Prognostic Relevance of CXCL13 and IL-8 Biomarkers in Predicting the Transition from Clinically Isolated Syndrome to Multiple Sclerosis

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October 5, 2023

Abstract

Aims: The initial phase of multiple sclerosis (MS), often known as clinically isolated syndrome (CIS), is a critical period for identifying individuals at high risk of progressing to full-blown MS and initiating timely treatment. In this study, we aimed to evaluate the prognostic value of CXCL13 and IL-8 as potential markers for CIS patients' conversion to MS. Methods: Our study encompassed patients with CIS, those with relapsing-remitting MS (RRMS), and control subjects, with sample analysis conducted on both cerebrospinal fluid (CSF) and serum. Patients were categorized into four groups: CIS-CIS (no MS development within two years), CIS-RRMS (conversion to RRMS within two years), RRMS (already diagnosed), and a control group (CG) with non-inflammatory CNS disorders. Results: Results showed significantly elevated levels of CXCL13 in CSF across all patient groups compared to the CG ($p < 0.0001$, Kruskal-Wallis test). Although CXCL13 concentrations were slightly higher in the CIS-RRMS group, statistical significance was not reached. Similarly, significantly higher levels of IL-8 were detected in CSF samples from all patient groups compared to the CG ($p < 0.0001$, Kruskal-Wallis test), with comparable levels among patient groups. ROC analysis in the CIS-RRMS group identified both CXCL13 (AUC = 0.959) and IL-8 (AUC = 0.939) in CSF as significant predictors of CIS to RRMS conversion. Conclusion: In conclusion, our study suggests a trend toward elevated CXCL13 levels in CIS patients progressing to RRMS. These findings emphasize the importance of identifying prognostic markers to guide appropriate treatment strategies for individuals in the early stages of MS.

INTRODUCTION

The initial phase of multiple sclerosis (MS) typically manifests as clinically isolated syndrome (CIS), which constitutes the first clinical episode bearing resemblance to MS. Predominantly observed in young adults, CIS impacts the optic nerve, brainstem, or spinal cord. Specifically, CIS is characterized by an acute or subacute occurrence of neurological symptoms, persisting for a duration exceeding 24 hours [1]. The subsequent progression of the disease post-CIS exhibits significant variability. While around a third of patients exhibit a benign trajectory marked by minimal disability, another half develops secondary progressive MS [2]. Within the CIS stage, the identification of patients at heightened risk of MS development stands as a pivotal objective. The potential progression of the disease can significantly influence the formulation of treatment strategies. Consequently, numerous prognostic markers, such as chitinase 3 like 1, neurofilament heavy chains, and interleukins, have been subject to investigation for their predictive value [3].

Cerebrospinal fluid (CSF) analysis, in conjunction with the assessment of the patient's clinical state and MRI results, holds a crucial role in multiple sclerosis diagnosis. A defining feature of CSF-related alterations is the presence of limited oligoclonal bands (OCBs), observable in the vast majority of MS patients [4]. The role of B cells in connection with MS and its progression has been substantiated through the scrutiny of B cell-associated biomarkers in CSF [5, 6]. Among these markers, one such example is CXCL13, a chemotactic molecule that attracts B cells, which are deemed pivotal in MS pathogenesis. CXCL13 is generated within ectopic lymphoid follicles, linked to inferior MS outcomes. Elevated CXCL13 levels might aid in predicting the conversion of patients with clinically isolated syndrome (CIS) to full-fledged MS [7, 8].

An additional noteworthy marker tied to MS activity is IL-8, produced by various cell types such as monocytes, lymphocytes, granulocytes, astrocytes, and epithelial cells. IL-8 is an inflammatory chemokine primarily responsible for attracting and activating neutrophilic granulocytes, basophilic granulocytes, and specific lymphocyte subsets. Notably, IL-8 also exerts robust angiogenic effects [9]. Research has demonstrated significantly raised levels of CSF IL-8 in MS patients [10].

Our study aimed to assess the significance of CXCL13 and IL-8 as prognostic indicators in patients diagnosed with CIS, in comparison with a control group. The evaluation occurred at the juncture when the initial clinical manifestations of the disease were registered.

MATERIAL AND METHODS

Ethical statement

The study was carried out in accordance with the Helsinki Declaration of 1964 (2013 revision). This study was approved by the ethics committee of Palacký University in Olomouc. All patients signed an informed consent form for a lumbar puncture for diagnostic reasons, no healthy volunteers were involved.

Patients and sampling

Cerebrospinal fluid (CSF) samples were obtained from patients who had been diagnosed with clinically isolated syndrome (CIS), patients with relapsing-remitting multiple sclerosis (RRMS), and individuals in a control group (CG). The control group comprised patients with non-inflammatory central nervous system (CNS) involvement, such as headaches, back pain, and vertigo, for whom lumbar puncture was indicated for differential diagnostic purposes, in order to exclude pathological processes in the CNS.

The patient cohort was subsequently categorized into four distinct groups. The first group, labeled CIS-CIS, consisted of individuals who did not progress to multiple sclerosis (MS) within two years following the collection of cerebrospinal fluid (CSF) samples. The second group, designated CIS-RRMS, encompassed patients who transitioned to relapsing-remitting multiple sclerosis (RRMS) within the same two-year period following CSF collection. The third group, denoted as RRMS, comprised patients for whom marker analysis was conducted at the point of confirmed disease diagnosis. The fourth and final group constituted the control group (CG). Assessment of disability was performed using the Kurtzke Expanded Disability Status Scale (EDSS) by experienced neurologists affiliated with the Department of Neurology at Olomouc University Hospital and Palacký University in Olomouc. Diagnosis was established in accordance with the revised McDonald diagnostic criteria.

Cerebrospinal fluid (CSF) was acquired through a lumbar puncture procedure using an atraumatic needle with patients positioned in a seated posture. The puncture was targeted at the L4/5 intervertebral space. A volume of 10 milliliters of CSF was collected from each patient into a sterile tube devoid of any additives. The sample underwent initial evaluation for cell count and qualitative cytology, followed by centrifugation (at 1100 g for 10 minutes at 4°C) for further biochemical and immunological analysis.

CXCL13 and IL-8 analysis

The concentration of CXCL13 in cerebrospinal fluid (CSF) was quantified utilizing a sandwich Enzyme-Linked Immuno Sorbent Assay (ELISA) technique, employing the Euroimmun CXCL13 ELISA CE kit (Euroimmun, Lübeck, Germany), with a catalog number EQ 6811-9602-L. The analysis was executed in

compliance with the manufacturer's guidelines, where values below 20 pg/ml were deemed within the normal range.

To measure IL-8 levels in CSF, an enzyme-labeled solid-phase chemiluminescent sequential immunometric assay was employed, specifically, the IMMULITE® 1000 IL-8 (Euroimmun, Lübeck, Germany) kit, catalog number LK8P1. The analysis was conducted following the manufacturer's protocol, with CSF concentrations of up to 62 pg/ml considered as falling within the normal range.

Statistical analysis

The Shapiro-Wilks normality test showed that the data do not have a normal distribution. Therefore, the data were expressed as median, minimum, and maximum values, and independent samples were compared using the Kruskal-Wallis test. The differences for all variables were statistically significant. Graphically, the distribution of data is shown in box graphs. The distribution of the measured values is shown in a box graph. The horizontal line in the box shows the median value, the lower edge of the box the value of the 1st quartile (25th percentile), the upper edge the value of the 3rd quartile (75th percentile). The terminals show the maximum and minimum measured values. Outliers (values that are more than 1.5 times the interquartile range from the quartiles) are plotted in circles. Extremes (values that are more than 3 times the interquartile range from the quartiles) are plotted with asterisks. All tests were performed at a significance level of 0.05. IBM SPSS Statistics for Windows, Version 23.0 statistical software was used for statistical processing. Armonk, NY: IBM Corp.

In this study, ROC analysis was employed to assess the diagnostic performance of the tested method. ROC curves were constructed to evaluate the trade-off between sensitivity and specificity across different threshold values. The optimal threshold point was determined to maximize the diagnostic accuracy. The area under the ROC curve (AUC) was calculated to quantify the overall discriminatory power of the method. ROC analysis was used to aid in the evaluation and selection of the most suitable diagnostic criteria for our study.

RESULTS

Patients and controls

The study encompassed 124 patients, who were categorized into four distinct groups, comprising 37 individuals in the CIS-CIS group (consisting of 7 males and 30 females, with a mean age of 34.0), 14 patients in the CIS-RRMS group (comprising 2 males and 12 females, with a mean age of 35.0), 18 patients in the RRMS group (comprising 4 males and 14 females, with a mean age of 38.0), and 55 individuals in the control group (CG) (comprising 9 males and 46 females, with a mean age of 33.0). These groups exhibited homogeneity with regard to both gender and age. Detailed data is presented in **Figures 1 and 2.**

All patients underwent examinations for CXCL13, IL-8, cerebrospinal fluid (CSF) cell count, qualitative CSF cytology, oligoclonal bands (OCB) of IgG, and the Expanded Disability Status Scale (EDSS). A summary of the data is presented in **Figures 3 and 4** . At the time of collection, patients exhibited no clinical or laboratory indications of neuroinfection, and there were no ongoing systemic inflammatory processes.

Group comparison

Following the statistical analysis, markedly elevated levels of IL-8 were observed in all examined patient groups when compared to the control group (CIS-CIS: median 51.40 pg/ml vs. 36.60 pg/ml; CIS-RRMS: median 55.85 pg/ml vs. 36.60 pg/ml; RRMS: 55.70 pg/ml vs. 36.60 pg/ml; $p < 0.0001$, Kruskal-Wallis test). No statistically significant distinctions were noted among the individual patient groups, as the levels of IL-8 in these groups were comparable, as illustrated in**Box Plot 1** . A summary of the data is presented in**Figure 3** .

Significantly elevated levels of the CXCL13 marker were also observed in all groups when compared to the control group (CIS-CIS: median 16.82 pg/ml vs. 0.10 pg/ml; CIS-RRMS: median 32.30 pg/ml vs. 0.10 pg/ml; RRMS: median 5.37 pg/ml vs. 0.10 pg/ml; $p < 0.0001$, Kruskal-Wallis test). Notably, within the CIS-RRMS group, the concentration of CXCL13 was higher in comparison to the other groups (CIS-CIS

median 16.82 pg/ml; CIS-RRMS median 32.30 pg/ml; RRMS median 5.37 pg/ml). However, these differences did not attain statistical significance, as indicated by **Box Plot 2** . A summary of the data is presented in**Figure 3** .

ROC analysis

In the CIS-RRMS group, ROC analysis identified both CXCL13 (AUC = 0.959) and IL-8 (AUC = 0.939) in cerebrospinal fluid (CSF) as significant predictors for the conversion from clinically isolated syndrome (CIS) to relapsing-remitting multiple sclerosis (RRMS).

The optimal cut-off value for IL-8 in CSF was determined to be 45.6 pg/ml, as calculated by the Youden's J statistic, which maximizes the sum of sensitivity (0.9) and specificity (0.836). For CXCL13 in CSF, the optimal cut-off value was 0.210 pg/ml, also determined by Youden's J statistic, with sensitivity of 0.95 and specificity of 0.873. This test showed higher sensitivity. Alternatively, if a higher cut-off value (1.795) were selected, both sensitivity and specificity would be more balanced, both approximately at 0.9, with SE = 0.900 and SP = 0.909. The data are summarized in the **Figure 4** and depicted in the

Graph 3.

DISCUSSION

The diagnosis of MS requires a comprehensive approach and is based on the evaluation of the patient's clinical condition and magnetic resonance imaging (MRI) findings. However, MRI is not an infallible diagnostic tool, especially at the CIS stage. CSF examinations plays an irreplaceable role in the diagnosis of MS. Many studies have recently been conducted that focus on the use of new prognostic markers such as neurofilament light chains (NfL) or chitinase-3 like-protein-1 (CHI3L1). They may be beneficial in the diagnosis and prognosis of MS [17]. In our research, we focused on the use of the markers CXCL13 and IL-8 in assessing CIS to MS conversion.

IL-8 is a chemoattractant, a cytokine produced by many tissue cells and leucocytes. It attracts and activates neutrophilic granulocytes and other immune cells in inflammatory areas [13]. In CSF, its levels are elevated during inflammatory processes of the CNS. IL-8 is responsible for opening the blood-brain barrier allowing immune cells to migrate to the CNS [9]. In our study, we identified heightened IL-8 levels in patients diagnosed with clinically isolated syndrome (CIS) and multiple sclerosis (MS), thereby affirming the presence of ongoing inflammatory processes. Previous investigations, including those by Matejčíková et al., have detected increased CSF levels of IL-8 coinciding with the initial clinical manifestations of MS, while concurrently observing decreased IL-8 levels in the serum [10, 20]. Studies by Bartošík-Psujek and Stelmasiak and those by Stelmasiak et al. have further substantiated significant rises in CSF IL-8 levels during relapse episodes [18, 19].

Within our cohort, IL-8 levels consistently displayed elevation across all groups. Specifically, for the CIS-RRMS group, a ROC analysis was conducted, and it revealed that IL-8 in cerebrospinal fluid (CSF) served as a noteworthy predictor for the transition from clinically isolated syndrome (CIS) to relapsing-remitting multiple sclerosis (RRMS), with an AUC of 0.939. The optimal threshold for IL-8 in CSF was identified as 45.6 pg/ml, as determined by Youden's J statistic, which maximizes the amalgamation of sensitivity (0.9) and specificity (0.836).

These observations imply that IL-8 may prove to be a suitable indicator for forecasting disease prognosis. However, owing to the akin IL-8 concentration levels in CSF also noticed in the other groups (CIS-CIS and RRMS), the practicality of IL-8 in predicting disease progression remains unverified. However in a study conducted by Rossi et al., heightened IL-8 levels were linked to an increased risk of transitioning from CIS to RRMS and a heightened frequency of relapses during the initial two years of the disease [21].

CXCL13 is a chemokine that exerts chemotactic effects on B cells, which play a crucial role in the pathogenesis of multiple sclerosis (MS). This chemokine is produced by macrophages and follicular dendritic cells, and monitoring changes in its concentration can provide insights into the condition of MS patients. CXCL13

interacts with the CXCR5 receptor, acting as a chemoattractant that guides B cells to secondary lymphatic organs. Furthermore, CXCL13 promotes cytokine secretion, facilitating humoral immune responses and the migration of plasma cells and B cells to the cerebrospinal fluid (CSF) [14, 9, 16]. Elevated CXCL13 levels are commonly observed in patients with neuroborreliosis [8]. Numerous studies have investigated the levels and prognostic significance of CXCL13 in patients with clinically isolated syndrome (CIS) and MS. For instance, a study by Khademi et al. in 2011 associated CXCL13 with disease exacerbation and poor prognosis in relapsing-remitting MS (RRMS). High CXCL13 levels were predictive of the conversion from CIS to MS [21]. In a prospective study by Bretschneider et al. in 2010, CXCL13 was found to be relevant in CIS for predicting conversion to MS, underlining its role in the inflammatory cascade linked to the intrathecal B cell response [23]. Lepennetier et al. confirmed CXCL13 as a reliable marker and its utility in predicting disease activity in MS and diagnosing Lyme neuroborreliosis (LNB) and central nervous system (CNS) lymphoma [24]. Additionally, Ferraro et al. in 2015 established a correlation between CSF CXCL13 levels and markers of CNS inflammation, suggesting that CXCL13 levels were associated with earlier conversion to MS and a more aggressive disease course [25]. A study by DiSano et al. in 2020 emphasized the predictive value of the CXCL13 index in comparison to oligoclonal bands (OCBs) and cerebrospinal fluid (CSF) neurofilament light chain in patients with clinically and radiologically isolated syndrome and MS. The CXCL13 index demonstrated significant increases in MS patients with active disease, surpassing both OCB and CSF NfL in terms of sensitivity, specificity, and the identification of future disease activity [26].

Our study findings indicate elevated CXCL13 levels in patients with CIS and MS. Within our cohort, we observed a tendency toward higher CXCL13 levels in patients transitioning from CIS to RRMS. In the CIS-RRMS group, ROC analysis identified CXCL13 in cerebrospinal fluid as a highly significant predictor for the transition from clinically isolated syndrome to relapsing-remitting multiple sclerosis, with an impressive AUC (area under the curve) value of 0.959. The optimal cut-off value for CXCL13 in CSF was determined to be 0.210 pg/ml, based on Youden's J statistic, providing a sensitivity of 0.95 and specificity of 0.873. Notably, this test demonstrated notably high sensitivity. Alternatively, if a higher cut-off value of 1.795 were chosen, both sensitivity and specificity would be more evenly balanced, each approximately at 0.9, with SE (sensitivity) = 0.900 and SP (specificity) = 0.909.

These findings are in line with previous research underlining the significance of CXCL13 as a biomarker in patients with multiple sclerosis. CXCL13 levels in CSF hold the potential to serve as an additional tool for identifying patients at a heightened risk of transitioning to MS, thereby assisting clinicians in determining the need for early intervention. Nevertheless, further investigation involving a larger patient cohort is imperative to validate these observations.

CONCLUSION

Based on the analysis of the collected data, it appears that IL-8 may not be a suitable prognostic marker for the conversion from clinically isolated syndrome (CIS) to multiple sclerosis (MS). Conversely, the CXCL13 marker shows greater promise in this regard. We observed an inclination towards higher CXCL13 levels in patients who transitioned from CIS to relapsing-remitting MS (RRMS). These findings are consistent with previously published studies that have highlighted the prognostic significance of CXCL13 in patients with CIS.

Considering the evidence from our results and those of other studies, it is conceivable that CXCL13 holds potential as an indicator of inflammatory activity in individuals with MS, and its inclusion in routine clinical practice should be contemplated. Nonetheless, these findings should be validated through further investigation involving a larger patient cohort.

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Figure 1 – characteristic of the group in terms of age

	CIS - CIS (n = 37)	**CIS - CIS (n = 37)**	**CIS - CIS (n = 37)**	**CIS - RRMS (n = 14)**	**CIS - RRMS**
	Min	Median	Max	Min	Median
Age	22	34	49	20	35

CIS – clinically isolated syndrome, RRMS - relapsing remitting multiple sclerosis

Figure 2 – characteritics of the group in terms of gender

	Sex	Sex	Total
	M	**F**	
CIS-CIS	7	30	37
CIS-RRMS	2	12	14
RRMS	4	14	18
Controls	9	46	55
Total	22	102	124

CIS – clinically isolated syndrome, RRMS - relapsing remitting multiple sclerosis, M – male, F - female

Figure 3 – IL-8, CXCL13, OCB (IgG), cell count in patients in the study groups

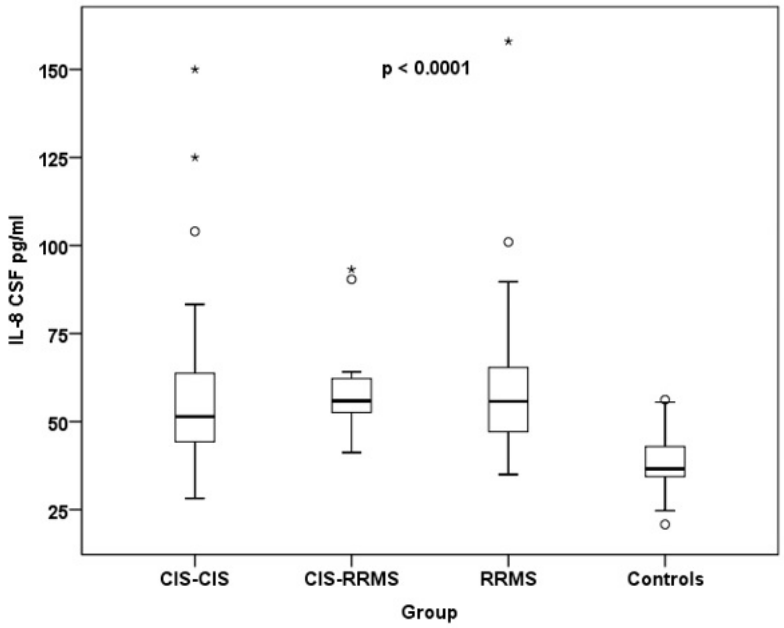
	Group	Group	Group	Group	Gr
	CIS-CIS (n = 37)	**CIS-CIS (n = 37)**	**CIS-CIS (n = 37)**	**CIS-RRMS (n = 14)**	**CI**
	Med	Min	Max	Med	M
IL-8 CSF (pg/ml)	51.4	28.2	150	55.85	41
CXCL13 CSF (pg/ml)	16.8	0.1	119.1	32.3	2.0
OCB (IgG CSF)	7	0	23	12	1
CSF cell count ($.10^6$/l)	4	0.6	17	6.45	1.3

CIS – clinically isolated syndrome, RRMS - relapsing remitting multiple sclerosis , CSF – cerebrospinal fluid, OCB – oligoclonal bands

Figure 4 – ROC (reciever operating characteristic) analysis

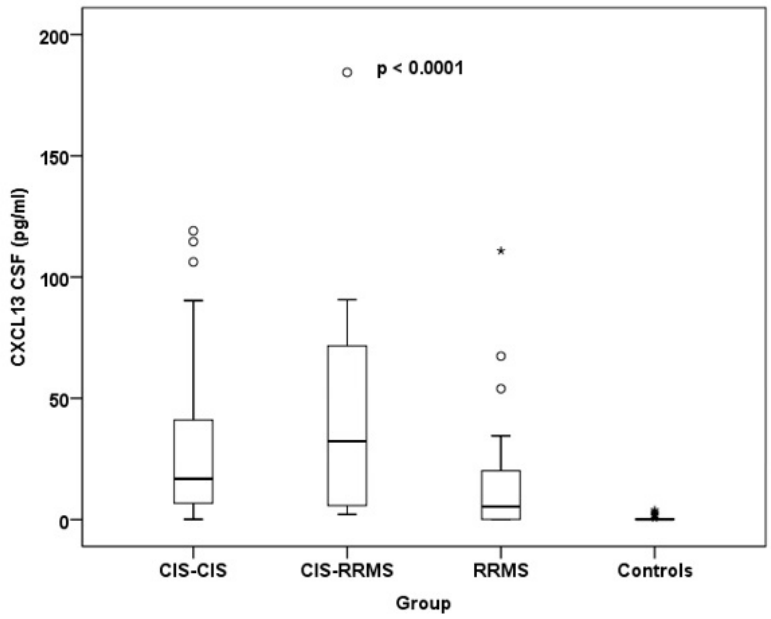
Test Result Variable(s)	AUC	p[b] (signifikance)	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
IL-8 CSF pg/ml	0,939	<0,0001	0,887	0,992
IL-8 S pg/ml	0,602	0,178	0,461	0,744
CXCL13 CSF (pg/ml)	0,959	<0,0001	0,897	1,000

[b] Null hypothesis: true area = 0.5; AUC – area under the curve



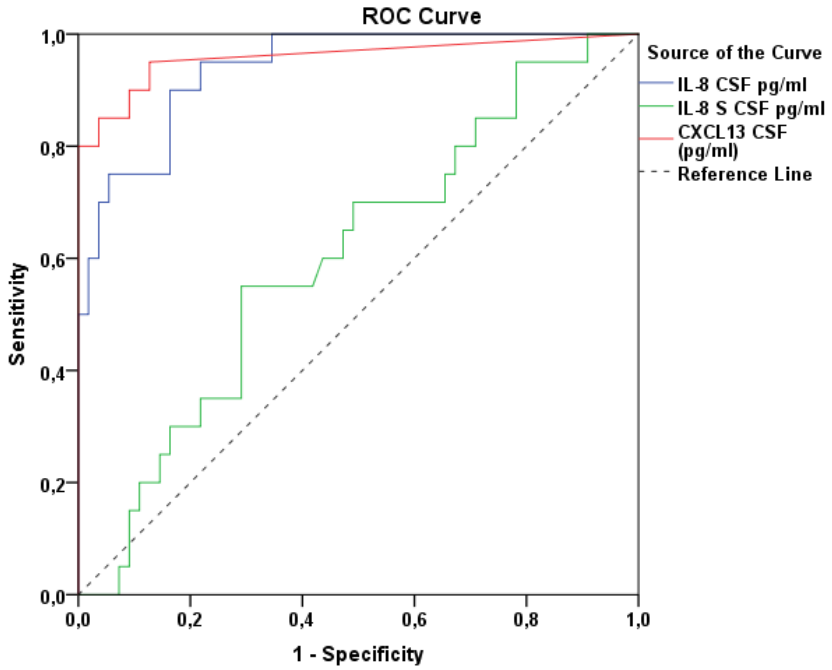
Graph 1 - comparison IL8

CIS – clinically isolated syndrome, RRMS - relapsing remitting multiple sclerosis , CSF – cerebrospinal fluid



Graph 2 – comparison CXCL13

CIS – clinically isolated syndrome, RRMS - relapsing remitting multiple sclerosis , CSF – cerebrospinal fluid



Graph 3 – ROC analysis, CIS – RRMS prediction