Predictive markers for response to interferon therapy in patients with multiple sclerosis

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ABSTRACT

Background: Prolonged therapy with interferon β (IFNβ) often leads to the development of anti-IFNβ binding antibodies (BAbs). A subset of the BAbs is of a neutralizing nature (neutralizing antibodies, NAbs) and is associated with reduced clinical efficacy of therapy. Myxovirus-resistance-protein A (MxA) has proven to be a reliable biomarker of IFNβ bioactivity. We analyzed the prognostic value of MxA mRNA, NAbs, and BAbs on the risk of having a new relapse in IFNβ-treated patients.

Methods: A 3-year study was conducted in 137 IFNβ-treated patients. Blood samples for BAbs, NAbs, and MxA mRNA measurements were taken after 12 ± 3 months of therapy. Analysis of relapse-free survival (RFS) was performed for all measures by using known thresholds, generating "positive" and "negative" groups. Also, time between sampling and following relapse and risk of new relapses were calculated.

Results: The MxA-negative group showed poorer RFS rates than the MxA-positive group [$p < 0.0001$, hazard ratio (HR) = 2.87]. Likewise, the NAb-positive group showed poorer RFS rates than the NAb-negative group ($p = 0.0013$; HR = 2.49). On the contrary, BAb measurement did not show a clear clinical significance.

Conclusions: Findings indicate that measurements of both myxovirus-resistance-protein A (MxA) and neutralizing antibodies (NAbs) predict the risk of new relapses; however, the slightly stronger prognostic significance of MxA mRNA and the easier method for it measurement make MxA mRNA the preferred biomarker for monitoring interferon β (IFNβ)-treated patients. This information can be used to better tailor treatment to the individual patient with MS.

Neurology® 2008;70:1119–1127

GLOSSARY

BAb = binding antibody; **CPE** = cytopathic effect; **EDSS** = Expanded Disability Status Scale; **GAPDH** = glyceraldehyde phosphate dehydrogenase; **HR** = hazard ratio; **IFN** = interferon; **MxA** = myxovirus-resistance-protein A; **NAb** = neutralizing antibody; **RFS** = relapse-free survival; **ROC** = receiver operating characteristic; **TRU** = 10-fold reduction units.

Interferon beta (IFNβ) therapy modifies the clinical course of relapsing MS and reduces the accumulation of new brain lesions and brain atrophy.[1-3] As with other protein drugs, some patients develop anti-IFNβ antibodies with chronic administration of IFNβ products.[4,5] Anti-IFNβ antibodies are referred to as binding antibodies (BAbs), and they may or may not interfere with binding of IFNβ to its receptor or alter its receptor-mediated functions in some other manner. A specific subset of the BAbs is of a neutralizing nature (NAbs), as they interfere with the receptor-mediated functions of IFNβ. Recently a number of reports have addressed the biologic significance of the latter on clinical measures, showing decreased treatment efficacy of IFNβ in patients in whom NAbs had developed.[4-7] Due to the risk of decreased treatment efficacy, the existence of alternative

Supplemental data at www.neurology.org

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e-Pub ahead of print on February 13, 2008, at www.neurology.org.

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Supported in part by a grant from the European Community under its 6th Framework, for a specific project on neutralizing antibodies in MS (NABINMS Project, Contract 018926). Also supported by the Fondazione per la Ricerca Biomedica ONLUS and the S. Luigi Gonzaga ONLUS. Funding sources had no role in data collection, data analysis, data interpretation, or writing of this paper.

Disclosure: The authors report no conflicts of interest.

therapies (i.e., glatiramer acetate, mitoxantrone, natalizumab), and the high costs of therapy with IFNβ, demand is increasing to introduce into the clinical practice an effective monitoring strategy for individual treatment response to IFNβ.

Management of NAbs is a new challenge for clinicians who care for patients with MS, but it is also an opportunity to improve the individualization of treatment. Against this background, specific European guidelines were recently published.[8] These guidelines state that immunogenicity of IFNβ products must be one of the factors that neurologists consider when treating patients with MS.

Binding assays are commonly used to screen patients for the presence of BAbs, whereas NAb positivity is defined by the ability of a serum sample to neutralize in vitro biologic activity of IFNβ [e.g., the antiviral activity of IFNβ in a cytopathic effect (CPE) assay]. In addition, in the last few years several studies have shown that myxovirus-resistance protein A (MxA), an antiviral protein exclusively induced by type 1 IFNs, is a sensitive measure of the in vivo biologic response to therapeutically applied IFNβ and of its reduced activity due to the development of BAbs and NAbs.[9-17] Indeed, induction of MxA indicates binding of IFNβ to its receptor, activation of intracellular signal transduction, and production of protein that is a hallmark of the biologic function of IFNβ. Hence, abolition of MxA induction can indicate reduced biologic activity of the administered cytokine. It is important to note that this reduced biologic activity may be due not only to the presence of BAbs or NAbs, but also to other factors such as noncompliance and soluble circulating IFNβ receptors.[18] Because MxA gene expression does not represent a measure of the specific mechanism inhibiting biologic activity, but rather a specific measure of biologic activity, the MxA assay in vivo seems to be much more sensitive than other bioassays.

While NAb measurement has been shown to have prognostic value in determining the risk of having a new relapse,[19-22] there is still no clear concept of the prognostic information given by both BAbs and MxA mRNA as well as by the concurrent measurement of BAbs, NAbs, and MxA mRNA. In the present report, we describe a 3-year study of the development of BAbs and NAbs and their relationship to MxA mRNA levels in patients with MS treated with one of the currently available IFNβs.

Our first aim was to determine whether a single ongoing measurement of BAbs, NAbs, or MxA mRNA, performed after 1 year of IFNβ therapy, can provide valuable prognostic information about future patient outcomes. This was achieved by comparing all measurements with relapse-free survival (RFS) and time to first relapse. We also aimed to determine whether the simultaneous measurement of BAbs, NAbs, and MxA mRNA has a higher prognostic value than each measure singularly quantified.

METHODS

Patients and study design. In planning this study, we had available matched measurements of BAbs, NAbs, and MxA mRNA of 386 patients. Because blood specimens had been obtained at different time points in treatment follow-up, to reduce possible bias due to differences in treatment duration we considered only samples obtained after 12 months (±3 months) of treatment with IFNβ. In this way, 137 patients (92 women and 45 men) with definite MS according to the McDonald criteria[23] were retrospectively included in the study. Patients had been treated with one of the IFNβ products for at least 3 years, had not switched type of IFNβ, had an Expanded Disability Status Scale (EDSS) score of ≤6.5 (inclusive), and had no viral infection at least 4 weeks before and after blood sampling. Before enrollment, all aspects of the study protocol were reviewed with each patient and informed consent was obtained.

Patients had been evaluated for the presence of BAbs and NAbs, as well as for gene expression levels of MxA at treatment entry (baseline) and again after 12 months of therapy with IFNβ. The latter blood samples were obtained 12 ± 1.4 hours (range 9-15) after the last injection of IFNβ.

For clinical monitoring, subjects were required to visit the clinic for a baseline evaluation and every 6 months for neurologic examination, with completion of the EDSS and recording of relapses and adverse effects. Relapses were assessed by neurologic examination and were defined as the appearance of a new symptom or worsening of an old symptom over at least 24 hours that could be attributed to MS and was preceded by stability or improvement for at least 30 days.[24] Relapses were accompanied by an increase of at least one point in the score for at least one of the EDSS functional systems. All of those relapses were treated with high-dose IV methylprednisolone and, therefore, scheduled. Statistical analyses were conducted, considering both time to first relapse and confirmed progression in disability, defined as an

Table 1 Baseline characteristics of patients divided based on their treatment

	IM IFNβ-1a	SC IFNβ-1b	SC IFNβ-1a, 22 μg	SC IFNβ-1a, 44 μg
No. of patients	39	29	37	32
Age, mean ± SD	38.0 ± 10.6	39.2 ± 12.3	33.7 ± 9.1	32.0 ± 10.1
Range	14–61	19–64	17–60	14–59
Women/men	25/14	18/11	27/10	22/10
EDSS score, mean ± SD	1.1 ± 1.0	2.2 ± 1.4	1.9 ± 1.6	1.6 ± 1.4
Range	0–3.5*	0–5.0	0–6.5	0–6.5
Disease duration, mean ± SD	69.9 ± 74.6	96.5 ± 111.3	70.2 ± 88.5	58.5 ± 74.7
Range	2–274	1–582	1–417	2–228
RRMS/SPMS/RPMS	39/0/0	18/8/3	37/0/0	32/0/0

Age refers to age (in years) at start of IFNβ treatment. Disease duration refers to the months since disease onset to start of IFNβ treatment.
*Patients treated with IM IFNβ-1a had a lower mean EDSS score than patients treated with IFNβ-1b ($p = 0.0008$).
IFNβ = interferon β; EDSS = Expanded Disability Status Scale; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; RPMS = relapsing progressive multiple sclerosis.

increase in EDSS score of at least one point sustained over at least 6 months.

The study protocol was approved by the local institutional review boards and carried out according to the Declaration of Helsinki.

NAb measurement by CPE assay. The antiviral CPE assay was used to detect NAbs because this assay has been recommended by the World Health Organization (WHO).[25] The assay was performed as previously reported[11,17,20]. Briefly, A549 cells (ECACC) were plated and incubated with one of the three IFNβ preparations and then encephalomyocarditis murine (EMC) virus. After culture, the cells were stained and the absorbance was read.

The neutralization titer of a serum sample was calculated according to Kawade's formula[26,27] and expressed in 10-fold reduction units per milliliter (TRU/mL).[28]

Because IFNβ-1b was shown to bind approximately three times more NAb molecules than IFNβ-1a (based on an MIU-to-MIU comparison), to avoid false-negative results NAbs were tested against the type of IFNβ for individual patients.[29,30] The IFNβ preparations used in the CPE assay were IFNβ-1b (Betaferon, Schering AG), IM IFNβ-1a (Avonex, Biogen Idec), and subcutaneous IFNβ-1a (Rebif, EMD Serono), which are commercially available and intended for clinical use.

BAb measurement by cELISA. BAbs were measured with a cELISA assay as described elsewhere.[31,32] In brief, microtiter plates were coated overnight with the monoclonal anti-human IFNβ IgG antibody BO2 (Yamasa-Shoyu). After plate washing and blockade with nonfat dry milk, wells were coated with either buffer or one of the three IFNβ preparations. The presence of bound antibody was detected using a peroxidase-conjugated goat antiserum to human immunoglobulin-G (IgG), followed by the addition of substrate. Results were obtained in optical density (OD) units by spectrophotometric analysis and were converted to units by comparison with a standard curve from a known positive specimen.

MxA gene expression analysis. MxA gene expression analysis was performed as previously described.[17,33,34] In brief, peripheral blood mononuclear cells (PBMCs) were collected from edetic anticoagulated whole blood by centrifugation over Lymphoprep separation medium (Axis-Shield). PBMCs were then subjected to RNA extraction and total RNA was reverse-transcribed. Finally, cDNA was used as a template for the real-time PCR analysis based on the 5′ nuclease assay with the ABI Prism 7000 Sequence Detection System (Applied Biosystems). Transcriptional expression of the MxA gene was normalized using glyceraldehyde phosphate dehydrogenase (GAPDH) as a reference gene. Applied Biosystems's TaqMan Assay-on-Demand gene expression products were used as primers and probes.

The relative expression of MxA gene was calculated by the comparative cycle threshold (Ct) method outlined in User Bulletin no. 2 provided by Applied Biosystems.

Statistical analyses. The effects of NAbs, BAbs, and low MxA gene expression were evaluated by comparing patients for RFS and time between blood sampling and first relapse. Kaplan-Meier statistics and a log-rank test with first relapse after blood sampling as the end point were used for RFS analyses.

Fisher's exact test was used to compare the number of relapse-free patients.

The optimal cutoff value for mxa gene expression was confirmed by receiver operating characteristic (ROC) analysis.

All statistical analyses were performed using GraphPad Prism software, Version 4.0 (GraphPad Software). All reported p values are based on two-tailed statistical tests, with a significance level of 0.05.

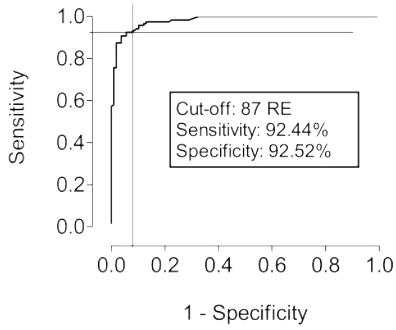
RESULTS

Patients. Treatment among the 137 patients was the following: subcutaneous IFNβ-1b 250 μg every other day (n = 29), IM IFNβ-1a 30 μg once weekly (n = 39), and subcutaneous IFNβ-1a (n = 69) either 22 μg (n = 37) or 44 μg (n = 32) three times weekly. Demographic and clinical characteristics of patients are shown in table 1 and table e-1 on the *Neurology*® Web site at www.neurology.org.

Baseline evaluations. At baseline, all patients scored negative for both NAbs and BAbs and had levels of gene expression for MxA similar to that of healthy controls (data not shown). The mean level ± SD of MxA-specific transcript was 31.13 ± 31.44 relative expression compared with GAPDH (RE) (median = 30.4; range 1 to 162).

An optimized cutoff for equally important sensitivity and specificity was determined through standard ROC analysis. The threshold was calculated at 87 RE; MxA gene expression levels above or below 87 RE correctly predicted the biologic response to IFNβ injection with a sensitivity of 92.44% and a specificity of 92.52%. The characteristics of the ROC analysis (AUC 0.9816; 95% CI 0.9683 to 0.9950; $p < 0.0001$) are shown in figure 1.

Figure 1 Predictive discrimination of myxovirus-resistance-protein A mRNA levels in 137 untreated patients with multiple sclerosis vs 120 neutralizing antibody-negative treated samples (12 hours after the last interferon β injection)



The remaining 17 patients were not considered in the receiver operating characteristic analysis because of neutralizing antibody positivity. RE = relative expression compared with GAPDH.

Based on previously reported studies, a level of 20 TRU/mL was considered the threshold for NAb positivity,[26-28] whereas a level of 8 U was considered the threshold for BAb positivity.[14-16]

NAb, BAb, and MxA status after 12 months of IFNβ therapy. To estimate the relation among time to first relapse, NAbs, BAbs, and MxA mRNA, we analyzed patients based on their positive or negative status for each measure at 12 ± 3 months after treatment initiation.

Individuals were classified as MxA-positive when showing an increase (≥87 RE) in MxA gene expression, MxA-negative without increase (<87 RE) in MxA expression, NAb-positive or BAb-positive with samples positive for either NAbs or BAbs, and NAb-negative or BAb-negative with no NAbs or BAbs.

MxA and NAbs were tested in all 137 patients, whereas BAb measurement was not performed in two patients because of the paucity of their serum samples. Results for each measure are reported in table 2, and results obtained by the combination of the three measures are summarized in table 3.

Clinical significance of BAbs. To determine the utility of testing BAbs alone, we assessed the clinical outcomes for BAb-positive and BAb-negative patients using a threshold for positivity, 8 U, that has been validated by Pachner.[14-16] Fifty-eight (54%) of 108 BAb-negative patients were relapse-free, as well as 12 (44%) of 27 BAb-positive patients. There was no difference in the number of relapse-free patients ($p = 0.1531$), but there was a slight difference in the RFS analysis [$p = 0.0208$, hazard ratio (HR) = 1.86] according to the BAb status. The BAb-positive group showed a median time to first relapse of 10 months, whereas this value was undefined in the BAb-negative group (table e-2, figure 2A).

Relation between time to first relapse and MxA and NAb status. A two-step analysis was conducted. First, MxA and NAbs were singularly considered and patients were divided into two groups according to the respective nominal categorization. Analysis for RFS was performed by using the thresholds above. Time to first relapse after blood sampling was calculated, and the risk of experiencing a relapse according to each measure was analyzed. Afterward, to evaluate whether the association of the two measures increased predictive value, patients were divided into two groups: MxA-positive/NAb-negative patients had MxA gene expression above the threshold for positivity (≥87 RE) and NAbs below the cutoff (<20 TRU/mL), and MxA-negative/NAb-positive patients had MxA mRNA levels <87 RE and were positive for NAbs (≥20 TRU/mL). Again, the risk of new relapse was calculated for both groups.

According to our first analysis, both MxA and NAbs showed a predictive value, as time to first relapse was significantly increased in both MxA-positive compared with MxA-negative patients and in NAb-negative compared with NAb-

Table 2 MxA, NAb, and BAb evaluation after 12 ± 3 months of IFNβ treatment

	MxA mRNA		NAbs		BAbs	
	No. of patients	%	No. of patients	%	No. of patients	%
IM IFNβ-1a						
Positive	34	87.2	2	5.1	4	10.5
Negative	5	12.8	37	94.9	34	89.5
SC IFNβ-1b						
Positive	20	69	6	20.7	15	51.7
Negative	9	31	23	79.3	14	48.3
SC IFNβ-1a, 22 μg						
Positive	33	89.2	4	10.8	5	13.5
Negative	4	10.8	33	89.2	32	86.5
SC IFNβ-1a, 44 μg						
Positive	26	81.2	5	15.6	3	9.7
Negative	6	18.8	27	84.4	28	90.3
Total						
Positive	113	82.5	17	12.4	27	20
Negative	24	17.5	120	87.6	108	80

MxA-positive: ≥87 relative expression compared with GAPDH (RE); NAb-positive: ≥20 10-fold reduction units (TRU)/mL; BAb-positive: ≥8 U.
IFNβ = interferon β; BAb = binding antibody; MxA = myxovirus-resistance-protein A; NAb = neutralizing antibody.

Table 3 Patient categorization based on the combination of measures of MxA, BAbs, and NAbs

Treatment/MxA response	No. of pts.	%	NAb/BAb categorization	No. of pts.	% (based on MxA response)
IM IFNβ-1a					
MxA-positive	34	87.2	NAb+/BAb+	1	2.9
			NAb−/BAb−	31	91.2
			NAb−/BAb+	1	2.9
			NAb−	1	2.9
MxA-negative	5	12.8	NAb+/BAb+	1	20
			NAb−/BAb−	3	60
			NAb−/BAb+	1	20
SC IFNβ-1b					
MxA-positive	20	69	NAb+/BAb+	—	—
			NAb−/BAb−	14	70
			NAb−/BAb+	6	30
MxA-negative	9	31	NAb+/BAb+	6	66.7
			NAb−/BAb−	—	—
			NAb−/BAb+	3	33.3
SC IFNβ-1a, 22 μg					
MxA-positive	33	89.2	NAb+/BAb+	1	3
			NAb−/BAb−	30	91
			NAb−/BAb+	1	3
			NAb+/BAb−	1	3
MxA-negative	4	10.8	NAb+/BAb+	2	50
			NAb−/BAb−	1	25
			NAb−/BAb+	1	25
			NAb+/BAb−	—	—
SC IFNβ-1a, 44 μg					
MxA-positive	26	81.2	NAb+/BAb+	—	—
			NAb−/BAb−	25	96.1
			NAb+/BAb−	1	3.9
MxA-negative	6	18.8	NAb+/BAb+	2	33.3
			Nab−/BAb−	1	16.7
			Nab−/BAb+	1	16.7
			NAb+/BAb−	1	16.7
			NAb+	1	16.7

MxA-positive: ≥87 relative expression compared with GAPDH (RE); BAb-positive: ≥8 U; NAb-positive: ≥20 10-fold reduction units (TRU)/mL.
IFNβ = interferon β; BAb = binding antibody; MxA = myxovirus-resistance-protein A; NAb = neutralizing antibody.

positive patients. The median time to first relapse was 7 months for the MxA-negative group, whereas this value for the MxA-positive group was so long as to be undefined in this 3-year study ($p < 0.0001$, HR = 2.87) (table e-2, figure 2B). Likewise, the median time to first relapse was shown to be 8 months for the NAb-positive group and undefined in the NAb-negative group ($p = 0.0013$, HR = 2.49) (table e-2, figure 2C).

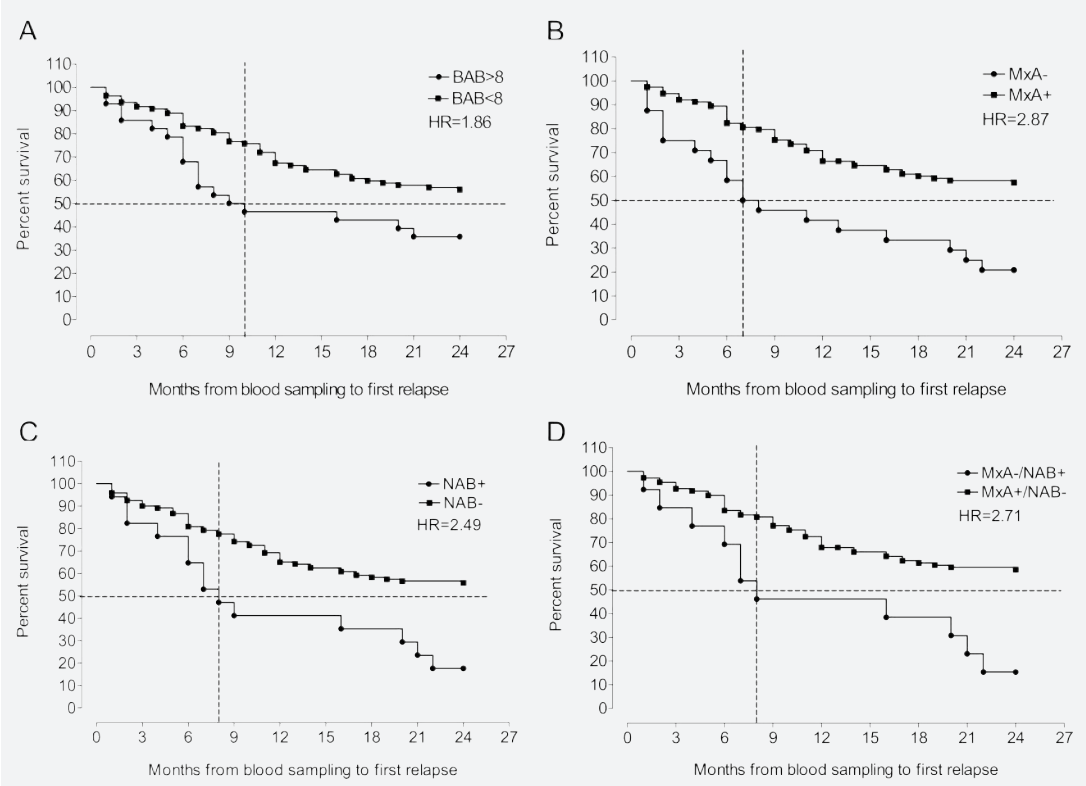
Because time to first relapse was shorter in MxA-negative patients than in NAb-positive patients, the log-rank test gave a lower p value when comparing MxA-positive and MxA-negative patients than when comparing NAb-positive and NAb-negative patients, and the HR value was higher when comparing MxA expression than comparing NAbs, we conclude that MxA measurement gives stronger predictive information than NAb measurement (table e-2).

As expected, by correlating MxA and NAb measurements, a shorter time to first relapse was shown in patients with NAbs and without MxA gene expression (MxA-negative/NAb-positive) compared with the group of patients with no NAbs and with MxA gene expression (MxA-positive/NAb-negative) (median time = 8 months vs undefined; $p = 0.0023$; HR = 2.71) (figure 2D).

Relapse-free patients. Among all patients, 70 (51%) were relapse-free. Of these, 65 (93%) were MxA-positive and five (7%) were MxA-negative. More MxA-positive patients were relapse-free compared with MxA-negative patients (57.5% vs 21%; $p < 0.0001$). A comparable result was found for NAb status, as 55.8% (67/120) of NAb-negative patients were relapse-free compared with 17.6% (3/17) of NAb-positive patients ($p = 0.0038$).

DISCUSSION Currently there is no consensus on the definition of a patient nonresponsive to IFNβ treatment, and criteria used to judge poor treatment outcomes often include observation of different clinical measures such as relapse rate, EDSS status, and EDSS increase at different time points.[35,36] From a theoretical view, nonresponsive patients can be divided into two subgroups: pathogenetic nonresponders and pharmacological nonresponders. Pathogenetic nonresponders show a lack of clinical efficacy, although IFNβ is still biologically active. In these patients, the drug is not able to antagonize the pathology because of its aggressive pathogenetic characteristics. Although, in recent years, several studies have demonstrated that MRI is an acceptable surrogate marker for monitoring treatment response and gives significant prognostic information on the clinical outcome,[22] for pathogenetic nonresponders there are still no validated instruments for early detection. In contrast, pharmacological nonresponders show a lack of clinical efficacy due to presence of serological factors, inhibiting IFNβ biologic activity. For early detection of these nonresponders, a major effort is currently focused on identifying biologic markers for disease activity and IFNβ treatment response.

Figure 2 RFS of patients according to BAbs (A), MxA gene expression (B), NAbs (C), and the concurrent measurement of MxA and NAb (D)



(A) Using a threshold of 8 U, the BAb-positive group (median time to first relapse = 10 months) showed poorer RFS rates than the BAb-negative group (median time = undefined; log-rank test $p = 0.0208$). (B) Likewise, the MxA-negative group (median time = 7 months) showed significantly poorer RFS rates than the MxA-positive group (median time = undefined, log-rank test $p < 0.0001$). (C) Accordingly, the NAb-positive group (median time = 8 months) showed significantly poorer RFS rates than the NAb-negative group (median time = undefined; log-rank test $p = 0.0013$). (D) By correlating MxA mRNA and NAbs, a poorer RFS rate was observed in the MxA-negative/NAb-positive group (median time = 8 months) than in the MxA-positive/NAb-negative group (median time = undefined; log-rank test $p = 0.0023$).

The aim of the present study was to clinically validate some of the most common biologic markers used for early detection of IFNβ pharmacological nonresponders in MS (i.e., MxA mRNA, NAbs, and BAbs). In other words, we have evaluated whether a single measurement of MxA mRNA, as well as measurements of NAbs and BAbs, has a predictive value on the clinical responsiveness to IFNβ therapy in terms of risk of new relapses.

A number of studies have already demonstrated that the presence of NAbs causes a reduction in clinical and MRI efficacy of IFNβ therapy[4-7] and that NAb measurement has a prognostic value in predicting clinical response.[19] Accordingly, specific European guidelines have been published recently about the use of NAb measurement in MS.[8] However, the role of both MxA mRNA and BAbs on IFNβ clinical efficacy had not previously been investigated.

Along with several authors,[9,13-16] we have shown that MxA gene expression is a sensitive measure of the biologic response to IFNβ[10] and is abolished by the presence of IFNβ-induced NAbs.[11,12,17,33,34] Nevertheless, there are issues regarding the use of MxA mRNA as a measure of the clinical efficacy of IFNβ in this context.

At present, no clear consensus exists regarding the level of MxA expression that can be considered biologically and clinically relevant. Thus, a cutoff of 87 RE has been chosen through standard ROC analysis; this model postulates that the biologic effect of IFNβ injection occurs only when postinjection values are above this expression threshold. On these grounds, we have shown that MxA mRNA levels did not exceed the upper reference limit in 17.5% of IFNβ-treated patients and that, in these patients, the clinical efficacy of IFNβ therapy was reduced. In fact, there was a higher number of relapse-free patients in the MxA-positive group than in the MxA-negative group; moreover, compared with MxA-positive patients, MxA-negative patients had a higher risk of experiencing a new relapse.

A similar reduction in clinical efficacy also was correlated with the presence of NAbs. In the present cohort, NAbs were detected in 12.4% of IFNβ-treated patients, in whom a significantly higher risk for new relapses was calculated. This is in line with several previous studies showing that the presence of NAbs against IFNβ reduces the clinical efficacy of the drug.[19-22]

Conversely, data from our work show that BAbs do not have the same clinical significance as NAbs and MxA. In fact, considering BAbs, the difference in relapse-free patients did not reach significance, whereas RFS analysis showed only a small level of statistical significance. This discrepancy could be due to the ambiguous function of BAbs; in some patients, while BAbs are present IFNβ biologic activity is preserved, whereas in other patients IFNβ biologic activity is abolished.[14,15] Nevertheless, BAb measurement might be useful as a confirmation test, indicating that the decrease of the biologic effects is due to specific anti-IFNβ antibodies.

Additional considerations include the timing of sampling and management options. Ideally, all patients with MS being treated with IFNβ should be routinely tested in clinical practice. At present, however, this is not practical because there are no standardized, economical assays available to most neurologists. Therefore, from a clinical perspective patients might be tested at least once. Previous data show that NAbs develop during the first year of IFNβ treatment and the negative clinical effects of NAbs become evident after 18 to 24 months.[19] In the latter study, patients who were NAb-positive at 1 year were at a significantly higher risk for more relapses during NAb-positive periods than during NAb-negative periods (i.e., NAbs abrogated the effect of IFNβ on relapses). This means that measuring NAbs after 1 year of treatment can provide valuable prognostic information about the future outcome of patients. On this ground, in the present study samples were analyzed once at 12 $\pm$ 3 months after the beginning of IFNβ treatment, applying the same timing not only for NAbs, but also for MxA mRNA and BAbs.

Data from the present study show that measurement of BAbs alone, after 1 year of treatment, does not give clear prognostic information on the clinical outcome of IFNβ-treated patients. This observation confirms that the mere presence of anti-IFNβ antibodies does not interfere with the therapeutic efficacy of the cytokine. On the contrary, anti-IFNβ neutralizing activity is of great importance, as demonstrated by the strong prognostic information given by both MxA mRNA measurement and NAbs detection. Among these two measures, MxA is the most sensitive, as patients without MxA increase show a median time to first relapse of 7 months and an HR of 2.87. In addition, patients positive for NAbs show a median time of 8 months and an HR of 2.49. Considering a combination of the two measures, results were comparable with the above data regarding individual measurement of NAbs. This is probably an understatement of the impact of lack of biologic activity because this latter analysis excludes some of the MxA-negative patients in whom NAbs are not detectable.

The binding of IFNβ to its receptor results in the specific induction of MxA gene, and any serological factor interfering in this binding affects MxA gene expression. NAbs are the most popular inhibiting factors, but mechanisms other than NAbs can also reduce MxA gene expression. Other explanations for reduced expression might be noncompliance, increased concentrations of soluble circulating IFNβ receptor,[18] and IFNβ-receptor saturation.[37] It is remarkable that 12.8% of patients treated with IM IFNβ-1a were MxA-negative, although the majority of them were both NAb- and BAb-negative. This was not the case with the other, more immunogenic IFNβ preparations (i.e., subcutaneous IFNβ-1a and IFNβ-1b), where pharmacological nonresponsiveness was much more closely related to BAbs and NAbs.

The present study demonstrated for the first time that MxA mRNA quantification after 1 year of treatment with IFNβ has a strong prognostic value for the risk of experiencing new relapses. The prognostic value obtained using MxA mRNA is higher than that given by NAbs measurements. Therefore, to improve efficacy, the practice of MxA measurement might be the first choice in a monitoring strategy for IFNβ-treated patients. In addition, NAb and (less importantly) BAb evaluation might be used to predict the persistency of loss of biologic activity, distinguishing between reduced drug efficacy due to persistent inhibitory factors or treatment failure due to poor compliance.

ACKNOWLEDGMENT

The authors thank Rita Guerrieri, Marina Panealbo, Giuliana Savoldi, and Angela Zaccaria for their nursing assistance with this study, and Anna Messina and Daniele Dell'anna for their administrative support.

Received June 15, 2007. Accepted in final form October 22, 2007.

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