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The cross-reactivity of binding antibodies with different interferon beta formulations used as disease-modifying drugs in multiple sclerosis patients

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Abstract

Interferon beta (IFNb) preparations are commonly used as first-line therapy in relapsing-remitting multiple sclerosis (RRMS). They are, however, characterized by limited efficacy, partly due to the formation of anti-IFNb antibodies in patients.

In this pilot study, we assessed with the ELISA method the presence of the binding antibodies (BAbs) against interferon beta after 2 years of therapy with subcutaneous interferon beta 1a (Rebif) in 49 RRMS patients. Antibody levels were established again within 1 year after treatment withdrawal. We used 3 interferons that are commercially available for MS therapy, namely Avonex (Biogen Idec Limited), Rebif (Merck Serono), and Betaferon (Bayer Pharma AG), as antigens.

BAbs reacting with Rebif were found in 24.4% to 55% of patients, depending on the units of their expression. The levels of anti-Rebif antibodies remained high in 8 patients and in 4 patients they dropped significantly. Strong correlations were obtained in all assays (anti-Rebif-anti-Avonex, anti-Rebif-anti-Betaferon, and anti-Betaferon-anti-Avonex) and the existence of cross-reactivity in the formation of antibodies against all the tested formulations of interferon beta was confirmed. The levels of BAbs remain significant in the clinical context, and their assessment is the first choice screening; however, methods of BAbs evaluation can be crucial for further decisions. More studies are needed to confirm our results; specifically it would be of interest to evaluate methods of neutralizing antibodies identification, as we only assessed the binding antibodies. Nevertheless, our results support the concept that in interferon nonresponders, that are positive for binding antibodies, switching the therapy to alternative disease-modifying agent (for example glatiramer acetate, fingolimod, or natalizumab) is justified, whereas the switch to another interferon formulation will probably be of no benefit.

Abbreviations: AU = arbitrary units, AUC = area under the curve, BAbs = binding antibodies, EDSS = Expanded Disability Status Scale, IFN = interferon, IFNb = interferon beta, MRI = magnetic resonance imaging, MS = multiple sclerosis, NAbs = neutralizing antibodies, O.D. = optical density, PBS = phosphate-buffered salt, RRMS = relapsing-remitting multiple sclerosis.

Keywords: beta interferon, binding antibodies, disease modifying therapies, immunology, multiple sclerosis

1. Introduction

Interferon beta (IFNb) immunomodulatory treatment in patients with relapsing-remitting multiple sclerosis (RRMS) demonstrates the average reduction of the relapse rate by 30%, with considerable variations between patients. The effect on radiological activity of the disease was confirmed in phase III clinical trials with interferons and glatiramer acetate preparations, where the ~30% reduction in the number of new T2 lesions on magnetic resonance imaging (MRI) was demonstrated as compared with the placebo-treated patients.[1–4] One of the factors that

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determine an incomplete response to treatment could be the production of antibodies against these drugs in patients. This phenomenon has been investigated from the beginning of the use of immunomodulatory therapy. Therefore, it is recommended to assess the patient's response to treatment after the first year of immunomodulation.[5] Patients can produce binding antibodies (BAbs) and neutralizing antibodies (NAbs) against IFNb. The former are more common and predict development of the latter.[6] Although only NAbs are able to neutralize IFNb function in bioassays, while BAbs represent all antibodies binding to the interferon molecule, it has been shown that BAbs decrease IFNb bioavailability independently of NAbs.[6] Moreover, these antibodies are capable of forming cross-reactions with other interferons. All of the known mechanisms of IFNb immunomodulatory activity are based on its binding to a target receptor on the surface of immune cells. This process activates the intracellular tyrosine kinase cascade leading to transcription of a number of genes. It may be disrupted when the antibodies change the normal structure of the protein, in this case interferon, which is necessary for the interaction with its receptor.[7] According to Gneiss et al, patients who present with NAbs during IFN treatment will not benefit from continuous therapy with any subcutaneous IFNb preparation.[8] It is generally not recommended to apply the drug of the same group (i.e. interferon) in case when neutralizing antibodies presence is confirmed; however, these recommendations are based on single studies only.[7,9]

Few studies were conducted with regard to cross-reactivity between 2 different interferon preparations used in clinical practice.[10,11] In our pilot study, we wanted to determine whether any immunological cross-reactions occur between interferons in a full spectrum of IFN formulations that are commercially available.

2. Material and methods

2.1. Patient population

Fourty-nine RRMS patients receiving IFNb 1a in a dose of 44 μg 3 times a week for 2 years were included in this prospective observational pilot study. Patients were enrolled from the outpatient MS Clinic by the Department of Neurology and Cerebrovascular Disorders, Poznan University of Medical Sciences. Written informed consent to participation in the study was obtained from all subjects. The study protocol was approved by the Internal Review Board at the Poznan University of Medical Sciences.

The study group consisted of 35 women (71.4%) and 14 men (28.6%), with the mean age of 32.8 ± 7.9 years and the mean disease duration of 7.5 ± 4.2 years. The median Expanded Disability Status Scale (EDSS) score in the group was 1.0, with a minimum of 0.0 and a maximum of 4.0, and the mean total number of relapses was 3 (min 1, max 4). All patients enrolled in the study met the diagnostic criteria for MS according to Polman et al.[12]

2.2. Laboratory protocol

Serum samples were tested for the presence of antibodies binding IFNb (BAbs) in patients after 2 years of immunomodulatory treatment and the same assay was repeated after 1 year from interferon treatment cessation. The reaction of patients' serum with commercially available formulations of IFNb-1a (Rebif and Avonex) and beta-1b (Betaferon) was tested to reveal interferon binding antibodies. BAbs assessment was carried out in the Department of Neurochemistry and Neuropathology, Chair of Neurology, Poznan University of Medical Sciences Poznan, Poland, by means of home-made indirect ELISA. 96-well ELISA plates (Nunc, Roskilde, Denmark) were coated with interferon beta preparations diluted in 0.05 M sodium bicarbonate (pH = 9.6) to obtain final concentration of 1 μg/mL per well. The plates were incubated for 12 hours at room temperature for antigen immobilization. After careful washes with phosphate-buffered salt (PBS) with 0.05% (vol/vol) Tween 20, the nonspecific binding sites were blocked with 1% bovine serum albumin solution in PBS-0.05% Tween 20. Washing with PBS-0.05% Tween 20 was followed by addition of blind samples (PBS added instead of serum), rat serum serving as negative control, goat anti-human interferon antibodies (Sigma-Aldrich) as the standard (in decreasing dilutions 1:20, 1:50, 1:100, 1:200 and 1: 400); sera from treatment-naive MS patients and study group sera tested at a dilution of 1:100, respectively. Room temperature incubation, PBS-0.05% Tween 20 washes were followed by the addition of secondary antibodies (goat ant-rabbit IgG, Sigma-Aldrich, or rabbit anti-human IgG conjugated with alkaline phosphatase, Sigma-Aldrich, respectively). P-nitrophenyl phosphate was used as the substrate (Sigma-Aldrich) and the reaction was stopped with 1 M HCl. The absorbance was measured with the ELISA reader ELx800 (Bio-TEK) at the wavelength of 405 nm.

The above-mentioned procedure was carried out consecutively for plates coated with all the tested interferon preparations—Rebif, Betaferon, and Avonex.

The results were expressed as the optical density (O.D.), arbitrary units AU/mL (AU—arbitrary units), and reciprocal serum dilution (RSD). Arbitrary units were calculated according to the formula: (10 × absorbance of tested sample)/absorbance of the cut-off values. The cut-off was established as 95th percentile of absorbance for each series of tested samples (with Rebif, Betaferon, Avonex as antigens) performed in immunomodulatory treatment naive MS patients. Cut-off values in tests using Rebif as the antigen were 0.180, for Avonex—0.208 and Betaferon—0.074. Reciprocal serum dilution was calculated according to the formula: $[1: x]^{-1}$, where x is dilution of standard antibodies obtained from the fitting of standard curve. The standard curve fitting was based on the calculation of log–log regression between absorbance and reciprocal standard dilution ($R^2 = 0.9777$). Then, the absorbances of tested samples were substituted to the regression formula and reciprocal serum dilutions were calculated. Thus, the values were validated against standard anti-interferon antibodies. Statistical analysis was performed with the use of MedCalc software ver.11.0.1.0. The results were considered statistically significant when P was ≤ 0.05.

3. Results

In our study, we have analyzed the sera from patients treated with Rebif for the presence of BAbs by means of ELISA, using Rebif, Avonex or Betaferon formulations as immobilized antigens. Different methods of magnitude of quantity expressions were evaluated for their clinical significance. In the group of 49 patients receiving interferon beta 1a, the presence of antibodies binding to Rebif (anti-Rebif) was found after 24 months of treatment in 12 patients (24,4%), when the cut-off was exceeded. The cut-off was established as the 95th percentile of O.D. in the group of treatment naive MS patients. Within a year of the treatment discontinuation the levels of the antibodies did not drop significantly (see Table 1).

Table 1

BAbs levels measured after 2 years of Rebif therapy and 1 year after Rebif treatment termination expressed in optical density units (O.D.), arbitrary units (AU), and reciprocal serum dilution ([1: ×]$^{-1}$), where *x*– is dilution.

	Sampling 1 (after 2 years of Rebif therapy)		Sampling 2 (1 year after completing the Rebif therapy)
[O.D.] Median	0.092		0.068
[O.D.] Minimum–maximum	0.012–0.996		0.017–0.99
[O.D.] Interquartile range	0.046–0.174		0.046–0.152
P-value		$P=0.6265$	
[AU/mL] Median	5.14		3.75
[AU/mL] Minimum–maximum	0.67–55.37		0.95–55.03
[AU/mL] Interquartile range	2.55–9.68		2.54–8.48
P-value		$\boldsymbol{P}=0.25$	
[reciprocal serum dilution] mean	133		140
[reciprocal serum dilution] standard deviation	83		78
P-value		$\boldsymbol{P}=0.4431$	

Continuously high levels of antibodies were found in 8 patients, whereas 4 patients had a significant reduction in their level during the observation period.

Moreover, BAbs' level expressed as reciprocal serum dilution was higher (mean 167 ± 12 vs 130 ± 12, $P=0.0428$) in patients who manifested worsening from EDSS score evaluated after completion of IFNb treatment to EDSS score evaluated 1 year after therapy cessation than in subjects with a stable disability score. Such an effect was not observed when the BAbs level was expressed as O.D. or in arbitrary units. Thus, worsening at least in EDSS score can be used as indication of BAb's testing.

We have analyzed ROC curves for BAbs as markers of clinical worsening during IFNb treatment. As a classifier we have used hard clinical endpoints like increase in the EDSS score or relapse. When coexisting worsening in the EDSS score together with any relapse during IFNb therapy was used as clinical indicator of disease activity, BAbs identified after 2 years of Rebif therapy were relevant. In such a group of patients BAb's expressed as reciprocal serum dilution, showed area under the curve (AUC) of 0.671 with $P=0.0321$ and a cut-off criterion of >126 (see Fig. 1). Moreover, the presence of BAb's expressed as reciprocal serum dilution and evaluated 1 year after INFb treatment termination (AUC$=0.655$, $P=0.0258$, cut-off criterion >122, Fig. 2) and after 2 years of Rebif therapy cessation (AUC$=0.637$, $P=0.0626$, cut-off criterion >140, Fig. 3) predicted the worsening from EDSS evaluated after completion of IFNb treatment to

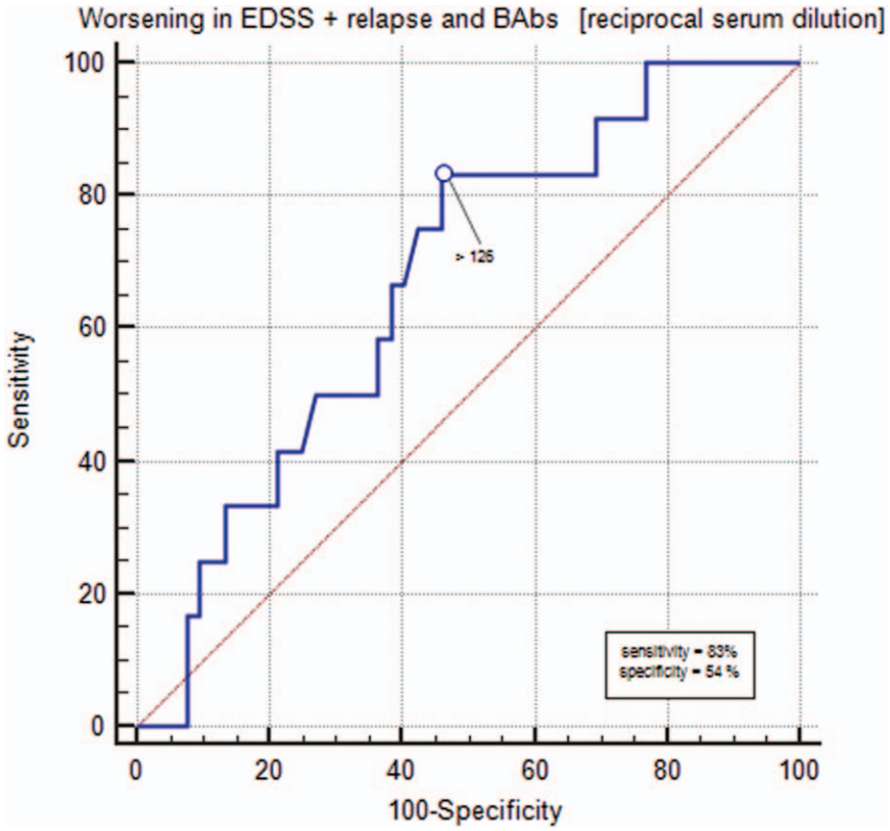

Figure 1. ROC curve for BAbs as markers of clinical worsening during IFNb treatment. Increase in EDSS score and relapse during IFNb therapy were used as classifiers. BAbs = binding antibodies, EDSS = Expanded Disability Status Scale, IFNb = interferon beta.

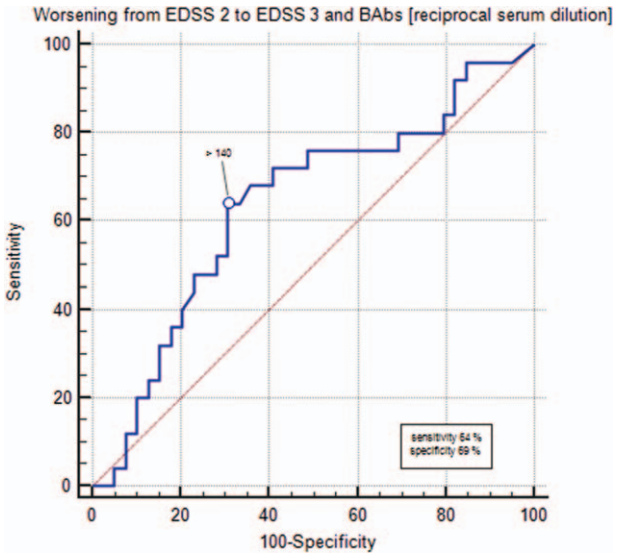

Figure 2. ROC curve for BAbs identified 1 year after IFNb termination and worsening from EDSS evaluated after completion of IFNb treatment (EDSS2) to EDSS score evaluated 1 year after therapy cessation (EDSS3) used as the classifier. BAbs = binding antibodies, EDSS = Expanded Disability Status Scale, IFNb = interferon beta.

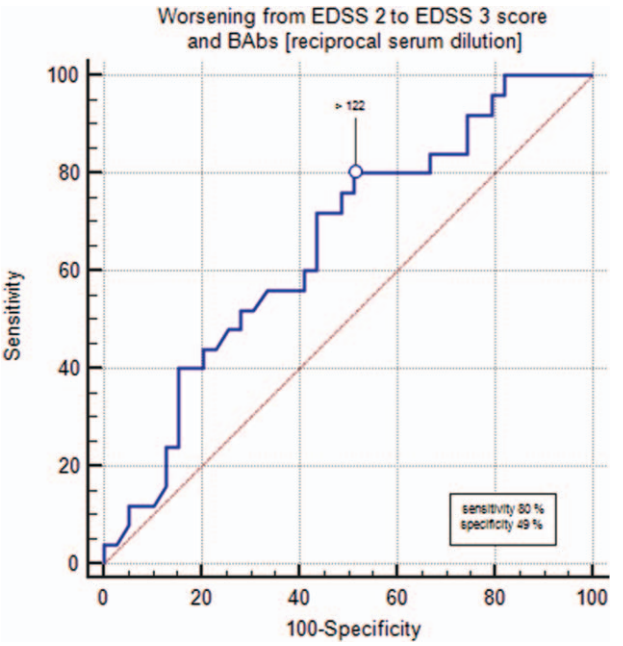

Figure 3. ROC curve for BAbs evaluated after 2 years of INF treatment and worsening from EDSS evaluated after completion of IFNb treatment (EDSS2) to EDSS score evaluated 1 year after therapy cessation (EDSS 3) used as the classifier. BAbs = binding antibodies, EDSS = Expanded Disability Status Scale, IFNb = interferon beta.

EDSS score evaluated 1 year after therapy cessation. Such effect was not found when BAbs were expressed as O.D. or arbitrary units.

With the use of cut-off value exceeding 140 of reciprocal serum dilution, 45% of patients tested BAbs positive after 2 years of IFNb treatment, and 55% tested positive 1 year after IFNb therapy termination.

The change of currently used INFb formulation can be considered in patients who do not respond to therapy. For this reason we have tested the cross-reactivity of BAb's. The analysis of correlation between levels of different antibodies (anti-Rebif/anti- Betaferon, anti-Rebif/anti-Avonex and anti-Betaferon/anti-Avonex) for the study group was carried out. Statistically significant ($P1 = 0.0002$ and $P2 < 0.0001$) correlation between the level of anti-Rebif antibodies with the level of anti-Betaferon antibodies (r1 correlation coefficient = 0.4578 and $r2 = 0.8095$; slope1 = 0.6179 and slope2 = 1.0823) was observed as illustrated in Fig. 4.

The level of anti-Rebif antibodies also correlated (r1 = 0.6531 and r2 = 0.6781; slope1 = 0.5945 and slope2 = 0.3695) with the level of anti-Avonex antibodies ($P1 < 0.0001$; $P2 < 0.0001$); see Fig. 5.

A strong correlation ($r1 = 0.8255$ and $r2 = 0.6819$; slope1 = 1.2367 and slope2 = 1.5244) between levels of anti-Betaferon and anti-Avonex antibodies ($P1 < 0.0001$; $P2$ $P < 0.0001$) was determined (Fig. 6).

4. Discussion

In this study, we have confirmed that binding antibodies are formed in a significant (24.4% to 55%, depending on the time of evaluation and units used for their expression) proportion of IFNb-1a treated patients, which is consistent with other publications. According to the literature, BAbs are present in

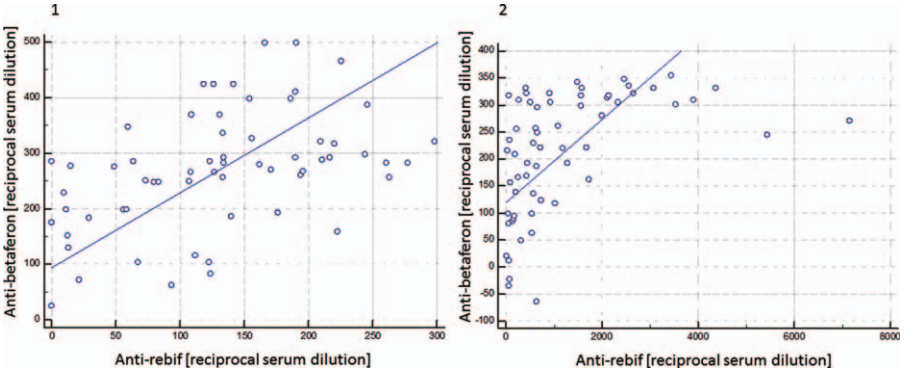

Figure 4. Correlation between anti-Rebif antibodies and anti-Betaferon antibodies: (1) after 2 years of Rebif therapy, (2) 1 year after Rebif treatment termination. Reduced major axis (RMA) regression was used to present the data. In RMA regression measurement errors are taken into account for both dependent (*Y*-axis) and independent (*X*-axis) variables.RMA = reduced major axis.

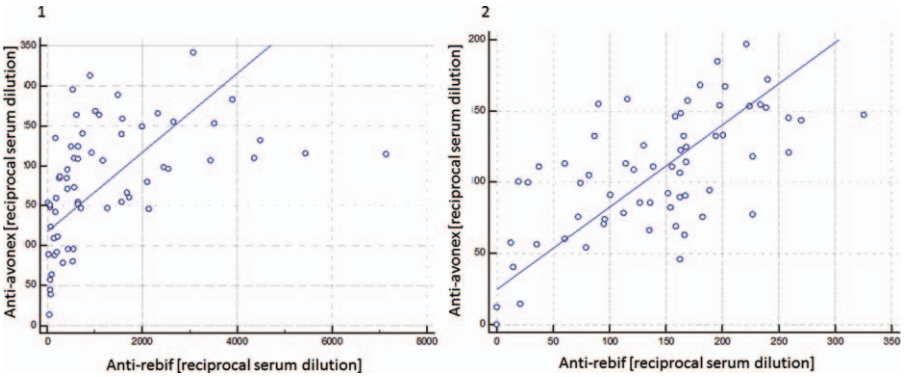

Figure 5. Correlation between anti-Rebif antibodies and anti-Avonex antibodies: (1) after 2 years of Rebif therapy, (2) 1 year after Rebif treatment termination. Reduced major axis (RMA) regression was used to present the data. In RMA regression measurement errors are taken into account for both dependent (*Y*-axis) and independent (*X*-axis) variables. RMA = reduced major axis.

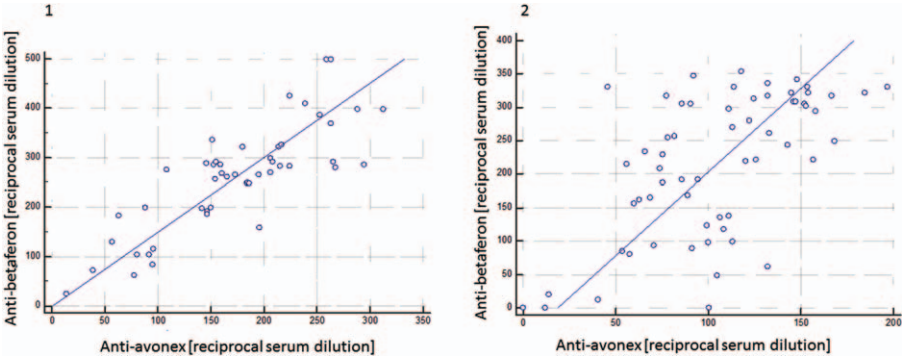

Figure 6. Correlation between anti-Betaferon antibodies and anti-Avonex antibodies: (1) after 2 years of Rebif therapy, (2) 1 year after Rebif treatment termination. Reduced major axis (RMA) regression was used to present the data. In RMA regression measurement errors are taken into account for both dependent (*Y*-axis) and independent (*X*-axis) variables. RMA = reduced major axis.

~5% to 30% of patients on Avonex, 25% to 45% in patients on Rebif and 50% to 80% in those treated with Betaferon.[13–17] In comparison, neutralizing antibodies occur in ~2% to 6% of patients treated with Avonex, 12% to 28% in those treated with Rebif, and in 28% to 47% of patients on Betaferon.[18,19] Pharmacological forms of beta interferons differ in their immunogenicity; therefore, there is a different proportion of patients with elevated levels of antibodies for different formulations.[18,20]

A golden-standard method for detection of anti-interferon antibodies is ELISA, which has also been applied in our study.[20–23] Importantly, the United States and Canadian authors demonstrated that the test reliability for the individual patient is better when one uses as the antigen the drug with which the patient has been treated.[20] All of the commercially available preparations of IFNb (at the time of patient recruitment these included: Rebif, Avonex i Betaferon) were used in this study.

It should be noted that in our study, we only assessed the levels of BAbs, and not NAbs. In our opinion, this does not diminish the significance of the results since international recommendations describe the assessment of BAbs as a first choice screening in RRMS patients treated unsuccessfully with interferons. It has been demonstrated that BAbs titers reliably predict the presence of NAbs.[6] In the same study it was shown that BAbs diminish IFNb bioavailability independently from NAbs, as measured by reduced protein concentrations of CXCL-10 and sTRAIL. In patients with elevated BAbs it is reasonable to expand the analysis to include the assessment of NAbs.[22] However, it is recommended to stop IFN-b treatment already at the moment when BAbs are present.[6]

The type of expression of BAbs level may play a role in its clinical significance and can be responsible for observed discrepancies between studies. Most of the methods used for BAbs evaluation and analyzed in EFNS guidelines for anti-IFN measurements[24] are based on O.D. or AU and only one on the standard curve. Thus, the limitations attributed to BAbs analyses may, additionally to biological effects, result from the units used for their expression. In our study we have shown the differences in clinical significance of BAbs, which depended on the type of magnitude of quantity. To our knowledge, there are no other studies comparing the effects of BAbs measurements expression on clinical evaluations. Moreover, we used ROC curve analysis since EDSS and relapse number are clinical hard endpoints revealing MS activity. Thus, clinical outcomes of MS patients evaluation in everyday clinical practice are based on dichotomous results (stable/worsened EDSS; relapse / no relapse). The concept of ROC curve uses the notion of a separator variable, which defines such outcomes. For this reason, we have tested a novel mode of expression of BAb's results (reciprocal serum dilution) using ROC curve analysis.

Interestingly, in our study we did not find any statistically significant differences between serum antibody levels after 2 years

of treatment with subcutaneous interferon beta 1a, and 1 year after treatment cessation. This suggests a continuously high level of antibodies reached within 2 years of treatment for at least another year.

Another interesting finding in our study is the presence of strong correlations between antibody levels for all the tested interferon formulations. These correlations were found between anti-Rebif and anti-Betaferon, anti-Rebif and anti-Avonex, as well as anti-Avonex and anti-Betaferon antibodies (Figs. 3–5).

In a very small earlier study by Khan and Dhib-Jalbut[11] 3 out of 10 patients treated with IFN beta 1a developed BAbs and NAbs, both of which demonstrating cross-reactivity with interferon beta 1b. Similarly, antibodies in 6 patients treated with IFNb 1b cross-reacted with IFNb 1a. This study confirmed the results of previous observations that cross-reactivity between IFNb 1a and endogenous IFNb occurs in patients with high levels of antibody binding.[10] Similar results were obtained in subsequent studies[25,26] and were consistent with the earlier findings.[10,11] However, to our knowledge, so far the cross-immunogenicity of all the different commercially available interferons has not been estimated.

This work is a pilot study and was conducted on a relatively small group of patients. A definite advantage of this study is the follow-up antibody levels' assessment after a year since therapy withdrawal. We could, therefore, demonstrate the persistence of elevated anti-interferon antibody levels in the majority of patients after treatment discontinuation.

We have also managed, with the use of ROC curve analysis, to show that BAbs could be useful markers of clinical worsening during IFNb treatment. We believe these results have important implications for the clinical practice as they relate to commonly used IFNb formulations and confirm that in patients diagnosed with high levels of antibodies against any of the interferons one should expect high levels of BAbs against other interferon preparations.[7,9]

To conclude, if antibodies against any interferon are found, switching therapy to alternative disease-modifying agent should be considered.

Such therapeutic strategy could be effective even within the first-line therapy, as it has been proved recently. The results of the recent COPTIMISE trial demonstrated that switching

RRMS patients who did not benefit from initial IFNb therapy to glatiramer acetate is associated with a positive treatment outcome.[27]

In light of the above-mentioned findings, different methods of expression of BAbs' levels may be crucial for clinical purposes, and switching therapy from one interferon to another is not likely to benefit the patient.

References

[1] The IFN-b Multiple Sclerosis Study, GroupInterferon beta-1b is effective in relapsing-remitting multiple sclerosis. Neurology 1993;43:662–7.

[2] PRISM, GroupRandomised double-blind placebo-controlled study of interferon b- 1a in relapsing/remitting multiple sclerosis. Ann Neurol 1996;39:285–94.

[3] Kappos L, Traboulsee A, Constantinescu C, et al. Long-term subcutaneous interferon beta-1a therapy in patients with relapsing-remitting MS. Neurology 2006;67:944–53.

[4] Jacobs LD, Cookfair DL, Rudick RA, et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). Ann Neurol 1996;39:285–94.

[5] International Working Group of Treatment Optimization in MSTreatment optimisation in multiple sclerosis: report of an international consensous meeting. Eur J Neurol 2004;11:43–7.

[6] Hegen H, Millonig A, Bertolotto A, et al. Early detection of neutralizing antibodies to interferon-beta in multiple sclerosis patients: binding antibodies predict neutralizing antibody development. Mult Scler J 2014;20:577–87.

[7] Bertoletto A. Implication of neutralizing antibodies on therapeutic efficacy. J Neurol Sci 2009;277:S1S29–32.

[8] Gneiss C, Koudouovoh-Tripp PM, Ropele S, et al. Influence of interferon-beta therapy switching on neutralizing antibody titres: results from the Austrian Switch Study. Mult Scler 2009;15:1481–8.

[9] Polman Ch, Bertoletto A, Deisenhammer F, et al. Recommendation for clinical use of data on neutralising antibodies to interferon-beta therapy in multiple sclerosis. Lancet Neurol 2010;9:740–50.

[10] Kivisakk P, Alm GV, Tian WZ, et al. Neutralising and binding anti-interferon beta-I-b (IFN-beta-Ib) antibodies during IFN-beta-Ib treatment of multiple sclerosis. Mult Scler 1997;3:184–90.

[11] Khan O, Dhib-Jalbut S. Neutralizing antibodies to interferon β-1a and interferon β-1b in MS patients are cross reactive. Neurology 1998;51:1698–702.

[12] Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69:292–302.

[13] Fernandez O, Mayorga C, Luque G, et al. Study of binding and neutralizing antibodies to interferon-β in two groups of relapsing-remitting multiple sclerosis patients. J Neurol 2001;248:383–8.

[14] Francis G, Rice G, Alsop J. Interferon-β 1a in MS. Results following development of neutralizing antibodies in PRISMS. Neurology 2005;65:48–55.

[15] Kinisakk P, Alm G, Fredrikson S, et al. Neutralizing and binding anti-interferon-beta (IFN-beta) antibodies. A comparison between IFN-β-1a and IFN-β-1b treatment in multiple sclerosis. Eur J Neurol 2000;7:27–34.

[16] Perini P, Calabrese M, Biasi G, et al. The clinical impact of interferon beta antibodies in relapsing-remitting MS. J Neurol 2004;251:305–9.

[17] Scagnolari C, Bellomi F, Turrziani O, et al. Neutralizing and binding antibodies in IFN-β: relative frequency in relapsing-remitting multiple sclerosis patients treated with different IFN-β products. J Interferon Cytokine Res 2002;22:207–13.

[18] Bertoletto A, Deisenhammer F, Gallo P, et al. Immunogenicity of interferon beta: differences among products. J Neurol 2004;251(suppl 2):II/15–24.

[19] Menge T, Hartung H-P, Kieseier BC. Neutralizing antibodies in interferon beta treated patients with multiple sclerosis: knowing what to do now. J Neurol 2011;258:904–7.

[20] Prince H, Lape-Nixon M, Audette C. Van Horn Identification of interferon-beta antibodies in a reference laboratory setting: Findings for 1144 consecutive sera. J Neuroimmunol 2007;190:165–9.

[21] Sorensen PS, Deisenhammer F, Duda P, et al. Guidelines on use of anti-IFN-β antibody measurements in multiple sclerosis: report of an EFNS Task Force on IFN-β antibodies in multiple sclerosis. Europ J Neurol 2005;12:817–27.

[22] Oger J, Gibbs E. Binding antibodies: Vancouver's perspective. Mult Scler 2007;13:S36–43.

[23] Pachner A, Oger J, Palace J. The measurement of antibodies binding to IFNβ in MS patients treated with IFNβ. Neurology 2003;61(suppl 5):S18–20.

[24] Blackwell Publishing Ltd, Sorensen PS, Deisenhammer F, Duda P. Gilhus NE, Barnes MP, Brainin M, et al. Use of anti-interferon beta antibody measurements in multiple sclerosis. European Handbook of Neurological Management. Vol 1 2nd ed.2011.

[25] Bertoletto A, Malucchi S, Milano E, et al. Interferon beta neutralizing antibodies in multiple sclerosis: neutralizing activity and cross-reactivity with three different preparations. Immunopharmacology 2000;48:95–100.

[26] Antonelli G, Simeoni E, Bagnato F, et al. Further study on specificity and incidence of neutralizing antibodies to interferon (IFN) in relapsing remitting multiple sclerosis patients treated with IFN beta-1a or IFN beta-1b. J Neurol Sci 1999;168:131–6.

[27] Ziemssen T, Bajenaru OA, Carra A, et al. A 2-year observational study of patients with relapsing-remitting multiple sclerosis converting to glatiramer acetate from other disease-modifying therapies: the COPTIMIZE trial. J Neurol 2014;261:2101–11.