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Tumor necrosis factor alpha (TNF-α) and its soluble receptors are associated with disability, disability progression and clinical forms of multiple sclerosis

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Abstract

Background The association between tumor necrosis factor (TNF)-α, soluble TNF receptor (sTNFR)1 and sTNFR2 with clinical characteristics of multiple sclerosis (MS) remains unclear.

Objective To examine whether TNF-α, sTNFR1 and sTNFR2 are associated with MS diagnosis, disability, disability progression and clinical forms of MS.

Materials and subjects The study included 147 patients with relapsing–remitting MS (RRMS), 21 with progressive clinical forms (ProgMS) and 70 controls. Expanded Disability Status Scale (EDSS) evaluated disability as mild (EDSS < 3.0) or moderate/high (EDSS ≥ 3.0). Multiple Sclerosis Severity Score (MSSS) evaluated disability progression as no progression (MSSS < 5) and progression (MSSS ≥ 5). Baseline data of subjects and plasma levels of TNF-α, sTNFR1, sTNFR2 were obtained.

Results The MS diagnosis explained 44.6% and 12.3% of TNF-α and sTNFR2 levels, respectively. Moderate/high disability and disability progression were best predicted by sTNFR1 and age (positively) and ProgMS were best predicted by sTNFR1 (positively) and sTNFR2 (negatively), coupled with age and sex. A composite score reflecting the sTNFR1/sTNFR2 ratio showed a positive association with ProgMS after adjusting for age and sex.

Conclusion Increased sTNFR1 and age were positively associated with disability and disability progression, whereas increased sTNFR1 (positively) and sTNFR2 (negatively) were associated with ProgMS, suggesting a distinct role of them in the immunopathological mechanisms of MS.

Keywords Multiple sclerosis · Tumor necrosis factor alpha · sTNFR1 · sTNFR2 · Disability · MSSS

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating immune-mediated disease, which affects the central nervous system (CNS). The etiology is multifactorial with a complex interrelation between genetic, epigenetic, hormonal, immunological, and environmental factors. Accumulating evidence suggests that the autoimmune inflammation in early MS is primarily mediated by an adaptive immune response and involves autoreactive T helper (Th)1, Th17, and B cells, as well as autoantibodies with reactivity against epitopes of the myelin proteins. However, the later chronic stages of the disease are characterized by a compartmentalized innate immune response in the CNS with activated microglia and macrophages [1].

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Different mechanisms may explain the heterogeneity of MS regarding the clinical symptoms, clinical forms, disability, disability progression and therapeutic response presented by the affected patients. Cytokines and their receptors are among the possible mediators of this heterogeneity [1]. TNF-α is essential in triggering healing mechanisms, but can initiate both cell death or survival pathways depending on the pathophysiological conditions. This cytokine is initially synthesized as a transmembrane TNF-α (tmTNF-α) that is cleaved by the TNF-α-converting enzyme (TACE) to release soluble TNF-α (solTNF-α) [2]. The cellular functions of TNF-α are mediated by TNF receptor (TNFR)1 and TNFR2, which differ in expression, ligand affinity, structure, and downstream signaling pathways. As a result, solTNF-α signals only through TNFR1-mediating primarily inflammation and apoptosis [3], while tmTNF-α signals through both TNFR1 and TNFR2, promoting cell survival, resolution of inflammation, immunity and myelination [4, 5]. Unlike TNFR1 that has a ubiquitous expression, TNFR2 is expressed by some immune cells, preferentially by a fraction of T regulatory cells (Treg), some endothelial cells, and cells of the nervous tissue [6].

In MS, TNF-α may exert a dual role through the link with its receptors TNFR1 and TNFR2. While the proinflammatory and neurodegenerative effects of TNF-α are mediated by solTNF-α, and therefore, TNFR1, tmTNF-α signaling that predominantly occurs via TNFR2 is mainly neuroprotective and favors tissue homeostasis and regeneration [7]. Though TNFR2, TNF-α promotes remyelination and oligodendrocyte precursor cells (OPCs) proliferation after cuprizone-induced demyelination [7].

The membrane-bound forms of both receptors are also substrate for proteolytic cleavage by TACE, producing soluble TNF-α receptor (sTNFR). This process is an important self-regulatory mechanism to prevent exaggerated damage and may contribute to the regulation of cellular TNF-α responsiveness. Increased release of ectodomain has two consequences: on one hand, receptor cleavage can counteract the circulating TNF-α bioactivity, thereby sequestering it, so that sTNFR will act as an intrinsic TNF-α inhibitor; on the other hand, the process will decrease the number of cell-surface signaling competent receptors and cause transient TNF-α desensitization [8].

TNF-α, sTNFR1 and sTNFR2 have been investigated in MS [5, 9–14]. High levels of TNF-α were found in blood samples of MS patients as compared with controls [5, 10] and in cerebrospinal fluid (CSF) of the patients with chronic progressive clinical forms of MS (ProgMS), suggesting that TNF-α could be associated with the diagnosis of MS as well as the progression of the disease over time [11, 12]. Previously, we demonstrated that patients with ProgMS with disease progression presented higher levels of TNF-α than those with ProgMS but without disease progression [13].

However, conflicting results have been reported regarding the levels of sTNFR1 and sTNFR2 in patients with MS with different clinical forms of the disease. A previous study reported that levels of sTNFR1 in both serum and CSF were higher in patients with relapsing–remitting MS (RRMS) and correlated with disease status [14], whereas in another study, sTNFR1 levels were similar in healthy subjects and MS patients [15]. Moreover, a selective increase of sTNFR2 levels were observed in patients with primary progressive MS (PPMS) compared with patients with other clinical forms of MS and healthy controls. However, these authors observed a lack of association between radiological variables, measuring inflammation and atrophy, and serum levels of sTNFR2 among the PPMS patients, probably indicating that the sTNFR2 levels may not reflect the whole extent of CNS damage [16].

TNF-α-targeting immunobiologicals are extremely successful in treating a number of immune-mediated diseases; however, clinical trials using anti-TNF-α monoclonal antibodies as therapy in MS patients resulted in disease exacerbation [17, 18]. It is hypothesized that these paradoxical effects of anti-TNF-α are due to inhibition of TNFR2 signaling [19].

Considering that the association between TNF-α and sTNFR1 and sTNFR2 with clinical characteristics of MS patients remains unclear, the aim of this study was to verify whether TNF-α and its soluble receptors are associated with MS diagnosis, disability, disability progression and clinical forms of MS.

Methods

Subject characteristics

The study included 168 patients with MS enrolled from the Neurology Outpatient of the University Hospital, Londrina, Paraná, Brazil. The diagnosis of MS was defined according to the McDonald criteria [20]. RRMS was diagnosed in 147 (87.5%) patients and ProgMS in 21 (12.5%), including 4 (2.4%) with primary progressive MS (PPMS) and 17 (10.1%) with secondary progressive MS (SPMS). At the baseline visit, the disability was evaluated using the Expanded Disability Status Scale (EDSS) [21] and the patients were divided into those with mild disability (EDSS < 3.0) or moderate/high disability (EDSS ≥ 3.0) [12]. The disability progression was evaluated using the Multiple Sclerosis Severity Score (MSSS) [22] and score ≥ 5.0 denoted higher than average speed of disability accumulation [23]. As controls, 70 healthy individuals were selected among blood donors of the Regional Blood Bank of Londrina, from the same geographic region of the patients. None of the participants presented clinical symptoms or laboratory

markers of heart, thyroid, kidney, hepatic, gastrointestinal, or oncologic diseases.

Demographic, epidemiological and anthropometric data (patients and controls), as well as clinical history and the use of therapy for MS before the inclusion at this study (for patients) were obtained using a standard questionnaire at the admission of the individuals, as previously described [10]. Briefly, body mass index (BMI) was calculated as weight (kg) divided by height (m) squared and the ethnicity was self-reported as Caucasian and non-Caucasian. Smoking was considered when the individuals were current smokers. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured and the mean of two measurements was used in the analysis [10]. Use of antihypertensive medication was an indication of systemic arterial hypertension (SAH) [24]. Diabetes mellius type 2 (DM) was defined as a fasting serum glucose ≥ 126 mg/dL, a non-fasting serum glucose ≥ 200 mg/dL and/or use of hypoglycemic medication [25]. Dyslipidemia and metabolic syndrome (MetS) were defined as previously reported [26].

The protocol was approved by the Institutional Research Ethics Committees of University of Londrina, Paraná, Brazil (CAAE: 22290913.9.0000.5231) and all of invited individuals were informed in detail about the research and gave written informed consent. The patients and their blood samples were consecutively identified by number to guarantee the confidentiality.

Laboratory biomarkers

At admission, venous blood samples were drawn under fasting state, with and without anticoagulant. Plasma and serum were obtained through centrifugation (2500 rpm for 15 min) and aliquots were stored at − 80 °C until analysis. A panel of TNF-α, sTNFR1 and sTNFR2 was determined using customized immunofluorimetric assay (Novex Life Technologies, Frederick, USA) for the Luminex® Platform in MAGPIX instrument.

Statistical analysis

Analyses of contingency tables (χ^2 test) checked the associations between categorical variables and diagnostic groups. The Kolmogorov–Smirnov test assessed normality of distribution. Logarithmic (Ln) transformation of continuous data was used in the analysis when the variables were not normally distributed or when there was heterogeneity of variance as assessed with the Levene test. We assessed the differences in continuous variables between groups using analyses of variance (ANOVA). Categorical variables were expressed as absolute number (n) and percentage (%) and continuous variables were expressed as mean ± error standard of main (SEM). The correlations between TNF-α, sTNFR1 and sTNFR2 were assessed using Spearman correlation coefficients. Multivariate general linear model (GLM) analysis assessed the effects of explanatory variables (including diagnosis) on dependent variables (TNF-α, sTNFR1, and sTNFR2) while controlling for sex, age, ethnicity, and BMI. Tests for between-subject effects assessed the univariate effects of significant predictor variables on the dependent variables. Box's M statistic was used to test for homogeneity of covariance matrices.

The association between TNF-α, sTNFR1 and sTNFR2 and MS were evaluated using automatic stepwise binary logistic regression analysis controlled for covariates that may confound the association of interest. Four different models of automatic stepwise binary logistic regressions analysis were employed to delineate TNF-α, sTNFR1 and sTNFR2 and the most significant variables that were associated with disability (EDSS < 3 versus ≥ 3), disability progression (MSSS < 5 versus MSSS ≥ 5) and clinical forms (RRMS versus ProgMS). The values of sTNFR1 and sTNFR2 were also transformed as z score and a composite score reflecting the sTNFR1/sTNFR2 ratio (namely z Ln sTNFR1-z Ln sTNFR2) was computed. The analysis also included the odds ratio (OR) and 95% confidence interval (CI) evaluated using the z scores. All statistical analyses were performed using IBM SPSS windows version 25. Tests were two-tailed and an alpha level of 0.05 indicated statistically significant results.

Results

Characteristics of the subjects

The mean age at MS diagnosis and disease duration were 34.6 years (± 0.85) and 7.8 years (± 0.54), respectively; 87 (55.1%) patients presented moderate/high disability and 71 (44.9%) patients presented mild disability. When the disability progression was evaluated, 79 (54.9%) patients showed progression and 65 (45.1%) showed no progression. According to the MS therapy, 14 (8.4%) patients were without treatment, 96 (57.5%) were treated with interferon β, 46 (27.5%) were treated with glatiramer acetate, 10 (6.0%) were using natalizumab and 1 (0.6%) was using fingolimod (data not shown).

Table 1 shows the baseline data of the MS patients and controls. We did not use p corrections to interpret the multiple results of univariate tests presented in Table 1 as these results were used to delineate the most significant predictor variables to be used as independent explanatory variables in the binary logistic regression analyses. Compared to controls, MS patients did not differ in the variables, age, sex, ethnicity, BMI, smoking, SAH, the presence of MetS and the levels of sTNFR1, but showed higher levels of TNF-α,

Table 1 Socio-demographic, clinical and inflammatory biomarker data in patients with multiple sclerosis and controls

Characteristics	Controls ($n=70$)	MS ($n=168$)	F/X^2	df	P value
Age (years)	45.16 (0.97)	42.02 (1.06)	3.17	1/236	0.076
Sex					
Male/female	17 (24.3)/53 (75.7)	49 (29.2)/119 (70.8)	0.587	1	0.443
Ethnicity					
C/NC	54 (77.1)/16 (22.9)	132 (78.6)/36 (21.4)	0.059	1	0.808
BMI (kg/m^2)	26.25 (0.50)	25.53 (0.39)	1.063	1/235	0.304
Smoking (Y/N)	7/63	19/147	0.11	1	0.746
Diabetes mellitus (Y/N)	13/44	10/158	13.17	1	<0.001
SAH (Y)	13/44	33/135	0.26	1	0.609
MetS (Y)	17 (24.3)	43 (25.9)	0.068	1	0.794
TNF-α (pg/mL)[a]	1.20 (0.60)	60.55 (26.09)	336.24	1/236	<0.001
sTNFR1(pg/mL)[a]	981.33 (56.43)	1047.66 (31.02)	0.344	1/195	0.588
sTNFR2 (pg/mL)[a]	1435.86 (68.75)	2020.18 (52.82)	21.55	1/195	<0.001
IL-6 (pg/mL)[a]	7.16 (2.24)	20.65 (8.50)	369.10	1/175	<0.001
IL-17 (pg/mL)[a]	0.55 (0.29)	31.41 (14.95)	141.07	1/175	<0.001
IFN-γ (pg/mL)[a]	1.50 (0.27)	12.11 (6.44)	5.88	1/175	0.016
IL-2 (pg/mL)[a]	0.91 (0.22)	8.82 (5.32)	152.74	1/175	<0.001
IL-10 (pg/mL)[a]	0.29 (0.20)	25.30 (9.52)	469.10	1/175	<0.001
IL-4 (pg/mL)[a]	1.51 (1.00)	71.22 (47.99)	280.76	1/175	<0.001

All results of analyses of variance (F values). X^2: results of analyses of contingency tables. Continuous variables were expressed as mean and SEM and categorical variables were expressed as absolute number (n) and percentage (%)

BMI body mass index, *SAH* systemic arterial hypertension, *MetS* metabolic syndrome, *TNF-α* tumor necrosis factor α, *sTNFR1* soluble tumor necrosis factor receptor 1, *sTNFR2* soluble tumor necrosis factor receptor 2, *IL-6* interleukin 6, *IL-17* interleukin 17, *IFN-γ* interferon γ, *IL-2* interleukin 2, *IL-10* interleukin 10, *IL-4* interleukin 4

[a]These variables were processed in Ln transformation

sTNFR2 than controls. It should be stressed that these biomarker results were not adjusted for possible extraneous variables including age, sex, BMI, and ethnicity. As shown in Fig. 1, patients with moderate/high disability showed higher levels of TNF-α, sTNFR1 and sTNFR2 than those with mild disability ($P=0.029$, $P=0.002$ and $P=0.019$, respectively). Moreover, patients with disability progression showed higher levels of sTNFR1 and sTNFR2 than those with no disability progression ($P<0.001$ and $P=0.005$, respectively), but only patients with ProgMS showed higher levels of sTNFR1 when compared to those with RRMS ($P=0.038$).

Associations with MS diagnosis

Table 2 shows the results of a multivariate GLM analysis with TNF-α, sTNFR1 and sTNFR2 as dependent variables and diagnosis as the primary explanatory variable while adjusting for age, sex, BMI, and ethnicity. We found that MS diagnosis, age, and BMI had significant effects on the levels of TNF-α and its receptors, whereas sex and ethnicity did not. Tests for between-subject effects showed that MS diagnosis was positively associated with TNF-α with an effect size of 44.6% and sTNFR2 with an effect size of 12.3%. BMI showed a modest positive effect on both sTNFR1 and sTNFR2, and age was positively associated with sTNFR2. The GLM model showed equivalence of covariance matrices ($P=0.066$) and the dependent variables presented equal variances ($P>0.05$). In Table 3, we showed the estimated marginal mean values (in z scores) of TNF-α, as well as sTNFR1 and sTNFR2 in MS patients and controls. TNF-α and sTNFR2 were significantly higher in patients with MS than controls, while sTNFR1 did not differ from controls.

Multivariate GLM analysis showed that there were no significant effects of SAH ($F=0.98$, df = 3/184, $P=0.403$, $n=35$), smoking ($F=0.32$, df = 3/185, $P=0.296$, $n=21$), DM ($F=0.24$, df = 3/182, $P=0.866$, $n=12$), MetS ($F=1.22$, df = 3/184, $P=0.302$, $n=46$), and the drug state of the patients ($F=1.51$, df = 12/471, $P=0.117$) on the three TNF data (data not shown).

To delineate the best biomarkers associated with MS, we have carried out different hierarchical logistic regression analyses. We found that TNF-α is the single best predictor of MS diagnosis ($x^2=107.18$, df = 1, $P<0.001$, Nagelkerke = 0.742) with an OR of 20.10 and 95% CI of 12.98–193.30 (lower and upper limit); 96.9% of all subjects

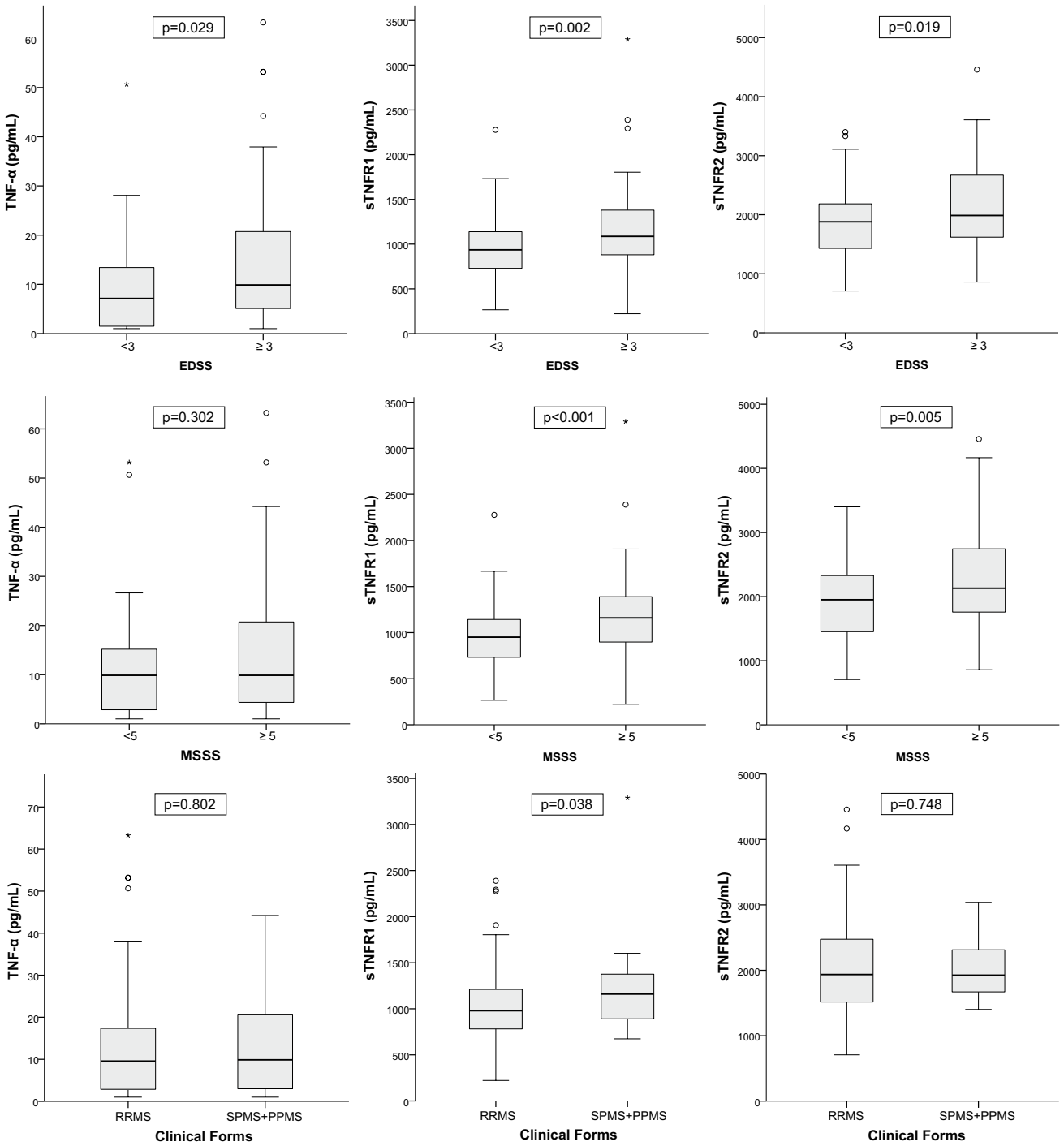

Fig. 1 Plasma levels of TNF-α, sTNFR1 and sTNFR2 according to the disability, disability progression and clinical forms of multiple sclerosis (MS). *EDSS* Expanded Disability Status Scale, *< 3* mild disability, ≥ moderate/high disability, *TNF* tumor necrosis factor, *sTNFR1* soluble tumor necrosis factor receptor 1, *sTNFR2* soluble tumor necrosis factor receptor 2, *MSSS* Multiple Sclerosis Severity Score, *< 5* no progression, *≥ 5* progression, *RRMS* relapsing–remitting multiple sclerosis, *SPMS* secondary progressive multiple sclerosis, *PPMS* primary progressive multiple sclerosis

Table 2 Results of multivariate GLM analysis with tumor necrosis factor (TNF)-α and TNF receptor sTNFR1 and sTNFR2 as dependent variables

Type test	Dependent variables	Explanatory variables	F/X^2	df	*P* value	Partial η^2
Multivariate	TNF-α	Diagnosis	62.61	3/188	<0.001	0.500
	sTNFR1	Sex	0.12	3/188	0.948	0.002
	sTNFR2	Age	6.05	3/188	0.001	0.088
		BMI	3.80	3/188	0.011	0.057
		Ethnicity	0.77	3/188	0.513	0.012
Between-subject effects	TNF-α	Diagnosis (+)	153.04	1/190	<0.001	0.446
	sTNFR1	BMI (+)	5.98	1/190	0.015	0.031
	sTNFR2	Diagnosis (+)	26.67	1/190	<0.001	0.123
		Age (+)	18.21	1/190	<0.001	0.087
		BMI (+)	9.26	1/190	0.003	0.046

All the results of analyses of variance (*F* values)

df degree of freedom, *BMI* body mass index, *TNF-α* tumor necrosis factor α, *sTNFR1* soluble tumor necrosis factor receptor 1, *sTNFR2* soluble tumor necrosis factor receptor 2

Table 3 Model-generated estimated marginal means in *z* scores (SE) of TNF-α, sTNFR1 and sTNFR2 in multiple sclerosis (MS) patients and controls. These results were obtained by the GLM analyses shown in Table 2

	Controls	MS	*F*	df	*P* value
(Ln) TNF-α (*z* scores)*	−1.163 (0.131)	0.498 (0.064)	68.89	1/126	<0.001
(Ln) sTNFR1 (*z* scores)*	−0.038 (.194)	0.087 (0.095)	2.18	1/126	0.112
(Ln) sTNFR2 (*z* scores)*	−0.731 (.175)	0.196 (0.086)	6.51	1/126	0.012

TNF-α tumor necrosis factor α, *sTNFR1* soluble tumor necrosis factor receptor 1, *sTNFR2* soluble tumor necrosis factor receptor 2

*These variables were processed in Ln transformation; the analysis was adjusted for age, sex, body mass index and ethnicity

were correctly classified with a sensitivity of 100% and a specificity of 79.3% (data not shown).

Associations with disability, progression of disease and clinical forms of MS

To delineate the best biomarkers associated with the disability (EDSS ≥ 3 versus EDSS < 3), disability progression (MSSS ≥ 5 versus MSSS < 5) and clinical forms (RRMS versus ProgMS), we have carried out different automatic stepwise regression analyses with disability, progression and clinical subtypes as dependent variables. Table 4 regression #1 shows that EDSS ≥ 3 was best predicted by sTNFR1 and age (both positively), while TNF-α and sTNFR2 had no significant effects. With this model, 73.6% of all cases were correctly classified with a sensitivity of 74.6% and a specificity of 73.8% (R^2 Nagelkelke = 0.262, $x^2 = 34.37$, df = 2, $P < 0.001$). Entering drug state, BMI, smoking, HAS, ethnicity, DB and MetS showed that these variables were not significant predicting EDSS ≥ 3 (data not shown).

Logistic regression #2 shows that MSSS ≥ 5 was best predicted by sTNFR1 and age (both positively) and that TNF-α and sTNFR2 have no significant effects. With this model, 66.0% of all cases were correctly classified with a sensitivity of 58.5% and specificity of 72.2%, (R^2 Nagelkelke = 0.170, $x^2 = 19.62$, df = 2, $P < 0.001$). Entering drug state, BMI, smoking, HAS, DM, ethnicity and MetS showed that smoking was a significant predictor ($F = 5.69$, df = 1, $P = 0.017$) and that the effects of sTNFR1 remained significant ($F = 8.18$, df = 1, $P = 0.004$) (data not shown).

Regression #3 shows that the ProgMS was best predicted by increased sTNFR1 (positively) and increased sTNFR2 (negatively), coupled with age and sex. With this model, 86.9% of all cases were correctly classified with a sensitivity of 66.7% and specificity of 89.8%, (R^2 Nagelkelke = 0.285, $x^2 = 27.43$, df = 4, $P < 0.001$). Consequently, we have computed a score reflecting the sTNFR1/sTNFR2 ratio. Table 4 regression #4 shows that ProgMS was significantly and positively associated with this ratio after adjusting for age and sex. With this model, 86.3% of all cases were correctly classified with a sensitivity of 66.7% and specificity of 89.1%, (R^2 Nagelkelke = 0.285, $x^2 = 27.43$, df = 3, $P < 0.001$). Moreover, 1000 bootstraps showed that the composed score zLn sTNFR1–zLn sTNFR2 had a significant effect ($P = 0.019$) with a *B* value of 0.693, bias of 0.066 and standard error (SE) of 0.354

Table 4 Result of binary logistic regression analysis with an increased disability (EDSS score ≥ 3), disease progression (MSSS ≥ 5) and progressive clinical forms of multiple sclerosis as dependent variables

Dependent variables	Explanatory variables	Wald	df	*P* value	OR	95% CI
Model # 1 EDSS ≥ 3	Age	19.82	1	< 0001	1.069	1.038–1.101
	sTNFR1	7.36	1	0.007	1.653	1.150–2.377
Model # 2 MSSS ≥ 5	Age	4.82	1	0.028	1.031	1.003–1.059
	sTNFR1	9.92	1	0.002	1.90	1.274–2.835
Model # 3 ProgMS	Age	14.20	1	< 0.001	1.084	1.039–1.130
	Male sex	4.75	1	0.029	3.219	1.125–9.214
	sTNFR1	3.88	1	0.049	2.048	1.003–4.179
	sTNFR2	4.01	1	0.045	0.495	0.251–0.986
Model # 4 ProgMS	Age	16.32	1	< 0.001	1.084	1.043–1.128
	Male sex	4.76	1	0.029	3.222	1.126–9.218
	zsTNFR1_zsTNFR2	5.09	1	0.024	2.000	1.095–3.655

EDSS Expanded Disability Status Scale, *MSSS* Multiple Sclerosis Severity Score, *ProgMS* progressive clinical forms of multiple sclerosis, *df* degree of freedom, *OR* odds ratio, *CI* confidence interval, *sTNFR1* soluble tumor necrosis factor receptor 1, *sTNFR2* soluble tumor necrosis factor receptor 2, sTNFR1 and sTNFR2 are entered in *z* scores of Ln transformations; zsTNFR1_zsTNFR2 is computed as *z* score of Ln sTNFR1 − *z* Ln sTNFR2. *Model 1#* Sensitivity = 74.6, Specificity = 73.8%, correct cases = 73.6%, R^2 Nagelkelke = 0.262 ($X^2 = 19.62$, df = 2, $P < 0.001$), *Model 2#* Sensitivity = 58.5%, Specificity = 72.2%, correct cases = 66.0%, R^2 Nagelkelke = 0.170 ($X^2 = 19.62$, df = 2, $P < 0.001$), *Model 3#* Sensitivity = 66.7%, Specificity = 89.8%, correct cases = 86.9%, R^2 Nagelkelke = 0.285 ($X^2 = 27.43$, df = 4, $P < 0.001$), *Model 4#* Sensitivity = 66.7%, Specificity = 73.8%, correct cases = 89.1%, R^2 Nagelkelke = 0.285 ($X^2 = 27.43$, df = 3, $P < 0.001$)

(95% CI 0.180–1.526). Entering drug state, BMI, smoking, HAS, DM, ethnicity and MetS, the analysis showed that BMI had a protective effect on the ProgMS ($F = 4.81$, df = 1, $P = 0.028$) and that the effects of zLn sTNFR1–zLn sTNFR2 remained significant ($F = 6.37$, df = 1, $P = 0.012$).

Discussion

The main findings of the present study are that increased TNF-α and sTNFR2 levels are associated with MS, as well as increased levels of sTNFR1 coupled with age are positively associated with moderate/high disability and disability progression. Moreover, increased sTNFR1 levels combined with lower sTNFR2 levels, increasing age and male sex increase risk towards the ProgMS, suggesting that high levels of sTNFR1 may be a risk factor while high levels of sTNFR2 may exert a protective role modulating the immunopathological mechanisms of MS. As such, a newly proposed score reflecting the sTNFR1/sTNFR2 ratio could be an important biomarker to evaluate the development of the ProgMS.

Our results also demonstrated that the MS diagnosis can exert a strong effect of 44.6% in the levels of TNF-α. The higher levels of TNF-α observed in the present cohort of MS patients compared to controls are consistent with previous studies [5, 13, 27], as well as regarding to sTNFR1 and sTNFR2 [10]. TNF-α is considered the principal mediator of inflammatory response and is increased in CSF and peripheral mononuclear cells during relapses of MS suggesting that this cytokine exerts an important effect in the pathogenesis and progression of the disease [1].

The higher levels of sTNFR2 observed in MS patients of the present study, as well as its association with the MS diagnosis show the vital role of this receptor in the modulation of the immune response, most likely through its interactions with Treg cells [28]. The importance of TNFR2 signaling for neuroprotection and tissue regeneration was demonstrated in the reversible demyelination cuprizone model. In this model, oligodendrocyte progenitor cell (OPC) proliferation and remyelination are significantly late in TNF-α and TNFR2 knockout mice, thus demonstrating that tissue regeneration is dependent on TNF-α signaling via TNFR2 [7, 29]. Some of the protective and regenerative effects of TNFR2 activation could be explained by the fact that this receptor promotes the release of anti-inflammatory and neurotrophic factors from astrocytes and microglia. In particular, astrocyte-derived factors, namely chemokine CXCL12 and leukemia inhibiting factor (LIF), which promote oligodendrocyte differentiation and may thus favor remyelination [30–32]. In this way, our results suggest that sTNFR1 and sTNFR2 may have an opposite role in the development of ProgMS, while sTNFR1 may act as a harmful factor for neurodegeneration, sTNFR2 may act as a protective factor involved in the remyelination [7, 29].

Using binary logistic regression analysis to predict the models for high disability in MS patients, we observed that older patients with higher levels of sTNFR1 showed increased moderate/severe disability, as well as more disability progression than younger participants with lower

levels of sTNFR1. These results underscore age as one independent factor for disability and disability progression [33, 34]. The positive association between sTNFR1 with increased disability in MS patient is in agreement with previous study [10], underscoring that TNF-α could be signaling via sTNFR1. These results could be explained by the fact that sTNFR1 can exacerbate neurodegeneration mediated by activation of nuclear factor kappa B (NF-B) signaling pathway in endothelial cells and can induce the expression of cell adhesion molecules thereby promoting the transmigration of immune cells into the CNS parenchyma [35]. Moreover, in glial cells, in particular, microglia and astrocytes, enhanced sTNFR1 signaling will thus greatly enhance the inflammatory response thereby promoting neurodegeneration [29, 36]. TNF-α, via sTNFR1, can also in vitro mediate direct apoptosis of neurons by activation of caspase 8 [37] and elevated levels of TNF-α and activated caspase 8 in spinal cord injury models further support the notion of TNF-α mediated direct neuronal cell death in vivo [38]. In this way, our results support the hypothesis that sTNFR1 is a factor that is more associated with disability progression in MS patients and that therapy targeting this receptor could be useful for the treatment of them [19].

The positive associations between sTNFR1 levels and disability, disability progression and ProgMS, as well as the negative association between sTNFR2 and ProgMS, may underscore the key role of these molecules in the immune-inflammatory pathophysiology of MS [39, 40]. Although sTNFR1 and sTNFR2 are strongly correlated with each other, they have distinct roles in immune responses. TNFR2 is expressed on cells within specific lymphocyte populations, including Treg cells [6] and has an important role in apoptotic cell death and in thymocyte and cytotoxic T cell proliferation [41, 42]. When the TNFR2 signaling pathway is activated, it increases Treg stability, expansion and function. Under inflammatory conditions, the membrane TNFR2 can be shed into the plasma (where the receptor is measurable as the sTNFR2) thereby attenuating TNF-α signaling [28].

Furthermore, the results of the present study showed that age and sTNFR1 were the best predictors of ProgMS, suggesting that sTNFR2 may exert a protective role in these clinical forms. We also showed that age was associated with the ProgMS, while male sex was positively associated with this condition. Although men have a lower risk of developing MS than women, previous studies have suggested that male sex is a risk factor for a progressive onset of the disease, poor recovery after initial attacks, more rapid accrual of disability and an overall more malignant course. Conversely, females are more likely to manifest benign MS outcome [42, 43].

ProgMS is characterized clinically by the accumulation of neurological disability, independent of relapses, and can present as the initial disease course (PPMS) or more commonly after an initial relapsing phase of the disease (SPMS) [44]. Degeneration of chronically demyelinated axons is a prominent feature of the progressive MS brain and a major cause of irreversible neurological disability, besides the damage or dysfunction of astrocytes and microglia activation [1]. Whereas the proinflammatory and neurodegenerative effects of TNF-α are primarily mediated by solTNF-α and thus by TNFR1, signaling via tmTNF-α, predominantly via TNFR2, is mainly neuroprotective and supports tissue homeostasis and regeneration [29].

sTNFR1 and sTNFR2 play an important role in the regulation of TNF-α activity under physiologic conditions through neutralizing the function of TNF-α without invoking inflammatory effects. sTNFR1 and sTNFR2 have the ability to bind TNF-α, acting as an inhibitor that competes with a membrane receptor. At low concentrations, they exhibit agonistic activity with respect to TNF-α, while at high concentrations, they act antagonistically, binding excessive TNF at the site of inflammation [45]. In various pathologic states, the production and release of sTNFR1 and sTNFR2 may mediate host response and determine the course and outcome of disease by interacting with TNF-α and competing with cell surface receptors. Therefore, the determination of sTNFRs in body fluids such as plasma or serum is a new tool to gain information about immune processes and provides valuable insight into a variety of pathological conditions [46].

The proposed mechanism for the modulatory effects of sTNFR1 and sTNFR2 during inflammatory demyelination involves blocking proinflammatory effects of TNF-α as well as regulation of lymphocyte clearance by apoptosis. sTNFR2 levels are increased in the serum of ProgMS patients during IFN-β therapy and this TNFR2 binds and neutralizes TNF-α and TNF-β, thus inhibiting these two proinflammatory cytokines. Therefore, the increased sTNFR2 may be one mechanism by which IFN-β mediates its effects in the treatment of MS [47, 48]. Other study reported that MS patients receiving treatment with IFN-β showed higher sTNFR2 serum levels during the follow-up period compared with those of placebo group. Moreover, a strong correlation at 24 months was observed between levels of sTNFR2 and disability scores in the placebo group and a trend for negative association was found between changes in sTNFR2 and percentage change in T2-weighted lesion load at 24 months in the IFN-β treated group [49].

The apparent conflicting results of TNF-α obtained in the present study with those reported previously by Sharief and Hentges [11] who showed high levels of TNF in CSF samples of patients with ProgMS and these values correlated with the severity of disease, may be explained by some reasons, such as the design of the study and the method used for the TNF-α measurements. While Sharief and Hentges [11] carried out a 24-month prospective study

and measured the serum and CSF levels of TNF-α using enzyme-linked immunosorbent assay (ELISA), we carried out a cross-sectional study and used a customized immunofluorimetric assay to measured plasma TNF-α levels, a method with high sensitivity and specificity than ELISA. Other reason could be that these authors [11] excluded the patients who received treatment with immunossuppressive medications at any time. In our study, the patients with MS were treated with different MS therapies and the results obtained were after adjusted by these therapies. Other explanation could be the type of controls that they included in the study. While they used patients with other neurological diseases that collected cerebrospinal fluid, we included healthy individuals. Moreover, our individuals are from Brazilian population, a genetically more heterogeneous than the individuals included in the Sharief and Hentges study [11]. Genetic variations may result in different immune response regarding the gene expression and regulation taken into account several variants in the *TNFA* and *TNFB* genes that play a role in the TNF-α expression [reviewed in [50]]. Moreover, the presence of the *TNFB2/B2* genotype was associated with increased TNF-α levels in Brazilian patients with MS [51].

Furthermore, genetic variants in the *TNFRSF1A* may also explain apparent conflicting results of plasma levels of sTNFR1 obtained in different studies. A single nucleotide variant identified in the largest MS genome-wide association study [52] has been reported as occurring in the *TNFRSF1A* gene that encodes TNFR1, and has been shown to be associated with an increased susceptibility to MS development. This genetic variant results in the expression of a sTNFR1, which can block pan TNF-α signaling [53]. Therefore, treatment using an anti-TNFR1 as strategy might also neutralize the detrimental effects of this sTNFR1 variant.

The present results should be discussed taking into account some limitations. First, the cross-sectional design of the study does not allow to make causal inferences. Second, the sample size of patients with ProgMS was rather modest. However, the study used the MSSS scale, which is currently the best available rating scale to measure speed of disability accumulation in MS [23]. To our knowledge, the present study is the first to evaluate the association between TNF-α, sTNFR1 and sTNFR2 with disability, disability progression, and clinical forms of MS.

Taken together, our results support the hypothesis of the distinct role of TNF-α and its receptors in the immunopathogenesis of MS, with a clear evidence of a neurodegenerative role of sTNFR1 in the disability, disability progression, and clinical forms, while high levels of sTNFR2 associated with the presence of MS, as well as with ProgMS, may be a compensatory and protective response to modulate the immunopathological mechanisms involved in the disease. These results also suggest that the specific blockage of TNFR1 could be a possible therapy approach for the treatment of patients with MS, mainly those with ProgMS who do not respond to the current therapies. Moreover, enhancing TNFR2 signaling in the CNS may provide a viable therapeutic option to boost remyelination and achieve neuroprotection, halting, or even reverting, the progression of the disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent All the participants included in this study provided written informed consent.

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