

The increased serum level of sRAGE is associated with multiple sclerosis but not with disability progression

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Abstract

Objective: It is still unclear as to why there are such large inter-individual variations in the risk and rate of disease disability progression of multiple sclerosis (MS). MS is a chronic autoimmune inflammatory disease of the central nervous system, with the involvement of autoimmunity, and inflammatory and neurodegenerative processes. In these processes, the important biologic function of receptors for advanced glycation end products (RAGE) could be altered by its circulating soluble form (sRAGE). The aim of our study was to investigate the possible role of sRAGE in the etiopathogenesis and disability progression of MS.

Methods: The serum level of sRAGE was determined using enzyme-linked immunosorbent assay (ELISA) in 44 patients with MS (22 rapidly progressing and 22 slow progressing) and 32 healthy controls from Slovakia.

Results: We showed a significantly increased serum level of sRAGE in patients with MS in comparison with the controls. We found no statistically significant differences in serum levels of sRAGE between the MS subgroup with rapid and slow disease disability progression, which was measured using the MSSS score.

Conclusion: Our results suggest a role of sRAGE in the etiopathogenesis of MS, but there was no association with disease disability progression. Further studies in larger cohorts of patients are necessary to clarify the exact mechanisms of the role of sRAGE in MS and its progression.

Keywords: Multiple sclerosis, disability progression, MSSS, sRAGE, serum level, biomarker

INTRODUCTION

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS). The formation and activation of myelin reactive T-lymphocytes with altered functions is the key immunopathologic mechanism of MS development (1). The etiopathogenesis of the disease is very complex. Lymphocytic infiltration triggers the cascade of processes resulting in injury of myelin and axons. In the early stages, the disease can be described by attacks of neurologic worsening (relapses) that usually recover. However, the following pathologic changes may cause chronic neurodegeneration, which closely manifests and correlates with the progression of disability (2). It is still unclear as to why there are such large interindividual variations in the rate of disease disability progression.

Neurodegenerative mechanisms that are present in chronic demyelination plaques are believed to be initiated by oxidative stress. The formation of reactive oxygen species (ROS) is probably primarily orchestrated by immunoinflammatory mechanisms, but there are some mechanisms of neuronal degeneration and demyelination independent from inflammation (3). Lower antioxidant capacity and increased levels of oxidative stress markers have been found in the plasma of patients with MS, which can be in relation to the onset of a relapse or to disability progression (4, 5). ROS are connected with the activity of the receptor for advanced glycation end products (RAGE). This receptor is expressed on many types of cells, binds a large group of ligands and mediates responses to cellular injury and stress conditions. RAGE has been implicated in the activation of cascades related to acute and chronic inflammatory reaction (6). Soluble RAGE (sRAGE) is believed to act as a decoy receptor that can sequester circulating ligands and thus, its proportion can influence RAGE function (7, 8). The role of RAGE was confirmed in the pathogenesis of a number of diseases such as diabetes

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mellitus, atherosclerosis, cancer, infectious diseases, as well as in autoimmune and neurodegenerative disorders (7, 9). Few studies have focused on the role of RAGE in the pathogenesis of MS. The functional gene polymorphism with effect on transcriptional activity of RAGE was found to be involved in the development of MS (10). However, the results of studies that measured serum levels of sRAGE in patients with MS are divergent (11, 12)

In light of these facts, several studies suggest the possible pathophysiologic relevance of sRAGE in a number of diseases including MS. It is important to find biomarkers that could help to identify individuals early with a higher MS risk or even rapid progression of disability and possibly to choose the appropriate preventive or therapeutic strategy in predisposed individuals. Therefore, the aim of the present study was to assess the possible differences in serum sRAGE levels in a group of Central European Slovak patients with MS and healthy controls. Moreover, we tried to investigate whether there was any association between serum levels of sRAGE and the disease disability progression rate in MS.

METHODS

Patients and Controls

Our study consisted of 44 patients diagnosed with MS and 32 healthy control individuals, all of them Slovak people residing in the central-northern part of Slovakia. The study was approved by the ethics committee of Comenius University, Bratislava, Jessenius Faculty of Medicine, Martin. Informed consent was given by all participating individuals. The age-matched control group consisted of healthy volunteers with no disease of the CNS. For the patients with MS, a clinically definitive MS diagnosis was established according to the McDonald's criteria in The Centre for Demyelinating Diseases at the Clinic of Neurology, Jessenius Faculty of Medicine, Martin, and Martin University Hospital, Slovak Republic (13, 14). Clinical data and blood samples were collected from January 2014 until November 2014 only at regular medical check-ups to reduce trauma to patients. At the time of blood sample collection, no acute clinical attacks were present in any of the patients involved in the study. An individual Multiple Sclerosis Severity Score (MSSS) for each patient was ascertained from the table of Roxburgh et al. using the Expanded Disability Status Scale (EDSS) score and disease duration data (15, 16). To be able to assess the association of sRAGE serum levels with the MS disability progression, we chose 22 patients with MS with slow progressing disease (MS-1, MSSS <3) and 22 patients with MS with rapidly progressing disease (MS-3, MSSS >6). The clinical descriptions of the patients with MS and controls are shown in Table 1.

Measurement of sRAGE Serum Level

Blood samples were drawn from peripheral veins and dispensed into 5 mL blood collection tubes prefilled with gel and clot activator (Vacutest Kima S.r.l., Arzgergrande, Italy). The samples were centrifuged at 4000 g, at 4°C for 5 min, aliquoted, and stored at -80°C until use. The levels of sRAGE in sera

were measured in duplicates using an enzyme-linked immunosorbent assay (ELISA) and commercially available kits (Human sRAGE ELISA, BioVendor, Czech Republic) following the manufacturer's guidelines. Sera were incubated with polyclonal anti-human sRAGE Antibody present in ELISA wells. After that, biotinylated polyclonal anti-human sRAGE antibody was added to bind with the caught sRAGE. After incubation, streptavidin-horseradish peroxidase (HRP) conjugate was added to bind the biotinylated antibody. Subsequently, the HRP conjugate reacted with added tetramethylbenzidine, and the reaction was later stopped using acidic solution. The yellow product absorbance was measured by spectrophotometer at 450 nm and unknown concentrations of samples were calculated using a standard curve.

Statistical Analysis

Statistical analysis was done using SVS 7 software (SNP & Variation Suite v7.6.11, Golden Helix, Bozeman, Montana, USA). We used non-parametric methods for the comparison of the serum levels of sRAGE because the data showed non-normal distribution. To test the statistical differences in sRAGE levels between the studied groups, we used the Mann-Whitney test. In all performed tests, the results were deemed statistically significant when two-tailed p was less than or equal to 0.05. Levels of sRAGE, patient's age, disease duration, EDSS, MSSS, and progression index are expressed showing the mean ± standard deviation.

RESULTS

The serum levels of sRAGE that were measured in 22 MS patients with slow disease disability progression (MS-1), in 22 patients with MS with rapid disease disability progression (MS-3), and in 32 healthy control subjects are summarized in Table 2.

First, we detected significantly increased serum levels of sRAGE in MS patients (1071.2 ± 519.0 pg/mL) when compared with controls (797.4 ± 460.3 pg/mL) ($p=0.003$) (Figure 1).

Figure 1. Serum level of sRAGE in patients with MS and controls

CTL: controls; MS: multiple sclerosis; SD: standard deviation

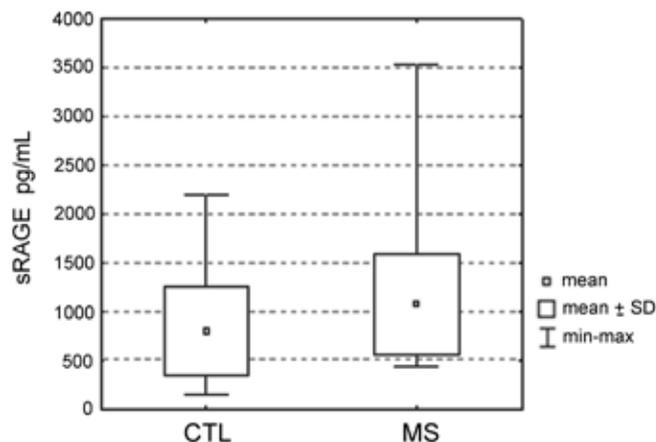


Table 1. Characteristics of examined individuals

	Patients (n=44)		Controls (n=32)	
	Sex	16 men (36.4%)	28 women (63.6%)	12 men (37.5%)
Age (years)	40.8±10.0	46.8±7.6	40.3±7.9	46.1±7.2
		44.6±8.9		43.9±7.9
Age range (years)	27-60	30-59	30-51	37-62
		27-59		30-62
Age at first symptoms (years)	31.8±10.3			
Disease duration (years)	12.9±6.6			
EDSS (points)	3.7±2.1			
MSSS (points)	4.43±2.98			
Progression index (EDSS / years)	0.389±0.289			
MS phenotype				
Relapsing-remitting	n=31 (70.5%)			
Secondary progressive	n=13 (29.5%)			
Rate of disease disability progression				
Slow progressing MS (MS-1)	n=22 (50%)			
MSSS score	1.61±0.70			
Rapidly progressing MS (MS-3)	n=22 (50%)			
MSSS score	7.24 ±1.07			

MS: multiple sclerosis; EDSS: expanded disability status scale; MSSS: multiple sclerosis severity score

Table 2. Serum levels of sRAGE in patients with MS with rapid and slow disability progression and in controls

	Serum level of sRAGE (pg/mL)	MS-1 vs. CTL		MS-3 vs. CTL		MS vs. CTL		MS-1 vs. MS-3	
		Z _{adj}	p _(M-W)						
CTL (n=32)	797.4±460.3								
MS (n=44)	1071.2±519.0								
MS-1 (n=22)	1081.4±638.3	-2.271	0.023	-2.65	0.008	-2.941	0.003	-0.48	0.630
MS-3 (n=22)	1060.9±379.5								

CTL: controls; MS: multiple sclerosis; MS-1: slow progressing MS; MS-3: rapidly progressing MS; p(M-W): p value (Mann-Whitney test)

When compared with the controls (797.4±460.3 pg/mL), we observed significantly higher levels serum of sRAGE in the MS subgroup with slow disability progression (MS-1; 1081.4±638.3 pg/mL) (p=0.023) (Figure 2) and with rapid disability progression (MS-3; 1060.9±379.5 pg/mL) (p=0.008) (Figure 3).

Due to the proposed functions of sRAGE in the regulation of immune response, inflammation, and neurodegeneration, we hypothesized that sRAGE could also affect disease progression. Accordingly, we compared the serum levels of sRAGE

in the defined subgroups of MS patients with the different disability progression rate. We identified no statistically significant differences in serum levels of sRAGE in patients with MS with rapid progression of disability (1060.9±379.5 pg/mL) when compared with patients with slow progression of disability (1081.4±638.3 pg/mL) (p=0.630) (Figure 4).

In summary, our results showed sRAGE serum levels to be significantly increased in patients with MS when compared with controls, but we found no differences between the MS subgroups with different rates of disease disability progression.

Figure 2. Serum level of sRAGE in patients with MS with slow disability progression (MS-1) and controls

CTL: controls; MS-1: slow progressing multiple sclerosis; SD: standard deviation

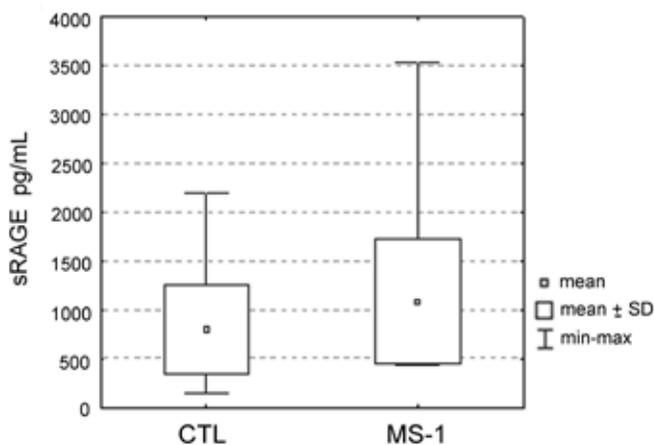
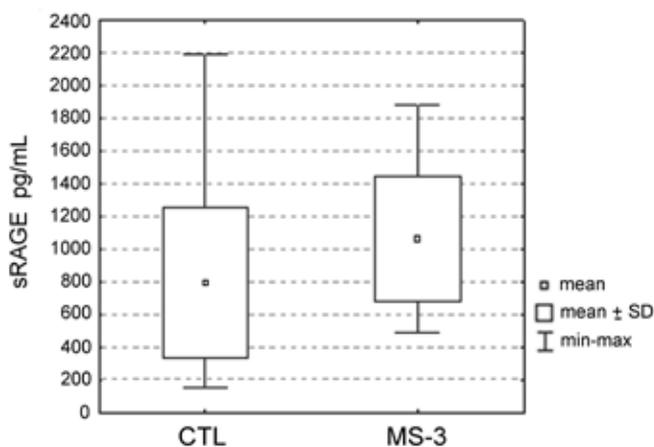


Figure 3. Serum level of sRAGE in patients with MS with rapid disability progression (MS-3) and controls

CTL: controls; MS-3: rapidly progressing multiple sclerosis; SD: standard deviation



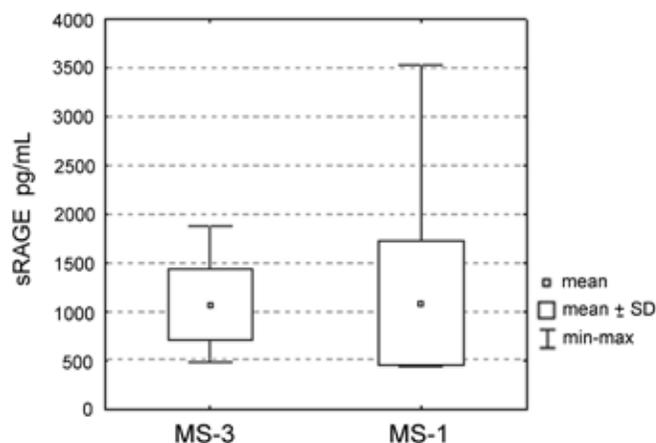
DISCUSSION

This is the first study to assess the association of serum levels of sRAGE with the pathogenesis of MS in the population of Slovakia. Until now, only few studies have described the possible role of sRAGE in MS, but no studies have reported an association between serum levels of sRAGE and MS progression as measured using the MSSS score (11, 12). In our present study, we investigated serum levels of sRAGE as one of the proposed markers that could be involved in the development and disability progression of MS.

In this study, we detected significantly increased serum levels of sRAGE in 44 patients with MS (1071.2 ± 519.0 pg/mL) in comparison with 32 healthy control subjects (797.4 ± 460.3 pg/mL). The previous study of Glasnovic et al. enrolled a group of 27 patients with MS at clinical onset and 30 healthy controls (11). In contrast with our findings, the authors did not observe any difference in serum levels of sRAGE between patients with MS

Figure 4. Serum level of sRAGE in patients with MS with rapid disability progression (MS-3) and slow disability progression (MS-1)

MS-3: rapidly progressing multiple sclerosis; MS-1: slow progressing multiple sclerosis; SD: standard deviation



and healthy controls, which was around 550 pg/mL in both groups. This could be explained by the different time of serum sample collection; samples were taken at the disease onset in their study, whereas in our study, the mean disease duration at the time of sampling was 12.9 ± 6.6 years. It is known that the predominant pathophysiologic processes that occur in MS change over time. Newer, more radical concepts suggest that in progressive stages of MS, a secondary inflammatory reaction can be trapped behind a closed blood-brain barrier (BBB) and can superimpose and augment the neurodegeneration already present from earlier stages of the disease (17). We think that the ongoing inflammatory process and oxidative stress inside the CNS of our patients with MS could result in the upregulation of RAGE in neuronal cells, microglial cells and BBB. In those conditions, sRAGE could be produced and released extracellularly by pericytes and endothelial cells as the result of balancing feedback and immune surveillance in RAGE signal transduction pathways (7, 12).

On the other hand, Glasnovic et al. detected that cerebrospinal fluid (CSF) levels of sRAGE lower than 7.39 pg/mL were able to distinguish patients with MS from controls with high diagnostic sensitivity and specificity (72.22% and 87.50%, respectively) (11). According to the tissue-specific distribution of sRAGE, authors assumed that a decreased sRAGE level in CSF might be a result of endothelial dysfunction, which is deeply characterised in MS (18).

Contrarily, Sternberg et al. detected significantly decreased serum levels of sRAGE in 37 patients with MS when compared with 22 healthy controls (998 ± 52.6 pg/mL vs. 1292 ± 77.1 pg/mL, $p=0.005$) (12). They found the prevalence of individuals with levels of sRAGE lower than 776 pg/mL to be increased in the MS group when compared with controls. The sRAGE serum level in patients with MS examined in that study was only slightly lower than in our study. This small difference could be

explained by the different disease duration (9.09 ± 7.98 years) or more likely by the divergence in ratios of patients with secondary progressive MS in the analyzed cohorts (16.2% vs. 29.5% in our study). It is known that in progressive forms of MS, the processes triggered by the chronic inflammatory process differ from those present in the initial or relapsing-remitting course of the disease (19). Variations between the serum levels of sRAGE in control groups might be based on differences in methodologies of control probands recruitment or due to silent non-detected immunologic aberrations in the control group.

When considering the disease progression, our results did not show any association of sRAGE serum levels with the rate of disability progression in MS. We found no difference in serum levels of sRAGE in 22 patients with MS with rapid disability progression in comparison with 22 patients with slow disability progression. Concordantly, the study of Sternberg et al. showed no association of sRAGE serum levels with the disease progression index, which considers EDSS score together with disease duration, the same parameters that were used in our study to obtain MSSS scores (12, 15). On the other hand, Sternberg et al. reported an inverse correlation between sRAGE serum level and EDSS score and thus they speculated that serum concentrations of sRAGE might correlate with brain tissue damage in patients with MS with BBB leakage. It is important to point out that EDSS scores only evaluate the degree of neurologic impairment of the functional systems, and does not regard the duration of the disease course, which was considered in our study (16).

To better understand the functions and mechanisms of RAGE actions, we have to note that it is a transmembrane immunoglobulin protein, coded in class III major histocompatibility complex, and synthesized in neuronal, microglial, and endothelial cells. When present in cells, it serves as a receptor for "advanced glycation end products" (AGEs), β -amyloid fibrils (A β), amphotericin, integrin Mac-1, and other molecules. It is potentially able to enhance immune and inflammatory responses by its interaction with S100/calgranulin proteins and consequent cytokine expression on lymphocytes, mononuclear cells, and the blood vessel wall (7, 20). The interaction of ligands with RAGE amplifies the signal pathways of inflammatory cytokines, adhesion molecules, and matrix metalloproteinases (MMPs) (21). During autoimmune reactions, RAGE has been found to have a role in the process of activation and differentiation of T-helper 1 lymphocytes and in the regulation of BBB integrity (22). In MS, microglial RAGE is probably related to neuroinflammation-related neurotoxicity. The linkage with NF- κ B transcriptional activity involves RAGE in the regulation of gene expression of MAP kinases, tumor necrosis factor- α , differentiation antigens of leukocytes, cytokines, and vascular endothelial growth factor (9, 23). Moreover the blockage of RAGE function was found to suppress the development of experimental autoimmune encephalomyelitis in

mice and can decrease the migration of immune and inflammatory cells to the CNS (24).

RAGE in cells is produced in various isoforms, including full-length RAGE, membrane-bound RAGE, and soluble sRAGE. Soluble sRAGE consists of the extracellular ligand-binding domain without a transmembrane segment and exists in 2 types. One of them is endogenous secretory RAGE (esRAGE), which is predominant in endothelial cells and encoded by the splice variant. The second form is cleaved RAGE (cRAGE), predominant in serum and yielded by metalloproteinase cleavage of full-length RAGE (7). The amount of the soluble form of the receptor is presumed to affect the regulation of RAGE-mediated functions during inflammation. Pullerits et al. detected a lower serum level of sRAGE in patients with rheumatoid arthritis compared with healthy controls and patients with noninflammatory joint disease (871 ± 66 pg/mL vs. 1290 ± 78 pg/mL and 1569 ± 168 pg/mL, respectively), which can be caused by an increased consumption of this molecule or by formation of in vivo complexes with HMGB1. Serum sRAGE has also been found to be associated with other diseases (8). Falcone et al. showed low levels of sRAGE to be associated with acute coronary syndrome and regarded it as a putative risk factor for atherosclerosis in humans (25). Emanuele et al. found serum levels of sRAGE lower than 776 pg/mL to be associated with Alzheimer's disease (AD) and vascular dementia (26). In AD, RAGE is probably implicated in the mediation of A β toxicity by high-affinity A β binding and its transport from blood to brain and vice versa (27).

In our study, we found an increased serum level of sRAGE in MS patients who were in the remission phase of the disease, with no clinical signs of acute disease attack. Due to this fact, we can speculate that this level of serum sRAGE could be related to self-regulatory pathways, resulting in increased sRAGE formation to slow the ongoing inflammatory process present in MS (28). This is supported by findings in animal models of collagen-induced arthritis, where RAGE antagonism appears to interfere with the disease effector stage rather than the initial priming of lymphocytes (20). A similar mechanism was described by Pullerits et al., who identified a positive relation of the leucocyte count and synovial levels of sRAGE in patients with rheumatoid arthritis (8). They suggested that synovial endothelial cells secreted extracellular sRAGE to limit the infiltration of inflammatory cells into the articular cavity by direct binding of the leukocyte integrin Mac-1.

Another possible explanation for our findings is that the increased sRAGE level in our patients with MS could be the result of enhanced RAGE expression, which was found to be present in neurons and inflammatory cells derived from spinal cords tissue of patients with MS, and its subsequent cleavage to sRAGE by MMPs (24). In mice, the matrix metalloproteinase MMP-9 was shown to release cell-bound RAGE into the cell environment (29). The increased level of MMPs in brain,

serum, and CSF of patients with MS has been found to be caused by their increased synthesis by neurons and infiltrating white blood cells. The upregulated MMPs, mainly MMP-9, have several potentially harmful roles in the CNS, including the enhancement of neuroinflammation, disintegration of the BBB, demyelination, and axonal and neuronal toxicity (30).

According to our findings and the results of the previous studies, we hypothesize that the higher serum levels of sRAGE detected in our patients MS could be produced to decrease the propensity towards inflammation and to worsen the access of RAGE ligands to cell membrane-bound receptors. In future studies, it could be interesting to analyze serum sRAGE levels in patients with MS during periods of clinical relapse.

The significantly increased serum level of sRAGE in patients with MS found in our study suggests the likely association of sRAGE with etiopathogenesis of MS. In spite of that, no statistically significant association of sRAGE serum level with the disease disability progression rate in our cohort was proved. To explain the detailed mechanisms of the role of sRAGE in MS and its progression, and to elucidate whether the change in its serum levels is the result of the disease or a factor that contributes to the disease, further studies with larger cohorts of patients are necessary.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of the Comenius University in Bratislava, Jessenius School of Medicine in Martin.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - J.L., D.C.; Design - D.C., J.L.; Supervision - J.L., D.D.; Resources - J.L., D.D.; Materials - E.K., J.M., E.K.; Data Collection and/or Processing - J.M., E.K., D.C.; Analysis and/or Interpretation - S.H., M.S., D.C.; Literature Search - D.C., M.S.; Writing Manuscript - D.C.; Critical Review - J.L., E.K.

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