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JC virus antibodies in Portuguese multiple sclerosis patients: JUSTIFY study results

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ABSTRACT

Objective: To confirm anti–JC virus (JCV) antibody seroprevalence in Portuguese patients with relapsing-remitting multiple sclerosis (RRMS) and to determine their anti-JCV antibody index.
Methods: JUSTIFY was a retrospective, multicentre study that included 655 RRMS patients tested at least once with the anti-JCV antibody assay STRATIFY JCV DxSelect. Demographic data, multiple sclerosis history and results of the anti-JCV antibody test were collected, along with physicians' reasons for requesting the test and the impact of the results.
Results: Overall anti-JCV antibody seroprevalence was 60.8% (95% confidence interval, 56.9–64.5). Seroprevalence was associated with higher age ($P = .030$) and was lower in natalizumab-treated patients ($P < .001$). The mean anti-JCV antibody index of immunosuppressant-naive patients was 1.5 ± 1.3 ($n = 378$). The main reasons for performing the test were clinical characterization (35.5%) and medication change (26.2%). In patients who switched treatments ($n = 109$), fingolimod (47.7%) and natalizumab (26.6%) were the most commonly chosen new treatments.
Conclusions: The study confirmed the high anti-JCV antibody prevalence in Portuguese RRMS patients and its association with age. These data can be used to better understand the benefit-risk profile of natalizumab treatment in Portuguese patients and to support progressive multifocal leukoencephalopathy risk management strategies.

1. Introduction

Multiple sclerosis (MS) is a chronic, inflammatory and degenerative demyelinating disease of the central nervous system (CNS) with a multifactorial aetiology that has not yet been fully elucidated [1,2]. With an estimated global prevalence of 33 per 100,000, MS is in many countries the leading cause of nontraumatic neurologic disability among young adults, and it has a significant social and economic impact [3–5]. In Portugal, MS is estimated to affect approximately 40–50 per 100,000 inhabitants [6–8].

Progressive multifocal leukoencephalopathy (PML) is a rare opportunistic infection of the CNS that can result in death or severe disability [9,10]. PML is caused by JC virus (JCV), a human polyomavirus that individuals are commonly exposed to early in life and that usually results in a subclinical infection [9,10]. JCV infection results in production of anti-JCV antibodies detectable in the blood or serum [11]. JCV activation and PML generally occur only in patients who are immunocompromised or being treated with certain immunosuppressive/immunomodulatory treatments.

Natalizumab (Tysabri®, Biogen), a monoclonal antibody approved as therapy for active relapsing MS patients [12], has been associated with an increased risk of PML [12–14]. Three risk factors for natalizumab-associated PML have been identified: the presence of anti-JCV antibodies, the duration of natalizumab treatment (particularly > 2 years) and the prior use of immunosuppressants [13,14]. To stratify PML risk in natalizumab-treated patients, Biogen developed an

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analytically validated enzyme-linked immunosorbent assay (the STRATIFY JCV™ assay, Focus Diagnostics, Cypress, CA, USA) to detect the presence of JCV antibodies in serum [15,16]. An enhanced assay (STRATIFY JCV DxSelect™) in a new, easier-to-use kit format has been available since March 2012 [17]. This test has improved sensitivity to facilitate detection of anti-JCV antibody positive patients with low JCV antibody levels [17]. More recently, the level of anti-JCV antibodies in serum, as indicated by anti-JCV antibody index determined using the STRATIFY JCV DxSelect assay, has been shown to further delineate PML risk in patients without prior immunosuppressant use [14,18].

Rates of anti-JCV antibody seropositivity in MS patients show substantial variation, ranging from approximately 50% to 60% depending upon assay methodology, sample size, the age of the individuals studied and the types of populations studied [15,19–29]. The JCV Antibody Epidemiology in MS (JEMS) study, which was conducted using the STRATIFY JCV assay, found a global anti-JCV antibody prevalence of 57.1% in MS patients from Europe, Canada and Australia [28]. In Portugal, JEMS enrolled 131 patients and found an anti-JCV antibody prevalence of 69.5% [29], the highest level among European countries studied. This suggested a need for further studies of anti-JCV antibody prevalence in Portugal using a larger sample size and the newer STRATIFY JCV DxSelect assay.

The objectives of the JUSTIFY study were to determine the seroprevalence of anti-JCV antibody in Portuguese relapsing-remitting multiple sclerosis (RRMS) patients, to determine these patients' mean anti-JCV antibody index and to estimate the distribution of these patients by index threshold. In addition, the study aimed to identify Portuguese physicians' main reasons for requesting the anti-JCV antibody assay and the impact of the results in clinical practice.

2. Materials and methods

This retrospective, multicentre epidemiological study included 655 RRMS patients and was conducted in 24 centres in all regions of Portugal between August 2014 and July 2015. The study was approved by all the centres' administrations and ethic committees and by the Portuguese Commission for Data Protection. The study was conducted according to the Declaration of Helsinki and International Conference of Harmonisation, Good Clinical Practice and Good Epidemiological Practice guidelines (as applicable).

Eligible patients were over 18 years of age, had a documented diagnosis of RRMS according to the 2010 McDonald criteria [30], had been tested at least once with the STRATIFY JCV DxSelect assay [17] before their study centre evaluation visit, understood the purpose of the study and gave informed consent for participation.

The primary endpoint in JUSTIFY was the prevalence of anti-JCV antibodies, calculated as the number of patients with anti-JCV antibodies detected in serum divided by the total number of patients for whom a serum sample was evaluated. Secondary endpoints included anti-JCV antibody prevalence in subpopulations defined by age, gender, MS duration and use of prior MS therapies (differentiated by duration). At the initial study visit, demographic data (age, gender, place of birth and residence), MS history (comorbidities, duration, treatment, number of relapses in the year before the STRATIFY JCV DxSelect test and Expanded Disability Status Scale [EDSS] score at or before the date of the test) and test data (date, type, result, anti-JCV antibody index, reason for administration of the anti-JCV antibody assay and its resultant impact on therapy selection) were collected. Only the last test before the initial study visit was considered.

Anti-JCV antibody tests are considered positive for anti-JCV antibodies with anti-JCV antibody index > 0.4 and negative with index < 0.2; tests with index ≥ 0.2 and ≤ 0.4 required the second step of the assay to be conducted to determine positive or negative status [17].

2.1. Statistical analysis

Prevalence values are presented along with their 95% confidence intervals (CIs). As anti-JCV antibody index has been associated with PML risk only in patients without prior immunosuppressant use [14,18], only patients with no prior history of classical immunosuppressive therapies (azathioprine, methotrexate, mycophenolate mofetil, mitoxantrone or cyclophosphamide) were considered when determining the mean anti-JCV antibody index and the patient distribution by index threshold. Patients were stratified into ≤ 0.9, > 0.9 to ≤ 1.5 and > 1.5 index cohorts.

Continuous variables were presented as mean ± standard deviation (SD), minimum and maximum. Categorical variables were described by the absolute and relative frequencies. Differences between independent categorical variables were tested using Pearson's chi-squared test. Student's *t*-test for independent samples was used to assess the statistical significance of differences between independent continuous variables. Results were considered significant at a 95% confidence level. All analyses were performed using R (version 3.1.0).

3. Results

3.1. Patients

A total of 655 patients were included in the study, 69.2% of whom were female. At study evaluation visit, the mean age was 40.8 ± 11.0 years (range, 18–74 years), and MS duration varied from 0 to 36 years, with a mean of 7.7 ± 6.0 years. The mean age at MS diagnosis ($n = 596$) was 31.9 ± 10.3 years (range, 13–68 years), and most patients (93.3%) were under 50 years of age at diagnosis.

The anti-JCV antibody tests considered for this analysis were performed between 1 March 2012 and 22 January 2015. The test was administered for the first time in 365 of 655 patients (55.7%) and as a retest in 290 of 655 patients (44.3%).

At the time of the anti-JCV antibody tests considered for this analysis, the mean age was 39.5 years old, with the majority of patients under 40 years old (Table 1). Patients had an MS diagnosis for a mean of 6.6 years and EDSS scores prior to the JCV antibody serostatus evaluation of 0.0–7.5, with a mean of 2.8 ($n = 619$).

At the time of the test, 31.5% of patients had been treated for ≤ 3 years, 41.7% had taken only one therapy for MS and 91.1% had no history of immunosuppressive treatment (Table 1). The most common therapy prior to or concurrent with the test was interferon beta-1a, followed by natalizumab and interferon beta-1b. Average treatment duration on natalizumab was 2.6 ± 1.7 years ($n = 247$).

3.2. The prevalence of anti-JCV antibodies

Of the 655 included patients, 398 tested anti-JCV antibody positive, for an overall prevalence of 60.8% (95% CI, 56.9–64.5). The association between seropositivity and clinical and demographic features is shown in Table 2. Anti-JCV antibody seroprevalence was significantly higher in older patients, whereas it was not significantly related to gender (Table 2) or the patient's current or former area of residence (data not shown). It was also not significantly related to the duration of the disease or of treatment, both of which were similar in seropositive and seronegative patients. There was no significant difference in the prevalence of positive anti-JCV antibodies between immunosuppressant-naive and non–immunosuppressant-naive patients. Patients who had taken natalizumab before or at the time of the test were significantly less likely to be seropositive, though natalizumab treatment duration did not differ significantly between seronegative and seropositive patients (Table 2). Positive tests were also significantly less frequent among retested patients than among those tested for the first time (51.4% [95% CI, 45.5–57.2] vs 68.2% [95% CI, 63.1–72.9]; $P < .001$).

Table 1
Disease and treatment characteristics at the time of the STRATIFY JCV DxSelect test ($N = 655$).

Characteristic	Overall study population
Age, mean, years	39.5 ± 11.0
Age, *n* (%)	
18–39 years	357 (54.5)
40–49 years	166 (25.3)
≥50 years	131 (20.0)
Time since MS diagnosis, years	
Mean	6.6 ± 5.9[a]
Median (range)	5.0 (0–34.0)
Time since MS diagnosis, *n* (%)	
0–3 years	206 (31.5)
4–6 years	133 (20.3)
7–9 years	97 (14.8)
≥10 years	148 (22.6)
Unknown	71 (10.8)
Number of relapses in the previous year, *n* (%)	
0	382 (58.3)
1	186 (28.4)
2	50 (7.6)
≥3	37 (5.7)
EDSS score, mean	2.8 ± 1.9[b]
EDSS score, *n* (%)	
0.0–1.0	53 (8.1)
1.0–1.5	154 (23.5)
2.0–2.5	123 (18.8)
3.0–3.5	116 (17.7)
4.0–4.5	64 (9.8)
5.0–5.5	41 (6.3)
6.0–6.5	58 (8.9)
7.0–7.5	10 (1.5)
Unknown	36 (5.5)
MS treatment duration, *n* (%)	
0–3 years	213 (32.5)
4–6 years	153 (23.4)
7–9 years	113 (17.3)
≥10 years	132 (20.2)
Unknown	44 (6.7)
Number of current and prior MS treatment therapies, *n* (%)	
0	37 (5.7)
1	273 (41.7)
2	177 (27.0)
3	99 (15.1)
≥4	69 (10.5)
Immunosuppressive therapy use, *n* (%)	
Yes	45 (6.9)
No	597 (91.1)
Unknown	13 (2.0)
Treatment history (current and prior), *n* (%)	
Interferon beta-1a	305 (46.6)
Natalizumab	248 (37.9)
Interferon beta-1b	245 (37.4)
Glatiramer acetate	191 (29.2)
Fingolimod	21 (3.2)
Azathioprine	18 (2.8)
Cyclophosphamide	18 (2.8)
Immunoglobulin	15 (2.3)
Mitoxantrone	13 (2.0)
Other (≤5 cases)	22 (3.4)
Natalizumab treatment duration, *n* (%)	
< 1 year	53 (21.4)
1–2 years	46 (18.6)
> 2 years	148 (59.7)
Unknown	1 (0.4)

JCV, JC virus; MS, multiple sclerosis; EDSS, Expanded Disability Status Scale; NA, not available; SD, standard deviation.
Mean values are presented as mean ± SD.
[a] Data were missing for 71 patients.
[b] Data were missing for 36 patients.

3.3. Anti-JCV antibody index

At the time of this study, physicians had to specifically request to receive information on anti-JCV antibody index. Thus, index data were not available for all patients; such data were collected for 430 of 655 patients in this study. In this subset of patients, 299 tested positive for anti-JCV antibodies (288 with index > 0.4 and 11 with an indeterminate result [≥0.2 and ≤0.4] confirmed as positive) and 131 tested negative (81 with an index < 0.2 and 50 with an indeterminate result confirmed as negative).

3.4. Anti-JCV antibody index in immunosuppressant-naive patients

Anti-JCV antibody index was available for 378 of 597 patients (63.3%) with no prior immunosuppressant use. For seropositive anti-JCV antibody immunosuppressant-naive patients ($n = 270$), 24.1% had an index ≤0.9, 12.2% had an index > 0.9 and ≤1.5 and 63.7% had an index > 1.5 (Fig. 1). Mean index values for negative ($n = 108$) and positive ($n = 270$) tests were 0.2 ± 0.1 and 2.1 ± 1.2, respectively. In the overall population of immunosuppressant-naive patients (including seronegative patients), 45.8% of patients had an index ≤0.9, 8.7% had an index > 0.9 and ≤1.5 and 45.5% had an index > 1.5.

Most anti-JCV antibody tests in immunosuppressant-naive patients (337 of 597; 56.4%) were being administered for the first time; most tests of patients with prior immunosuppressant use (29 of 45; 64.4%) were retests.

3.5. Reasons for and influence of anti-JCV antibody tests in clinical practice

The most commonly reported reasons for performing the test were clinical characterization and the need to change medication (Table 3). Of those tests motivated by a decision to change medication in which the clinician also predicted a potential next treatment ($n = 169$), natalizumab was the predicted next treatment in most cases (62.7%), followed by fingolimod (14.8%), 'second-line treatment' (11.2%), 'natalizumab or fingolimod' (7.1%), interferon beta-1a or glatiramer acetate (1.1%) and 'to be decided' (3.0%).

The test result had no impact on the subsequent treatment in 74.0% of cases based on physician records (Table 3). In those cases in which the test did influence treatment choice (Fig. 2), the most common subsequent treatment (when known) was fingolimod (47.7%) or natalizumab (26.6%). Fingolimod was also most frequently chosen when a new treatment was being selected for a treatment-naive patient based on the test (5 of 13 patients). Three of the four patients who suspended their previous treatment based on test results were taking natalizumab; the fourth patient was taking glatiramer acetate. Most of the patients who changed medication after a positive test were taking interferon beta or natalizumab, and most of them changed to fingolimod (24 of 37 and 14 of 31 patients, respectively). Eleven of 80 patients who changed medication after a positive test switched to natalizumab (nine of whom switched from interferon beta or glatiramer acetate).

4. Discussion

The overall prevalence of anti-JCV antibody positivity in our study was 60.8% (95% CI, 57.0–64.7). Our calculations of anti-JCV antibody prevalence among Portuguese patients are lower than those identified in the JEMS study (69.5% [95% CI, 61.6–77.4]) [28,29], though the CIs partially overlap. Similarly, the results of our Portugal-wide study are slightly lower than those of a recent single-centre Portuguese study conducted in 371 patients from the Coimbra region, which reported an anti-JCV antibody seroprevalence of 68.2% [31]. However, our results showed a similar rate of seroprevalence when considering only the

Table 2
JCV antibody seroprevalence by demographic, clinical and treatment characteristics in the overall study population ($N = 655$).

	Positive anti-JCV antibody test			Negative anti-JCV antibody test		*P*
	n	%	95% CI	*N*	%	
Gender						
Male	132	65.3	58.3–71.8	70	34.7	0.129
Female	266	58.7	54.0–63.3	187	41.3	
Age, mean, years	398	40.0 ± 10.9	–	257	38.6 ± 11.1	0.073
Age cohort						
18–39 years	202	56.6	51.3–61.8	155	43.4	0.030
40–49 years	114	68.7	61.0–75.6	52	31.3	
≥50 years	81	61.8	52.9–70.1	50	38.2	
Time since MS diagnosis, mean, years[a]	354	6.5 ± 6.0	–	230	6.8 ± 5.7	0.283
Time since MS diagnosis cohort[a]						
0–3 years	128	62.1	55.1–68.7	78	37.9	0.878
4–6 years	82	61.7	52.8–69.9	51	38.3	
7–9 years	56	57.7	47.3–67.6	41	42.3	
≥10 years	88	59.5	51.1–67.4	60	40.5	
MS treatment duration, mean, years[b]	385	5.4 ± 4.7	–	250	6.1 ± 4.9	0.060
MS treatment duration cohort[b]						
0–3 years	132	62.0	55.1–68.5	81	38.0	0.060
4–6 years	95	62.1	53.9–69.7	58	37.9	
7–9 years	64	56.6	47.0–65.8	49	43.4	
≥10 years	75	56.8	47.9–65.3	57	43.2	
Immunosuppressive therapy use, *n* (%)[c]						
Yes	24	53.3	38.0–68.1	21	46.7	0.382
No	365	61.1	57.1–65.1	232	38.9	
Treatment with natalizumab, *n* (%)						
Yes	120	48.4	42.0–54.8	128	51.6	< 0.001
No	269	68.3	63.4–72.8	125	31.7	
Natalizumab treatment duration, mean, years	119	2.7 ± 1.6	–	128	2.5 ± 1.7	0.336
Natalizumab treatment duration cohort						
< 1 year	20	37.7	25.7–52.2	33	25.8	0.202
1–2 years	22	47.8	33.1–62.9	24	18.8	
> 2 years	77	52.0	43.7–60.3	71	55.5	

JCV, JC virus; MS, multiple sclerosis; SD, standard deviation.
Mean values are presented as mean ± SD.
[a] Data were missing for 71 patients.
[b] Data were missing for 44 patients.
[c] Data were missing for 13 patients.

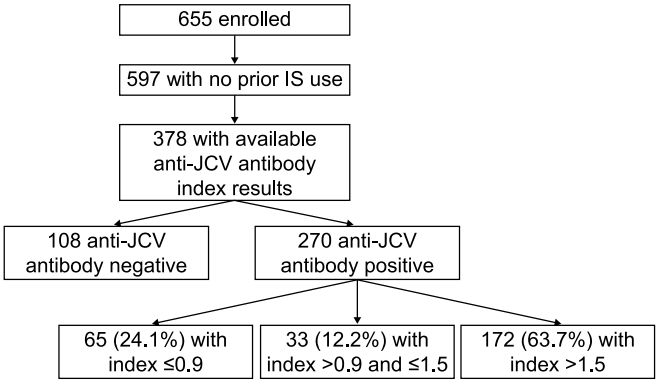

Fig. 1. Anti-JCV antibody index in immunosuppressant-naive patients. IS, immunosuppressant; JCV, JC virus.

region included in that study (data not shown).

Although the anti-JCV antibody seroprevalence in this study was lower than in previous Portuguese studies [28,29,31], we would still classify Portugal as a country with a high prevalence of anti-JCV antibody seropositivity. In JEMS, Portugal had the highest anti-JCV antibody seroprevalence among the 10 included European countries; using the estimates from our study, Portugal would rank fourth, after Germany, the Netherlands and Austria [28]. The estimated proportion of seropositive patients in this study is similar to the reported data from two multinational studies in Germany (59.1% and 60.0%) [22,28] and one of two studies in Sweden (59.0%) [22]. However, comparisons with these studies, as with the JEMS study, are limited by the use of the STRATIFY JCV assay in those studies and STRATIFY JCV DxSelect in the current study. While these two generations of the assay generally produce consistent results, STRATIFY JCV DxSelect has greater reproducibility and enhanced ability to detect low anti-JCV antibody responses [17]. Furthermore, the current study was a retrospective analysis of patients whose clinicians were interested in their anti-JCV antibody status. The population may therefore have been biased toward

Table 3
Motives for and impact of the anti-JCV antibody test.

Patient motives and impacts	Number of patients
Motive for JCV test, *n* (%)	$N = 654$
Clinical characterization	232 (35.5)
Change of medication	171 (26.2)
Retest of a negative patient[a]	150 (22.9)
Retest of a positive patient to request the anti-JCV antibody index[a]	55 (8.4)
Retest of a positive patient to assess anti-JCV antibody index evolution[a]	26 (4.0)
Other	20 (3.1)
Impact of test on changing medication, *n* (%)	$N = 655$
None	484 (74.0)
Changed to a new treatment	150 (22.9)
New MS treatment on a treatment-naive patient	17 (2.6)
Suspended previous treatment	4 (0.6)

JCV, JC virus; MS, multiple sclerosis.
[a] For patients with a retest, results from any preceding tests were not considered in this study.

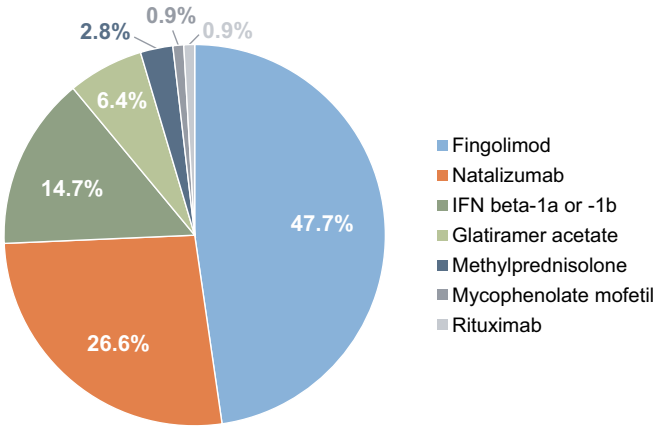

Fig. 2. Selected treatment when switching to a new treatment (n = 109). IFN, interferon.

patients who were receiving or were candidates for natalizumab treatment, limiting the seropositivity comparisons that can be made with populations in other studies.

A recent multicentre study in a Spanish cohort of 1061 MS patients found an anti-JCV antibody seropositivity rate of 58.2% [26]. In another multicentre study in Spain, an overall anti-JCV antibody seroprevalence of 55.3% was reported; however, when only the samples analysed by the STRATIFY JCV DxSelect assay were considered, the prevalence (60.5%) was equivalent to the estimate in our cohort [27]. These two studies show apparent similarity in MS patients between two geographically proximate countries.

As in other studies, anti-JCV seropositivity was associated with greater age [19,20,22–27] and was lower in women [19,22–25,28,29], though we did not find a statistically significant difference between genders, as was observed in other studies [27,31]. Consistent with the published results, anti-JCV antibody positivity was unrelated to MS duration [23,24,26,28,29] or the number or duration of MS treatments [23,26,28,29]. Consistent with the published data that generally show no difference in seropositivity prevalence between immunosuppressant-naive and non-naive treated patients [19,25,26,29], the prevalence of positive anti-JCV antibodies did not differ between these two groups in our study.

In our study, patients who had taken natalizumab before or at the time of the test were significantly less likely to be seropositive than those who had not taken natalizumab. This is most likely due to the selection bias; as anti-JCV antibody positivity is a known PML risk factor, patients with a positive result are less likely to continue natalizumab and are therefore less likely to have available anti-JCV antibody tests.

In our study, 45.5% of patients without prior treatment with immunosuppressants had an anti-JCV antibody index > 1.5. When conducting a risk-benefit assessment of natalizumab, it is important to consider the anti-JCV antibody index level in patients without prior immunosuppressant use, since PML risk can be further differentiated in these patients [14]. In patients with no prior immunosuppressant use before starting natalizumab, the anti-JC virus antibody index level relates to the level of risk of PML. The risk of PML is < 1 per 1000 across all index groups during the first 2 years of treatment, and this risk remains low at antibody index ≤0.9, whereas it is substantially higher in patients with index > 1.5 who have been treated with natalizumab for > 2 years [14].

It may not be necessary to exclude treatments with clinical impact, like natalizumab, in anti-JCV antibody positive patients. Indeed, many patients (40.1%) in this study were receiving or had previously received natalizumab at the time of the test. Most of those on natalizumab treatment had received it for > 2 years. In addition, natalizumab was initiated in some patients with a positive anti-JCV antibody test. Together, these findings suggest that some Portuguese clinicians did not exclude natalizumab as a treatment option for anti-JCV antibody positive patients. However, as the landscape of MS treatments continues to change with new therapies becoming available, treatment practices are also likely to shift. Careful consideration of each patient's anti-JCV antibody index along with other PML risk factors will allow informed assessment of the risks and benefits of natalizumab treatment on an individualized basis.

These results were collected prior to the introduction of the updated PML risk estimates [14], which may influence clinical practice. The updated risk estimates incorporate multiple risk factors, including both anti-JCV antibody index and natalizumab treatment duration in patients without prior immunosuppressive treatment. In anti-JCV antibody positive patients receiving natalizumab, anti-JCV antibody index should be monitored regularly, and patients should receive routine magnetic resonance imaging (MRI) monitoring [32], in line with the updated monitoring recommendations from the European Medicines Agency [33].

Our study has limitations that restrict the interpretation of some results. In addition to the potential selection bias mentioned above, we included only the last anti-JCV antibody test results using the STRATIFY JCV DxSelect assay, which did not allow us to evaluate longitudinal changes in seroprevalence and the effects of specific treatments.

5. Conclusions

We found a 60.8% seroprevalence of anti-JCV antibodies in 655 Portuguese RRMS patients, a seroprevalence lower than that reported in Portuguese patients in JEMS but in line with other studies using the same anti-JCV antibody assay. These results demonstrate the need for individualized PML risk assessment and consideration for MRI monitoring for Portuguese patients receiving or considering initiation of natalizumab treatment.

Contributors

All authors contributed to study design, data collection and interpretation of the results. All authors critically revised the manuscript and approved the final version for submission.

Declaration of Competing Interest

MJS has received consulting fees from Bayer, Biogen, Merck Serono, Novartis, Roche, Sanofi Genzyme and Teva. CCN has received compensation from Biogen, Merck, Novartis and Sanofi Genzyme. AMdS has received consulting fees from Bayer, Biogen, Merck Serono, Novartis, Roche, Sanofi Genzyme and Teva. PM is an employee of Biogen and may hold stock and/or stock options in Biogen. JP-M has received consulting fees from Biogen, Merck and Sanofi Genzyme.

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