Short Communication. Does the serum IL-17 titer influence the efficacy of interferon-β treatment in multiple sclerosis patients?

Nivelul seric al IL-17 influențează eficacitatea tratamentului cu beta interferon la pacienții cu scleroză multiplă?

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Abstract

Background. *The IL-17 is a proinflammatory cytokine that plays an important role in the pathogenesis of multiple sclerosis (MS). Interferon-β (IFN-β) is the major immunomodulatory treatment for relapsing remitting MS (RRMS). Up to two thirds of patients respond to treatment. Criteria to classify patients into responders and non-responders to IFN-β therapy are usually applied after 1 or 2 years follow-up.* ***Objective.*** *To detect the level of serum IL-17A in MS patients treated with IFN-β and find if it plays a role in MS patients' response to IFN-β.* ***Material and methods.*** *150 patients with MS (mean age: 41.8±9.2; mean EDSS: 2.8±1.87; 71.3% RRMS, 28.7% SPMS), who had at least 18 months of IFN-β treatment, underwent a serological IL-17A test. IL-17A was tested using ELISA-indirect method, values over 1.6pg were pathological. Patients had at least 30 days without steroids. For each patient, the following were recorded: epidemiological, clinical and treatment features (early vs. late treatment, responder vs. nonresponder, duration of treatment). Mann-Whitney test and Spearmann correlation was used, p values of ≤0.05 were significant.* ***Results.*** *27 patients (18%) had titers of IL-17 above 1.6pg/ml, mean 3.38 (±13.5). We found correlations between the IL-17A titer and both number of MS relapses ($p<0.05$) and EDSS ($p=0.05$). In nonresponders the titer of IL-17 was significantly higher ($p=0.03$). None of the other parameters correlated.* ***Conclusions.*** *High serum IL-17 concentration in MS treated patients plays a role in nonresponsiveness to IFN-β therapy. Serum IL-17 might be a sensitive biomarker to be used but it must be correlated with other markers for disease activity or response to treatment.*

Keywords: *multiple sclerosis, interleukine 17, interferon beta*

Rezumat

Introducere. *Interleukina 17 (IL-17) este o citokină proinflamatoare cu rol important în patogenia sclerozei multiple (SM) . Interferonul-β (IFN-β) reprezintă terapia imunomodulatoare în tratamentul formei recurrent remissive de SM (RRSM). Maxim 2/3 din pacienții cu SM răspund la acest tratament. Criteriile de clasificare ale pacienților în "responderi" şi "nonresponderi" la tratamentul cu IFN-β sunt greu de aplicat şi necesită 1-2 ani de urmărire.* ***Obiective.*** *Determinarea nivelului seric de IL-17A la pacienții cu SM tratați cu IFN-β şi găsirea unui eventual rol al acesteia în răspunsul terapeutic la IFN-β.* ***Material şi metode.*** *La 150 de*

pacienţi cu SM (vârstă medie: 41.8±9.2; EDSS mediu: 2.8±1.87; 71.3% RRSM, 28.7% SPSM) care au primit minimum 18 luni IFN-β, s-a determinat nivelul seric de IL-17A . IL-17A a fost determinată folosind metoda ELISA-indirectă, valori peste 1.6pg au fost considerate patologice. Pacienţii nu au primit corticoterapie minimum 30 de zile înaintea testării. La fiecare patient s-au notat: date epidemiologice, clinice şi terapeutice (tratament precoce vs tardiv, responder vs nonresponder, durata tratamentului). S-a utilizat testul Mann-Whitney şi corelaţia Spearmann, valori p≤0.05 au fost considerate semnificative. ***Rezultate.*** *27 pacienţi (18%) au avut titruri crescute de IL-17 (peste 1.6pg/ml). Media titrului seric a fost 3,38 (±13,5) Am găsit corelaţii ale titrului IL-17A cu: numărul de recurenţe de SM (p<0.05) şi scorul EDSS (p=0.05). La "nonresponderi" titrul de IL-17 a fost crescut semnificativ (p=0.03). Nici un alt parametru nu s-a corelat cu IL-17.* ***Concluzii.*** *La pacienţii cu SM, titrurile de IL-17 sunt crescute la "nonresponderi" la terapia cu IFN-β. Titrul seric de IL-17 poate fi un biomarker sensibil care trebuie utilizat în corelaţie cu alţi markeri ai activităţii SM sau ai răspunsului la terapie.*

Cuvinte cheie: *scleroză multiplă, interleukina 17, interferon beta*

Introduction

Studies have shown that T cells and inflammatory cytokines play an important role in central nervous system (CNS) autoimmune disease pathogenesis, including multiple sclerosis (MS) lesions (1).

MS is a progressive neurological disease of unknown origin characterized by the progressive accumulation if inflammation and degeneration in the grey and white matter of the CNS.

The IL-17 is a proinflammatory cytokine that activates T cells and other immune cells to produce a variety of cytokine, chemokines and cell adhesion molecules. Large amounts of IL-17 are secreted by a lineage of T cells named Th17 cells. The Th17-chemokines pathways are essential for the development of CNS autoimmune disease such as MS (2).

Interferon-β (IFN-β) is the major immunomodulatory treatment for relapsing remitting MS (RRMS). However, maximum two thirds of patients with RRMS respond to treatment. Criteria to classify patients into responders and nonresponders to IFN-β therapy are usually applied after 1 or 2 years follow-up using disability progression or relapse rate (3).

T-helper and Th17 lymphocytes are involved in experimental autoimmune encephalomyelitis (EAE), the model of MS. Evidence that an expansion of peripheral Th17 cells can infiltrate brain parenchyma and damage cells is associated with disease activity in MS. A high IL-17 concentration in the serum of people with relapsing-remitting MS (RRMS) is associated with nonresponsiveness to therapy with IFN-β.

The identification of biomarkers to predict disease course and treatment response is a major challenge in MS research. Successful therapeutically strategies in MS might require characterization of the immune response in a given patient to design effective treatment protocols on an individual basis (4).

In the era of early therapeutic intervention in MS with disease modifying drugs and after several alternative options have become available, the issue of early identification of patients who are not responding to a given treatment has become more important (5).

Material and methods

Patients

This is a prospective study which included MS patients diagnosed and treated in First Neurological Department of the University Emergency County Hospital Targu Mures. The informed consent of all participating subjects was obtained and the study was approved by the local ethics committee and was carried out according to the Declaration of Helsinki.

Blood samples were collected from 150 patients with MS treated for at least 18 months with the same type of IFN-β. None of the patients had been treated with glucocorticoids within 4 weeks of study entry, and none of the patients had ever been treated with immunosuppressive drugs

such as azathioprine, methotrexate, mitoxantrone, cyclophosphamide. Patients were tested at least 24 hours after the last IFN-β injection. IL-17 serum titer was determined in twenty healthy blood donors (70% were women and 30% men, median age 36.7 years, range 25 - 46).

Peripheral blood was collected and serum was obtained by centrifugation at 500g for 15 minutes and stored at -70°C until all samples were obtained to avoid loss of bioactive human IL-17A. The duration of the blood collection and IL-17A measurements was from 1st July 2010 until 10th March 2011.

The DRG International IL-17A ELISA kit (EIA-4840) was used for the quantitative detection of human IL-17A. The method was an indirect sandwich type ELISA. Anti-human IL-17A coating monoclonal antibody adsorbed onto microwells binds human IL-17A present in the sample or standard. A biotin-conjugated anti-human IL-17A antibody is added and binds to human IL-17A captured by the first antibody. A colored product is formed by Streptavidin HRP conjugated to biotinylated anti-IL 17A antibody, in proportion to the amount of human IL-17A present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm.

In healthy subjects, there were no detectable human IL-17A levels. As the kit instructions provided, elevated human IL-17A levels were considered if the value of 1.6 pg/ml was exceeded. The value of serum IL-17A from each patient was noted.

All patients were neurologically examined 12 months before IL-17A testing. In addition, for each patient we collected demographic data, took a medical history, recorded the age, clinical form of MS, the score for neurological deficit EDSS (Expanded Disability Status Score), the change in the EDSS within the last 12 months of treatment, the number of relapses in the last year, the moment of initiation of IFN-β: "early treatment" was considered if the immunomodulatory treatment was initiated within the first year after MS was diagnosed or after a first relapse while "late treatment" was considered when the patient started IFN-β therapy after at least 18 months of clinically definite MS. The patients were classified according to treatment response in "responders" (no relapse or maximum a rise of 0.5 points on EDSS in the previous year under IFN-β) and "nonresponders" (minimum 1 relapse or an increase of at least 1 EDSS point in the last year).

Inclusion/exclusion criteria

Eligible patients had multiple sclerosis according to the McDonald criteria [6-8]. Further inclusion criteria were: age 18-60 years; RRMS and SPMS (secondary progressive MS); minimum 18 months under constant IFN-β treatment (Avonex, Rebif or Betaferon). All patients had negative blood test for other autoimmune or infectious diseases: lupus, antiphospholipid syndrome, HIV infection, syphilis, Lyme disease, etc.

Patients were excluded if they had been treated previously in the last 30 days with methilprednisolon; change of IFN-β preparation within 18 months. Also, we did not include any patients with other chronic disease associated to MS, nor those previously treated with immunosuppresive agents.

Objectives

The primary objective was to detect the level of serum IL-17A in MS patients already treated with IFN-β for at least 18 months.

The secondary objectives were: a) to find if the IL-17 serum level correlates with the response to IFN-β; b) determine whether early starting of treatment in newly diagnosed MS patients influence the production of IL-17; c) find correlations between these titers and other patients' MS characteristics (age, sex, clinical form of MS, number of MS relapses, EDSS, change in the EDSS score in the previous year, type of IFN-β administered).

Last, but not least, we present the first Romanian study and one of the few in the literature to determine the plasmatic level of IL-17 in MS patients treated with IFN-β.

Table 1. Demographics, clinical and therapeutically characteristics of MS patients

MS age at onset; at testing	36.7 (9.4); 41.1 (9.2)
Women/men	106 (70.6%) / 48 (29.4%)
EDSS	2.83 (1.8)
Change in EDSS in one year	0.8 (0.9)
Number of relapses within previous year	0.43 (0.61)
RRMS	107(71.3%)
SPMS	43 (28.7%)
Patients on IFN-β 1b; 1a s.c.; 1a i.m.	69; 55; 26
Patients "early treated"	54 (36%)
Patients "late treated"	96 (64%)
"Responders"	114 (76%)
"Nonresponders"	36 (24%)

Data are presented as mean (SD), or number of patients (%)

Statistical methods

Groups were compared using Mann-Whitney test and correlation analyses were conducted using Spearman rank correlation coefficient. Correlation and regression analysis was used to determine patient characteristics asociated with IL-17+ and IL-17- status. Before using the above tests, we applied the Kolmogorov-Smirnov Normality Test that showed a nongaussian distribution of our data, thus nonparametric statistical tests were required. All reported p values are based on two tailed statistical tests, with a significance level of 0.05. Diagrams were presented using Statistica (StatSoft) program.

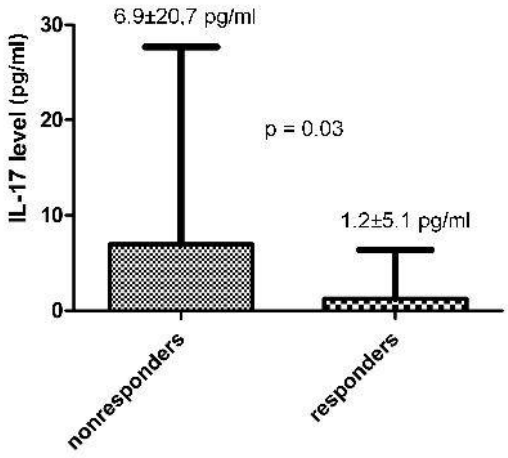

Figure 1. IL-17 serum level in responder and nonresponder groups

Results

One hundred and fifty patients fitted the inclusion criteria and were tested for IL-17A. *Table 1* shows baseline demographics, clinical and therapeutically characteristics.

A total number of 27 patients (18%) had titers of IL-17 above 1.6 pg/ml. Mean value for the titer was 3.38 pg/ml.

By dividing the patients into "responders" and "nonresponders", we found a significant higher level of serum IL-17 in the "nonresponders" group (p=0.03) (*Figure 1*).

Comparing the two groups of patients, respectively IL-17+ and IL-17- we found significant correlation with the number of relapses in the previous year (p=0.04).

No other differences regarding the sex, age (both of MS onset and at time of the study), disease duration, early or late start of the treatment, clinical form of MS, EDSS were found between the 2 groups of patients.

When we analyzed the correlation between the titer of serum IL-17A with different characteristics, we found interesting data. The mean IL-17 plasmatic value correlates signifi-

cantly and directly with the number of relapses ($p<0.05$, $r=0.25$) (*Figure 2)*.

A statistical correlation was seen between the level of IL-17 and the aggravation of EDSS score in the previous year ($p=0.05$, $r=0.13$).

Even though not statistically significant but with a serious trend, the plasmatic level of IL-17 correlated directly with EDSS at the moment of inclusion in the study.

Also a slightly trend was noticed between serum levels of IL-17 and the late start of disease modifying therapy like IFN-β, as a "late treated" patient had higher titers of IL-17.

The mean level of IL-17 did not correlate with: age at MS onset or age at inclusion, clinical form of MS.

Regardless of the type of IFN-β used, no correlations have been found between the levels of serum IL-17 in these patients.

A limitation of our results that we are aware of, is the big value of standard deviation regarding the titers of IL-17 found. This fact is the consequence of semiquantitative ELISA test used for IL-17 detection. For statistical reasons, we artificially considered as 0 the value of IL-17 that was not detected in the serum. Even if such transformation of data was necessary, due to the type ELISA test, we concluded that the statistical significance found is true.

Discussions

Among the disease modifying treatments for MS, IFN-β is the most widely used. A major limitation with IFN-β is that 50-70% of RRMS patients are considered "nonresponders", according to proposed criteria of response (9).

There are many purposed mechanisms of action of IFN-β: a) reduces the Th1 pathogenesis blocking the proinflammatory properties of IFN-γ, IL-12, IL-23; b) inhibits the Th17 cell differentiation by reducing IL-1β, IL-23 and TGF-β (which induces Th17 differentiation) and by increasing IL-10, IL-27, IL-12 and IL-4 (which suppress Th17 differentiation) (10).

A systematic analysis of IL-17+ cells in the brains of MS patients revealed a significant increase in the number of IL-17+ T cells in active areas of MS lesions. In addition, the authors find IL-17 production by many CD8+ cells, astrocytes

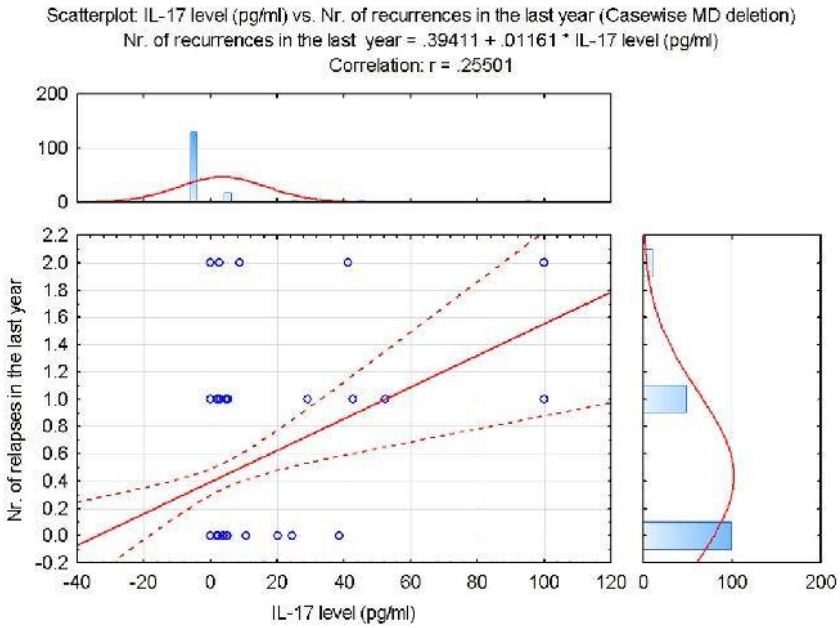

Figure 2. Correlation between the number of MS relapses in the last year and IL-17 serum level (pg/ml)

and some oligodendrocytes, which spotlight IL-17 as a critical cytokine in MS (11-14).

The relapsing remitting phase of MS may involve waves of proinflammatory Th1 and Th17 cells that infiltrate the nervous system, provoking a clinical attack. The Th17 cells percentage increases around seven fold in active MS compared with inactive MS or healthy subjects. The progressive phase of MS is believed to be secondary to neurodegenerative changes triggered by inflammation (15, 16).

Axtell et al (17) examined whether the cytokine networks regulated by INF-β influence its effectiveness as a therapy for RRMS. They induced EAE by transferring myelin-autoimmune T cells of either Th1 or Th17 type into healthy mice and found that both Th1, Th17 lineages mediated disease, but Th1 cells seem to produce more severe EAE. In a key experiment, treatment with recombinant human IFN-β exacerbated Th 17-mediated EAE but dampened Th1-mediated disease. Next, the authors examined a group of patients with RRMS according with their relapse rates after IFN-β therapy. When the authors analyzed the cytokine profiles of serum samples taken from these subjects before IFN-β treatment, they found that serum concentrations of both IL-17 and endogenous IFN-β were elevated in those who did not respond to IFN-β compared to responders. Non-responders had worse disease evolution with more steroid usage and more relapses than did responders. In the opinion of the authors, a high IL-17 concentration in the serum of patients with RRMS is associated with nonresponsiveness to IFN-β therapy.

As Axtell et al (17) we found the strongest correlation with a high level of significance between the serum level of IL-17 and the relapse rate and with EDSS score, both giving the profile of "nonresponders" to IFN-β. The novelty of our study consists in the fact that we tested patients under IFN-β treatment for 18 months. The reason for choosing this minimum time for treatment is that the immunogenicity of IFN-β expressed by the development of neutralizing antibodies (NAbs) is settled within first 12-18 months of treatment (18).

One can assume that the presence of high levels of IL-17 in the blood of MS treated patients is on account of the presence of NAbs. But our study showed no difference between patients irrespective the type of IFN-β used, while NAbs are much more frequent in patients treated with IFN-β 1b (19-21). This leads to the future research aiming to perform a study in which both NAbs and IL-17 must be determined and to search other pathological mechanisms of IL-17 production and raise in the presence of an active therapy with IFN-β in MS patients. Researchers at Stanford found that in people with RRMS, there seem to be 2 types: one type characterized by high levels of IL-17 in the blood and the other type with a low level of IL-17. It is possible that interferon-based disease modifying drugs (Avonex, Rebif, Betaferon) have different effects on patients: in those with high levels of IL-17 have a proinflamatory effect, worsening the disease.

Clinical parameters, such as relapse rate and disability progression assessing with EDSS scale, may represent two useful indicators for therapeutic response.

Based on their biological characteristics, nonresponsive patients can be divided into three subgroups: genetic, pharmacological and pathogenetic nonresponders.

IFN-β stimulates the expression of a number of genes (ISG), following interaction with a specific membrane receptor (IFNAR). Thus, misregulation of at least one ISG might be responsible for the failed response to the therapy.

Similarly, pharmacological nonresponders show a lack of clinical efficacy due to presence of serological factors (e.g. anti-IFN-β antibodies), inhibiting IFN-β biological activity. Thus, when the interaction between IFN-β and its receptor is blocked, there can be no clinical activity. Because measurement of the clinical benefit of IFN-β in patients can be difficult, the

quantification of molecules up- and down-regulated by IFN-β represents an alternative. Another cause of pharmacological nonresponsiveness might be anomalous patterns and low expression levels of the IFNAR subunits, based on the hypothesis that the biological response to a specific ligand is dependent on the receptor expression profile in that tissue. A well known phenomenon linked to receptor stimulation is tachyphylaxis during IFN-β therapy both from clinical and biological perspectives (chronic and prolonged treatment with IFN-β in MS patients significantly decreases MxA expression, which is known to be regulated exclusively by IFN). It is noteworthy that pharmacological non-responsiveness develops over the course of treatment (at IFN-β initiation, all patients are biological responders).

On the other hand, pathogenic nonresponders show a lack of clinical efficacy, although IFN-β is still biologically active. In these patients, the drug is not able to antagonize the pathology due to its aggressive pathogenetic characteristics (22, 23).

The clinical course of MS is unpredictable and, as a consequence, clinicians who treat patients with MS are facing different clinical conditions that differ not only from one patient to another, but also in the same patient as the disease progresses (22).

Specific aspects of MS not only complicate the design and conduct of clinical trials, but also present challenges when assessing the effect of a chronic administered therapy in an individual patient. MS is known to be variable. A "bad" year with three relapses might be followed by a "good" year with no relapses, simply because of the variable disease course. MS can respond differently to the same treatment as a result of change in disease duration, disease category, or age of the patient (5).

We did not find any significant difference between the levels of IL-17 and RRMS or SPMS stages of evolution. It is well known that processes of brain atrophy and accumulation of neurological deficits in the late stage of MS does not have in the first place an immunological mechanism like secretion of IL-17 (5).

Axtell et al (17) analyzed a subset of RRMS nonresponders. These patients had high serum concentrations of both Il-17 and endogenous IFN-β before IFN-β therapy was initiated, compared with responders. This correlation between high IL-17 and IFN-β concentrations in the serum suggests a tight biological association between these two cytokines. Two hypotheses, which may not be mutually exclusive, could explain this phenomenon. One hypothesis is that a non-responsive have aggressive Th17-mediated disease, and, to counteract inflammation, their immune system upregulate IFN-β. Since endogenous IFN-β expression is already high, IFN-β treatment is ineffective. A second hypothesis is that IFN-β is proinflammatory during Th-17-skewed disease. Not only would IFN-β treatment be ineffective, it could worsen symptoms.

The data presented in our study, together with others demonstrate a phenomenon often seen with cytokines. IFN-β has opposing effects in different contexts (17).

The identification of immunological parameters associated to clinical subtypes, disease activity and treatment responsiveness become a major goal in research on MS (24).

Fortunately, there are patients who are fully responsive to IFN-β treatment. Among them, it is possible that a proportion of patients may have a mild clinical course, but the majority are patients constitutively responsive to IFN-β treatment.

Conclusions

We found significant correlations between IL-17A titer and both number of MS relapses and neurological EDSS handicap score. This conclusion might mean that in nonresponders to IFN-β treatment, high IL-17 detected predicts a more severe evolution unless other therapies emerge.

Since several drugs active against MS are available, identification of patients for different therapies represents a key point to maximize the management of this chronic disease.

High IL-17 concentration in the serum of MS treated patients could play a role in non-responsiveness to IFN-β therapy. Serum IL-17 might be a biomarker assay sensitive enough to be used at bedside but it must be correlated with other surrogate markers for disease activity (neopterin, myelin basic protein-like, β2-microglobulin, etc) or response to immunomodulatory treatment. Thus assessing that a patient is not responding to a certain treatment still relies on the clinical sensibility of the neurologist. In the near future, drug-specific biomarkers will be developed that might have predictive value in assessing the response to a therapy or MS.

Aknowledgments

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Abbreviations

CNS – central nervous system
EAE – experimental autoimmune encephalomyelitis
EDSS - Expanded Disability Status Score
IFN – interferon
IL – interleukin
i.m. – intramuscular
MS – multiple sclerosis
MxA – myxovirus type A
NAbs – neutralizing antibodies
RRMS – recurrent remitting multiple sclerosis
s.c. – subcutaneous
SP – secondary progressive multiple sclerosis
TGF – tumor growth factor

References

1. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol 2005; 23: 683-747.
2. Korn T. Pathophysiology of multiple sclerosis. J Neurol 2008; 255: 2-6.
3. Rio J, Nos C, Tintoré M, Borrás C, Galán I, Combella M, et al. Assessment of different treatment failure criteria in a cohort of relapsing-remitting multiple sclerosis patients treated with interferon-β: implications for clinical trials. Ann Neurol 2002; 52: 400-406.
4. El-behi M, Rostami A, Ciric B. Current views on the roles of Th1 and Th17 cells in experimental autoimmune encephalomyelitis. J Neuroimmune Pharmacol 2010; 5: 189-197.
5. Rudick RA, Polman CH. Current approaches to the identification and management of breakthrough disease in patients with multiple sclerosis. Lancet Neurol 2009; 8: 545-559.
6. McDonald WI, Compson A, Edan G, Goodkin D, Hartung H-P, Lubin FD, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001; 50: 121-127.
7. Polman CH, Reingold SC, Edan G, Filippi M, Hartung H-P, Kappos L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 2005; 58: 840-846.
8. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the "McDonald Criteria". Ann Neurol 2011; 69: 292-302.
9. Pozzilli C, Prosperini L. Clinical markers of therapeutic response to disease modifying drugs. Neurol Sci 2008; 29: S211-S213.
10. Durelli L, Conti L, Clerico M, Boselli D, Contessa G, Ripellino P, et al. T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon-β. Ann Neurol 2009; 65: 499-509.
11. Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity 2004; 21: 467-476.
12. Fort MM, Cheung J, Yen D, Li J, Zurawaski SM, Lo S, et al. Il-25 induced Il-4, Il-5 and Il-13 and Th2-associated pathologies in vivo. Immunity 2001; 15: 985-995.
13. Weaver CT, Hatton RD, Mangan PR, Harrington LE. Il-17 family cytokines and the expanding diversity of effectors T cell lineages. Annu Rev Immunol 2007; 25: 821-852.
14. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM, et al. Interleukin-17 production in central nervous system-infiltrating T cells in glial cells is associated with active disease in multiple sclerosis. Amer J Pathol 2008; 172: 146-155.
15. Weiner HL. A shift from adaptative to innate immunity: a potential mechanism of disease progression in multiple sclerosis. J Neurol 2008; 255: 3-11.
16. Brucklacher-Waldert V, Stuerner K, Kolster M, Wolthansen J, Tolosa E. Phenotypical and functional characterization of T helper 17 cells in multiple sclerosis. Brain 2009; 132: 3329-3341.
17. Axtell RC, de Jong BA, Boniface K,Van der Voort L, Bhat R R, De Sarno P, et al. T helper type 1 and 17 cells determine efficacy of interferon-β in multiple sclerosis and experimental encephalomyelitis. Nat Med 2010; 16: 406-412
18. Bălaşa R, Huţanu A, Feier C, Bajko Z, Pascu I. Incidence and clinical significance of binding and neutralizing antibodies induced by interferon-β treatment in patients with multiple sclerosis. Rev Rom Med Lab 2010; 19: 39-50.
19. Van der Voort L, Kok A, Visser A, Oudejans CBM, Caldono M, Gilli F, et al. Interferon-beta bioactivity measurement in multiple sclerosis: fesability for routine clinical practice.

Multiple Sclerosis 2009; 15: 212-218.
20. Sominanda A, Hillert J, Fogdell- Hahn A. In vivo bioactivity of interferon-beta in multiple sclerosis with neutralizing antibodies is titer-dependent. J Neurol Neurosurg Psychiatry 2008; 79: 57-62.
21. Sørensen PS, Koch-Henriksen N, Ross C, Clemmesen KM, Bendtzen K, Danish Multiple Sclerosis Study Group. Appearance and disappearance of neutralizing antibodies during interferon-beta therapy. Neurology 2005; 65: 33-39.
22. Bertolotto A, Gilli F. Interferon-beta responders and non-responders. A biological approach. Neurol Sci 2008; 29 : S216-S217.
23. Gilli F, Marnetto F, Caldano M, Sala A, Malucchi S, Di Sapio A, et al. Biological responsiveness to first injections of interferon-betain patients with multiple sclerosis. J Neuroimmunol 2005; 158 : 195-203.
24. Furlan R.Definition of nonresponders: biological markers. Neurol Sci 2008; 29 : S214-S215