oligoclonal band detection

CSF and serum samples were analyzed for the presence of IgMOB by means of agarose gel isoelectric focusing (IEF) followed by immunoblotting with polyclonal specific anti-human IgM antibodies (Dako), according to the method proposed by Villar et al (Villar et al., 2001), with the following modifications: for Agarose IEF gel we used 2 ml of Pharmalyte (GE Healthcare), pH 5-8 and 1 ml of Pharmalyte, pH 3-10; the electrode strips were soaked with 1 M NaOH and 0,01 M H₂SO₄; ten-microliter paired samples were applied on a sample application strip; the PVDF membrane was previously wetted in ethanol and then washed in two changes of saline for the total migration time; the membrane was incubated with a polyclonal rabbit anti-human IgM (Dako Cytomation) diluted 1:500 in 0,1% TWEEN 20 in saline for 30 minutes and then with a polyclonal swine anti-rabbit IgG/AP (Dako Cytomation) diluted 1: 500 in 0,1% TWEEN 20 in saline solution for 30 minutes.

The presence of CSF IgMOB (at least two) was blindly assessed by two independent neurologists (DF and PS) and by a biologist (RB) in case of discrepancies.

At the time of the diagnostic spinal tap patients signed an informed consent permitting the storage and the use for research purposes of their serum and CSF samples. The Ethics Committee of the province of Modena, Italy, approved the study (protocol nr. 116/09).
~~Ethics committee approval was obtained for this study.~~

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2.3 Statistical methods

We calculated absolute frequencies and percentages for categorical variables and mean \pm standard deviation and median for continuous variables. Correlations between CXCL13 levels and collected variables were analyzed using Spearman's rank test and Wilcoxon's rank sum test for categorical variables. Mann-Whitney's test and equalitu of medians test was used to explore differences between groups. Wilcoxon's rank sum test was used to explore the null hypothesis that the AUC=0.5.

Receiver Operating Characteristics (ROC) analysis, calculating the area under the curve (AUC), was used to examine the accuracy of CXCL13 values in differentiating patients who remained with a CIS diagnosis from those who acquired a diagnosis of MS/CDMS.

Youden Index was useds to determine the best cut-off values

Sensitivity was calculated as $(\text{true-positive}/[\text{true-positive} + \text{false-negative}])$, specificity was calculated as $(\text{true-negative}/[\text{true-negative} + \text{false-positive}])$. The positive predictive value (PPV) was calculated as $(\text{true-positive}/[\text{true-positive} + \text{false-positive}])$, and the negative predictive value (NPV) as $(\text{true-negative}/[\text{true-negative} + \text{false-negative}])$.

The impact of baseline characteristics on the risk of an early diagnosis of MS/CDMS (i.e. within one year) was analyzed using logistic regression and the risk of a diagnosis of MS/CDMS throughout the follow-up period was analyzed using survival analysis (Kaplan-

Meier survival curves followed by Log-rank test). The impact of the recorded variables on the risk of a MS/CDMS diagnosis was analyzed using a univariate and multivariate Cox analysis.

P-values below 0.05 were considered significant. Data were analyzed using STATA 11 (StataCorp, Texas, USA).

3. Results

3.1 Patients

Of 120 evaluated CIS patients, five were excluded from the analysis because spinal tap was not carried out (due to patients' refusal or to concomitant anticoagulant therapy in one case), two because baseline MRI was not carried out (due to claustrophobia) and three because the spinal tap had been carried out elsewhere. One-hundred and ten patients were included in the study. Patients' characteristics are shown in Table 1. Conversion to CDMS was defined by the occurrence of new or worsening neurological symptoms lasting at least 24 hours, associated with at least a one-point increase in an EDSS functional score, not accompanied by fever or infection. Baseline MRI was carried out after a mean period of 24 days from onset (range 0-8 months). Follow-up MRI was carried out after a mean period of 4 months (range 1-12). By the end of the follow-up period (mean: 41 ± 18 months, median: 40 months; range: 4-72), a diagnosis of MS was established in 94 (86%) patients were diagnosed with MS. Of these, 49 (45%) patients had experienced a relapse (diagnosis of CDMS), while in 45 patients (41%) patients, MS diagnosis was established based on MRI criteria of DIS and DIT, according to in accordance with the 2010 McDonald's criteria (Polman et al., 2011), while a diagnosis of CDMS was established in 49 (45%) patients by the end of the follow-up period (mean: 41 ± 18 months, median: 40

~~months; range: 4-72~~. An early diagnosis, i.e. within one year, occurred in 74 (67%) and an early relapse in 34 (31%) patients.

3.2 CXCL13 values

Mean CSF CXCL13 levels were $31\text{pg/ml} \pm 55$ (range: 0-357), median value was 13.5pg/ml. Mean CXCL13 levels were significantly higher in patients who acquired a MS diagnosis in accordance with McDonald's criteria throughout the follow-up period versus those who did not (35.8pg/ml versus 2.5pg/ml; $p < 0.0001$). Figure 1 shows a dotplot of CSF CXCL13 values in the two groups of patients and indicates median values. Mean CXCL13 levels were also significantly higher in patients with CSF IgGOB (34.8pg/ml versus 24.9pg/ml; $p < 0.0001$), in patients with IgMOB (44.7pg/ml versus 23.7pg/ml ; $p = 0.009$) and in those with an increased CSF cell count (49.9pg/ml versus 16.3pg/ml; $p < 0.0001$).

3.3 CXCL13 diagnostic accuracy

ROC analyses were carried out for CSF CXCL13 levels in relation to an early MS/CDMS diagnosis (within one year from onset) (Figures 1a and 1b, respectively) and also in relation to the occurrence of a diagnosis of CDMS throughout the follow-up period (figure not shown).

For a MS diagnosis within one year, the optimal CSF CXCL13 cut-off value was 8.7pg/ml, which yielded a sensitivity of 66.2% and a specificity of 63.9%. The area under the curve (AUC) was 71% (95% confidence interval: 60-82) (Figure 24a). For a CDMS diagnosis within one year, the cut-off value of 8.7pg/ml yielded a sensitivity of 74% and a specificity of 51.3% (Figure 24b), with an AUC of 64% (95% confidence interval: 54-75), while the

optimal cut-off of 15.4pg/ml increased its specificity (sensitivity: 64.7%; specificity: 63.1%) (Figure 1b). Similarly, for a CDMS diagnosis throughout the follow-up period (figure not shown), the cut-off of 15,4pg/ml revealed a sensitivity of 61.2% and a specificity of 67.1%, with an AUC of 64%. Figure 24 displays the ROC analysis for MS/CDMS diagnosis within one year, showing two different cut-off values (8.7pg/ml and 15.4pg/ml).

~~Table 2 shows sensitivity, specificity, PPV and NPV for the two different CSF CXCL13 cut-off values (8.7 and 15.4pg/ml), for the presence of CSF-restricted IgGOB, and for the presence of at least one lesion on T2-weighted images on baseline MRI scan in relation to an early MS/CDMS diagnosis and to a CDMS diagnosis throughout the follow-up period.~~

We chose to further discuss the cut-off value of 15.4pg/ml since it combines the best sensibility/specificity values for an early CDMS diagnosis.

3.4 CXCL13 correlation analyses

CSF CXCL13 values correlated with CSF cell count ($R=0.6$; $p<0.0001$) (Figure 32), CSF total protein ($R=0.5$; $p<0.0001$), CSF IgG Index ($R=0.3$; $p=0.008$), (figures not shown) and with the presence of CSF IgGOB ($p<0.0001$) and IgMOB ($p=0.009$).

3.5 Survival analysis

Of all the collected clinical, neuroradiological and immunological variables, only an elevated CSF cell count (HR: 1.04; 95%CI:1.01-1.07; $p=0.006$), a positive baseline MRI (defined by the presence of at least one lesion on T2-weighted images) (HR:5; 95%CI: 1.2-21.3; $p=0.029$), a positive baseline spinal cord MRI (HR:1.7; 95%CI: 1.1-2.7; $p=0.017$) and CSF CXCL13 levels ≥ 15.4 pg/ml increased the risk of a MS diagnosis (HR: 3; 95%CI: 1.8-4.9; $p<0.001$). Cox analysis using raw CXCL13 values also yielded significant results

(HR:1; 95%CI: 1-1.007; p=0.012), although significance was lost at multivariate analysis.

When combined in a multivariate model, only a positive baseline brain MRI (p=0.01) and high-CSF CXCL13 levels $> 15.4\text{pg/ml}$ (p<0.001) maintained a statistical significance.

The risk of a CDMS diagnosis throughout the follow-up period at survival analysis was only increased by the presence of CSF-restricted IgGOB (HR:7.3; 95%CI: 1-53.1; p=0.049) and by CXCL13 levels $\geq 15.4\text{pg/ml}$ (HR:2.9; 95%CI: 1.2-3.9; p=0.007). In a multivariate Cox model, only elevation of CXCL13 values maintained a statistical significance (p=0.045 for levels greater than 15.4pg/ml).

Figure 43 shows Kaplan Meier relapse-free survival in patients with CSF CXCL13 levels greater or lower than 15.4pg/ml. The log-rank test revealed a p-value of 0.005. Similar curves (data not shown) and an even lower p-value (<0.001) was obtained for diagnosis-free survival in patients with CXCL13 levels above or below the cut-off of 15.4pg/ml.

3.6 Logistic regression: risk factors for an early MS/CDMS diagnosis

Logistic regression analysis showed that, amongst other clinical, CSF and MRI variables CSF CXCL13 levels of at least 15.4pg/ml increased the risk of an early diagnosis (OR: 3.6; 95%CI:1.5-8.5; p=0.005) (Table 23). Similar results were obtained by analyzing the risk of an early relapse (OR: 2.7; 95%CI: 1,2-6.4; p=0.017) for CXCL13 levels $\geq 15.4\text{pg/ml}$.

Discussion

The main findings of this study are that elevated CSF CXCL13 levels in CIS patients are associated with other CNS inflammatory markers (cell count, total protein, Link's Index, CSF-restricted IgGOB) and negative prognostic markers (CSF-restricted IgMOB) and that they increase the risk of a MS/CDMS diagnosis and of an early MS/CDMS diagnosis, i.e., within one year from onset.

Our results confirm previously published data by Khademi et al (Khademi et al., 2011), reporting that CSF CXCL13 levels were higher in 31 CIS patients who converted to CDMS within two years from their first episode, compared to 48 CIS patients who did not. Moreover, within the group of patients who converted to CDMS, higher CXCL13 levels exhibited a trend to an earlier conversion. Similarly, Brettschneider et al (Brettschneider et al., 2010) determined CSF CXCL13 values in a prospective 2-year study, including 91 CIS patients and 30 controls. These authors found that CSF CXCL13 was significantly higher in patients who converted to CDMS (45/91, 49.5%) compared to those who remained CIS and controls ($p < 0.001$). They also found CXCL13 levels greater than 7.7pg/ml (optimal cut-off value obtained by applying the Youden Index) to have a sensitivity of 62% and a specificity of 76% for a MS diagnosis, and to have the best PPV (70%) of all single parameters investigated, being superior to Barkhof's criteria, IgGOB and MRZR (positive polyspecific intrathecal B cell response against measles, rubella and varicella zoster virus). In the present study, we found a similar optimal cut-off value (8.7pg/ml), able to differentiate those patients who remained CIS from those who converted to MS within one year (see Figure [21](#) and [Table 2](#)). However, increasing the cut-off value to 15.4pg/ml notably increased its specificity in identifying patients at risk of an early conversion to MS/CDMS. Compared to other known diagnostic/prognostic factors, such as the presence of CSF-restricted IgGOB and to MRI parameters, elevated CSF CXCL13 levels show a lower sensitivity and a lower NPV but a much higher specificity (IgGOB: 31%, MRI: 36%, CXCL: 75%) and the highest PPV (IgGOB: 75%, MRI: 67%, CXCL: 81%) for a MS diagnosis within one year from onset.

Results obtained this far, therefore, imply that elevated CSF CXCL13 should highly encourage an early preventive treatment while a negative result is less informative.

Confirmatory studies are necessary to determine the optimal CSF CXCL13 cut-off value.

Elevated CSF CXCL13 levels do not only increase the risk of a MS diagnosis and of a clinical relapse at survival analysis, but they also predict an early MS diagnosis (i.e. within one year) and an early clinical relapse at logistic regression analysis, alongside other clinical, CSF and MRI parameters (see table 23 for variables associated with an early MS diagnosis). In particular, the presence of CSF IgGOB and of at least one lesion on brain MRI, factors which are known to increase the risk of developing MS in CIS patients (Optic and Treatment, 2008; Tintoré et al., 2008, 2006), presented the highest OR for a MS diagnosis within one year (OR:32 and 21, respectively), followed by [positivity of spinal cord MRI \(OR: 6.8\) and](#) CSF CXCL13 levels of at least 15.4pg/ml (OR: 3.6).

Furthermore, in multivariate survival analysis models on the risk of a MS diagnosis, only MRI data and [elevated CSF CXCL13 values > 15.4pg/ml](#) maintained a statistical significance while CSFCXCL13 levels alone maintained a statistical significance on the risk of a relapse throughout the follow-up period.

We found an association between CSF CXCL13 levels and the presence of CSF IgMOB. 23/38 (61%) IgMOB-positive patients showed CSF CXCL13 levels ≥ 15.4 pg/ml (chi-square test: $p=0.014$), and IgMOB-positive patients have significantly higher mean CSF CXCL13 levels ($p=0.009$). There is increasing evidence on the association between intrathecal IgM/lipid-specific IgM synthesis and a more aggressive disease course (Mandrioli et al., 2008; Thangarajh et al., 2008; Villar et al., 2005, 2003, 2002). This may be due to its multimeric structure being an efficient complement activator. Complement activation can, in turn, cause extensive demyelination and axonal loss (Mead et al., 2002). Villar et al (Villar et al., 2010) investigated the immunological mechanisms associated with intrathecal

IgM production: they found a close correlation between IgM index and CSF CD5+ B cells, which are postulated to be responsible for intrathecal IgM synthesis. Increased CD5+ B cells also correlated with CSF CXCL13, which probably induced the CD5+ B cell migration into the central nervous system (CNS). Elevated CD5+ expression on B lymphocytes was ~~moreover shown to~~ correlated with MS disease activity, number of gadolinium-enhancing lesions on MRI and ~~to be was~~ inversely correlated with disease duration (Seidi et al., 2002). Our data is in agreement with that of Villar et al (Villar et al., 2010), who found higher CSF CXCL13 levels in patients with intrathecal IgM production, especially during relapses, than in patients without intrathecal IgM synthesis.

In accordance with previous studies (Festa et al., 2009; Khademi et al., 2011; Krumbholz et al., 2006; Kuenz et al., 2008; Ragheb et al., 2011; Sellebjerg et al., 2009; Senel et al., 2014), we found a positive correlation of CXCL13 levels with other markers of CNS inflammation, including CSF cell count, total protein, IgG index and CSF IgG. An elevated CSF cell count ~~has been shown~~ was found to be associated with short-term conversion to MS in CIS (Ferraro et al., 2013; Gout et al., 2011). We could not find a correlation with EDSS, as previously reported by Alvarez et al (Alvarez et al., 2013) and correlation with number of MRI lesions and number of gadolinium-enhancing lesions did not reach statistical significance ($p=0.07$ and $p=0.08$, respectively).

Elevated CSF CXCL13 levels are not specific for MS, since they have been reported in other different inflammatory conditions of the CNS, including neuroborreliosis, bacterial and viral infections, CNS lymphoma, and neuromyelitis optica (Alvarez et al., 2013). In the CNS, CXCL13 is expressed by cells with macrophage morphological features in the perivascular, inflammatory lesions, and in scattered parenchymal cells (Krumbholz et al., 2006). It has been ~~found to be~~ implicated in the pathogenesis of Experimental

Autoimmune Encephalitis (EAE), as CXCL13-deficient mice exhibited a mild, self-limited form of disease (Bagaeva et al., 2006), and intracerebral expression of CXCL13 is associated with the development of lymphoid follicle-aggregates in the meninges of mice with relapsing EAE (Magliozzi et al., 2004). In MS CXCL13 is produced in actively demyelinating MS lesions but not in chronic inactive lesion or in the CSF of controls (Krumbholz et al., 2006). CSF CXCL13 levels decrease following treatment with high-dose methylprednisolone, natalizumab (Sellebjerg et al., 2009), and rituximab (Piccio et al., 2010).

Taken together, data from animal models and from MS populations, showing a relationship between CSF CXCL13 levels and disease activity, relapse rate and disability, in addition to their reduction in response to treatment with natalizumab or rituximab, suggest an important role for CXCL13 in MS pathogenesis. CXCL13 is important for attracting and maintaining B and T cells in inflamed CNS lesions and inflammatory CNS can provide a B-cell-friendly environment which can contribute to the long-term survival of B cells and plasmablasts (Corcione et al., 2004).

CIS patients with elevated CSF CXCL13 levels at onset have a high probability of presenting an early diagnosis/clinical relapse. Higher CXCL13 levels have been found to correlate with relapse rate in 52 Relapsing-Remitting MS (RRMS) patients who were followed-up for 5 years (Khademi et al., 2011). Since a higher relapse rate in the first two years (Leray et al., 2010) and a shorter first inter-attack interval seem to predict future disability (Scalfari et al., 2010), patients with elevated CSF CXCL13 levels may be at higher risk of long-term disability.

Conclusion

CSF CXCL13 levels correlate with markers of CNS inflammation including CSF cells, total protein, IgGOB, and with the presence of IgMOB, which have been shown to be associated with an earlier conversion to CDMS and with a more aggressive disease course.

Furthermore, we found CXCL13 values ≥ 15.4 pg/ml to have a high PPV and specificity (greater than the PPV and specificity of CSF IgGOB and of a positive baseline MRI scan) for a MS/CDMS diagnosis and for an early MS/CDMS diagnosis. (i.e. within one year from onset). CSF CXCL13 levels could be considered as an additional tool for treatment decisions by identifying patients at high risk of an early relapse, who could be encouraged to start an early preventive treatment.

Figure Titles and legends:

Figure 1 Title: CSF CXCL concentrations in patients who remained CIS (CIS) versus those who converted to MS (MS) throughout follow-up

Horizontal bar indicates median value: 0.1pg/ml (range: 0-14; 25%-75% percentile: 0-0.4) (CIS) versus 15.6pg/ml (range: 0-357.7; 25%-75% percentile: 4-38.8) (MSCIS), p=0.003

Figure 24 Title: Receiver operating characteristic (ROC) curve for CSF CXCL13 levels in relation to a MS/CDMS diagnosis within one year from onset.

Legend: ROC analysis of CSF CXCL13 levels in relation to a MS (Figure 24a) and a CDMS (Figure 24b) diagnosis within one year from onset. Two different cut-off values (8.7pg/ml and 15.4pg/ml) are shown. P<0.001 for a MS diagnosis within one year, and p=0.017 for a CDMS diagnosis within one year (null hypothesis; AUC = 0.5).

Figure 32 Title: Correlations between CSF CXCL13 levels and CSF cell count

Legend: R= 0,6; p<0,0001

Figure 34 Title: Time to clinical relapse

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Legend: Kaplan Meier relapse-free survival in patients with CSF CXCL13 levels greater or lower than 15.4pg/ml (Log-rank test: $p=0.005$).

Conflict of Interest statement

The Authors declares that there is no conflict of interest.

References

- Alvarez, E., Piccio, L., Mikesell, R.J., Klawiter, E.C., Parks, B.J., Naismith, R.T., Cross, A.H., 2013. CXCL13 is a biomarker of inflammation in multiple sclerosis, neuromyelitis optica, and other neurological conditions. *Mult. Scler.* 19, 1204-8. doi:10.1177/1352458512473362
- Bagaeva, L. V, Rao, P., Powers, J.M., Segal, B.M., 2006. CXC chemokine ligand 13 plays a role in experimental autoimmune encephalomyelitis. *J. Immunol.* 176, 7676–85.
- Boscá, I., Magraner, M.J., Coret, F., Alvarez-Cermeño, J.C., Simó-Castelló, M., Villar, L.M., Casanova, B., 2010. The risk of relapse after a clinically isolated syndrome is related to the pattern of oligoclonal bands. *J. Neuroimmunol.* 226, 143–6. doi:10.1016/j.jneuroim.2010.05.032
- Brettschneider, J., Czerwoniak, A., Senel, M., Fang, L., Kassubek, J., Pinkhardt, E., Lauda, F., Kapfer, T., Jesse, S., Lehmensiek, V., Ludolph, A.C., Otto, M., Tumani, H., 2010. The chemokine CXCL13 is a prognostic marker in clinically isolated syndrome (CIS). *PLoS One* 5, e11986. doi:10.1371/journal.pone.0011986
- Corcione, A., Casazza, S., Ferretti, E., Giunti, D., Zappia, E., Pistorio, A., Gambini, C., Mancardi, G.L., Uccelli, A., Pistoia, V., 2004. Recapitulation of B cell differentiation in the central nervous system of patients with multiple sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 101, 11064–9. doi:10.1073/pnas.0402455101
- Ferraro, D., Simone, A.M., Bedin, R., Galli, V., Vitetta, F., Federzoni, L., D'Amico, R., Merelli, E., Nichelli, P.F., Sola, P., 2013. Cerebrospinal fluid oligoclonal IgM bands predict early conversion to clinically definite multiple sclerosis in patients with Clinically Isolated Syndrome. *J. Neuroimmunol.* 257, 76–81. doi:10.1016/j.jneuroim.2013.01.011
- Festa, E.D., Hankiewicz, K., Kim, S., Skurnick, J., Wolansky, L.J., Cook, S.D., Cadavid, D., 2009. Serum levels of CXCL13 are elevated in active multiple sclerosis. *Mult. Scler.* 15, 1271–9. doi:10.1177/1352458509107017
- Gout, O., Bouchareine, A., Moulignier, A., Deschamps, R., Papeix, C., Gorochov, G., Héran, F., Bastuji-Garin, S., 2011. Prognostic value of cerebrospinal fluid analysis at

the time of a first demyelinating event. *Mult. Scler.* 17, 164–72.
doi:10.1177/1352458510385506

- Khademi, M., Kockum, I., Andersson, M.L., Iacobaeus, E., Brundin, L., Sellebjerg, F., Hillert, J., Piehl, F., Olsson, T., 2011. Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. *Mult. Scler.* 17, 335–43. doi:10.1177/1352458510389102
- Kim, C.H., Rott, L.S., Clark-Lewis, I., Campbell, D.J., Wu, L., Butcher, E.C., 2001. Subspecialization of CXCR5+ T cells: B helper activity is focused in a germinal center-localized subset of CXCR5+ T cells. *J. Exp. Med.* 193, 1373–81.
- Krumbholz, M., Theil, D., Cepok, S., Hemmer, B., Kivisäkk, P., Ransohoff, R.M., Hofbauer, M., Farina, C., Derfuss, T., Hartle, C., Newcombe, J., Hohlfeld, R., Meinl, E., 2006. Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment. *Brain* 129, 200–11. doi:10.1093/brain/awh680
- Kuenz, B., Lutterotti, A., Ehling, R., Gneiss, C., Haemmerle, M., Rainer, C., Deisenhammer, F., Schocke, M., Berger, T., Reindl, M., 2008. Cerebrospinal fluid B cells correlate with early brain inflammation in multiple sclerosis. *PLoS One* 3, e2559.
- Langenkamp, A., Nagata, K., Murphy, K., Wu, L., Lanzavecchia, A., Sallusto, F., 2003. Kinetics and expression patterns of chemokine receptors in human CD4+ T lymphocytes primed by myeloid or plasmacytoid dendritic cells. *Eur. J. Immunol.* 33, 474–82. doi:10.1002/immu.200310023
- Leray, E., Yaouanq, J., Le Page, E., Coustans, M., Laplaud, D., Oger, J., Edan, G., 2010. Evidence for a two-stage disability progression in multiple sclerosis. *Brain* 133, 1900–13. doi:10.1093/brain/awq076
- Magliozzi, R., Columba-Cabezas, S., Serafini, B., Aloisi, F., 2004. Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 148, 11–23. doi:10.1016/j.jneuroim.2003.10.056
- Mandrioli, J., Sola, P., Bedin, R., Gambini, M., Merelli, E., 2008. A multifactorial prognostic index in multiple sclerosis. Cerebrospinal fluid IgM oligoclonal bands and clinical features to predict the evolution of the disease. *J. Neurol.* 255, 1023–31. doi:10.1007/s00415-008-0827-5
- Mead, R.J., Singhrao, S.K., Neal, J.W., Lassmann, H., Morgan, B.P., 2002. The membrane attack complex of complement causes severe demyelination associated with acute axonal injury. *J. Immunol.* 168, 458–65.
- Meinl, E., Krumbholz, M., Hohlfeld, R., 2006. B lineage cells in the inflammatory central nervous system environment: migration, maintenance, local antibody production, and therapeutic modulation. *Ann. Neurol.* 59, 880–92. doi:10.1002/ana.20890

- Optic, F., Treatment, N., 2008. Multiple Sclerosis Risk After Optic Neuritis. *Brain* 65, 727–732.
- Piccio, L., Naismith, R.T., Trinkaus, K., Klein, R.S., Parks, B.J., Lyons, J.A., Cross, A.H., 2010. Changes in B- and T-lymphocyte and chemokine levels with rituximab treatment in multiple sclerosis. *Arch. Neurol.* 67, 707–14. doi:10.1001/archneurol.2010.99
- Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J. a, Filippi, M., Fujihara, K., Havrdova, E., Hutchinson, M., Kappos, L., Lublin, F.D., Montalban, X., O'Connor, P., Sandberg-Wollheim, M., Thompson, A.J., Waubant, E., Weinschenker, B., Wolinsky, J.S., 2011. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann. Neurol.* 69, 292–302. doi:10.1002/ana.22366
- Ragheb, S., Li, Y., Simon, K., VanHaerents, S., Galimberti, D., De Riz, M., Fenoglio, C., Scarpini, E., Lisak, R., 2011. Multiple sclerosis: BAFF and CXCL13 in cerebrospinal fluid. *Mult. Scler.* 17, 819–29. doi:10.1177/1352458511398887
- Scalfari, A., Neuhaus, A., Degenhardt, A., Rice, G.P., Muraro, P. a, Daumer, M., Ebers, G.C., 2010. The natural history of multiple sclerosis: a geographically based study 10: relapses and long-term disability. *Brain* 133, 1914–29. doi:10.1093/brain/awq118
- Seidi, O. a, Semra, Y.K., Sharief, M.K., 2002. Expression of CD5 on B lymphocytes correlates with disease activity in patients with multiple sclerosis. *J. Neuroimmunol.* 133, 205–10.
- Sellebjerg, F., Börnsen, L., Khademi, M., Krakauer, M., Olsson, T., Frederiksen, J.L., Sørensen, P.S., 2009. Increased cerebrospinal fluid concentrations of the chemokine CXCL13 in active MS. *Neurology* 73, 2003–10. doi:10.1212/WNL.0b013e3181c5b457
- Senel, M., Tumani, H., Lauda, F., Presslauer, S., Mojib-Yezdani, R., Otto, M., Brettschneider, J., 2014. Cerebrospinal fluid immunoglobulin kappa light chain in clinically isolated syndrome and multiple sclerosis. *PLoS One* 9, e88680. doi:10.1371/journal.pone.0088680
- Sola, P., Mandrioli, J., Simone, A.M., Ferraro, D., Bedin, R., Annecca, R., Venneri, M.G., Nichelli, P.F., Merelli, E., 2011. Primary progressive versus relapsing-onset multiple sclerosis: presence and prognostic value of cerebrospinal fluid oligoclonal IgM. *Mult. Scler.* 17, 303–11. doi:10.1177/1352458510386996
- Thangarajh, M., Gomez-Rial, J., Hedström, a K., Hillert, J., Alvarez-Cermeño, J.C., Masterman, T., Villar, L.M., 2008. Lipid-specific immunoglobulin M in CSF predicts adverse long-term outcome in multiple sclerosis. *Mult. Scler.* 14, 1208–13. doi:10.1177/1352458508095729
- Tintoré, M., Rovira, a, Río, J., Nos, C., Grivé, E., Téllez, N., Pelayo, R., Comabella, M., Sastre-Garriga, J., Montalban, X., 2006. Baseline MRI predicts future attacks and disability in clinically isolated syndromes. *Neurology* 67, 968–72. doi:10.1212/01.wnl.0000237354.10144.ec

- Tintoré, M., Rovira, A., Río, J., Tur, C., Pelayo, R., Nos, C., Téllez, N., Perkal, H., Comabella, M., Sastre-Garriga, J., Montalban, X., 2008. Do oligoclonal bands add information to MRI in first attacks of multiple sclerosis? *Neurology* 70, 1079–83. doi:10.1212/01.wnl.0000280576.73609.c6
- Villar, L.M., Espiño, M., Cavanillas, M.L., Roldán, E., Urcelay, E., de la Concha, E.G., Sádaba, M.C., Arroyo, R., González-Porqué, P., Alvarez-Cermeño, J.C., 2010. Immunological mechanisms that associate with oligoclonal IgM band synthesis in multiple sclerosis. *Clin. Immunol.* 137, 51–9. doi:10.1016/j.clim.2010.06.007
- Villar, L.M., González-Porqué, P., Masjuán, J., Alvarez-Cermeño, J.C., Bootello, a, Keir, G., 2001. A sensitive and reproducible method for the detection of oligoclonal IgM bands. *J. Immunol. Methods* 258, 151–5.
- Villar, L.M., Masjuan, J., González-Porqué, P., Plaza, J., Sádaba, M.C., Roldán, E., Bootello, A., Alvarez-Cermeño, J.C., 2002. Intrathecal IgM synthesis predicts the onset of new relapses and a worse disease course in MS. *Neurology* 59, 555–9.
- Villar, L.M., Masjuan, J., González-Porqué, P., Plaza, J., Sádaba, M.C., Roldán, E., Bootello, A., Alvarez-Cermeño, J.C., 2003. Intrathecal IgM synthesis is a prognostic factor in multiple sclerosis. *Ann. Neurol.* 53, 222–6. doi:10.1002/ana.10441
- Villar, L.M., Sádaba, M.C., Roldán, E., Masjuan, J., González-porqué, P., Villarrubia, N., Espiño, M., García-trujillo, J.A., Bootello, A., 2005. Intrathecal synthesis of oligoclonal IgM against myelin lipids predicts an aggressive disease course in MS. *J. Clin. Invest.* 115, 187–194. doi:10.1172/JCI200522833.The

Figure

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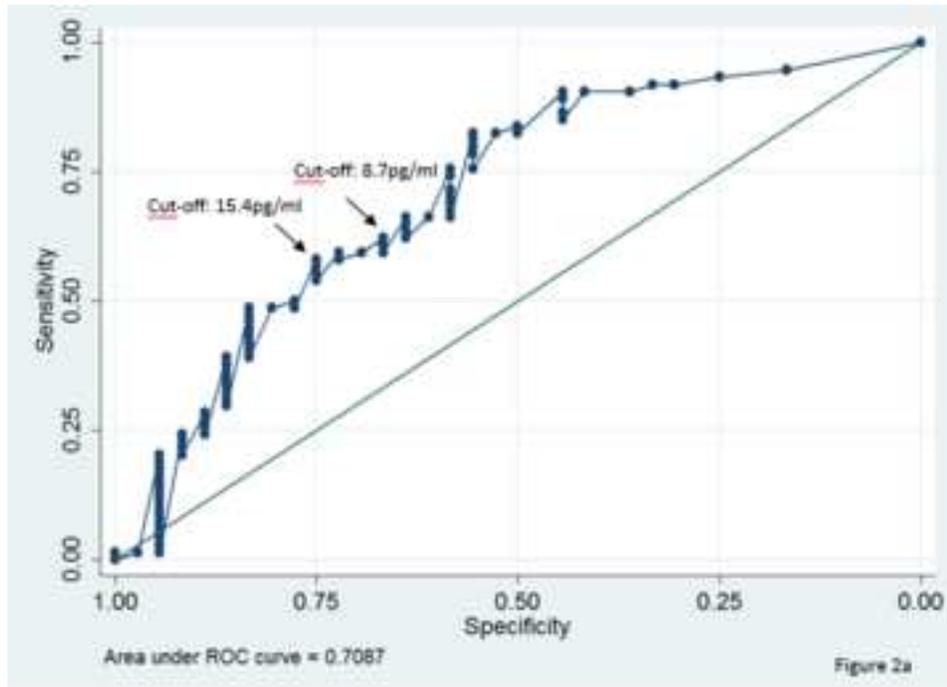


Figure 2a

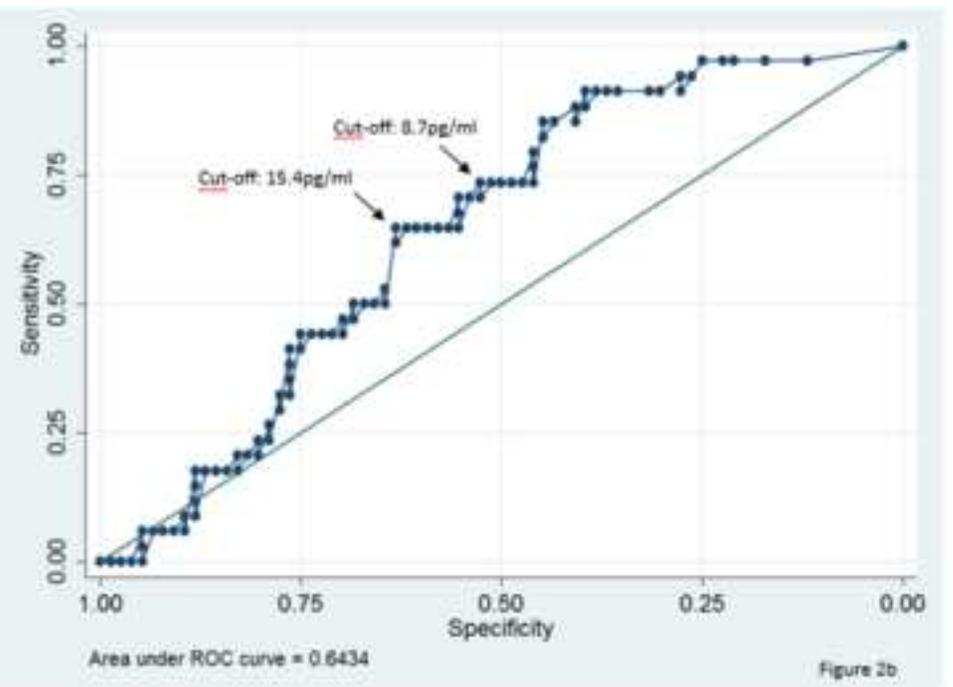


Figure 2b

Figure

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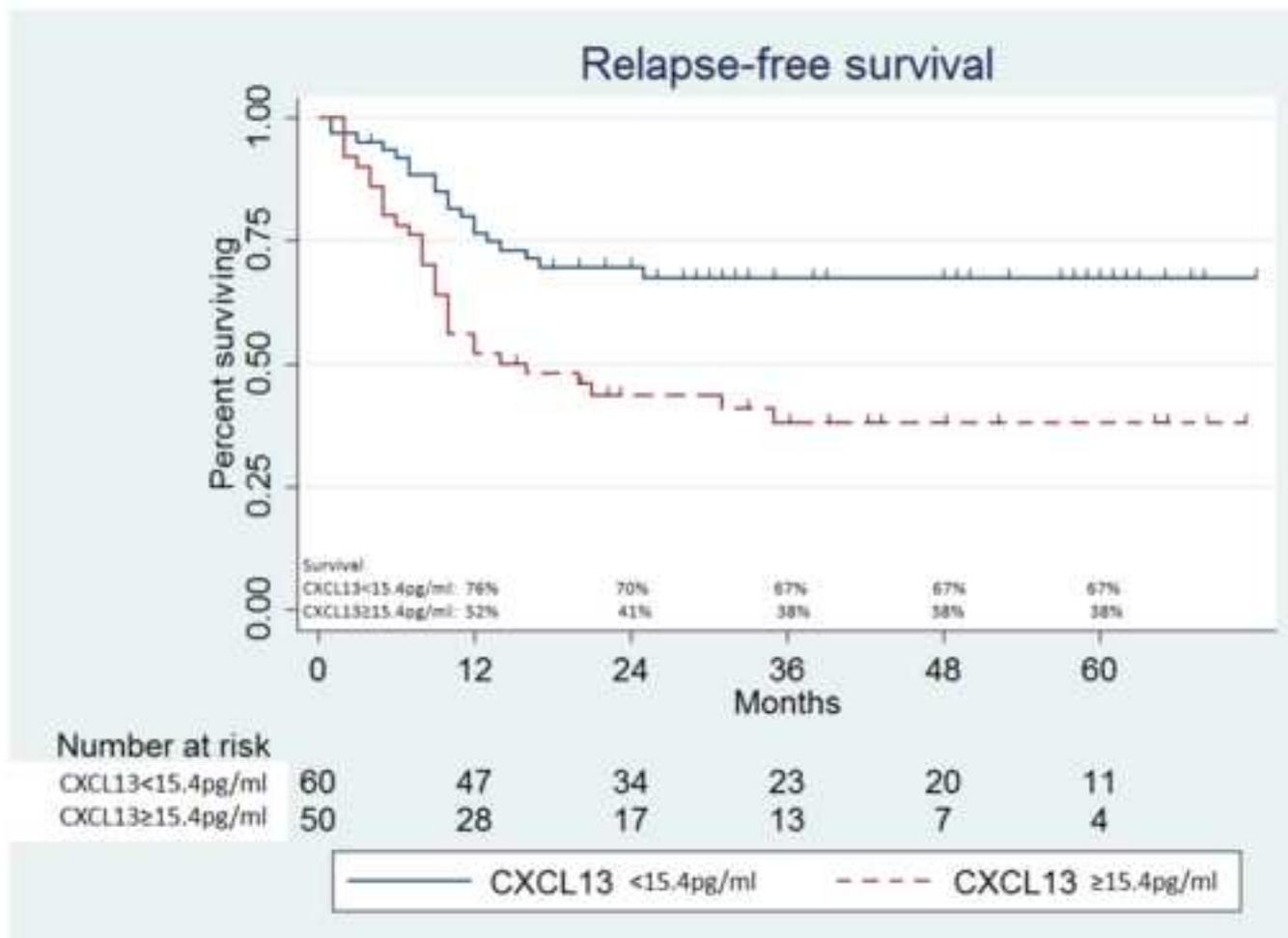


Table 1 Patient characteristics at baseline (nr=110)

Variable	Nr/total
Sex	
M, n(%)	40/ <u>110</u> (36)
F, n(%)	70/ <u>110</u> (64)
Age at onset , years*	35 ± 10
<i>Symptoms at onset</i>	
ON**, n(%)	26/ <u>110</u> (24)
Sensory, n(%)	25/ <u>110</u> (23)
Motor/sensory-motor, n(%)	24/ <u>110</u> (22)
Brainstem/cerebellum, n(%)	29/ <u>110</u> (27)
Other, n(%)	6/ <u>110</u> (5)
<i>Spinal onset</i>	
Yes, n(%)	39/ <u>110</u> (35)
No, n(%)	71/ <u>110</u> (65)
EDSS at onset*	2 ± 0.7
Complete recovery, n(%)	83/ <u>110</u> (75)
<i>Baseline brain MRI</i>	
Positive (at least one lesion), n(%)	101/ <u>110</u> (92)
Negative, n(%)	9/ <u>110</u> (8)
<i>Baseline brain MRI</i>	
0 lesions, n(%)	9/ <u>110</u> (8)
1-2 lesions, n(%)	10/ <u>110</u> (9)
3-8 lesions, n(%)	33/ <u>110</u> (30)
>9 lesions, n(%)	58/ <u>110</u> (53)
Baseline brain MRI scan: presence of CE§, n(%)	42/74 (57)

<i>Baseline spinal cord MRI</i>	
Positive (at least one lesion), n(%)	61/74(82)
Negative, n(%)	13/74(18)
CSF proteins (g/dl) *(range)	41 ± 16 (18-89)
Link's IgG index* (range)	1.1 ± 0.6 (0.3-4.5)
Patients with CSF IgGOB, n (%)	98/ <u>110</u> (89 90)
Patients with CSF IgMOB, n (%)	38/ <u>110</u> (35)
Mean CSF cell count (normal value ≤ 4) *	6.1 ± 7.1 (0-39)
Patients with increased cell count, n (%)	48/ <u>110</u> (44)
Diagnosis at onset (MRI with DIS and DIT§§), n(%)	36 (33)
Diagnosis after follow-up MRI (DIS and DIT§§), n(%)	37 (34)
Diagnosis of MS within 1 year, n(%)	74/ <u>110</u> (67)
Diagnosis of CDMS within 1 year, n(%)	34/ <u>110</u> (31)
Diagnosis of MS by end of follow-up period, n(%)	94/ <u>110</u> (86)
Diagnosis of CDMS by end of follow-up period, n(%)	49/ <u>110</u> (45)

* Values expressed as mean ± SD; **ON=optic neuritis; §CE= contrast enhancement;

§§ DIS and DIT=dissemination in space and dissemination in time, in accordance with McDonald's 2010 criteria.

Table 23

Descriptive Assessment of the association between patient baseline clinical, MRI and CSF characteristics and risk of MS diagnosis at one year (univariate logistic regression results)-

and diagnosis of Multiple Sclerosis at one year

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Variable	<u>CIS</u> <u>(n=36)</u>	<u>MS</u> <u>(n=74)</u>	OR	95%CI	P
Sex (Male/female)	<u>16/20</u>	<u>24/50</u>	0.6	0.6-1.4	0.221
<u>Age (years) ± SD</u>	<u>39±11</u>	<u>33±9</u>	<u>0.94</u>	<u>0.89-0.98</u>	<u>0.007</u>
Age (>30/<=30)	29/7	41/33	0.3	0.1-0.77	0.012
CSF IgGOB (Present/Absent)	<u>25/11</u>	<u>73/1</u>	32.1	3.9-261.5	0.001
CSF IgMOB (Present/Absent)	<u>15/21</u>	<u>23/51</u>	<u>0.6</u>	<u>0.2-1.4</u>	<u>0.275</u>
<u>CSF cell count (µl) ± SD</u>	<u>4±7</u>	<u>7±7</u>	<u>1.1</u>	<u>1-1.17</u>	<u>0.06</u>
CSF cell count (Elevated/Normal)	<u>10/26</u>	<u>38/36</u>	2.3	1.2-6.4	0.021
<u>CSF protein count (mg/dl) ± SD</u>	<u>40±14</u>	<u>41±17</u>	<u>1</u>	<u>1-10.3</u>	<u>0.598</u>
CSF protein count (Elevated/Normal)	<u>6/30</u>	<u>19/55</u>	1.7	0.6-4.8	0.294
<u>CSF CXCL13 levels (>8.7pg/ml/≤8.7pg/ml)</u>			<u>3.5</u>	<u>1.5-8</u>	<u>0.003</u>
<u>CSF CXCL13 levels (pg/ml)± SD</u>	22	35	1	1-1.02	0.253
CSF CXCL13 levels (>15.4pg/ml/≤15.4pg/ml)	<u>9/27</u>	<u>40/34</u>	3.6	1.5-8.5	0.005
Recovery (No/Yes)	<u>7/29</u>	<u>20/54</u>	1.5	0.6-4.1	0.388
Spinal onset (Yes/No)	<u>13/23</u>	<u>26/48</u>	1	0.4-2.2	0.920
Brain MRI (Pos*/Neg)	<u>28/8</u>	<u>73/1</u>	<u>20.9</u>	<u>2.5-174.4</u>	0.005

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Presence of brain CE§ (Yes/No)	<u>15/19</u>	<u>47/25</u>	2.3	1-5.5	0.041
Spinal cord MRI (Pos*/Neg) (<u>74 patients</u>)	<u>16/9</u>	<u>45/4</u>	6.8	1.8-25.1	0.004
Infratentorial lesions (Yes/No)	<u>13/23</u>	<u>40/34</u>	2.1	0.9-4.7	0.08

CIS = patients who remained CIS at one year

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MS = CIS patients who converted to MS within one year

Statistically significant results in bold.

* Positive= at least one lesion

§CE= contrast enhancement