

John Cunningham Virus seroconversion during natalizumab treatment

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ABSTRACT

Introduction: Multiple sclerosis is a progressive disease that is difficult to predict, originating in cases of disability. Natalizumab is a highly effective disease-modifying therapy but is associated with greater John Cunningham virus (JCV) reactivation and consequent increased risk of developing Progressive Multifocal Leukoencephalopathy (PML). **Objective:** To analyze JCV seroconversion in patients treated with natalizumab. **Methods:** A retrospective study was conducted involving patients diagnosed with multiple sclerosis, between January 2012 and December 2021. To assess seroconversion during treatment with natalizumab, patients were considered seronegative at the beginning of treatment and who had at least one result in the period of medication use. The study was approved by the Human Research Ethics Committee under protocol 3,177,442. **Results:** Sixty-two patients treated with Natalizumab were included, with a seroprevalence of 67.7%. At the start of treatment, 41.9% (26/62) of the patients were negative for anti-JCV, of which 23.1% (6/26) were seroconverted. The mean time to seroconversion was 2.5 years. The baseline index of anti-JCV antibodies was statistically significant with the age of the patients. Among patients with a negative anti-JCV antibody result at baseline, 82.6% (19/23) remained negative throughout monitoring. Treatment was discontinued in 53.2% (33/62) of patients, and 72.7% (24/33) due to anti-JCV positivity with a consumption index >1.5 in 41.9% of cases. **Conclusion:** Knowing how to monitor the anti-JCV antibody index and treatment approaches in our patient cohort may be useful in future clinical decisions in treating MS.

Keywords: Multiple Sclerosis; Leukoencephalopathy, progressive multifocal; Natalizumab; JC Virus; Seroconversion; therapeutics.

INTRODUCTION

Multiple sclerosis (MS) is a chronic progressive disease characterized by acute episodes of demyelination, axonal damage, and progressive neurodegeneration of the central nervous system (CNS), leading to long-term disability¹. Natalizumab (Tysabri®), a humanized anti-alpha 4 integrin monoclonal antibody, acts by preventing the adhesion

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and migration of activated T lymphocytes to the central nervous system. It has proven to be a highly effective disease-modifying therapy (DMT) and is prescribed for patients with high-activity relapsing-remitting MS².

Although phenomenally successful in controlling highly active MS, its use is associated with increased reactivation of John Cunningham virus (JCV), a polyomavirus found exclusively in humans, capable of replicating in immunosuppressed individuals, which can lead to the development of Progressive Multifocal Leukoencephalopathy (PML)³. This is a rare infectious demyelinating disease of the CNS^{1,4} characterized by deficits affecting neurobehavioral, motor, language, and visual functions, which can be severe and potentially fatal. In 2020, the global incidence of LEMP was 3.94/1000 patients treated with natalizumab⁵, with a mortality rate of 22%⁶.

Monitoring the risk of PML using the anti-JCV antibody index, length of treatment and previous use of immunosuppressants in patients taking natalizumab is essential for the doctor to assess the individualized risk-benefit profile for the patient⁷. Although there are inherent concerns regarding anti-JCV antibody positivity among MS patients starting natalizumab therapy, there are few studies on the stability of anti-JCV antibody indices over time⁸.

This study aimed to analyze JCV seroconversion in patients treated with natalizumab.

METHODS

This was a longitudinal, retrospective study based on data analysis of patients diagnosed with MS. Patients followed up at the *Ambulatório de Doenças Desmielinizantes do Hospital Universitário Maria Aparecida Pedrossian* (HUMAP), under treatment with natalizumab 300mg from January 2012 to December 2021, were included. Patient data was collected from the medical records and laboratory information.

Patients who had only one anti-JCV antibody result during treatment were considered for seroprevalence calculation, while patients who had at least two serological status and/or anti-JCV antibody index results were monitored for seroconversion or seroreversion assessment.

The results of the serological status and anti-JCV antibody index, determined by a two-step enzyme-linked immunosorbent assay, conducted in a reference laboratory (Unilabs, Copenhagen, Denmark), using STRATIFY JCV™, were identified. An anti-JCV antibody index >0.40 denotes positivity for anti-JCV antibodies and an index <0.20 denotes negativity for anti-JCV antibodies. For samples with an index ≥0.20 but ≤0.40 (intermediate response), additional evaluation in the confirmatory test (second stage) is required⁹.

The different anti-JCV antibody index categories are defined for the risk stratification of PML and are classified according to

indices (index ≤0.9, >0.9 ≤1.5, and >1.5)⁸. Because of this important clinical application, we also used these categories and investigated the percentage of patients who switched between these different index categories.

Seroconversion was defined as a positive result for anti-JCV antibodies at least once during treatment, remaining positive for one more test if the baseline serological status was negative. If the patient's serological status went from positive to negative during treatment and remained negative for anti-JCV antibodies for one more test, it was considered seroreversion. Seroprevalence was obtained by considering the number of seropositive patients for anti-JCV antibodies (JCV⁺) at the end of the period.

Regarding demographic characteristics, continuous variables were expressed as mean and standard deviation or median and interquartile range, and categorical variables as frequencies and percentages. The normality of the quantitative variables was assessed using the Kolmogorov-Smirnov test. The change in the anti-JCV antibody index over time was assessed using the Friedman test. Spearman's correlation was used to assess correlations between age, gender, length of illness, previous use of DMT, and the anti-JCV antibody index. Statistical significance was p-value <0.05. The data was analyzed using the statistical program SPSS V.22.0.

The study was approved by the ethics committee of the Federal University of Mato Grosso do Sul (protocol no. 3,177,442).

RESULTS

The study included 62 patients and seroprevalence at the end of the period was 67.7% (42/62). The clinical and demographic data are described in Table 1.

At the start of treatment, 41.9% (26/62) of patients were negative for anti-JCV antibodies and 12.9% (8/62) had no serological status result.

Fifty-two patients had at least two serological status and/or anti-JCV antibody index results and were used to assess seroconversion or seroreversion. 44.2% (23/52) were positive, 46.2% (24/52) were negative and 9.6% (5/52) had no anti-JCV antibody result before starting treatment. The average time taken to monitor the JCV antibody index was 41.8 ± 27.7 months. Seroconversion occurred in 25.0% (6/24) of patients at least once during monitoring and no cases of seroreversion were observed. The median time for seroconversion was 2.5 years. Ten MS patients who were not monitored had only one serological status result and were not considered for follow-up.

In 45.2% (28/62) of the patients who started treatment with natalizumab, the serological status was positive, with a mean anti-JCV antibody index of 2.29 ± 0.98. There were no cases of seroreversion.

Three (50.0%) of the six seroconverts remained with an anti-JCV antibody index ≥1.5. One patient (16.7%) remained with an index ≤0.9, and two patients (33.36%) seroreverted to negative

Table 1: Demographic, clinical, and serological characteristics of patients treated with Natalizumab 300mg between January 2012 and December 2021.

Characteristics of the cohort (n=62)			
Age at diagnosis, mean (SD)		31.8	9.3
Age at onset, mean (SD)		37.1	10.2
Diagnosis time in years, median (IQR)		3.5	1-9
Sex, n (%)	Male	13	21.0%
	Female	49	79.0%
Treatment time in months, median (IQR)		32.5	14.7-60.5
EDSS, median (IQR)	Start NTZ	5,0	2.5-5.0
JCV status onset, n (%)	Negative	26	41.9%
	Positive	28	45.2%
	Unknown	8	12.9%
Previous use of immunosuppressants, n (%)			
	Azathioprine	2	3.2%
Previous use of DMT, n (%)			
	Betainterferone 1A	36	58.1%
	Glatiramer Acetate	26	41.9%
	Fingolimod	6	9.7%
No DMT use, n (%)		19	30.6%

Patient characteristics. Abbreviations: SD: standard deviation; MS: Multiple Sclerosis; DMT: Disease Modifying Therapy; IQR: interquartile range; EDSS: Expanded Disability Status Scale.

status. Thus, 79.1% (19/24) of patients with a negative anti-JCV antibody result at baseline remained negative throughout monitoring. The percentages of patients who changed from lower to higher anti-JCV antibody index categories are shown in Figure 1.

The baseline anti-JCV antibody index showed a statistically significant correlation with patient age ($R=0.32$, $p=0.009$; figure 2), with no difference between men and women, disease duration, or previous exposure to DMT, whether immunomodulatory or immunosuppressive.

The anti-JCV antibody index did not vary significantly during the monitoring period. There was no difference between the baseline serological status and that at the end of the monitoring period about age, gender, duration of the disease, or previous use of DMT.

Treatment was discontinued in 53.2% (33/62) of patients, predominantly women (75.8%) with a mean age of 43 ± 9.9 years, discontinued treatment. The average treatment time until discontinuation was 30 ± 19.5 months, ranging from 3 to 79 months.

Negative baseline status was observed in 24.3% (8/33) of patients who discontinued treatment, two patients who discontinued had no additional anti-JCV index result, and 9.1% (3/33) remained negative until the time of discontinuation. In 51.4% (17/33) of the patients, the baseline status was positive, and 24.3% (8/33) did not have baseline status assessed.

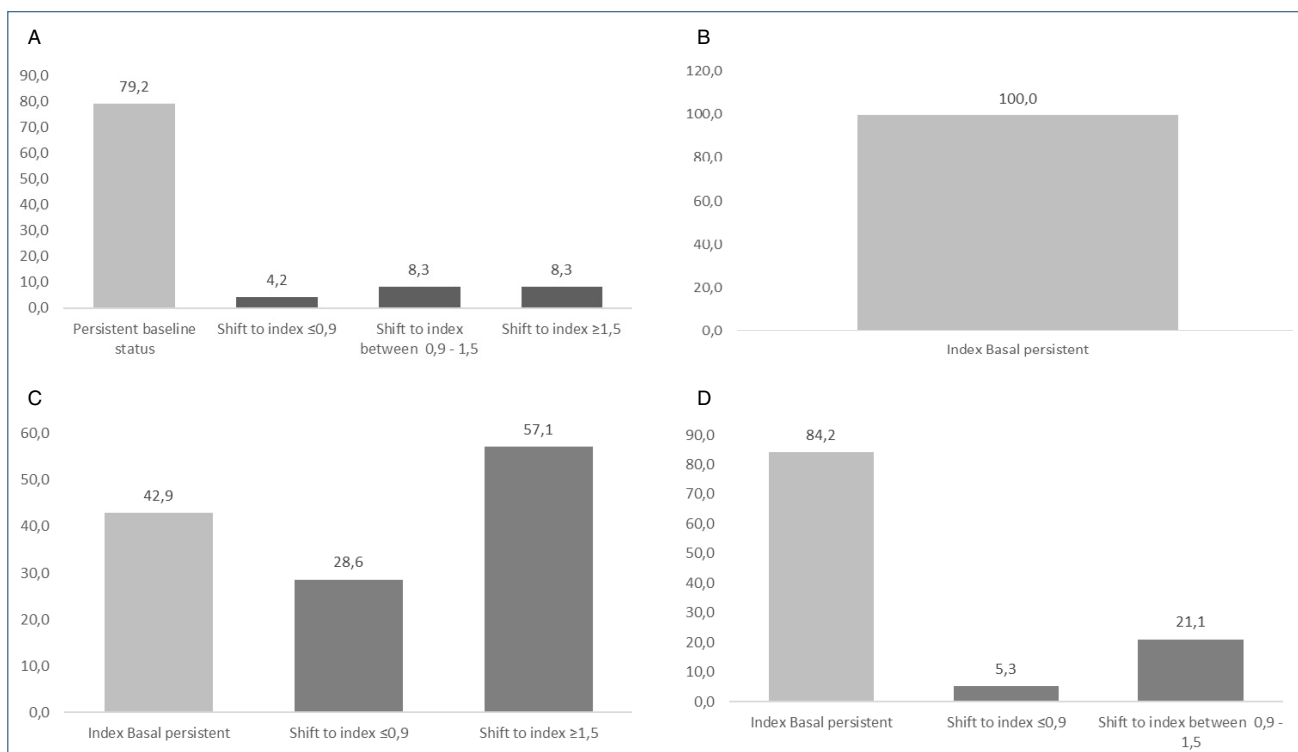


Figure 1: Percentages of patients who switched between different anti-JCV antibody index categories. (A) Percentage of patients with negative anti-JCV antibody index at baseline (n=24 - 46.2%) who switched to higher index categories at least once during treatment (B). Percentage of patients with positive anti-JCV serological status and antibody index ≤ 0.9 (n=2 - 3.8%) at baseline, there were no patients who changed category. (C) Percentage of patients with anti-JCV antibody index >0.9 and ≤ 1.5 (n=7 - 13.5%) at baseline who changed to higher or lower index categories at least once during treatment. (D) Percentage of patients with an anti-JCV antibody index >1.5 (n=19 - 36.5%) at baseline who switched to lower index categories at least once during treatment.

Of the patients who discontinued treatment, 72.7% (24/33) were due to JCV positivity, 18.2% (6/33) due to therapeutic failure, and 9.1% (3/33) due to post-infusion adverse reactions.

Only one patient did not start another DMT after discontinuation. Fingolimod and ocrelizumab were the main drugs, in 43.7% (14/32) and 31.3% (10/32) of cases, respectively. Alentuzumab was prescribed for 12.6% (4/32) of patients, followed by teriflunomide (3,2%) and glatiramer acetate (3,2%).

DISCUSSION

In Brazil, there are few studies on the seroprevalence of anti-JCV¹⁰⁻¹² antibodies in MS patients, changes in serological status and the stability of these indices over time, as well as the relationship between clinical and demographic factors.

Analysis of the seroprevalence data in this study was in line with the literature, which describes values ranging from 40 to 69.5%^{10,13-16}. We observed a seroconversion rate like other studies, which ranged from 13 to 26.7%¹⁷⁻²⁰ even with the long monitoring period of our cohort.

Elevated levels of anti-JCV antibodies at the start of treatment with natalizumab is a crucial point to consider. This is because this therapy reduces CD4, CD8, and Th17 cells and immunoglobulin levels in the cerebrospinal fluid, contributing to a decline in immunity against JCV throughout treatment, which is associated with a higher risk of PML^{9,17,21}. In addition, previous use of immunosuppressants and prolonged treatment with natalizumab in JCV-positive patients are additional risk factors^{3,7,17}.

This study found a correlation between patient age and index values. Similarly, as observed by Bonek et al.²², they identified a correlation between increased prevalence of anti-JCV antibodies at older ages. Analysis of seroprevalence categories showed that patients with anti-JCV indices >1.5 make up the largest group, corresponding to 34.6% of patients. Comparable results in the Polish, Portuguese, and Austrian cohorts, and higher than in other European countries (Germany, France, United Kingdom)^{8,20,22-26}. In some countries on the Asian continent, such as Japan and South

Korea, the rates are higher than those found in this study^{27,28}. The possible hypothesis responsible for the differences in seroprevalence and anti-JCV antibody index between Asian and Western countries may be the interaction of viral and host genetic factors²⁷.

Knowing the prevalence of patients with a high-risk index for PML is important for clinical practice since in this group of patients it is risky to conduct a high number of infusions, and clinical and radiological monitoring is recommended to detect the disorder early²⁸. According to the algorithm for estimating the risk of PML⁷, patients with an anti-JCV antibody index >1.5 being treated with natalizumab are at elevated risk of developing PML. Thus, caution is needed when selecting therapies, especially with highly effective drugs, since the relationship between JCV risk stratification and PML has only been validated for patients treated with natalizumab, and no studies are identifying this relationship in other therapies^{29,30}.

Serological tests for detecting antibodies can be false-positive due to cross-reaction. In the case of the STRATIFY JCV test, this can occur with antibodies to Cytomegalovirus (CMV), Human Immunodeficiency Virus (HIV), or Herpes Simplex Virus (HSV)³⁰. Thus, the cases of seroreversion observed in our study may be due to cross-reaction since the serological status of these cases remained negative for more than three consecutive analyses.

Patients who experienced seroreversion could be considered technical seroconverters since their antibody levels were low. Schwab et al.²⁰ demonstrated that patients who experienced serologic reversion had fluctuations in the anti-JCV index of around 0.4, posing an extremely minimal risk of developing PML. Biological seroconversion occurs when antibody levels rise to index values above 0.9, where the risk of PML is higher. Biological seroconversion is considered a rare event with questionable significance, as it cannot be extrapolated to clinical practice. These results corroborate the findings of this study, given that patients alternate between the distinct categories in the risk stratification and can return to the category at the start of treatment.

In those treated with natalizumab³¹, the seroconversion rate is higher than in the general population, ranging from 2% to 26%^{15,17,19-21}. Therefore, for both patients with negative and positive baseline serological status, periodic monitoring of serological status and anti-JCV antibody index is necessary during treatment with natalizumab^{7,22}.

Corroborating the results found in the literature, we found that the main reason for discontinuing treatment with natalizumab was JCV positivity and the consequent risk of PML^{32,33}. The average discontinuation time is in line with what is described in the literature since remaining on treatment for longer than 24 months considerably increases the risk of developing PML³⁴. In patients with an antibody index >1.5, the estimated risk increases from 9 to 30 in 10,000 patients³². However, studies are comparing the efficacy and safety of extended-dose and standard-dose natalizumab,

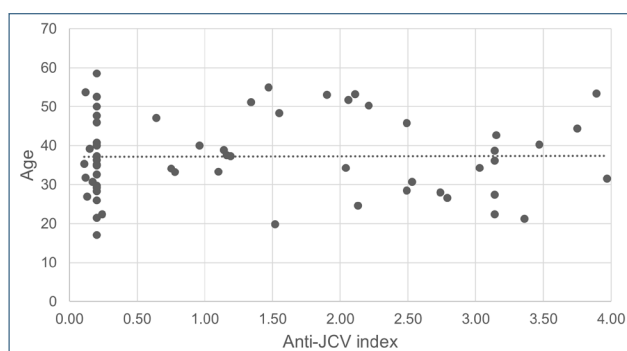


Figure 2: Spearman's correlation between the anti-JCV antibody index and patient age.

suggesting that infusions more than 4 weeks apart maintain efficacy and reduce the risk of PML^{35,36}.

Changing the highly effective pharmacological strategy in the treatment of MS is often a cause for concern and uncertainty. Although there is a cumulative risk of PML, therapy with fingolimod or ocrelizumab after discontinuation of natalizumab is the alternative used at our center. Data from the literature corroborates the use of these drugs after discontinuation of natalizumab for adequate suppression of the disease, proving to be safe and effective³⁷⁻⁴⁰. Although transitional PML can still occur, this needs to be weighed against the risk of recurrence of disease activity.

Although rare, bridging PML represents a risk for patients treated with JCV-positive natalizumab after switching to DMT with long-term immunosuppressive effects³⁴.

In conclusion, the results highlight the importance of constantly monitoring the serological status of patients being treated with natalizumab since there has been seroconversion in these patients over time. In addition, the observation between the age of patients and the rate of anti-JCV antibodies suggests the need to consider demographic factors when assessing risk. The decision to continue or discontinue treatment with natalizumab should be carefully weighed, considering the risk and efficacy of alternative therapy.

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