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## Incidence and clinical significance of binding antibodies and their relationships with neutralizing antibodies, both induced by interferon- $\beta$ treatment in multiple sclerosis patients

### Incidența și importanța clinică a anticorpilor legați și a relația lor cu anticorpii neutralizanți, induși de tratamentul cu $\beta$ interferon la pacienții cu scleroză multiplă

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#### Abstract

**Purpose:** During interferon (IFN $\beta$ ) treatment of multiple sclerosis (MS), the therapy induced binding antibodies (BAbs) and neutralizing antibodies might decrease the clinical efficacy of IFN $\beta$ . We evaluated: differences in immunogenicity of different IFN $\beta$  products using BAbs; the impact of BAbs on clinical efficacy of IFN $\beta$ . We present the first Romanian experience on this issue. **Material and methods:** 229 MS patients treated for at least 12 months with either subcutaneous IFN $\beta$ -1b 250 $\mu$ g every other day (Betaferon 132 patients), subcutaneous IFN $\beta$ -1a 44 $\mu$ g three times a week (Rebif 71 patients) or intramuscular IFN $\beta$ -1a 30 $\mu$ g once weekly (Avonex, 26 patients) were tested for BAbs using quantitative dELISA. The groups were clinically and demographically alike. According to the results, each group was divided into: 1. BAbs+; 2. BAbs-. Subgroups and titer of BAbs were compared with disability score (EDSS) variation in the last year and number of relapses during IFN $\beta$  treatment (Mann-Whitney and Spearman correlation). **Results:** The percentage of BAbs+ patients was: Betaferon 64.4%, Rebif 18.3%, Avonex 15.4%. In the Rebif group, a correlation was found between presence and value of BAbs and EDSS change ( $p=0.05$ ) but not with the number of relapses. No correlation was found between the presence or value of BAbs and relapses or EDSS change in Betaferon or Avonex group. **Conclusions:** (i) Betaferon is more immunogenic than Rebif or Avonex; (ii) BAbs do not abrogate the clinical effects of Betaferon or Avonex regardless the parameter used; (iii) assays for BAbs are relatively inexpensive and have a role in following Rebif treatment.

**Keywords:** binding antibodies, neutralizing antibodies, multiple sclerosis, immunomodulatory treatment

#### Rezumat

**Scop:** În timpul terapiei cu interferon (IFN $\beta$ ) a pacienților cu scleroză multiplă (SM), apariția anticorpilor legați (BAbs) și a celor neutralizanți ai IFN $\beta$  poate scădea eficacitatea IFN $\beta$ . Am evaluat: diferențele imunogenice ale diferitelor tipuri de IFN $\beta$  folosite în SM prin determinarea BAbs; impactul prezenței BAbs asupra

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eficacității terapeutice a IFN $\beta$ ; prezentăm prima experiență românească. **Material și metodă:** Am selectat 229 pacienți cu SM tratați minimum 12 luni cu același tip de IFN $\beta$ : IFN $\beta$ -1b 250 $\mu$ g subcutan la 2 zile (Betaferon 132 pacienți), IFN $\beta$ -1a 44 $\mu$ g subcutan de 3 ori pe săptămână (Rebif 71 pacienți) sau IFN $\beta$ -1a 30 $\mu$ g intramuscular săptămânal (Avonex 26 pacienți). Grupurile au fost asemănătoare clinic și demografic. BAbs au fost măsurate utilizându-se un test ELISA direct cantitativ, iar în funcție de rezultat, grupurile au fost divizate în: 1.BAbs+; 2.BAbs-. Prezența BAbs și titrul acestora au fost comparate cu modificarea scalei de handicap EDSS, cu numărul de recurențe în ultimele 12 luni de tratament cu IFN $\beta$  (testele Mann-Whitney și Spearman). **Rezultate:** Procentajul de pacienți BAbs+ a fost: Betaferon 64.4%, Rebif 18.3%, Avonex 15.4%. În grupul Rebif, am găsit o corelație între prezența și titrul BAbs și modificarea EDSS ( $p=0.05$ ) dar nu cu numărul de recurențe. Nu am găsit nici o corelație între prezența sau valoarea BAbs și recurențele sau EDSS în grupurile Betaferon sau Avonex. **Concluzii:** (1) Betaferon este mai imunogen decât Rebif sau Avonex; (2) BAbs nu reduc eficiența clinic a Betaferonului sau a Avonex; (3) Determinarea BAbs este relativ ieftină și poate fi utilizată în urmărirea terapiei cu Rebif.

**Cuvinte cheie:** anticorpi anti-interferon, scleroza multiplă, tratament imunomodulator

## Introduction

Interferon $\beta$  (IFN $\beta$ ) is a first line therapy for patients with multiple sclerosis (MS). A potential consequence of IFN $\beta$  treatment is the development of binding antibodies (BAbs) and neutralizing antibodies (NABs). Both BAbs and NABs bind to the IFN $\beta$  molecule. BAbs may bind to the IFN $\beta$  molecule at a variety of locations, and some of these interactions result in blocking the receptors of IFN $\beta$  and appearance of NABs. Thus, NABs are a subset of Babs. (1-10). Several publications have concordantly reported that BAbs and NABs occur during the treatment with recombinant IFN $\beta$  products. The biological and clinical meaningfulness of BAbs induction still needs to be defined with more precision, but the presence of NABs has been associated with reduction in the clinical effectiveness of IFN $\beta$  and a decrease in IFN $\beta$ -related side effects (11-18).

The frequencies and titers of anti-IFN $\beta$  antibodies vary considerably depending on: a) the type of IFN $\beta$  preparation; b) the frequency and route of administration; c) the type of assay being used. Assays for BAbs activity are often used as a screening for identifying patients with possible NABs activity. The development of BAbs precedes that of NABs, and both can often be detected in the same patients after several months. BAbs can also be detected in patients who never express NABs.

NABs, however, do not appear in patients with BAbs negative tests (19-22).

The common assays for determining BAbs are: a) enzyme-linked immunosorbent (ELISA); b) radioimmunoprecipitation (RIPA); c) affinity chromatography (ACA). The measurement of NABs can be performed by: a) cytopathic effect (CPE); b) myxovirus resistance protein A (MxA). Until now there is no clear method to establish a common titer for quantifying BAbs positive (BAbs+) or NABs positive (NABs+) results (23-33).

## Material and methods

### Patients and demonstration of BAbs

This study evaluated the presence of BAbs in MS patients treated for at least one year with 30  $\mu$ g intramuscular (IM) IFN $\beta$ -1a (Avonex) single dose weekly, subcutaneous (SC) IFN $\beta$ -1b (Betaferon) 250  $\mu$ g every other day and SC IFN $\beta$ -1a (Rebif) 44  $\mu$ g 3 times weekly. BAbs were tested using direct ELISA test.

Before enrolment, all aspects of the study protocol were reviewed in each subject and informed consent was obtained. The study protocol was approved by the local Ethic Committee and was carried out according to the Declaration of Helsinki.

Patients were tested at 24 hours after the last IFN $\beta$  injection. Peripheral blood was collected and serum was obtained by centrifugation.

gation at 2000 x for 15 min and stored at -70°C until all samples were obtained.

The Buhlmann anti-IFN $\beta$  antibodies ELISA kit was used for the direct and quantitative in vitro diagnostic determination of IgG antibodies to therapeutically administered IFN $\beta$  in human serum. The standards of the anti-IFN $\beta$  BAbs ELISA kit were calibrated against an internal reference. The Buhlmann Titer Units (BTU) were established as follows: the dilution at which the reference pool falls short of the cut-off of the control samples, corresponds to the titer, expressed in BTU. Patients were considered BAbs+ if they had a positive sample with an optical density (OD) that was higher than the mean +3SD of the OD of the control sample. Patients were enrolled into 1 of 6 groups based on BAbs status and type of treatment: BAbs-positive (BAbs+) and BAbs-negative (BAbs-) for each type of treatment received, respectively, Avonex, Betaferon and Rebif. Due to the fact that most patients were treated with Betaferon, this BAbs+ group was subdivided according the titer of BAbs (maximum 1: 500 binding units-BTU and >1: 500 BTU) into mild BAbs+ (mBAbs+) and high BAbs+ (hBAbs+).

In addition, for each patient was registered the age, the change of Expanded Disability Status Score (EDSS) in the last year before BAbs testing and number of relapses in the 12 months before screening for BAbs.

#### **Inclusion/exclusion criteria**

Patients were included if they were aged between 18 and 65 years, diagnosed with relapsing remitting and secondary progressive form of MS and treated with the same type IFN $\beta$  for at least 12 months, had no methylprednisolone treatment in the previous 45 days to BAbs testing. Patients were excluded if they had undergone a prior combination of IFN $\beta$  and any immunosuppressant other than IV corticosteroids.

#### **Objectives**

The primary objective was to compare the immunogenicity of different types of IFN $\beta$ .

The secondary objective was to evalu-

ate the impact of BAbs on clinical efficacy using clinical outcome measures, including clinically documented relapses and change in EDSS score. A relapse was defined as the appearance of a new symptom or the worsening of a pre-existing symptom, lasting more than 24 hours and producing a 1.5 points modification in the corresponding functional system of the EDSS. We analyzed the number of relapses that appeared within 12 months prior to testing. All relapses were treated with high dose of methylprednisolone (1g/day for maximum 5 days).

Another objective was to determine if in BAbs+ Betaferon treated patients, age and MS activity differ significantly in high titers BAbs+ group compared with mild titers of BAbs+ group.

Last, but not least, we present the first Romanian experience on determining the presence and the value of BAbs in an IFN $\beta$  treated MS population.

#### **Statistical methods**

The groups were compared using a rank-based analysis of variance and correlation analyses were conducted using Spearman rank correlation coefficient and Mann-Whitney test. Logistic regression analysis was used to determine patient characteristics associated with BAbs+ and BAbs- status. All reported p values are based on two-tailed statistical tests, with a significance level of 0.05.

"NAb+ and NAb-" in Pachner2009bis

#### **Results**

A total of 229 patients completed the laboratory analysis. A hundred and thirty two patients received IFN $\beta$ -1b 250  $\mu$ g SC every other day, 71 patients received SC IFN $\beta$ -1a 44  $\mu$ g three times weekly and 26 patients received IM IFN $\beta$ -1a 30 $\mu$ g once weekly for minimum one year. Although the patients were not randomized to treatment, no significant differences were observed among the three groups with regard to age, gender, age at disease on-

**Table 1. Patient demographics and disease characteristics at the moment of BAbs testing**

Characteristics	IFN $\beta$ -1b	SC IFN $\beta$ -1a	IM IFN $\beta$ -1a
Sex, F/M	89/43	51/20	18/8
Age, years, mean $\pm$ SD	34.5 $\pm$ 7	32.4 $\pm$ 5	30.8 $\pm$ 8
Disease duration years, mean $\pm$ SD	9.3 $\pm$ 7.4	8.1 $\pm$ 6.0	5.9 $\pm$ 4.5
EDSS, mean $\pm$ SD	3.9 $\pm$ 1.9	3.3 $\pm$ 0.9	2.3 $\pm$ 1.1
Relapses in the last year, mean $\pm$ SD	1.6 $\pm$ 1.2	1.2 $\pm$ 0.9	1.3 $\pm$ 1.0

set, disease duration and number of relapses during one year before BAbs testing (Table 1).

Overall, 102 of the 229 (44.5%) MS patients randomized to the three treatment groups, developed BAbs (85/132 IFN $\beta$ -1b, 13/71 SC IFN $\beta$ -1a, 4/26 IM IFN $\beta$ -1a).

In the SC IFN $\beta$ -1a treated patients, BAbs were + in 18.3% of the cases (13 patients).

In the IM IFN $\beta$ -1a treated patients, BAbs+ were 4 cases (15.4%).

The presence of 85 BAbs+ patients was significantly higher in the Betaferon treated group than in others two ( $p < 0.01$ ). In the IFN $\beta$ -1b treated patients, 85 cases (64.4%) were BAbs+. Among the BAbs+ patients, those with hBAbs+ represented 36.5% (31 cases).

Regardless of the therapy used, no correlations have been found between the presence of BAbs and any of the following measures: age of patient, gender, disease duration, relapse rate, mean EDSS. In the BAbs+ group, the mean relapse rate was 1.8 and in the BAbs- group was 1.2. The percentage was in the favor of the BAbs+ group but without statistical difference (Table 2).

Comparing the two subgroups of BAbs+ IFN $\beta$ -1b patients (mBAbs+ vs hBAbs+), we found no significant difference regarding the age of patients, the number of relapses nor the change of EDSS in the last year (Table 3).

In the Rebif treated group, a correlation was found between the presence and the value of BAbs and the EDSS change ( $p = 0.05$ ) but not with the number of relapses. In this group, BAbs+ patients had a significant higher

EDSS change than the BAbs- population. No correlation was found between the presence of BAbs and relapses or EDSS change in neither the Betaferon nor the Avonex group.

In general, the number of patients BAbs+ treated with IFN $\beta$ -1a, either given three times a week or once a week, was significantly lower than those treated with IFN $\beta$ -1b ( $p < 0.01$ ). Patients treated with IM IFN $\beta$ -1a had the lowest incidence of BAbs+.

## Discussions

We used in our study BAbs without continuing with NAbs from technical reasons, but Pachner et al (30) discuss that NAbs have some important disadvantages: they are time consuming, expensive and only indirectly measure antibodies. Regarding BAbs, ELISA methods have been used, but direct adhesion of the antigen (IFN $\beta$ ) to the plate has resulted in false-negative and false-positive results, presumably because of changes in antigenicity. This limitation is circumvented by using capture ELISA in which a first antibody is used to capture the antibody and hold it in an antigenic position, mimicking that of IFN $\beta$  in its natural state. Assays for BAbs are relatively inexpensive.

In our study, BAbs developed in 64.4% of patients receiving 250  $\mu$ g SC IFN $\beta$ -1b while in the series of Pachner et al [30] they were present in 70%. This difference is explicable by the time of collecting the samples, BAbs in Betaferon-treated patients tend to decrease over time.

**Table 2. Patient demographics and disease characteristics in the BAbs+ and BAbs- groups of patients. Comparison of the two groups**

Characteristics	BAbs+	BAbs-	p value
Sex, F/M	71/31	87/40	ns
Age, years, mean, $\pm$ SD	33.5 $\pm$ 6.2	38.3 $\pm$ 6.9	ns
Disease duration years, mean, $\pm$ SD	8.5 $\pm$ 5.9	8.8 $\pm$ 6.7	ns
EDSS, mean, $\pm$ SD	4.3 $\pm$ 1.4	3.8 $\pm$ 1.1	ns
Relapses in the last year, mean $\pm$ SD	1.8 $\pm$ 1.1	1.2 $\pm$ 1.0	ns

**Table 3. Patients BAbs+ treated with Betaferon: demographics and disease characteristics in mBAbs+ and hBAbs+ subgroups**

Characteristics	mBAbs+	hBAbs+	p value
Sex, F/M	30/24	8/5	ns
Age, years, mean	30.5 $\pm$ 7.4	38.3 $\pm$ 3.6	ns
Disease duration years, mean	8.7 $\pm$ 6.7	9.8 $\pm$ 7.1	ns
EDSS, mean	4.1 $\pm$ 1.2	3.6 $\pm$ 2.0	ns
Relapses in the last year, mean	1.5 $\pm$ 1.1	1.7 $\pm$ 1.3	ns

Our patients' disease characteristics reveal a more severe and ancient disease than other groups because we did not select the recurrent remitting forms of MS. We tested all patients treated with IFN $\beta$  for at least one year. In other cohorts, the mean age, disease duration and EDSS are less than ours (40).

Pachner et al (30) found that despite of relatively lower incidence of BAbs in the Rebif group, anti-IFN $\beta$  antibodies appeared to persist more in the Rebif group than in the Betaferon group. This finding might be an explanation for our statistical significant correlation between the presence of BAbs+ and the EDSS change in Rebif treated group. The mean MS duration in our group was 8.1 when in most cases the disease has a progressive course, less relapses and an increased EDSS.

Our finding that Betaferon is more immunogenic than the rest of IFN $\beta$  correlates with other studies and the explanation consists in the IFN $\beta$  dose (the higher, the more immunogenic), frequently administered (multiple times per week), the route of administration

(subcutaneously). The discrepancies among the published data and our results may be explained by differences in both methodology and number of patients analyzed. Moreover, in the majority of the published studies, only NAbs+ patients were considered. BAbs were not determined because they were considered "irrelevant antibodies". However, Perini et al (40) tested both BAbs and Nabs and they found that the presence of high titers of both types of antibodies correlates with disease activity and progression, being responsible for the diminution in therapeutic benefit of IFN $\beta$ . Also, NAbs do not appear in patients who test for BAbs was negative.

In the pivotal trials of the three IFN $\beta$  preparations (Betaseron/Betaferon, Avonex, Rebif), the frequency of NAbs has varied from 7% to 40%, while BAbs have been found in up to 78% of MS patients (15; 17; 20-22; 34; 35).

Direct comparison studies have shown that NAbs develop more frequently during treatment with IFN $\beta$ -1b (Betaferon) than IFN $\beta$ -1a and that in the IFN $\beta$ -1a products, NAbs develop

more frequently during treatment with Rebif than Avonex. NABs develop faster during treatment with Betaseron/Betaferon than with Rebif, but after 12 months the proportions of NAb+ patients treated with Betaseron/Betaferon or Rebif were similar. Therefore, the immunogenicity of IFN $\beta$  is: Betaseron/Betaferon > Rebif > Avonex (13; 19; 30; 36-42).

NABs to Avonex and Betaseron/Betaferon cross-react, both in the binding and in the biologic assays. This suggests that switching to alternate IFN $\beta$  preparation in patients who develop NABs may not be clinically beneficial (42).

Gibbs et al (22) showed that BAb levels could be detected as early as one to three months after initiation of treatment with IFN $\beta$ -1b. The expression of BAb peak at about four to six months, while the peak in NABs expression occurred at 13-18 months. Perini et al (40) demonstrated that of the total number of patients treated with one type of IFN $\beta$ , ~60% had been BAb+ after three months, while NABs began to appear after six months and ~40% of patients had become NABs+ after 12 months of treatment. In a reference laboratory, antibody status was measured at screening and then BAb+/NAB+ status were measured at baseline ( $\leq 8$  weeks after screening) and 6 months after baseline in patients with relapsing forms of MS treated with Avonex, Rebif or Betaseron. The authors provide evidence that high titers of NABs+ abolish in vivo response to IFN $\beta$ , but frequencies of BAb+ and NABs+ were lower in the Avonex group than in the Rebif and Betaseron groups (41). The conversion of BAb into NABs depends to some degree on BAb titer. Once NABs have developed, the bioavailability of IFN $\beta$  is completely inhibited (43).

In some NABs+ patients, NABs may subsequently disappear during continuous therapy with IFN $\beta$ . More than 50% of all NABs+ patients treated with IFN $\beta$ -1b had reverted to NABs- status four to six years after

becoming NABs+. In addition, patients who had high NABs titers after two years of treatment remained NABs+, but the titres reduced significantly, even though treatment was still ongoing (1;10; 26; 35; 43; 44).

Use of a higher dose of IFN $\beta$ -1b (375  $\mu$ g) than the currently approved 250  $\mu$ g dose is associated with an increased probability of persistence of IFN $\beta$  effect even if NABs are present. Rebif New Formulation, produced without fetal bovine serum and without human serum albumin, improved overall immunogenicity and safety profiles (including lower levels of NABs) compared with original formulation (45; 46).

In a study measuring the appearance and disappearance of NABs, Sørensen et al (47) demonstrated that 34% of patients who became NAB+ with low levels (neutralizing capacity  $\leq 20\%$ ) subsequently became NABs+ over a period of 48 months. They also demonstrated that: a) the probability of reverting to NABs+ status increased with continued treatment; b) the patients treated with Betaferon reverted to NABs+ more frequently and earlier than patients treated with Rebif. In a subset of patients with high levels of NABs+ (neutralizing capacity  $\geq 80\%$ ), the same authors found that only 23% reverted to NABs- status over 48 months. Moreover, patients who remained NABs- during the first 24 months of treatment remained NABs free for the rest of the treatment. After reversion to the NABs- state, patients regained the full effect of IFN $\beta$ -1b therapy (48).

Reversion of NABs status largely depends on the titer. Patients with low NABs titers are likely to revert to NABs-, whereas patients with high titers ( $\geq 200$  NU/ml with IFN $\beta$ -1b and  $> 500$  NU/ml with IFN $\beta$ -1a) rarely become NABs- within a time span of 2-3 years. It is not known exactly at which NABs titer antibody-mediated decreased bioactivity becomes significant nor how much the bioactivity should be before all beneficial effects of IFN $\beta$  are abrogated (9; 49; 50).

"decreased" in Sorensen2008

Data from NAb<sup>+</sup> patients who discontinued therapy indicate that NAb<sup>+</sup> may persist for long periods after cessation of treatment (51).

Giovannoni (5) reviewed the strategies to treat and prevent the development of NAb<sup>+</sup>: a) immunosuppressive therapy; b) induction immune tolerance; c) removal of NAb<sup>+</sup>; d) switching to less immunogenic IFN $\beta$  preparations; e) deimmunization. Add-on therapy with methylprednisolone administered monthly intravenously reduced the frequency of patients that had developed NAb<sup>+</sup> after 12 months, but did not restore biological response in MS patients with NAb<sup>+</sup>. Treatment with azathioprine and/or cyclic methylprednisolone for 6 months has a little or no effect on bioactivity in NAb<sup>+</sup> patients with MS (52-54).

While most of these studies have linked the appearance of NAb<sup>+</sup> to reductions in the clinical efficacy of IFN $\beta$  treatment, few have demonstrated that this reduction outweighs the continued clinical benefits of IFN $\beta$  therapy. It is also important to consider that not all patients develop BAb<sup>+</sup> or NAb<sup>+</sup>, and of the patients who do, some revert to seronegative status. The majority of patients are likely to experience breakthrough symptoms at some point, but these are not necessarily an indication that the patient's response to treatment has changed (3; 55-57).

In an analysis of NAb<sup>+</sup> development and the impact on clinical outcomes from the PRISMS study in patients treated with Rebif, the authors discovered that NAb<sup>+</sup> patients had significantly more relapses than did NAb<sup>-</sup> patients during years 3 and 4 of treatment. The PRISMS-4 study showed that the persistent NAb<sup>+</sup> in Rebif MS patients' treatment was associated with reduced efficacy (43; 58).

Kappos et al (59) have investigated MS patients with treated 48 months with Avonex and have demonstrated that the annual relapse rate of NAb<sup>+</sup> patients was 39% higher than that of NAb<sup>-</sup> patients.

Sabbagh (60) showed that relapse rate and MRI measures in patients with Rebif treat-

"no clear" in Chiu 2009. It reverses the meaning of sentence

ment were higher in NAb<sup>+</sup> than NAb<sup>-</sup> patient, but both demonstrated significant improvement versus placebo or delayed treated patients. On the other hand, Chiu et al (37) demonstrated that the association between neutralizing antibody profile and MRI response was evident.

Sørensen et al (61) and Boz et al (62) showed that NAb<sup>+</sup> patients experienced more relapses than did NAb<sup>-</sup> patients. The authors also showed that the time to first relapse was significantly longer for patients who were NAb<sup>-</sup> rather than NAb<sup>+</sup> at 12 months after beginning IFN- $\beta$  treatment.

In contrast, the North American Placebo Control Randomized Study of IFN $\beta$  in secondary progressive MS patients for three years, Panitch et al [63] showed that the presence of NAb<sup>+</sup> did not consistently affect clinical outcomes. The results obtained by Goodin et al (55) in a large patient cohort with MS patients receiving IFN $\beta$ -1b suggested that NAb<sup>+</sup> are not responsible for poor clinical responses and that NAb status is of little clinical value. None of the pivotal trials in relapsing-remitting MS showed an effect of NAb<sup>+</sup> on disease progression and neither did any of the trials in the secondary progressive MS. However, all the trials were not able to show an effect of NAb<sup>+</sup> because IFN $\beta$  by itself had no or only minor effect on disease progression (17; 58; 64; 65).

The presence of NAb<sup>+</sup> was associated with a higher risk of developing disability during the subsequent 3-5 years in a long-term follow-up study of patients receiving IFN $\beta$  and MS patients positive for NAb<sup>+</sup> need to be shifted to alternative treatments (66; 67).

A negative effect of NAb<sup>+</sup> against Rebif could be observed in some MRI endpoints in the two-year PRISMS study and NAb<sup>+</sup> also caused a clear reduction of efficacy in MRI endpoints in the third and the fourth year (58). The pivotal phase III placebo-controlled trial of Avonex reported a trend towards more gadolinium-enhancing lesion in NAb<sup>+</sup> patients

"of 250 µg IFN-1b" in Panitch2004

(68). In the European secondary progressive MS study, NAb<sup>s</sup>+ patients showed a higher percentage increase from baseline in T2-lesion volume compared with NAb<sup>s</sup>- patients (56; 64). In the EVIDENCE study, NAb<sup>s</sup> to Betaseron were detected in 23% MS patients, but their presence did not consistently affect clinical or MRI outcomes (68).

On the contrary, Kappos et al found that NAb<sup>s</sup> to Avonex reduce the therapeutic benefits measured by MRI activity (59). A three-year open follow-up study of patients treated with Betaferon for relapsing-remitting MS showed that NAb<sup>s</sup>+ patients had significantly more gadolinium-positive lesions and a higher T2-lesion load compared to NAb<sup>s</sup>- patients (69). Sørensen et al (70) showed that high-level NAb<sup>s</sup>+ patients had more MRI activity than NAb<sup>s</sup>- patients. MRI activity and NAb occurrence during the first 6 months of IFN-β treatment were reliable predictors of long term clinical response, particularly when combined. Patients with negative predictors showed a less than 10% risk of developing clinical activity. Patients with positive predictors showed a 50% risk of further clinical activity (71). The Betaseron Copaxone in Multiple Sclerosis with triple-dose Gadolinium and 3-Tesla MRI Endpoints (BECOME) study, a head-to-head study of IFN-1b vs. glatiramer acetate with a primary endpoint of enhancing lesions on MRI, provided an excellent opportunity to determine the effect of NAb<sup>s</sup> on MRI activity. The authors of this study have demonstrated that the high levels of NAb<sup>s</sup> are correlated with reduced therapeutic efficacy of IFNβ as manifested by diminished reductions in enhancing lesions (73).

Little information is available regarding NAb<sup>s</sup> IFN-β in the pediatric MS population, although one small study has suggested that positive NAb<sup>s</sup> might be less commonly seen in pediatric MS than in adult MS population (72).

The purpose of the Impact of Neutralizing Antibodies on Interferon Responsive

Genes Highlights Biomarker Response (INSIGHT) study was to examine the effect of antibodies to IFNβ on in vivo response of 3 biomarkers: MxA (myxovirus resistance protein), IFIT-1 (interferon-induced protein with tetratricopeptide 1) and viperin. The data provide evidence that high titers of NAb<sup>s</sup> abolish the in vivo response to IFNβ. The same authors found that patients aged 50 years or older were more likely to be NAb<sup>s</sup>+ compared with patients aged <50 years. We couldn't find any correlation between the age of the patients and NAb<sup>s</sup> presence or titre irrespectively the type of IFNβ received (74).

Today, there isn't any agreement between the European Guidelines on the use of anti-IFNβ antibody measurements in MS, produced by an European Federation of Neurological Societies Task Force, and the American Academy of Neurology report on NAb<sup>s</sup> to IFNβ and assessment of their clinical and radiographic impact, produced by a working group under the Therapeutics and Technology Subcommittee of the American Academy of Neurology.

The European Guidelines recommend:

a) measurements of NAb<sup>s</sup> for IFNβ antibody screening before performing a NAb<sup>s</sup> assay (Level A); b) measurement of NAb<sup>s</sup> should be performed in specialized laboratories with validated cytopathic effect assay or MxA production assay using serial dilution of the test sera (Level A); c) the NAb titer should be calculated using the Kawade formula (Level A); d) tests for the presence of NAb<sup>s</sup> should be performed in all patients at 12 and 24 months of therapy (Level A); e) in the patients who remain NAb<sup>s</sup>- during this period measurements of NAb<sup>s</sup> can be discontinued (Level B); f) in patients with NAb<sup>s</sup>, measurements should be repeated, and therapy with IFNβ should be discontinued in patients with high titers of NAb<sup>s</sup> sustained at repeated measurements with 3- to 6-month intervals (Level A) [75].

The North American Report concluded: a) treatment of patients with MS with



IFN $\beta$  (Avonex, Betaseron or Rebif) is associated with the production of NABs (Level A); b) NABs in the serum are probably associated with a reduction in the radiographic and clinical effectiveness of IFN $\beta$  treatment (Level B); c) the rate of Nab production is probably less with IFN $\beta$ -1a treatment than with IFN $\beta$ -1b treatment, although the magnitude and persistence of this difference is difficult to determine (Level B); d) it is probable that there is a difference in seroprevalence due to variability in the dose of IFN $\beta$  injected or in the frequency or route of its administration (Level B); e) it seems clear that IFN $\beta$ -1a (as it is currently formulated for intramuscular injection) is less immunogenic than the current IFN $\beta$  preparations (either IFN $\beta$ -1a or IFN $\beta$ -1b) given multiple times per week subcutaneously (Level A); f) because NABs disappear in some patients even with continued IFN $\beta$  treatment (especially in patients with low titers), the persistence of this difference is difficult to determine (Level B); g) although the finding of sustained high-titer NABs (>100 to 200 NU/ml) is associated with a reduction in the therapeutic effects of IFN $\beta$  on radiographic and clinical measures of MS disease activity, there is insufficient information on the utilization of Nab testing to provide specific recommendations regarding when to test, which test to use, how many tests are necessary or which cutoff titer to apply (Level U) (76).

## Conclusions

Given the overall clinical efficacy of IFN $\beta$  in patients with MS, the inconsistent data regarding the effect of NABs to IFN $\beta$  on treatment outcome and unpredictable course of disease, treatment decisions should be based on patient response to therapy, not to NABs status.

As MS is also a degenerative disease and interferons appear to treat only the inflammatory component, the majority of patients would be expected to have a breakthrough in disease activity at some time.

According to our data, BAbS do not abrogate the clinical effect of Betaferon or Avonex, regardless of the parameter used. Assays for BAbS are relatively inexpensive and have a role in following IFN $\beta$  treatment and select the candidates for further NABs testing.

Future studies are required to better understand the dynamics of BAbS and NABs biology and which risk factors may be most important in considering how patients will react to IFN $\beta$  therapy.

## Disclosure

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