Eosinophilia during natalizumab treatment: incidence, risk factors and temporal patterns

Milad-Kazava Keshvari[1,2*], Frederick van Someren[1,2*], Saima Sheikh[1,2*], Ian Galea[1,2]

[1] Clinical Neurosciences, Clinical & Experimental Sciences, Faculty of Medicine, University of Southampton

[2] Neurology Department, Wessex Neurological Centre, Southampton General Hospital

* these authors contributed equally to this work

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Corresponding author:

Ian Galea

Professor of Clinical & Experimental Neurology

Clinical Neurosciences, Clinical & Experimental Sciences

Faculty of Medicine, University of Southampton

Mailpoint 806, Level D

Southampton General Hospital

Southampton SO16 6YD

UK

I.Galea@soton.ac.uk

Abstract

Eosinophilia is common during natalizumab treatment for multiple sclerosis but risk factors are unknown. We aimed to identify demographic, clinical and laboratory characteristics predicting eosinophilia. Sustained eosinophilia occurred in 16.8%. Risk factors for sustained eosinophilia included baseline pre-treatment eosinophilia, medical conditions potentially associated with eosinophilia including allergies, and suboptimal compliance. One temporal profile was associated with the highest and most rapidly developing eosinophilia, and was less likely to resolve: in one such case, eosinophilia was symptomatic. Changes in eosinophil and lymphocyte counts were only weakly correlated, suggesting factors other than Very Late Antigen-4 (VLA-4) inhibition drive eosinophilia.

1. Introduction

Natalizumab is a humanised monoclonal antibody against α4β1-integrin (very late antigen-4, VLA-4) and α4β7-integrin, and has been shown to be highly effective in the treatment of relapsing-remitting multiple sclerosis (RRMS) (Pucci et al., 2011). It is indicated for rapidly evolving severe RRMS and highly active disease despite a full and adequate course of treatment with at least one disease modifying therapy (Biogen, 1-May-2021). Natalizumab's primary mechanism of action in MS is by blocking the binding of VLA-4 on lymphocytes to endothelial cell receptors (vascular cell adhesion protein 1, VCAM-1), thus hindering lymphocyte migration across the blood-brain barrier (Yednock et al., 1992).

The most significant side effect of natalizumab is an increased risk of progressive multi-focal leukoencephalopathy (PML) in people carrying the JC virus (McGuigan et al., 2016). A slightly increased risk of infection with herpetic viruses occurs (Luna et al., 2020). Fifteen cases of primary central nervous system lymphoma have been associated with natalizumab treatment (Sartori and Grundmark, 2018). Other side effects include drug-induced liver injury (Antezana et al., 2015), hypersensitivity reactions, and anaphylaxis. Haematopoietic abnormalities are observed but these are non-consequential (van Rossum et al., 2019). Natalizumab treatment results in increases in circulating numbers of lymphocytes, eosinophils and sometimes monocytes (Bridel et al., 2015; Kaufmann et al., 2018) and these haematological abnormalities are usually reversible on stopping natalizumab (Plavina et al., 2017).

Eosinophilia is defined as the elevation of blood eosinophil count $>0.5 \times 10^9$ per litre, whilst hypereosinophilia is defined as eosinophil count $>1.5 \times 10^9$ per litre for longer than six months (Butt et al., 2017). A persistently raised eosinophil count occurs in several eosinophilic infiltrative diseases sometimes accompanied by multi-systemic end organ damage. Asymptomatic eosinophilia and hypereosinophilia during natalizumab treatment is not uncommon (Abbas et al., 2011). The mechanisms driving natalizumab-associated eosinophilia have not been studied, but it is thought that expression of VLA-4 by eosinophils plays a major role. In some cases, natalizumab-associated eosinophilia is associated with symptomatic tissue infiltration. Pulmonary eosinophilia may present with cough, wheezing, fever, dyspnoea and/or respiratory failure with pulmonary infiltrates on lung imaging and eosinophils in bronchoalveolar lavage or transbronchial biopsy (Curto et al., 2016; Diem et

al., 2021; Yasuda et al., 2019). Generalized skin eruption (Andre et al., 2010) and eosinophilic fasciitis (Bujold et al., 2014) have been reported. It is important to note that any degree of eosinophilia (not just hypereosinophilia) may accompany symptomatic tissue infiltration during natalizumab treatment e.g. pulmonary eosinophilia has been reported with a blood eosinophilia of 0.49 x 10^9 per litre (Curto, 2016). In these cases, it was not clear whether symptomatic tissue infiltration developed after a period of asymptomatic eosinophilia or hypereosinophilia.

Some studies have followed eosinophil counts with time in aggregate (Bridel, 2015; Kaufmann, 2018; Plavina, 2017); none studied temporal profile patterns in individuals. A recent study found that natalizumab eosinophilia had an incidence of 38%, much higher compared to untreated people with MS (PwMS) (3%), and the eosinophil level was related to symptoms (Diem, 2021). The study authors emphasize that the risk factors for development of natalizumab-associated eosinophilia are unknown (Diem, 2021). There are no guidelines regarding the appropriate frequency of blood counts in natalizumab-treated PwMS and several centres monitor blood counts regularly. For these reasons, we evaluated our natalizumab infusion service to determine patterns of eosinophilia in individual PwMS and identify risk factors for eosinophilia to help with reducing cost, human resource use and anxiety related to frequent full blood count monitoring. The timeliness of this study is underscored by the recent approval of a subcutaneous formulation of natalizumab, which will increase its use due to ease of administration and patient acceptability.

2. Methods

2.1. Study design

This was a retrospective observational service evaluation of blood test monitoring of PwMS receiving intravenous natalizumab at the Wessex regional MS service at the Wessex Neurological Centre at the University Hospital Southampton National Health Service Foundation Trust. Eligibility criteria included: a diagnosis of RRMS according to McDonald criteria (Thompson et al., 2018), administration of at least three doses of natalizumab in the period August 2007 to April 2019. PwMS receiving natalizumab were identified by consulting the MS service database and searching electronic records. Demographic and clinical data were collected, as well as all blood count results performed during the period starting from six months prior to the first natalizumab infusion until the last infusion or

January 2020. Full blood counts were performed at least every three months, or more frequently, on a Sysmex XN10 analyser.

2.2. Definitions

MS duration was defined as time from development of the first symptom associated with RRMS until initiation of treatment with natalizumab. Pre-natalizumab medical conditions which may be potentially associated with eosinophilia were defined as those listed in the British Society for Haematology's 2017 guideline for the investigation and management of eosinophilia (Butt, 2017). Allergies were divided into non-drug allergy (e.g. food allergy) and drug allergy (e.g. penicillin); a group "any allergy" included both types of allergy. Mobility, employed as a surrogate measure of disability, was categorized into four groups as independent, and requiring unilateral support, bilateral support or a chair. Eosinophilia was defined as present if eosinophil count was greater than 0.5×10^9 per litre (Butt, 2017), subdivided into 'mild' if greater than 0.5×10^9 per litre but less than 1.5×10^9 per litre, 'moderate' if greater than 1.5×10^9 per litre but less than 5×10^9 per litre, and 'severe' if greater than or equal to 5×10^9 per litre. Since symptomatic eosinophilic tissue infiltration may be associated with any level or duration of eosinophilia, persistent eosinophilia was defined as a function of eosinophil level and duration, in three ways: greater than 0.5×10^9 per litre for at least three months, greater than 1.5×10^9 per litre for at least three months, and greater than 1.5×10^9 per litre for six months or more. We defined isolated peaks as a single eosinophil count greater than 0.5×10^9 per litre occurring only once throughout treatment, and isolated troughs as a single eosinophil count less than 0.5×10^9 per litre occurring only once throughout treatment. Lymphocytosis was defined as a lymphocyte count greater than 4×10^9 per litre and lymphopenia was defined as a lymphocyte count less than 1.5×10^9 per litre, according to our local laboratory reference range. Persistent lymphocytosis was defined as a function of lymphocyte level and duration, in two ways: as greater than 4×10^9 per litre for three months or more, and six months or more.

2.3. Statistical methods

SPSS (version 27.0.0.0, IBM Corp., Armonk, NY) was used for all statistical analyses. Descriptive statistics were conducted on all the study population (n=196). Data distribution was assessed for normality, so that parametric and non-parametric tests could be conducted as indicated. To identify baseline risk factors for natalizumab-induced persistent eosinophilia (defined as eosinophil count of $>0.5 \times 10^9$ per litre for ≥90 days), the *a priori* planned

primary analysis was multivariable logistic regression in cases who had received natalizumab for at least a period equivalent to the median time to eosinophilia. A sensitivity logistic regression analysis using the whole study population was also conducted. Univariable logistic regression was performed first, and variables with $p<0.1$ were taken forward into multivariable logistic regression. Within-subject correlation coefficients were calculated from the sum of squares of analysis of covariance, correcting for subjects (Bland and Altman, 1995). Null hypotheses were rejected at $p < 0.05$.

3. Results

3.1. Baseline characteristics

A total of 202 PwMS were identified during the study period and 196 cases were available for analysis (Figure 1).

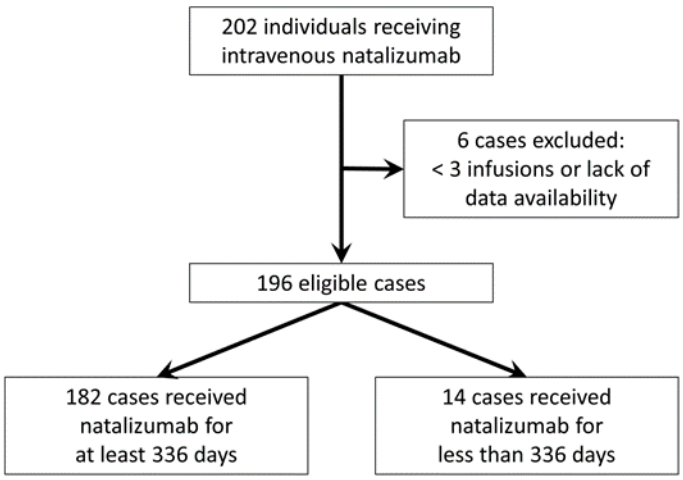

Figure 1. Flowchart of cases in study.

Baseline demographic and clinical characteristics are shown in Table 1.

Age (years)*	37.0 (29-44)
MS duration (years)*	5.0 (1.5-10.1)
Sex (female)[#]	141 (71.9%)
Disease-modifying treatments prior to natalizumab[#]	99 (51.3%)
Anti-JC virus antibody positive prior to natalizumab[#]	13 (6.7%)

Mobility[#]	
Bilateral help required	15 (7.7%)
Unilateral help required	37 (18.9%)
Chair required	6 (3.1%)
Walk unaided	138 (70.4%)
Comorbidity associated with eosinophilia[#]	24 (12.4%)
Allergies[#]	
Drug allergy	31 (15.8%)
Non-drug allergy	52 (26.6%)
Any allergy	69 (35.2%)
New MS lesion(s) whilst on NTZ[#]	22 (12.4%)
Smoking[#]	
Smoker	49 (25.0%)
Ex-smoker	35 (17.9%)
Never smoked	112 (57.1%)
Duration of natalizumab treatment (years)*	4 (2.1-5.9)
Number of natalizumab doses missed*	0 (0-0)
PwMS on alternate infusions of natalizumab (bimonthly, vs monthly)[#]	6 (3.1%)
JC seroconversion after starting natalizumab[#]	56 (29.0%)
PwMS who missed doses of natalizumab[#]	44 (22.4%)
Frequency of full blood count monitoring*	3.2 (2.6-4.1)

Table 1. Baseline demographics and clinical characteristics of the whole cohort (n=196). Values are median (inter-quartile range)* or number of PwMS (percentage)[#]. Information

was available for 188, 193, 193 and 177 PwMS, for MS duration, previous disease-modifying treatment, anti-JC virus antibody positivity prior to natalizumab, and new lesions whilst on natalizumab respectively. The range for the number of natalizumab doses missed was 0-40.

3.2. Incidence of eosinophilia

Eosinophils prior to and during natalizumab treatment in the whole cohort were analysed (Table 2). Eosinophil count increased after starting natalizumab, compared to baseline (Wilcoxon signed rank test, $p<0.001$). The number of PwMS with any level of eosinophilia at any time increased from 10 (5.1%) prior to starting natalizumab to 72 (36.7%) during natalizumab treatment ($\chi^2=5.017$, $p=0.025$). Eosinophilia persisted at some level for longer than three months in 16.8% (33 PwMS). Of these 33 cases, 5 (15%) had pre-existent eosinophilia prior to starting natalizumab. Hypereosinophilia was encountered in two individuals, with the highest count being 8.3×10^9 per litre. In one of these individuals hypereosinophilia became symptomatic after treatment with natalizumab infusions for 4.5 years (38 doses, 70% adherence to a planned four weekly schedule), during which a slow-rising eosinophilia was noted. Symptoms consisted of a three-month history of progressive dyspnoea, myalgia, malaise and gastrointestinal disturbance. At that point, eosinophil count had increased from 5.4×10^9 per litre to a peak of 8.3×10^9 per litre. Chest X-ray and pulmonary function tests were normal. Natalizumab therapy was stopped, resulting in a complete resolution of symptoms within two months, and complete normalization of eosinophil count within nine months. In the other case with hypereosinophilia, eosinophil levels fluctuated between 1.5 and 5.1×10^9 per litre, the PwMS remains asymptomatic and natalizumab treatment continues with monitoring.

Median eosinophil count during the six months prior to natalizumab*	0.13 (0.1-0.25)
Median eosinophil count on natalizumab*	0.27 (0.2-0.4)
Any eosinophilia during the six months prior to natalizumab[#]	10 (5.1%)
Mild eosinophilia during the six months prior to natalizumab[#]	9 (4.6%)
Moderate eosinophilia during the six months prior to natalizumab[#]	1 (0.5%)
Severe eosinophilia during the six months prior to natalizumab[#]	0 (0%)

Any eosinophilia on natalizumab[#]	72 (36.7%)
Mild eosinophilia on natalizumab[#]	64 (32.7%)
Moderate eosinophilia on natalizumab[#]	6 (3.1%)
Severe eosinophilia on natalizumab[#]	2 (1.0%)
Persistent eosinophilia on natalizumab >0.5 for ≥ 90 days[#]	33 (16.8%)
Persistent eosinophilia on natalizumab >1.5 for ≥ 90 days[#]	5 (2.6%)
Persistent eosinophilia on natalizumab >1.5 for ≥ 180 days[#]	2 (1.0%)
Isolated trough on natalizumab (eosinophil count < 0.5)[#]	7 (3.6%)
Isolated peak on natalizumab (eosinophil count > 0.5)[#]	24 (12.2%)

Table 2. Eosinophil counts during the six months preceding natalizumab treatment and during natalizumab treatment in the whole cohort. Values are median (inter-quartile range) of individual means* or number of PwMS (percentage)[#].

3.3. Temporal profile of eosinophilia

The median time to onset of eosinophilia relative to starting natalizumab was 336 days (inter-quartile range: 672 days), equivalent to 11 doses of natalizumab. Peak eosinophil count was reached within a median of 588 days (inter-quartile range: 1024). The eosinophil count temporal profile of each individual PwMS over the duration of their treatment was visually assessed. No single pattern could be found with some PwMS peaking at the start, some at the end or some with a relative steady increase (Figure 2): an "early peak" group (n = 13, peak eosinophil count <19 months after commencing natalizumab), a "slow rise - late peak" group (n = 9, peak eosinophil count >19 months after commencing natalizumab), and a "stable eosinophilia" group (n = 9, low variation in eosinophil count after establishment of eosinophilia, irrespective of peak). The temporal profile of two PwMS with eosinophilia did not fall into any of these three categories. Typically, individuals with an "early peak" exhibited eosinophilia early in their treatment course which would promptly resolve, whereas individuals in the "slow rise - late peak" group appeared less likely to achieve a resolution of their eosinophilia, as indicated by their slopes (Figure 2). The rate of change of eosinophilia over time (the slopes of temporal profiles from individual PwMS) was steepest and most

positive in the “slow rise - late peak” pattern. The “slow rise - late peak” pattern was associated with the highest level of eosinophilia (Mann-Whitney U, p=0.024 versus other patterns). Of the two PwMS with hypereosinophilia (>1.5 x 10^9 per litre), only the individual with symptoms exhibited a “slow rise - late peak” pattern.

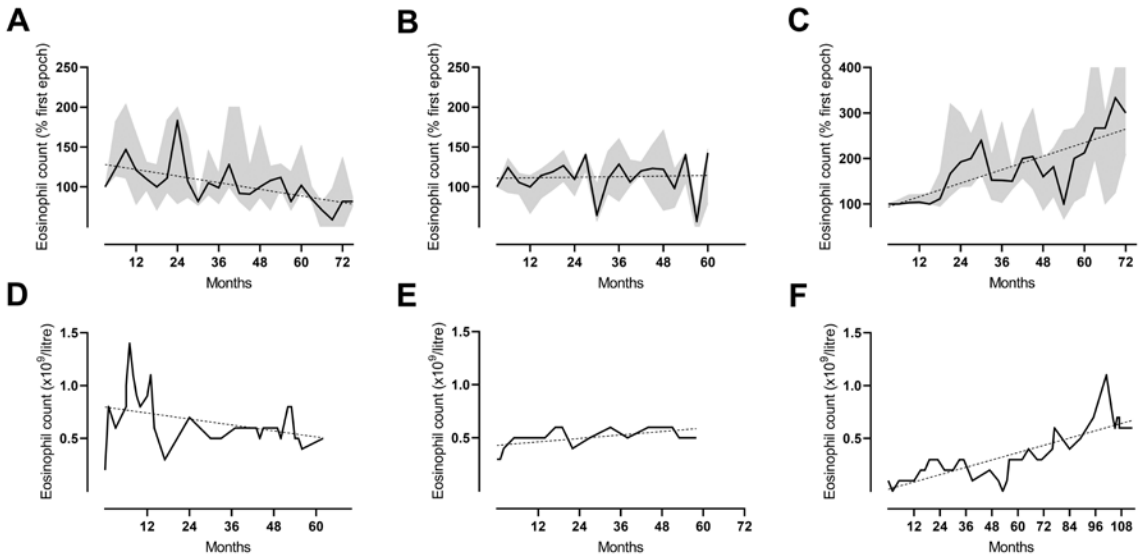

Figure 2. Eosinophilia temporal profiles on natalizumab treatment. Profiles fall into three patterns: “early peak” (A, D), “stable” (B, E), and “slow rise - late peak” (C, F). The upper row of panels (A, B, C) shows the median and inter-quartile range of post-natalizumab eosinophil counts of individuals with eosinophilia within each temporal pattern. To account for variation of initial eosinophil count values and blood test timing between individuals, data is expressed as percentage of the eosinophil count in the first three month epoch. The lower row of panels (D, E, F) shows an example individual from each pattern. The dotted line in each figure represents the trend.

3.4. Relationship of eosinophilia with lymphocytosis

Since lymphocytosis is also common during natalizumab treatment, this was also studied (Supplementary Table 1), with the aim of establishing whether it was correlated with eosinophilia within individuals. We reasoned that if it was indeed the case that VLA-4 inhibition was the only factor driving eosinophilia, one would expect to find concordance between eosinophilia and lymphocytosis within individuals, similar temporal profiles of eosinophil and lymphocyte counts, and a strong correlation between the levels of these two white cell populations. Lymphocyte count increased after starting natalizumab treatment

(Wilcoxon signed rank test, p<0.001). The number of individuals with any level of lymphocytosis increased from 12 (6.1%) prior to starting natalizumab to 96 (49.2%) on natalizumab treatment (χ^2=13.046, p<0.001). Lymphocytosis persisted at some level for longer than three months in 32.1% (63 PwMS). The highest lymphocyte count encountered was 6.0 x 10^9 per litre. Natalizumab was never discontinued because of lymphocytosis.

Of the 33 PwMS who developed persistent eosinophilia (greater than 0.5 x 10^9 per litre for at least three months), 15 did not have persistent lymphocytosis (greater than 4 x 10^9 per litre for three months or more); the discordance was significant (χ^2=9.131, p=0.003). The median time to onset of lymphocytosis was 137 (inter-quartile range: 492) days after starting natalizumab i.e. before that of eosinophilia (336 days). Lymphocytes reached their peak within a median of 675.5 (inter-quartile range: 1229) days i.e. after that of eosinophilia (588 days) and exhibited a flat profile in comparison to eosinophils (Supplementary Figure 1). Within subject eosinophil and lymphocyte counts were only weakly correlated (r=0.23, p<0.001, n=196). In individuals with eosinophilia, the rate of change of eosinophil and lymphocyte counts were only moderately correlated (r=0.543, p<0.001).

3.5. Predictors of eosinophilia

Baseline demographic, clinical and laboratory characteristics were investigated for their role to predict eosinophilia, in those PwMS who had natalizumab for at least 11 months (the median time to onset of eosinophilia). Supplementary Table 2 shows the results of univariable logistic regressions for each characteristic. In order of effect size, baseline eosinophilia, medical conditions potentially associated with eosinophilia including allergies, and higher percentage of missed natalizumab doses, were more likely to be associated with eosinophilia. In multivariable logistic regression (Table 3) the same variables were associated with post-natalizumab eosinophilia. Similar results were observed in a sensitivity analysis including all 196 PwMS, irrespective of natalizumab treatment duration (Supplementary Tables 3 & 4).

Independent variable	**p**	**Odds ratio**	**95% confidence interval**

Number of disease-modifying treatments prior to natalizumab	0.347	0.713	0.353 to 1.443
Percentage of natalizumab doses missed	**0.005**	1.058	1.017 to 1.101
Non-drug allergy	**0.019**	3.433	1.226 to 9.612
Comorbidity associated with eosinophilia	**0.014**	4.359	1.346 to 14.125
Mean eosinophil count six months prior to natalizumab	**<0.001**	326.104	19.481 to 5458.854

Table 3. Multivariable logistic regression for predictors of natalizumab-associated eosinophilia (>0.5 x 10^9 per litre) sustained ≥90 days, in PwMS who had received natalizumab for at least 11 months (n=179, Hosmer Lemeshow test = 0.300, Nagelkerke pseudo-R square = 0.348).

4. Discussion

In this study, we show that eosinophilia during natalizumab treatment is common, occurring in 36.7% which is strikingly similar to a recent study (Diem, 2021). It persisted for three months or more in 16.8% of cases. Median time to onset of 11 months is consistent with a study of 48 PwMS followed for 18 months (Bridel, 2015). Whilst eosinophilia is often mild and self-limiting, it has the rare potential to cause morbidity and disrupt treatment as seen here in one case.

Three distinct temporal profiles of eosinophilia were identified, namely an “early peak”, a “slow rise - late peak” and a “stable eosinophilia”. The “early peak” was the most prevalent pattern, in 39% of cases, with eosinophilia commencing early in treatment and reaching its peak rapidly, most often in the first year of treatment, followed by a decline in eosinophil count, possible reflecting desensitization. The “early peak” and “stable eosinophilia” appeared indolent, the need for routine full blood count monitoring in these cases is questionable, and the recognition of these patterns prevents unnecessary referrals for haematological investigation. On the other hand, the “slow rise - late peak pattern” was associated with a gradual rise in eosinophils, the highest level of eosinophilia, and the

steepest positive slope, possibly reflecting an acquired sensitisation. In these cases, enquiry about systemic symptoms and regular blood count monitoring is advised.

Risk factors for the development of sustained eosinophilia included baseline pre-treatment eosinophilia, comorbidities potentially associated with eosinophilia including allergies, and a higher percentage of missed natalizumab doses. These findings were robust to multivariable regression and sensitivity analyses. These risk factors will help formulate guidance regarding frequency of full blood count monitoring.

The association of eosinophilia with intermittent natalizumab treatment, but not extended interval dosing, is interesting. A causative association is possible since an allergic response to natalizumab is more likely to occur with intermittent treatment compared to continuous drug administration (Strand et al., 2017), akin to prime-boost vaccination. On the other hand, reverse causation is possible i.e. non-adherence to planned treatment schedule secondary to subtle unrecognized symptoms related to eosinophilia.

The mechanism of natalizumab-associated eosinophilia is thought to be related to VLA-4 expression by eosinophils (Barthel et al., 2008). However we suspected that other factors may be at play based on various observations in the literature. For instance, inhibition of VLA-4 by natalizumab would not explain eosinophilic infiltration of peripheral tissues in some cases (Andre, 2010; Bujold, 2014; Curto, 2016; Diem, 2021; Yasuda, 2019). Resolution of refractory eosinophilic esophagitis has been noted with natalizumab use in one case report (Beales, 2019), presumably by blocking the interaction between the α4β7-integrin and mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1). Natalizumab-associated pulmonary eosinophilia (Curto, 2016; Diem, 2021; Yasuda, 2019) occurs despite the presence of VCAM-1 in the lung and the recognition of its importance for eosinophilic infiltration in this tissue (Barthel, 2008). Hence other mechanisms, such as a susceptibility to allergy, enhanced eosinophil survival after engagement of α4β7-integrin (Meerschaert et al., 1999), or other undefined mechanisms, may co-exist. To gain some insight into the role of VLA-4 in natalizumab-associated eosinophilia, we reasoned that examination of the correlation between eosinophilia and lymphocytosis in individual PwMS would be informative since both eosinophils and lymphocytes express VLA-4. We observed a clear discordance between eosinophilia and lymphocytosis, and between within-subject eosinophil and lymphocyte counts, suggesting factors other than VLA-4 inhibition play a role. Indeed, we found that a

history of allergy predisposes to natalizumab-associated eosinophilia. In keeping with this, eosinophilia occurs with multiple monoclonal antibody treatments (not just those inhibiting extravasation), including blockade of TNFα (Malisiewicz et al., 2011), IL-5 (Faiz et al., 2019), IL-6 (Morrisroe and Wong, 2015), and IL-2 (Di Palma-Grisi et al., 2019), in the latter case as part of DRESS (drug reaction with eosinophilia and systemic symptoms), suggesting a generic drug-induced adverse event.

This study's strengths include its sample size, length of follow-up and the availability of blood tests throughout, with only six cases excluded from analysis. Detailed medical history was also available, enabling an assessment of risk factors for natalizumab-associated eosinophilia for the first time. However, there were several limitations. It was a single centre study, although the clinical service covers a catchment of four million people on the southern coast of England. The study was retrospective, so data recorded in medical notes was not documented with this study in mind. The sample size for eosinophilia was small (n=33) and while three patterns of eosinophil kinetics were easily discernible their sample sizes were even smaller, so other patterns may have been missed and the prognostic relevance of individual patterns will need larger sample sizes in multi-centre studies.

5. Conclusions

In conclusion, eosinophilia is common during natalizumab therapy, and in the vast majority is non-consequential. This study establishes temporal patterns and risk factors for eosinophilia, which will help formulate guidance for blood count monitoring during natalizumab treatment. Any level of eosinophilia of 0.5×10^9 per litre or above (i.e. equal to or above the upper limit of the reference range), if accompanied by symptoms suggestive of tissue infiltration in any organ (lung, skin, muscle, gut or joint) in the context of natalizumab treatment, should be investigated. Blood counts are performed regularly but may not be needed in PwMS in the absence of new symptoms suggestive of eosinophilic tissue infiltration, especially in those without risk factors for natalizumab-associated eosinophilia defined here. This study is timely in view of the recent approval of a subcutaneous formulation of natalizumab, which will extend its usability. Observations made in this study indicate that natalizumab-associated eosinophilia may include mechanisms distinct from VLA-4 blockade.

Data sharing statement

Data is available from the corresponding author, upon reasonable request, subject to institutional agreements and ethical approvals.

Declarations of interest

None

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SUPPLEMENTARY MATERIAL FOR:

Eosinophilia during natalizumab treatment: incidence, risk factors and temporal patterns

Milad-Kazava Keshvari[1,2*], Frederick van Someren[1,2*], Saima Sheikh[1,2*], Ian Galea[1,2]

[1] Clinical Neurosciences, Clinical & Experimental Sciences, Faculty of Medicine, University of Southampton
[2] Neurology Department, Wessex Neurological Centre, Southampton General Hospital

* these authors contributed equally to this work

Supplementary Tables

Median lymphocytes during the six months prior to natalizumab*	2.0 (1.6-2.5)
Median lymphocytes on natalizumab*	3.3 (2.8-4.0)
Lymphocytosis during the six months prior to natalizumab[#]	12 (6.1%)
Lymphopenia during the six months prior to natalizumab[#]	57 (29.1%)
Lymphocytosis on natalizumab[#]	96 (49.2%)
Lymphopenia on natalizumab[#]	21 (10.7%)
Persistent lymphocytosis on natalizumab > 4 for ≥ 90 days[#]	63 (32.1%)
Persistent lymphocytosis on natalizumab > 4 for ≥180 days[#]	57 (29.1%)

Supplementary Table 1. Lymphocyte counts prior to and during natalizumab treatment in the whole cohort. Values are median (inter-quartile range) of individual means* or number of cases (percentage)[#].

Independent variable	n	p	Odds ratio	95% confidence interval
Age (years)	182	0.667	0.991	0.953 to 1.031
Male gender	182	0.241	1.612	0.726 to 3.579
Current smoking	182	0.413	1.416	0.615 to 3.259
MS duration (years)	174	0.841	0.993	0.930 to 1.061
Disease-modifying treatments prior to natalizumab	179	0.377	0.707	0.327 to 1.527
Number of disease-modifying treatments prior to natalizumab	179	0.086	0.587	0.319 to 1.079
Anti-JC virus serology positivity	180	0.656	1.187	0.557 to 2.529
Percentage of natalizumab doses missed	182	**0.031**	1.039	1.004 to 1.076
At least 80 percent natalizumab doses received	182	**0.014**	0.267	0.093 to 0.766
Natalizumab bimonthly (versus monthly)	182	0.060	4.867	0.937 to 25.286
Mobility	182	0.369	N/A	N/A
Help required with mobility	182	0.916	0.955	0.410 to 2.224
New MS lesion(s) on MRI whilst on natalizumab	169	0.184	2.022	0.716 to 5.711
Any allergy	182	0.056	2.107	0.980 to 4.534
Non-drug allergy	182	**0.001**	4.159	1.754 to 9.863
Drug allergy	182	0.943	0.969	0.402 to 2.332

Comorbidity associated with eosinophilia	182	**0.002**	4.705	1.762 to 12.558
Mean eosinophil count six months prior to natalizumab	182	**<0.001**	138.869	11.160 to 1728.022
Any eosinophilia six months prior to natalizumab	182	**0.008**	6.473	1.636 to 25.618
Mild eosinophilia six months prior to natalizumab	182	**0.004**	8.690	1.964 to 38.459
Moderate eosinophilia six months prior to natalizumab	182	1.000	0.000	0.000
Severe eosinophilia six months prior to natalizumab	182	NA	NA	NA

Supplementary Table 2. Univariate logistic regression for predictors of natalizumab-associated eosinophilia (>0.5 x 109 per litre) sustained ≥90 days, in PwMS who had received natalizumab for at least 11 months. Mobility was grouped into four categories: able to walk unaided, unilateral help, bilateral help and wheelchair. NA= not applicable.

Independent variable	n	p	Odds ratio	95% confidence interval
Age (years)	196	0.609	0.990	0.953 to 1.029
Male gender	196	0.247	1.595	0.724 to 3.514
Current smoking	196	0.684	1.192	0.511 to 2.780
MS duration (years)	188	0.876	0.995	0.931 to 1.063
Disease-modifying treatments prior to natalizumab	193	0.459	0.749	0.349 to 1.608
Number of disease-modifying treatments prior to natalizumab	193	0.119	0.618	0.337 to 1.132
Anti-JC virus serology positivity	193	0.573	1.241	0.586 to 2.630
Percentage of natalizumab doses missed	196	**0.019**	1.042	1.007 to 1.079
At least 80 percent natalizumab doses received	196	**0.008**	0.243	0.085 to 0.695
Natalizumab bimonthly (versus monthly)	196	**0.046**	5.333	1.027 to 27.691
Mobility	196	0.673	1.106	0.692 to 1.770
Help required with mobility	196	0.749	0.872	0.378 to 2.013
New MS lesion(s) on MRI whilst on natalizumab	177	0.148	2.152	0.763 to 6.074
Any allergy	196	0.083	1.953	0.916 to 4.165
Non-drug allergy	196	**0.001**	4.331	1.841 to 10.189
Drug allergy	196	0.744	0.865	0.363 to 2.062
Comorbidity associated with eosinophilia	196	**0.006**	3.700	1.457 to 9.398

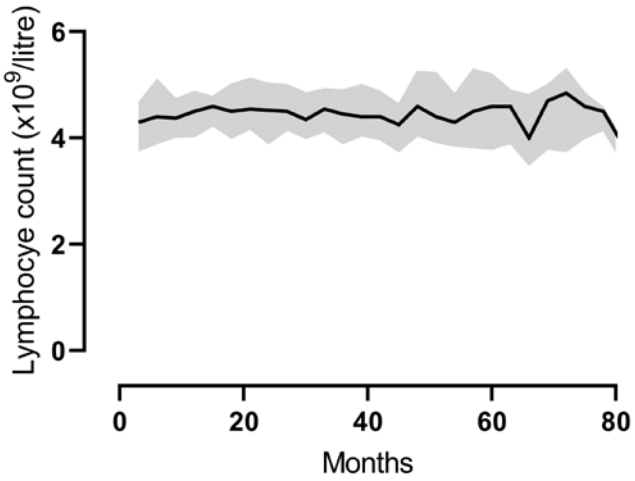
Mean eosinophil count six months prior to natalizumab	196	**0.000**	98.519	9.227 to 1051.854
Any eosinophilia six months prior to natalizumab	196	**0.009**	5.643	1.533 to 20.770
Mild eosinophilia six months prior to natalizumab	196	**0.005**	7.098	1.795 to 28.068
Moderate eosinophilia six months prior to natalizumab	196	1.000	0.000	0.000
Severe eosinophilia six months prior to natalizumab	182	NA	NA	NA

Supplementary Table 3. Sensitivity univariate logistic regression for predictors of natalizumab-associated eosinophilia (>0.5 x 109 per litre) sustained ≥90 days, in all cases (n=196). Mobility was grouped into four categories: able to walk unaided, unilateral help, bilateral help and wheelchair. NA= not applicable.

Independent variable	p	Odds ratio	95% confidence interval
Percentage of natalizumab doses missed	**0.004**	1.060	1.019 to 1.103
Non-drug allergy	**0.002**	4.669	1.754 to 12.431
Comorbidity associated with eosinophilia	**0.027**	3.346	1.143 to 9.793
Mean eosinophil count six months prior to natalizumab	**0.000**	196.789	15.545 to 2491.208

Supplementary Table 4. Sensitivity multivariable logistic regression for predictors of natalizumab-associated eosinophilia (>0.5 x 10^9 per litre) sustained ≥90 days, in all cases (n=196). Hosmer Lemeshow test = 0.628. Nagelkerke R square = 0.323

Supplementary Figures



Supplementary Figure 1. Lymphocytosis during natalizumab treatment. Temporal profile of median lymphocyte count (inter-quartile range) in cases who developed lymphocytosis.