CSF Levels of CXCL12 and Osteopontin as Early Markers of Primary Progressive Multiple Sclerosis

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Abstract

Background and Objectives
To evaluate the extent of intrathecal inflammation in patients with primary progressive MS (PPMS) at the time of diagnosis and to define markers and a specific inflammatory profile capable of distinguishing progressive from relapsing-remitting multiple sclerosis (RRMS).

Methods
Levels of 34 pro- and anti-inflammatory cytokines and chemokines in the CSF were evaluated at the diagnosis in 16 patients with PPMS and 80 with RRMS. All patients underwent clinical evaluation, including Expanded Disability Status Scale assessment and a 3T brain MRI to detect white matter and cortical lesion number and volume and global and regional cortical thickness.

Results
Higher levels of CXCL12 (odds ratio [OR] = 3.97, 95% CI [1.34–11.7]) and the monocyte-related osteopontin (OR = 2.24, 95% CI [1.01–4.99]) were detected in patients with PPMS, whereas levels of interleukin-10 (IL10) (OR = 0.28, 95% CI [0.09–0.96]) were significantly increased in those with RRMS. High CXCL12 levels were detected in patients with increased gray matter lesion number and volume (p = 0.001, r = 0.832 and r = 0.821, respectively). Pathway analysis confirmed the chronic inflammatory processes occurring in PPMS.

Conclusions
At the time of diagnosis, a specific CSF protein profile can recognize the presence of early intrathecal inflammatory processes, possibly stratifying PPMS with respect to RRMS. Elevated CSF levels of CXCL12 and osteopontin suggested a key role of brain innate immunity and glia activity in MS. These molecules could represent useful candidate markers of MS progression, with implications for the pathogenesis and treatment of progressive MS.

Classification of Evidence
This study provides Class III evidence that CXCL12 and monocyte-related osteopontin may be correlated with PPMS, and IL-10 may be related to RRMS. It is may be correlated due to Bonferroni correction negating the statistical correlations found in the study.

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Multiple sclerosis (MS) is the most common immune-mediated disorder of the CNS. The disease course is usually characterized by an initial relapsing-remitting MS (RRMS) phase, defined by the occurrence of new neurologic symptoms and subsequent disability. Later in the disease course, most patients enter a secondary progressive MS (SPMS) phase defined by progressive accumulation of disability, mostly independent of relapses. About 10%–15% of patients exhibit a progressive disease course from the onset (primary progressive MS [PPMS]), with superimposed relapses in few cases. Both focal and diffuse white matter (WM) and gray matter (GM) damage characterize MS pathology. GM damage occurs since earlier disease stages and is a negative prognostic factor for MS-related disability. Inflammation is a major driving force in progressive MS: inflammatory infiltrates, particularly compartmentalized in the meningeal spaces, persist in both SPMS and PPMS associating with GM subpial demyelination and a severe disease course. In a combined ex vivo and in vivo study, CSF analysis revealed an inflammatory profile that associates with GM pathology and predicts MS disease activity in the first years after diagnosis. Here, we evaluated the presence and levels of inflammatory markers in the CSF of patients with PPMS at the time of diagnosis, with the aim to identify molecules capable of distinguishing PPMS from RRMS. This will shed some light on the pathogenesis of progressive MS and, at the same time, will define in vivo the extent of intrathecal inflammation in PPMS and its association with disease severity.

Methods

Patient Cohort

Sixteen consecutive treatment-naive patients with PPMS and 80 with RRMS from the MS Center of Verona University Hospital (Italy) were evaluated at diagnosis between September 2014 and February 2017. Age and disability were different between the 2 groups, whereas the disease duration was similar. Detailed demographic, clinical, and MRI characteristics of patients with MS are shown in Table 1. All patients had a confirmed MS diagnosis according to the most recent diagnostic criteria. They underwent a neurologic evaluation, including the Expanded Disability Status Scale (EDSS) assessment, a brain 3T MRI, and CSF examination. A group of 13 age- and sex-matched (with patients with PPMS) controls with noninflammatory neurologic disorders (2 myopathy, 2 ischemic stroke, 2 peripheral neuropathy, 1 idiopathic tremor, 1 migraine, 1 fibromyalgia, 1 spondylotic myelopathy, 1 amyotrophic lateral sclerosis, 1 idiopathic spastic paraparesis, and 1 endocranial hypertension) that underwent neurologic evaluation and lumbar puncture was also included in the study.

MRI Protocol and Analysis

Three-Tesla MRI equipped with a 8-channel head coil was performed at the Radiology Unit of the University Hospital of Verona using a Philips Achieva 3T MRI Scanner, on each patient at least 1 month from the last relapse. MRI sequences were acquired as previously described. The following sequences were acquired: (a) 3D T1-weighted turbo field echo (repetition time (TR)/echo time (TE) = 8.4/3.7 ms, voxel size of 1 x 1 x 1 mm), total acquisition time of 5:51 minutes; (b) 3D double inversion recovery (DIR) (TR/TE = 5,500/292 ms, inversion times (TI) T1/T12 = 525 ms/2530 ms voxel size of 1 x 1 x 1 mm), turbo spin echo (TSE) readout with an optimal variable flip angle scheme, number of excitations 3, the total acquisition time of 10:49 minutes; and (c) 3D fluid attenuated inversion recovery (FLAIR) (TR/TE = 5,500/292 ms, TI = 1650 ms voxel size of 1 × 1 × 1 mm), total acquisition time of 10:49 minutes.
× 1 × 1 mm), same TSE readout as the DIR sequence, number of excitations 1, the total acquisition time of 5:44 minutes

WM Lesion Detection and Lesion Load Assessment
To identify and segment WM lesions, thus obtaining a T2 hyperintense WM lesion volume (WMLV) at baseline, a semiautomatic thresholding technique, included in MIPAV software, was adopted to calculate the total CL volume.

Cortical Lesion Number and Volume
The number of cortical lesions (CLs) was assessed on DIR images following the recent recommendations for CLs scoring in patients with MS.17 Such number included both intracortical and mixed (WM/GM) lesions, whereas subpial were not counted due to technical difficulties. A semiautomatic thresholding technique based on a Fuzzy C-mean algorithm18 included in MIPAV software was adopted to calculate the total CL volume.

Cortical Thickness Evaluation
FreeSurfer (release v5.3.0), semiautomatic software based on a T1-weighted structural volumetric image (surfer.nmr.mgh.harvard.edu/) was adopted to obtain global and regional measurements of the cortical thickness (CTh) and a semiautomatic procedure with lesion filling, was used to correct topological defects in the cortical surface due to intracortical lesions. Regions included in the correlation analysis were cingulate, cuneus, insula, precentral gyrus, precuneus, superior frontal gyrus, and hippocampus. The mean of the left and right hemispheres for each region of interest of the FreeSurfer parcellation was considered for the analysis.19

Primary Research Question
Can CSF inflammatory markers distinguish PPMS from RRMS at the time of diagnosis? This study provides Class III evidence that chemokine (C-X-C motif) ligand (CXCL12) and monocyte-related osteopontin may be correlated with PPMS, and interleukin-10 (IL-10) may be related to RRMS. It is may be correlated due to Bonferroni correction negating the statistical correlations found in the study.

Statistical Analysis
Differences among PP and RR patients’ groups were initially assessed with the Mann-Whitney test and Fisher exact test. Inflammatory molecules were divided into subgroups according to their main immunologic function. For each pathway identified, the associations between CSF protein levels at diagnosis and the disease course (PPMS and RRMS) were assessed with logistic regression, adjusted for age at onset. CSF levels were log transformed to obtain reliable odds ratio (OR). The log base 2 allowed a more intuitive interpretation of ORs,
Table 2  Levels of the Cytokines and Chemokines Detected in the CSF of Patients With MS

|                               | Total MS | PPMS       | RRMS       | PPMS/RRMS |
|--------------------------------|----------|------------|------------|-----------|
| **T-cell pathway**             |          |            |            |           |
| IFN gamma                      | 14.7 ± 24.0 | 10.7 ± 14.1 | 15.5 ± 25.6 | 0.69      |
| IFN alfa2                      | 14.2 ± 18.7 | 16.2 ± 15.1 | 13.8 ± 19.4 | 1.18      |
| IFN lambda2                    | 191.3 ± 470.1 | 259.7 ± 544.8 | 177.7 ± 456.4 | 1.46      |
| IL12(p40)                      | 13.1 ± 21.8 | 7.4 ± 13.4  | 14.2 ± 23.0 | 0.52      |
| CXCL8                          | 61.7 ± 79.3 | 56.3 ± 69.0 | 62.8 ± 81.6 | 0.90      |
| IL 22                          | 33.5 ± 30.8 | 32.4 ± 21.6 | 33.8 ± 32.5 | 0.96      |
| CCL19                          | 96.4 ± 93.2 | 64.0 ± 59.2 | 102.9 ± 97.6 | 0.62      |
| CCL20                          | 1.0 ± 1.6   | 1.0 ± 1.2   | 1.0 ± 1.6   | 1.02      |
| CCL21                          | 1,599.9 ± 1,000.5 | 1,300.3 ± 968.5 | 1,659.9 ± 1,001.9 | 0.78      |
| CCL25                          | 123.3 ± 81.6 | 141.0 ± 115.7 | 119.8 ± 73.4 | 1.18      |
| IL4                            | 19.9 ± 18.1 | 25.4 ± 19.6 | 18.7 ± 17.7 | 1.35      |
| **B-cell pathway**             |          |            |            |           |
| CXCL12                         | 2087.1 ± 1924.6 | 2,765.7 ± 2,173.0 | 1951.4 ± 1856.3 | 1.42      |
| CXCL13                         | 11.2 ± 25.4 | 5.3 ± 9.3   | 12.4 ± 27.4 | 0.43      |
| BAFF                           | 9,894.8 ± 6,333.8 | 11,387.0 ± 10,110.0 | 9,596.3 ± 5,319.2 | 1.19      |
| IL10                           | 19.5 ± 18.6 | 12.0 ± 8.8  | 21.1 ± 19.6 | 0.57      |
| IL35                           | 286.4 ± 204.8 | 270.1 ± 198.5 | 289.7 ± 207.1 | 0.93      |
| GMCSF                          | 87.4 ± 99.8 | 76.7 ± 90.0 | 89.6 ± 102.0 | 0.86      |
| **Monocyte/macrophage pathway**|          |            |            |           |
| IL1beta                        | 2.5 ± 3.5  | 2.0 ± 2.7   | 2.5 ± 3.6   | 0.80      |
| IL6                            | 26.5 ± 49.2 | 23.5 ± 39.0 | 27.1 ± 51.1 | 0.87      |
| CCL2                           | 526.2 ± 598.0 | 717.4 ± 674.2 | 488.0 ± 578.6 | 1.47      |
| CCL8                           | 183.5 ± 587.0 | 157.3 ± 430.7 | 188.7 ± 615.6 | 0.83      |
| CX3CL1                         | 348.4 ± 248.4 | 325.6 ± 260.4 | 353.0 ± 247.4 | 0.92      |
| CXCL10                         | 431.6 ± 507.3 | 421.4 ± 425.8 | 433.6 ± 524.4 | 0.97      |
| CXCL11                         | 64.6 ± 499.6 | 10.6 ± 26.9  | 75.4 ± 547.1 | 0.14      |
| sCD163                         | 49,534.7 ± 33,284.3 | 51,226.7 ± 23,913.1 | 49,196.3 ± 34,970.7 | 1.04      |
| MMP1                           | 708.2 ± 927.3 | 739.4 ± 616.9 | 702.0 ± 980.6 | 1.05      |
| MMP2                           | 1,165.5 ± 3,189.4 | 478.3 ± 556.0 | 1,302.9 ± 3,472.7 | 0.37      |
| Osteopontin                    | 84,163.9 ± 95,433.9 | 130,158.0 ± 139,428.3 | 74,965.1 ± 82,139.4 | 1.74      |
| **TNF pathway**                |          |            |            |           |
| TNF                            | 39.7 ± 38.4 | 55.2 ± 43.1 | 36.6 ± 36.9 | 1.51      |
| sTNFR1                         | 4,300.7 ± 2,653.3 | 5,294.0 ± 3,304.1 | 4,102.0 ± 2,480.6 | 1.29      |
| sTNFR2                         | 1,062.4 ± 886.4 | 1,604.3 ± 1,227.6 | 954.1 ± 766.4 | 1.68      |
| APRIL                          | 54,318.8 ± 61,277.7 | 63,853.0 ± 62,885.8 | 52,411.9 ± 61,175.9 | 1.22      |
| LIGHT                          | 318.2 ± 434.0 | 585.6 ± 511.1 | 264.7 ± 399.5 | 2.21      |
| Tweak                          | 2,060.4 ± 1,979.0 | 2,337.3 ± 2,014.9 | 2,005.0 ± 1,979.9 | 1.17      |

Abbreviations: APRIL = A proliferation-inducing ligand; CX3CL1 = chemokine (C-X3-C motif) ligand; IFN = interferon; LIGHT = tumor necrosis factor ligand superfamily member 14; PPMS = primary progressive MS; RRMS = relapsing-remitting MS; sCD163 = soluble CD163. Values are expressed as pg/mL; mean ± SD are reported. In the PPMS/RRMS column, values are reported as fold change between the 2 groups.
as each unit in log base 2 (protein level) corresponds to a doubling in protein level. Before performing the logistic regression models, the multicollinearity among independent variables was checked using the variance inflation factor (VIF). No multicollinearity was detected (VIF < 10). The receiver operating characteristic (ROC) analysis (Youden index method) was used to identify both CXCL12 and osteopontin cutoff that maximize specificity and sensitivity of identifying patients with PPMS from RRMS. Sensitivity, specificity, accuracy, and area under curve with 95% CI were reported. Logistic regression analysis was used to evaluate the association of CXCL12 and osteopontin levels with a PPMS disease course and noninflammatory controls. A false discovery rate correction with significance level of 0.05 was applied. Pathway analysis revealed these molecules as mainly involved in regulation of dendritic cell (DC) processes, calcium ion import, leukocyte apoptotic processes, immunologic synapse formation, regulation of endothelial cell development and extravasation, and regulation of glial cell apoptotic process (Table 3).

**Results**

**Differences in the CSF Profile Between PPMS and RRMS**

Increased levels of the lymphoid chemokine CXCL12 (fold change 1.42) and of molecules related to monocyte/macrophage recruitment and innate immunity activity as chemokine (C-C motif) ligand (CCL2) and osteopontin (1.47 and 1.74, respectively) were detected in patients with PPMS compared with RRMS. Patients with PPMS had increased levels of many other cytokines, in particular tumor necrosis factor (TNF) (fold change 1.51), its soluble TNF receptors (sTNFRs) sTNFR1 and sTNFR2 (fold change 1.29 and 1.68, respectively) and the TNF superfamily member TNF ligand superfamily member 14 (fold change 2.21). Among other molecules, levels of IFN lambd and IL4 were increased in patients with PPMS with a fold change of 1.46 and 1.35, respectively. Conversely, patients with RRMS showed higher levels of matrix metalloproteinase (MMP) 2 (fold change 2.72), IL12 (1.91), IL10 (1.73), CCL19 (1.61), CXCL11 (7.1), and CXCL13 (2.34). Levels of all the examined molecules in each MS group are reported in Table 2.

**Logistic Regression and ROC Analysis**

The multivariate logistic regression analysis showed that the CSF level at diagnosis of CXCL12 (OR = 3.97, 95% CI [1.34–11.7]) and osteopontin (OR = 2.24, 95% CI [1.01–4.99]) independently predicted a higher probability of a primary progressive course of the disease. Conversely, elevated IL10 levels were significantly associated with the diagnosis of RRMS (OR = 0.28, 95% CI [0.09–0.96]). ROC curve analysis estimated the optimal cutoff values of 2063.58 and 184,736.4 pg/mL for CXCL12 and osteopontin, respectively (Figure 1). Both CXCL12 and osteopontin levels were found increased compared with control group patients with noninflammatory neurologic disorders (OR = 1.10, 95% CI [1.05–3.15], and OR = 1.38, 95% CI [1.08–5.23], respectively).

**Correlations and Pathway Analysis in PPMS**

Patients with increased CSF CXCL12 showed a concurrent increment of the monocyte chemoattractant protein CCL2 levels (r = 0.647, p = 0.040; Figure 2A). Despite a not significant correlation after adjusting for multiple comparisons, the same patients displayed high levels of B-cell activating factor (BAFF) (r = 0.597, p = 0.064; Figure 2A), IL4 (r = 0.653, p = 0.106), the lymphoid chemokine CCL19 (r = 0.603, p = 0.129), and both the TNF receptors, sTNFR1 (r = 0.515, p = 0.186) and sTNFR2 (r = 0.559, p = 0.147). Pathway analysis revealed these molecules as mainly involved in regulation of dendritic cell (DC) processes, calcium ion import, leukocyte apoptotic processes, immunologic synapse formation, regulation of endothelial cell development and extravasation, and regulation of glial cell apoptotic process (Table 3).

**Data Availability**

Deidentified data will be shared on request from a qualified investigator.

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**Figure 1** Results of ROC Curve Analysis

For each protein, AUC, sensitivity, specificity, and accuracy in discriminating between PPMS and RRMS are reported. The optimal threshold for each protein is reported in the curve (red dot) and in the text. Acc = accuracy; AUC = area under the curve; PPMS = primary progressive MS; ROC = receiver operating characteristic; RRMS = relapsing-remitting MS; Sens = sensitivity; Spec = specificity.
Increased osteopontin levels were detected in PPMS patients with higher expression of MMP2 ($r = 0.747$, $p = 0.013$), sTNFR1 ($r = 0.829$, $p = 0.002$; Figure 2B), and CXCL10 ($r = 0.674$, $p = 0.040$), molecules mainly involved in regulation of endothelial cell development and endothelial barrier, regulation of sphingolipid and ceramide biosynthesis, cell death regulation, regulation of cyclic adenosine monophosphate-mediated signaling, regulation of epithelium morphogenesis, and T-cell chemotaxis (Table 3).

Finally, patients with high IL10 levels were also characterized by increased TNF ($r = 0.747$, $p = 0.012$), IL1beta ($r = 0.829$, $p = 0.002$), and chemokine (C-X3-C motif) ligand 1 ($r = 0.650$, $p = 0.040$) CSF levels (Figure 2C). The pathway analysis revealed that these molecules are linked with cell adhesion, regulation of fever generation, regulation of endothelial cell development, cytokine secretion, regulation of membrane protein, response to corticosteroids, and regulation of acute inflammatory response (Table 3). The entire correlation panel is graphically shown in Figure 2D.

**Correlations Between CSF Inflammation and Disease Severity in PPMS**

No correlations were found between the selected inflammatory molecules and EDSS (not shown). Both cortical lesion number
and cortical lesion volume \((r = 0.821, p = 0.001)\) were increased in patients harboring higher intrathecal CXCL12, whereas a similar trend was not observed regarding osteopontin and IL10. No significant associations were observed between CXCL12 and osteopontin levels and WM lesion number or WMLV. CXCL13 levels were particularly

| Name                                                                 | \(p\) Value | OR    | Combined score |
|----------------------------------------------------------------------|-------------|-------|----------------|
| **CXCL12 and related molecules**                                      |             |       |                |
| (1) Negative regulation of the dendritic cell apoptotic process (GO:2000669) | 0.000001574 | 952.38 | 12,725.67      |
| (2) Regulation of the dendritic cell apoptotic process (GO:2000668)   | 0.000003371 | 666.67 | 8,400.14       |
| (3) Positive regulation of calcium ion import (GO:0090280)           | 0.000003371 | 666.67 | 8,400.14       |
| (4) Negative regulation of the leukocyte apoptotic process (GO:2000107) | 0.000004943 | 555.56 | 6,787.49       |
| (5) Positive regulation of dendritic cell antigen processing and presentation (GO:0002606) | 0.001799 | 555.56 | 3,511.45       |
| (6) Immunologic synapse formation (GO:0001771)                       | 0.001799    | 555.56 | 3,511.45       |
| (7) Regulation of endothelial cell development (GO:1901550)           | 0.001799    | 555.56 | 3,511.45       |
| (8) Negative regulation of cellular extravasation (GO:0002692)       | 0.001799    | 555.56 | 3,511.45       |
| (9) Positive regulation of antigen processing and presentation (GO:0002579) | 0.001799 | 555.56 | 3,511.45       |
| (10) Regulation of the glial cell apoptotic process (GO:0034350)      | 0.001799    | 555.56 | 3,511.45       |
| **Osteopontin and related molecules**                                 |             |       |                |
| (1) Regulation of endothelial cell development (GO:1901550)           | 0.001799    | 555.56 | 3,511.45       |
| (2) Positive regulation of the sphingolipid biosynthetic process (GO:0090154) | 0.002098 | 476.19 | 2,936.47       |
| (3) Death-inducing signaling complex assembly (GO:0071550)            | 0.002098    | 476.19 | 2,936.47       |
| (4) Positive regulation of the ceramide biosynthetic process (GO:2000304) | 0.002098 | 476.19 | 2,936.47       |
| (5) Positive regulation of cAMP-mediated signaling (GO:0043950)      | 0.002398    | 416.67 | 2,513.82       |
| (6) Regulation of morphogenesis of an epithelium (GO:1905330)        | 0.002697    | 370.37 | 2,190.93       |
| (7) T-cell chemotaxis (GO:0010818)                                   | 0.002997    | 333.33 | 1936.76        |
| (8) Regulation of T-cell chemotaxis (GO:0010819)                    | 0.002997    | 333.33 | 1936.76        |
| (9) Regulation of the ceramide biosynthetic process (GO:2000303)     | 0.003296    | 303.03 | 1731.85        |
| (10) Regulation of establishment of the endothelial barrier (GO:1903140) | 0.003595 | 277.78 | 1,563.39       |
| **IL10 and related molecules**                                        |             |       |                |
| (1) Positive regulation of heterotypic cell-cell adhesion (GO:0034116) | 4.948e-10   | 1,363.64 | 29,218.41   |
| (2) Positive regulation of fever generation (GO:0031622)             | 4.499e-7    | 1,666.67 | 24,357.23   |
| (3) Regulation of endothelial cell development (GO:1901550)          | 4.499e-7    | 1,666.67 | 24,357.23   |
| (4) Positive regulation of cell-cell adhesion (GO:0022409)           | 6.138e-12   | 606.06  | 15,646.39     |
| (5) Regulation of heterotypic cell-cell adhesion (GO:0034114)         | 2.905e-9    | 789.47  | 15,518.51     |
| (6) Regulation of cytokine secretion involved in immune response (GO:0002739) | 0.000001079 | 1,111.11 | 15,265.62 |
| (7) Regulation of membrane protein ectodomain proteolysis (GO:0051043) | 4.617e-9    | 681.82  | 13,086.55     |
| (8) Response to corticosteroid (GO:0031960)                           | 0.000001649 | 909.09  | 12,104.88     |
| (9) Regulation of establishment of the endothelial barrier (GO:1903140) | 0.000001979 | 833.33  | 10,944.26     |
| (10) Positive regulation of acute inflammatory response (GO:0002675) | 0.00003147  | 666.67  | 8,446.00      |

Abbreviations: OR = odds ratio; PPMS = primary progressive MS; cAMP = cyclic adenosine monophosphate.
increased in those patients with lower CTh of cuneus ($r = -0.762$, $p = 0.024$), hippocampus ($r = -0.678$, $p = 0.063$), cingulate gyrus ($r = -0.652$, $p = 0.063$), and insula ($r = -0.633$, $p = 0.063$). All correlations are graphically shown in Figure 3.

Discussion

The analysis of the CSF profile in PPMS revealed that higher CXCL12 and osteopontin are associated with a progressive disease course and suggest that a chronic intrathecal inflammatory process, although partly different from RRMS, also occurs in patients with progressive MS, since the time of diagnosis and independently of relapses.

Such findings are in line with the idea that inflammation exerts a major role in progressive MS, including PPMS. Whether PPMS and RRMS/SPMS represent similar physiopathologic entities has been debated; differences between SPMS and PPMS seem to be quantitative rather than qualitative.\(^8\)\(^{21}\) Furthermore, the similar age at onset and rates of disability progression indicate that a similar process underlies the 2 entities.\(^7\)\(^{22}\)\(^{23}\) Although based on a small cohort, our preliminary results highlighted 2 molecules as associated with the primary progressive disease course: the CXCL12 and the osteopontin.

Besides modulating neuronal activity through multiple regulatory pathways, CXCL12 is a potent chemoattractant molecule for different immune cells, including monocytes, T cells, B cells, and plasma cells.\(^{24}\) Elevated levels of CXCL12 have been detected both in active and inactive lesions and have been suggested as contributors to the maintenance of immune cells within the CNS.\(^{25}\) Along with CXCL13, it mediates germinal center organization in lymphoid tissue, and possibly, a similar process could occur in the CNS of patients with MS, thus contributing to the persistence of B cells and plasma cells in inflamed meninges and perivascular spaces.

In line with previous findings, we have found CXCL12 strictly associated with the local production by astrocytes of the B-cell survival factor BAFF\(^25\) and with CLs, thus confirming the link between B cell–associated CSF inflammation and the adjacent cortical pathology.\(^12\)\(^{13}\) Probably due to the low sample size, a significant correlation between the CXCL12 and the CTh was not observed. On the contrary, after correction for multiple comparisons, CXCL13 levels correlated with the CTh of many brain regions that are early involved in PPMS pathology.\(^26\)\(^{27}\) Such observations could have clinical prognostic value, as cortical pathology has been recognized as a crucial substrate for the progression and irreversible clinical and cognitive disability in MS.\(^7\)\(^{28}\)

Osteopontin, which in the brain is mainly released by endothelial cells, microglia, macrophages, and DCs, has a role in mediating MS severity and progression.\(^29\)\(^{32}\) CSF levels of osteopontin are higher than in plasma, further suggesting a contribution from CNS resident or infiltrating cells as intrathecal sources of the molecule.\(^33\) It is increased in lesional brain tissue from patients with progressive MS\(^34\) and in the CSF of patients with MG\(^33\)\(^{35}\) and decreases after treatments.\(^36\) Of interest, patients with SPMS showed stable and continuously increased osteopontin levels.\(^37\) Osteopontin mainly reflects activation of innate immune system and exerts an essential role in inflammation and immune response, influencing T helper differentiation 1-type and 2-type responses, as well as regulating DC migration at multiple levels.\(^38\)\(^{39}\) Among others, it enhances interferon and IL12 proinflammatory activity and reduces IL10-mediated responses, all cytokines involved in MS and other inflammatory diseases.\(^38\)
Osteopontin increases myelin-reactive T-cell survival and induces relapses and progression in experimental autoimmune encephalomyelitis; accordingly, osteopontin-deficient mice show a greater number of remissions, less progression, and higher IL10 levels in a preclinical MS model. All these shreds of evidence further point to a detrimental role of osteopontin in MS progression since the early stages.

As suggested by pathway analysis, the molecules identified as associated with PPMS in this study are mainly related to several inflammatory processes of both innate and adaptive immune responses that occur in the CNS. Indeed, as MS progresses, inflammation becomes increasingly compartmentalized within the CNS beyond a relatively intact blood-brain barrier, with lesions showing less prominent focal inflammation but persisting microglia activity at the edge of chronic active demyelinating plaques. A major contribution to MS progression is given by meningeal inflammation: diffuse and clustered meningeal and perivascular infiltrates, enriched in B cells, associated with subpial GM pathology, have been detected in both SPMS and PPMS postmortem brains, although, in the case of PPMS, they appeared less extensive and not clustered in tertiary lymphoid follicles.

The current study is mainly limited by the low number of PPMS cases evaluated, whose follow-up is still ongoing. This implies that our observations are preliminary and that further validation in a larger cohort is needed. The analysis of CSF intrathecal profile in a larger cohort could confirm the markers capability to early identify patients with severe progressive MS course that could benefit from an immunosuppressant approach.

In summary, our work showed that (1) intrathecal inflammation is significant in the CSF of patients with PPMS at the time of diagnosis; (2) the CSF profile of patients with PPMS is characterized explicitly by higher levels of CXCL12 and osteopontin when compared with patients with RRMS and associate with a specific proinflammatory innate immune profile; (3) as observed in RRMS, also in PPMS, the lymphoid chemokines CXCL12 and CXCL13 are associated with an increased level of GM pathology already at the time of diagnosis. Our observations highlight the potential differential signature of intrathecal inflammation in PPMS, suggesting possible clinical implications for the diagnosis and treatment of PPMS that warrant further investigations.

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**Disclosure**

The authors report no disclosures relevant to this manuscript. Go to Neurology.org/NN for full disclosures.

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| Name                        | Location                        | Contribution                                                                 |
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