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Exploring the relationship between Endothelin-1 and peripheral inflammation in multiple sclerosis

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ABSTRACT

Background: Identifying pathways linking neuroinflammation and neurodegeneration is essential to help prevent disability progression in people with multiple sclerosis (MS). Endothelin-1 (ET-1) is a potent vasoconstrictor thought to contribute to cerebral hypoperfusion and tissue damage in MS. Its link with the neuroinflammatory process remains poorly investigated.

Objectives: To determine plasma ET-1 levels in treatment-naïve people with MS and controls, and the relationship between ET-1 and other peripheral immune mediator levels as potential markers of the disease process.

Methods: This is a retrospective study that included specimens previously collected from 35 treatment-naïve patients with clinically isolated syndrome highly suggestive of MS or definite MS and 35 sex- and age-matched controls. ET-1 plasma levels were measured by enzyme-linked immunosorbent assay (ELISA), and plasma cytokine levels [interleukin (IL)-1beta, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12(p70), IL-13, interferon (IFN)-γ and tumor necrosis factor (TNF)-α] were simultaneously measured by Multiplex assay.

Results: ET-1 levels were significantly increased in MS patients compared to controls. No significant difference in cytokine levels between the groups were found. However, a significant increase in IFN-γ/IL-4 ratio was observed in patients with MS in comparison with controls, suggestive of Th1 skewed response. Binary logistic regression was performed to ascertain the effects of age, sex, ET-1 and cytokine levels on the likelihood of MS diagnosis. In the final model, ET-1, IL-4 and IFN-γ levels remained as predictors of MS. There was no significant correlation between ET-1 and cytokine levels.

Conclusions: Patients with MS presented increased levels of ET-1 and an immune response biased towards a Th1 profile. Although both ET-1 and Th1 cytokine profile were predictors of MS diagnosis, ET-1 levels were not associated with peripheral immune markers, suggesting that these changes may occur independently.

1. Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS), with great variability in clinical manifestations, involving motor, sensory, visual and autonomic systems (Compston and Coles, 2008). Depending on the clinical course, MS can be divided into four different phenotypes: i) clinically isolated syndrome (CIS): a first clinical episode with patient-reported symptoms associated with inflammatory demyelination that could be MS, but has yet to fulfill criteria of dissemination in time; ii) relapsing-remitting MS (RRMS): the most common disease course, characterized by relapses (attacks of new or increasing neurologic symptoms) with stable neurological disability between episodes; iii) primary progressive MS (PPMS): characterized by worsening neurologic function (accumulation of disability) from the onset of symptoms, without early relapses or remissions; and iv) secondary progressive MS (SPMS): a progressive course following an initial relapsing-remitting course (Thompson et al., 2018).

The peak age of disease onset is between 20 and 40 years, making MS one of the most common causes of neurological disability among young adults. The pathological hallmarks of MS are focal lesions characterized by demyelination, inflammation and diffuse axonal degeneration throughout the CNS. Although MS is pathologically well

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characterized, its pathogenesis remains poorly understood. The most accepted hypothesis is that the destruction of the CNS myelin results from autoimmune processes. However, little is known about the mechanisms linking inflammatory insult and neuroaxonal damage in vivo (Dendrou et al., 2015; Pérez-Cerdá et al., 2016).

Recent epidemiological and neuroimaging studies have reported vascular abnormalities in patients with MS [reviewed in (D'Haeseleer et al., 2011)]. Patients with MS have an increased risk of developing ischemic stroke (Tseng et al., 2015). Imaging studies reported a decrease in cerebral blood flow (CBF) that affects widespread areas including normal-appearing white and grey matter (Aviv et al., 2012, D'Haeseleer et al., 2011, Debernard et al., 2014, Narayana et al., 2014, Vitorino et al., 2016). Cerebral hypoperfusion in MS has been associated with chronic hypoxia, focal lesion formation, diffuse axonal degeneration, and clinical symptoms such as cognitive impairment and fatigue. There is mounting evidence that elevated levels of the potent vasoconstrictor peptide endothelin-1 (ET-1) in the cerebral circulation may drive CBF changes (D'Haeseleer et al., 2013; D'Haeseleer et al., 2015). ET-1 is normally produced by vascular endothelial cells and abluminally released to regulate local vascular tone. A small amount of ET-1 is released intraluminally, contributing to ET-1 levels in the circulating blood. Under some conditions such as inflammation and hypoxia, other cells also produce ET-1, thus increasing its levels in the blood (Flammer and Konieczka, 2017; Hostenbach et al., 2016; Houde et al., 2016), and studies have shown increased levels of ET-1 in MS patients in comparison with controls in tandem with vascular dysregulation (D'Haeseleer et al., 2013; Pache et al., 2003).

It has become critical to identify pathways linking neuroinflammation and neurodegeneration to help prevent disability progression in people with MS. Although ET-1 is thought to contribute to cerebral hypoperfusion and tissue damage in MS, its relationship with the inflammatory/immune process remains poorly investigated. We hypothesized that ET-1 changes may be associated with the Th1 shifted immune response observed in MS. This study was designed to investigate the relationship between ET-1 and peripheral cytokine levels in patients with MS.

2. Methods

2.1. Subjects and specimens

This retrospective study included specimens from 35 patients diagnosed with CIS highly suggestive of MS or definite MS according to the 2005 revision of the McDonald criteria (Polman et al., 2005), and a group of 35 sex- and age-matched healthy controls. Plasma samples were previously obtained from our collection of banked specimens from treatment-naïve patients with CIS or MS followed in the Multiple Sclerosis Research Group, McGovern Medical School, UTHealth. Specimens were stored in aliquots at −80 °C until assayed. All specimen collection was approved by the Committee for Protection of Human Subjects at UTHealth.

Patients had not experienced a clinical relapse or received any systemic steroid therapy during the past 3 months. Controls were recruited from the community and included if they had no previous diagnosis of neurological disorder or autoimmune disease. Demographic information was available for enrolled participants, as well as disease phenotype and Expanded Disability Status Scale (EDSS) scores (Kurtzke, 1983) for MS patients.

2.2. Endothelin-1 and cytokine level assessment

Plasma samples were thawed and the levels of ET-1 and cytokines were measured as routinely performed in our laboratory.

ET-1 levels were measured by enzyme-linked immunosorbent assay (ELISA), according to the procedures supplied by the manufacturer (Endothelin-1 Quantikine ELISA Kit, R&D systems, Minneapolis, MN).

Multiple cytokines [interleukin (IL)-1beta, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12(p70), IL-13, interferon (IFN)-γ and tumor necrosis factor (TNF)-α] were simultaneously measured by Multiplex assay, using the Bio-Plex Precision Pro Human Cytokine 10-Plex Panel (Bio-Rad Laboratories, Hercules, CA).

The detection limits were 0.2 pg/mL for ET-1 and ≤ 1 pg/mL for the cytokines. Concentrations are expressed as pg/mL.

2.3. Statistical analysis

Associations between dichotomous variables were assessed with the Fisher's exact-test. All continuous variables were tested to assess whether they follow a Gaussian distribution using the Shapiro-Wilk normality test. Two groups (MS vs. controls) were compared using the Student's t-test or the Mann–Whitney *U* test when data were determined to follow or not a normal distribution, respectively. Spearman's correlation analyses were performed to examine the relationship between age and plasma levels of ET-1 or cytokines. Lastly, a binary logistic regression (backward stepwise approach) was performed to ascertain the effects of age, sex, ET-1 and cytokine levels on the likelihood of MS diagnosis. The goodness of fit of the logistic regression model was assessed by a Receiver Operating Characteristic (ROC) curve.

3. Results

Sociodemographic, clinical features, and plasma levels of ET-1 and cytokines in patients with MS and controls are shown in the Table 1. Patients with MS presented increased levels of ET-1 in comparison with controls (Fig. 1A). There was no difference between patients with MS and controls regarding the levels of the evaluated cytokines (Table 1). Contrary to our initial hypothesis, ET-1 levels were not associated with cytokine levels. In addition, neither age at disease onset nor disease duration correlated with ET-1 or cytokine levels.

Then, we performed a binary logistic regression using a backward stepwise approach to evaluate which variables could be considered significant predictors of MS diagnosis in a multivariate analysis. All variables described in Table 1 were included in the starting model. In the final model (step 11), higher ET-1 levels, lower levels of IL-4 and higher levels of IFN-γ remained as predictors of MS diagnosis. The results are presented in Table 2. The logistic regression model can be considered fair since the predicted variability resulted in an area under the curve (AUC) of 0.793 in the ROC analysis. Corroborating the binary logistic results, we found a significant increase in the IFN-γ/IL-4 ratio in patients with MS in comparison with controls (Fig. 1B, $p = .009$, Student's *t*-test). In addition, higher EDSS scores were associated with increased IL-12/IL-13 (rho = 0.408, $p = .017$) and IL-6/IL-10 (rho = 0.480, $p = .004$) ratios.

4. Discussion

Although the pathogenesis of MS is not completely understood, immune/inflammatory processes have been consistently associated with demyelination. The other pathological hallmark of MS, axonal degeneration, may be related to cerebral hypoperfusion, which in turn, is thought to be associated with increased levels of the potent vasoconstrictor ET-1 (D'Haeseleer et al., 2015). So far, the link between neuroinflammation and neurodegeneration in MS has not been well characterized. In the current study, we investigated whether ET-1 levels were associated with cytokines levels in samples from patients with MS and controls. In line with current knowledge, we found that patients with MS present increased levels of ET-1 and an immune response biased towards a Th1 profile. These results were confirmed by multivariate analysis since a shift to a Th1 response (i.e., decreased levels of IL-4 and increased levels of IFN-γ) was a significant predictor of MS diagnosis. Despite remaining as one of the predictors for MS diagnosis in the final model of the binary logistics regression, ET-1 did not reach

Table 1
General characteristics and plasma levels of endothelin-1 and cytokines in treatment naïve patients with multiple sclerosis (MS) and controls.

	Controls (*N* = 35)	MS (N = 35)	*P* value
Age in years [mean ± SD (median)]	36.99 ± 10.79 (33.00)	37.57 ± 11.19 (35.50)	0.883[a]
Females, N (%)	24 (68.57)	24 (68.57)	1.000[b]
EDSS score [mean ± SD (median)]	–	2.67 ± 2.55 (2.00)	–
Age at disease onset [mean ± SD (median)]	–	31.54 ± 9.02 (30.67)	–
Disease duration in years [mean ± SD (median)]	–	5.93 ± 8.17 (2.58)	–
MS phenotype: CIS	–	9 (25.71%)	
MS phenotype: RRMS [N (%)]	–	21 (60.00%)	–
MS phenotype: SPMS [N (%)]	–	3 (8.57%)	–
MS phenotype: PPMS [N (%)]	–	2 (5.72%)	–
ET-1 levels	**1.35 (1.26–1.58)**	**1.56 (1.32–1.90)**	**0.034**[a]
IL-1β levels	37.52 (25.35–46.10)	37.52 (30.97–55.00)	0.390[a]
IL-2 levels	137.39 (94.63–163.81)	132.37 (112.80–177.87)	0.401[a]
IL-4 levels	16.94 (11.62–22.06)	17.78 (14.18–24.15)	0.453[a]
IL-5 levels	282.36 (174.95–355.87)	304.31 (219.86–427.78)	0.384[a]
IL-6 levels	424.20 (332.80–588.87)	559.25 (365.75–721.56)	0.113[a]
IL-10 levels	176.13 (113.45–255.10)	218.71 (157.99–357.25)	0.083[a]
IL-12p70 levels	16.05 (8.08–39.16)	20.75 (10.70–38.75)	0.316[a]
IL-13 levels	63.50 (45.70–79.36)	68.42 (52.22–114.85)	0.274[a]
IFN-γ levels	119.16 (81.29–153.34)	134.15 (98.12–194.59)	0.256[a]
TNF-α levels	9.79 (8.17–15.58)	11.32 (8.17–16.99)	0.661[a]

Abbreviations: EDSS = Expanded Disability Status Scale; ET = endothelin; IFN = interferon; IL = interleukin; CIS = clinically isolated syndrome; MS = multiple sclerosis; PPMS = primary progressive MS; RRMS = relapsing-remitting MS; SD = standard deviation; SPMS = secondary progressive MS; TNF = tumor necrosis factor.

[a] Mann-Whitney test;

[b] Fisher's exact test. ET-1 and cytokine levels are given in pg/mL [median (25th – 75th percentiles)].

statistical significance (p = .060). Contrary to our initial hypothesis, ET-1 levels were not associated with peripheral immune markers levels, suggesting that these changes may occur independently.

Recent evidence suggests that ET-1 plays an important role in MS pathogenesis. The first study conducted in this regard reported a significant increase in cerebrospinal fluid (CSF) levels of ET-1 in MS patients experiencing an acute relapse, in comparison to those in a stable phase, controls or subjects with other neurological diseases (Speciale et al., 2000). Taking into account the signs of vascular dysregulation in MS patients (e.g., tendency to have cold extremities and high frequency of migraine), Haufschild et al. (2001) hypothesized that patients with MS had increased levels of ET-1. Confirming their initial hypothesis, they found a marked increase in plasma levels of ET-1. However, contrary to the former study results, the different forms and stages of MS had no significant influence on the results. They did not find any association between ET-1 levels and disease duration or EDSS scores (Haufschild et al., 2001). The same group confirmed findings of increased levels of ET-1 in an independent cohort of patients with MS in tandem with a significant reduction of extraocular blood flow, indicating a potential role for ET-1 on vascular dysregulation in MS (Pache et al., 2003).

The involvement of ET-1 in cerebral hypoperfusion was supported by a study that found increased levels of ET-1 in both peripheral and internal jugular vein blood from patients with MS in comparison with controls. The ratio of ET-1 level in jugular vein/peripheral vein was higher in patients with MS than in controls, suggesting that ET-1 was released from the brain to the circulation in MS (D'Haeseleer et al., 2013). The same study performed immunohistochemistry stains in postmortem brain tissues showing that reactive astrocytes are likely the main source of ET-1 in MS plaques. In addition, MS patients presented

Table 2
Final logistic regression model (step 11) to predict MS diagnosis.

Predictive variable							95% CI for Odds Ratio	
	B	SE	Wald	df	*P* value	Odds Ratio	Lower	Upper
ET-1 levels	1.363	0.724	3.542	1	0.060	3.908	0.945	16.155
IL-4 levels	−0.308	0.144	4.563	1	0.033	0.735	0.554	0.975
IFN-γ levels	0.036	0.016	4.808	1	0.028	1.037	1.004	1.070

Variables entered on step 1: age, sex, ET-1, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL12-p70, IL-13, IFN-γ, TNF-α.
Abbreviations: CI = confidence interval; df = degrees of freedom; ET = endothelin; IFN = interferon; IL = interleukin; SE = standard error; TNF = tumor necrosis factor.

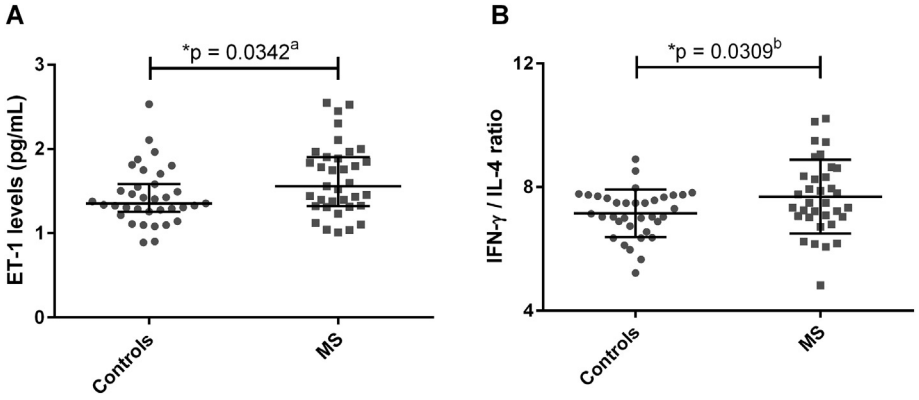

Fig. 1. Plasma levels of endothelin-1 (ET-1) and the Th1/Th2 response in patients with multiple sclerosis (MS) and controls. Patients with MS exhibited increased levels of ET-1 (A) and an immune response shifted to a Th1 profile, as observed by the increased IFN-γ/IL-4 ratio (B) in comparison with controls. Horizontal bars represent the median and interquartile range (A) or the mean and standard deviation (B). *p < .05. [a]Mann-Whitney test; [b]Student's t-test.

lower CBF in comparison with controls, as observed using arterial spin-labeling magnetic resonance imaging. After administration of the ET-1 antagonist bosentan in MS patients, CBF increased to control values (D'Haeseleer et al., 2013). Altogether, these results suggest a relationship between cerebral hypoperfusion and astrocytic reactivity, an early phenomenon in MS pathology (Ponath et al., 2018).

A recent study has shown increased serum levels of ET-1 in patients with MS compared to those with acute inflammatory demyelinating polyneuropathy (AIDP), Alzheimer's disease and controls. Interestingly, similar levels of ET-1 were seen in MS and chronic inflammatory demyelinating polyneuropathy (CIDP) patients. In this study, ET-1 levels correlated with disability in both MS and CIDP, but not in AIDP (Chang et al., 2017). These results suggest that ET-1 may be associated with long-term immune activation/dysregulation rather than with acute changes.

Patients with MS in our study presented an immune response biased towards a Th1 profile, as previously reported (Martin et al., 1998). Our multivariate analysis showed that decreased levels of IL-4 (Th2) and increased levels of IFN-γ (Th1) are associated with MS diagnosis. The Th1 shifted immune pathway in MS was confirmed by the increased IFN-γ/IL-4 ratio observed in patients with MS in comparison with controls. In addition, greater EDSS scores were associated with higher IL-12/IL-13 ratios, an indicative of Th1 shifted response. EDSS also correlated positively with the IL-6/IL-10 ratio, helpful for the evaluation of pro/anti-inflammatory profile. Previous research showed that Th1/proinflammatory cytokines such as INF-γ and TNF-α are related to increase inflammation, leading to disease progression and worsening of symptoms in MS. Concurrently, Th2-related/anti-inflammatory cytokines such as interleukin IL-4 and IL-13, have been associated with inflammation reduction and improvement of symptoms in MS patients (Imitola et al., 2005; Miller et al., 2004; Sharief and Hentges, 1991). The modulation of Th1/Th2 balance towards a Th2-shifted response has been regarded as a marker of treatment response in MS (Oreja-Guevara et al., 2012).

Experimental data has linked ET-1 to inflammation. Mice with endothelial or astrocytic ET-1 overexpression developed more severe experimental autoimmune encephalomyelitis (EAE) with increased serum levels of IL-6, IL-17A, IFN-γ and TNF-α. They also presented increased splenic lymphocyte production of Th1 and Th17 cytokines (Guo et al., 2014). In addition, intrastriatal injection of TNF-α in rats resulted in a dose-dependent reduction of cerebral blood volume that is mediated by ET-1, and was ameliorated by pretreatment with a non-specific ET-1 receptor antagonist (Sibson et al., 2002). These experimental findings suggest that ET-1 and immune mediators jointly contribute to the pathogenesis of MS. We did not find any significant association between peripheral levels of ET-1 and cytokines in plasma samples of MS patients, though ET-1, IL-4 and IFN-γ levels were considered predictors for MS diagnosis in our multivariate analysis. We suspect that changes in ET-1 and peripheral inflammatory mediators in MS may occur independently, or that the nature and strength of this relationship may vary over the course of the disease. More studies are needed to clarify this point.

Limitations of our study include the sample size and the cross-sectional design. In addition, measurement of inflammatory markers from peripheral blood does not necessarily mirror CNS pathology, or local upregulation of such markers. This must be considered when interpreting our data. The fact that we did not correct for multiple comparisons should also be taken into account as a limitation of our study results. By contrast, the use of samples from drug-naïve patients can be regarded as a strength of our study.

Our data together with previous studies corroborate the hypothesis that both ET-1 and a peripheral immune response biased towards a Th1 profile are involved in the pathophysiology of MS. These processes, however, might occur independently contributing to both demyelination and neurodegeneration observed in MS.

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Declarations of interest

None.

References

Aviv, R.I., Francis, P.L., Tenenbein, R., O'Connor, P., Zhang, L., Eilaghi, A., et al., 2012. Decreased frontal lobe gray matter perfusion in cognitively impaired patients with secondary-progressive multiple sclerosis detected by the bookend technique. AJNR Am. J. Neuroradiol. 33, 1779–1785.
Chang, C.W., Wu, H.C., Lyu, R.K., Lo, Y.S., Chen, C.M., Ro, L.S., et al., 2017. Elevated serum levels of endothelin-1 in patients with chronic inflammatory demyelinating polyneuropathy. Clin. Chim. Acta 476, 49–53.
Compston, A., Coles, A., 2008. Multiple sclerosis. Lancet 372, 1502–1517.
Debernard, L., Melzer, T.R., Van Stockum, S., Graham, C., Wheeler-Kingshott, C.A., Dalrymple-Alford, J.C., et al., 2014. Reduced grey matter perfusion without volume loss in early relapsing-remitting multiple sclerosis. J. Neurol. Neurosurg. Psychiatry 85, 544–551.
Dendrou, C.A., Fugger, L., Friese, M.A., 2015. Immunopathology of multiple sclerosis. Nat. Rev. Immunol. 15, 545–558.
D'Haeseleer, M., Cambron, M., Vanopdenbosch, L., De Keyser, J., 2011. Vascular aspects of multiple sclerosis. Lancet Neurol. 10, 657–666.
D'Haeseleer, M., Beelen, R., Fierens, Y., Cambron, M., Vanbinst, A.M., Verborgh, C., et al., 2013. Cerebral hypoperfusion in multiple sclerosis is reversible and mediated by endothelin-1. Proc. Natl. Acad. Sci. U. S. A. 110, 5654–5658.
D'Haeseleer, M., Hostenbach, S., Peeters, I., Sankari, S.E., Nagels, G., De Keyser, J., et al., 2015. Cerebral hypoperfusion: a new pathophysiologic concept in multiple sclerosis? J. Cereb. Blood Flow Metab. 35, 1406–1410.
Flammer, J., Konieczka, K., 2017. The discovery of the Flammer syndrome: a historical and personal perspective. EPMA J. 8, 75–97.
Guo, Y., Chung, S.K., Siu, C.W., Kwan, S.C., Ho, P.W., Yeung, P.K., et al., 2014. Endothelin-1 overexpression exacerbate experimental allergic encephalomyelitis. J. Neuroimmunol. 276, 64–70.
Haufschild, T., Shaw, S.G., Kesselring, J., Flammer, J., 2001. Increased endothelin-1 plasma levels in patients with multiple sclerosis. J. Neuroophthalmol. 21, 37–38.
Hostenbach, S., D'Haeseleer, M., Kooijman, R., De Keyser, J., 2016. The pathophysiological role of astrocytic endothelin-1. Prog. Neurobiol. 144, 88–102.
Houde, M., Desbiens, L., D'Orleans-Juste, P., 2016. Endothelin-1: biosynthesis, signaling and vasoreactivity. Adv. Pharmacol. 77, 143–175.
Imitola, J., Chitnis, T., Khoury, S.J., 2005. Cytokines in multiple sclerosis: from bench to bedside. Pharmacol. Ther. 106, 163–177.
Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33, 1444–1452.
Martin, R., Ruddle, N.H., Reingold, S., Hafler, D.A., 1998. T helper cell differentiation in multiple sclerosis and autoimmunity. Immunol. Today 19, 495–498.
Miller, A., Glass-Marmor, L., Abraham, M., Grossman, I., Shapiro, S., Galboiz, Y., 2004. Biomarkers of disease activity and response to therapy in multiple sclerosis. Clin. Neurol. Neurosurg. 106, 249–254.
Narayana, P.A., Zhou, Y., Hasan, K.M., Datta, S., Sun, X., Wolinsky, J.S., 2014. Hypoperfusion and T1-hypointense lesions in white matter in multiple sclerosis. Mult. Scler. 20, 365–373.
Oreja-Guevara, C., Ramos-Cejudo, J., Aroeira, L.S., Chamorro, B., Diez-Tejedor, E., 2012. TH1/TH2 Cytokine profile in relapsing-remitting multiple sclerosis patients treated with Glatiramer acetate or Natalizumab. BMC Neurol. 12, 95.
Pache, M., Kaiser, H.J., Akhalbedashvili, N., Lienert, C., Dubler, B., Kappos, L., et al., 2003. Extraocular blood flow and endothelin-1 plasma levels in patients with multiple sclerosis. Eur. Neurol. 49, 164–168.
Pérez-Cerdá, F., Sánchez-Gómez, M.V., Matute, C., 2016. The link of inflammation and neurodegeneration in progressive multiple sclerosis. Mult. Scler. Demyelinating Disord. 1, 9.
Polman, C.H., Reingold, S.C., Edan, G., Filippi, M., Hartung, H.P., Kappos, L., et al., 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann. Neurol. 58, 840–846.
Ponath, G., Park, C., Pitt, D., 2018. The role of astrocytes in multiple sclerosis. Front. Immunol. 9, 217.
Sharief, M.K., Hentges, R., 1991. Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis. N. Engl. J. Med. 325, 467–472.
Sibson, N.R., Blamire, A.M., Perry, V.H., Gauldie, J., Styles, P., Anthony, D.C., 2002. TNF-alpha reduces cerebral blood volume and disrupts tissue homeostasis via an endothelin- and TNFR2-dependent pathway. Brain 125, 2446–2459.
Speciale, L., Sarasella, M., Ruzzante, S., Caputo, D., Mancuso, R., Calvo, M.G., et al., 2000. Endothelin and nitric oxide levels in cerebrospinal fluid of patients with multiple sclerosis. J. Neuro-Oncol. 6 (Suppl. 2), S62–S66.
Thompson, A.J., Banwell, B.L., Barkhof, F., Carroll, W.M., Coetzee, T., Comi, G., et al., 2018. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 17, 162–173.
Tseng, C.H., Huang, W.S., Lin, C.L., Chang, Y.J., 2015. Increased risk of ischaemic stroke among patients with multiple sclerosis. Eur. J. Neurol. 22, 500–506.
Vitorino, R., Hojjat, S.P., Cantrell, C.G., Feinstein, A., Zhang, L., Lee, L., et al., 2016. Regional frontal perfusion deficits in relapsing-remitting multiple sclerosis with cognitive decline. AJNR Am. J. Neuroradiol. 37, 1800–1807.