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# Prediction of antibody persistency from antibody titres to natalizumab

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## Abstract

**Background:** In a subgroup of patients with multiple sclerosis natalizumab therapy causes generation of anti-natalizumab antibodies that may be transient or persistent. It is recommended to discontinue natalizumab therapy in persistently antibody-positive patients.

**Objective:** To use titres of anti-natalizumab antibodies to predict persistency of antibodies.

**Patients and methods:** In 525 consecutive natalizumab treated patients tested for anti-natalizumab antibodies 43 (8.2%) were antibody-positive. Thirty of the antibody-positive patients, who were tested both at three and at six months after treatment start, had antibody titres in blood measured using an extended ELISA method.

**Results:** Samples from persistently positive patients (N=18) had higher titre values than samples from transiently positive patients (N=12). A cut-off value for high titre values was generated, above which patients may discontinue natalizumab therapy after three months. The method had a sensitivity of 0.83, a specificity of 1.00 and a diagnostic accuracy of 0.90.

**Conclusion:** An extended ELISA method for measuring anti-natalizumab antibody titres in multiple sclerosis patients on natalizumab therapy may be used for evaluation of antibody persistence. A test at three months may identify patients with high titres, who should discontinue natalizumab therapy, and patients with transient low-titre antibodies, who may continue natalizumab therapy despite development of antibodies.

## Keywords

antibodies, antibody titre, multiple sclerosis, natalizumab, Tysabri, enzyme-linked immunosorbent assay

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## Introduction

Multiple sclerosis (MS) is a chronic, immune-mediated demyelinating disease of the central nervous system (CNS) in which migration of activated autoreactive T-cells from the periphery to the CNS is of pathogenetic importance. Natalizumab is a monoclonal antibody to the  $\alpha_4$ -integrin chain (CD49d) of the  $\alpha_4\beta_1$  integrin (VLA4) and  $\alpha_4\beta_7$  integrin expressed on the surface of lymphocytes and monocytes.<sup>1</sup>  $\alpha_4\beta_1$  and  $\alpha_4\beta_7$  integrins are mediators of transendothelial leukocyte migration, and by binding to the  $\alpha_4$ -integrin chain natalizumab blocks the migration of T-cells into the CNS.<sup>2</sup> The beneficial effects of natalizumab in the treatment of relapsing–remitting MS are well documented, both in the setting of clinical trials<sup>3–7</sup> and in general practice.<sup>8–10</sup>

However, anti-natalizumab antibodies are generated in about 9% of the MS patients treated with natalizumab, and their presence results in reduced serum levels of natalizumab, decreased bioefficacy, and abrogated therapeutic efficacy.<sup>4–6,11</sup> Anti-natalizumab antibodies in MS patients

typically develop during the first months after initiation of treatment.<sup>5–7</sup> Discontinuation of natalizumab therapy is recommended in patients with persistent antibodies.

Anti-natalizumab antibodies are detected by an ELISA-method, whereby samples are classified as either positive or negative for anti-natalizumab antibodies. This classification is of clinical relevance, as it denotes an amount of

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antibody sufficient for the neutralization of natalizumab to an extent that will have significant effects on the efficacy of treatment [Biogen Idec protocol 'Assay Procedure to Determine Natalizumab (Tysabri) Immunogenicity (CST02-180AP-R.3)].

The antibody titre at given time points, however, may be of importance in that it may provide an early identification of patients who will become persistently antibody-positive. We therefore performed repeated quantitative measurements of natalizumab-binding antibodies at given time points in 30 antibody-positive MS-patients, so that the development of antibodies could be followed over time.

We hypothesized that a measure of titres of natalizumab-binding antibodies at an early time point would be predictive for patients being transiently or persistently antibody-positive: low titres predicting transient and high titres persistent antibodies. An early shift to another treatment strategy may be beneficial in patients with high anti-natalizumab antibody titres, whereas patients with low titres might continue treatment.

## Patients and methods

### Patients and blood sampling

In Denmark patients were, according to treatment guidelines issued by the National Board of Health, eligible for natalizumab therapy if they, on therapy with a first-line disease modifying drug, had two relapses in one year or three relapses in two years, or had suffered a relapse-related increase in EDSS-score of two steps or more. Further, patients on treatment with mitoxantrone could be switched to natalizumab therapy. From June 2011 the European medicines agency criteria for treatment with natalizumab were adopted. It is mandatory, as a part of the national guidelines issued by The National Board of Health, that all patients treated with natalizumab have measurements of anti-natalizumab antibodies performed.

The study comprised all patients treated with natalizumab from December 2006 to December 2009. The patients did not receive any other disease-modifying treatment. Blood sampling for antibody measurements was performed three, six and 12 months after initiation of natalizumab therapy. If a patient tested positive for antibodies at three months another sample was taken for analysis after five months of treatment. Patients were regarded as antibody negative if they had at least two negative tests and no positive tests.

Using data from the Danish MS Treatment Registry, which contains prospectively collected information about clinical disease activity in all Danish patients treated with natalizumab, we calculated the proportion of patients with relapses in the period from three to six months after initiation of therapy, and the relapse rates were calculated as number of relapses per 365 days of observation using all

available data, also outside the three to six month interval. Relapse rates in three patient groups defined by the natalizumab antibody titres were compared using the Pearson contingency table test counting numbers of months with and without relapse. The proportion of patients with relapses in months 3–6 was assessed in antibody negative patients ( $N=482$ ), in low/medium titre patients ( $N=12$ ) (defined as patients with at least one positive test with low titres  $<600$  arbitrary titre units (ATU), but no tests with high titres  $>600$  ATU), and in high titre patients ( $N=7$ ) (having at least one test with high titres  $>600$  ATU).

The proportions of patients analysed for annualized relapse rates were  $N=482$  for antibody negative patients,  $N=17$  for low/medium titre patients and  $N=13$  for high titre patients.

The ethical perspectives were covered by the mandatory routine screening for antibodies to natalizumab and reporting the results to the Danish MS Treatment Registry according to the National Guidelines for Natalizumab Therapy in Denmark.

### Anti-natalizumab antibody ELISA

All patient samples were screened for antibody positivity by the ELISA procedure described in the Biogen Idec protocol 'Assay Procedure to Determine Natalizumab (Tysabri) Immunogenicity (CST02-180AP-R.3). All controls [Tysabri Reference Standard, biotinylated-Tysabri, high-positive quality control 1 (QC1;  $3\mu\text{g/ml}$  of monoclonal antibody 12C4)] and low-positive quality control 2 (QC2;  $0.5\mu\text{g/ml}$  of monoclonal antibody 12C4) were obtained from Biogen Idec (Maine Biotechnology Services Inc., Portland, ME, USA). A negative control consisted of a pool of untreated healthy people serum samples [Pel-Freez Clinical Systems, WI, USA (cat. no. 34005-100)]. All buffers and reagents used were made in accordance with the Biogen Idec protocol. Microtitre plates (cat. no. 3590) were from Corning Laboratories, NY, USA. For washing the plates a Thermo Wellwash AC (Thermo Electron Corporation, USA) was used and absorbance at 450 nm was read on a Biotek Synergy HT (Biotek, USA). All samples and controls were tested in a 1:10 dilution in the ELISA. A competition sample, where an excess of natalizumab was added to neutralize all binding antibodies in the sample, was run for all samples. The requirements for a positive sample were that the sample OD value was greater than or equal to that of  $0.5\mu\text{g/ml}$  of the positive control sample (QC2), and that the ratio of the OD of the competition sample divided by the OD of the screening sample was lower than or equal to 0.5.

In antibody-positive samples titres were measured with a modification of the routine procedure. All materials and chemicals used in the titre protocol were the same as for the screening protocol described. The titre results given are in ATUs and were calculated as follows. The sample tested

**Table 1.** Patient characteristics of the negative, transiently and persistently antibody-positive patients.

|                                                  | Transiently antibody-positive patients<br>(N= 12)* | Persistently antibody-positive patients<br>(N= 18)* | All antibody-positive patients<br>(N= 30)* | All antibody-negative patients<br>(N= 482) |
|--------------------------------------------------|----------------------------------------------------|-----------------------------------------------------|--------------------------------------------|--------------------------------------------|
| Age, years; median (range)                       | 38 (27–64)                                         | 41 (20–50)                                          | 39 (20–64)                                 | 39 (16–64)                                 |
| Sex, male/female                                 | 3/9                                                | 5/13                                                | 8/22                                       | 161/321                                    |
| Disease duration, years; median (range)          | 7 (0–17)                                           | 6 (1–13)                                            | 7 (0–17)                                   | 8 (0–37)                                   |
| Years on disease-modifying drugs; median (range) | 4 (0–10)                                           | 3 (1–6)                                             | 3 (0–10)                                   | 4 (0–13)                                   |
| EDSS; median (range)                             | 4.0 (2.0–6.5)                                      | 4.3 (0.0–6.5)                                       | 4.3 (0.0–6.5)                              | 4.0 (0.0–8.0)                              |
| Relapses in previous year; median (range)        | 3 (0–6)                                            | 2 (1–4)                                             | 2 (0–6)                                    | 2 (0–9)                                    |

\*Only patients who were included in the titration analysis.

was diluted in five 10-times dilution steps starting at a dilution of 1:10. The corresponding dilution series of competition samples were generated for subtraction from the results of the sample dilutions. A curve was then generated with the dilution values of the patient sample ( $x; y = [\log(1/\text{dilution})]$ ; KP/SP). KP and SP are, respectively, competition and screening samples for each dilution of the patient sample. A linear equation was calculated from an adapted line. The points of the equation were distributed on each side of the KP/SP=0.5 point of the ordinate. The abscissa on the curve was the logarithms of the inverse dilution factors [ $\log(1/\text{dilution})$ ]. From each equation the abscissa-value was calculated by including the ordinate-value of 0.5 in the respective equation. Further, we calculated the plate control-value of QC2/NC, which was used to correct the values of the equations for the final ATU-values. Thereby the ATU of the sample was obtained that denotes the corrected relative titre of antibodies bound to natalizumab. A control for the NC is that the CV-value should be less than 20% between the average values of QC1-C and NC for the plate of measurements. When screening of samples for antibodies was negative (KP/SP value above 0.5 at the dilution 1:10), no titration-value was calculated. All the negative samples were set to 1 ATU.

### Statistical analysis

The SPSS program version 19.0 was used. The Mann–Whitney test was performed for comparison between the groups of patients.

### Results

Among 525 consecutive patients analysed, 43 (8.2%; 95% confidence interval, CI, 6.1–10.9) tested antibody-positive at any time during the first 12 months of natalizumab therapy. Five of these were only tested once and could not be evaluated as persistently or transiently positive, and the remaining 38 patients had at least two tests performed and were classified as either persistently positive [23 patients (4%)] or transiently positive [15 patients (3%)]. Of the 38

positive patients a further eight were excluded from the titration study since the titration results from either the three or six months samples were missing, leaving 30 patients for the titration study (12 transiently positive and 18 persistently positive patients). No significant clinical or demographic differences between the groups of transiently and persistently positive patients were observed (Table 1).

Examples of the results of KP/SP-values for each dilution value of four selected patients (denoted patients A–D) tested at three different time points for antibodies to natalizumab are shown in Figure 1. All samples of patients A, B and C were positive at all three time points of measurements and therefore defined as persistently positive, while patient D was positive at three months and negative thereafter and defined as transiently positive. The linear parts of the curves crossing the KP/SP-value of 0.5 are shown for each of the four patients (Table 2). Patient A showed high antibody titres as seen in persistently positive patients. Patient B showed a pattern of median positive titres and patient C illustrates a case of repeatedly low titres in persistently positive patients. Patient D was transiently positive with a low titre at three months and negative tests at six and 12 months. Only the titre of the first sample of patient D was calculated as that was the only positive sample from that patient.

The titre values measured at three months in 30 patients varied widely, ranging from 4 to 3293 ATU for all of the patient samples (Table 3). Twelve patients were transiently positive with a median ATU of 11 and a range of 4–134 ATU, while 18 patients were persistently positive with a median of 908 ATU and a range of 10–3293 ATU. The titre values were significantly different between the two groups ( $p < 0.001$ ). Except for three, all titre values in the persistently positive group were higher than those in the transiently positive group. Using a cut-off value of 150 for predicting persisting antibody positivity, the method had a sensitivity of 0.83, a specificity of 1.00 and a diagnostic accuracy of 0.90.

The titre values in the transiently positive patients were equal to or below 150 ATU after three months of treatment. With the exception of two patients, who were also positive after five months, the antibodies were undetectable in these

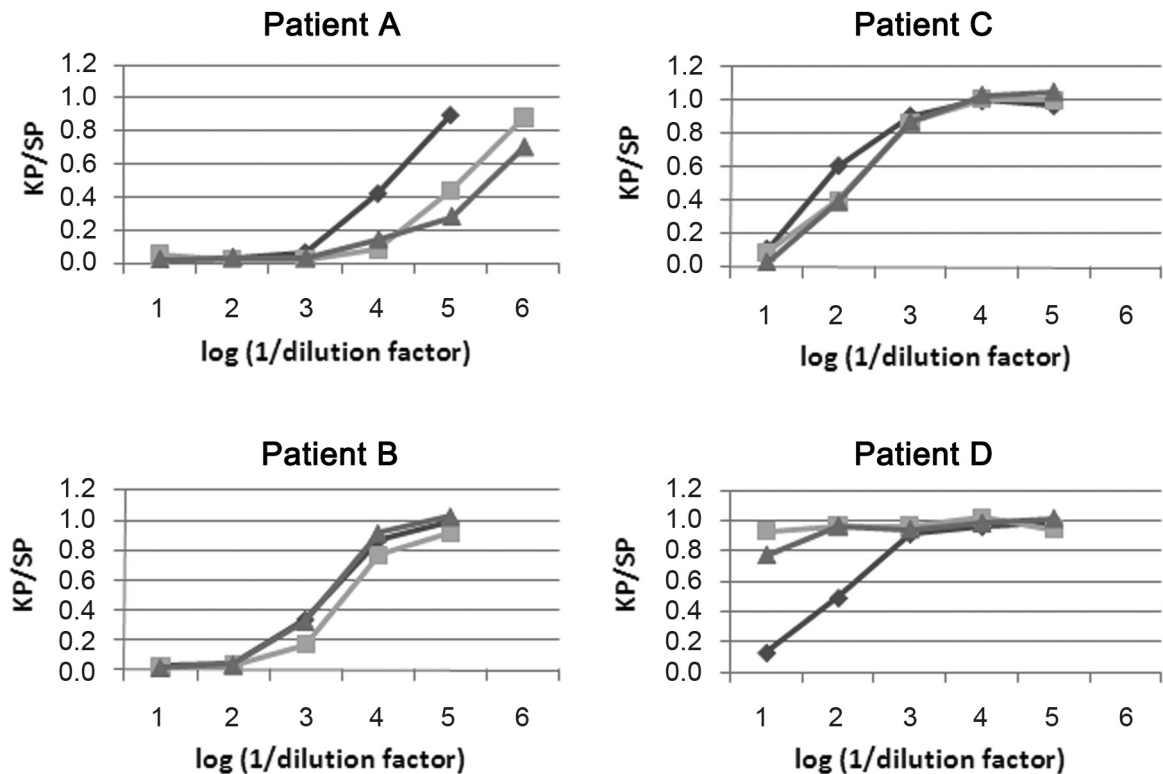


**Figure 1.** Dilution curves for four patients positive for antibodies at the three months time points. The curves are (diamonds) three months, (squares) five months and (triangles) six months after start of treatment with natalizumab. Table 2 includes the corresponding equations for the linear part crossing  $KP/SP=0.5$  of the curves, which were calculated from two or three points with  $R^2>0.97$ .

**Table 2.** The equations for the linear part crossing at  $KP/SP=0.5$  of the four selected patient examples (Figure 1), calculated from two or three points with  $R^2>0.97$ .

| Patient | Sample number | Linear equations      | QC2/NC-values | ATU   |
|---------|---------------|-----------------------|---------------|-------|
| A       | 1             | $y=0.48 \times -1.50$ | 5.1           | 2878  |
|         | 2             | $y=0.40 \times -1.53$ | 5.9           | 20534 |
|         | 3             | $y=0.42 \times -1.82$ | 5.1           | 65500 |
| B       | 1             | $y=0.53 \times -1.25$ | 5.1           | 393   |
|         | 2             | $y=0.60 \times -1.63$ | 5.1           | 696   |
|         | 3             | $y=0.59 \times -1.44$ | 5.9           | 329   |
| C       | 1             | $y=0.50 \times -0.39$ | 5.9           | 10    |
|         | 2             | $y=0.47 \times -0.54$ | 5.9           | 28    |
|         | 3             | $y=0.48 \times -0.57$ | 3.0           | 56    |
| D       | 1             | $y=0.39 \times -0.27$ | 5.1           | 18    |

patients after five months (Figure 2(a)) and all patients were negative at six months.

The three persistently positive patients with titres equal to or less than 150 ATU after three months are shown in Figure 2(b). In one patient only, followed for one year, the antibody-titre increased dramatically, while two other patients stopped natalizumab treatment after six to seven months due to three

positive antibody samples and were not tested for antibodies after one year. The patterns of development of antibodies in six persistently positive patients with three months titres from 150 to 900 ATU were mixed, that is, three of the patients had increasing titres, two had similar titres and one patient had decreasing titres (Figure 2(c)). Nine persistently positive patients were found with antibody-titres higher than 900 ATU at three months (Figure 2(d)); all had persistently high antibody titres. Two patients were tested at 1 and 1.5 years, respectively, after treatment initiation even though they discontinued natalizumab treatment after six to seven months of treatment. One patient still had a high antibody titre of 1020 ATU after 12 months, whereas in the other one the antibody titre was reduced to 21 ATU after 18 months.

In 68 of the 512 cases (see Table 1), information on relapses within the period from three to six months after initiation of treatment was missing. Of the remainder, 38/424 antibody-negative patients (9.8%; 95% CI 7.3–13.2) had at least one relapse within the period. For the low-medium positive, the proportion was 1/14 (7.7%; 95% CI 1.3–31.5) and for the high positive, the proportion was 1/6 (16.7%; 95% CI 3.0–56.4), which was not statistically significant from the antibody-negative patients ( $p=0.78$ ). We also compared annualized relapse rates among the three groups, and

**Table 3.** The antibody titres (in ATU) of 30 antibody-positive patients at three months ( $\pm 0.2$ ) time points.

| Patient number | ATU (three months) | Positivity |
|----------------|--------------------|------------|
| 1              | 4                  | Transient  |
| 2              | 7                  | Transient  |
| 3              | 8                  | Transient  |
| 4              | 8                  | Transient  |
| 5              | 9                  | Transient  |
| 6              | 10                 | Persistent |
| 7              | 10                 | Transient  |
| 8              | 12                 | Transient  |
| 9              | 16                 | Transient  |
| 10             | 18                 | Transient  |
| 11             | 25                 | Transient  |
| 12             | 36                 | Persistent |
| 13             | 55                 | Transient  |
| 14             | 113                | Persistent |
| 15             | 134                | Transient  |
| 16             | 156                | Persistent |
| 17             | 176                | Persistent |
| 18             | 393                | Persistent |
| 19             | 418                | Persistent |
| 20             | 433                | Persistent |
| 21             | 887                | Persistent |
| 22             | 929                | Persistent |
| 23             | 1002               | Persistent |
| 24             | 1040               | Persistent |
| 25             | 1523               | Persistent |
| 26             | 2212               | Persistent |
| 27             | 2236               | Persistent |
| 28             | 2442               | Persistent |
| 29             | 2677               | Persistent |
| 30             | 3293               | Persistent |

in this analysis we did not restrict the counting of relapses to the three to six months period. The 482 antibody negative patients encountered 526 relapses with an annualized relapse rate of 0.50 (95% CI 0.46–0.54). In the 17 low–medium-positive patients, the number of relapses was 11 and the annualized relapse rate 0.31 (95% CI 0.16–0.56). The 13 high-positive patients experienced four relapses in their short observation period, resulting in an annualized relapse rate of 0.76 (95% CI 0.20–1.95). The difference was not statistically significant with a *p*-value of 0.21.

Among all 43 antibody positive patients, allergic infusion reaction was recorded in six cases as opposed to six cases among the 482 antibody negative cases (OR=12.9; 95% CI 5.0–32.8; *p*<0.0001).

## Discussion

The ELISA assay performed in accordance with the protocol developed by Biogen Idec classifies the blood sample

as positive or negative, but yields no information about the antibody titre in the patient sample. Our study of a group of 30 anti-natalizumab antibody-positive patients using a quantitative ELISA assay shows that the titres of natalizumab-binding antibodies give an early indication of the development of persistent antibody positivity in MS patients. Our results confirm the hypothesis that the titre of antibodies against natalizumab in patients on natalizumab therapy is a major determinant for persistence of antibodies over time. High antibody titres are associated with persistent antibodies, whereas low titres indicate that the patients may be only transiently positive.

The titre at three months seems to predict whether a patient will become transiently or persistently antibody-positive. In fact, among 525 MS patients screened for antibodies we did not encounter any antibody-positive MS patients on treatment with natalizumab that were not antibody-positive at the three months time point after initiation of treatment. Calabresi et al. found patients (11% of the positive patients) who developed antibodies later than after three months of treatment.<sup>6</sup> The reason for this difference from our results is not clear.

In the AFFIRM<sup>4</sup> and SENTINEL<sup>5</sup> trials binding anti-natalizumab antibodies were measured using the ELISA method also used in the present study.<sup>6</sup> The concentration of natalizumab antibody binding was calculated from a titre value. The details concerning the use of sample background and type of equations for calculations are not described and that makes it difficult to compare the method with that of our study. However, transiently positive patients had titres in the range 0.59–9.18  $\mu\text{g/ml}$ , whereas the range of titres in the persistently positive patients was 1.01–600  $\mu\text{g/ml}$ .<sup>6</sup> We have made calculations by a similar method (not shown), and found comparable results: the ranges for transiently positive were 0–11  $\mu\text{g/ml}$  and persistently positive patients had 3–394  $\mu\text{g/ml}$ . The main differences between the two methods are the use of the background values measured for each sample in our method and the correction for the inter-assay variation between plate measurements. By direct use of absorbance values to calculate the concentrations of antibodies in a sample, uncertainties of the result may arise if the sample has a high background value, which is corrected for in our method by using KP/SP calculations.

Our results suggest that measuring the titres of antibodies to natalizumab after three months allows identification of patients with high titres, in whom it might be considered to discontinue natalizumab treatment already after three months of treatment. All samples with values higher than 150 ATU originated from persistently positive patients, whereas the majority of samples with titres equal to or below 150 ATU represented transiently antibody-positive patients and only a few persistently antibody-positive patients. Patients with titres below 150 ATU may be further followed with measurements of antibody titres up to 12

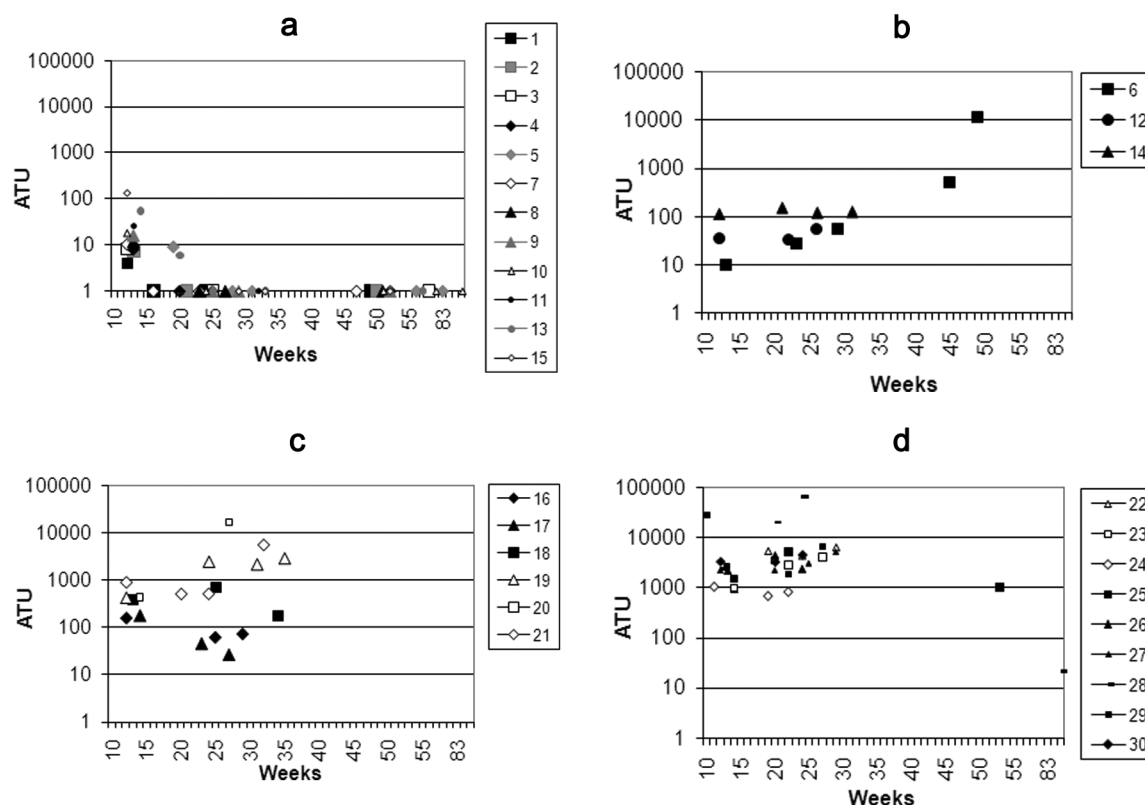


**Figure 2.** Development of antibody titres for antibody-positive patients. (a) Transiently antibody-positive patients. Negative values are set to 1 to be seen on the graph. (b) Persistently antibody-positive patients with titre values below 150 ATU (low titres) at three months. (c) Persistently antibody-positive patients with titre values between 150 and 900 ATU (medium titres) at three months. (d) Persistently antibody-positive patients with titre values higher than 900 ATU (high titres) at three months.

months of therapy before the decision of discontinuation or continuation of treatment on natalizumab is made.

It is of particular interest that after having converted to negative antibody status, none of the transiently positive patients became positive at later time points despite the continued treatment with natalizumab. For the persistently positive patients with low titres (less than 150 ATU) or medium titres (150–900 ATU) about half continued to have low/moderate titres over the year, but a few even had decreasing titres. These patients were not followed after the discontinuation on natalizumab treatment, generally after six to seven months of treatment, when the third positive antibody sample was obtained. It is not known whether patients with low titres have preserved some therapeutic effects, and our study cannot answer this question. We could not demonstrate any statistically significant difference in the proportion of patients who suffered relapses between months 3 and 6 or in the relapse rates in the full observed period between anti-natalizumab antibody negative patients, low/median titre positive patients and high titre positive patients, but the power of these analyses was low and the 95% CI was very wide for the latter two groups. Hence, low titre positive patients at three months, even those who had another positive titre and therefore were

classified as persistently positive patients, might benefit from continued natalizumab treatment, as the titres continued to stay low within the following months and the majority of these patients reverted to antibody negative status within the first year of therapy.

In conclusion, by using an extension of the Biogen Idec protocol it is possible to obtain an antibody titre that can be incorporated in the evaluation of patients after three months of treatment with natalizumab as a predictor of persistence of antibodies.

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### Conflict of interest statement

PEHJ has nothing to declare. NK-H has received honoraria for lecturing and participation in advisory councils, travel expenses for attending congresses and meetings and financial support for monitoring the Danish MS Treatment Register from

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## References

1. Frenette PS and Wagner DD. Adhesion molecules – Part 1. *N Engl J Med* 1996; 334: 1526–1529.
2. Stüve O and Bennett JL. Pharmacological properties, toxicology and scientific rationale for the use of natalizumab (Tysabri) in inflammatory diseases. *CNS Drug Rev* 2007; 13: 79–95.
3. Miller DH, Khan OA, Sheremata WA, et al. A controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2003; 348: 15–23.
4. Polman CH, O'Connor PW, Havrdova E, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2006; 354: 899–910.
5. Rudick RA, Stuart WH, Calabresi PA, et al. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. *N Engl J Med* 2006; 354: 911–923.
6. Calabresi PA, Giovannoni G, Confavreux C, et al. The incidence and significance of anti-natalizumab antibodies: Results from AFFIRM and SENTINEL. *Neurology* 2007; 69: 1391–1403.
7. Hutchinson M, Kappos L, Calabresi PA, et al. The efficacy of natalizumab in patients with relapsing multiple sclerosis: Subgroup analyses of AFFIRM and SENTINEL. *J Neurol* 2009; 256: 405–415.
8. Oturai AB, Koch-Henriksen N, Petersen T, et al. Efficacy of natalizumab in multiple sclerosis patients with high disease activity: A Danish nationwide study. *Eur J Neurol* 2009; 16: 420–423.
9. Putzki N, Yaldizli Ö, Bühler R, et al. Natalizumab reduces clinical and MRI activity in multiple sclerosis patients with high disease activity: Results from a multicenter study in Switzerland. *Eur Neurol* 2010; 63: 101–106.
10. Sangalli F, Moiola L, Bucello S, et al. Efficacy and tolerability of natalizumab in relapsing–remitting multiple sclerosis patients: A post-marketing observational study. *Neurol Sci* 2011; 31(Suppl. 3): S299–S302.
11. Sorensen PS, Jensen PE, Haghikia A, et al. Occurrence of antibodies against natalizumab in relapsing multiple sclerosis patients treated with natalizumab. *Mult Scler* 2011; 17: 1074–1078.