Assessing the values of circulating immune complexes in multiple sclerosis patients following immunomodulator or corticosteroid treatment

ANY DOCU AXELERAD[1], ALINA ZORINA STROE[1], ANCA ELENA GOGU[2] and DANIEL DOCU AXELERAD[3]

[1]Department of Neurology, Faculty of General Medicine, 'Ovidius' University of Constanta, 900470 Constanta; [2]Department of Neurology, Faculty of General Medicine, Victor Babeș University of Medicine and Pharmacy Timișoara, 300041 Timisoara; [3]Department of Sports, Faculty of Physical Education and Sports, 'Ovidius' University of Constanta, 900470 Constanta, Romania

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Abstract. Multiple sclerosis is defined as an immune-mediated disease that affects the central nervous system, and also is characterized by the presence of immune cells and mediators which contribute to the subsidiary neuroinflammation associated with multiple sclerosis. Throughout the evolution of multiple sclerosis, it has been observed that circulating immune complexes (CICs) have higher values in these patients, especially in the acute phase of the disease. Thus, the aim of the present study was to observe, if in acute attack, relapsing-remitting multiple sclerosis patients still present high values of CICs after treatment with glatiramer and prednisone. We divided 70 patients with multiple sclerosis with high values of CICs into two treatment groups, one treated with glatiramer (Copaxone) (immunomodulatory treatment) and the other with prednisone (corticosteroid treatment). After three months of treatment, we assessed the levels of CICs of the two multiple sclerosis groups and we observed that the patients that followed the immunomodulatory treatment had lower values of CICs than the group that followed the corticosteroid treatment. In addition, another observation established was that the glatiramer treatment group had higher levels of vitamin D in the serum than the prednisone group of multiple sclerosis patients. To conclude, better outcomes, from the point of view of the results obtained from the comparative analysis of the values of CICs and vitamin D, were demonstrated by following immunomodulatory treatment.

Correspondence to: Dr Alina Zorina Stroe, Department of Neurology, Faculty of General Medicine, 'Ovidius' University of Constanta, 1 Aleea Universității, 900470 Constanta, Romania
E-mail: zorina.stroe@yahoo.com

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Introduction

Multiple sclerosis presents as a chronic central nervous system inflammatory disease which determines processes of demyelination and neurodegeneration. Multiple sclerosis is characterized by impairments in motor and sensory function caused by immune-mediated inflammation, demyelination and subsidiary axonal damage (1). The evolution of the disease is defined by episodes of neurological impairments and deficits that can be succeeded by a stage of remission or progression. The prevalence consists of 2.5 million people worldwide who are afflicted with multiple sclerosis, and these reports also have areal variability (1).

Multiple sclerosis is defined by the invasion of T lymphocytes, B lymphocytes, macrophages, and natural killer cells and is accompanied by demyelination and axonal damage (1).

Relapsing-remitting multiple sclerosis is defined as the most prevalent form of the disease and is characterized by relapses or acute attacks (aggravation of neurological symptoms during 24 h or longer, without the presence of fever). In evolution, the attack can proceed to remission automatically or consequent to the treatment with corticosteroids or pulse therapy (2). The majority of patients are initially diagnosed with relapsing-remitting multiple sclerosis. The most generally detected symptoms include optic neuritis, motor or sensory deficits of the limbs, motor impairments of coordination, loss of balance, myelitis, sphincter dysfunction and cognitive-behavioral impairment, either separately or in association (3,4). Subsequent to relapsing-remitting multiple sclerosis, the majority of patients traverse into a secondary progressive course with continuous intensification of impairment (3).

The principles of treatment are based on the usage of disease modifying therapies or immunomodulators that target impediment of the progression of relapsing-remitting multiple sclerosis and prevent relapse by diminishing circulating immune cells (CICs) or by limiting these cells from passing the blood-brain barrier, by decreasing the inflammatory response (5,6).

The immunological development in the pathophysiology of multiple sclerosis has been determined through the results

of experimental studies established using murine configurations and specimens from multiple sclerosis patients (7-9). Primarily, in the peripheral lymph nodes, the stimulation of autoreactive T cells and B cells occurs through activation and differentiation toward effector cells. The crucial part in the pathogenesis of multiple sclerosis is executed by effector $CD4^+$ T cells, T helper 1 and T helper 17 cells. The numbers of the mentioned subpopulations are higher in the peripheral blood and central nervous system, especially in the cerebrospinal fluid and the perivascular space (7-9).

Activated T and B cells proceed to the blood-brain barrier, the interruption of which indicates the incipient phase of the disease, and these cells enter the central nervous system, where they are additionally stimulated by local antigen-presenting cells (10,11). In the central nervous system, macrophages and activated $CD4^+$ and $CD8^+$ T cells target the myelin constituents and generate cytokines and chemokines that engage additional autoreactive cells having as source the peripheral blood (4,12,13). The mentioned cells also activate B cells, that develop into antibody-generating plasma cells; cause, preserve and restimulate $CD4^+$ T cells; and generate proinflammatory cytokines (13,14). In general, the mechanisms stated before increment inflammation produce demyelination and axonal destruction (15,16). In the determinative stages of the disease, the inflammatory response is reinstated with microglial activation and chronic neurodegeneration (17,18).

In the medical literature, the presence of CICs has been reported in the serum of multiple sclerosis patients since 1976 (19).

Patients and methods

Patients. Circulating immune complexes (CICs) were analyzed from blood samples of 70 multiple sclerosis patients with relapsing-remitting form. The patients were recruited during the period of January 1, 2017 to August 1, 2020 at Constanta County Hospital in Romania. A total of 70 multiple sclerosis patients participated in this study. A total of 44 subjects (62.86%) were women and 26 (37.14%) were men. The mean age of the patients was 28.3 years, and the age range was 26-40 years. The current study was approved by the Ethics Committee of the Constanta Clinical Hospital, Romania. Written informed consent was obtained from all subjects.

Inclusion criteria were patients diagnosed with multiple sclerosis relapsing-remitting form, diagnosed by a neurologist. Exclusion criteria were i) the presence of other immunological diseases, such as rheumatic diseases (systemic lupus erythematosus, mixed connective tissue disease, rheumatoid arthritis, Sjoegren's syndrome, nodular periarteritis, Felty's syndrome, Reiter's syndrome, Bechterew's disease); ii) the presence of neoplastic diseases infectious type including viral infections (hepatitis B, cytomegalovirus infection, Ebstein Barr, subacute sclerosing panencephalitis) and bacterial infections (infectious endocarditis, disseminated gonorrhea, syphilis, streptococcal and meningococcal infection); and parasitosis (malaria, schistosomiasis, trypanosomiasis, toxoplasmosis); and iii) the presence of other chronic pathologies including glomerulonephritis, ulcerative colitis, Crohn's disease, idiopathic interstitial pneumonia, cystic fibrosis, multiple sclerosis and thrombotic purpura thrombocytopenic hepatitis.

Patient groups and treatment. In the present study, we aimed to assess the values of CICs in the relapsing-remitting multiple sclerosis patients in acute attack and we divided 70 patients with multiple sclerosis with high values of CICs into two treatment groups: One treated with glatiramer (Copaxone) (immunomodulatory treatment) and the other with prednisone (corticosteroid treatment). After three months of treatment, we assessed the levels of CICs in the two multiple sclerosis treatment groups. In addition, we assessed the levels of vitamin D in both of the groups to observe if any difference was visible between the levels of vitamin D in both groups. Vitamin D is related to the bone health and calcium metabolism, along with the general immunity level of the organism, currently being of general interest in multiple sclerosis.

The therapeutic strategy for each patient was decided upon by the treating doctor on the basis of the best possible solution for each patient. Glatiramer acetate represents a synthetic protein which simulates myelin basic protein, a component of the myelin that isolates nerve fibers located in the brain and spinal cord. This drug functions by blocking myelin-damaging T-cells using a mechanism which is not entirely understood. Prednisone represents one of a group of corticosteroids that contribute to relieve inflammation in different parts of the body. Corticosteroids are used in multiple sclerosis for their ability to seal the damaged blood-brain barrier and decrease inflammation located in the central nervous system. Patients from the prednisone treatment group received 1 milligram Prednisone per kilogram of body weight per day, during the three months. The patients from the Copaxone treatment group received three injections of Copaxone (40 mg/ml solution for injection in pre-filled syringe) per week during the three months.

CIC serum test method. The venous blood collected was treated according to the protocols of the hospital's analysis laboratory, using the ELISA technique, with the following methodology. CICs in the patient's serum were fixed by the Fc fragment on C1q from the microplate; in order to quantify the presence of CIC-IgG complexes, peroxidase-conjugated anti-IgG antibodies and then tetramethylbenzidine substrate were afterwards added. The color intensity obtained was directly proportional to the level of C1q-CIC complexes related to the solid phase. The venous blood collected was treated according to the protocols of the hospital's analysis laboratory, using the immunochemical method with detection by electrochemiluminescence. In addition, total vitamin D was measured (vitamin D3, vitamin D2 and other hydroxylated metabolites of vitamin D).

Statistical analysis. All statistical analyses were performed using IBM SPSS Statistics 20 (IBM Corp.). Data from the patients were compared using the non-parametric Mann-Whitney's U test. P-values <0.05 were considered to indicate statistical significance.

Results

A Mann-Whitney U test was run to determine whether there were differences in the results of the initial CIC serum test performed on the multiple sclerosis patients treated

Table I. Results of the CIC serum test in the multiple sclerosis patients (N=70).

Group_Treatment	Number	Mean rank	Sum of ranks
CIC_Initial			
Glatiramer acetate	35	36.83	1,289
Prednisone	35	34.17	1,196
Total	70		
CIC_Final			
Glatiramer acetate	35	18	630
Prednisone	35	53	1,855
Total	70		

Table II. Statistical analysis of the results obtained by the multiple sclerosis patients (N=70) for the CIC serum test.[a]

Statistical variables	CIC_Initial	CIC_Final
Mann-Whitney U	566	0.000
Wilcoxon W	1,196	630
z-value	-0.561	-7.241
Asymp. Sig. (2-tailed)	0.575	0.000

[a]Grouping variable: Group_Treatment. CIC, circulating immune complex.

Table III. Median results of the assessment of vitamin D between the two treatment groups of multiple sclerosis patients (N=70).

	Vitamin D		
Group_treatment	Number	Mean rank	Sum of ranks
Glatiramer acetate	35	53	1,855
Prednisone	35	18	630
Total	70		

Table IV. Statistical results of the assessment of vitamin D between the two treatment groups of multiple sclerosis patients (N=70).[a]

Statistical variables	Vitamin D
Mann-Whitney U	0.000
Wilcoxon W	630
z-value	-7.211
Asymp. Sig. two-tailed)	0.000

[a]Grouping variable: Group_Treatment.

with glatiramer acetate vs. the patients treated with prednisone. Distributions of the results for both of the groups of patients were similar, as assessed at a first glance. CIC dosing results were not statistically significant between the glatiramer acetate treatment group (mean rank=36.83) and the prednisone treatment group of multiple sclerosis patients (mean rank=34.17) (U=566, z=-0.516, P=0.575). The results are provided in Tables I and II.

A Mann-Whitney U test was run to determine whether there were differences in the results of the final CIC serum test (3 months following the initial test) performed on the multiple sclerosis patients treated with glatiramer acetate and on the multiple sclerosis patients treated with prednisone. Distributions of the results for both of the groups of patients were not similar, as assessed at a first glance. The results from the CIC serum test in the glatiramer acetate treatment group (mean rank=18) were statistically significantly lower when compared to the results from CIC serum test in the prednisone treatment group of multiple sclerosis patients (mean rank=53) (U=0.000, P<0.001). The results are provided in Tables I and II.

A Mann-Whitney U test was run to determine whether there were differences in the results of the vitamin D serum test performed on the multiple sclerosis patients treated with glatiramer acetate and on the multiple sclerosis patients treated with prednisone. Distributions of the results for both of the groups of patients were not similar, as assessed by at a first glance. The results from the vitamin D serum test in the glatiramer acetate treatment group (mean rank=53) were statistically significantly higher when compared with the results from the vitamin D serum test in the prednisone treatment group of multiple sclerosis patients (mean rank=18) (U=0.000, P<0.001). The results are provided in Tables III and IV.

In our study performed on 70 patients with multiple sclerosis relapsing-remitting form in the period of active disease, with 35 patients treated with glatiramer acetate immunomodulator (Copaxone) and 35 patients treated with prednisone only in the active phase of the disease, we observed that the CICs were relatively the same at the active phase of the disease, before initiation of the medication.

Following three months of treatment, we observed that the immunomodulator treated group of multiple sclerosis patients obtained statistically lower values for the CIC test than the group of patients that was treated with prednisone only in the acute phase.

Another observation of our study was that the levels of vitamin D were statistically higher in the group of patients treated with immunomodulators than in the group of patients treated with corticosteroids.

Discussion

In the present study, we were interested in ascertaining whether our 70 patients with multiple sclerosis obtained high values of circulating immune complexes (CICs) in the active phase and if the high values of CICs were influenced by the category of the treatment chosen.

Immune complex formation is a hallmark of both infectious and autoimmune disorders. Tanaka *et al* examined the serum

of 21 multiple sclerosis patients for CICs and anti-endothelial cell antibodies and revealed higher titres of CICs in the serum of patients with multiple sclerosis, especially the patients with active disease (20).

In a previous study, CICs were more frequently detected in patients with active disease than in patients that were recovering from an exacerbation and patients whose disease showed no progression in the last years (21).

The influence of immune complexes has been demonstrated related to the impairments that CICs experience in the blood-brain barrier and this factor is especially important in multiple sclerosis (22).

In the evolution of multiple sclerosis, pathological disruption of the blood-brain barrier was identified (23) through studies *in vivo* using contrast induced CT scan of the lesions in multiple sclerosis (24-27).

Dasgupta *et al* reported the prevalence of CICs in the serum of 254 multiple sclerosis patients, showing a 35% positivity of CICs in multiple sclerosis. The incidence of CICs in acute relapse, progressive, remission, and stable state of multiple sclerosis was 33.3, 30.2, 26.1 and 23.1, respectively, compared with 7.75 and 8.82% among normal and neurologic controls (21).

In another study, Dasgupta *et al* studied the prevalence of myelin basic protein which is an antigenic component of CICs in patients with multiple sclerosis, demonstrating that myelin basic protein was found to be an antigenic component in some CICs isolated from the sera of some multiple sclerosis patients. From the 22 samples of multiple sclerosis patients, 15 were CIC-positive and 6 of 7 samples from patients in relapse and of the 15 CIC-positive serum, 9 samples were also positive for myelin basic protein (28).

Myelin basic protein was discovered and dosed in the cerebrospinal fluid of patients with active demyelination, including multiple sclerosis (29,30). In addition, myelin basic protein antibodies have been discovered in the cerebrospinal fluid of patients with multiple sclerosis (31).

In a study by Tachovsky *et al*, CICs were determined in 49% of serum from patients with multiple sclerosis, but the association between the presence of CICs and the severity of the disease was not established (32).

In a study by Patzold *et al*, CICs were detected in the sera of 46 (33.3%) of 138 multiple sclerosis patients investigated. In cerebrospinal fluid, immune complexes were found in 24 (19.4%) of the 124 samples of multiple sclerosis patients that were tested (33).

In a study by Jans *et al,* immune complexes were found in serum from 17 of the 32 multiple sclerosis patients with progressive form and in CSF from 9 of 31 multiple sclerosis patients in progressive form (34).

In a study by Wajgt *et al*, the levels of CICs in multiple sclerosis patients were higher than that in the control group and following prednisone therapy, a significant decrease in CIC level was found in the CSF of the multiple sclerosis patients (35).

The insufficiency of vitamin D represents a risk factor in the development of multiple sclerosis and is linked to increased disease activity in patients already diagnosed with multiple sclerosis (36,37).

Research has revealed that patients with multiple sclerosis present a lower rise in serum 25-hydroxyvitamin D levels when compared with healthy controls, following treatment with the same amount of oral cholecalciferol supplementation (38).

These results provide evidence for the importance of the treatment elected for multiple sclerosis patients that can beneficially affect the level of CICs; the most effective treatment being an immunomodulator.

Moreover, this study observed higher levels of vitamin D among the multiple sclerosis patients that followed the immunomodulator treatment, compared to the multiple sclerosis patients that followed corticoid medication. Further studies on this issuer are required to formulate a justified theory.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ADA, AEG and AZS conceived and designed the study. AZS performed the literature research. ADA, AEG, AZS and DDA were involved in the interpretation of the results. ADA, AEG, AZS and DDA were involved in the writing of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The current study was approved by the Ethics Committee of the Constanta Clinical Hospital, Romania (no. 26/15.09.2020) and all the patients gave informed consent and signed a statement to that effect.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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