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A Novel Approach to Discriminate Subgroups in Multiple Sclerosis

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ABSTRACT

Multiple sclerosis (MS) is an autoimmune disease of central nervous system. Since different types of immune cells are involved in MS pathogenesis, in this study we aimed to evaluate serum levels of several immunological components including soluble CD4 (sCD4), sCD8, sCD163, and immunoglobulins as markers of activity of T-cells, macrophages, and B-cells in different types of MS.

Serum levels of sCD4, sCD8, and sCD163 of patients with relapsing-remitting MS (RRMS, n=61), primary progressive MS (PRMS, n=31), secondary progressive MS (SPMS, n=31), clinical isolated syndrome (CIS, n=31) and neuromyelitis optica (NMO, n=31), and healthy controls (n=49) were measured using enzyme-linked immunosorbent assay (ELISA). Serum levels of Ig-G, Ig-M, and Ig-A were determined using nephelometric technique.

Serum levels of sCD4, sCD8, sCD163, Ig-G, Ig-M, and Ig-A were significantly different in five groups of cases ($p<0.05$). Furthermore, application of stepwise method of discriminant analysis yielded 4 significant discriminant functions of classification due to the presence of six levels of categorical variables in the analysis. The most important function explained 85.5% of the total variance with the correlation value of 0.79.

Taken together, our preliminary analysis suggests that although we found some functions to discriminate most of the patients, further studies will be required to individuate immunological markers characterizing the different type of MS including RRMS, PPMS, SPMS, CIS and NMO as proved by the data on sCD4, sCD163, Ig-M, and Ig-G in blood.

Keywords: Multiple sclerosis; Soluble CD4; Soluble CD8; Soluble CD163; Immunoglobulins

INTRODUCTION

Reactive microglia/macrophages have been suggested as active participants in inflammatory multiple sclerosis (MS) lesions.[1] It has been shown that clusters of activated macrophages are tissue

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Specific monocytes with versatile roles even before demyelination in MS.[2] Infiltrating macrophages and activated microglia are the predominant cell types in active MS lesions in both stages of activation including pro-inflammatory and anti-inflammatory.[3] Macrophages have been implicated in inducing neural pathology in MS by myelin phagocytosis, secretion of a wide variety of cytokines, antigen presentation, and lymphocyte stimulation.[4] CD163, a member of the scavenger receptor cysteine-rich family class B, is found on macrophages in the CNS, including perivascular macrophages (PVMs) and microglia.[5] Macrophages play a dual role in the CNS as they are implicated in both neuroprotective and anti-inflammatory actions in the healthy and injured CNS and they can also fuel neuroinflammation and promote secondary neurodegenerative events.[6,7] It has been shown that serum levels of soluble CD163 (sCD163) as a marker of macrophage activity is inversely associated with membrane CD163 (mCD163). Presence of this factor in serum, which is related to the different inflammatory disorders, can be measured using enzyme-linked immunosorbent assay (ELISA).[8,9] Presence of self-antigens in the CNS is a major target for migration and therefore an infiltration of many subtypes of immune cells such as CD4, CD8, and macrophages into the white matter in MS patients.[10] It has been shown that depletion of lymphocytes population in MS patients can prevent formation of new lesions; however, depletion of only CD4 cells using anti-CD4 cells failed to reduce relapse rate or formation of new lesions.[11,12] Both CD4 and CD8 cells have a role in formation of MS lesions; as CD4 cells are observed in acute lesions and predominance of CD8 cells is observed in chronic lesions.[13]Although there is considerable evidence with respect to the key role of CD4 cells in pathogenesis of MS, there are many arguments on the role of CD8 cells. Taken together, both of them are implicated in MS pathogenesis; CD4 by response to the viral infection and myelin antigens and CD8 by causing death in oligodendrocytes.[14,15] Another evidence in this regard is that, activated myelin reactive CD4 cells were detected in blood and CSF of MS patients, while only non-activated myelin reactive T cells were present in blood of controls.[16] Previously, serum concentration of sCD8 was suggested as an indicator of suppressor/cytotoxic activity of T cells.[17] Levels of sCD4 and sCD8 has been suggested as activity markers of T-cells which can be measured in serum using ELISA as performed previously in other disorders such as rheumatoid arthritis, systemic sclerosis, and systemic lupus erythematous.[18-20] In addition, there is extensive evidence suggesting the role of B cells in MS pathogenesis. In this regard, presence of B cells in MS lesions with higher levels of them in acute demyelination has been reported.[21,22] Presence of autoantibodies such as anti-myelin basic protein (MBP) and proteolipid protein (PLP) in MS lesions suggest other evidence for the involvement of B cells in MS pathogenesis.[23]

In this study we aimed to investigate serum levels of several immunological markers including sCD4, sCD8, sCD163, Ig-G, Ig-M, and Ig-A in different types of MS; relapsing-remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS), clinical isolated syndrome (CIS), and neuromyelitis optica (NMO) to compare possible role of them in each type of MS. Therefore, in this study, a stepwise discriminant analysis was preliminarily applied to study immunological markers able to discriminate amongst the five groups of MS disorders.

MATERIALS AND METHOFDS

Study Subjects

This case-control study was performed to assess the role of innate, cellular و and humoral immunity in MS patients who reside in Isfahan, one of the most populated provinces of Iran. From June 2013 to July 2014, 219 patients were admitted to MS clinic of Alzahra hospital, Isfahan, Iran. We performed further diagnostic investigations in order to find eligible cases according to the study protocol shown in Figure 1.

34 patients were excluded from the study and 185 patients met the inclusion criteria. In addition, 49 matched healthy subjects were chosen from individuals referred to Isfahan Transfusion Organization for sample collection (control group).

Patients included in the study were divided into five subgroups: RRMS, PPMS, SPMS, CIS and NMO.

Patients were excluded if they had other neurologic or autoimmune diseases, had some other medical disease, had been undergoing immunemodulating treatment within the month preceding sampling, or if data were missing. At the time of study, none of the

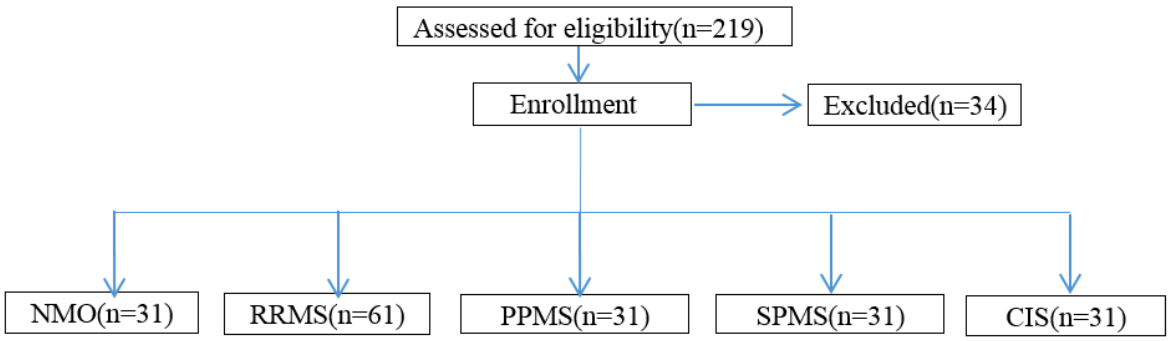

Figure 1. Flowchart demonstrating protocol of the study: 34 patients were excluded from the study and 185 patients met the inclusion criteria. Patients enrolled in the study were divided into five subgroups including: neuromyelitis optica (NMO), relapsing remitting multiple sclerosis (RRMS), primary progressive multiple sclerosis (PPMS), clinically isolated syndrome (CIS).

patients with RRMS and CIS were in the clinically active stage of the disease and all of the RRMS patients were in remission. Our participants were mostly clinically inactive patients with SPMS and PPMS who were not under any treatment within the 3 months prior to the study and among the 10 clinically active MS patients; two were on interferon-β, one on natalizumab, and one on fingolimod treatments. None of the participating subjects had received corticosteroids in the previous 3 months.

All patients had been clinically diagnosed as a case of MS or NMO according to the Mcdonalds or Wingerchuk criteria. For evaluation of disability in cases, expanded disability status scale (EDSS) was used. In the month preceding the study, all patients underwent pre-study evaluation to record demographic data, complete neurologic and medical history, and the findings of physical and neurologic examinations and magnetic resonance imaging (MRI) scans of all patients from both brain and spinal cord were collected. Laboratory tests including cerebrospinal fluid (CSF), oligoclonal bands (OCB), and NMO-IgG antibody detection were performed in our laboratory. Informed written consents were collected from all participants of study. Protocol of study was approved by ethnical committee of Multiple Sclerosis and Neuroimmunology Research Center, Isfahan, Iran (No.MS-1264).

Determination of sCD16, sCD4, sCD8, Ig-G, Ig-M, and Ig-A Levels

Peripheral blood samples were obtained from patients and healthy controls according to the routine venipuncture method, and then sera were immediately frozen at –80 ºC and stored at this temperature until use. Level measurement of sCD163 was carried out using the enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN, USA) with a detection limit of1.56 ng/mL, according to the manufacturer's instructions. Concentrations of sCD4 and sCD8 were also determined with commercially available ELISA kits (T Cell Diagnostics, Inc., Woburn, MA, USA). Automated system (nephelometric determination) with COBAS INTEGRA700 analyzer (Roche Diagnostics, Mannheim, Germany) was used for measuring levels of immunoglobulin M, G, and A in the sera of both healthy subjects and patients.

Statistical Analysis

After collection of all data, they were inputted in SPSS software (version 20.0, Chicago, IL, USA). Kolmogorov-Smirnov test was used for assessment of normal distribution of data. Analysis of variance (ANOVA) was performed for comparison between groups of cases and also healthy controls with respect to the serum levels of sCD163, sCD4, sCD8, Ig-G, Ig-M, and Ig-A. Furthermore, we used discriminant analysis with six level namely, CIS, RRMS, SPMS, PPMS, NMO, and healthy control, as well as, 8 variables including 6 serum concentrations being applied. All tests were two-tailed and $p \leq 0.05$ was deemed as a significant threshold.

RESULTS

Characteristics of the Study Subjects

This case-control study included 31 CIS patients (23 women and 8 men), 31 SPMS patients (23 women and 8 men), 31 PPMS patients (23 women and 8 men), 61 RRMS patients (41 women and 20 men), 31 NMO patients (18 women and 13 men), and also 49 healthy subjects (30 women and 19 men) as the control group with the mean age of 35.61±7.57, 36.96±4.76, 38.96±5.31, 37.91±9.30, 37.96±9.87, and 35.26±7.97, respectively. Since groups of cases and healthy controls were matched with respect to the age and sex, no significant differences were observed between them (p=0.27 and p=0.61, respectively). Other clinical features and paraclinical characteristics of cases and healthy controls are shown in Table 1.

One-way analysis showed significant differences for all immunological factors including sCD4, sCD8, Ig-G, and Ig-M (p<0.05) except for serum levels of Ig-A, which failed to show statistically significant differences between groups of study (p=0.37) (Table 2).

Table 1. Clinical and paraclinical characteristics of patients with RRMS, SPMS, PPMS, CIS, NMO, and healthy controls

Characteristics		RRMS	SPMS	PPMS	CIS	NMO	Healthy control	*p*-value
No. of subjects		n=61	n=31	n=31	n=31	n=31	n=49	-
Gender M/F		41/20	23/8	23/8	23/8	18/13	30/19	0.61
Age		37.91±9.30	36.96±4.76	38.96±5.31	35.61±7.57	37.96±9.87	35.26±7.97	0.27
EDSS		2.62±1.42	3.35±1.09	3.96±1.47	1.82±1.28	2.72±1.32	-	0.00
Duration of Disease		4.73±4.42	14.32±2.94	5.45±3.77	0.16±0.32	6.22±5.14	-	0.00
Relapse number (during last year)		0.70±0.69	0.19±0.40	-	0.80±0.60	0.74±0.57	-	0.00
Brain MRI	Positive	60(98.4%)	30(96.8%)	30(96.8%)	28(90.3%)	12(38.7%)	0(0.0%)	0.00
	Negative	1(1.6%)	1(3.2%)	1(3.2%)	3(9.7%)	19(61.3%)	49(100%)	
Spinal MRI	Positive	46(75.4%)	28(90.3%)	26(83.9%)	22(71%)	31(100%)	0(0%)	0.01
	Negative	15(24.6%)	3(9.7%)	5(16.1%)	9(29%)	0(0%)	49(100%)	
CSF-OCB	Positive	44(72.1%)	17(54.8%)	18(58.1%)	20(64.5%)	3(9.7%)	-	0.19
	Negative	17(27.9%)	14(45.2%)	13(41.9%)	11(35.5%)	28(90.3%)	-	
NMO-Ig-G	Positive	4(6.6%)	0(0.0%)	2(6.5%)	1(3.2%)	11(35.5%)	-	0.00
	Negative	57(93.4%)	31(100%)	29(93.5%)	30(96.8%)	20(64.5%)		

Except for age, gender, and CSF-OCB, statistically significant differences in demographics and paraclinical characteristics were found between groups of patients and healthy controls.

M/F: male/female; RRMS: relapsing remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis; PPMS: primary progressive multiple sclerosis; CIS: clinically isolated syndrome; NMO: neuromyelitis optica; MRI: magnetic resonance imaging; OCB: oligoclonal band; Ig-G: Immunoglobulin-G; sCD163: soluble CD163; sCD4: soluble CD4; sCD8: soluble CD8; Ig-M: immunoglobulin-M; Ig-A: immunoglobulin-A.

Table 2. Serum levels of immunological factors in different types of multiple sclerosis

Characteristics	RRMS	SPMS	PPMS	CIS	NMO	Healthy control	*p*-value
sCD163	2.16±1.14	2.72±1.26	1.98±0.53	2.39±1.42	2.47±1.19	1.45±0.73	0.00
sCD4	36.04±8.07	38.03±6.79	32.00±6.69	28.35±8.08	34.70±5.94	22.48±5.16	0.00
sCD8	378.39±285.94	330.51±100.04	312.19±106.15	300.16±99.82	270.32±94.34	279.97±94.78	0.02
Ig-G	1036.95±180.47	1196.32±365.34	1006.38±171.60	955.03±160.19	1397.19±173.22	927.97±187.16	0.00
Ig-M	158.50±43.11	180.41±42.34	171.45±49.19	140.70±44.98	191.19±51.47	110.48±36.83	0.00
Ig-A	169.12±87.72	181.83±70.06	201.06±93.54	169.12±87.72	209.64±106.83	178.22±75.18	0.37

RRMS: relapsing remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis; PPMS: primary progressive multiple sclerosis; CIS: clinically isolated syndrome; NMO: neuromyelitis optica

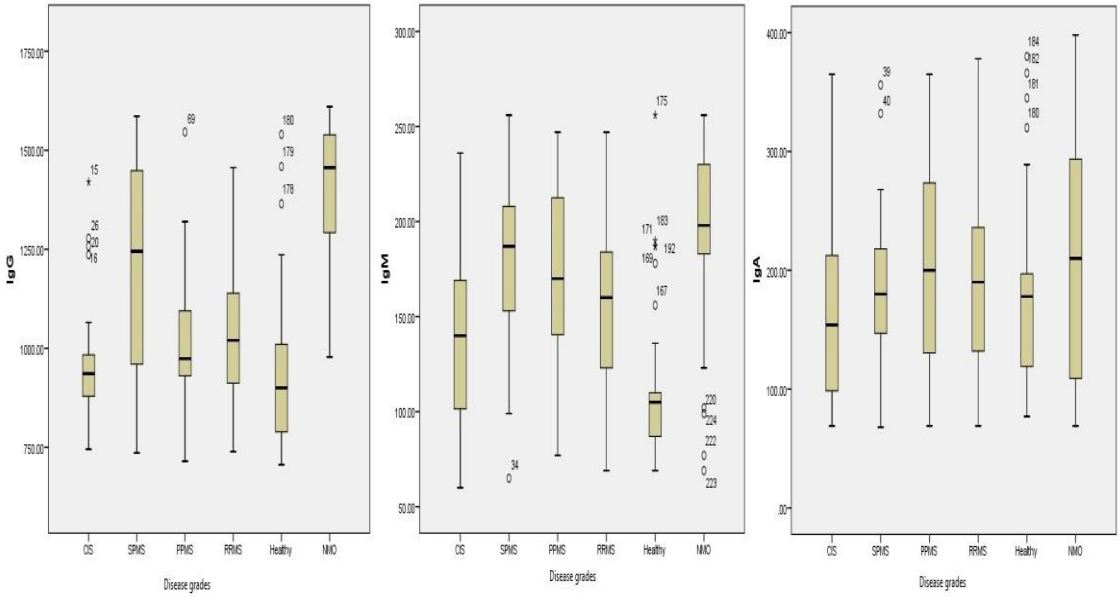

Figure 2. Boxplot of Ig-G, Ig-M, and Ig-A concentrations (mg/dL) in serum of subtypes of multiple sclerosis
RRMS: relapsing remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis; PPMS: primary progressive multiple sclerosis; CIS: clinically isolated syndrome; NMO: neuromyelitis optica

Each group of patients showed significantly higher serum levels of sCD163 compared to healthy controls (p<0.05). However, when we only analyzed the groups of patients, we found no significant difference between them regarding the serum levels of sCD163 (p>0.05). Similar to sCD163, groups of patients showed significant differences compared to healthy controls regarding the serum levels of sCD8 (p<0.05). According to the post hoc analysis, there are significant differences between some groups, but taken together, we cannot statistically assign each marker to a specific group of patients. The serum levels of immunoglobulins and also CD4, CD8, and CD163 in the serum of patients with different types of MS and healthy controls are shown in Figure 2 and 3.

Correlations between Serum Markers and Clinical Features of Patients

All possible correlations between serum markers and some clinical features of patients and controls including sCD4, sCD8, sCD163, Ig-G, Ig-M, Ig-A, relapse number, and EDSS were analyzed. We found that serum levels of sCD163 are significantly correlated with EDSS (p=0.00 and R=0.63) (figure 4). Serum level of Ig-A was associated with serum level of sCD4 (p=0.02 and R=0.18). Furthermore, we found that serum level of Ig-M is significantly in line with number of relapses in patients (p=0.01 and R=0.21).

Discriminant Analysis

Discriminant analysis was conducted to determine which factors best discriminated between six groups of the study. The best combination of immunological markers was selected by stepwise discriminant analysis using the minimum Wilk's lambda test. In this regard, we used discriminant analysis with six levels, namely, CIS, RRMS, SPMS, PPMS, NMO, and healthy control, as well as 8 variables including 6 serum concentrations applied as independent variables. To remove superfluous data from our analysis we used stepwise method, which yielded 4 significant discriminant functions of classification due to the presence of six levels of categorical variable in the analysis. Moreover, 4 variables, i.e., sCD4, sCD163, Ig-M, and Ig-G were selected. Other details of model were summarized in Table 3.

Each function is applied to maximize the difference between levels or groups of patients which can also be shown as an equation that discriminate between groups with a strong power. Discriminant

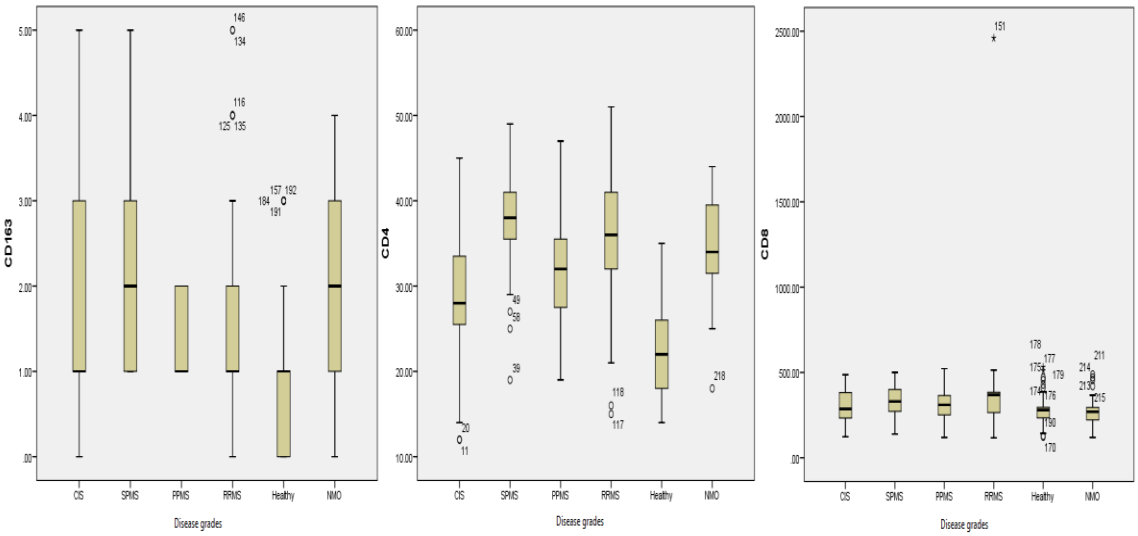

Figure 3. Boxplot of sCD163 (mg/L), sCD4 (U/mL), and sCD8 (U/mL) concentrations in serum of subtypes of multiple sclerosis

RRMS: relapsing remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis; PPMS: primary progressive multiple sclerosis; CIS: clinically isolated syndrome; NMO: neuromyelitis optica

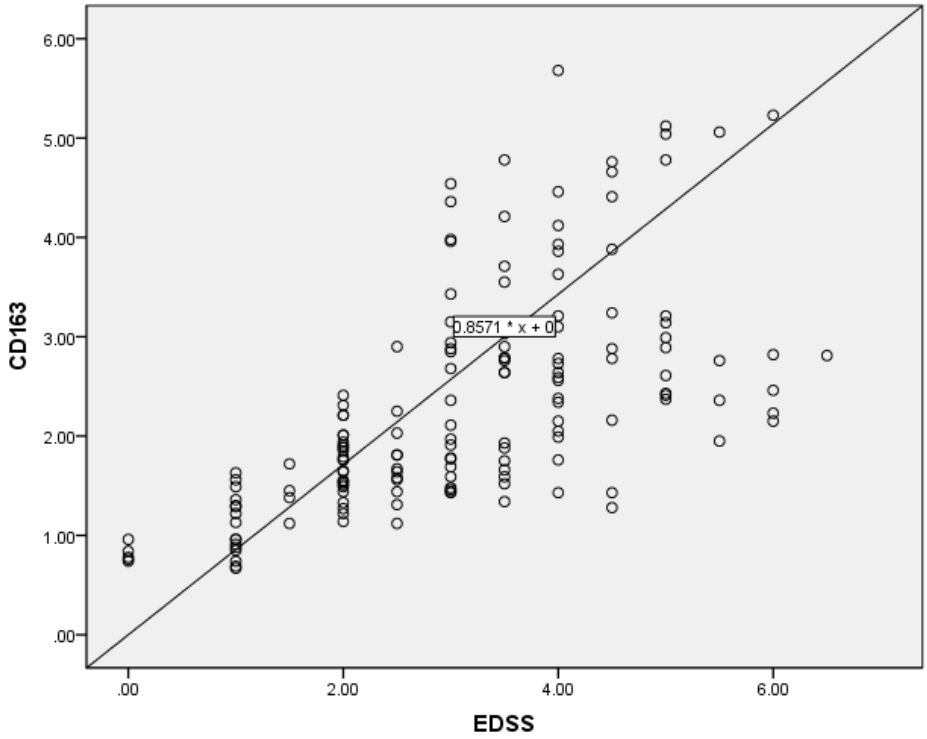

Figure 4. Correlation between serum levels of sCD163 (mg/L) and EDSS in multiple sclerosis patients

EDSS: expanded disability status scale

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Table 3. Matrix structure coefficients, percentage of variance, eigenvalues, canonical correlations and Wilks' Lambda of the classification model of multiple sclerosis patients

Immunological markers	Function 1	Function 2	Function 3	Function 4
CD4	0.559	0.630*	-0.309	-0.441
CD163	0.252	0.128	0.954*	0.100
Ig-G	0.528	-0.816*	-0.030	-0.233
Ig-M	0.454	0.037	-0.225	0.861*
% of variance	85.3%	11.3%	1.8%	1.5%
Eigenvalues	1.768	0.235	0.038	0.032
Canonical correlation	0.799	0.436	0.192	0.176
Wilk's Lambda	0.273	0.756	0.933	0.969

This table shows statistical importance of selected categorical variable in discriminant functions of classification.

function=a+b1V1+b2V2+…+biV, where b= weight for that variable or the discriminant coefficient which also shows ability of each variable for discrimination between groups and a= a constant. Finally, we can use these functions to calculate to which group, CIS, RRMS, SPMS, PPMS, NMO or healthy control, each case mostly belonged. In other words, each case is classified to a specific group for which it had the highest classification score of the functions.

Discriminant analysis for classifying patients into six groups illustrated that two canonical discriminant functions 1 and 2 cumulatively accounted for 96.6% of the total variance. As it is shown in Table 3, function 1 explains 85.5% of the total variance with the correlation value of 0.79 indicating it as the best discrimination between healthy controls, CIS, RRMS, PPMS, SPMS, and NMO patients. The function 1 as the most important discriminating one was strongly correlated to high concentrations of serum levels of sCD4 (0.680) and Ig-G (0.602) which means that cases with positive score on the function 1 tended to have higher concentration of former markers. The serum levels of immunological markers in predicting dependent variables are shown in Table 4, in other words, the standardized discriminant coefficients were applied to compare the relative importance of the independent variables.

The standardized discriminant coefficients show loadings for each variable in the discriminant function.

The second function explained 11.5% of the total variance with the canonical correlation value of 0.43. Similar to the function 1, serum levels of sCD4 and Ig-G had the most important role in discrimination of function 2, but in contrast with the function 1, cases with positive score on the function 2 tended to have lower concentrations of Ig-G. On the other hand, function 3 and 4 revealed only a slight increase in the total variance (function 3+ function 4= 3.3%) with low correlation values (0.19 and 0.17, respectively); they are considered in the classification procedure, but are not graphically represented. Application of the functions resulted in the correct classification of 70.3% of original cases; while, cross validation of the functions resulted in the correct classification of 51.7% of cases. Indeed, these percents demonstrate that the discriminant analysis provides classification rates far higher than those that could be obtained by chance. In this regard, Table 5 shows classification matrix, which reveals the ability of model to correctly classify each of the six groups of participants.

Table 4. Standardized discriminant coefficients for whole blood in multiple sclerosis

Immunological markers	Function 1	Function 2	Function 3	Function 4
CD163	0.350	0.178	0.927	0.050
CD4	0.680	0.573	-0.211	-0.439
Ig-M	0.471	0.069	-0.222	0.852
Ig-G	0.602	-0.752	-0.027	-0.292

Table 5. Classification matrix in multiple sclerosis

Type of classification		Predicted group membership						Total
	Group	CIS	SPMS	PPMS	RRMS	Healthy	NMO	
Original CIS		11(35.5%)	1(3.2%)	6(19.4%)	4(12.9%)	9(29.0%)	0(0.0%)	31(100%)
	SPMS	0(0.0%)	10(32.3%)	5(16.1%)	5(16.1%)	0(0.0%)	11(35.5%)	31(100%)
	PPMS	5(16.1%)	4(12.9%)	16(51.6%)	2(6.5%)	3(9.7%)	1(3.2%)	31(100%)
	RRMS	7(11.5%)	10(16.4%)	11(18%)	27(44.3%	1(1.6%)	5(8.2%)	61(100%)
	Healthy	3(6.1%)	0(0.0%)	4(8.2%)	1(2.0%)	40(81.6%)	1(2.0%)	49(100%)
	NMO	1(3.2%)	4(12.9%)	1(3.2%)	0(0.0%)	0(0.0%)	25(80.6%)	31(100%)
Cross-validated	CIS	10(32.3%)	1(3.2%)	6(19.4%)	5(16.1%)	9(29.0%)	0(0.0%)	31(100%)
	SPMS	0(0.0%)	7(22.6%)	5(16.1%)	6(19.4%)	0(0.0%)	13(41.9%)	31(100%)
	PPMS	5(16.1%)	4(12.9%)	16(51.6%)	2(6.5%)	3(9.7%)	1(3.2%)	31(100%)
	RRMS	8(13.1%)	10(16.4%)	11(18.0%)	26(42.6%)	1(1.6%)	5(8.2%)	61(100%)
	Healthy	5(10.2%)	0(0.0%)	4(8.2%)	1(2.0%)	38(77.6%)	1(2.0%)	49(100%)
	NMO	1(3.2%)	5(16.1%)	1(3.2%)	0(0.0%)	0(0.0%)	24(77.4%)	31(100%)

RRMS: relapsing remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis; PPMS: primary progressive multiple sclerosis; CIS: clinically isolated syndrome; NMO: neuromyelitis optica

Furthermore, these classifications are represented in the scatter plot of Figure 5 for the function 1 and 2.

Figure 5 shows that the main contribution of function 1 is that it separates NMO and SPMS patients (who are characterized by high CD4, CD163, Ig-G, and Ig-M serum levels) from the other groups, while function 2 mainly discriminates NMO and RRMS patients from the other groups.

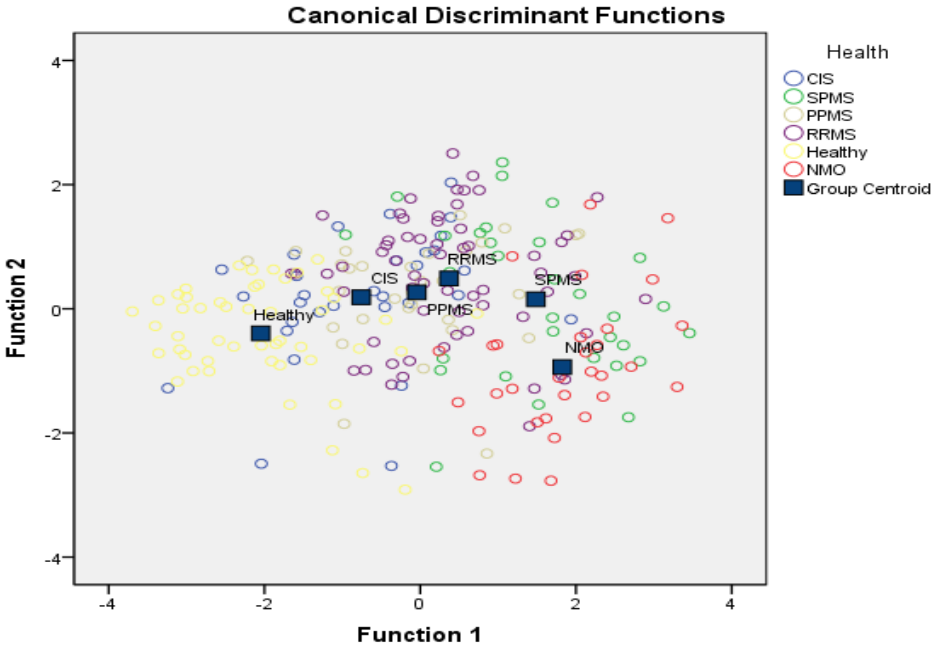

Figure 5. Scatter plot for the two most important functions in multiple sclerosis.
This figure shows the importance of function 1 and 2 which explain 96.6% of the total variance.
RRMS: relapsing remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis; PPMS: primary progressive multiple sclerosis; CIS: clinically isolated syndrome; NMO: neuromyelitis optica

The generated canonical variable formula was validated on an additional group of 59 individuals including patients with; RRMS, PPMS, SPMS, CIS, NMO, and also healthy subjects. A correct classification was obtained in 86.4% of subjects. Sensitivity and specificity for the validation group were 83.7% and 93.8% respectively, with the correlation value of 0.74.

DISCUSSION

In this case-control study we investigated levels of some potential immunological markers in sera of different types of MS in addition to the healthy controls. Each marker is indicating higher activity of special subtypes of immune cells. Our preliminary analysis gives evidence of a possible application of discriminant analysis to individuate immunological markers characterizing the RRMS, SPMS, PPMS, CIS, NMO, and healthy controls as proved by the data on sCD4, sCD163, Ig-M, and Ig-G in sera. Although in this study we mostly aimed to investigate possible application of these immunological markers for differentiation of different types of MS from each other, we can also use these markers for establishing new protocols for classification types of MS into different immune-based groups including innate MS and adaptive MS, which consist of T-cell MS, and B-cell MS. However, for approaching this aim we need to study immunological factors further in sera of MS patients and in different populations. In addition, it should be mentioned that we assessed serum levels of these immunological markers, therefore, for better conclusion, investigation of these markers on the surface of different immune cells in all types of MS will be required. In an interesting study, Stilund et al.[24] measured sCD163 as a marker of macrophage activity in serum and CSF of MS patients and symptomatic controls. Patients were divided into five subgroups including: RRMS (n=45), PPMS (n=15), SPMS (n=4)), CIS (n=27) and, SC (n=39) with either normal or unspecific MRI and levels of sCD163 in serum and CSF all cases and controls could be measured using ELISA method. They found that median levels of sCD163 were statistically decreased in serum and significantly elevated in CSF in patients with RRMS and PPMS. On the other hand, they showed significantly increased levels of sCD163 in the CSF of MS patients, which was in agreement with pathophysiology of lesions in MS patients as a dense presence of them were detected in active lesions.[25] Another study in this regard was performed by Febriek et al.[3] who found that sCD163 is upregulated in MS patients in comparison with healthy controls. There are 3 possible explanations for controversy between our results and what Stilund et al. reported with respect to the lower serum levels of sCD163 in MS patients in comparison with symptomatic controls: 1) Our controls group is different with theirs as they selected symptomatic controls with either normal or unspecific MRI, but we chose healthy subjects as control group; 2) They removed patients who had been treated with methylprednisolone, or other immunomodulating, but we also included patients with immunomodulating treatments; 3) They included newly diagnosed MS patients in the case group, but case group of our study consisted of both newly diagnosed MS patients and MS patients who had been diagnosed many years before initiation of study. Some studies reported no infiltration of inflammatory cells in progressive course of MS, while, others indicated presence of inflammation and neurodegeneration during all courses of MS.[3,26,27] In a study by Tsukada et al. level measurement of sCD4 and sCD8 in sera of MS patients as case group and patients with T lymphotropic virus type 1 (HTLV-1)-associated myelopathy (HAM) as control group was performed by ELISA.[28] They found that serum levels of sCD8 of cases was elevated in comparison with control group (p<0.001), in addition, they showed that MS patients with exacerbation of acute relapses have higher levels of this marker in their sera in comparison with controls or patients in remission stage (p<0.01 and p<0.001, respectively). In this study, serum level of sCD4 was not significantly different from that of the controls. Franciotta et al.[29] assessed serum levels of sCD8 in patients with MS, non-inflammatory neurological disorders (NIND), and healthy controls before and after treatment with 6-methylprednisolone. Serum levels of sCD8 did not significantly differ from that of the patients with NIND and healthy controls, while after treatment, serum levels of sCD8 were higher in the MS group (p=0.05).In a similar study, serum levels of immunoglobulins including Ig-G, Ig-M, and Ig-A before and after treatment with high-dose methylprednisolone were measured.[30] Their results revealed that serum levels of immunoglobulins cannot

be influenced by steroid treatment. Chen et al.[31] investigated serum levels of immunoglobulins (Ig-G, Ig-M, and Ig-A) in 88 definite cases of NMO and 44 controls. They reported increased levels of Ig-G in MS patients in comparison with controls though both levels of Ig-M and Ig-A were not significantly different from those of the controls (p<0.01 was considered as significant threshold). Furthermore, they found that only serum levels of Ig-G was increased in patients with acute phase of NMO than those who passed the stage. Studies on serum levels of immunoglobulins have been also carried out in other autoimmune disorders such as rheumatoid arthritis (RA). For example, a study on rheumatoid arthritis patients was carried out by Aho et al.[32], which was a case-control study within a Finnish cohort of 19072 adults who had neither arthritis nor a history of it at the baseline examination during 1973–1977. They diagnosed 124 cases of rheumatoid arthritis, and rheumatoid factor (RF) test was positive in 89 of them. They also chose three control subjects for each case who were matched well regarding age and sex. Their results revealed that serum levels of Ig-G were higher among future incident cases of RF positive RA than their controls without a significant difference. Furthermore, they found that the mean concentrations of Ig-A and Ig-M did not differ in either RF positive or RF negative RA and their controls. Although serum concentrations of immunogolobulins did not differ significantly from their controls, serum levels of Ig-G and Ig-A was found to be directly proportional to the risks of RF positive RA. In a study which was performed in RA patients, concentration of sCD4 in serum and other clinical parameters of cases were assessed.[33] The levels of sCD4 was higher in rheumatoid patients than controls (p<0.001). In addition, this marker was correlated with other clinical parameters including the erythrocyte sedimentation rate (ESR), the C-reactive protein (CRP), and RF; while, in our study, only serum levels of sCD163 was significantly correlated with EDSS. In this regard, there are many studies in which increased serum levels of sCD8 were reported in other autoimmune disorders such as bullous pemphigoid, type-1 diabetes, and systemic lupus erythematous.[34-36]

Taken together, our preliminary analysis suggests that although we found some functions to discriminate most of the patients, further studies will be required to individuate immunological markers characterizing the different type of MS including RRMS, PPMS, SPMS, CIS, and NMO as proved by the data on sCD4, sCD163, Ig-M, and Ig-G in blood.

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