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Title: Anti-JC virus antibody titres increase over time with natalizumab treatment

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Abstract

Background: Anti-JC virus antibody status is a risk factor for progressive multifocal leukoencephalopathy after natalizumab treatment in multiple sclerosis. Previous studies have used a cross-sectional approach to conclude that the presence and duration of natalizumab treatment does not influence anti-JCV Ab seropositivity.

Objectives: Using a longitudinal approach, to measure change in anti-JCV Ab results after natalizumab treatment.

Methods: Anti-JCV Ab results (n=1154) from the second-generation STRATIFY JCV™ DxSelect™ test were analysed from an observational cohort of MS patients on natalizumab (n=485; n=340 with repeat testing; n = 657 repeat tests on natalizumab).

Results: Across sequential paired tests, seroconversion rate was greater than seroreversion rate (40/364 (11.0%) vs 18/293 (6.1%); $p < 0.05$). Moreover, anti-JCV Ab index increased across longitudinal paired tests (m_{A-B} 0.102; paired $t(656) = 5.0$; $p < 0.0001$). This magnitude of Ab level increase far exceeds that expected due to increasing age alone.

Conclusion: Our data suggest that natalizumab therapy is associated with a significant and substantial increase in anti-JC virus antibody index over time. Further work should focus on the underlying mechanisms of this phenomenon, and the clinical relevance to risk stratification.

Introduction

Natalizumab is a disease modifying therapy licensed for the treatment of relapsing-remitting MS.^{1, 2} Its use is complicated by the risk of progressive multifocal leukoencephalopathy (PML).³ PML is fatal in approximately 20% of affected individuals and can also result in severe disability.⁴ There are three well-established risk factors for PML in MS patients: previous exposure to immunosuppressive drugs, duration of treatment with natalizumab and anti-JC virus (JCV) antibody (Ab) status and titre.⁵ Biogen Idec's second-generation STRATIFY JCV™ DxSelect™ ELISA (STRATIFY Gen2; analysed at UNILABS) is a two-step ELISA, used to stratify risk of PML.^{6, 7} A seronegative result is assigned if the first-step anti-JCV Ab titre ('index') is <0.20; a seropositive result is assigned if >0.40. Samples with intermediate index values (0.20-0.40) undergo a second confirmatory step.⁶

Previous studies have used a cross-sectional approach to conclude that the presence and duration of natalizumab treatment does not influence anti-JCV Ab seropositivity.⁸⁻¹¹ By analysing longitudinal change in anti-JCV Ab index after natalizumab treatment, we show that natalizumab exposure is associated with a significant and substantial increase in anti-JCV Ab titre over time.

Materials and Methods

Patients treated with natalizumab were tested for anti-JCV Ab serostatus, as part of normal clinical practice at Imperial College Healthcare NHS Trust, UK. Anti-JCV Ab serostatus and anti-JCV Ab titres were retrospectively collated from all patients

who were tested using STRATIFY Gen2 between February 2012 and December 2014. Results from other anti-JCV Ab assays (including STRATIFY Gen1) were not included.

Anonymised data were analysed as part of a clinical audit, registered at Imperial College Healthcare NHS Trust (audit number 1987-2015).

Statistical Analysis

Study cohort characteristics are presented as mean $\pm$ standard deviation, while anti-JCV Ab results are presented as mean $\pm$ standard error of the mean. For patients with more than one anti-JCV Ab result, longitudinal change in anti-JCV Ab index between ‘paired sequential samples’ was tested using Student’s paired t-test. Difference between seroconversion and seroreversion rates was tested using Fisher’s exact test. To assess differences in ordered categorical variables, the χ^2 test for trend was used. Difference in mean values between multiple groups was tested using repeated measure ANOVA. All parametric tests were performed after assessing for normality. Correlation of anti-JCV Ab titre with baseline age was measured using linear regression analysis, and tested using the non-parametric Spearman’s rank correlation coefficient. All tests were two sided, and P values were considered significant if <0.05 .

Results

Study cohort

1154 anti-JCV Ab results were available for 485 patients (194 male), with a mean age of 41.7 ± 10.7 . Of 485 patients, 340 had more than one anti-JCV Ab test; there were 1009 results from this cohort, and therefore 669 “pairs of sequential tests”. 12 of these ‘pairs of tests’ happened while the patient was not on natalizumab. The remaining 657 ‘pairs of tests’ make up the longitudinal cohort of this study. The mean time between sequential tests was approximately 9 months, both for pairs of tests seronegative at baseline and seropositive at baseline. The mean duration of natalizumab treatment (from drug initiation until the patients’ last available anti-JCV Ab test) was 3.0 ± 1.9 years.

At baseline, 287 patients were anti-JCV Ab seropositive (59%) and 198 were anti-JCV Ab seronegative (41%). 162 of 485 patients had an anti-JCV Ab index >1.5 at baseline (33%).

Anti-JCV Ab index increases with natalizumab exposure

To investigate the effect of natalizumab on anti-JCV Ab levels, individual pairs of sequential tests were analysed for longitudinal change in anti-JCV Ab index values ($n = 657$ pairs). This found a highly significant mean change between sequential titres of $+0.102$ ($m_{A-B} 0.102 \pm 0.021$; paired $t(656) = 5.0$; $p < 0.0001$). By adjusting for the number of days between each pair of tests, the mean annualised change in anti-JCV Ab titre was calculated as $+0.136/\text{year}$.

This increase in longitudinal anti-JCV Ab index persists long-term, since it occurred in sequential tests performed during the periods 0-2, 2-4, and 4-7 years after initiation

of natalizumab treatment (Table 1). The rate of increase was greater in years 2-4 than in years 0-2 (0.070 ± 0.028 vs 0.167 ± 0.043 ; $t(466) = 2.0$; $p < 0.05$).

(Table 1)

Period of natalizumab treatment	n	Mean index change $\pm$ SEM between paired sequential tests	p value	Annualised increase in anti-JCV Ab titre
0-2 years	285	$+0.070 \pm 0.028$	<0.05	$+0.092$
2-4 years	193	$+0.167 \pm 0.043$	<0.0001	$+0.221$
4-7 years	189	$+0.081 \pm 0.039$	<0.05	$+0.114$
All	657	$+0.102 \pm 0.021$	<0.0001	$+0.136$

Table 1: Mean change in anti-JCV Ab index between paired sequential tests, as function of duration of natalizumab treatment when second test performed. p values are reported for paired Student's t-tests. Annualised increase in anti-JCV Ab titre calculated after adjusting for number of days between each pair of tests.

Next, paired tests were divided into groups defined arbitrarily by baseline anti-JCV Ab index. The mean increase in longitudinal anti-JCV Ab index was statistically significant in all baseline index groups, other than tests with baseline index >3.0 (Fig 1). This group with baseline index >3.0 had a mean decrease in anti-JCV Ab index across sequential tests, and the difference between this group and all others approached statistical significance with one-way ANOVA ($F(4,652) = 1.95$, $p = 0.10$; post hoc Tukey test: "Baseline Titre >3.0 group" different from 0-0.2 group, 0.2-0.4 group, 0.4-1.5 group, and 1.5-3 group, with p values <0.05 , 0.051, <0.05 , and 0.09, respectively).

One-Way ANOVA: $F(5,663) = 2.195$, $p=0.054$; post hoc Tukey test: “Baseline Titre >3.0 ” group different from “0-0.2”, “0.2-0.4”, and “0.4-1” groups, $p<0.05$; Fig 1).

Figure 1: Longitudinal change in anti-JCV Ab index between paired sequential tests ($n=657$), as function of baseline anti-JCV Ab index. Error bars represent mean $\pm$ standard deviation. Paired t test to assess difference between sequential tests: * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, ns = non-significant. Note the x-axis and y-axis on this Figure are non-linear.

When considering all pairs of tests, the overall mean increase in anti-JCV Ab index was driven both by disproportionate levels of small increases (versus small decreases)

and disproportionate levels of large increases (versus large decreases) in index (χ^2 test for trend: $p < 0.0001$; Fig 2).

Figure 2: Change in anti-JCV Ab index in pairs of sequential tests ($n=657$). Small increases outweigh small decreases, and large increases outweigh large decreases. Note the x-axis on this Figure is non-linear.

Anti-JCV Ab seroconversion increases with natalizumab use

Seroconversion rates were analysed for the 340 patients with two or more anti-JCV Ab tests. Of 157 patients seronegative at baseline, 35 seroconverted with repeat testing (22.3%). Of the 35 seroconversions, 12, 13, and 10 seroconverted during the periods 0-2, 2-4, and 4-7 years post-treatment, respectively. Of 183 patients seropositive at baseline, 9 seroreverted with repeat testing (4.9%). There was a statistical difference in seroconversion rates versus seroreversion rates ($p < 0.001$).

However, mean number of tests was 1.95 in those anti-JCV Ab seropositive at baseline and 2.99 in those anti-JCV Ab seronegative at baseline, reflecting increased duration of natalizumab use in those who were seronegative at baseline. To eliminate this source of bias, longitudinal seroconversion rates were analysed for each individual pair of sequential tests (n = 657 pairs). Of the seronegative tests (n = 364), the subsequent test became seropositive in 40 cases (11.0%), with an annualised seroconversion rate of 15.4%. Of the seropositive tests (n = 293), 18 became seronegative (6.1%), with an annualised seroreversion rate of 7.8%. This difference in seroconversion versus seroreversion rate was statistically significant ($p < 0.05$). The annualised seroconversion rates were 12%, 18%, and 16% respectively during years 0-2, 2-4, and 4-7 of natalizumab treatment, while annualised seroreversion rates were 6%, 8%, and 9% during these periods. Of the 18 seropositive tests that seroreverted, 16 had baseline anti-JCV Ab titres of ≤ 0.6 , the remaining two had baseline anti-JCV Ab titres of 0.68 and 3.09.

Increasing age does not account for increasing Anti-JCV Ab index

It has been well characterised that anti-JCV Ab seropositivity increases with age.^{9, 10} In our cohort, baseline age had only a weak effect on baseline anti-JCV Ab titre (Anti-JCV Ab titre = $0.006 \times \text{Age} + 0.908$; r^2 0.003; $p=0.2$). This increase of 0.006/year is far less than the 0.136/year observed between paired longitudinal tests in those treated with natalizumab.

A cross-sectional approach is insufficient to replicate these findings

When the same data described thus far were re-analysed in a cross-sectional manner, correlating all 1154 anti-JCV Ab titres with duration of treatment using Spearman's

rank correlation coefficient, no significant relationship between natalizumab use and anti-JCV Ab titre were found ($r_s = 0.015$, $p=0.6$). 139 of 245 tests performed prior to natalizumab use were seropositive (57%), whereas 466 of 909 tests performed after natalizumab use were seropositive (51%). If taken alone, without the longitudinal results, these cross-sectional data could be interpreted as showing a trend towards *lower* anti-JCV Ab seropositivity rates after natalizumab treatment ($p=0.13$).

PML cases

Two patients in this cohort developed PML, with confirmed JCV on CSF PCR. The first patient had started natalizumab in 2012, and had been seropositive on STRATIFY Gen1. STRATIFY Gen2 anti-JCV Ab titres were 3.2, and 3.0 six months later. The second patient had started natalizumab in 2010, and had been seronegative on STRATIFY Gen1. STRATIFY Gen2 anti-JCV Ab titres went from 2.5 to 3.4 to 4.4 in the year prior to clinical or radiographic PML presentation and diagnosis.

Discussion

This is the first study to report longitudinal increase in anti-JCV Ab index in a cohort of patients treated with natalizumab. We find that anti-JCV Ab index increases with natalizumab exposure by 0.136/year, a level far greater than that which would be expected due to age alone.

Cross-sectional studies have compared patients on natalizumab and those not on natalizumab, and found no relationship with anti-JCV Ab serostatus.⁸⁻¹¹ These studies also found no relationship between duration of natalizumab treatment and anti-JCV

Ab serostatus,^{8, 10} with one exception.¹² One cross-sectional study compared the duration of natalizumab treatment with anti-JCV Ab titres (rather than serostatus), and found no significant relationship.¹³ The cross-sectional approach makes selection bias more prominent, since anti-JCV Ab seropositive patients are less likely to actually commence natalizumab, or to continue natalizumab long-term. Indeed, when the data described in our manuscript were re-analysed in a cross-sectional manner, no significant relationships were found.

Outteryck et al. (2014) compared longitudinal serostatus in patients treated with natalizumab. However, the baseline test was analysed with STRATIFY Gen1 assay and the follow-up test analysed with STRATIFY Gen2 assay.¹³ This study found increased seroconversion compared with seroreversion, but concluded that this was most likely the consequence of different sensitivities between assays. Longitudinal change in anti-JCV Ab titre could not be measured, given the different units used between the two STRATIFY assays.^{6, 13} A longitudinal approach has also been conducted by Biogen Idec, whereby 6-monthly serostatus was determined over a 2.5 year period for 538 patients enrolled in the pivotal AFFIRM randomised controlled trial, using both STRATIFY Gen1 and STRATIFY Gen2 assays.¹⁴ In contrast to our results, this study found equivalent levels of longitudinal seroconversion (3%) versus seroreversion (2%) using STRATIFY Gen2, while 5% of patients were classified as “intermittent positive”. However, this study does not state whether participants were on the placebo arm of AFFIRM, or the natalizumab arm; if patients on placebo were included this could explain why longitudinal seroconversion rates were considerably lower than those found in our study. Change in longitudinal anti-JCV Ab Index was not reported.

Natalizumab's mechanism of action involves limiting leucocyte extravasation across the blood-brain-barrier.¹⁵ This increases the peripheral sequestration of leucocytes, and could theoretically cause non-specific increased widespread peripheral immune activity, including increased anti-JCV antibody titres.¹⁶ A second putative mechanism is that natalizumab could promote mobilisation of peripheral-blood mononuclear cells from the bone marrow, some of which may harbour latest JCV infection.¹⁷⁻¹⁹ This study observed many "large increases" in prospective anti-JCV Ab index, disproportionate to the effect that would be expected from increasing age alone, perhaps in support of this second putative mechanism. If true, one might hypothesise that natalizumab could increase risk of PML through mechanisms additional to decreased immune surveillance of the brain.

The results of this study have clinical implications. With increasing anti-JCV Ab indexes over time, patients' risk of PML will increase.⁷ The observed mean increase of 0.136/year months is substantial, in the context of the index values used to define STRATIFY Gen2 serostatus (seronegative = 0-0.2; intermediate = 0.2-0.4; seropositive = >0.4).⁶ The mean increase in anti-JCV Ab index occurs regardless of baseline index, other than those with extreme high index (>3).. Given the rate of anti-JCV Ab level increase observed in this study, we believe patients on natalizumab should be re-tested for anti-JCV Ab levels every 6 months, regardless of whether they are anti-JCV Ab seronegative or seropositive. Patients can be counselled that "one out of seven seronegative patients will seroconvert in one year of treatment"; more data are needed to confirm whether the rate is greatest in years 2-4 post-treatment. One can also advise that "one out of thirteen seropositive patient will serorevert",

although this is mainly in those with anti-JCV Ab titre ≤ 0.6 . Further work is needed using larger datasets of those who develop PML, to investigate whether ‘rate of change’ of anti-JCV Ab index may affect PML risk.

Natalizumab is a very effective disease modifying therapy for MS, and shall continue to have an important role in treating patients with severe relapsing-remitting MS. This study implies that given enough time on natalizumab, clinicians might expect the majority of their patients to develop positive anti-JCV Ab titres.

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Conflicts of Interest Statement

Biogen Idec provided the STRATIFY Gen2 assay free-of-charge, as per normal clinical practice. JR and AG have no conflicts of interest. RN has received grants, personal fees and non-financial support from Novartis, grants, personal fees and non-financial support from Biogen Idec, personal fees from Genzyme, personal fees from Roche, outside the submitted work. OM has received travel grants to educational meetings from Biogen Idec and honoraria from Biogen Idec, which have been used exclusively for clinical research, outside the submitted work.

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