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Title**: Kinetics and incidence of anti-natalizumab antibodies in multiple sclerosis patients on treatment for 18 months**

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Abstract

Natalizumab is a monoclonal antibody shown to be highly effective in the treatment of relapsing–remitting multiple sclerosis (RRMS). Patients treated with natalizumab can develop antibodies directed against this agent that may affect the efficacy and safety of the drug. In this observational study, the kinetics of the appearance and the incidence of antinatalizumab antibodies were followed prospectively for 18 months in a cohort of 64 consecutive patients treated with natalizumab for relapsing MS. Blood samples were drawn immediately before starting natalizumab therapy and each month afterwards. The presence of antibodies against natalizumab was assessed by enzyme-linked immunosorbent assay (ELISA) in all patients. Anti-natalizumab antibodies were detected in nine (14.1%) natalizumab-treated patients, three (4.68%) of whom were transiently positive while six (9.37%) were persistently positive (these patients discontinued natalizumab). All positive titres were observed during the first 4 months of treatment. One patient with a hypersensitivity reaction also had persistent antibodies. We conclude that antibodies against natalizumab develop early, within the first 6 months of therapy with natalizumab. Although no antibodies were detected after 4 months of therapy in this particular study, this does not rule out their development later on in exceptional cases.

Keywords: antibodies, multiple sclerosis, natalizumab, observational study

1. Introduction

TYSABRI is a recombinant humanized IgG4κ monoclonal antibody that binds to the α_4 subunit of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrin on the surface of leukocytes and interferes with binding to endothelial receptors. This action is thought to ultimately hinder the migration of leukocytes across the blood–brain barrier into the central nervous system (CNS).[1] The efficacy of natalizumab has been demonstrated in large phase III clinical trials.[2,3] At the standard dose of one 300mg intravenous infusion every month, the drug is eliminated in 6–9 days, but more than 80% of α_4 integrin receptors remain saturated 1 month later.[4] As in other multiple sclerosis (MS) therapies, antibodies to natalizumab have been reported and are clinically relevant, since there is a possible correlation with decreased natalizumab serum concentrations and loss of efficacy and increased infusion-related adverse events.[2,5] However, little is known about the kinetics of antibody production in natalizumab-treated patients.

The aim of this study was therefore to investigate the kinetics and incidence of anti-natalizumab antibodies in a cohort of relapsing MS patients – relapsing–remitting MS (RRMS) and relapsing secondary progressive MS (SPMS) – followed prospectively over 18 months of continuous natalizumab therapy.

Materials and methods

This prospective observational study enrolled consecutive patients treated with natalizumab according to the labelling and usual standard of care at Carlos Haya Hospital, Malaga, Spain. Patients were followed prospectively for 18 months. The Institutional Review Board of Carlos Haya Hospital approved the study and all the subjects gave written informed consent. A detailed medical history was recorded, including history of MS and treatments for MS.

Sample collection

For each subject, a sample of 5 ml of peripheral blood was collected in clot activator tubes (Vacutainer® Becton, Dickinson and Co, USA) basally and each month after starting treatment, just before the next infusion, for 18 months, to obtain the serum to determine the immunogenicity of natalizumab. Patients with a positive sample for antibodies stopped therapy and the test was repeated after 1 month. If the result was again positive for antibodies (persistent antibodies), the patient was withdrawn from therapy definitively. If the result was negative in the retest (transiently positive antibodies), the therapy was reintroduced and we proceeded exactly as before, month by month. Special attention was paid for an adverse reaction during the infusions, in which case the antibodies were retested before the next infusion.

Natalizumab antibody detection

The detection and confirmation of antibodies to natalizumab in human serum was made by indirect enzyme-linked immunosorbent assay (ELISA). The ELISA was run in the Research Laboratory, Hospital Regional Universitario Carlos Haya and IMABIS Foundation, Malaga, Spain. 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. The ELISA performed was a qualitative assay that detected the presence or absence of antibodies. Microtitre plates were coated with natalizumab (0.25 μl/ml) and incubated at ambient temperature for 12–28 h. Screening controls and samples were added to the blocked plate. Competition controls and samples in a final concentration of natalizumab of 100 μg/ml were also included for the confirmation assay. The presence of a high concentration of natalizumab binds the antibodies and when added to the wells, the antibody/drug complex is blocked and no signal is detected. Next, biotinylated natalizumab at 1 μg/ml was added and incubated for 1 h. After washing, streptavidin-conjugated horseradish peroxidase (SA-HRP) was added which binds to the captured biotinylated natalizumab. Finally, the HRP substrate was dispensed. The ensuing colour development reaction was then stopped at a defined 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.

The assay was accepted when the controls (QC1/NC, QC2/NC and QC1/QC1C) were within the acceptance criteria established by Biogen Idec Inc. 133 Boston Post RoadWeston, MA 02493, USA. A sample was considered positive when its screening OD was higher than the OD of QC2 and the ratio OD competition/OD screening was ≤0.5.

Statistical analysis

Patients were classified into three categories: antibody negative (no post-baseline positive determination), transiently positive (a single positive determination post-baseline) and persistently positive (two or more positive

determinations separated by at least 1 month). Means and standard deviations were calculated for quantitative variables and percentages for qualitative variables. Analysis of variance was used to compare quantitative variables and the chi-square test for qualitative variables. All reported p values correspond to two-tailed tests, with $p<0.05$ considered as statistically significant. The statistical analysis was performed using the SPSS software program, version 14.5.

Results

The study included 64 MS patients. The demographic and clinical characteristics of the patients are summarized in Table 1. All patients were followed up over 18 months for the presence of natalizumab antibodies. There were no drop-outs during this period, with all of the patients completing the follow-up as established in the protocol.

Fifty-five patients (85.9%) were negative at all times while nine (14.1%) had at least one positive determination. Three of these antibody-positive patients (4.7%) were transiently positive while six were persistently positive (9.37%) and thus had to stop the treatment, though they were still followed up in order to collect the clinical data. All the transiently positive patients reverted to negative status and remained antibody negative for the rest of the study. All positive findings occurred during the first 4 months of therapy (Figure 1). Five of the six persistently positive patients developed antibodies after the first month of treatment while the remaining patient had the first positive finding after the third month of treatment. Transiently positive patients continued therapy, while persistently positive patients discontinued natalizumab.

The clinical form, time from onset, presence of relapses and change in Expanded Disability Status Scale (EDSS) after 1 year of natalizumab treatment were analysed according to antibody status. No significant differences between positive and negative patients were found in any of the variables.

Of the patients included in the study, 60 (93.7%) had previously been treated with at least one other MS drug, and only four (6.3%) received natalizumab as

first treatment. All nine positive patients had been previously treated with an immunomodulator – one with glatiramer acetate and eight with interferon-beta (IFN-β). All IFN-β-treated patients were negative for IFN-β antibodies.

Thirty-one patients (48.4%) experienced no adverse drug reactions during the study. In the 33 patients (51.6%) with at least one adverse drug reaction, the most frequent reaction was headache (23.4%), followed by fatigue (10.9%) and urinary tract infections (10.9%). There were no differences according to antibody status. The one patient with a hypersensitivity reaction (during the second infusion) also had persistent antibodies.

Discussion

As with other therapeutic proteins, patients may develop antibodies against natalizumab during therapy. Similarly to what happens in patients who develop IFN-β antibodies,[6] the presence of natalizumab antibodies leads to a reduced treatment efficacy.[5] Moreover, in the case of natalizumab these antibodies might be associated with post-infusion hypersensitivity reactions.[2,3]

The incidence of these antibodies in our series of patients was 9/64 (14.1%) – slightly higher than the 9% and 12% reported in the Natalizumab Safety and Efficacy in Relapsing Remitting Multiple Sclerosis (AFFIRM) and the Safety and Efficacy of Natalizumab in Combination with Interferon Beta-1a in Patients with Relapsing Remitting Multiple Sclerosis (SENTINEL).[2,3] In our study, all three transiently positive patients continued the treatment with natalizumab. These patients were tested for the presence of antibodies each month but remained negative throughout the 18 months of follow-up. Most of the patients who developed antibodies were persistently positive and discontinued treatment. The percentage of persistently positive patients (9.3%) was also slightly higher than in AFFIRM and SENTINEL (6% in both). In both those studies, the presence of antibodies was correlated with a reduction in the serum concentration of natalizumab.[5]

The data published previously and the results obtained in this independent study highlight that the appearance of antibodies occurs early after the initiation of treatment. In our experience no patient developed antibodies after the fourth infusion, suggesting that measurement of antibody titre after this time is of little value, except in patients with adverse drug reactions or hypersensitivity

reactions (which are also uncommon after the early infusions).[7] Clearly, although we did not detect any anti-natalizumab antibodies after the fourth month of therapy in our study, this does not imply that they may not be detected in isolated cases in a larger patient population.

While it has been shown that the presence of antibodies decreases the activity of the drug,[5] the clinical implications are not always so clear. We found no association between antibody status and relapses or disability progression after treatment, although the study was not powered to detect such differences and possibly, too, because we stopped natalizumab therapy as soon as persistent antibodies were detected. In the SENTINEL study, analysis of EDSS progression showed no difference between persistently positive and negative patients, in contrast to the AFFIRM trial.[5]

The most common adverse drug reactions in our study were headache, fatigue and urinary tract infections in both antibody-positive and -negative patients. No serious adverse events were reported, except for hypersensitivity in one patient with persistently positive antibodies.

According to our experience, most patients are negative for natalizumab antibodies but it is important – from a clinical point of view – to detect these antibodies in the event that they occur. We would therefore suggest a strategy of testing for the presence of antibodies 4–6 months after starting treatment. This would detect most patients who become antibody positive, avoiding unnecessary exposure to the drug and reducing significantly the costs associated with MS therapy while at the same time avoiding unnecessary testing. Patients with two confirmed consecutive determinations or those

with hypersensitivity reactions should discontinue treatment with natalizumab.

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Table I. Demographic and clinical characteristics of multiple sclerosis patients

N	64
Age (years)	38.6 (9.59)
Female/male	46/18 (71.9%/28.1%)
Duration of disease (years)	11.6 (7.02)
Age at disease onset	27.0 (9.42)
EDSS	3.3 (2.04)
Number of relapses in the previous year	1.0 (1.06)
Clinical form	
RRMS	57 (89.06%)
Relapsing SPMS	7 (10.93%)

Data presented as mean ± SD unless otherwise indicated.
EDSS: Expanded Disability Status Scale, RRMS: relapsing–remitting multiple sclerosis, SPMS: secondary progressive multiple sclerosis.

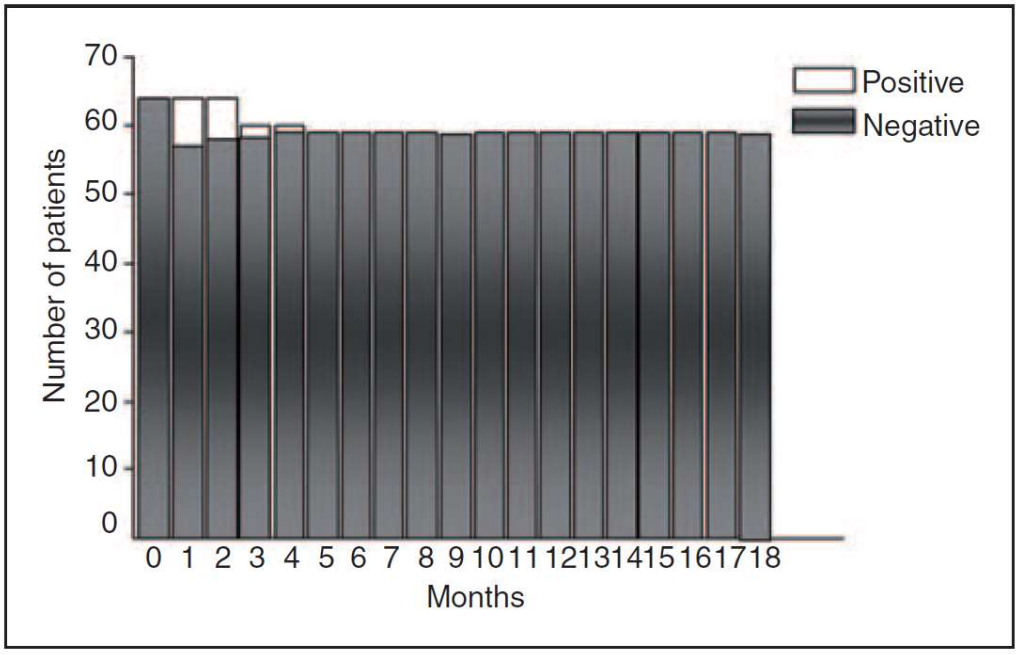

Figure I. Month of appearance and number of patients with antibodies against natalizumab.