Early development of anti-natalizumab antibodies in MS patients

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Abstract The purpose of this study is to monitor the development of anti-natalizumab antibodies to evaluate their first appearance in multiple sclerosis patients, since their presence has been associated with a reduction in the efficacy of the treatment and an increase of adverse events. A total of 134 multiple sclerosis patients were included in the trial. Anti-natalizumab antibodies were monthly detected by ELISA up to the first year of treatment and subsequently, a determination was made at 18 months. 15.7 % of the patients were positive, being 7.5 % transiently positive and 8.2 % persistently positive. The first appearance of anti-natalizumab antibodies occurred after the first month of treatment onset in 72 % of positive patients; 18 % did so after the second month, and 9.7 % after the third month. Antibodies were never detected for the first time after the fourth infusion. The development of anti-natalizumab antibodies occurs very early after treatment onset. This observation should be considered when standardizing the follow up of patients treated with this drug in order to minimize the risks and optimize the treatment.

Keywords Multiple sclerosis · Natalizumab treatment · Antibodies

Introduction

Natalizumab (TYSABRI) is a recombinant humanized IgG4j monoclonal antibody approved for the treatment of multiple sclerosis (MS). This antibody binds to the integrin a4b1 (adhesion molecule very late activation antigen 4, VLA-4) on the surface of lymphocytes, blocking the binding with its receptor VCAM-1 (vascular cell adhesion molecule 1) present in the endothelium, and thereby interfering with lymphocyte trafficking into the central nervous system and so reducing the pathogenic processes of MS [1].

The efficacy of natalizumab treatment has been demonstrated in two large, randomized controlled trials [2, 3]. An important aspect taken into account was the development of antibodies against natalizumab. About 9 % of the natalizumab-treated patients generated antibodies, being 3 % transiently positive and 6 % persistently positive. Their presence has been related with a reduction in the drug serum concentration, and a loss of therapeutic efficacy [2, 4]. But these trials provided no information about the development of antibodies just after treatment initiation.

We previously described the appearance and the incidence of anti-natalizumab antibodies in a cohort of 64 MS patients followed up to month 18 after treatment onset. The most important finding was that all of the antibodies appeared within the first 4 months of treatment, since no antibodies were detected after 4 months of therapy onset in any of the patients included [5]. Due to the impact that this fact could have in the clinical practice, our current aim is to evaluate the first appearance of antibodies and validate our

previous findings in a larger cohort of MS patients monthly followed for the development of anti-natalizumab antibodies.

Materials and methods

Patients and study design

The follow-up trial enrolled 134 patients (64 patients from the original cohort and 70 new patients in the validation cohort) treated with natalizumab. The Ethics Committee of Carlos Haya Hospital (Ma´laga, Spain) approved the trial and all of the subjects gave written informed consent.

To determine the immunogenicity of natalizumab, 5 ml of peripheral blood were collected in clot activator tubes (Vacutainer; Becton, Dickinson and Co., USA) at baseline before the administration of the first dose of natalizumab and each month after starting treatment, right before the next infusion, to obtain the serum. The determination of anti-natalizumab antibodies was performed each month up to the first year of treatment and subsequently, a determination was made at 18 months.

Patients with a positive sample for antibodies discontinued the therapy, and the assessment of antibodies was repeated 1 month later. If the antibodies were positive again (persistent antibodies), the therapy was definitively withdrawn. If the result was negative in the retest (transiently positive antibodies) therapy was reintroduced and we proceeded exactly as before, with a monthly evaluation of the antibodies.

Anti-natalizumab antibodies detection by ELISA

The detection and confirmation of natalizumab antibodies in human serum was made by a sandwich/bridging ELISA developed by Biogen Idec. In this procedure, samples were run in both a screening and a confirmation assay (to demonstrate the specificity of the binding interactions in the antibody/drug complex) on the same plate. A control set was also included in each run.

Microtiter plates were coated with Natalizumab and incubated at room temperature for 12–28 h. After washing the plate once with wash buffer, blocking buffer was added to the plate and incubated for 1–4 h at room temperature. During incubation time, screening controls (QC1, QC2 and NC) and samples were diluted 1:10 in assay diluent. Competition control and samples were also diluted 1:10 in assay diluent supplemented with natalizumab at a final concentration of 100 lg/ml. After 75 min at room temperature, 100 ll of each one were added to the blocked plate. Controls were run in triplicate and samples in duplicate. After 2 hours incubating, and once the plate was washed four times with wash buffer, 100 ll of dilute biotinylated-natalizumab were added and incubated for 1 h. After washing, streptavidin horseradish peroxidase (SA-HRP) was added (in an appropriate working dilution), which binds to the captured biotinylated-natalizumab. After a final washing step, HRP substrate was added. The ensuing colour development reaction was stopped at a specific time point by the addition of a dilute acid solution. The optical density (OD) was then measured, and was considered directly proportional to the amount of anti-natalizumab antibodies present in the serum specimen. To consider a sample as positive, its screening OD needs to be higher than the OD of QC2, and the ratio $OD_{competition}/OD_{screaming}$ has to be B0.5.

Statistical analysis

Patients were classified in three categories: antibodies negative (none of them having a positive determination post-baseline), transiently positive (patients having a single positive determination post-baseline) and persistently positive (patients having at least two positive determinations separated by 1 month).

Descriptive statistics included means and standard deviations for quantitative variables, and percentages for qualitative variables. Student's t-test was used to compare quantitative variables and a chi-square test for qualitative variables. All reported P values represented two-tailed tests, with P values \0.05 being considered statistically.

Results

A total of 134 patients were included in the trial. One hundred and sixteen were followed up to 18 months for the presence of natalizumab antibodies. The 18 remaining patients had been on therapy for more than 6 months, but had not yet completed a year of treatment with natalizumab. The demographic and clinical characteristics of the patients are summarized in Table 1.

Firstly, we analyzed both cohorts separately in order to validate our previous work [5], and then we proceeded to perform the analysis in all of the patients followed.

In the independent analysis of the validation cohort, we obtained a higher percentage of positive patients than in the original cohort, mainly due to a higher percentage of transiently positive patients (Table 2). Significantly, this cohort supports the fact that all of the positive patients develop antibodies during the first 4 months of treatment (from the first to the third infusion), as we previously described [5].

In the joint analysis of both the original and the validation cohort, 113 patients (84.3 %) remained negative

Table 1 Demographic and clinical characteristic of multiple sclerosis patients

N	Original Cohort (64)	Validation cohort (70)	*P* value[t]	Joint analysis (134)
Age (years)	38.75 (9.58)	36,39 (9.22)	0.154	37.59 (9.28)
Female/male (%)	46/18 (71.9/28.1)	51/19 (72.9/27.1)	0.526	97/37 (72.4/27.6)
Disease duration (years)	11.95 (7.01)	12.08 (8.21)	0.932	12.01 (7.91)
Age at disease onset	27.03 (9.42)	26.15 (8.85)	0.598	26.63 (9.12)
EDSS Score at present	3.4 (2.25)	3.03 (2.18)	0.352	3.2 (2.1)
Number of relapses (Year before treatment onset)	1 (0.17)*	1 (0.1)*	0.970	1 (0,1)*
Clinical form at present (%)			0.07	
RR	38 (59.3 %)	53 (75.7 %)		91 (67.91 %)
SP	24 (37.45 %)	16 (22.85 %)		40 (29.85 %)
PR	2 (3.12 %)	1 (1.4 %)		3 (2.2 %)

Quantitative data are presented as mean (standard deviation)

EDSS Expanded disability status scale, *PR* progressive relapsing, *RR* relapsing–remitting, *SP* secondary progressive

* Median (interquartile range)

[t] *P* value obtained from comparing the original cohort and the validation cohort

Table 2 Percentage of positive patients for anti-Natalizumab antibodies in each cohort and in the joint analysis

	Cohort 1*	Cohort 2	Joint analysis
Total positive patients	14.1 % (9)	17.1 % (12)	15.7 % (21)
Transient antibodies	4.7 % (3)	10.0 % (7)	7.5 % (10)
Permanent antibodies	9.4 % (6)	7.1 % (5)	8.2 % (11)

Values in round braces are absolute number of positive patients

* Data previously published [5]

during the follow-up period, 10 patients (7.5 %) were transiently positive and 11 (8.2 %) were classified as persistently positive (Table 2).

It is important to highlight that all transiently positive patients became negative in the following determination made a month later and remained so throughout the observation period. All of them continued their treatment with natalizumab. On the other hand, all permanently positive patients had to discontinue the treatment and were excluded from the trial.

Table 3 shows the timing of the first appearance of antibodies both in each cohort and in the joint analysis. Most patients who developed antibodies did so after the first month of treatment, and in none of the patients were the antibodies detected after the fourth infusion.

Natalizumab was the first treatment for 25 patients (18.8 %), while 109 patients (81.2 %) had been previously treated with at least one of the first line drugs for MS (70.6 % with IFNß and 29.4 % with Copaxone). Only one patient previously treated with IFNß -and who presented persistently high titers of antibodies against IFNß- also developed antibodies to natalizumab.

The patients categorized by an anti-natalizumab antibodies status did not differ at baseline in the clinical characteristic (age, disease duration, Expanded Disability Status Scale [EDSS] and number of relapses). After 1 year of treatment, no significant differences in the presence of relapses and changes in the EDSS were observed between positive and negative patients, but it must be taken into account that in persistently positive cases the treatment with natalizumab was withdrawn.

Table 3 First appearance of antibodies after natalizumab treatment onset

	Original cohort (64 patients)			Validation cohort (70 patients)			Joint analysis (134 patients)		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
Month 1	7	10.9	77.7	8	11.4	66.6	15	11.2	72.15
Month 2	1	1.56	11.1	3	4.28	25	4	2.98	18
Month 3	1	1.56	11.1	1	1.42	8.33	2	1.49	9.71
Month 4	0	0	0	0	0	0	0	0	0

(1) Absolute number of positive patients

(2) Relative percentage to the total of patients included in the trial

(3) Relative percentage to the total of patients developing antibodies

Only one patient from the original cohort developed a hypersensitivity reaction during the second infusion, which was associated with the presence of persistent antibodies. Other adverse events like headache, fatigue, and urinary tract infections were reported in 36.7 % of the patients. Among these patients, the most frequent reaction was a headache (25.4 %), followed by fatigue (12.5 %) and urinary tract infections (12.5 %).

Discussion

The presence of antibodies against therapeutic proteins observed in some patients is an important aspect to consider for the optimization of the treatment. Our previous study described that all positive patients develop antibodies during the first 4 months of treatment (from the first to the third infusion), a fact that has been now reconfirmed in an independent cohort. Moreover, the present study shows that the first appearance of antibodies occurs after the first infusion in a very high percentage of positive patients.

The slightly higher percentage of positive patients in our cohorts compared with the percentages reported in the AFFIRM and SENTINEL studies [2, 3] and the one reported by Sorensen et al. [6] could be explained by considering the timing in which sampling was performed. Mentioned studies stated that anti-natalizumab antibodies were developed 12 weeks after treatment initiation and blood samples were obtained at baseline every 12 weeks to determine the presence of antibodies [2–4]. What differentiates our study is that we have prospectively searched the appearance of antibodies against natalizumab from the first to the 18th month after treatment onset, every month, while other studies describe what happens from the sixth month of treatment. This design allows us to know that the first appearance of antibodies mainly occurs during the first month of treatment, as in 72 % of positive patients antibodies were detected after the first dose.

There seems to be no relation between the previous development of antibodies against IFNß and the development of antibodies against natalizumab, since only one patient was positive for both, as we have previously described [8, 9].

Some authors state that an early testing of patient (prior to the sixth month of treatment) may lead to an erroneous determination of clinically meaningful antibodies, given the proportion of patients with transient antibodies [4]. This is partly true, but a second, confirmatory assessment of antibodies after 4 or 5 weeks, as in our monitoring design, allows us to discriminate those patients who develop transient antibodies, observing that all of them became negative in the following determination and remained so throughout the monitoring period, up to 18 months. It is noteworthy that all transient positive patients became negative between the second and fourth month of therapy.

It has to be taken into account that the presence of two consecutive positive antibody determinations is probably a basis to recommend the discontinuation of the therapy, since it has been associated with a reduction in the benefits of natalizumab treatment and also with an increase in the risk of adverse events [4]. In our practice, all permanent positive patients were excluded from the treatment, that being the most common reason for treatment discontinuation in our hospital [7]. We suggest a regular determination of anti-natalizumab antibodies 1 month after the third infusion. According to our data, a determination made at this point would allow the detection of all permanent positive patients (although many of these patients could have developed antibodies after the previous infusions), but not of transient positive patients, because by that time, all of them would have reverted to a negative status.

As a conclusion, the early development of natalizumab antibodies observed in this study should be considered when standardizing the follow up of patients treated with this drug, in order to minimize the risks and optimize the patient^{0}s treatment.

Conflicts of interest B. Oliver reports no disclosures. T. O´ rpez reports no disclosures. P. Urbaneja reports no disclosures. R. Maldonado-Sanchez reports no disclosures. L. Leyva reports no disclosures. Dr. Ferna´ndez has received honoraria as a consultant in advisory boards, and as chairman or lecturer in symposia, and has also taken part in clinical trials and other research projects promoted by Biogen-Idec, Bayer-Schering, Merck-Serono, Teva, Novartis, Almirall, Allergan and Genzyme.

Ethical standard The ethics committee approved the follow up of Natalizumab treated patients, the clinical and laboratory exams and the related study on 20/05/2010 (CTS507).

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