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Clinical importance of neutralising antibodies against interferon beta in patients with relapsing-remitting multiple sclerosis

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Summary

Background Interferon beta is the first-line treatment for relapsing-remitting multiple sclerosis, but the drug can induce neutralising antibodies against itself, which might reduce effectiveness. We aimed to assess the clinical effect of neutralising antibodies.

Methods We measured neutralising antibodies every 12 months for up to 60 months in 541 patients with multiple sclerosis, randomly selected from all patients who started treatment with interferon beta between 1996 and 1999. Patients left the study if they changed or discontinued therapy. Antibodies were measured blindly, using antiviral neutralisation bioassays with high, medium, and low sensitivity, and with different neutralising capacities as cutoff value for definition of a neutralising-antibody-positive result.

Findings Patients developed neutralising antibodies independent of age, sex, disease duration, and progression index at start of treatment. Relapse rates were significantly higher during antibody-positive periods (0·64–0·70) than they were during antibody-negative periods (0·43–0·46; p<0·03). When comparing the number of relapses in the neutralising-antibody-positive and neutralising-antibody-negative periods we found odds ratios in the range 1·51 to 1·58 (p<0·03). Time to first relapse was significantly increased by 244 days in patients who were antibody-negative at 12 months (log rank test 6·83, p=0·009). During this short-term study, presence of neutralising antibodies did not affect disease progression measured with the expanded disability status scale.

Interpretation Our findings suggest that the presence of neutralising antibodies against interferon beta reduces the clinical effect of the drug. In patients who are not doing well on interferon beta, the presence of such antibodies should prompt consideration about change of treatment.

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Introduction

Interferon beta is the first-line treatment for relapsing-remitting multiple sclerosis, and several large randomised trials have shown that the drug reduces the frequency and severity of clinical relapses, slows the progression of disability, and suppresses signs of disease activity on MRI.[1–10] A drawback of this treatment is the induction of anti-interferon antibodies, which, in high concentrations, have been associated with reduction of treatment response. In the pivotal trials of different interferon beta preparations, the frequencies of neutralising antibodies against interferon beta in multiple sclerosis have varied from 7% to 42%, while binding antibodies have been found in up to 78% of patients treated with interferon beta.[2,11–13] The measured frequency of neutralising antibodies depends not only on the type of interferon beta preparation, dosage, dose frequency, and route of administration, but also on the methods used to detect antibodies, emphasising the need for standardised measurement techniques. We previously reported that up to 80% of serum samples from patients treated for more than 1 year with a commercial preparation of interferon beta contained measurable amounts of neutralising antibody when assessed with an optimised assay.[14] Neutralising antibodies were detected more frequently and at higher concentrations in patients who were given interferon beta-1b than in those treated with interferon beta-1a. Frequent administration led to an increased rate of positive samples, and subcutaneous administration induced antibodies more frequently than intramuscular administration.[14]

However, the clinical importance of these antibodies is still the subject of much controversy. To assess the effect of different concentrations of neutralising antibodies against interferon beta in serum on therapeutic efficacy, we measured these antibodies in prospectively sampled serum from patients with relapsing-remitting multiple sclerosis in Denmark who began treatment with interferon beta during the years 1996–99. We also assessed the effects of the sensitivity of the bioassay.

Our aims were: (1) to follow the development of neutralising antibody for up to 5 years of treatment with different interferon preparations; (2) to set a standard for the definition of neutralising-antibody-positivity, by use of differences in relapse rates, to find which of three assay sensitivities and levels of neutralising capacity are clinically most appropriate to dichotomise the quantitative level into positive and negative; (3) to ascertain whether sex or pretreatment clinical features affect occurrence of neutralising antibodies; and (4) to estimate the effect of such antibodies on clinical outcome measures.

Materials and methods

Patients

When interferon beta-1b was approved in Denmark for the treatment of relapsing-remitting multiple sclerosis, the Danish National Board of Health endorsed common

	Treatment				
	Rebif once a week (n=103)	Rebif three times a week (n=162)	Avonex (n=82)	Betaferon (n=194)	All (n=541)
Age (years)	37·5 (20–55)	39·2 (16–67)	40·3 (14–67)	39·3 (19–62)	38·1 (14–67)
Sex ratio (female:male)	2·22:1	2·24:1	2·15:1	1·77:1	2·04:1
EDSS	2·85 (0–5·5)	2·99 (0–6·5)	2·56 (0–5·5)	2·80 (0–5·5)	2·84 (0–6·5)
Relapses*	3·16 (2–10)	2·80 (2–8)	2·52 (1–6)	2·95 (0–8)	2·88 (0–10)
Disease duration (years)	7·34 (0–35)	5·88 (0–27)	7·41 (0–24)	7·66 (0–51)	7·04 (0–51)

Data are mean (range) except for sex ratio. *During past 2 years.

Table 1: **Baseline characteristics of patients**

treatment criteria and guidelines for the management of the treatment. In accordance with these recommendations, treatment with the drug was restricted to departments of neurology, and as part of the Danish national interferon beta project, all patients starting treatment with the drug were recorded in a national multiple sclerosis treatment database.[15] The database also contains records with clinical information from all 6-month control visits (such as number of relapses in the preceding control period, expanded disability status score [EDSS], side effects, and information about treatment withdrawal).

By December, 1999, 1074 patients had started treatment with interferon beta. We obtained frozen serum samples from all these patients, but due to restricted capacity in the laboratory, we have assessed samples from only 555 patients to date. These samples were selected at random from among patients who were treated with the same preparation throughout the entire observation period. The investigators in the laboratory had no knowledge of the patients' disease characteristics. Age, sex, disease duration, EDSS, and relapse rate during the 2 years before treatment did not differ between patients who were selected for analysis and the total population of patients. The patients were followed up until June, 2002. 14 patients were excluded because they had not been followed up clinically for at least 12 months, leaving 541 patients in the study.

The 541 patients included 209 of the 303 patients who between June, 1996, and October, 1997, took part in an open-label, national randomised trial comparing interferon beta-1b (8 MIU Betaferon, Schering AG, Berlin, Germany; subcutaneously every other day) with interferon beta-1a (22 μg Rebif, Serona, Geneva, Switzerland subcutaneously once weekly).[15] The results of this study will be published separately. Patients who refrained from randomisation or started treatment after October, 1997, were treated with an approved interferon preparation (8 MIU of Betaferon subcutaneously every other day, available from 1996; interferon beta-1a [Avonex, Biogen, Cambridge, USA] 30 μg intramuscularly once weekly, available from 1997; or Rebif 22 μg three times weekly, available from 1998).

Treatment among the 541 patients was Betaferon 8 MIU every other day in 194 patients, Rebif 22 μg weekly in 103 patients, Rebif 22 μg three times weekly in 162 patients, and Avonex 30 μg once weekly in 82 patients. The baseline characteristics of the patients are shown in table 1.

Observation of the patients for this study terminated for the following reasons: censored at follow-up (346); change of treatment to another preparation (126); and cessation of treatment (69). Clinical characteristics at baseline, treatment response, and occurrence of neutralising antibodies in these patients are shown in table 2. Censored patients were older, had longer disease duration, and had a lower relapse rate during treatment than patients who changed preparation or stopped treatment, but there was no difference in the proportion of patients who developed neutralising antibodies between the three groups. Table 3 shows the reasons for changing preparation or stopping treatment.

Procedures

Clinical data were collected prospectively at follow-up visits at 3 and 6 months after start of treatment and, thereafter, at intervals of 6 months, including neurological examination with EDSS and recording of relapses and adverse effects. Standard laboratory tests were undertaken at all visits. Relapses were assessed by history and physical examination and were defined (in accord with Schumacher and colleagues[16]) as the appearance of a new symptom or worsening of an old symptom attributable to multiple sclerosis, accompanied by an appropriate new neurological abnormality or focal neurological dysfunction lasting at least 24 h in the absence of fever, and preceded by stability or improvement for at least 30 days. We collected blood samples for measurements of neutralising antibodies against interferon beta at 0, 3, 6, 12, 18, 24, 36, 48, and 60 months of treatment, but in this study we only used the yearly results. If, however, a result was missing, it was substituted by the interpolation of the result 6 months before and 6 months after that date. Blood was obtained by venepuncture 48 h after injection of interferon beta, and the samples were isolated and stored at −20°C until assay.

Antiviral neutralisation bioassay was undertaken essentially as previously described.[17–19] Briefly, MC-5 cells, a subclone of A549 cells, were seeded in microtrays at a concentration of 10 000 cells per well and incubated at 37°C in a 5% CO_2 atmosphere for 24 h. Interferon beta preparations at different concentrations were preincubated for 1 h with diluted serum at 5% concentration in a volume of 100 μL and then transferred to the MC-5 cells; interferon beta was added at concentrations of 3, 10, and 100 LU per mL, corresponding to 0·9, 3, and 30 IU per mL (1 LU is the amount of interferon inducing 50% protection against challenge virus). After another 24 h, the antiviral effect of interferon was measured by use of nitroblue

	Mean age at end of treatment or follow-up	Sex ratio (female: male)	Mean EDSS at start of treatment	Mean duration of treatment (years)	Annual relapse rate during treatment	Mean EDSS at end of treatment or follow-up	Mean neutralising capacity at end of treatment or follow-up	Proportion neutralising-antibody-positive at end of treatment or follow-up
Censored (n=346)	43·5	1·91:1	2·81	3·5	0·46	3·21	28·8	33·2
Changed preparation (n=126)	40·5	2·07:1	2·87	2·3	0·64	3·45	20·9	25·4
Stopped treatment (n=69)	39·2	2·83:1	2·91	2·7	0·58	3·49	26·5	29·0
p*	0·000	0·41	0·74	0·000	0·0001	0·23	0·011	0·25

*For difference between censored and other groups.

Table 2: **Comparability of patients who were censored at follow-up, changed preparation, or dropped out of treatment**

	Changed preparation	Treatment stopped
Side effects	11	20
Treatment failure	15	9
Neutralising antibodies	1	1
Lack of compliance/injections undesirable	0	4
Pregnancy	0	18
New preparations becoming available	51	1
2-year treatment in randomised study completed	36	1
Other/unknown	12	15
Total	126	69

Table 3: **Reasons for changing preparation or dropping out of treatment**

tetrazolium assay.[17,18] To avoid false-positive and false-negative results, controls for endogenous antiviral activity and serum toxicity were included in each assessment. Each sample was tested with three different sensitivities of the neutralising assay: (1) high sensitivity (addition of 3 LU/mL interferon beta), (2) medium sensitivity (10 LU/mL), and (3) low sensitivity (100 LU/mL). The neutralising capacity—ie, the percentage of the added interferon that was neutralised by the neutralising antibody—of each serum sample was measured.

To establish a clinically appropriate definition of neutralising-antibody-positivity, we calculated how the odds ratios for relapse versus non-relapse by neutralising antibody status depended on the chosen sensitivity of the assay and cutoff value of neutralisation capacity. Numbers of months of observation with and without relapses were counted in all antibody-positive and antibody-negative periods and were pooled for all patients. If a blood test taken at a particular time was denoted neutralising-antibody-positive according to the current definition, the patient was judged to be positive during a full 12-month period, including 6 months on either side of the time of the blood sample. A period was omitted from the analysis if the clinical information or the blood test was missing and could not be substituted by interpolation. On this basis we calculated odds ratios corresponding to all three sensitivity assays and cutoff values of neutralising capacities ranging from 5% to 95% at 5% intervals.

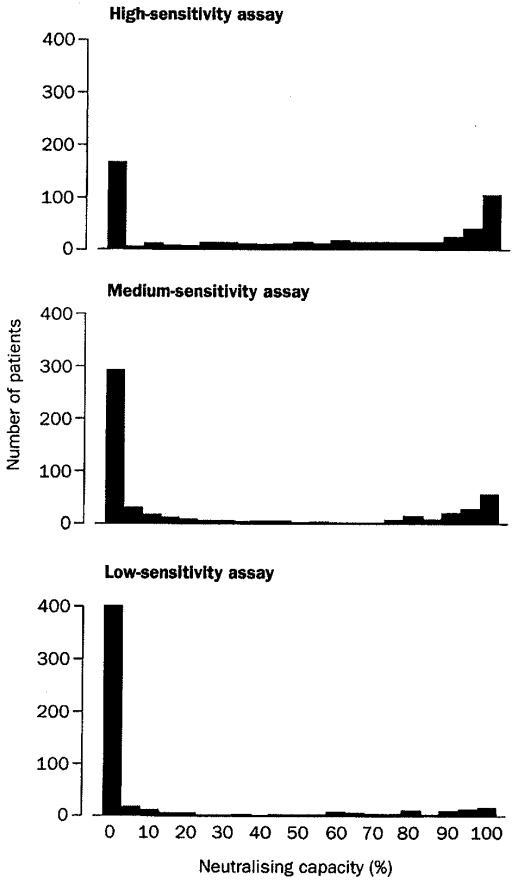

Figure 1: **Distribution of patients according to serum neutralising capacity at 12 months in neutralising assays of low, medium, and high sensitivity**

We measured time to first relapse, proportion of relapse-free patients, mean changes in EDSS, and confirmed progression in disability, defined as an increase in EDSS of at least one point sustained over at least 6 months. In the analyses we defined neutralising-antibody-positive patients according to the neutralising-antibody status at 12 months after the start of treatment with interferon beta. The study was undertaken as a per protocol analysis, in which patients were censored if they changed or terminated treatment before meeting the endpoint.

Statistical analyses

For each level of sensitivity and cutoff value of neutralising capacity, a four-fold table analysis was used to calculate the odds ratios for relapse versus non-relapse, and standard statistical methods were used to calculate 95% CIs for the odds ratios. Since many patients stopped observation after varying periods of treatment, simply because they were censored at follow-up, we used Kaplan Meier statistics and log rank test with first relapse and sustained progression as endpoints. Additionally, we compared the proportions of neutralising-antibody positive and negative patients who met their endpoint during the study period, with the Pearson test. EDSS scores were compared with the Mann-Whitney *U* test, and comparison of more than two independent ordinal scale variables was done with the Kruskal Wallis χ^2 test

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

The distribution curve of the neutralising capacity had a characteristic U-shape (figure 1), especially for the high and medium sensitivity assays. Data tended to cluster at zero or very low values, or at very high values near 100% with a ceiling effect. A 100% neutralisation capacity suggests that all the added interferon was neutralised, but there could still be an excess of neutralising antibody even after addition of 100 LU of interferon in the low sensitivity assay.

Development of neutralising antibodies to interferon beta, as measured with the medium sensitivity assay, is shown in table 4. Neutralising antibodies were present in several patients treated with interferon beta-1b, but after 36 months of therapy, a significantly lower proportion were neutralising-antibody-positive than after 12 months of treatment ($p=0{\cdot}023$, χ^2 test). When interferon beta-1a (Rebif) was administered once weekly, significantly fewer patients became antibody-positive at 12 months of treatment than when the drug was given three times

	12 months*	24 months*	Changed status between 12 and 24 months†		36 months*	Changed status between 24 and 36 months†		48 months*	Changed status between 36 and 48 months†		60 months*	Changed status between 48 and 60 months†	
			neg→pos	pos→neg		neg→pos	pos→neg		neg→pos	pos→neg		neg→pos	pos→neg
Treatment													
Rebif 22 μg×1 weekly	15/103 (15%)	18/82 (22%)	8/72	0/10	5/38 (13%)	0/33	0/5	6/24 (25%)	2/20	0/4	2/6 (33%)	0/4	0/2
Rebif 22 μg×3 weekly	74/162 (46%)	51/123 (42%)	6/72	6/51	21/36 (58%)	3/18	0/18	1/1 (100%)	0/0	0/1	..	0/0	0/0
Avonex 30 μg×1 weekly	7/82 (9%)	5/67 (8%)	2/62	2/5	2/34 (6%)	0/32	0/2	0/8	0/8 (0%)	0/0	..	0/0	0/0
Betaferon 8 MIU/2 days	88/194 (45%)	67/153 (44%)	7/76	17/77	44/126 (35%)	5/75	12/51	21/75 (28%)	3/48	9/27	5/13 (39%)	1/9	0/4
All	184/541 (34%)	141/425 (33%)	23/282	25/143	72/234 (31%)	8/158	12/76	28/108 (26%)	5/76	9/32	7/19 (37%)	1/13	0/6

*Data are number positive/total tested (%). †Data are number who had a change in neutralising-antibody status/number at risk. neg→pos=converted from antibody-negative to antibody-positive. pos→neg=converted from antibody-positive to antibody-negative.

Table 4: **Development in numbers of antibody-positive patients (neutralisation capacity ≥20% in medium-sensitivity assay) during treatment**

weekly (p=0·000, Fisher's exact test). When interferon beta-1a (Avonex) was administered intramuscularly, significantly fewer patients became antibody-positive at 24 months of treatment compared with subcutaneous administration of interferon beta-1a (Rebif) once a week (p=0·022, Fisher's exact test).

The neutralising antibody status at 12 months was somewhat, but not fully, predictive of status later on. Table 4 shows, for each 12-month period, the number of neutralising-antibody-negative patients who developed antibodies, and the number of positive patients that reverted to negative status.

Development of neutralising antibodies (defined as 20% neutralising capacity as cutoff value in the medium sensitivity assay—see below) was not predicted by sex, by duration of multiple sclerosis at start of treatment, by number of relapses during the 2 years before treatment, or by progression index (EDSS at start of treatment divided by duration of disease). Table 5 shows the proportions of patients grouped according to clinical characteristics, who developed neutralising antibodies after 12 months.

In the medium and low sensitivity assays, the proportions of antibody-positive patients depended little on the cutoff value in the range 20–70% neutralising capacity, because most results were outside this interval, but in the high-sensitivity assay the proportion of antibody-positive results decreased as cutoff values were increased (figure 2).

	Number positive for neutralising antibodies at 12 months of treatment*	Odds ratio (95% CI)
Sex		0·98 (0·67–1·43)
Male	60/178 (34%)	
Female	124/363 (34%)	
Duration of multiple sclerosis†		0·82 (0·57–1·18)
<6 years	99/277 (36%)	
≥6 years	81/258 (31%)	
Unknown	4/6	
Relapses‡		0·91 (0·64–1·31)
<3	92/263 (35%)	
≥3	87/264 (33%)	
Unknown	5/14	
Progression index§		1·17 (0·81–1·68)
<0·4	75/236 (32%)	
≥0·4	103/292 (35%)	
Unknown	6/13	

*Data are number positive/number tested (% positive). †At treatment start. ‡During 2 years before treatment start. §EDSS divided by disease duration at treatment start.

Table 5: **Pretreatment clinical features and development of neutralising antibodies**

The presence of neutralising antibodies had a substantial effect on the relapse rate. Figure 3 shows odds ratios for relapse, comparing neutralising-antibody-positive and negative periods as functions of the cutoff values of neutralising capacity, when the medium-sensitivity assay was used. Table 6 shows the same data, along with data from the assays with high and low sensitivity, but restricted to cutoff values of 20% and 50% neutralising capacity. Occurrence of relapses was increased significantly during neutralising-antibody-positive periods, but was similar for the low and medium sensitivity assays and for all cutoff values for neutralising capacity. When data from the assays with medium or low sensitivity were used, we calculated yearly relapse rates of 0·64–0·70 in neutralising-antibody-positive periods compared with 0·43–0·46 in negative periods, yielding odds ratios from 1·51 to 1·58. The odds ratios were similar when we used data from the same assays with cutoff values for neutralising capacity between 20% and 60%, but with the low sensitivity assay and cutoff values at 75% or greater, the odds ratios for relapses were as low as 1·3. However, this value was still significantly different from unity, since with this extreme definition of positivity, many periods were misclassified as neutralising-antibody-negative in spite of high amounts of antibody in the serum. Data from the high-sensitivity assay yielded lower odds ratios with cutoff values in the lower range, because many patients with very low antibody concentrations were classified as neutralising-antibody-positive. For further analyses, we decided to define neutralising-antibody positivity as neutralisation capacity of 20% or greater in the medium sensitivity assay.

For analysis of the relation between time to first relapse and neutralising antibodies, we divided the patients into those who were positive and those who were negative for neutralising antibodies at 12 months after treatment start. The time to first relapse was significantly increased in neutralising-antibody-negative patients by Kaplan-Meier analysis of the probability of remaining exacerbation-free (log rank test 6·83, p=0·009). The median time to first exacerbation was 605 days in the negative group compared with 361 days in the positive group, a difference of 244 days (figure 4). The proportion of neutralising-antibody-negative patients who remained free of relapses throughout the study period was 39%, compared with 27% in the positive group (p=0·0064).

Our assessment of disease progression showed that mean EDSS was increased in neutralising-antibody-positive patients after 42 months (table 7). The differences between positive and negative patients were significant only at 42 months (p=0·049) and at 48 months (p=0·008) from

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