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Cerebrospinal fluid CXCL13 in clinically isolated syndrome patients: Association with oligoclonal IgM bands and prediction of Multiple Sclerosis diagnosis

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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).

In MS patients, elevated CSF CXCL13 levels 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 (Krumbholz et al., 2006; Kuenz et al., 2008; Festa et al., 2009; Sellebjerg et al., 2009; Khademi et al., 2011; Ragheb et al., 2011; Senel et al., 2014). Furthermore, they were 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 correlated with the number of CSF CD5+ B cells and with intrathecal IgM production (Villar et al., 2010). Intrathecal IgM synthesis (Villar et al., 2002, 2003; Mandrioli et al., 2008; Sola et al., 2011), and intrathecal lipid-specific IgM synthesis (Villar et al., 2005; Thangarajh et al., 2008) 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.

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

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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.

CXCL13 determination and IgMOB detection was 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 min 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 1.64 pg/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. (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 H_2SO; 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 min 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 min.

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).

2.3. Statistical methods

We calculated absolute frequencies and percentages for categorical variables and mean ± 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 equality 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 used to determine the best cut-off values.

Sensitivity was calculated as (true-positive / [true-positive + false-negative]), specificity was calculated as (true-negative / [true-negative + false-positive]). The positive predictive value (PPV) was calculated as (true-positive / [true-positive + false-positive]), and the negative predictive value (NPV) as (true-negative / [true-negative + 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 a 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 h, 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), 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). 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 pg/ml ± 55 (range: 0–357), median value was 13.5 pg/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 (35.8 pg/ml versus 2.5 pg/ml; $p < 0.0001$). Fig. 1 shows a dotplot of CSF CXCL13 values in the two groups of patients and indicates median