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Cerebrospinal fluid inflammatory markers in patients with multiple sclerosis: a pilot study

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Abstract Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. Autoimmune inflammation is common in the early stages of MS. This stage is followed by the neurodegenerative process. The result of these changes is axon and myelin breakdown. Although MS is according to McDonald's revised diagnostic criteria primarily a clinical diagnosis, paraclinical investigation methods are an important part in the diagnosis of MS. In common practice, magnetic resonance imaging of the brain and spinal cord, examination of cerebrospinal fluid (CSF) and examination of visual evoked potentials are used. There are an increasing number of studies dealing with biomarkers in CSF and their role in the diagnosis and treatment of MS. We hypothesized that the levels of some markers could be changed in MS in comparison with controls. We studied five inflammatory markers [interleukin-6 (IL-6), interleukin-8, interleukin-10 (IL-10), beta-2-microglobulin, orosomucoid]. CSF and serum levels of inflammatory markers were assessed in 38 patients with newly diagnosed MS meeting McDonald's revised diagnostic criteria and in 28 subjects as a control group (CG). Levels of beta-2-microglobulin and interleukin-8 in CSF were found to be significantly higher in MS patients in comparison to CG ($p < 0.001$ resp. $p = 0.007$). No differences in other CSF markers (IL-6, IL-10 and orosomucoid) and serum levels of all markers between both groups were found. The levels of two studied inflammatory markers were found to be increased at the time of first clinical symptoms of MS. Research on the role of inflammatory and neurodegenerative markers in MS should continue.

Keywords Interleukin-6 · Interleukin-8 · Interleukin-10 · Beta-2-microglobulin · Orosomucoid

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Introduction

Multiple sclerosis is an inflammatory demyelinating disease of the central nervous system. The inflammation is caused by autoimmune processes especially appearing in the early stages of MS. The neurodegenerative processes appear mainly in later stages of MS. The disease is characterized by many inflammatory infiltrates especially in the white matter, where T-lymphocytes and macrophages are activated (also fewer B-lymphocytes). T-lymphocytes are probably activated in the periphery. After activation these specific lymphocytes proliferate into the final tissue and initiate the inflammatory lesion by the production of many pro-inflammatory cytokines. The damage of myelin and oligodendrocytes is followed in this lesion. The demyelinating fibre loses the ability to spread electric impulses, and the cells of major histocompatibility complex I (MHC I) are also expressed. Through these processes the fibre becomes visible for CD8 cytotoxic lymphocytes, which damage this fibre. The neurodegeneration is caused by microglia's activation and mediated by oxidative stress and excitotoxicity. The axon loss in white and grey matter is

present in early stages of MS and correlates with the cognitive deficit and also with the permanent disability.

Generally, relapses are the clinical equivalent of the acute focal inflammation, and progression is caused by chronic neurodegeneration. The standard diagnostic test in patients with suspected MS is the examination of the intrathecal synthesis of IgG antibodies. However, it is important to discover the more detailed mechanism causing MS. One of these methods is the examination of CSF and levels of inflammatory and neurodegenerative markers in CSF.

The aim of our study was to ascertain levels of some inflammatory markers in MS patients in comparison to the control group. Based on the results of previous studies and the availability of testing in our laboratory, we chose five inflammatory markers (IL-6, IL-8, IL-10, beta-2 microglobulin, orosomucoid) to investigate their CSF levels at the time of first clinical symptoms of MS.

Methods

The study was approved by the Institutional Ethics Committee, and all patients have signed an informed consent to lumbar puncture.

The study was based on CSF and serum examination in patients with MS and the control group. In the control group were patients with non-inflammatory disease of CNS (e.g., headache, vertigo, back pain, etc.). The lumbar puncture was indicated from differential diagnostic reasons, to exclude other pathological processes of CNS. In the MS patients, the lumbar puncture was indicated and performed for diagnostic purposes in cases suggestive of MS, at the time of the first clinical symptoms compatible with MS. The diagnosis of MS was confirmed in all patients included in MS group. All patients included in the MS group fulfilled the McDonald's revised diagnostic criteria. None of our patients had been treated by corticosteroids before the lumbar puncture.

The lumbar puncture was performed by means of atraumatic needle under aseptic conditions. About 10 ml of CSF was taken along with around 10 ml of blood from each participant. The serum and CSF were the subject of haematological, immunological and biological testing. The protein CSF level and cell count were normal in all patients, without signs of neuroinflammation. There were also no signs of any other inflammation at the time of lumbar puncture.

Biomarker selection and measurement

CSF and serum were analyzed to assess the levels of five inflammatory markers: interleukin-6, interleukin-8, interleukin-10, beta-2-microglobulin, orosomucoid, available for testing in our laboratory at the study onset.

The principle of measurement of IL-6 levels used in our study was ELISA (solid-phase, enzyme-labelled, chemiluminescent sequential immunometric assay, IMMULITE®/IMMULITE® 1000 IL-6, Catalogue Number: LK6PZ, LK6P1). Incubation Cycles: 2 × 30 min. Analytical sensitivity: 2 pg/ml. Reference limits: CSF 0–18 pg/ml, serum 0–5.9 pg/ml.

For measurement of IL-8 ELISA was also used (IMMULITE®/IMMULITE® 1000 IL-8, Catalogue Number LK8P1). Incubation Cycles: 1 × 30 min. Analytical sensitivity: 2 pg/ml. Reference limits: CSF 0–62 pg/ml, serum 0–62 pg/ml.

The same method was used also for measurement levels of IL-10. IMMULITE®/IMMULITE® 1000 IL-10, Catalogue Number: LKXPZ, LKXP1. Incubation Cycles: 1 × 60 min. Analytical sensitivity: 1 pg/ml. Reference limits: CSF 0–39 pg/ml, serum: 0–9.1 pg/ml.

Levels of beta-2-microglobulin were measured by particle-enhanced immunonephelometry using the BN* II and BN ProSpec® System. Reference limits: CSF 0.2–2 mg/l, serum 1–3 mg/l. Coefficients of variation (CV): CSF 2.7 %, serum 2.7 %.

Levels of orosomucoid were also measured by particle-enhanced immunonephelometry using the BN* II and BN ProSpec® System. Reference limits: CSF 1.8–4.5 mg/l, serum 0.3–1.3 g/l. Coefficients of variation (CV): CSF 3.3 %, serum 3.8 %.

Statistical analysis

Then, the results were statistically processed using SPSS software version 15 (SPSS Inc., Chicago, USA). The categorical data were compared by Fisher's exact test.

The Mann–Whitney *U* test was used to compare quantitative parameters. The age was compared by the Student's *t* test. The normality of data was checked by the Shapiro–Wilk test. Tests were made at the significance level of 0.05.

Results

We investigated a group of 66 patients. CSF and serum examinations were performed in 38 patients with newly diagnosed MS meeting McDonald's revised diagnostic criteria (25 females, 13 males; mean age 35.8 ± 9.1 years) and in 28 control group patients (20 females, 8 males; mean age 39.7 ± 13.0 years). No statistically significant differences in demographic data between patients and control group were found. The demographic data are shown in Table 1.

Significantly higher CSF levels of IL-8 (52.8 (41.6–68.3); $p < 0.001$, Mann–Whitney *U* test) and beta-2-

microglobulin (1.26 (1.03–1.37); $p = 0.007$, Mann–Whitney U test) in MS patients group were found. No differences in other CSF markers (IL-6, IL-10 and orosomucoid) and in serum levels of all markers between both groups were found (Tables 2, 3; Figs. 1, 2).

Table 1 Characteristic of patients

	MS ($n = 38$)	Controls ($n = 28$)	p
Females	25 (65.8 %)	20 (71.4 %)	0.790
Age	35.8 ± 9.1	39.7 ± 13.0	0.182

Discussion

We identified increased levels of IL-8 and beta-2 microglobulin in CSF. These results confirm the presence of inflammatory processes at time of the first clinical symptoms of MS.

There have been multiple studies investigating the levels of inflammatory markers in the cerebrospinal fluid in MS. Their results are not entirely consistent, which probably results from the great clinical diversity of the studied populations. There are studies which examine a wider range of biomarkers, which are not included in our research and have interesting results. Martins et al.

Table 2 Levels of investigated markers in CSF

Levels	Multiple sclerosis Median ± SD (min–max)	Control group Median ± SD (min–max)	p
IL-6 (pg/ml)	2.1 ± 1.48 (2.0–9.1)	2.1 ± 0.83 (2.0–5.8)	0.607
IL-8 (pg/ml)	52.8 ± 15.65 (32.5–92.3)	36.8 ± 9.11 (24.1–60.2)	<0.0001
IL-10 (pg/ml)	5.0 ± 0.58 (5.0–8.4)	5.0 ± 0.12 (5.0–5.6)	0.841
Beta-2-microglobulin (mg/l)	1.26 ± 0.22 (0.91–1.82)	0.96 ± 0.34 (0.6–2.03)	0.007
Orosomucoid (mg/l)	4.57 ± 2.57 (0.0–9.29)	5.00 ± 2.44 (0.0–10.1)	0.348

SD standard deviation

Table 3 Levels of investigated markers in serum

Levels	Multiple sclerosis Median ± SD (min–max)	Control group Median ± SD (min–max)	p
IL-6 (pg/ml)	2.0 ± 1.54 (2.0–9.5)	2.0 ± 0.78 (2.0–5.0)	0.489
IL-8 (pg/ml)	7.7 ± 36.74 (5.0–232.0)	7.2 ± 7.16 (5.0–42.3)	0.896
IL-10 (pg/ml)	5.0 ± 2.36 (5.0–18.1)	5.0 ± 1.09 (5.0–10.7)	0.228
Beta-2-microglobulin (mg/l)	1.53 ± 0.63 (1.26–5.12)	1.61 ± 0.56 (1.02–3.97)	0.626
Orosomucoid (g/l)	0.76 ± 0.24 (0.41–1.28)	0.77 ± 0.24 (0.47–1.58)	0.311

SD standard deviation

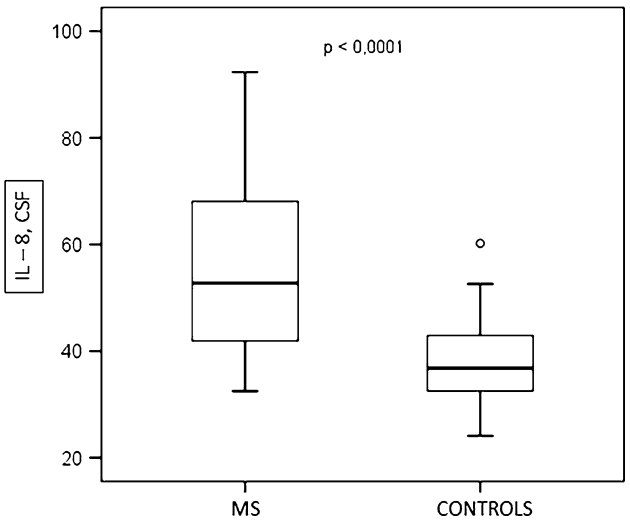

Fig. 1 Box graph showing statistically significantly higher levels of interleukin-8 in MS patients in comparison to controls

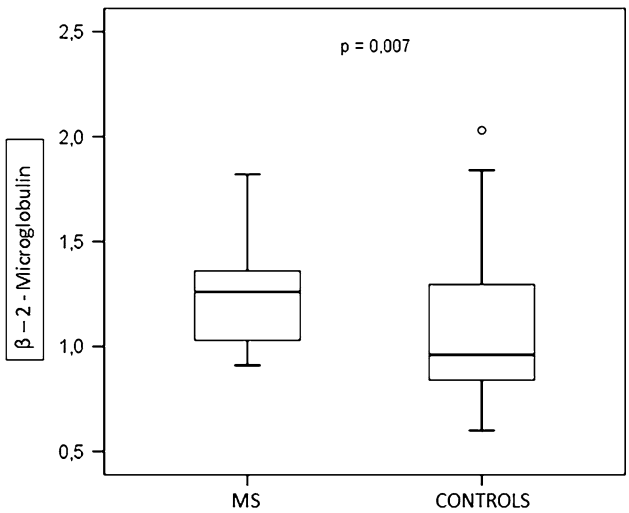

Fig. 2 Box graph showing statistically significantly higher levels of beta-2-microglobulin in MS patients in comparison to controls

(2011) analyzed the serum concentrations of 13 proinflammatory and anti-inflammatory markers. Significant increases between patients and control subjects were found in seven of them. Another frequently discussed marker is the cytokine interleukin-23 (IL-23). IL-23 was found significant elevated in study by Wen et al. (2012). The study by Vaknin-Dembinsky et al. (2006) reported an increase in the levels of IL-23 secreted from monocyte-derived dendritic cells as well as increased mRNA production of both IL-23p19 in MS patients. There is also a more comprehensive study by Modvig et al. (2013) dealing with the interrelation among biomarkers of inflammation, demyelination and neurodegeneration in acute optic neuritis and to assess their association with MS risk. In that study, it was found that the CSF biomarkers of leukocyte infiltration (CXCL13, MMP-9, CXCL10) are associated strongly with MS-risk parameters, whereas CHI3L1 and MBP correlated with MRI findings (dissemination in space), but not with CSF MS-risk parameters and osteopontin and NF-L do not correlate with any MS-risk parameters. However, in the following discussion, we will focus on the markers we have included in our study.

Beta-2 microglobulin is the part of histocompatibility antigens on cell surfaces. It plays an important role in intercellular interactions and is used as a cell turnover marker. It is mostly found on white blood cells (especially B lymphocytes). Beta-2- microglobulin has been found to correlate with disease activity in several autoimmune disorders. It is also used as a pharmacodynamic marker of interferon beta, which is used for treating MS patients at the present time (Bagnato et al. 2003; Bjerrum et al. 1988). This outcome correlates with our conclusion. However, no other studies showed differences in serum and CSF levels of beta-2 microglobulin in MS patients in comparison to controls (Sladkova et al. 2011; Carrieri et al. 1992). As a result, there are inconsistent results concerning beta-2-microglobulin in MS patients today. Additional studies of beta-2-microglobulin are needed.

Interleukin-8 (IL-8) is an inflammatory chemokine. It attracts and activates neutrophils and a subpopulation of lymphocytes. It also has a strong angiogenic effect. Interleukin-8 is produced by many cells such as monocytes, lymphocytes, granulocytes, fibroblasts, endothelial cells and astrocytes. It is elevated in CSF in inflammatory processes of CNS (Bielekova et al. 2012). Interleukin-8 is also responsible for blood–brain barrier disruption and the migration of immune cells to the CNS. According to one very interesting study on the effect of high-dose corticosteroids on levels of interleukin-8, the blood levels of interleukin-8 decrease after therapy (Mirowska-Guzel et al. 2006). The serum levels are also decreased in MS patients receiving interferon beta 1a therapy in comparison to untreated MS patients (Lund et al. 2004). Two other studies also confirm our results, and their authors found a significant rise of CSF levels of IL-8 in MS during relapse (Bartosik-Psujek and Stelmasiak 2005; Franciotta et al. 2001). The fact that IL-8 is associated with disease activity was used in other research. Neuteboom et al. (2009) researched levels of IL-8 in MS patients during pregnancy. High serum levels of IL-8 during the first trimester were associated with a high risk of postpartum relapses. Another study compared levels of interleukins in two types of MS (opticospinal and conventional). Elevated levels of IL-8 were found in both groups. However, in opticospinal MS the levels were significantly higher than in conventional MS. According to this study, elevated levels of IL-8 also correlate with Expanded Disability Status Scale (EDSS) (Ishizu et al. 2005). All of these studies suggest that IL-8 is an important chemokine in the inflammatory phase of MS. The results of our study also confirm these findings.

We found no statistically significant levels of the other three biomarkers (IL-6, IL-10, orosomucoid) in our study.

Interleukin-6 (IL-6) is the proinflammatory cytokine. It might be responsible for immunoglobulin (Ig) synthesis in the CNS (Maimone et al. 1991). Low levels of IL-6 were found by Maimone et al. (1991) in most sera from MS patients and also in patients with non-inflammatory neurological disease. Other results of this study indicate increased CSF levels of IL-6 in MS patients and also in patients with inflammatory neurological disease, without correlation to intrathecal synthesis of IgG. Some degree of dependence between serum IL-6 concentration and the level of EDSS were ascertained by Stelmasiak et al. (2000).

Interleukin-10 (IL-10) is an anti-inflammatory cytokine. B cells producing IL-10 regulate autoimmune diseases, including MS (Hirotani et al. 2010). There are some studies dealing with therapy of MS by interferons and glatiramer acetate (GA) and their effect on the production of some cytokines, including IL-10. The study by Pul et al. (2011) shows the effect of GA on CNS. Among other things, increased secretion of IL-10 after therapy by GA was found.

The small molecule of orosomucoid is able to transgress the blood–brain barrier (BBB). Its concentration increases in malignant CNS processes (Sladkova et al. 2011). The increased CSF levels can occur in some MS patients (50–60 %) after several years of disease duration (Adam et al. 2003). Decreased levels of orosomucoid in secondary progressive MS were found in the same study.

In the future, we intend to broaden the spectrum of available inflammatory markers including, e.g., IL-23 and also to include comparison with patients suffering from other inflammatory and infectious diseases of the nervous

system, such as meningitis, encephalitis and inflammatory demyelinating polyradiculoneuropathy.

Conclusion

The levels of two studied inflammatory markers were found to be increased at the time of first clinical symptoms of MS. The increased levels of IL-8 were also confirmed in other studies. Thus, IL-8 seems to be an important chemokine in the inflammatory phase of MS. The second marker with elevated levels was beta-2-microglobulin, for which, however, other studies in MS patients have yielded inconsistent results. As the aetiology of MS is still unknown, research on inflammatory and neurodegenerative markers in MS should continue.

Conflict of interest The authors declare that they have no conflicts of interest.

References

Adam P, Sobek O, Táborský L, Hildebrand T, Tutterová O, Zácek P (2003) CSF and serum orosomucoid (alpha-1-acid glycoprotein) in patients with multiple sclerosis: a comparison among particular subgroups of MS patients. Clin Chim Acta 334(1–2): 107–110

Bagnato F, Durastanti V, Finamore L, Volante G, Millefiorini E (2003) Beta-2 microglobulin and neopterin as markers of disease activity i multiple sclerosis. Neurol Sci 24:S301–S304

Bartosik-Psujek H, Stelmasiak Z (2005) The levels of chemokines CXCL8, CCL2 and CCL5 in multiple sclerosis patients are linked to the activity of the disease. Eur J Neurol 12(1):49–54

Bielekova B, Komori M, Xu Q, Reich DS, Wu T (2012) Cerebrospinal fluid IL-12p40, CXCL13 and IL-8 as a combinatorial biomarker of active intrathecal inflammation. PLoS ONE 7(11): e48370

Bjerrum OW, Bach FW, Zeeberg I (1988) Increased level of cerebrospinal fluid beta 2-microglobulin is related to neurologic impairment in multiple sclerosis. Acta Neurol Scand 78(1): 72–75

Carrieri PB, Indaco A, Maiorino A, Buscaino GA, Ponticiello A, Liberti A, Perrella O (1992) Cerebrospinal fluid beta-2-microglobulin in multiple sclerosis and AIDS dementia complex. Neurol Res 14(3):282–283

Franciotta D, Martino G, Zardini E, Furlan R, Bergamaschi R, Andreoni L, Cosi V (2001) Serum and CSF levels of MCP-1 and IP-10 in multiple sclerosis patients with acute and stable disease and undergoing immunomodulatory therapies. J Neuroimmunol 115(1–2):192–198

Hirotani M, Niino M, Fukazawa T, Kikuchi S, Yabe I, Hamada S, Tajima Y, Sasaki H (2010) Decreased IL-10 production mediated by Toll-like receptor 9 in B cells in multiple sclerosis. J Neuroimmunol 221(1–2):95–100

Ishizu T, Osoegawa M, Mei FJ, Kikuchi H, Tanaka M, Takakura Y, Minohara M, Murai H, Mihara F, Taniwaki T, Kira J (2005) Intrathecal activation of the IL-17/IL-8 axis in opticospinal multiple sclerosis. Brain 128(Pt 5):988–1002

Lund BT, Ashikian N, Ta HQ, Chakryan Y, Manoukian K, Groshen S, Gilmore W, Cheema GS, Stohl W, Burnett ME, Ko D, Kachuck NJ, Weiner LP (2004) Increased CXCL8 (IL-8) expression in multiple sclerosis. J Neuroimmunol 155(1–2): 161–171

Maimone D, Gregory S, Arnason BG, Reder AT (1991) Cytokine levels in the cerebrospinal fluid and serum of patients with multiple sclerosis. J Neuroimmunol 32(1):67–74

Martins TB, Rose JW, Jaskowski TD, Wilson AR, Husebye D, Seraj HS, Hill HR (2011) Analysis of proinflammatory and anti-inflammatory cytokine serum concentrations in patients with multiple sclerosis by using a multiplexed immunoassay. Am J Clin Pathol 136(5):696–704

Mirowska-Guzel DM, Kurowska K, Skierski J, Koronkiewicz M, Wicha W, Kruszewska J, Czlonkowski A, Czlonkowska A (2006) High dose of intravenously given glucocorticosteroids decrease IL-8 production by monocytes in multiple sclerosis patients treated during relapse. J Neuroimmunol 176(1–2): 134–140 Epub 2006 May 24

Modvig S, Degn M, Horwitz H, Cramer SP, Larsson HB, Wanscher B, Sellebjerg F, Frederiksen JL (2013) Relationship between cerebrospinal fluid biomarkers for inflammation, demyelination and neurodegeneration in acute optic neuritis. PLoS One 8(10):e77163. doi:10.1371/journal.pone.0077163

Neuteboom RF, Verbraak E, Voerman JS, van Meurs M, Steegers EA, de Groot CJ, Laman JD, Hintzen RQ (2009) First trimester interleukin 8 levels are associated with postpartum relapse in multiple sclerosis. Mult Scler 15(11):1356–1358

Pul R, Moharregh-Khiabani D, Škuljec J, Skripuletz T, Garde N, Voss EV, Stangel M (2011) Glatiramer acetate modulates TNF-α and IL-10 secretion in microglia and promotes their phagocytic activity. J Neuroimmune Pharmacol 6(3):381–388

Sladkova V, Mares J, Lubenova B, Zapletalova J, Stejskal D, Hlustik P, Kanovsky P (2011) Degenerative and inflammatory markers in the cerebrospinal fluid of multiple sclerosis patients with relapsing–remitting course of disease and after clinical isolated syndrome. Neurol Res 33(4):415–420

Stelmasiak Z, Kozioł-Montewka M, Dobosz B, Rejdak K, Bartosik-Psujek H, Mitosek-Szewczyk K, Belniak-Legieć E (2000) Interleukin-6 concentration in serum and cerebrospinal fluid in multiple sclerosis patients. Med Sci Monit 6(6):1104–1108

Vaknin-Dembinsky A, Balashov K, Weiner HL (2006) IL-23 is increased in dendritic cells in multiple sclerosis and down-regulation of IL-23 by antisense oligosincreases dendritic cell IL-10 production. J Immunol 176(12):7768–7774

Wen SR, Liu GJ, Feng RN, Gong FC, Zhong H, Duan SR, Bi S (2012) Increased levels of IL-23 and osteopontin in serum and cerebrospinal fluid of multiple sclerosis patients. J Neuroimmunol 244(1–2):94–96