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Original research

Low natalizumab trough concentrations are associated with reduced seroconversion of the John Cunningham virus in natalizumab-treated patients with multiple sclerosis

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ABSTRACT

Background Natalizumab is a highly effective drug for patients with relapsing-remitting multiple sclerosis (MS). A disadvantage of this treatment is the risk of progressive multifocal leukoencephalopathy in patients who are seropositive for the John Cunningham virus (JCV). JCV seroconversion rates increase under natalizumab treatment compared with non-natalizumab using controls. The aim of this study was to assess whether lower natalizumab trough concentrations are associated with reduced JCV seroconversion compared with higher natalizumab trough concentrations.

Methods Two overlapping cohorts of patients treated with intravenous natalizumab in the Netherlands were combined for this study. JCV seroconversion was assessed during periods of high (≥15 µg/mL) and low (<15 µg/mL) natalizumab trough concentrations. Low trough concentrations were mainly the result of trough concentration guided personalised extended interval dosing (EID). The seroconversion rates during high and low trough concentrations were compared using a generalised linear mixed model with a Poisson link function.

Results A total of 357 patients from 21 hospitals in the Netherlands were included. The annual seroconversion rate of 8.4% observed in patients during periods of high trough concentrations (n=226) was 2.32 times higher than the seroconversion rate of 4.8% in patients during periods of low trough concentrations (n=252) (95% CI=1.32 to 4.08, p=0.0035).

Conclusions The seroconversion rate observed in patients with MS with low trough concentrations was substantially lower compared with those with high trough concentrations during natalizumab treatment. This emphasises the importance of personalised EID, where intervals between infusions are prolonged to achieve lower natalizumab trough concentrations, to increase drug safety.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Patients with multiple sclerosis (MS) treated with natalizumab are at a higher risk of becoming carriers of the John Cunningham virus (JCV) compared with those not treated with natalizumab. Given that JCV positivity often leads to discontinuation of natalizumab treatment, this study aimed to assess whether extended interval dosing (EID), which leads to lower natalizumab trough concentrations, results in reduced JCV seroconversion rates.

WHAT THIS STUDY ADDS

⇒ This study found a significantly reduced JCV seroconversion rate in natalizumab-treated patients with MS with lower trough concentrations in serum, primarily resulting from personalised EID.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This new finding underscores the importance of personalised EID for patients treated with natalizumab, as treatment with lower trough concentrations lowers the risk of JCV seroconversion, which will enable patients to continue natalizumab therapy safely for a longer period of time.

INTRODUCTION

Natalizumab, a monoclonal antibody targeting the α4β1-integrin receptor on lymphocytes, is a highly effective treatment for patients with relapsing remitting multiple sclerosis (MS). By blocking the adhesion of the α4β1-integrin receptor to its ligand, vascular cell adhesion molecule-1 on the endothelium, natalizumab prevents lymphocytes from migrating across the blood-brain barrier into the central nervous system. This significantly

reduces the frequency of relapses and MRI activity among patients with MS.[1] Natalizumab is administered at a standard dose of 300 mg every 4 weeks, either intravenously (approved by the Food and Drug Administration and the European Medicines Agency (EMA)) or subcutaneously (approved only by the EMA).

Natalizumab is the only highly effective MS treatment currently available that does not induce systemic immune suppression. However, its major drawbacks include the risk of progressive multifocal leukoencephalopathy (PML), a potentially fatal infection of the brain caused by the John Cunningham virus (JCV) and the high cost. In patients who are JCV seropositive, several factors increase the risk of PML, including prior use of immunosuppressant therapy, longer duration of natalizumab treatment and a higher anti-JCV antibody index.[2] Therefore, screening for anti-JCV antibodies in serum is conducted both before and during natalizumab treatment.

JCV, whose transmission route remains elusive, is widespread in the human population with seroprevalence of 50% in the third decade of life, increasing to 70% in the sixth decade of life.[3] In healthy individuals, seroconversion rates are estimated at 1–2% per year.[3] However, several studies have reported that natalizumab-treated patients with MS have annual JCV seroconversion rates ranging from 7% to 11%, which is significantly higher than the healthy population.[4–8] One of these studies, conducted by Schwab *et al*, showed that the seroprevalence of JCV is higher in cohorts of patients with MS treated with natalizumab compared with those not treated with natalizumab. Additionally, they found that the rise in seroprevalence over time in patients treated with natalizumab is higher than would be expected due to ageing alone, indicating that natalizumab is the underlying factor increasing the risk of JCV seroconversion.[5] Given the association between positive JCV status and the risk of developing PML, JCV seroconversion under natalizumab treatment often prompts a switch to another disease modifying therapy (DMT).

Extended interval dosing (EID) of natalizumab has gained popularity in recent years. Studies have shown that natalizumab trough concentrations are still substantially high in most patients at the end of a 4-week interval (mean 26.1±14.1 µg/mL), whereas the minimal effective serum concentration of natalizumab is estimated to be 1–2 µg/mL.[9 10] Additionally, natalizumab trough concentrations after a 4-week treatment interval vary significantly between individuals (0.1–80 µg/mL), although intraindividual trough concentrations are relatively stable.[11] These findings have led to trials exploring EID of natalizumab by prolonging the interval between infusions. Such studies, including the NEXT-MS (a prospective trial of natalizumab personalised EID by therapeutic drug monitoring in patients with MS) and the NOVA trial (a randomised, controlled, open-label phase 3b trial, comparing switching to 6-week dosing of natalizumab versus continuing with 4-week dosing in patients with MS), have demonstrated that EID of natalizumab can be done safely without compromising efficacy.[12 13] Moreover, EID of natalizumab greatly reduces the risk of developing PML in JCV positive patients.[14]

Although the underlying mechanism for higher seroconversion rates in patients with MS treated with natalizumab is not yet understood, it raises the question of whether giving infusions less frequently, resulting in lower serum concentrations, could decrease JCV seroconversion rates in patients with MS. This study aims to assess if lower trough concentrations in serum, which can be achieved through EID, are associated with reduced JCV seroconversion rates.

METHODS

Study protocols

Patients of two overlapping cohorts, the NEXT-MS trial and the Amsterdam MS Cohort (AMSC), were included in this study. The NEXT-MS trial was an investigator-initiated multicentre prospective open-label non-randomised study conducted in the Netherlands.[12] Adult patients with MS who received at least six natalizumab infusions were included and were offered personalised EID of natalizumab based on trough concentrations in serum. Patients could choose to participate in a personalised dosing group aiming for a trough concentration of approximately 10 µg/mL, a personalised dosing group aiming for a trough concentration of approximately 5 µg/mL, or the standard interval group (every 4 weeks). Throughout the study, trough concentrations were frequently measured, JCV serostatus data were collected every 6 months and treatment intervals were registered. In the personalised dosing groups, treatment intervals were shortened if a trough concentration dropped below 2 µg/mL.

The AMSC is an observational cohort of patients with MS treated at the MS Center Amsterdam at the Amsterdam University Medical Center in the Netherlands. All past and current natalizumab-treated patients in this cohort were selected for this study. In standard care for patients with MS in the Netherlands, JCV serostatus is determined before initiating natalizumab and every 6 months during treatment, and for patients treated on an extended interval regimen, natalizumab trough concentrations are routinely monitored. Additionally, trough concentrations of natalizumab-treated patients were available in the AMSC cohort from previous measurements conducted for other research purposes.[11 15] For this study, data on patient demographics, JCV serostatus, treatment intervals and trough concentrations were extracted from the AMSC database.

Participants and observation periods

Patients from both cohorts were included in this study if they met the following criteria: (1) treated with natalizumab intravenously (Tysabri), (2) at least two JCV serostatus tests, with the first test showing a negative result; and (3) at least one trough concentration measured during natalizumab treatment. Included patients were observed for JCV seroconversion from their first to their last JCV test during natalizumab treatment, or until their first positive JCV test. Consequently, for most patients of the AMSC, the observation period began at the start of natalizumab treatment. However, for patients of the NEXT-MS study, the observation period began on enrolment in the NEXT-MS study, as JCV serostatus data could only be obtained through the study.

Examples of how the observation periods of each patient were defined are illustrated in figure 1. For each patient, it was determined whether their total observation period contained only high trough concentrations (≥15 µg/mL), only low trough concentrations (<15 µg/mL), or both. Both high and low trough concentrations were typically seen in patients who started EID after a period of treatment on standard intervals. In these patients, the specific observation periods with high and low trough concentrations were defined by identifying when the trough concentrations first fell below 15 µg/mL for two consecutive measurements.

In addition to patients treated with personalised EID, the cohort also included patients who received standard interval dosing of every 4 weeks throughout the entire observation period. Of these patients, 39 patients had only one trough concentration measurement during the entire observation period. Given

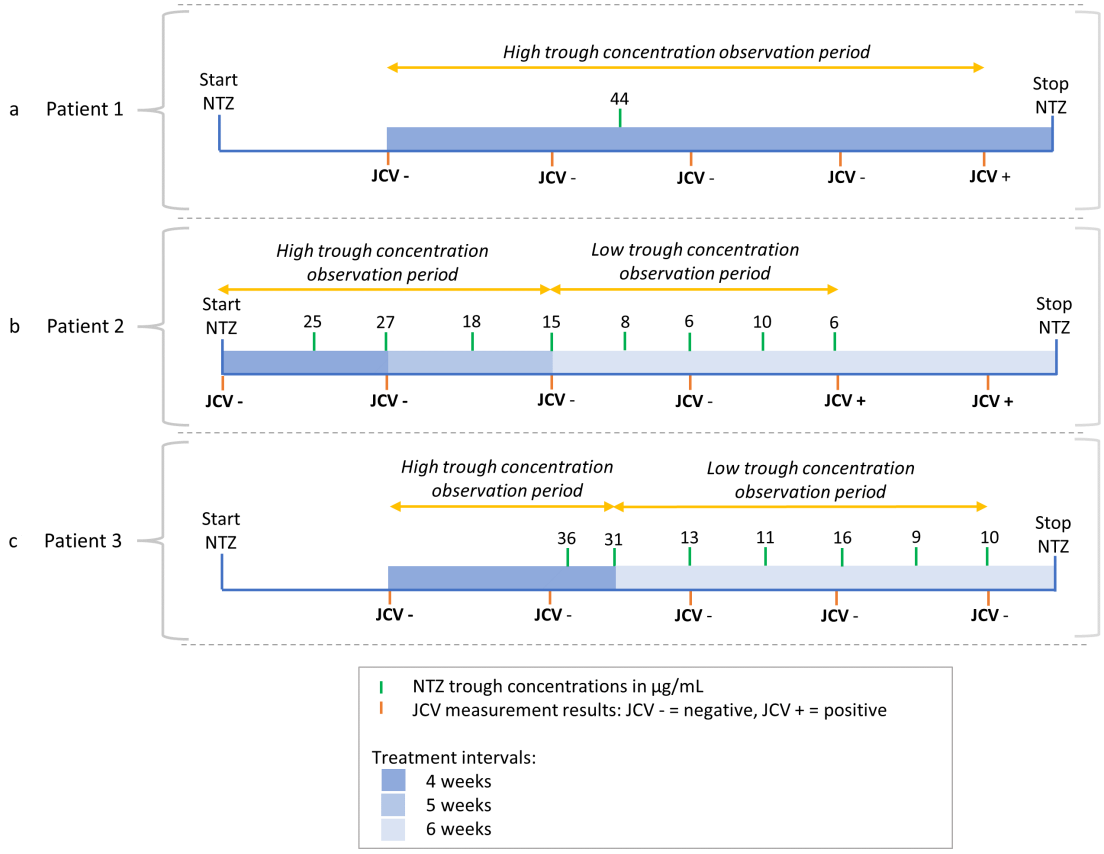

Figure 1 Examples of how the observation periods of each patient were defined. The first example (a) represents a case in which only one trough concentration was measured. Since the treatment interval remained unchanged throughout the observation period, this period is considered a high trough concentration observation period. JCV tests are usually done every 6 months, resulting in an observation period of 24 months for this patient. The second example (b) is a patient observed from the start of natalizumab treatment. Initially, the patient had high trough concentrations, which decreased as the treatment intervals were extended. If two consecutive measurements below 15 µg/mL were observed, the observation period with low trough concentrations was considered to have started at the last measurement showing a trough concentration of 15 µg/mL or higher, since extended interval dosing was always initiated right after a trough concentration measurement. Therefore, the trough concentration of 15 µg/mL, after which the 6-week interval regimen was started, marks the transition from the high to the low trough concentration observation period in this patient. A threshold of 15 µg/mL was chosen because most patients in the NEXT-MS trial were participating in the personalised dosing group aiming for a trough concentration of approximately 10 µg/mL.[12] In this study group, treatment intervals of patients were extended if a trough concentration was ≥15 µg/mL. The third example (c) shows a similar trend of decreasing trough concentrations as the treatment intervals were extended. It demonstrates that a single measurement above 15 µg/mL during the low trough concentration period is acceptable, as long as the mean trough concentration throughout the period remains below 15 µg/mL. An observation period starts with two consecutive measurements below or above 15 µg/mL. JCV, John Cunningham virus; NEXT-MS, Natalizumab personalized EXTended interval dosing in MS; NTZ, natalizumab.

that intraindividual trough concentrations remain stable in a set treatment interval,[11] this single measurement was considered as a representative value for the entire observation period for these patients. For patients with more than one trough concentration measured during the observation period, the mean trough concentration was calculated.

Measurements

All natalizumab trough concentration measurements in serum samples were performed by Sanquin Diagnostic Services, Amsterdam, using a bridging ELISA using polyclonal rabbit anti-natalizumab fragments and mouse anti-IgG_4 monoclonal antibodies.[16] All JCV measurements in serum samples were conducted by Unilabs in Copenhagen, Denmark, using an ELISA. This study only includes JCV serostatus results from the second-generation STRATIFY JCV DxSelect assay.[17] Only definitive JCV test results (negative or positive) were included in this study; index values were not considered.

Statistical analyses

JCV seroconversion is defined as a positive JCV test result in patients who were initially seronegative at the start of the observation period, with no subsequent negative test results. Patients with variable JCV serostatuses, meaning alternating positive and negative test results, were evaluated individually: patients with negative results after positive results were defined as seronegative, but if consecutive positive results followed the variable results, this was considered as positive seroconversion. Descriptive data are presented as frequencies with percentages for dichotomous variables and as means with SD or medians with IQRs for continuous variables. Seroconversion rates were calculated by dividing the proportion of patients who experienced JCV seroconversion by the mean duration of the observation period in years. To model JCV seroconversion rates, a generalised linear mixed model with a Poisson link function was performed, with the log-transformed duration of the observation period as an offset in the model. Model assumptions were assessed, and

no overdispersion was detected (dispersion parameter=0.91). The model was adjusted for age, sex, treatment duration and whether the patient was observed during COVID-19. Variables body mass index (BMI) and prior DMT use were only explored for associations with JCV seroconversion due to missing data. Adjustment for COVID-19 was done because a study by Dwyer *et al*, conducted in Australia, found a significant reduction of JCV seroconversion during the COVID-19 period, assumingly due to social distancing measures.[18] In contrast, a study by Krieger *et al*, conducted in a large cohort of natalizumab-treated patients with MS in the USA, did not find a decrease in JCV seroconversions during COVID-19.[19] Given that many patients in our study were observed for JCV seroconversion during COVID-19 in the Netherlands, we examined whether this acted as an effect modifier in our analyses. No such effect was found and therefore, we did not stratify our results but only adjusted for the COVID-19 period in the Poisson regression analysis. An overview of JCV seroconversions in our cohort before, during and after COVID-19 restrictions is presented in the online supplemental table 1. Kaplan-Meier survival curves were used to visualise JCV seroconversion within both study groups of high and low natalizumab trough concentrations. Statistical significance was defined as p values below 0.05. All analyses were conducted with R V.4.2.1.

RESULTS

A total of 357 patients were included, from 21 hospitals in the Netherlands. Reasons for exclusion from the study are presented in figure 2. Of the included patients, 105 were observed with only high natalizumab trough concentrations (≥15 µg/mL), 131 were observed with only low natalizumab trough concentrations (<15 µg/mL) and 121 were observed with both high and low natalizumab trough concentrations (and were therefore included in both groups based on the observation periods with high and low trough concentrations). The total observation period of the cohort included 1304.8 patient-years, with a mean observation period of 3.7 years. 85 patients (23.8%) seroconverted from negative to positive, resulting in an overall annual seroconversion rate of 6.5 per 100 person-years (6.5%). 15 patients (4.2%) showed variable JCV serostatuses and were considered as JCV negative. There were no cases of PML in this cohort.

Periods with high natalizumab trough concentrations were observed in 226 patients, while periods with low natalizumab trough concentrations were observed in 252 patients. Patient characteristics of these two groups are described in table 1. The difference in natalizumab exposure before the start of the observation periods can be attributed to the standard practice of starting the treatment with standard intervals, with EID introduced later during treatment. The total observation period of high trough concentrations was 631.2 years, with a

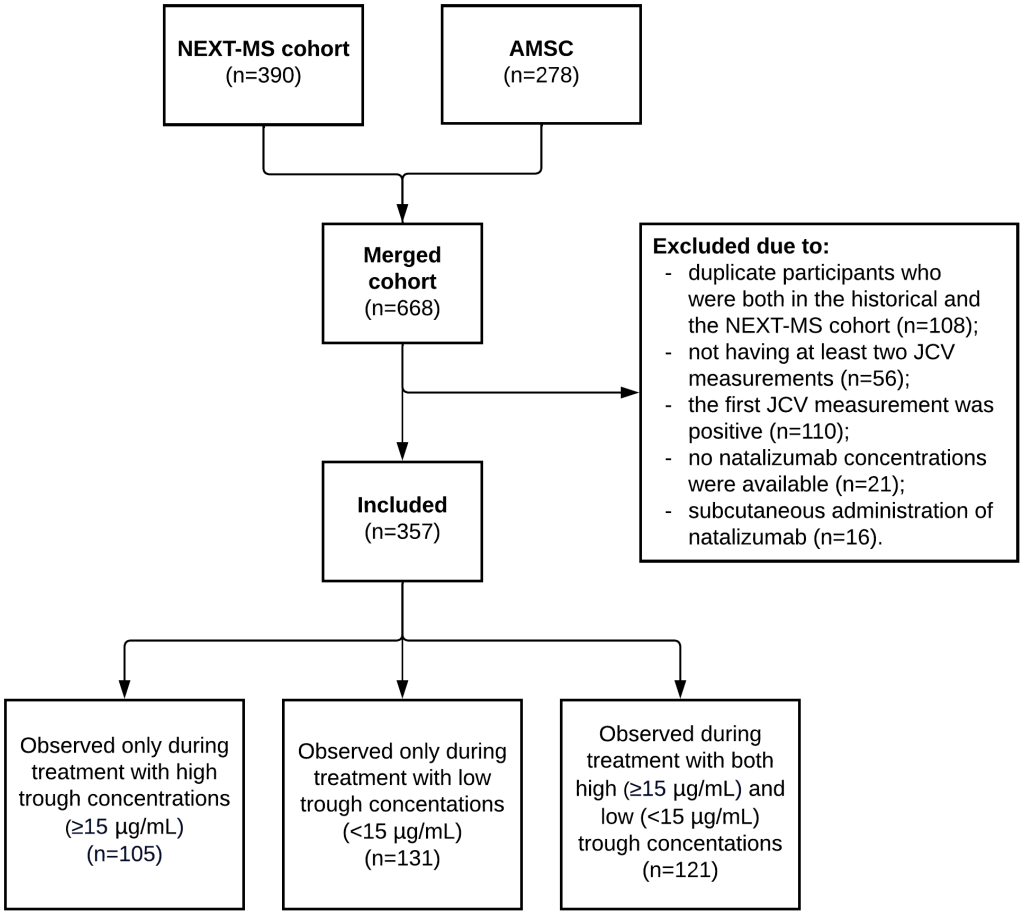

Figure 2 Inclusion and exclusion process. AMSC, Amsterdam Multiple Sclerosis Cohort; JCV, John Cunningham virus; NEXT-MS, Natalizumab personalized EXTended interval dosing in MS.

Table 1 Patient characteristics of the overlapping study groups

	Patients with observation periods during high natalizumab trough concentrations (n=226)*	Patients with observation periods during low natalizumab trough concentrations (n=252)
Sex, female, n (%)	176 (77.89)	207 (82.14)
Age at start observation period in years, median (quartiles)	36 (29–45)	39 (32–48)
Body mass in kg, mean (SD)†	72.86 (14.66)	75.53 (15.89)
BMI in kg/m^2, mean (SD)‡	24.43 (4.62)	25.18 (5.02)
Exposure to natalizumab until start observation period in years, median (quartiles)	0.99 (0–4.4)	3.19 (1.00–6.72)
Trough concentrations, median (quartiles)§	24.00 (19.75–31.31)	9.59 (7.51–11.49)

*In total, 357 patients were included in this study. Of these, 121 patients had both high and low trough concentrations within their total observation period. These patients were added to both of the study groups in this table, resulting in the total number of patients in this table exceeding the number of individual inclusions in the study.
†Body mass: 29 missing values (NA's) in the total cohort: 24 NA's in the group with high trough concentrations and 5 NA's in the group with low trough concentrations.
‡BMI: 34 NA's in the total cohort: 27 NA's in the group with high trough concentrations and 7 NA's in the group with low trough concentrations.
§Includes the mean trough concentrations during the observation periods.
BMI, body mass index; kg, kilograms.

mean observation period of 2.79 years per patient. 53 patients (23.5%) experienced a positive JCV seroconversion during the observation period, resulting in an annual seroconversion rate of 8.40%. The total observation period of low trough concentrations was 673.6 years, with a mean observation period of 2.67 years per patient. 32 patients (12.7%) experienced a positive JCV seroconversion during the observation period, resulting in an annual seroconversion rate of 4.75%. Comparing these rates using Poisson regression, the seroconversion rate among patients with high trough concentrations is 2.32 times higher than among those with low trough concentrations (95% CI=1.32 to 4.08, p=0.0035). The difference in JCV seroconversion over time is demonstrated in figure 3.

Age, sex and treatment during COVID-19 were not associated with JCV seroconversion in our analysis. Furthermore, BMI and prior DMT use were not found to be associated with JCV seroconversion. Additionally, we did not find an association between natalizumab treatment duration and JCV seroconversion (β=0.02, 95% CI=−0.05 to 0.10), suggesting that the risk of seroconversion does not increase or decrease with longer treatment duration.

DISCUSSION

The results of this study, derived from a large overlapping cohort of patients treated with natalizumab at multiple centres in the Netherlands, indicate that lower trough concentrations are associated with reduced JCV seroconversion. This suggests that patients treated with natalizumab could continue natalizumab treatment safely for a longer period of time when receiving EID.

The overall annual seroconversion rate found in this cohort of 6.51% is slightly lower compared with the seroconversion rates found in other cohorts, possibly due to the fact that it includes many patients on EID. Vennegoor *et al* previously assessed JCV seroconversion in 179 patients with MS treated with natalizumab at the MS Center in Amsterdam, with a median follow-up of 52.5 months and reported an annual seroconversion rate of 7.1%.[6] Schwab *et al* reported annual seroconversion rates of 8.5% in a French cohort of 243 seronegative patients whose seroconversion was monitored in the first 2 years of natalizumab treatment, and 10.3% in a German cohort of 339 initially seronegative patients with a mean observation period of 14.8 months.[5] Additionally, Dwyer *et al* reported annual seroconversion rates of 7.1% in an Australian cohort of 309 initially seronegative patients and 8.4% in a Brazilian cohort of 39 patients with a mean observation period of 3.3 and 3.1 years, respectively.[8] The patients in these studies were all treated with natalizumab on standard interval dosing. Given our finding that lower trough concentrations lead to reduced JCV seroconversion, it logically follows that the lower seroconversion rate observed in our cohort is due to the EID regimen, which results in lower trough concentrations.

For the first time, we found a reduced JCV seroconversion rate in patients with lower trough concentrations. Whether natalizumab trough concentrations and JCV seroconversion are associated has previously been studied in a cohort of 135 natalizumab-treated patients. Natalizumab trough concentrations were compared among three groups based on their serostatus: seropositive, seronegative and those who seroconverted from negative to positive.[20] In that cohort, natalizumab trough concentrations of patients who seroconverted (N=28) were comparable to the other groups. However, since this study was conducted before the initiation of EID, most patients still had trough concentrations that are now considered as high (median trough concentration of 21 µg/mL). Due to the small number of patients with low trough concentrations and possibly also due to the small patient cohort, an association may not have been found at that time.

The underlying mechanism for the increased JCV seroconversion with natalizumab treatment is yet unknown. One hypothesis suggests that natalizumab affects the immune system of the gut mucosa by altering B and T-cell populations, potentially increasing vulnerability to an initial JCV infection.[6] This hypothesis suggests an oral-faecal transmission of the virus, which is plausible given the high levels of JCV found in sewage.[3] Regardless of what the underlying mechanism is, reducing natalizumab concentrations in serum by treating with EID might partially reverse the effects of natalizumab on the immune system that increase the risk of seroconversion, thereby making patients less vulnerable to JCV infection.

EID of natalizumab up to intervals of 8 weeks has been studied in several retrospective observational studies, which have shown that EID does not compromise treatment efficacy.[21–28] Moreover, a few prospective trials have studied EID of natalizumab, with similar results. The NEXT-MS study, where treatment intervals were extended based on individual trough concentrations, showed that treatment intervals could be extended up to 9 weeks.[12] The NOVA trial, a large study in which patients were randomised to receive natalizumab either every 4 weeks or every 6 weeks, showed that most patients could be safely treated every 6 weeks without compromising efficacy.[13] However, trough concentrations still vary among patients on EID, as shown by a study by Foley *et al*, where patients on EID with treatment

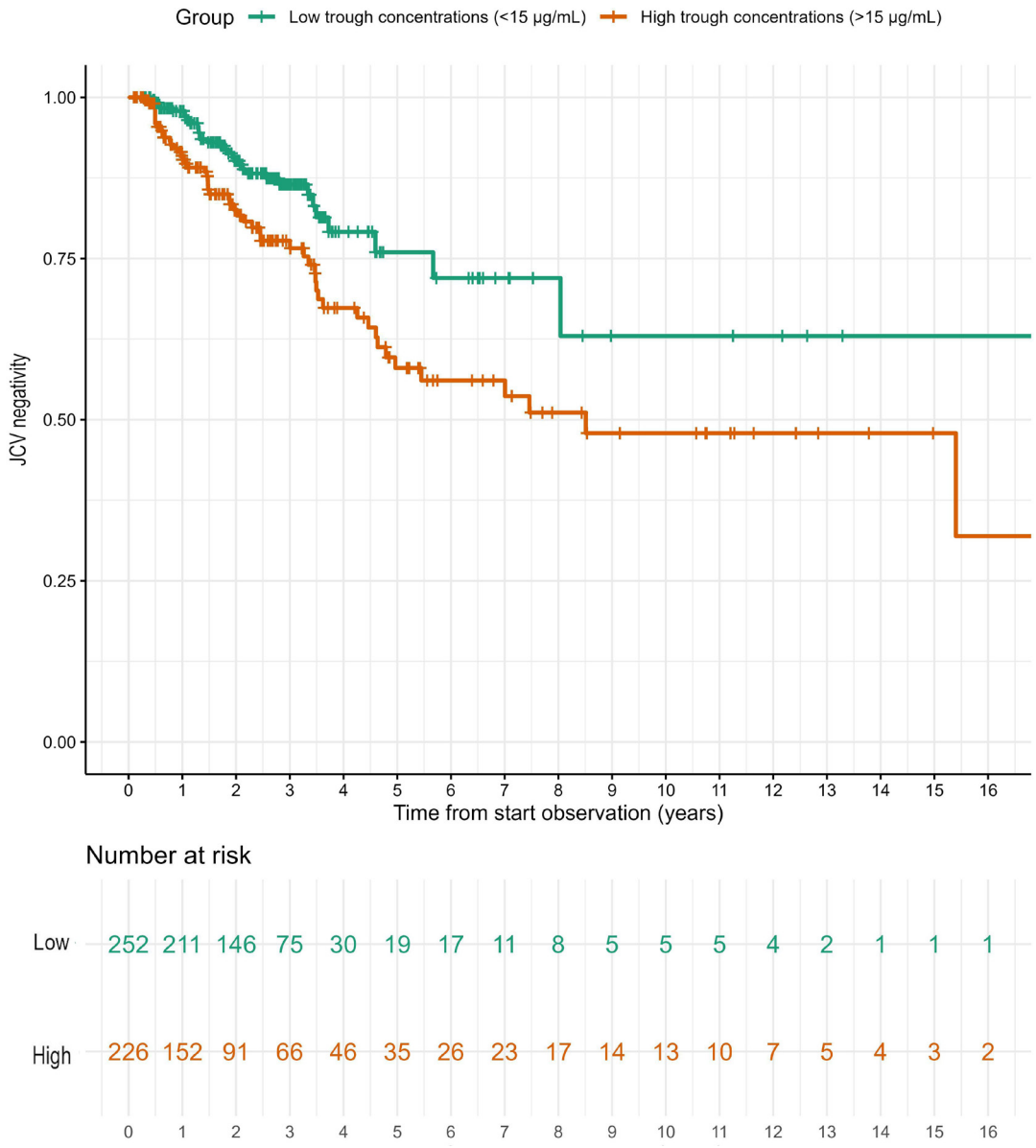

Figure 3 Kaplan-Meier survival curves to visualise differences in JCV seroconversion across both groups over time. In this figure, JCV seroconversion during the observation period is considered as the event. The graphs begin at 1.00, indicating that 100% of the patients started the observation period without JCV seroconversion. JCV, John Cunningham virus.

intervals ranging from 35 to 49 days (median 38 days) had natalizumab trough concentrations ranging from just above 0 to 65.4 µg/mL.[29] Therefore, it was decided to define the observation periods for our study based on the natalizumab trough concentrations rather than treatment intervals (standard or extended). Foley *et al* also demonstrated that BMI partially accounts for the variability in trough concentrations between patients, though not entirely.[29] In our cohort, we observed that patients with lower trough concentrations tended to have higher BMI and body mass compared with those with higher trough concentrations, as illustrated in table 1. Probably, patients with lower BMI need to extend their treatment intervals further than those with higher BMI to achieve similar low trough concentrations.

Based on the results of the NOVA trial, we recommend initiating EID with 6-week intervals and suggest further extending the intervals in patients who still have high trough concentrations at 6 weeks to reach a trough concentration below 15 µg/mL, for example, by aiming for a trough concentration between 5 and 10 µg/mL.[13] This strategy would minimise the risk of JCV seroconversion while preserving treatment efficacy by maintaining concentrations above the minimum effective level. Personalised EID of natalizumab starting at 6 weeks and targeting a trough concentration of 5 µg/mL is currently being studied in the next phase of the NEXT-MS trial, known as the SUPERNEXT (NCT04225312), with results expected in 2027.

This study has several limitations. First, possible selection bias might have been introduced, given that observation periods with low trough concentrations always followed after the observation periods with high trough concentrations. Second, JCV serostatus data were not available from the start of natalizumab for all patients, resulting in observation periods shorter than the treatment duration. However, previous research did not find different JCV seroconversion rates across different durations of natalizumab treatment, and similarly, we did not observe any association between treatment duration and JCV seroconversion in our cohort.[6 8] Third, the frequency of the measurements

of natalizumab trough concentrations was not standardised, resulting in a varying number of measurements between patients. The strengths of this study include the large cohort size and the exclusive use of the second-generation STRATIFY JCV assay.

In conclusion, we found a significantly reduced JCV seroconversion in patients with lower natalizumab trough concentrations during treatment with intravenous natalizumab. This new finding underscores the importance of personalised EID to achieve low but therapeutic natalizumab trough concentrations for patients with MS, enabling patients to continue natalizumab therapy even more safely for a longer period of time.

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Patient consent for publication Not applicable.

Ethics approval The NEXT-MS trial and the Amsterdam MS cohort were approved by the medical ethics committee: VUMC Ethics committee numbers 2019.552 and 2020.269, respectively. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Anonymised data will be shared upon reasonable request from any qualified investigator.

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