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Abnormalities and erythroblasts in peripheral blood of multiple sclerosis patients treated with natalizumab

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ABSTRACT

Background: In natalizumab treated patients several hematopoietic abnormalities including erythroblasts, myeloblasts and neutrophilic precursors in peripheral blood have been described. So far, long term effects of the hematopoietic changes have not been reported.
Objective: To describe hematopoietic abnormalities in longitudinally monitored MS patients treated with natalizumab. Patients treated with dimethyl fumarate, teriflunomide and fingolimod served as controls. Secondly, the relation between natalizumab serum levels and the occurrence of hematopoietic abnormalities was explored.
Methods: 212 natalizumab treated patients were included, 91 patients with available baseline samples (998 follow-up samples) were compared with patients with dimethyl fumarate (n = 166, 1154 samples), teriflunomide (n = 38, 228 samples) and fingolimod (n = 114, 853 samples). One hundred twenty one patients without baseline samples (1952 follow-up samples) were included in the follow-up group.
Results: More than half of all natalizumab treated patients developed hematopoietic abnormalities, almost a quarter had erythroblasts. Natalizumab use was associated with an increased risk of developing abnormalities in comparison to oral treatment, with a corrected hazard ratio of 2.3, 10.0 and 8.1 in comparison to fingolimod, dimethyl fumarate and teriflunomide respectively. No difference in developing abnormalities was observed in relation to natalizumab serum concentrations. None of the patients developed a hematologic malignancy during follow up.
Conclusion: Hematopoietic abnormalities are common during natalizumab treatment. Given the lack of consequences of this finding during long-term follow-up, it is generally justifiable to refrain from further diagnostic procedures when observing hematopoietic abnormalities in patients using natalizumab.

1. Introduction

During treatment with disease modifying therapy (DMT) for relapsing remitting multiple sclerosis (RRMS), peripheral blood is checked routinely (Pardo and Jones, 2017). In patients using natalizumab, abnormalities in the peripheral blood smear can be detected such as the presence of hematopoietic precursors that are not present in the peripheral blood under physiologic conditions. Erythroblasts are described in more than 90 percent of patients in small cross-sectional cohorts (La Gioia et al., 2016;Robier et al., 2014). One longitudinal study of 44 patients showed an incidence of erythroblasts of 16 percent during 18 months of follow up (Bridel et al., 2015). Myeloblasts and neutrophilic precursors were also found in this study (Bridel et al., 2015). Morphologic changes of red blood cells (RBCs) are less often described (La Gioia et al., 2016;Robier et al., 2014). Moreover, long term effects of the hematopoietic changes are not known.

One can hypothesize that the observed morphologic changes during natalizumab treatment are precursors for a hematologic malignancy such as myelodysplastic syndromes or myeloid neoplasms. In post marketing surveys hematologic malignancies are described during the use of natalizumab, but not with an increase of expected cases (Lebrun and Rocher, 2018). Several cases of central nervous system diffuse large B-cell lymphomas are identified in association with natalizumab. (Lebrun and Rocher, 2018; Nixon et al., 2018; Sartori and

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Grundmark, 2018) In previous studies on natalizumab treated patients with hematologic abnormalities, no evolution towards malignancy was observed. However, the groups were rather small or with a short follow-up. (La Gioia et al., 2016; Robier et al., 2014; Bridel et al., 2015)

Natalizumab is a humanized monoclonal IgG4 antibody directed against the lymphocyte alfa 4 subunit of the alfa 4 beta 1 (VLA-4) integrin. Natalizumab blocks the adhesion of lymphocytes to vascular cell adhesion molecule (VCAM-1) on endothelial cells, resulting in the inhibition of T lymphocyte migration over the blood brain barrier (Polman et al., 2006). The concentration of natalizumab corresponds with alfa 4 integrin receptor saturation (Muralidharan et al., 2017). Generally, the majority of natalizumab treated patients have high serum concentrations of natalizumab after a standard four week treatment interval and currently extended interval dosing is being explored (van Kempen et al., 2018). We hypothesized that abnormalities in hematopoietic cell lines could be related to the serum levels of natalizumab, and secondly that a prolonged treatment interval could lower the incidence of these abnormalities.

The aims of this study were to describe the hematopoietic abnormalities in a large cohort of MS patients treated with natalizumab and explore the consequences of these findings with long term follow up. We compared natalizumab treated patients with dimethyl fumarate, teriflunomide and fingolimod treated patients. Secondly, we studied the relation between serum levels of natalizumab and the incidence of hematopoietic abnormalities.

2. Materials and methods

We performed a retrospective observational chart study in which all blood samples performed between November 2011 and August 2018 were analyzed.

All patients in the observational cohort gave written informed consent for the use of the clinical, laboratory, and imaging data for research purposes. A waiver was obtained from our local institutional review board (IRB) stating that the requirements of the Medical Research Involving Human Subjects Act did not apply and that official IRB approval was not mandatory for the use of these data.

2.1. Patients

Study patients were selected from our local prospective observational cohort study including 243 relapsing remitting MS (RRMS) patients treated with natalizumab at the MS Center Amsterdam of the Amsterdam University Medical Centers. Patients were included in this study if a minimum of two samples were available. Patients with a baseline sample are categorized as the 'baseline group', patients with only follow-up samples as the 'follow-up group'. During treatment, patients routinely underwent blood sampling every three months. Blood analyses were automatically performed and standardized from November 2011 in our hospital.

2.1.1. Comparison between patients on different forms of disease modifying therapies

Study patients on natalizumab with a baseline sample (baseline group, $n = 91$) were compared to controls. Control patients consisted of patients who were treated with either fingolimod ($n = 114$), dimethyl fumarate ($n = 166$) or teriflunomide ($n = 38$),for at least three months between November 2011 and August 2018,with available baseline blood samples and at least one follow up sample. The baseline sample was taken from the start of the medication or most recent sample before start with a maximum of six months before. All available follow-up blood samples after start were analyzed, including samples up to three months after discontinuation of the medication.

2.1.2. Follow-up group

For evaluation of the effect of long term natalizumab treatment, patients with missing baseline samples were included in the separate follow-up group if they had at least two follow up samples and ongoing treatment between November 2011 and August 2018.

2.1.3. Natalizumab concentrations and prolonged treatment interval

Previous studies have shown that the majority of natalizumab treated patients have high serum concentrations of natalizumab after a standard four week treatment interval. In our clinic a study is ongoing about maintaining drug efficacy of natalizumab while extending dose intervals guided by drug concentrations (ClinicalTrials.gov Identifier: NCT03516526). Patients participating in this study with a baseline sample and one follow up sample, or two follow up samples if baseline was missing, were analyzed for abnormalities before and during prolonged interval.

Concentration measurements were performed at Sanquin Laboratory by using a cross-linking assay using polyclonal rabbit anti-NTZ F(ab)2 fragments for capture and a mouse anti-IgG4 monoclonal antibody for detection as described before (van Kempen et al., 2018; Rispens et al., 2011).

2.1.4. Switching from natalizumab to fingolimod

To evaluate a possible late effect of natalizumab after treatment switch, fingolimod treated patients with and without preceding natalizumab treatment in the year before fingolimod start were analyzed separately.

2.2. Blood analyses

Blood smears were automatically prepared and performed by the Sapphire Hematology Analyzer (Abbott, Illinois, USA) between November 2011 and December 2016. From December 2016 this was done by the XN-9000 Hematology Analyzer (Sysmex Europe GmbH, Norderstedt, Germany). If there was an automatic detection of abnormalities, manual evaluation was performed by a skilled hematology laboratory specialist according to the European consensus report (Zini et al., 2010). Blood films were screened for myeloblasts and abnormalities in the different cell lines of erythropoiesis, lymphopoiesis, thrombopoiesis and granulopoiesis.

2.3. Statistical analysis

All analyses were performed with IBM SPSS 22.0 software for Windows. Independent samples *t*-test or one way ANOVA were used to investigate the differences in age between the groups with different treatment duration of natalizumab, between the groups with different DMT, fingolimod patients with and without prior natalizumab use in the preceding year before fingolimod start, different concentrations, and patients with and without abnormalities in the peripheral blood smear. Chi square or Fisher's exact tests were used to investigate the difference between gender, number of restarts, abnormalities at baseline, total number of abnormalities, recurrent abnormalities, the different groups of hematological abnormalities, and available concentrations. The Mann–Whitney *U* test or Kruskal–Wallis test, were used to test for differences in time from onset, treatment duration, number of samples, time from natalizumab until fingolimod switch, mean samples a year and concentrations. McNemar test was used to determine differences in number of abnormalities and erythroblasts before and after extended dosing interval. Kaplan–Meijer analyses was used to measure the fraction of patients who developed abnormalities during treatment with natalizumab, fingolimod, dimethyl fumarate and teriflunomide, and separate analysis for fingolimod treated patients with and without prior natalizumab use in the preceding year. Age and disease duration at start were calculated using the first known start date of medication. Treatment duration was based upon the longest consecutive treatment duration in our hospital started or ongoing after November 2011. A Cox proportional hazard model was used to

calculate the hazard ratio to develop abnormalities for different types of disease modifying drugs, with and without correction for number of samples over time, by adding the categorized mean number of samples a year to the model. Natalizumab was used as reference. *P* values below 0.05 were considered statistically significant. If data were missing, calculations were performed over available data. Because the majority of patients have high serum natalizumab concentrations above 10 mcg/ml, we created and compared four groups based on quartiles of concentrations to evaluate the effect of these high concentrations on the abnormalities.

3. Results

Two hundred twelve of the 243 natalizumab treated patients complied with the inclusion criteria. Ninty one patients with available baseline blood samples were compared with controls, in this group a total of 998 follow-up samples were available, with a median of 9 samples per patient (interquartile range (IQR) 5–17) during a median follow up of 2.2 years (IQR 1.1–3.7). One hundred twenty one patients were included in the follow-up group, with a total of 1952 follow-up samples, with a median of 15 samples (IQR 8–26) during a median follow up of 6.4 years (IQR 3.6–8.8).

3.1. Natalizumab compared to other DMT

One hundred fourteen patients were treated with fingolimod, 166 with dimethyl fumarate and 38 with teriflunomide and were included as controls and compared to the 91 patients on natalizumab.

Baseline demographics and main hematopoietic outcome are described in Table 1.

Patients treated with natalizumab developed abnormalities more quickly (Fig. 1, 46 patients in total, $p < 0.001$ than patients treated with fingolimod (18 patients; hazard ratio (HR) 3.9, 95% CI: 2.3–6.8), dimethyl fumarate (8 patients; HR 12.8, 95% CI: 6.0–27.0) and teriflunomide (2 patients; HR 10.6, 95% CI: 2.6–43.5).

The number of samples taken during treatment and treatment duration was significantly higher for natalizumab ($p < 0.001$ and $p = 0.007$ respectively). However, after correcting for the mean number of samples, patients treated with natalizumab still had higher hazard to develop abnormalities, with a hazard ratio of 2.3 (95% CI: 1.2–4.4), 10.0 (95% CI: 4.5–22.2) and 8.1 (95% CI: 2.0–33.3) for fingolimod, dimethyl fumarate and teriflunomide respectively.

Erythroblasts were seen in 21 (23.1%) patients treated with natalizumab. In eight of them erythroblasts were also observed in follow up samples. Two patients had erythroblasts during fingolimod treatment after switch from natalizumab, both within two months after discontinuation of natalizumab. No erythroblasts were seen during treatment with dimethyl fumarate or teriflunomide.

Myeloblasts were only present in seven patients (7.7%) treated with natalizumab. During follow up all blood samples normalized. One patient had a second blood sample with a myeloblast. The follow up blood sample also normalized.

All of the other described abnormalities were significantly more pronounced during natalizumab treatment (see Table 1) except for abnormalities in thrombocytes.

In general, abnormalities were observed only once during treatment.

None of the patients treated with natalizumab with a baseline sample developed a hematologic malignancy during follow up (median treatment duration of 2.2 years). One patient treated with fingolimod - who did not use natalizumab preceding fingolimod - developed an intracerebral lymphoproliferative disorder, described in detail elsewhere (de Jong et al., 2018). No abnormalities in blood samples were observed preceding the diagnosis in this patient.

Table 1
Baseline characteristics and main hematopoietic outcome.

	Natalizumab *N* = 91	Fingolimod *N* = 114	Dimethyl fumarate *N* = 166	Teriflunomide *N* = 38	*P*
Female n (%)	63 (69.2)	73 (64.0)	119 (71.7)	28 (73.7)	0.52[f]
Age at first start (years)[c]	33.5 (9.9)	38.0 (9.6)	38.0 (10.4)	41.6 (9.8)	< **0.001**[g]
Time until first start of onset (years)[d]	5.7 (2.8–9.7)	10.2 (6.0–15.1)	7.7 (3.4–13.4)	12.8 (6.1–17.9)	< **0.001**[h]
Restarts analyzed period n (%)[a]	16 (17.6)	1 (0.9)	3 (1.8)	0	< **0.001**[f]
Treatment duration (years) [d]	2.2 (1.1–3.7)	2.8 (1.1–4.6)	2.4 (1.0–3.4)	1.7 (1.1–2.4)	**0.007**[h]
Number of samples (excl. baseline)[d]	9 (5–17)	7 (3–10)	7 (3–10)	6 (3–9)	< **0.001**[h]
Abnormalities at baseline n (%)	1 (1.1)	6 (5.3)	4 (2.4)	1(2.6)	0.34[f]
Total number of samples (excl. baseline)	998	853	1154	228	
Samples a year	3.9 (3.6–4.5)	2.7 (1.9–4.9)	3.2 (2.6–4.0)	3.6 (2.3–4.6)	**0.16**[h]
Abnormalities total n (%)	46 (50.5)	18 (15.8)	8 (4.8)	2 (5.3)	< **0.001**[f]
Recurrent abnormalities n (%)[b]	22 (47.8)	6 (33.3)	3 (37.5)	1 (50)	0.76[f]
Days until first abnormalities[d]	308 (98.8–706.3)	117.5 (40.5–355)	389.5 (167.3–552.5)	164 (56–272)[e]	0.10[h]
Lymphopoiesis n (%)	23 (25.3)	2 (1.8)	0	0	< **0.001**[f]
Thrombopoiesis n (%)	5 (5.5)	2 (1.8)	1 (0.6)	0	0.055[f]
Granulopoeisis n (%)	21 (23.1)	2 (1.8)	2 (1.2)	1 (2.6)	< **0.001**[f]
Erythropoeisis n (%)	40 (44)	16 (14)	7 (4.2)	1 (2.6)	< **0.001**[f]
Erythroblasts n (%)	21 (23.1)	2 (1.8)	0	0	< **0.001**[f]
Recurrent erythroblasts n (%)[b]	8 (38.1)	0	n.a.	n.a.	0.53[f]
Days until erythroblasts[d]	381 (224–788.5)	35 (31–39)[e]	n.a.	n.a.	0.38[i]
Blasts n (%)	7 (7.7)	0	0	0	< **0.001**[f]
Recurrent blasts n (%)[b]	1 (14.3)	n.a.	n.a.	n.a.	
Days until blasts[d]	980 (111–1799)	n.a.	n.a.	n.a.	

[a] Patients with temporarily interruption and restart of same therapy between November 2011 and August 2018.
[b] Percentages and *p* value calculated over number of abnormalities.
[c] Mean (standard deviation).
[d] Median (interquartile range)
[e] Median (min-max). (%) percentages of total.
[f] Chi square (or Fisher's exact test if applicable),
[g] One way ANOVA.
[h] Kruskall–Wallis test.
[i] Mann–Whitney *U* test.

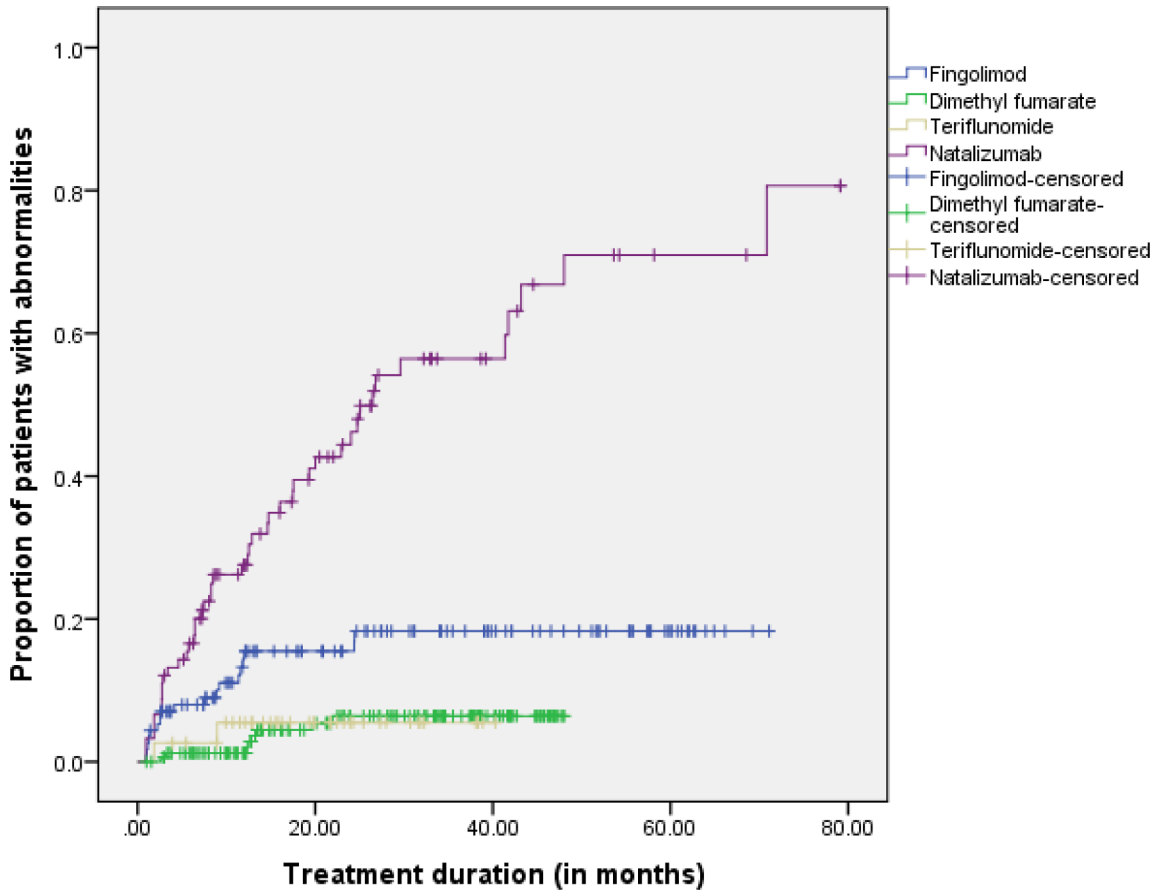

Fig. 1. Kaplan–Meier plot of proportion of patients with abnormalities during treatment with natalizumab, fingolimod, dimethyl fumarate and teriflunomide.

3.2. Natalizumab treatment follow-up group

In addition, 121 patients in the follow-up group with long term natalizumab treatment were analysed.

Of these 121, 102 had a start or restart before the availability of standardized testing. The median treatment duration was 6.4 years, 64 patients (52.9%) had abnormalities, 30 patients (24.8%) had erythroblasts. The number of patients with any of the abnormalities in one of the cell lines or with erythroblasts did not differ from the short treatment group. Recurrent abnormalities were more often seen in the follow-up treatment group. A significant difference was seen in the percentage of patients with myeloblasts two versus seven patients ($p = 0.040$), suggesting that myeloblasts occur early and normalize during treatment. None of the patients developed a hematologic malignancy during follow up.

3.3. Natalizumab concentrations and prolonged treatment interval

Of 174 patients natalizumab serum trough concentrations were available. When divided in subgroups we did not find any significant difference in baseline characteristics, nor for any of the abnormalities in the different cell lines (see Table 2).

Thirty seven patients switched to extended interval dosing with intervals varying between five and seven weeks (median 5 weeks). The median decrease in trough concentration from standard interval dosing to extended interval dosing was 17 mcg/l (interquartile range 11.4–25.1). Eighteen patients had abnormalities before extended interval dosing, 10 still had abnormalities while under extended interval dosing. Eight did not have abnormalities before but developed these during extended interval dosing. There was no significant difference in the proportion of patients with abnormalities before and after extended interval dosing ($p = 1.00$). Eleven patients had erythroblasts before extended interval dosing, of which two also had erythroblasts during extended interval dosing. Five patients without erythroblasts developed them during extended interval dosing. The proportion of patients with erythroblasts before and after extended interval dosing was not significant ($p = 0.42$).

3.4. Switch from natalizumab to fingolimod

Of the 114 fingolimod treated patients, 64 started with fingolimod treatment within one year after natalizumab discontinuation. Patients with prior natalizumab treatment were significantly older compared to patients without prior natalizumab use (mean age 39.9 years standard deviation (SD) 8.8 vs 35.5 years SD 10.1 $p = 0.016$), had a longer disease duration (11.4 years, interquartile range 8.0–16.0) vs 8.8 (interquartile range 5.3–12.3) $p = 0.016$), and more abnormalities at baseline (7.8% vs 2% $p = 0.038$). There was no significant difference in total number of abnormalities during fingolimod treatment between the two groups (18.8% vs 12.0%) nor for any of the different cell lines. There was no significant difference observed over time to develop abnormalities between the groups with and without prior natalizumab use ($p = 0.43$, see Fig. 2 for Kaplan–Meier survival curve).

Seventeen patients had abnormalities during treatment with natalizumab, after switch to fingolimod five of them had abnormalities in the same cell lines during treatment with fingolimod. Seven patients without abnormalities during natalizumab treatment developed abnormalities during treatment with fingolimod.

Erythroblasts during fingolimod treatment were only observed once after switch from natalizumab in two patients within two months after natalizumab discontinuation.

Table 2
Natalizumab concentrations.

Concentrations mcg/ml	0–15	16–23	24–35	> 35	*P*
N total	42	46	44	42	
Female%	27 (64.3)	32(69.6)	28 (63.6)	32 (76.2)	0.57[d]
Age at first start (years)[a]	35.2 (8.5)	35.1 (9.1)	34. (10.1)	34 (7.8)	0.88 [e]
Time until first start of onset (years)[b]	6.1 (3.4–12.4)	6.9 (3.3–11.6)	4.9 (3.0–10.1)	6.4 (2.8–10.7)	0.69[f]
Restarts in analyzed period n (%)	7 (16.7)	8 (17.4)	10 (22.7)	9 (21.4)	0.87[d]
Treatment duration in years[b]	4.8 (2.2–7.1)	4.5 (2.5–7.1)	4.9 (2.8–8.0)	4.6 (2.6–8.4)	0.73[f]
Samples[b]	12 (8–24)	14.5 (8.8–24)	15 (10–26)	14 (8–26)	0.68[f]
Abnormalities n (%)	19 (45.2)	25 (54.3)	28 (63.6)	22 (52.4)	0.39[d]
Recurrent abnormalities n (%)[c]	13 (68.4)	15(60)	16 (57.1)	15 (68.2)	0.80[d]
Lymphopoiesis n (%)	13 (31)	14 (30.4)	11 (25)	15 (35.7)	0.76[d]
Thrombopoiesis n (%)	2 (4.8)	2 (4.3)	2 (4.5)	2(4.8)	1.00[d]
Granulopoeisis n (%)	4 (9.5)	9 (19.6)	9 (20.5)	11 (26.2)	0.27[d]
Erythropoeisis n (%)	19 (45.2)	22 (47.8)	28 (63.6)	19 (45.2)	0.25[d]
Erythroblasts n (%)	11 (26.2)	10 (21.7)	14 (31.8)	11 (26.2)	0.76[d]
Recurrent erythroblasts n (%)[c]	3 (27.3)	3 (30)	5 (35.7)	7 (63.6)	0.32[d]
Blasts n (%)	3 (7.1)	2 (4.3)	2 (4.5)	2 (4.8)	0.94[d]
Recurrent blasts n (%)[c]	0	0	0	1 (50)	0.67[d]

[a] Mean, (standard deviation).
[b] Median (interquartile range).
[c] Percentages and *p* value calculated over number of abnormalities. (%) percentages of total.
[d] Chi square (or Fisher's exact test if applicable).
[e] One way ANOVA.
[f] Kruskall–Wallis test.

4. Discussion

More than half of all natalizumab treated patients developed hematopoietic abnormalities during treatment and almost a quarter of them had erythroblasts in our study. The proportion of abnormalities and erythroblasts during natalizumab treatment are significantly higher compared to patients on fingolimod, dimethyl fumarate and teriflunomide. No difference in developing abnormalities was observed in relation to natalizumab serum concentrations or to extended interval dosing.

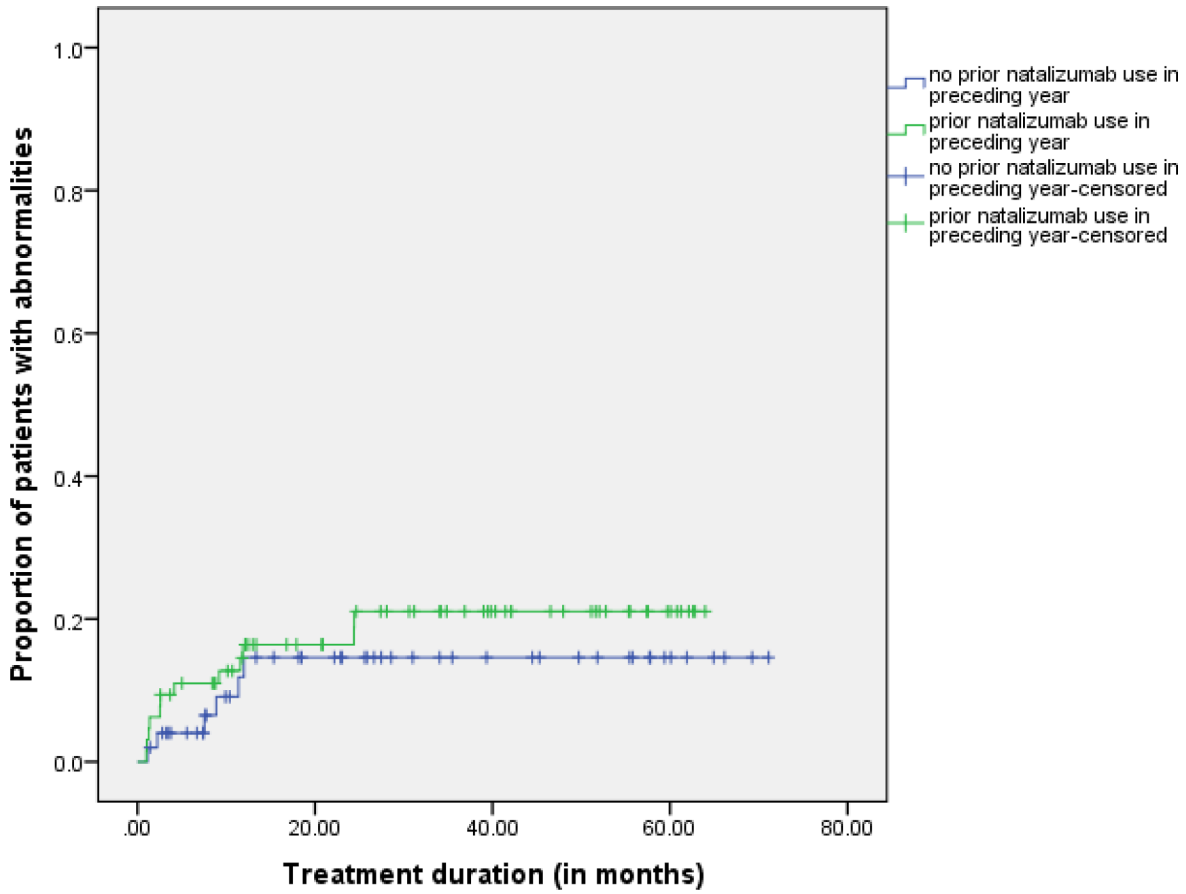

Fig. 2. Kaplan–Meier plot of proportion of patients with abnormalities during treatment with fingolimod for patients with and without prior natalizumab use in the preceding year.

As far as we know this is the largest study, including longitudinally monitored patients with extensive follow-up, addressing hematopoietic abnormalities and erythroblastaemia in peripheral blood during natalizumab treatment so far.

Several studies have described abnormalities in the peripheral blood during treatment with natalizumab (La Gioia et al., 2016; Robier et al., 2014; Bridel et al., 2015;Kobayashi et al., 2019). Most abnormalities were seen in erythropoiesis, mainly erythroblasts. We also found morphologic alterations in thrombopoiesis, granulopoiesis and lymphopoiesis.

Abnormalities in the peripheral blood can be part of a hematological malignancy such as acute leukemia or myelodysplastic syndrome. Peripheral blood is used for diagnosis in the field of hematology (Arber et al., 2016). In myelodysplastic syndromes, dysplastic lineages are seen as well as blasts in bone marrow and peripheral blood can occur (Arber et al., 2016; Boddu et al., 2018). If the percentages of precursor cells in the bone marrow is high, patients are diagnosed with acute myeloid leukemia (Arber et al., 2016; Boddu et al., 2018).

Theoretically, the presence of the different hematopoietic changes in peripheral blood can be associated with underlying hematologic disorders. However, none of our patients treated with natalizumab developed any hematologic malignancy during follow up. Although it cannot be excluded that patients at some point in life develop a hematologic malignancy, our data do not provide evidence for stopping natalizumab therapy when abnormalities in the peripheral blood smear are encountered. Also there is no indication to routinely perform invasive procedures such as a bone marrow biopsy to rule out hematologic disorders.

What causes natalizumab-associated hematopoietic abnormalities in MS? The beta 1 integrin VLA-4 is a heterodimer permitting adhesion of hematopoietic progenitors to different compounds of the bone marrow stroma. The VLA-4 mediated interaction between hematopoietic stem cells and bone marrow stroma is of functional relevance for hematopoiesis as well as for mobilization and homing of hematopoietic precursors cells (Neumann et al., 2009). Due to modifications of the bone marrow vascular niche and the interference of natalizumab with the homing of hematopoietic stem cells, the major hematologic findings in patient treated with natalizumab relate to the number of CD34+ cells which rapidly egress from the bone marrow into the peripheral blood (La Gioia et al., 2016). VLA-4 is essential for the terminal proliferation and differentiation of erythroid progenitor cells (La Gioia et al., 2016). Erythroid cells specifically express fibronection receptors alfa 4 beta 1, engagement of these receptors provides signals that are necessary for the terminal expansion of differentiating erythroblasts (La Gioia et al., 2016). In earlier studies of natalizumab it was already mentioned that this effect reflected a transient phenomenon (La Gioia et al., 2016; Robier et al., 2014; Polman et al., 2006). However, work by Bridel et al. observed that a proportion of patients with precursor cells and erythroblasts remained stable during 18 months of follow up (Bridel et al., 2015). We also observed a proportion of patients with recurrent abnormalities, the reason why is not quite clear. We hypothesized that patients with higher levels of natalizumab would have more and recurrent abnormalities. We did not find any evidence to confirm this hypothesis. Although the serum levels in our patients were not taken exactly at the same time of the abnormalities, this has probably not affected our results because natalizumab trough concentrations are stable in a set treatment interval and no association between treatment duration and serum concentrations is observed (van Kempen et al., 2018).

In line with the serum concentration findings, we did not observe a substantial change of abnormalities before and during prolonged treatment interval. Kobayashi et al. recently suggested that erythroblasts may correlate with therapeutic effect and could contribute to determination of the optimal amount of natalizumab and administration interval (Kobayashi et al., 2019). Although we did not correlate the abnormalities with therapeutic effect, our observations of emerging and disappearing abnormalities and erythroblasts during prolonged treatment intervals do not support this hypothesis.

In comparison to oral treatment, a higher proportion of natalizumab treated patients do have abnormalities.

We corrected for a possible bias due to different number of samples over time in patients with different follow-up by creating four categories based on the annualized number of samples per patient.. The numbers were categorised in one, two, three and four or more samples a year. After this correction we still observed a higher proportion of abnormalities in natalizumab treated patients.

No difference was observed in normalization of abnormalities between the different therapies. If patients had an abnormality in one of the cell lines and in a follow up sample, at any time, the abnormality persisted or an abnormality in another cell line emerged, during or within three months after discontinuation of treatment, this was considered as a recurrent abnormality. A possible bias of this assumption could not be ruled out, however due to the small numbers of recurrent abnormalities in oral treatment this is in our opinion negligible.

In conclusion, abnormalities in peripheral blood smears are common during natalizumab treatment, without any evidence for developing any related underlying hematological disorders during extensive follow up. There is no association between serum levels of natalizumab and these abnormalities.

If patients experience symptoms not related to their multiple sclerosis and other abnormalities are observed (such as persistent or increasing white blood cells, thrombocytosis, trombopenia, anemia, leukocytosis etc.) (Arber et al., 2016), it is recommended to do further diagnostics in close collaboration with the hematologist. Considering the frequency of the abnormalities with no evidence of developing any related underlying disorders, it is generally justifiable to refrain from further diagnostic procedures in asymptomatic patients.

Disclosures

J.A. van Rossum, Z.L.E. van Kempen, L. Schilder, B.I. Lissenberg-Witte declare no conflicts of interests.

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