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CSF inflammatory biomarkers responsive to treatment in progressive multiple sclerosis capture residual inflammation associated with axonal damage

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Abstract

Background—Development of treatments for progressive multiple sclerosis (MS) is challenged by the lack of sensitive and treatment responsive biomarkers of intrathecal inflammation.

Objective—To validate the responsiveness of cerebrospinal fluid (CSF) inflammatory biomarkers to treatment with natalizumab and methylprednisolone in progressive MS, and to examine the relationship between CSF inflammatory and tissue damage biomarkers.

Methods—CSF samples from two open-label phase II trials of natalizumab and methylprednisolone in primary and secondary progressive MS. CSF concentrations of 20 inflammatory biomarkers and CSF biomarkers of axonal damage (neurofilament light chain (NFL)) and demyelination were analysed using electrochemiluminescent and ELISA assays.

Results—Seventeen natalizumab and 23 methylprednisolone treated patients had paired CSF samples. CSF sCD27 displayed superior standardised response means and highly significant decreases during both natalizumab and methylprednisolone treatment, however post-treatment levels remained above healthy donor reference levels. Correlation analyses of CSF inflammatory biomarkers and NFL before, during and after treatment demonstrated that CSF sCD27 consistently correlates with NFL.

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DECLARATION OF CONFLICTING INTERESTS

Jeppe Romme Christensen has received speaker honoraria from Novartis, consultant honoraria from Biogen Idec, and has had travel expenses reimbursed by Novartis. Rikke Ratzer has had travel expenses reimbursed by Biogen Idec and Genzyme. Mika Komori contributed to this work as a former employee of NINDS, and the opinions expressed in this work do not represent her current affiliation (Eli Lilly Japan K.K., Kobe, Japan). Marina Rode von Essen reports no competing interest. Rikke Ratzer has had travel expenses reimbursed by Biogen Idec and Genzyme. Lars Börnsen has received support for congress participation from Genzyme and Novartis. Bibi Bielekova is co-inventor on several NIH patents related to daclizumab and CSF biomarkers and she received patent royalty payments from NIH only related to daclizumab patents. Finn Sellebjerg has served on scientific advisory boards, been on the steering committees of clinical trials, served as a consultant, received support for congress participation, received speaker honoraria, or received research support for his laboratory from Biogen, EMD Serono, Genzyme, Merck, Novartis, Roche, Sanofi and Teva.

Conclusions—These findings validate CSF sCD27 as a responsive and sensitive biomarker of intrathecal inflammation in progressive MS, capturing residual inflammation after treatment. Importantly, CSF sCD27 correlates with NFL, consistent with residual inflammation after anti-inflammatory treatment being associated with axonal damage.

Keywords

Primary progressive multiple sclerosis; secondary progressive multiple sclerosis; natalizumab; methylprednisolone; cerebrospinal fluid; inflammation; axonal damage

INTRODUCTION

Development of treatments for progressive forms of multiple sclerosis (MS) is a major unmet need and has been challenged by numerous negative phase 3 trials. The reason for this is thought to comprise a complex pathogenesis and several hurdles regarding trial design, including reliable clinical endpoints, trial size and duration, and the lack of validated biomarkers sensitive to the progressive MS pathology (1). Progressive MS pathogenesis continues to be debated, and although pathology studies demonstrate intrathecal inflammation involving both T- and B-cells and evidence of compartmentalised inflammation owing to the presence of ectopic lymphoid follicles and activated microglia, the relationship between inflammation and disease progression is unclear (2–4). In progressive MS brains, inflammation is clearly associated with axonal damage and demyelination (5;6). Nonetheless, the paucity of contrast-enhancing lesions (CEL) and the negative results of clinical trials of immunomodulatory and immunosuppressive treatments in progressive MS were suggested to reflect a diminished role of inflammation in progressive MS, and has promoted the study of neuroprotective agents instead (1). In relation to this assumption, it is noteworthy that current inflammatory biomarkers (CEL) do not capture progressive MS intrathecal inflammation well (3;7).

Recent studies have demonstrated the feasibility of using cerebrospinal fluid (CSF) biomarkers for studying the relationship between inflammation and ongoing tissue damage (8). In two open-label phase two trials we found these CSF biomarkers responsive to anti-inflammatory treatment with natalizumab or methylprednisolone in progressive MS. A trial with natalizumab demonstrated significant decreases in the primary endpoint, CSF osteopontin, and other CSF biomarkers of inflammation (CSF cell count, IgG index, CXCL13 and MMP9), neuronal/axonal damage (neurofilament light chain, NFL), demyelination (myelin basic protein, MBP) and improvement in secondary clinical and MRI outcomes (9). A similar trial of cyclic oral methylprednisolone demonstrated only significant decrease in IgG index and a trend for decreased MMP9 and NFL, while beneficial effects were seen on secondary clinical and MRI endpoints (10). Nevertheless, the CSF biomarkers of inflammation used in these trials have limitations, including diverse cellular origin, low abundance and possible interaction with glucocorticoid treatment. Soluble cellular CSF biomarkers of intrathecal inflammation have recently been validated, demonstrating CSF sCD27 as a superior biomarker of intrathecal inflammation compared to conventional biomarkers (CEL, CSF cell count, IgG index and oligoclonal bands) (7). Soluble cellular CSF biomarkers were validated to reflect distinct immune cell subsets (sCD27 = T-cells;

sCD21 = B-cells; sCD14 and sCD163 = myeloid cells), thus contributing essential information about the composition of intrathecal immune responses. Notably, progressive MS patients had increased sCD27/T-cell and sCD21/B-cell ratios, suggesting increased immune cell infiltration into the CNS parenchyma compared to relapsing remitting MS (RRMS), consistent with the concept of compartmentalised inflammation being a key element in the pathogenesis of progressive MS. Accordingly, these new biomarkers of intrathecal immune responses hold promise as biomarkers reflecting inflammatory pathogenic processes in progressive MS, and therefore may be used in phase 2 trials for identifying drug candidates for progressive MS. However, validation in longitudinal treatment cohorts is required to demonstrate responsiveness to treatment effects.

The first two positive phase 3 trials were reported for ocrelizumab in primary progressive MS (PPMS) and siponimod in secondary progressive MS (SPMS), but the treatment effects on clinical endpoints were modest (11;12). Accordingly, an emerging question is whether the modest clinical effects in progressive MS are explained by continued demyelination and axonal damage in the absence intrathecal inflammation or residual inflammation with ensuing demyelination and axonal damage.

We investigated the responsiveness of soluble cellular CSF biomarkers and other candidate CSF biomarkers of inflammation to treatment with natalizumab and methylprednisolone in progressive MS and quantified residual intrathecal inflammation on these therapies. Furthermore, we studied the relationship between CSF biomarkers of inflammation, axonal damage and demyelination before and after treatment to address the performance and relevance of the studied CSF inflammatory biomarkers. Lastly, we explored whether changes in CSF biomarkers were associated with changes in clinical endpoints.

METHODS

Patients

Baseline and 60 week follow-up CSF samples from PPMS and SPMS patients who had participated in two open-label phase 2 clinical trials of natalizumab (NCT01077466) and oral pulse methylprednisolone (NCT01305837) treatment in progressive MS were included for analysis (Table 1) (9;10). The storage time from the baseline sampling to analysis ranges from 1218 to 2061 days. Two patients who had participated in the natalizumab study were subsequently recruited for the methylprednisolone study, and therefore samples from these two patients from the latter study were excluded in pooled analyses of samples from the two trials. Written informed consents were signed before inclusion and the study protocols were in accordance with the Declaration of Helsinki and approved by the local ethics committee and Danish regulatory authorities.

CSF analysis

All CSF samples were run in duplicates. Cell-specific soluble biomarkers were analysed using electrochemiluminescent assays developed at NIH and described in (7), which includes assay parameters such as lower limits of detection. Upper reference levels for healthy donors for CSF sCD27 and sCD21 were calculated as mean + 2 standard deviations.

All other analyses were performed at DMSC using commercially available assays. Chitinase 3-like 1 (CHI3L1) was analysed using CHI3L1 Quantikine ELISA Kit (R&D, Abingdon, UK). CSF levels of cytokines and chemokines were analysed using V-PLEX Cytokine Panel 1, Chemokine Panel 1 and Proinflammatory Panel 1 (Meso Scale Diagnostics, Maryland, USA). Analytes which were undetectable, low abundant (detected in <50% of baseline samples) or had high (>12.5%) intra-assay coefficient of variance (CV) were excluded (Table 2). Data on CSF cell counts, IgG index, albumin quotient, CSF NFL and CSF MBP levels have been published previously in the individual studies (9;10). For the present study, we reanalysed CSF NFL and MBP with assays from the same batch using NF-Light Neurofilament ELISA (UmanDiagnostics, Umeå, Sweden) and Myelin Basic Protein ELISA (Beckman Coulter, Brea, USA). Upper reference values for cell counts and IgG index and are derived from the routine laboratory analysis at DMSC, while albumin quotient upper reference is derived from a previous study (13).

Statistical analysis

Statistical analyses were performed using IBM SPSS statistics version 22 (IBM Corp, Armonk, NY). For analysis of treatment effects, all available paired samples from patients who had completed the respective trials were included. Paired tests of significance were analysed using t-tests or Wilcoxon Signed Rank tests depending data distribution. Since data for baseline values and change during treatment were not all normally distributed, estimated means and 95% confidence intervals were generated using the bootstrap procedure. Standardised response means were used to estimate responsiveness and calculated as the mean of changes with standard deviation of changes (14). Analysis for correlation between levels of CSF biomarker levels were performed with Spearman's rank correlation coefficient. As the analyses involved multiple comparisons we applied the false discovery rate (FDR) method to calculate q-values, which were considered significant when $q < 0.05$ (15).

RESULTS

Baseline characteristics of the included patients from two previously published trials with similar inclusion and exclusion criteria are shown in Table 1 (9;10). The immunological baseline profiles were comparable with similar cell counts, IgG index and frequencies of CEL. No differences between PPMS and SPMS patient groups were observed regarding any of the inflammatory or tissue damage parameters (data not shown).

Technical performance of analytes

The majority of the CSF biomarkers were detectable in all baseline samples, although IL7, IL10, TNF- α , CCL11 and CCL22 were only detectable in the majority of the baseline samples (Table 2). Standardised response means during both treatments reached highest numerical values for sCD27 and IL7. None of the baseline sample biomarker levels correlated with storage time.

Treatment effects on CSF inflammatory biomarkers

After 60 weeks of natalizumab treatment we observed significantly reduced CSF concentrations of sCD27 (−6%), sCD21 (−31%), IL10 (−51%), IL12p40 (−79%), TNF-α (−1%) and CXCL10 (−40%), while IL5 (+18%) and IL7 (+197%) concentrations increased (Table 3). After 60 weeks of methylprednisolone treatment we observed significantly reduced sCD27 (−39%), sCD21 (−38%) and IL12p40 (−42%), and increased sCD163 (+44%), IL5 (+40%), IL7 (+35%), IL15 (+17%) and CCL2 (+14%) concentrations. During both natalizumab and methylprednisolone treatment, sCD27 and sCD21 demonstrated highly significant decreases. However, post-treatment levels of sCD27 remained above the upper reference level (sCD27 13.2 U/ml; sCD21 195 pg/ml) for healthy donors in the majority (77%) of patients. Analysis of difference in changes between natalizumab and methylprednisolone treatment revealed significant differences in changes of sCD163, IL7 and IL8.

Analysis of correlations between CSF biomarkers of inflammation and tissue damage

Figure 1 displays correlation analyses of CSF biomarkers of inflammation and CSF biomarkers of axonal damage (NFL) and demyelination (MBP). In the baseline samples, correlation analyses demonstrated the strongest significant correlations ($\rho = 0.50$ to 0.59) between NFL and sCD27, sCD14, CHI3L1, IL10, IL12p40 and TNF-α, while significant correlations with MBP were found for sCD14 ($\rho = 0.53$) and sCD163 ($\rho = 0.48$). Correlation analyses of changes during treatment with natalizumab and methylprednisolone showed strongest significant correlations between NFL change and sCD21, sCD27, IL10, IL12p40 and CXCL10 change ($\rho = 0.50$ to 0.64), whereas MBP change correlated strongest with IL7 change ($\rho = -.53$). In post-treatment samples after natalizumab or methylprednisolone treatment, the only CSF biomarkers consistently showing significant correlation with NFL were sCD27 ($\rho = 0.66$ for post-natalizumab samples; $\rho = 0.57$ for post-methylprednisolone samples) and IL10 ($\rho = 0.56$ for post-natalizumab samples; $\rho = 0.55$ for post-methylprednisolone samples).

Analysis of correlations between change during treatment in CSF biomarkers and in clinical outcomes

Correlation analysis of changes in CSF biomarker levels and changes in the clinical scales EDSS and MSIS during treatment showed no significant correlations with CSF inflammatory biomarkers, while CSF NFL change correlated significantly with MSIS change ($\rho = 0.42$; $p = 0.007$).

DISCUSSION

Development of treatments for progressive multiple sclerosis is challenged by the lack of validated surrogate markers, which would allow fast screening of candidate drugs in phase two trials of short duration. The present study validates CSF sCD27 as a highly treatment-responsive biomarker of intrathecal inflammation and with sensitivity to detect residual inflammation. Notably, sCD27 showed strong correlation with NFL, before and after treatment, consistent with compartmentalised intrathecal inflammation being associated with axonal damage after anti-inflammatory therapy in progressive MS.

The study included CSF samples prospectively collected using the same CSF handling protocol. Using high-sensitivity immunoassays we screened 33 CSF biomarkers of inflammation and omitted 13 due to suboptimal technical performance, leaving 20 technically robust biomarkers for further analysis. Using standardised response mean, we validated sCD27 and IL7 as biomarkers with superior responsiveness to the two anti-inflammatory treatments. Both natalizumab and methylprednisolone treatment of progressive MS resulted in significant decreases of several CSF markers of intrathecal inflammation. CSF biomarkers of T- and B-cell inflammation, sCD27 and sCD21, showed the most significant and consistent decreases during both treatments, thus corroborating responsiveness to anti-inflammatory treatments and demonstrating marked effect on T- and B-cell intrathecal inflammation. However, the levels of sCD27 did not decrease to levels of healthy controls, suggesting that substantial residual T-cell inflammation persists even after high efficacy anti-inflammatory treatment. Our observation of sCD27 as a CSF biomarker capable of detecting residual inflammation is consistent with previous studies demonstrating residual intrathecal inflammation after anti-inflammatory treatment in progressive MS, and the reduction of -6% to -9% of CSF sCD27 is comparable to a reduction of -1% after bone-marrow transplantation and -0% after IV/intrathecal rituximab treatment (16;17). The marked decreases in IL12p40 substantiate the anti-inflammatory effect during both treatments, as this reduction reflects an inhibitory effect on immune cells of the myeloid lineage (7).

Some differences between natalizumab and methylprednisolone treatment effects were observed. The decrease in sCD27 and sCD21 in the present study are consistent with an effect of methylprednisolone pulse treatment on intrathecal inflammation in progressive MS, which was not detected using other CSF inflammatory markers (10). Methylprednisolone treatment increased CSF sCD163, corroborating *in vitro* observations that CD163 and an anti-inflammatory macrophage phenotype can be induced by glucocorticoids, suggesting that pulse methylprednisolone treatment promotes an anti-inflammatory phenotype of microglia and macrophages (18–20). The majority of CSF sCD163 is produced intrathecally, and consequently this finding likely reflects intrathecal effects on myeloid cells (21). This conclusion is supported by the observation that CSF cell counts were unchanged during methylprednisolone treatment and by the fact that glucocorticoids are known to cross the blood-brain barrier (10;22). Accordingly, the effects on both adaptive and innate intrathecal inflammation indicate direct effects of methylprednisolone on immune cells in the intrathecal compartment.

The lymphoproliferative cytokines IL7 and IL15 together with CCL2 increased during both treatments, and IL7 and CCL2 correlated negatively with tissue damage biomarkers. Collectively, these findings may reflect less consumption by reduced numbers and activation state of CSF immune cells, which has been demonstrated for IL7 and CCL2 (23;24).

Analyses of correlations between CSF biomarkers of inflammation, axonal damage and demyelination revealed marked association between intrathecal inflammation and tissue damage in the baseline samples, supporting previous cross-sectional CSF studies and histopathology studies in progressive MS (5;8;25). During treatment, we also observed correlation between CSF biomarkers of inflammation and NFL, a marker of axonal damage,

suggesting that residual, compartmentalised inflammation not responsive to current immunomodulatory treatments contributes to CNS tissue destruction in progressive MS. Notably, the correlation between CSF inflammation and NFL was also evident in the follow-up samples, highlighting that residual inflammation after anti-inflammatory treatment remains associated with axonal damage, which may at least partly explain the modest effects of current anti-inflammatory treatments in progressive MS. This is in line with the finding of increased sCD27/T-cell and sCD21/B-cell ratios in SPMS and PPMS patients compared to RRMS and healthy controls, indicating that T- and B-cells may be sequestered in the CNS tissue in progressive MS (7). Moreover, the association between residual intrathecal inflammation and NFL emphasise the relevance of the concept of compartmentalised inflammation in progressive MS, and indicates that future CSF biomarker studies of treatment responses in progressive MS may help to advance the development of progressive MS treatments.

Analysis of correlation between changes in clinical endpoints and changes in CSF inflammatory biomarkers did not show significant correlations, whereas change in CSF NFL correlated significantly with change in MSIS, demonstrating that treatment effects on CSF NFL are associated with clinical treatment effects. This observation is consistent with a longitudinal study demonstrating prognostic value of CSF NFL in progressive MS, while other studies, likely owing to limited statistical power and poor responsiveness of EDSS scale, did not demonstrate this relationship (26–29). This corroborates CSF NFL as a clinically meaningful biomarker of axonal damage, and the consistent correlations between CSF NFL and sCD27 underscores that axonal damage occurs in association with intrathecal inflammation in progressive MS. Larger, longitudinal and preferably placebo-controlled studies are needed to determine whether CSF sCD27 may be used as a surrogate endpoint of disease progression. To what extent these findings may translate into positive phase 3 trials is currently uncertain since the phase III study of natalizumab in SPMS did not meet its primary clinical endpoint after two years (30).

Several limitations of the study should be noted. The lack of an untreated control group confers uncertainty whether the effects on CSF biomarkers are directly related to treatment, or if the changes may also be a result of regression to the mean or the natural course of progressive MS, which have been indicated in a cross-sectional study (35). However, previous longitudinal studies demonstrated stability of a proportion of CSF biomarkers tested in this study in the absence of therapeutic intervention (8;17). Furthermore, both of the previously published treatment studies found treatment effects on MRI endpoints reflecting inflammation and tissue damage (9;10). Although CSF NFL is widely accepted as a biomarker of axonal damage owing to its known function in the axonal cytoskeleton, association with tissue damage on MRI scans and increased levels in a variety of neurodegenerative diseases, NFL has not been validated in MS post-mortem studies (36). Accordingly, the correlations between CSF biomarkers of inflammation and axonal damage should be confirmed using validated biomarkers of neurodegeneration (brain atrophy or OCT). The study did not allow concurrent CSF cell immunophenotyping which precludes us to calculate sCD27/T cell and sCD21/B cell ratios; thus the conclusions regarding residual compartmentalised inflammation are based on the previously demonstrated increased ratios in progressive MS patients and the level of compartmentalisation in progressive MS patient

may determine the level of efficacy (7). On the other hand, residual inflammation in progressive MS may decrease further during treatment beyond one year of follow-up, as CSF sCD27 demonstrated incremental decreases from month 3 to month 12 during rituximab treatment in PPMS patients (17). Consequently, future studies could assess the sCD27/T cell and sCD21/B cell ratios with multiple CSF samples beyond one year. The lack of functional assays limits the understanding of the phenotype of the residual inflammation. The changes observed during methylprednisolone treatment on myeloid lineage phenotype reflected by selective increase in sCD163 and IL5 levels along with the unchanged CSF cell counts suggests that the observed decreases in sCD27 and sCD21 represent effects on the phenotype of intrathecal T and B cells. Accordingly, future studies should assess to what extent methylprednisolone may target intrathecal compartmentalised inflammation. Finally, analyses of correlations between changes in clinical and CSF biomarkers were limited by the small sample size and the lack of sensitivity of the used clinical scores, especially EDSS, which are particularly insensitive in progressive MS (29).

In conclusion, we demonstrate CSF sCD27 to be highly responsive and capable of capturing substantial residual intrathecal inflammation after natalizumab and methylprednisolone treatment. Importantly sCD27 correlate with NFL both before and after treatment, demonstrating that intrathecal compartmentalised inflammation contributes to CNS tissue destruction also after anti-inflammatory treatment. Moreover the findings support that CSF biomarkers can be successfully utilized in drug development for progressive MS, providing evidence of highly statistically significant pharmacodynamic effects in the intrathecal compartment in small cohorts of patients and support their use in future progressive MS clinical trials.

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Figure 1.

Correlation matrix plots of CSF biomarkers of inflammation and axonal damage (neurofilament light chain (NFL)) and demyelination (myelin basic protein (MBP)). Significance levels are corrected with the false discovery rate method. Baseline and change plots are based on data from both natalizumab (NTZ) and methylprednisolone (MP) treated patients. Post-treatment plots are based on data from the individual trials with NTZ and MP.

Baseline characteristics. Values of continuous variables are given as medians with interquartile ranges in brackets.

Table 1

		N	N completed	Female/male	Age (years)	Progressive phase duration (years)	
NAPMS	PPMS	12	10	6/6	47 (38–52)	4 (3–7)	
	SPMS	12	7	7/5	44 (42–49)	6 (2–8)	
COMTMS	PPMS	15	11	11/4	53 (50–57)	8 (4–16)	
	SPMS	15	14	7/8	49 (41–52)	4 (3–8)	

		N	CSF cell count increased (>5 per µL)	IgG index increased (>0.67)	CSF/serum albumin quotient increased (>10.2)	Patients with CEL (N)
NAPMS	PPMS	12	4	8	0	1
	SPMS	12	2	9	0	3
COMTMS	PPMS	15	6	7	2	0
	SPMS	15	10	9	0	3

Table 2

Overview of analytes and technical performance.

	Limit of detection	Intra-assay CV (median %)	Detection rate (baseline)	Standardised response mean (natalizumab)	Standardised response mean (methylprednisolone)
Soluble cellular markers (NIH)					
sCD27	0.4 U/ml	3.13	54/54	-1.03	-1.00
sCD21	5.8 pg/ml	2.16	54/54	-0.98	-0.54
sCD14	0.49 (ng/ml)	2.45	54/54	-0.44	-0.09
sCD163	0.85 (ng/ml)	2.66	54/54	-0.41	0.78
Chitinase (DMSC)					
CHI3L1	3.55 (pg/ml)	3.54	53/53	-0.46	-0.25
Cytokines (DMSC)					
IL5	0.22 (pg/ml)	10.51	53/53	0.79	0.57
IL6	0.06 (pg/ml)	3.89	54/54	-0.30	0.38
IL7	0.16 (pg/ml)	6.51	52/53	2.21	0.91
IL8	0.04 (pg/ml)	2.18	54/54	-0.60	0.13
IL10	0.03 (pg/ml)	10.50	39/53	-0.72	-0.28
IL12p40	0.39 (pg/ml)	6.32	53/53	-0.74	-0.61
IL15	0.17 (pg/ml)	3.40	53/53	0.51	0.99
IL16	2.83 (pg/ml)	4.24	53/53	-0.05	0.20
TNF- α	0.04 (pg/ml)	10.11	49/54	-0.74	-0.16
VEGF	1.12 (pg/ml)	4.80	53/53	-0.14	-0.12
Chemokines (DMSC)					
CCL2	0.09 (pg/ml)	3.48	54/54	0.57	0.56
CCL4	0.37 (pg/ml)	3.94	54/54	0.03	-0.28
CCL11	3.26 (pg/ml)	8.34	40/54	-0.24	0.12
CCL22	1.22 (pg/ml)	9.68	50/54	-0.52	-0.29
CXCL10	0.37 (pg/ml)	3.77	54/54	-0.74	-0.46
Excluded biomarkers					
Undetectable	GM-CSF, IL1A, IL1B, IL2, IL-4, IL-12p70, IL-13				
Low abundance	IL-17, INF-g, TNF-b				
High CV	CCL3, CCL17, CCL26				

Limits of detection values are determined in-house for the National Institute of Health (NIH) analyses and provided by the manufacturer for the Danish multiple Sclerosis Center (DMSC) analyses. Intra-assay coefficients of variation (CV) were determined from duplicate means and standard deviations.

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Table 3

CSF inflammatory biomarker levels at baseline and change during natalizumab and methylprednisolone treatment.

Natalizumab trial												Methylprednisolone trial					Change natalizumab vs methylprednisolone				
Paired samples (N)						Baseline Mean (95% CI)		Change Mean (95% CI)		p value change		q value change		Baseline Mean (95% CI)		Change Mean (95% CI)		p value change		q value change	
Soluble cellular markers (NIH)																					
sCD27 (U/ml)		17	77 (48 to 110)		-28 (-43 to -16)	0.00002*	0.0002	23	44 (31 to 57)		-17 (-24 to -10)	0.0000002*	0.00001	0.13*	0.15						
sCD21 (pg/ml)		17	192 (137 to 259)		-59 (-93 to -32)	0.00002*	0.0002	23	199 (143 to 274)		-76 (-151 to -30)	0.00001*	0.0001	0.81*	0.46						
sCD14 (ng/ml)		17	80 (65 to 94)		-7 (-15 to 1)	0.097	0.14	23	99 (88 to 111)		-1.2 (-7 to 4)	0.69*	0.48	0.22*	0.20						
sCD163 (ng/ml)		17	14 (12 to 16)		-1.5 (-2.9 to -0.2)	0.045	0.09	23	17 (15 to 20)		7.4 (3.9 to 12)	0.000006*	0.0001	0.000005*	0.0001						
Chitinases (DMSC)																					
CHI3L1 (pg/ml)		17	219 (144 to 323)		-45 (-102 to -1)	0.19*	0.22	21	194 (164 to 227)		-15 (-42 to 9)	0.30	0.29	0.32*	0.26						
Cytokines (DMSC)																					
IL5 (pg/ml)		15	0.33 (0.27 to 0.38)		0.06 (0.02 to 0.10)	0.01	0.03	21	0.65 (0.36 to 1.2)		0.26 (0.09 to 0.51)	0.0001*	0.001	0.05*	0.08						
IL6 (pg/ml)		17	1.2 (0.82 to 1.8)		-0.24 (-0.80 to 0.08)	0.24*	0.26	23	0.88 (0.73 to 1.1)		0.14 (-0.02 to 0.31)	0.09	0.14	0.06*	0.09						
IL7 (pg/ml)		15	0.65 (0.51 to 0.77)		1.28 (1.0 to 1.6)	0.000001	0.00002	21	0.63 (0.54 to 0.74)		0.22 (0.11 to 0.34)	0.001	0.005	0.000005	0.0001						
IL8 (pg/ml)		17	41 (36 to 45)		-5.0 (-9.0 to -1.1)	0.03	0.07	23	42 (35 to 51)		2.8 (-7.2 to 12)	0.22*	0.24	0.02*	0.03						
IL10 (pg/ml)		17	0.17 (0.08 to 0.28)		-0.09 (-0.16 to -0.03)	0.003*	0.01	23	0.17 (0.05 to 0.37)		-0.10 (-0.31 to 0.01)	0.35*	0.32	0.06*	0.08						
IL12p40 (pg/ml)		15	9.6 (4.1 to 16)		-7.6 (-14 to -2.2)	0.0003*	0.002	21	5.9 (3.6 to 9.0)		-2.5 (-4.3 to -0.81)	0.009*	0.028	0.20*	0.19						
IL15 (pg/ml)		15	1.9 (1.7 to 2.1)		0.21 (0.00 to 0.41)	0.08	0.13	21	1.9 (1.7 to 2.2)		0.32 (0.17 to 0.45)	0.0003	0.0019	0.4	0.30						
IL16 (pg/ml)		15	7.3 (5.9 to 8.7)		-0.12 (-1.3 to 1.0)	0.87	0.54	21	7.5 (6.4 to 8.5)		0.34 (0.41 to 1.1)	0.37	0.33	0.52	0.36						
TNF-a (pg/ml)		17	0.27 (0.18 to 0.37)		-0.11 (-0.18 to -0.04)	0.007*	0.02	23	0.24 (0.16 to 0.31)		-0.03 (-0.11 to 0.04)	0.60*	0.45	0.09*	0.12						
VEGF (pg/ml)		15	3.1 (2.6 to 3.8)		-0.10 (-0.5 to 0.2)	0.85*	0.54	21	3.0 (2.6 to 3.4)		-0.12 (-0.59 to 0.28)	0.89*	0.55	0.97*	0.51						
Chemokines (DMSC)																					
CCL2 (pg/ml)		17	307 (263 to 355)		36 (5 to 69)	0.04	0.08	23	385 (304 to 466)		52 (9.4 to 88)	0.01*	0.02	0.08*	0.11						
CCL4 (pg/ml)		17	15 (12 to 19)		0.12 (-1.7 to 2.2)	0.90	0.55	23	41 (15 to 90)		-23 (-72 to 0.5)	0.78*	0.51	0.85*	0.48						
CCL11 (pg/ml)		17	10 (5.8 to 16)		-2.2 (-6.9 to 2.2)	0.35	0.32	23	11 (8.0 to 14)		0.87 (-2.3 to 3.6)	0.55	0.43	0.24	0.22						
CCL22 (pg/ml)		17	49 (24 to 76)		-28 (-55 to -2.1)	0.06	0.11	23	28 (18 to 38)		-8.0 (-20 to 3.2)	0.18	0.21	0.21	0.20						
CXCL10 (pg/ml)		17	851 (504 to 1242)		-340 (-598 to -122)	0.004*	0.01	23	811 (567 to 1090)		-205 (-405 to -32)	0.26*	0.27	0.17*	0.18						

P-values are from parametric and non-parametric (*) tests. Q-values represent false discovery rate correction for multiple testing.