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MS treatment optimization: factors associated with poor clinical response in Nab positive patients

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ABSTRACT

Background and purpose. Interferon beta (IFN beta) belongs to the first line of disease modifying therapy drugs in the treatment of relapsing-remitting multiple sclerosis being widely used in the chronic treatment of this pathology. The serum presence of the neutralizing antibodies (Nabs) has been shown to alter the treatment response, its routine applicability being still debated.
In an observational study, we aimed to determine in the Nabs positive patients, correlations with other clinical factors which contribute to IFN beta decreased efficacy.
Methods. We measured Nabs in 104 patients who were on IFN beta therapy (29.8% on IFN beta 1a s.c., 27.88% on IFN beta 1a i.m. and 42,3 on IFN beta 1b s.c.) for at least one year in our clinic. Serum was collected at 24 h after treatment injection to avoid transitory antibody peak (12-18 h post administration). We considered positive the patients with a titer higher than 20 TRU.
Results. The prevalence of Nabs in our group of patients was 13.43% (42.85% IFN Beta 1b s.c., 50% IFN beta 1a s.c. and 7.14% IFN beta 1 a i.m.). Nabs positivity was associated with an increase in the relapse rate (for IFB beta 1a and 1b s.c. groups) and progression for IFN beta 1 b s.c. patient group.
Conclusions. The routine clinical testing for Nabs should impact the clinical decision of switching therapy in multiple sclerosis patients that present with an increased number of relapses, EDSS progression or a higher number of MRI T2 lesions.

Keywords: multiple sclerosis, neutralizing antibodies (Nabs), interferon beta (IFN beta)

Abbreviation list

ANOVA – analysis of variance
EDSS – Expanded disability Status Scale
GA – glatiramer acetate
IFN – interferon
IG – intermediate activity disease group
IP – intense positive
MANOVA – Multivariate analysis of variance
MS – multiple sclerosis
Nab – neutralizing antibodies
NEDA4 – no evidence of disease activity based on four parameters
NRG – non-responders group
P – positive
RG – responders group
RRMS – relapsing-remitting multiple sclerosis
SP – slightly positive
SPMS – secondary progressive multiple sclerosis
WHO – World Health Organization

INTRODUCTION

Interferon beta (IFN beta) and glatiramer acetate (GA) belong to the so-called first line therapeutic options in the treatment of multiple sclerosis, with proven value in positively influencing the course of the disease (in terms of relapse rate reduction and functional scores improvement), therefore considered disease-modifying therapies. Beside well-known factors, such as compliance, tolerability, adherence to the treatment (1), the clinical efficacy of IFN beta may be influenced by the development and presence in the serum of patients with neutralizing antibodies (Nabs).

The impact degree of reduced bioactivity of the medication is still a matter of debate, most of the studies indicating a titer-dependent effect in posi-

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tive patients with a titer exceeding 20 TRU/ml concentration of Nabs (2).

The association between the presence of Nabs and a reduced clinical response was assessed in a significant number of studies which found a variable correlation of clinical worsening and magnetic resonance imaging (MRI) (as a surrogate for disease progression); mostly related to higher titers of serum Nab (3).

The variability of Nabs positive patients ranged from 13.4 to 28.9% in the group of those with progression of the disease (4,5) with a higher variability in clinical studies (between 2 and 45%) (6).

We aimed to determine the correlation between the Nabs positive patients and the other clinical factors that contribute to IFN beta decreased efficacy in our patient group.

MATERIAL AND METHOD

Population and clinical assessment

We conducted an observational study to determine the presence of Nabs in the group of diagnosed MS patients included in the Romanian National MS Therapy Program, registered at Timisoara Center, treated for at least one year (therapy initiated between 2004-2011) with one of the three available forms of interferon: IFN beta – 1b 250 mcg sc. every other day, IFN beta – 1a 30 mcg i.m., once a week, IFN beta 1a 44 mcg, sc., three times a week. All patients have been diagnosed according to McDonalds 2005 or 2010 criteria. We included patients with relapsing-remitting (RR) and secondary progressive (SP) multiple sclerosis.

The subjects were assessed retrospectively (two years before baseline) and prospectively at six months or at each relapse for the following three years on a routine basis, at the Clinical Emergency County Hospital Timisoara, between December 2012 and December 2015. We collected retrospective demographic and MS history data.

The disease assessment comprised (Expanded Disability Status Scale) EDSS score and relapse numbers at baseline and during treatment, time until the second relapse, disease duration and progression.

We measured the time to the first relapse, time to reach the following EDSS disability scores: 4 (limited ambulation), 6 (unilateral support) and disease progression.

Progression was defined as at least 0.5 increase in EDSS score, sustained for six months. The measurements were done at baseline and every six months up to two years.

We correlated the impact of imaging parameters changes (number of MRI T2 lesion load), at baseline and 48 months after dosing to clinical evolution (EDSS scores and relapse rate) and antibody status.

CLINICAL RESPONSE

The clinical progression was confirmed by the increase of either 1 point if EDSS <5.0 or 0.5 points if EDSS ≥ 5.0. (7) We divided the patients' response according to their clinical and MRI imaging features into three groups: responders, intermediate (sub-optimal) responders and non-responders.

The responders' group (RG) were the patients who presented a stable EDSS score and no change in the number of T2 lesions on MRI at the assessment time. The intermediate (suboptimal) responder patients had one point EDSS score increase or a change in T2 lesion load (1-2 new T2 or Gd+ lesions) when reviewed, while the non-responders' group (NRG) presented multiple relapses or/and extensive MRI activity during the follow up period (7,8).

Nabs determination and status

Testing was performed in the laboratory of Motol University Hospital's Medical Microbiology, Medical University II Charles, Prague. The laboratory is accredited to test IFN beta antibody in agreement with the regulations of the World Health Organization (WHO) (9).

The titer of antibody status was determined using the cytopathogenic effect (CPE) method, which is recommended by WHO and the Nabs titer was calculated using the formula Kawada (level of recommendation A) (10). All samples of serum were allowed to clot for at a minimum of 30 minutes to a maximum of 2 hours, at the room temperature, then centrifuged at 3,000 rotation/min for 15 minutes and then stored at – 70°C.

The assessment was made once, after at least one year of therapy and the patient's status was considered positive at a concentration higher than >20 TRU/ml. The patients who had once a higher titer than 20 TRU/ml were considered "always positive" (11).

The baseline EDSS evaluation was done at the time of blood sampling. The patients did not receive any steroid therapy the month before the test, and sampling was done when at least 12 hours have elapsed from the last interferon injection.

Patients were classified by their Nabs titer as slightly positive (SP) with a titer of 20 to 100 TRU,

positive (P) with a titer of 100 to 300 TRU/ml and intense positive (IP) with a titer above 300 TRU/ml, (one patient sample had a value of 389 TRU/ml) (12).

STATISTICS

We used descriptive statistics (means, standard deviation, median, minimum and maximum) and frequency tables to characterize the study population. We considered P values < 0.05 as significant. We stratified the patients according to the duration of treatment and the last obtained clinical evaluation. We used Stata 14 for inferential statistical analysis of variance (ANOVA) to determine whether any important differences could be found between the means of two or more independent groups followed by simple or multilinear regression, as needed. Multivariate analysis of covariance (MANCOVA) was used to test the differences in outcome variables.

RESULTS

Baseline demographic and clinical data

The presence of Nabs was determined in 104 patients who were treated for at least one year with the following: 31.73% (33) IFN beta 1a s.c., 27.88% (29) IFN beta 1a i.m. and 42.3%(42) IFN beta 1b s.c. The data was collected retrospectively from the patients sampled. The population demographic characteristics, according to MS form, therapy received treatment duration, relapses at baseline (moment of sampling), was summarized in Table 1.

The IFN beta1b 250 mcg group had the longest mean treatment duration at the date of sampling and the highest mean of EDSS score at the initiation of therapy and baseline, while the IFN beta 1a 44 mcg had the longest period between the onset of the disease and treatment initiation (Fig. 1). The overall length of therapy before evaluating Nabs presence was 6.0 (+/- 2.06) ranging from 1.03 to 10.63 years.

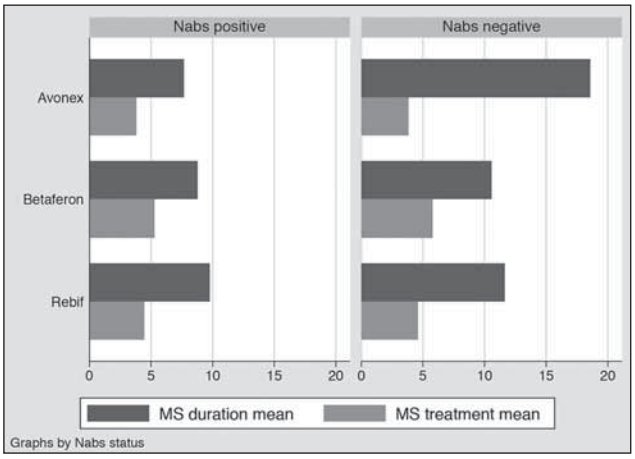

FIGURE 1. *MS and treatment duration in Nab positive and negative patients*

Nabs were evidenced in 14 patients (13.43%), who were distributed over medication categories as follows: 7.14% (1) IFN beta 1a i.m., 42.85% (6) IFN beta 1b s.c. and 50% (7) IFN beta 1a s.c (Table 2).

One of the particularities of our cohort was that all Nabs positive patients received disease-modifying treatment for more than four years. All Nabs positive patients were younger at the moment of the first MS relapse vs. Nabs negative 26.88 (sd 2.59) vs. 32.18 (sd 1.02), p = 0.06 – but yet not reaching statistical significance.

The age of diagnosis maintained the same tendency in Nabs positive patients: 30.92 years (2.56) – versus Nabs negative patients – 34.2 (1.09), with a p-value of 0.22.

The mean disease duration time before the sampling date was 8.71 years (sd 5.65), ranging from

TABLE 1. *Demographic characteristics of the population*

	IFN beta 1a i.m (30 mcg)		IFN beta 1b s.c. (250 mcg)		IFN beta 1a s.c. (44 mcg)	
	Male	Female	Male	Female	Male	Female
Age at onset	30.30 (10.81)	30.48 (11.01)	28.45 (9.72)	34.03 (9.95)	24.88 (5.13)	34.04 (8.53)
Age at treatment initiation	37.71 (10.19)	33.42 (11.10)	30.88 (9.82)	38.54 (10.06)	31.12 (5.72)	39.11 (7.66)
Baseline EDSS	2.44 (1.02)	2.14 (0.91)	2.20 (0.84)	3.02 (1.66)	2.00 (0.93)	2.48 (1.25)
Treatment duration at l	11.57 (7.10)	6.59 (4.59)	7.57 (3.08)	10.02 (10.02)	11.05 (9.03)	9.76 (6.67)
Number of relapse before treatment	1.94 (3.26)	2.13 (2.65)	3.58 (4.46)	2.94 (3.81)	1.42 (1.49)	2.15 (3.41)
Relapse rate	0.12 (0.23)	0.29 (0.44)	0.09 (0.20)	0.25 (0.45)	0.06 (0.18)	0.20 (0.32)
Total	8	21	16	26	8	25

(Statistics: mean and standard deviation in parantheses)

TABLE 2. *Baseline characteristics of the patients grouped according to the presence of Nabs*

	Nab Negative		Nab positive	
	Count (%)	Mean (sd)	Count (%)	Mean (sd)
	N= 90		N = 14	
Age		44.14 (10.52)		40.65 (8.38)
MS duration		8.90 (5.91)		10.56 (6.58)
Treatment duration		4.57 (2.15)		5.00 (1.28)
Male	25 (27.78%)		7 (50%)	
Female	65 (72.22%)		7 (50%)	
IFN beta 1a i.m.	28 (31.11%)		1 (7.1)	
IFN beta 1b s.c	36 (40%)		6 (42.85%)	
IFN beta 1a s.c.	26 (28.89%)		7 (50)	

1.19 to 24.94 for the Nabs negative patients and 11.59 years (sd 7.50), ranging from 3.69 to 26.08 for Nabs positive patients. There was no statistically significant difference in disease duration between treatment groups (p-value 0.375). Also, the presence of Nabs determined no significant variability between female and male. In the neutralizing antibodies positive group, the difference in the mean treatment duration was 11.8 (sd 0.69) years in the females group and 12.98 (sd 1.38) in the males group (p-value 0.4). In the Nabs negative group, the mean treatment duration for the female group was 16.1 (sd 3.32) years and 11.5 (sd 0.82) years in the males group (p-value 0.2).

IFN preparation types and treatment duration

In our cohort the patients treated with IFN beta 1a i.m (once a week) had a lower percent of Nabs positive, but the positive one had the highest titer (386 TRU/ml, intense positive) compared to the other two products (IFN beta 1a, three times per week and IFN beta 1b, every other day), which are both administered subcutaneously: 3.44% vs. 17.33% (p-value 0.031). The highest number of patients with a titer 100-300 TRU/ml was determined among the patients treated with IFN beta 1a s.c. 71.42% vs. 50% of the patients receiving IFN beta 1b s.c. Treatment duration before sample and Nabs status (p=0.3) presented no statistic value.

Nabs and the clinical response

We assessed the clinical features which might associate with antibodies status. We used repeated one-way analysis of variance (ANOVA) to test the relation between the number of relapses and EDSS progression in the previous two years which are the dependent variables, and Nab titer, the independent variable. We found a significant correlation between the number of relapses through 2011 and 2012 and Nabs categories F (2, 85) = 3.74, p-value = 0.02, which explains the 8.08% variability of positive patients. A shorter time to the second relapse correlated with significant differences among Nabs groups F (3, 91) = 5.08, p-value = 0.0027.

In the Nabs positive patients, one patient (7.1%) was a responder (completely free of the disease activity). The proportion of intermediate responders represented 21.42% (3 patients) while the non-responders group represented 71.42% (10 patients).

Out of the 104 tested patients, 13% of patients with intermediate (suboptimal) response to treatment and only 8% of the group with a progressing disease (non-responders) were positive for Nabs.

As a particularity of our cohort study, 76 patients (80%) of the negative patients and all 14 (13.43%) positive patients were tested, after at least three years of therapy, unlikely from most of the studies conducted after 2005 (13,14).

The clinical progression of the disease was calculated as the mean of the measured difference between EDSS scores from 2012 vs. 2015. The statistical analysis was obtained by running a Student's t-test, which indicated a weak evidence of EDSS progression in the positive group with a t (16.15) = – 1.316, p=0.10 and a mean difference within groups of 0.408 (sd = 0.310), and effect size of Cohen's d = – 0.421.

A multivariate analysis of covariance and variance (MANCOVA) estimated the relationship between the predictors Nab groups and gender (factorial variables), age at therapy initiation, treatment

***TABLE 3**. Multivariate analysis individual regression models*

Variables	Relapse rate	EDSS progression	MRI T2 lesions
SP group	0.391*(3.15)	-0.200 (-0.48)	-0.420 (-0.80)
P group	-0.0352 (-0.35)	0.548 (1.60)	0.0628 (0.15)
IP group	0.412 (1.53)	-0.0666 (-0.07)	0.977 (0.86)
Female	0.132*(2.08)	0.333 (1.55)	0.298 (1.12)
Age	-0.00727*(-2.26)	-0.00571 (-0.53)	0.00510 (0.38)
Treatment duration	-0.634*(-4.33)	-0.460 (-0.93)	-0.956 (-1.55)
MS duration	0.636* (4.31)	0.510 (1.02)	0.937 (1.51)
EDSS bsl	0.0487* (2.82)	-0.203*(-3.47)	0.0985 (1.36)
Relapse rate before bsl	0.192* (5.64)	0.198+ (1.72)	0.0309 (0.22)
_cons	1.120* (4.72)	0.569 (0.71)	2.977* (2.98)
N	95		

t statistics in parentheses
+ $p < 0.10$, * $p < 0.05$
(bsl – baseline, SP – slight positive, P – positive, IP – intense positive Nab patients groups)

and multiple sclerosis duration, EDSS score at baseline and the number of the relapses before baseline (continuous variables) and the three dependent variable EDSS progression, relapses rate, number of T2 lesions. The overall analysis indicated statistically significant effects of our model Pillay' = 0.93, F (27.0, 255) = 4.29, p-value = 0.0001. Table 3 displayed the data from the individual models although they had different statistical weight.

EDSS score progression correlates with EDSS score at baseline, as the most important and significant component of the model. It had an effect of 0.21 (95%CI; – 0.32 – 0.43; p < 0.001). The next as statistical relevance was the number of relapses in the previous two years, with an effect of 0.20 (95%CI; – 0.003 –0.43; p < 0.05). Other significant factors, although expressing less statistic strength (p-value < 0.11) were the positive antibodies status group of patients and the female gender which correlated with an impact coefficient of 0.54, respectively 0.33.

Prediction of relapse activity analysis [F (10; 94) = 13.69; p < 0.0001)] and R-squared = 0.59 demonstrated statistical significance for all the clinical variables studied (p < 0.05). The most important variables of the model were the treatment period as well as the MS disease duration with values of – 0.63 (95%CI; – (- 0.92 – 0.34); p < 0.0001), respectively 0.64 (95%CI; 0.34 – 0.93; p < 0.001). EDSS score at baseline was also a strong predictor for the relapse rate frequency, but although with a p < 0.003 despite the correlation coefficient of 0.28. The Nabs titer significantly predict relapse rate for the slight positive (SP) group with a coefficient of 0.40, a p-value of 0.002 [95%CI (0.15 – 0.64)] and for the intense positive (IP) group 0.42, 95%CI (-0.13 – 0.95) but with a p < 0.1 (Fig. 2).

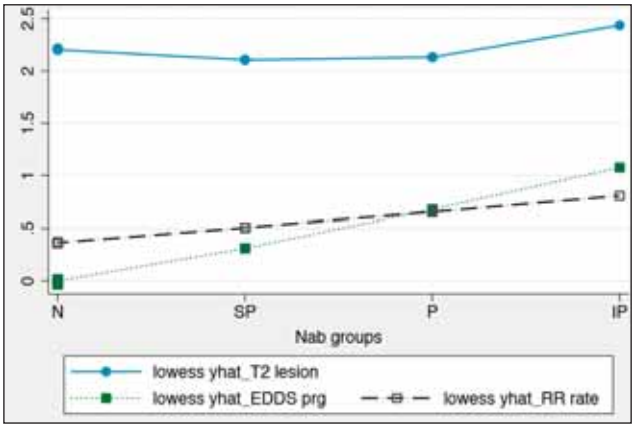

***FIGURE 2.** Correlation among EDSS progression, RR rate and MRI T2 lesion against Nab titer groups (N – negative, SP – slight positive, P – positive, IP – intense positive)*

The MRI T2 lesions regression model did not reach statistical significance for the included parameters (gender, age at therapy initiation, treatment and multiple sclerosis duration, EDSS score at baseline and the number of the relapses before baseline) (p = 0.2). On the other hand, the marginal analysis of means for the three Nabs positive groups (slight positive – SP, positive – P, intense positive – IP) in the setting of maintaining the other factor stable, had statistically significant results, indicating a level depending correlation (Fig. 3 and Table 4).

DISCUSSION

The results of our observational study in the frequency of (neutralizing antibodies) Nabs positive was within the range found in other studies, even if the range is quite large 2-46% (2, 13,14). A particularity of our group was that Nabs presence was acknowledged only in the relapsing-remitting MS form.

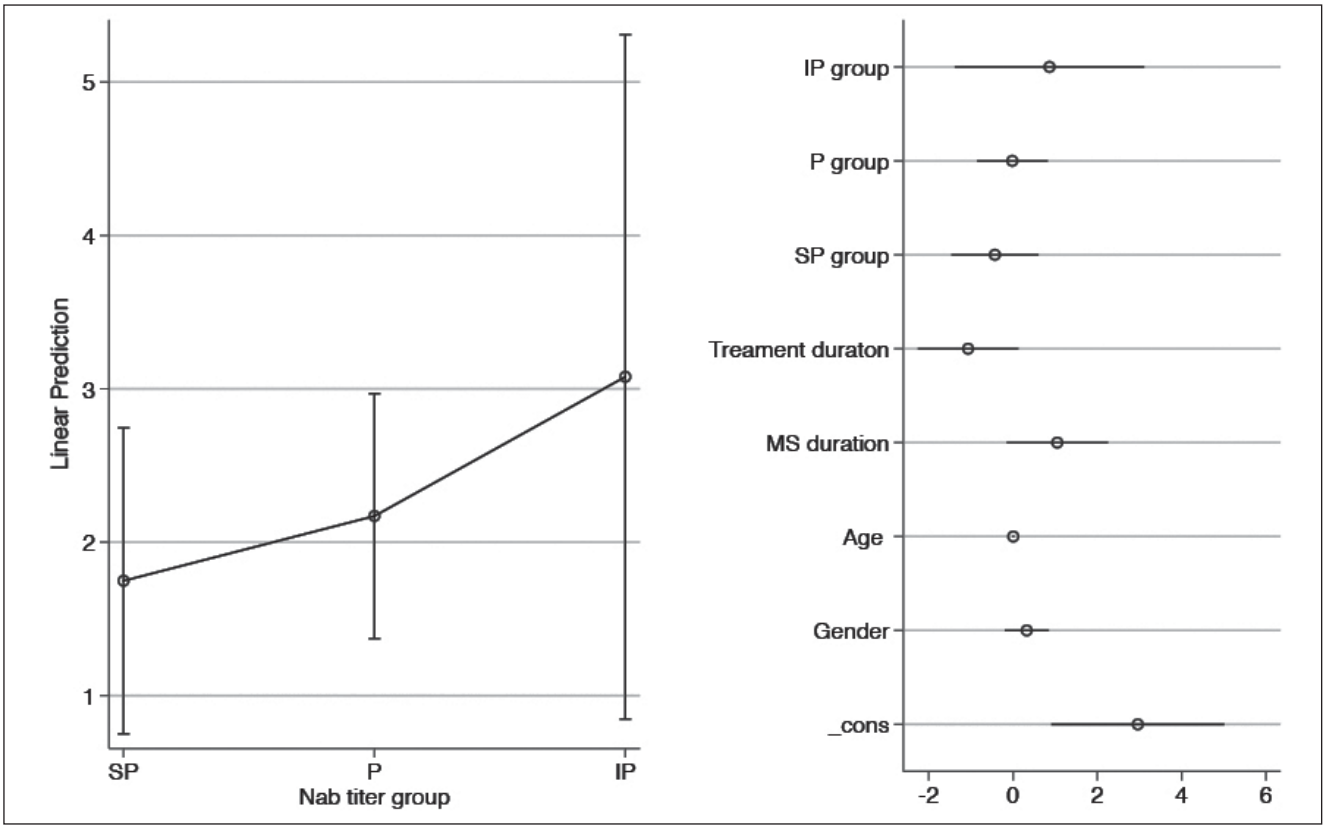

FIGURE 3. *T2 lesion load adjusted predictions against Nab groups and the plot coefficients of each depended variable and 95% confidence intervals in the regression model*

TABLE 4. *The marginal effect by Nabs groups evidencing the change of the outcome variable MRI T2 lesion number (N – negative serum antibodies group, SP – slight positive group, P – positive group, IP – intense positive group, N – the number of patients with MRI available data)*

Variables	MRI T2lesions
N group	2.164** (17.57)
SP group	1.744** (3.45)
P group	2.227** (5.52)
IP group	3.142* (2.79)
N	95

t statistics in parentheses
$^{+}$ $p < 0.10$, * $p < 0.05$, ** $p < 0.001$

A small percent of patients in our cohort exceeded the cut off threshold of 20 TRU/ml.

The fact that 83% of the antibody negative were suboptimal (intermediate) responders (56 patients – 53.8%) or non-responders (31 patients – 29.8%) suggests other reasons for low or no efficacy, mainly related to the limits of the IFN mechanism of action vs the patient's immune status vs the complexity of the pathogenic mechanisms of the disease. (7).

Our data regarding the antibody status variation depending on the route of administration of the IFN, i.m. vs s.c, is concordant with the results of other studies. This outcome demonstrated that IFN beta 1a i.m is the least immunogenic ranging from 2 to 6% (13), while for IFN beta 1a s.c and for IFN beta 1b s.c. the positivity of antibodies ranged between 12 and 30%, respectively 28 to 45% (14).

In our cohort of Nabs positive patients, the suboptimal clinical response was: six patients (85.72%) out of the Nabs positive patients that used IFN beta 1a s.c demonstrated an intermediate clinical response compared with one patient (7.18%) treated with IFN beta 1a i.m. and four patients (66.67%) of patients with IFN beta 1b s.c.. However, because of the small number of patients (small sample size), no significant correlation could be drawn between the type of medication and clinical progression in the presence of Nabs, p-values 0.15. Other studies found that immunogenicity was linked to the type of IFN preparation indicating IFN beta 1b s.c. as the most immunogenic followed by IFN beta 1a s.c. (15-17).

In the Nabs positive group, 21.42% (3 patients) experienced disease progression (clinical or MRI – T2) at three years of follow-up analysis. The percentage had a weak statistical evidence (p=0.261), due to the group size – small number of patients that were included in the study.

Within the last five years of treatment (two year were assessed retrospectively and three prospec-

tively), the EDSS disability score 4.0 was reached by 14.44% (13 patients) of negative and 14.29% (2 patients) of the positive Nabs (no statistical significance).

The EDSS 6.0 score was reached by one patient in each group (1% of Nabs negative, 7.14% in Nabs positive), with no statistical significant (p-value of 0.17), for the last five years of treatment.

In our study we found that a higher incidence of relapses was significantly related to the presence of antibodies. There was a stronger association between the increased number of relapses and slightly positive titer (20-99 TRU/ml).

The EDSS score progression (at least 0.5 points increase) did not show a strong correlation with the overall Nabs positivity (qualitative evaluation), but was correlated well with titers of antibodies higher than 100 TRU/ml (quantitatively).

The occurrence of serum antibodies reduced clinical efficacy of interferon treatment and associated with an increase number of T2 lesions on the MRI. Therefore they contribute to the disease activity as defined by the existing guidelines of NEDA4 (18).

The strength of this association depends on several clinical factors – most relevant covariance being treatment, MS duration and baseline EDSS score evaluation. Even if the significance of Nabs positivity is still greatly debated, Nabs presence should not be ignored as it can be used as a predictor of an increased potential treatment failure (the lesion load on MRI the increased number of relapses) which is one of the principal outcome measures of many studies that assessed the efficacy of disease-modifying therapies (DMTs).

CONCLUSION

The serum neutralizing antibodies status related to IFN therapy should be considered among the factors which might modulate treatment response failure. The routine clinical testing for the presence of serum Nabs should be considered, as it might be a factor in evaluating therapeutic response and eventually changing the therapy, mainly in patients that experience an increased number of relapses, EDSS progression or a higher number of MRI T2 lesions. A drawback of our study is the small group size but still our results are validated by being in line with data found in literature.

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Conflict of interest: none declared

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