

Lymphocytosis as a biomarker for natalizumab treatment response in multiple sclerosis: The link with anti-drug antibodies

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Abstract

Background: Natalizumab is a high-efficacy therapy drug for multiple sclerosis that inhibits lymphocyte migration into the central nervous system, increasing lymphocyte counts in the peripheral blood. Anti-drug antibody responses may limit drug efficacy, but current guidance recommends anti-drug antibody testing only after evidence of 'reduced efficacy'.

Objective: To assess the lack of relative lymphocytosis as a biomarker of anti-drug antibody responses.

Methods: Lymphocyte counts and immunogenicity data from the pivotal AFFIRM clinical trial ($n=627$ natalizumab vs $n=315$ placebo) and real-world data from the Cardiff MS register ($n=185$) were analysed to assess the relationship between lymphocyte counts, anti-drug antibody responses, drug levels and disease activity.

Results: Natalizumab treatment induced an absolute change in lymphocyte counts by week 12 (1.40 ± 0.92 vs $-0.05 \pm 0.62 \times 10^9$ cells/L in natalizumab vs placebo; $p < 0.0001$) that was absent in the persistent anti-drug antibody group ($-0.12 \pm 0.60 \times 10^9$ cells/L). Increases in lymphocyte counts by $\geq 0.8 \times 10^9$ cells/L from the baseline discriminated individuals without anti-drug antibody responses with high sensitivity ($\geq 84\%$) and specificity ($\geq 91\%$).

Conclusion: The absence of relative lymphocytosis is associated with anti-drug antibody responses and may provide a simple biomarker to prompt anti-drug antibody testing before disease breakthrough occurs.

Keywords

multiple sclerosis, natalizumab, immunogenicity, anti-drug antibody, immunotherapy

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Data Availability Statement is included at the end of the article.

Introduction

Multiple sclerosis (MS) is a major cause of non-traumatic disability in young adults, which develops following a peripheral immune attack on the central nervous system (CNS). Natalizumab is a humanised IgG4 monoclonal antibody that blocks alpha-4 integrin (CD49d) binding to the vascular cell adhesion molecule (VCAM-1; CD106) to inhibit lymphocytic infiltration into the CNS, lesion formation and clinical relapse.^{1,2} Saturation of CD49d occurs at a natalizumab blood concentration of 2–10 µg/mL, which occurs within 2 h and is maximal 24 h after infusion, and thus inhibits lesion formation within the first month of treatment.^{1,3} In this context, relative lymphocytosis in the peripheral blood is an expected feature of natalizumab treatment. By blocking alpha-4/beta-1 binding to VCAM-1 and alpha-4/beta-7 binding to the mucosal addressin cell adhesion molecule (MAdCAM), natalizumab prevents lymphocyte migration from the blood into tissues, causing lymphocytes to accumulate in the peripheral circulation.¹

Natalizumab is administered every 4–6 weeks to maintain clinical remission.^{4,5} Although natalizumab is generally a highly effective treatment to prevent MS relapses, the importance of anti-drug antibodies (ADAs) against therapeutics is increasingly being recognised as a risk factor for treatment failure,^{6,7} where neutralising antibodies block the biological effect of the drug by binding to the binding site and preventing interaction with its target. Combined data of the AFFIRM and SENTINEL pivotal trials show that 92.1% of the people developing functionally important levels of ADA do so within the first 12 weeks ($n=117/127$).^{8,9} The frequency of ADA does, however, wane over time with increasing numbers of infusions, which suggests the induction of immune tolerance to the drug, whereby potentially immunogenic proteins no longer trigger immunity.

Laboratory assays used to detect ADA are generated by manufacturers as part of the drug-development process, but these are often not readily and widely available. Reported ADA frequencies from trials and real-world studies vary based on the specific assay used, the reporting threshold and the timing of sample collection (Table 1). A bridging enzyme-linked immunosorbent assay (ELISA) was developed by the manufacturer of reference natalizumab, whereas an electrochemiluminescence (ECL) immunoassay was developed by the manufacturer of biosimilar natalizumab; importantly, the reporting thresholds of these assays are very different, where positive ADA responses are identified above a patented threshold of 500 ng/mL in ELISA,^{8,10} and 3.88 ng/mL in the ECL assay.¹¹ It is, therefore, unsurprising that ADA responses to reference natalizumab were reported to occur in 9–10% of people in the AFFIRM and SENTINEL pivotal phase III trials

(ClinicalTrials.gov: NCT00027300 and NCT00030966), whereas >70% were identified in the ANTELOPE trial (ClinicalTrials.gov: NCT04115488).^{8,11} A similar finding emerged with reference adalimumab (human anti-tumour necrosis factor), which was reported to induce ADA in only 13–26% of people within the label,¹² and yet was shown to induce ADA in 41–96% of people using assays developed by manufacturers of biosimilars.¹³

Of potential concern is that it seems that data from the pivotal reference natalizumab studies have been used to define clinical practice criteria in the label and summary of product characteristics (SmPC) of both the reference (Tysabri®) and biosimilar (Tyruko®) agents.^{4,5,40,41} These indicate that ADA should be tested following infusion reactions or evidence of ‘reduced efficacy’ after 6 months of treatment, and if ADAs are persistent for over 6 weeks, switching away from the drug may be needed.^{4,5,40,41} These switching criteria, based on the simple ‘presence’ of ADA in repeat samples, could be problematic, given the variability of reported persistent ADA responses based on the assay, which range from 27–55% using the ECL assay,^{11,42} compared to 6% using ELISA.^{2,8} This stark discrepancy highlights the need to establish standardised thresholds of clinically relevant levels of (neutralising) ADA responses. Increasing assay sensitivity improves the detection of a greater proportion of ADA responses, but the clinical importance of these responses remains uncertain, unless they are associated with significant drug neutralisation that reduces its efficacy.

Given the drug’s mechanism of action of limiting lymphocyte migration to the CNS, relative lymphocytosis occurs upon natalizumab treatment. Clinical trial data suggest that both reference and biosimilar natalizumab elevate circulating lymphocyte counts by a mean of $1.6\text{--}1.8 \times 10^9$ cells/L,⁴³ which occurs from the first natalizumab infusion. This effect persists during treatment and reverses about 8–16 weeks after treatment cessation, based on data from the RESTORE randomised controlled trial investigating the reversibility of natalizumab-led changes in people discontinuing the drug ($n=130$).⁴⁴ We hypothesised that ADA limiting drug action would result in the absence of relative lymphocytosis, and thus, the presence/absence of lymphocytosis could be used as a biomarker for the absence or presence of ADA responses, respectively. This follows a similar rationale to that used in cell-depleting monoclonal antibodies used in MS, in which lymphocyte and/or B cell reduction is recognised as an indicator of treatment efficacy,^{45,46} and failure to deplete under alemtuzumab treatment was observed in people with functional ADA.⁶ We suggest that the absence of relative lymphocytosis should prompt ADA testing, enabling the earlier identification of ADA responses and reducing the risk of unnecessary morbidity associated with the delayed recognition of treatment failure.

Table 1. Frequency of induced anti-drug antibody responses to natalizumab.

Natalizumab treatment	Timepoint of test	ADA-positive		ADA-persistent		References
		<i>n</i>	%	<i>n</i>	%	
Reference-natalizumab ADA assay						
i.v. Tysabri	Every 12 w	57/625	9.1	37/625	5.9	Polman et al. ² and Calabresi et al. ⁸
i.v. Tysabri	Every 12 w	70/589	11.9	38/589	6.5	Rudick et al. ⁹
i.v. Tysabri	At 3 and 6 mo, and every 6 mo after	8/215	3.7	7/215	3.3	Oturai et al. ¹⁴
i.v. Tysabri	After relapse	5/384	1.3			Outteryck et al. ¹⁵
i.v. Tysabri	Every 4 w	9/64	14.1	6/64	9.4	Oliver et al. ¹⁶
i.v. Tysabri	Every 12 w	221/4873	4.5	172/4873	3.5	Sørensen et al. ¹⁷
i.v. Tysabri	Every 12 w	43/525	8.2	18/525 ^a	3.4	Jensen et al. ¹⁸
i.v. Tysabri	Variable (at 2–4 mo), retest ≥ 6 w after	57/1379	4.1	19/1379	1.4	Lundkvist et al. ¹⁹
i.v. Tysabri	Every 4 w	12/70	17.1	5/70	7.1	Oliver-Martos et al. ²⁰
i.v. Tysabri	At 6 mo if suspected inefficacy or AE	9/793 ^b	1.1			Outteryck et al. ²¹
i.v. Tysabri	At 1 y	21/793	2.6			Outteryck et al. ²¹
i.v. Tysabri	Within 1 y	x/3440	6.0			Bachelet et al. ²² and Link et al. ²³
i.v. Tysabri ^c	Every 12 w	2/153	1.3	2/153	1.3	Trojano et al. ²⁴
i.v. Tysabri	Variable (at 1–25 + mo), retest 2–169 d after	153/1251	12.2	58/184	31.5	Ciano-Petersen et al. ²⁵
s.c. Tysabri	Variable (at 4, 6, and 8 w, and 6 and 8 mo)	6/26	23.1	1/26	3.8	European Medicines Agency ⁵ and Plavina et al. ²⁶
s.c. Tysabri ^c	Every 12 w	0/136	0.0	0/136	0.0	Trojano et al. ²⁴
Biosimilar-natalizumab ADA assay						
i.v. Tysabri	Every 4 w	76/103	73.8			Hemmer et al. ²⁷
i.v. Tyruko	Every 4 w	104/131	79.4			Hemmer et al. ²⁷
Other/undetermined ADA assay						
i.v. Tysabri	Not specified	9/77	11.7	6/77	7.8	Fernández et al. ²⁸
i.v. Tysabri	Not specified	3/30	10.0			Fernández-Megía et al. ²⁹
i.v. Tysabri	Variable (1–24 + mo)	52/1152	4.5	18/1152	1.6	Holmén et al. ³⁰
i.v. Tysabri	At 6 mo or after hypersensitivity reaction	2/109	1.8			Krysko et al. ³¹
i.v. Tysabri	Every 6 mo	39/1005	3.9	13/1005 ^a	1.3	Piehl et al. ³²
i.v. Tysabri	If suspected inefficacy or AE	19/190 ^b	10.0			Prosperini et al. ³³
i.v. Tysabri	At 6 mo	19/285	6.7			Sangalli et al. ³⁴
i.v. Tysabri	If suspected inefficacy or AE	34/1082 ^b	3.1			Fernández et al. ³⁵
i.v. Tysabri	If suspected inefficacy or recurrent AE	3/180 ^b	1.7			Melin et al. ³⁶
i.v. Tysabri	If relapse	0/44 ^b	0.0			Alroughani et al. ³⁷
i.v. Tysabri	Every 12 w	42/73	57.5			Vennegoor et al. ³⁸
s.c. Tysabri ^c	At baseline, following switch from i.v. to s.c.	0/15	0.0			Toorop et al. ³⁹

Note. A literature review was undertaken to determine the frequency of anti-drug antibody responses in studies using the bridging ELISA created by the reference natalizumab manufacturer, which reports patented thresholds of 500 ng/mL using the 12C4 antibody equivalent and an electrochemiluminescence (ECL) assay created by the biosimilar natalizumab manufacturer, with a reported sensitivity of 3.88 ng/mL, or other or undetermined assays. ADA: anti-drug antibody; AE: adverse event; d: day(s); i.v.: intravenous; mo: month(s); s.c.: subcutaneous; w: week(s); y: year(s).

^aSome patients were not tested due to missing samples. An 'x' indicates that the specific number has not been disclosed.

^bOnly participants who met the conditions were tested.

^cStudy participants were non-natalizumab naïve patients.

Materials and methods

Literature review

A literature review was performed using the search terms ‘natalizumab’ and ‘multiple sclerosis’, ‘neutralizing antibody’, ‘anti-drug antibody’ and ‘trial’. In addition, dosing, pharmacokinetic, efficacy and immunogenicity information relating to reference natalizumab (Tysabri) and biosimilar natalizumab-sztn (Tyruko) was extracted from information within the pivotal trials^{2,9,27} and regulatory submission documents, including the United States Food and Drug Administration (FDA) Purple Book database and drug labels, the European Medicines Agency (EMA) SmPC and the United Kingdom Medicines and Healthcare products Regulatory Agency (MHRA).

Clinical trial data analysis

Access to raw anonymous individual data from the pivotal phase III AFFIRM trial (ClinicalTrials.gov: NCT00027300) of natalizumab ($n=627$) versus placebo ($n=315$) was requested and accessed under agreement using the Vivli data sharing platform. Data relating to the incidence of relapses, T2-lesion formation, presence and titre of ADAs, absolute lymphocyte counts and drug dosing were requested and provided in spreadsheet format within a cloud-based remote research environment. Analysis was performed within this workspace using R, including the calculation of descriptive statistics (mean, standard deviation, median, frequency) and statistical tests. Participants with missing values for specific variables were excluded only from those individual analyses, rather than being removed them entirely from the study.

Real-world data analysis

A retrospective analysis of people treated with natalizumab in the Cardiff MS register was performed. This included a total of 185 patients, who were treated with natalizumab from January 2008 to January 2025. Data regarding the dates of natalizumab treatment initiation and cessation, as well as lymphocyte counts before, during and after treatment, were extracted and analysed. Baseline lymphocyte count measures were excluded from the analyses if the blood draw date occurred after, or could not be verified as prior to, natalizumab initiation.

Ethical approval

The analysis of clinical trial data did not require specific ethical review beyond the approvals and informed consents obtained during the original studies, and data were provided in a pseudonymised form. The real-world analysis of

data held in the Cardiff MS register was approved by an NHS Research Ethics Committee (REC 3; refs: 05/WSE03/111 and 19/WA/0289).

Statistical analysis

Statistical analyses were performed using R version 4.3.1 or GraphPad Prism version 11.0.2 for Windows (GraphPad Software, Boston, MA, USA). Group differences were assessed using an unpaired Welch's *t*-test when comparing two groups, and Brown–Forsythe and Welch's analysis of variance (ANOVA) tests when comparing three groups. Post hoc Dunnett's T3 pairwise comparisons were performed when appropriate. Only *p*-values <0.05 were considered statistically significant. Direct comparisons between lymphocytosis and outcomes were assessed using the Wilcoxon rank-sum (Mann–Whitney U) test. Receiver operating characteristic (ROC) curve analysis was performed to determine the threshold of lymphocyte count change that best discriminated the treated subjects without ADA from comparator groups (combining untreated subjects and treated subjects with persistent ADA, or treated subjects with persistent ADA only). The optimal threshold was defined by Youden's index ($J = \text{sensitivity} + \text{specificity} - 1$). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated at selected thresholds, and 95% confidence intervals were derived using DeLong's method for the area under the curve (AUC) and bootstrap resampling for sensitivity and specificity.

Results

ADA responses and evidence of disease activity under natalizumab

Published results from the ANTELOPE trial showed that both biosimilar and reference natalizumab successfully controlled clinical and magnetic resonance imaging (MRI) activity, but there was a transient signal of the early emergence of gadolinium (Gd^+)-enhancing T1 and new/enlarging T2 lesions at week 8 that coincided with the early period of high ADA frequencies, which were typically neutralising (Figures 1(a) and (b)).^{27,43} After peaking at week 8, the rates of ADA responses and MRI activity declined thereafter, where a steady decline in ADA rates was accompanied by a pronounced drop in MRI activity. To investigate the effects of persistent ADA responses, we compared the number of relapses and new/enlarging lesions in the AFFIRM clinical trial dataset. We observed that, over a 2-year period, the persistence of ADA removed the significant effect of natalizumab in reducing the number of new and newly enlarging T2 lesions and diminished its effect in reducing relapses (Figures 1(c) to (e)).

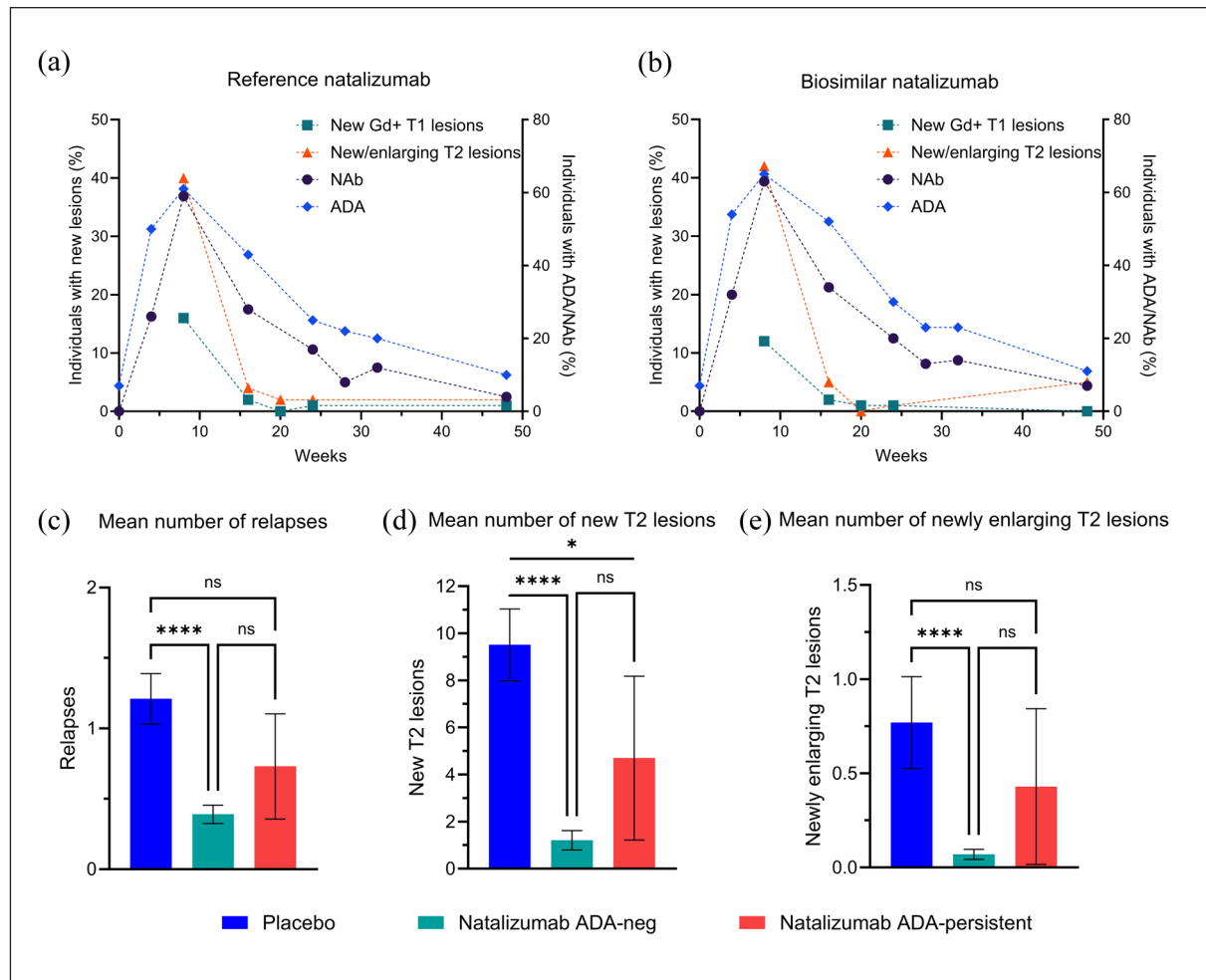


Figure 1. Anti-drug antibody responses under natalizumab treatment coincide with increased disease activity.

Note. The high frequency of natalizumab-induced anti-drug antibodies (ADAs) and neutralising antibodies (NAb) early after treatment initiation coincides with periods of higher frequencies of new MRI lesions for both (a) reference and (b) biosimilar natalizumab (data obtained from the biosimilar natalizumab regulatory documents⁴³ and the supplementary data in the ANTELOPE trial reports²⁷). Our secondary analysis of the AFFIRM clinical trial data showed that the mean number of (c) relapses, (d) new T2 lesions and (e) newly enlarging T2 lesions was decreased in people persistently negative for ADA (2-year period) ($n=568$) compared to placebo ($n=307$). Individuals with two or more positive ADA results at least 6 weeks apart were classified as 'persistent ADA' ($n=37$), as described previously.⁸ Mean $\pm$ SD values were calculated and plotted. Group differences were assessed using Brown–Forsythe and Welch's ANOVA tests, with Dunnett's T3 multiple comparisons test used for post hoc analysis.

ns = Not significant.

* $p < 0.05$; **** $p < 0.0001$.

Investigating the use of relative lymphocytosis as a biomarker of ADA responses

To investigate the impact of natalizumab treatment and ADA responses on lymphocyte counts, we analysed individual data from the AFFIRM clinical trial, sampled at 12-week intervals. As described previously,⁸ the treated individuals were stratified as no-ADA if they had no detectable antibodies at any measured timepoint; transient-ADA, if they had detectable antibodies at a single timepoint; or persistent-ADA, if they had multiple positive samples or one positive timepoint without available follow-up samples. Natalizumab quantification in plasma

showed that, at a population level, drug levels were above the receptor saturation level in people without ADA, but the presence of ADA delayed or prevented the maintenance of therapeutic drug levels (Figure 2(a)).

The absolute change in lymphocyte counts was calculated intraindividually by subtracting baseline measures from each timepoint measure, and the mean change values for each group across timepoints were plotted (Figure 2(b)). The comparison of the placebo and the treated group revealed that natalizumab initiation caused a marked increase in the levels of circulating lymphocytes by the first timepoint tested at 12 weeks (mean change in natalizumab 1.40 ± 0.92 ($n=572$) vs placebo -0.05 ± 0.62

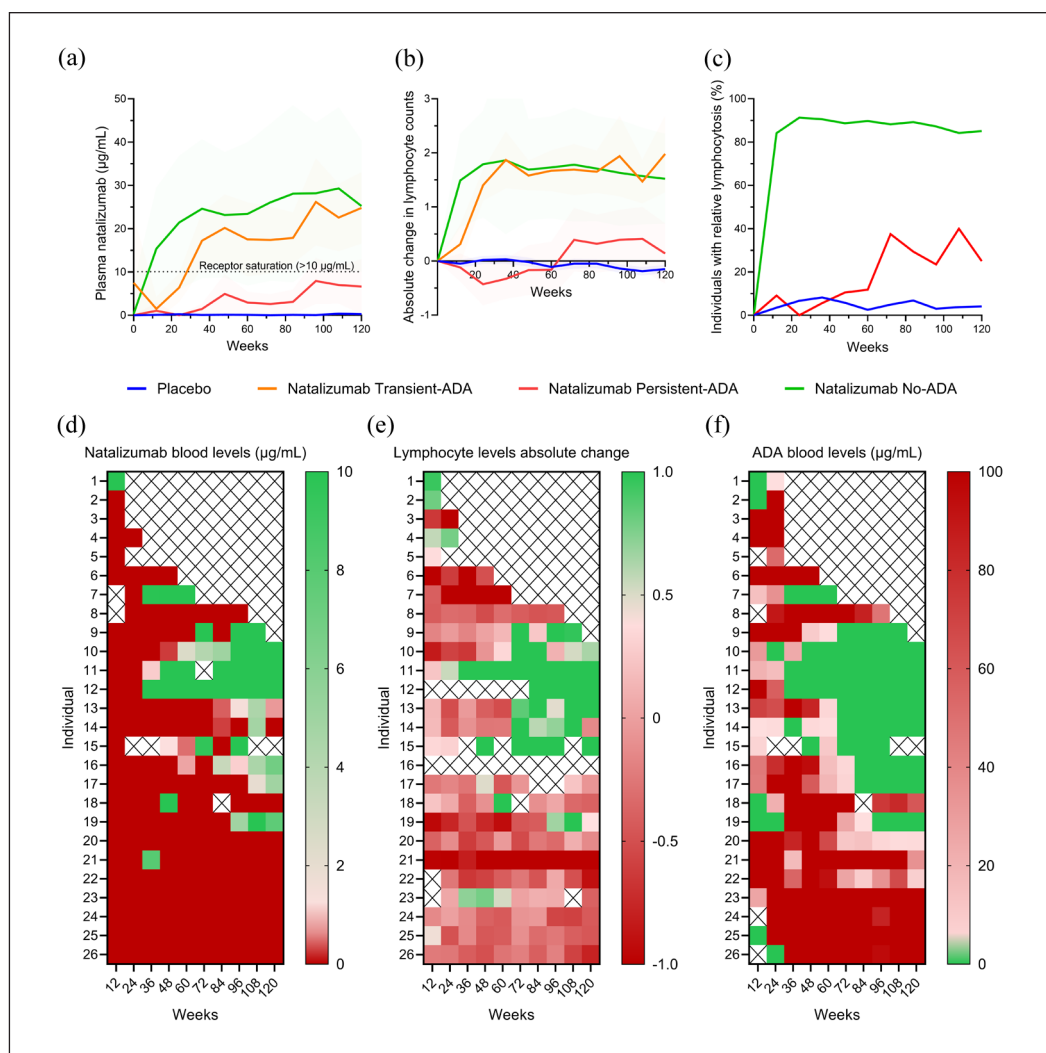


Figure 2. Natalizumab treatment induces an increase in lymphocyte counts that is inhibited by anti-drug antibody responses.

Note. Re-analysis of the AFFIRM clinical trial data, including lymphocyte absolute counts and anti-drug antibody levels, was performed. The results represent (a) mean trough circulating natalizumab levels, (b) absolute change in the lymphocyte count from the baseline and (c) percentage of individuals with relative lymphocytosis at or above the 0.8×10^9 cells/L threshold, at a group level (placebo ($n=307$), natalizumab without reported ADA (natalizumab-no ADA; $n=568$), transient ADA (natalizumab-transient ADA; $n=20$) and persistent ADA (natalizumab-persistent ADA; $n=37$)). Individual data for the $n=26$ individuals classified as 'persistent ADA' (according to the definition established previously)⁸ with available data on lymphocyte counts beyond the baseline, over time, are presented for (d) natalizumab blood levels, (e) absolute change in lymphocyte counts and (f) ADA levels. Participant number 16 did not have an available baseline measure for lymphocyte counts.

($n=288$); Welch's unpaired t -test $p < 0.0001$). This comparison did, however, mask the effect that ADA responses had on lymphocyte levels, as a three-way comparison of the placebo, transient-ADA and persistent-ADA groups revealed that, by the 12-week timepoint, there were no differences between these three groups regarding changes in lymphocyte counts (mean change in placebo -0.05 ± 0.62 ($n=288$) vs transient-ADA 0.31 ± 0.66 ($n=15$) vs persistent-ADA -0.12 ± 0.60 ($n=22$); Welch's ANOVA $p=0.11$). By week 24, however, differences between the groups emerged (Welch's ANOVA $p < 0.0001$). Post hoc Dunnett's T3 analysis revealed that the transient-ADA group experienced a significantly greater increase

in lymphocyte counts (1.40 ± 0.56 ($n=17$)) than both the placebo (0.02 ± 0.64 ($n=285$); adjusted $p < 0.0001$) and the persistent-ADA (-0.43 ± 0.87 ($n=21$); adjusted $p < 0.0001$) cohorts, while no significant difference was found between the placebo and persistent-ADA groups (adjusted $p=0.0759$). This effect was maintained over time, highlighting the absence of lymphocytosis in the context of ADA responses, which coincided with low levels of circulating drug in plasma (Figures 2(a) and (b)).

An ROC curve analysis was performed to identify the threshold at which an absolute change in lymphocyte counts best discriminated natalizumab-treated patients without ADA from a combined group of placebo-treated

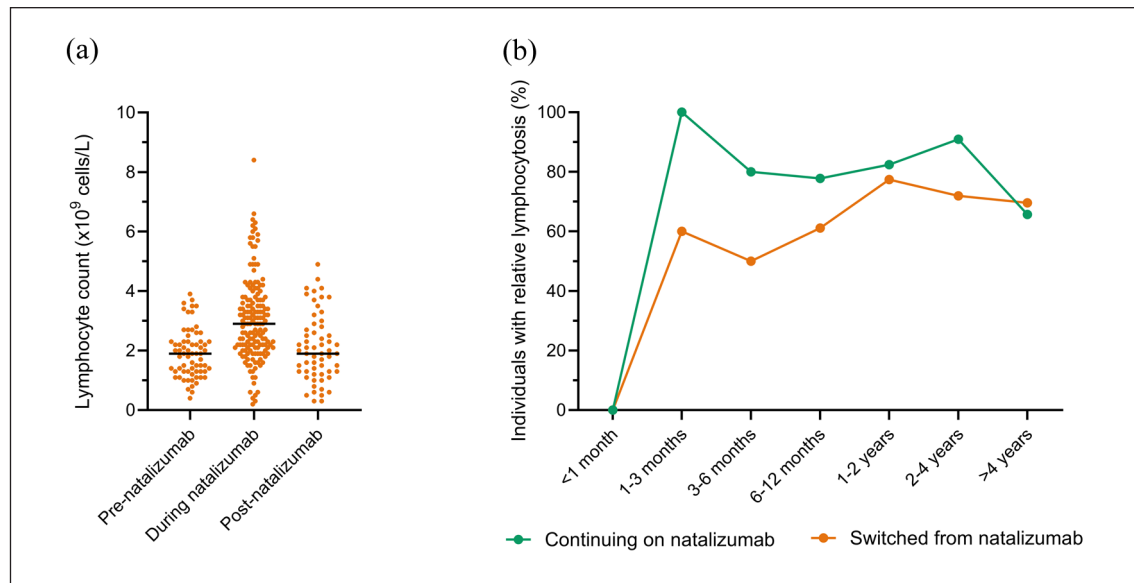


Figure 3. Real-world monitoring of lymphocyte counts in people with MS treated with natalizumab.

Note. A retrospective analysis of lymphocyte counts before, during and after natalizumab treatment was performed in $n = 185$ people with MS treated with natalizumab between 2008 and 2025 (Cardiff MS register). (a) Absolute lymphocyte counts increase upon natalizumab treatment initiation and decrease after natalizumab cessation. (b) The proportions of people with relative lymphocytosis were comparable to those seen in the analysis of the AFFIRM clinical trial in people who continued natalizumab treatment ($n = 67$), but people who ultimately switched from natalizumab during follow-up for any reason ($n = 68$) were less likely to have shown relative lymphocytosis, measured as an increase of $\geq 0.8 \times 10^9$ cells/L, during natalizumab treatment. All measurements shown were derived from samples taken while exposed to natalizumab. Suitable paired measurements for the relative lymphocyte count analysis were available for only $n = 135/185$ individuals.

patients and natalizumab-treated patients with persistent ADA. Thresholds were evaluated at weeks 12, 24 and 36, given that relative lymphocytosis is evident from week 12 in treated individuals (Figure 2(b)). At these timepoints, Youden's J statistic revealed the optimal thresholds to be 0.61 , 0.80 and 0.79×10^9 cells/L, respectively. As a single fixed threshold is more practical for clinical use than a timepoint-specific value, 0.8×10^9 cells/L was selected after the assessment of a range of candidate thresholds (0.6 – 0.9×10^9 cells/L; Supplemental Table S1), which performed consistently well across the tested timepoints (sensitivity 0.84 – 0.91 ; specificity 0.92 – 0.96 ; Supplemental Table S2). Based on this threshold, the peripheral increase in lymphocyte counts was sustained over time in the no-ADA group, while the persistent-ADA group showed increased frequencies of lymphocytosis over time (Figure 2(c)). This likely reflected the waning of ADA responses in some of the individuals classified as persistent-ADA, as shown in the individual-level data in which the emergence of ADA coincided with an absence of lymphocytosis and null/low circulating natalizumab levels (Figures 2(d) to (f)).

The inclusion of the placebo group in this comparator helps characterise the pharmacodynamic effect of active drug exposure, but it is not representative of ADA detection. We, therefore, repeated the ROC curve analysis restricted to the natalizumab-treated patients only (ADA-negative vs ADA-persistent), using the same fixed

threshold of 0.8×10^9 cells/L. Discrimination remained strong (AUC 0.96 – 0.99 ; sensitivity 0.84 – 0.91 ; specificity 0.91 – 1.00 ; Supplemental Table S2), but importantly, the precision of these estimates is limited by the small number of ADA-persistent patients with available data ($n = 22$, 21 and 18 at weeks 12, 24 and 36, respectively).

Absence of lymphocytosis and disease activity

Beyond its proposed use as a marker of potential ADA, we also examined whether a direct association between the absence of lymphocytosis and markers of clinical or neuroradiological activity existed. To investigate this, we mirrored the definition of 'persistence' from the ADA measures onto lymphocytosis, whereby the persistent absence of lymphocytosis was defined as failure to reach the 0.8×10^9 cells/L threshold in two or more assessment visits, or one if there was only one assessment measure available. Using the data from the AFFIRM clinical trial, participants classified as having a persistent absence of lymphocytosis over the 2-year study period ($n = 152$) had higher relapse counts (mean 0.49 vs 0.38 ; Wilcoxon's $p = 0.049$) and new T2 lesion counts (mean 1.93 vs 1.19 ; $p = 0.018$) compared to those without persistent absence ($n = 441$). A similar trend was observed for new enlarging T2 lesions (mean 0.13 vs 0.07), though this did not reach statistical significance ($p = 0.065$).

Monitoring of lymphocytes in the clinic

A retrospective analysis of lymphocyte counts before, during and after natalizumab treatment was performed in the Cardiff MS register ($n=185$, treated in 2008–2025). Mean lymphocyte counts before natalizumab initiation were 1.8×10^9 cells/L, increasing to 3.0×10^9 cells/L during treatment (measured at varying timepoints; mean: 38 months; range: 1–190 months after treatment initiation) and decreasing to 1.9×10^9 cells/L at least 1 month after natalizumab discontinuation (mean: 12.8 months; range: 1–51 months; all samples taken before the commencement of subsequent therapy) (Figure 3(a)). Data were further analysed to compare intraindividual changes in lymphocyte counts between individuals with suitable paired measurements who had discontinued natalizumab ($n=68$) and those who had not ($n=67$). Interestingly, people who ultimately stopped natalizumab treatment during follow-up were less likely to have had evidence of relative lymphocytosis (measured as an increase of $\geq 0.8 \times 10^9$ cells/L) during treatment, especially early after natalizumab initiation (Figure 3(b)). This effect was true for the entire switched population, irrespective of the reason for switching. Further analyses based on the reason for discontinuation could not be investigated, as this information was only available for $n=26/185$ individuals.

Discussion

The AFFIRM and SENTINEL trials showed that the development of persistent ADA under natalizumab treatment was prognostic for subsequent disease activity.⁸ In addition, there is a therapeutic lag upon natalizumab initiation,⁴⁷ which could be explained by the early incidence peak of ADA responses. While ADA responses wane over time in most people, a small minority of people never properly respond to natalizumab due to persistent ADA. According to current advice,^{4,5} it will take at least 6–9 months to detect and respond to a persistent ADA response. Improved guidance on ADA testing and monitoring could lead to more personalised decision-making, such as higher vigilance for breakthrough disease activity in people with persistent ADA.

Natalizumab-induced lymphocytosis has been previously suggested as a biomarker of natalizumab efficacy.⁴⁸ Our re-analysis of the AFFIRM clinical trial data showed that an increase in lymphocyte counts of $\geq 0.8 \times 10^9$ cells/L relative to the baseline was a useful marker of ADA responses, where the comparison of natalizumab-treated individuals without ADA and with persistent ADA responses had strong discriminative performance (AUC 0.96–0.99) and consistently high sensitivity (0.84–0.91) and specificity (0.91–1.00). Due to the small size of the ADA-persistent group, as well as the prevalence-dependent nature of these artificially constructed comparator

groups, these values and the positive/negative predictive values (PPV and NPV) should be interpreted as indicative rather than definitive, pending validation in larger, real-world cohorts. Nonetheless, it was evident that natalizumab induces relative lymphocytosis that is absent in individuals with ADA, and ADA responses coincided with the absence of relative lymphocytosis and low levels of the circulating drug. To improve treatment response monitoring, we propose an approach in which lymphocyte counts during natalizumab treatment are reviewed against pre-treatment baseline measures, and failure to demonstrate an increase in lymphocyte count on at least two consecutive measurements, particularly by week 12, should prompt ADA testing. This represents a provisional approach requiring prospective validation, rather than established clinical guidance. Further analyses on large, real-world datasets that incorporate other clinical practice standards (e.g. biosimilars, subcutaneous formulations, extended-interval dosing regimens) could help optimise and validate the lymphocyte change threshold. Analysis of samples taken at shorter intervals (e.g. every 4 weeks) may allow further refinement of recommendations. Moreover, it may be possible to further improve the value of lymphocyte monitoring by assessing B cell numbers. Despite increasing circulating levels of lymphocytes, total lymphocyte numbers often remain within the normal range (below the upper limit of normal) under natalizumab treatment, resulting in considerable overlap between untreated and treated individuals.⁴⁹ In addition, the effects of VLA-4 blockade driven by natalizumab are more pronounced on B cells than on the T cell pool.⁵⁰

The absence of lymphocytosis was independently associated with markers of clinical or neuroradiological activity (i.e. higher relapse and new T2 lesion counts but not new enlarging T2 lesions). For these exploratory analyses, we mirrored the definition of ADA ‘persistence’ onto lymphocytosis, defining the persistent absence of relative lymphocytosis as the failure to reach the 0.8×10^9 cells/L threshold in two or more assessment visits, or in one if there was only one measure available. However, unlike a positive ADA result, a single low-lymphocyte reading can arise from physiological fluctuation (e.g. infection, stress and other medications), and thus, a transient increase or decrease unrelated to the treatment effect may be incidentally captured. This is illustrated by the disparity in the number of individuals in the ADA-persistent and persistent lymphocytosis absence group classifications ($n=26$ vs $n=152$, respectively, in the 2-year study period). While these exploratory analyses provide some evidence that the persistent absence of relative lymphocytosis may be associated with greater disease activity, confirmation using prospectively defined sampling schedules and adjusted longitudinal analyses is warranted. It is also possible that a proportion of individuals with clinically significant ADA were missed in this dataset, as ADA frequencies are

assay- and threshold-dependent, and the ELISA used here has a reported false-positive rate of approximately 5% (Supplemental Figure S1),¹⁰ with false positives documented even in pre-dose samples.¹⁹ Conversely, factors independent of ADA, such as increased natalizumab clearance rates, may also contribute to suboptimal drug exposure and could be captured by lymphocyte monitoring in ADA-negative individuals.

Conclusion

The data presented here suggest that focused monitoring of lymphocyte counts, regularly undertaken as part of standard of care, may have the potential to guide ADA testing in people with MS treated with natalizumab. Evidence of the persistent absence of lymphocytosis across consecutive infusions could represent a candidate biomarker to prompt ADA testing before disease breakthrough occurs, potentially reducing the risk of unnecessary morbidity associated with the current guidance. Prospective validation of this biomarker in additional cohorts, particularly those reflecting current natalizumab use practices (e.g. extended interval dosing, subcutaneous formulations), could help clarify its utility in clinical practice in relation to ADA responses and clinical and MRI outcomes.

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Data availability statement

The dataset(s) from the Biogen trial(s) is/are made available through a controlled access method that follows best practices of sharing human-subjects clinical research data. This method protects participants' privacy and the request has been approved by an independent review panel. To request the data, researchers should apply using this same controlled access method via <https://vivli.org/>. The analysis code is supplied in the supplemental materials.

Supplemental material

Supplemental material for this article is available online.

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