Changes in Anti-JCV Antibody Status in a Large Population of Multiple Sclerosis Patients Treated with Natalizumab

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Abstract
Introduction Natalizumab (NTZ) can be associated with an opportunistic infection, progressive multifocal leukoencephalopathy (PML), caused by John Cunningham virus (JCV). High titer of anti-JCV antibody (JCV index) in patients treated with NTZ for over 2 years limit its use, leading to treatment discontinuation.

Objective Aim of the study was to investigate the JCV index changes pre, during and post NTZ treatment and describe the trend after a long period of NTZ discontinuation.

Methods Patients with relapsing–remitting multiple sclerosis (RR–MS) treated with NTZ between 2010 and 2018 were enrolled in this retrospective-prospective observational study. Inclusion criteria were: (1) diagnosis of RR–MS according to the McDonald criteria 2010, (2) at least six NTZ administrations, (3) at least two determinations of JCV Index during the follow-up period, (4) NTZ discontinuation period for more than 6 months. JCV index was determined by STRATIFY II. There were three different timepoints: NTZ initiation (T0), NTZ discontinuation (T1) and time after NTZ suspension (T2). Seroconversion was defined as changing status of serum JCV antibody. Main outcomes were the JCV index changes and the rate of seroconversion.

Results At baseline we enrolled 285 patients (208 JCV negative, 67 JCV positive, and 10 not available). There was a statistically significant increase of JCV index during NTZ treatment period (T0 vs T1, \( p = 0.0009 \)) and during NTZ discontinuation period (T1 vs T2, \( p = 0.04 \)). Patients seroconverted to a positive status more frequently during NTZ treatment than after discontinuation (\( p = 0.008 \)). Moreover, patients who shifted to fingolimod (FTY) as exit strategy after NTZ discontinuation, showed a statistically significant increase of JCV index.

Conclusion Our data confirmed that a high percentage of patients shift to or remain in a positive JCV status during NTZ treatment and after discontinuation. NTZ suspension seems not to be able to interfere on JCV status modification over an extended period. The choice of alternative treatment as exit strategy after NTZ discontinuation should be carefully considered because it could negatively influence the PML risk stratification of patients.

1 Introduction

Treatment options for multiple sclerosis (MS) have changed over the last two decades, bringing about a new category of drugs with more efficient profiles. Natalizumab...
(NTZ), a monoclonal antibody, is extremely effective in reduction of neuroinflammation in patients with relapsing–remitting MS (RR–MS) with high disease activity [1]. However, its use is limited due to risk of progressive multifold leukoencephalopathy (PML), an opportunistic infection caused by John Cunningham virus (JCV) which has a severe impact on patients’ disability course, functional outcome, and quality of life [2].

PML risk is associated with longer exposure to NTZ treatment, previous immunosuppressive therapies and presence of high titer of anti-JCV antibody (JCV index) [3, 4]. Therefore, determination of antibodies against JCV presence of high titer of anti-JCV antibody (JCV index) is an important tool for risk stratification in NTZ treated patients with RR–MS; based on these factors, a risk stratification algorithm has been created with the aim to estimate the PML risk for each patient [4].

The first use of serum antibodies to JC virus for PML risk stratification able to provide qualitative information (positive/negative status) was introduced in 2010 [5]. Since 2013, a second-generation test that delivers a JC antibody index value in addition to positive or negative serostatus has been validated and used [6]. The PML risk estimate for patients with an index value less than 0.9 has been reported to be significantly lower than the PML risk for patients with an index greater than 1.5 [7].

Prolonged use of NTZ is known to be associated with higher risk of PML [3, 8] because patients on therapy undergo to a JC virus seroconversion more frequently than control patients not on NTZ [9]. For these reasons, in patients who are negative or low positive for anti-JCV antibodies, at least six-monthly follow-up tests are recommended by consensus [10].

Generally, patients with high levels of JCV index treated for over 2 years tend to discontinue NTZ treatment, despite the risk of disease reactivation peaking during a “high risk period” between the second and the eighth month since stopping the drug [11]. This clinical practice is considered as a common strategy in order to reduce the JCV index values on long time and therefore to have the possibility of restarting treatment with NTZ, even if a consensus doesn’t exist.

On September 2019, the global overall incidence of PML in NTZ treated patients was 4.08 per 1000 patients (95% CI 3.80–4.36 per 1000 patients). There have been 825 confirmed PML cases, 822 of which in patients with MS (224 cases in US, 515 in European Economic Area, 86 in rest of world, respectively) and in the 76% of patients a moderate level of disability remained [Global Natalizumab (TYSABRI) Post-marketing PML Update. September 2019]. The administration of NTZ with extended interval dosing (EID) has been proposed as a strategy to potentially reduce the incidence of PML while maintaining its therapeutic efficacy [12]. However, even if NTZ discontinuation can lead to MS recurrence, the suspension of treatment and the shifting to a safer therapy represents the most common strategy to limit the risk of PML.

Some studies focused on the impact of disease modifying therapy (DMTs) on the longitudinal evolution of anti-JCV antibody index [13], but no studies have considered the interference of NTZ discontinuation on JCV status modification over an extended time, in order to refine treatment strategies.

Aim of the study was to investigate the anti-JCV antibody status pre, during and post-NTZ treatment and describe the trend of JCV index after a long period of NTZ discontinuation in order to identify possible alternative therapeutic strategies.

2 Methods

2.1 Study population

This retrospective-prospective observational study enrolled patients with diagnosis of RR–MS treated with NTZ and followed at the MS Centre of Catania University Hospital between January 2010 and December 2018. Data about patients was obtained retrospectively from the database iMED, a computerized medical record in which at each clinical follow-up physician of the MS centre collect demographic, clinical and laboratory information. This study protocol was approved by the local Ethical Committee of the University of Catania (Catania 1). Each patient participating to the study signed an Informed Consent specifically designed for the study.

Inclusion criteria were: (1) diagnosis of RR–MS according to the McDonald criteria 2010 [14], (2) at least six NTZ administrations, (3) at least two determinations of JCV Index during the follow-up period, (4) NTZ discontinuation period for more than 6 months. Patients eligible prospectively underwent to the anti-JCV antibody determination after a period of NTZ discontinuation. Main outcomes were the JCV index changes and the rate of seroconversion.

2.2 Sampling

JCV status was evaluated at baseline (T0), at the time of the NTZ discontinuation (T1) and at the last follow-up (T2).

At each timepoint, patients were divided into two groups based on their JCV status: negative JCV index and positive JCV index, with a JCV value between 0.9 and 1.5 (low positive JCV index) or > 1.5 (high positive JCV index) respectively.

Blood samples were collected by peripheral venous puncture. JCV index was determined through a only qualitative result (positive or negative) for patients screened before 2011 (STRATIFY JCV Dx Select, [7]), and by a two-step
enzyme-linked immunosorbent assay (STRATIFY II) for patients screened after 2011. Analysis was centrally performed at Unilabs in Copenhagen, Denmark. Qualitative (negative/positive) and, for anti-JCV antibody positive patients, semiquantitative results were obtained. An index value of less than 0.9 was considered as negative and equal to or greater than 0.9 as positive. Seroconversion was defined as changing status of serum JCV antibody.

2.3 Statistics

Statistical analysis was performed using STATA 12.1 software packages (StataCorp. 2011; Stata Statistical Software: Release 12; College Station, TX: StataCorp LP). The numerical data sets were tested for normal distribution with the Shapiro-Wilk test. Student’s t tests will be applied for parametric variables, while for non parametric variables, differences between subgroups are analyzed with a \( \chi^2 \) test. Nominal data were analyzed by Pearson’s Chi Square or Fisher’s exact test, where applicable. Differences in terms of JCV index value among each time point were calculated by Kruskall-Wallis test and one-way ANOVA, where applicable.

The correlation between clinical and laboratory variables was carried out using a bivariate correlation (Pearson’s or Spearman’s correlation). We considered a two-sided \( p \) value of <0.05 as statistically significant.

3 Results

Out of 332 patients screened referring to the MS centre who had a history of NTZ treatment during their disease course, 285 patients met inclusion criteria (mean age 41 years; female 201 [70.5%]).

In Fig. 1, the flow-chart shows how patients have been screened at different timepoints based on their JCV status.Demographic and clinical characteristics of the whole cohort at baseline are summarized in Table 1. Out of 285 patients, at T0 208 (73%) were JCV negative, and 67 patients (23.5%) were positive [33 (11.6%) in the low positive JCV index group and 34 (11.9%) in the high positive JCV index group, respectively]. Data were not available for the remaining 10 (3.5%) patients (female 7 and male 3, respectively).

Out of JCV positive patients at baseline, 3 (9.1% of 33) in low positive JCV index group and 9 (26.5% of 34) in high positive JCV index group were naive, while 55 (82.1% of 67) had already been treated with a DMT; in the group of JCV negative patients, 60 (28.8% of 208) were naive and 148 (71.2% of 208) had already been treated with a DMT. There were not statistically significant differences between groups in term of age and sex, years of disease, number of previous DMTs and previous use of immunosuppressors.

Fig. 1 Flow chart of patients screened and relative JCV index. JCV index – John Cunningham (JC) virus index negative (titer of anti-JCV antibody below 0.9), JCV index + John Cunningham (JC) virus index positive (titer of anti-JCV antibody above 0.9), T0 before natalizumab treatment initiation, T1 at the time of the natalizumab discontinuation, T2 last follow-up, n.a. not available, Tot. total
Table 1 Demographical and clinical characteristics of the study population at baseline

|                          | JCV index – N=208 (73%) | Low + JCV index (0.9 > JCV < 1.5) N=33 (11.6%) | High + JCV index (> 1.5) N=34 (11.9%) |
|--------------------------|-------------------------|---------------------------------------------|-------------------------------------|
| Age (mean ± SD)          | 40.3 ± 10.8             | 45.2 ± 8.4                                  | 41.4 ± 11.5                         |
| Female                   | n =152 (73.1%)          | n = 19 (57.6%)                              | n = 23 (67.6%)                      |
| Disease duration years (mean ± SD) | 12.4 ± 8.1            | 18.4 ± 8.2                                  | 13.4 ± 6.8                         |
| No. naïve patients (%)   | n = 60 (28.8%)          | n = 3 (9.1%)                                | n = 9 (26.5%)                       |
| No. previous DMTs (%)    | 1.2 ± 1.1               | 1.8 ± 1.1                                   | 1.3 ± 1.1                          |
| No. patients with previous use of IM (%) | 41 (19.7)             | n = 13 (39.4%)                              | n = 11 (32.4%)                     |

JCV index – John Cunningham (JC) virus index negative (titer of anti-JCV antibody below 0.9), Low + JCV index John Cunningham (JC) virus index positive (titer of anti-JCV antibody between 0.9 and 1.5), High + JCV index John Cunningham (JC) virus index positive (titer of anti-JCV antibody above 1.5), DMT disease modifying treatment, IM immunosuppressors, SD standard deviation

Out of 285 patients, 170 patients who remained with a negative JCV serology continued treatment, while after a mean of NTZ treatment of 31.1 ± 14.6 months (average of number of NTZ administrations: 26), 115 patients stopped NTZ because of high level of JCV index and progressive risk of PML, some of them shifting to a more safer DMTs.

Discontinuers stopped treatment for the following reasons: adverse events (2 patients, 1.7%), inefficacy as appearance of clinical or radiological relapse (4 patients, 3.5%), growing PML risk (89 patients, 77.4%), progression of disease (9 patients, 7.8%) or lost to follow-up (11 patients, 9.6%).

After a mean time of follow-up of 110.6 ± 115.1 days from NTZ discontinuation, most of the patients shifted to another DMTs, and in particular 34 (29.6% of 115) switched to a first-line therapy [16 (13.9%) to Copolymer/Glatiramer Acetate (COP) or Interferon (IFN), 12 (10.4%) to Dimethlulidimethyl-fumarate (DMF) and 6 (5.2%) to Teriflunomide (TER), respectively] and 60 (52.2%) switched to a second-line therapy [44 (38.3%) to Fingolimod (FTY), 9 (7.8%) to Alemuzumab (ATZ), 2 (1.7%) to Azathioprine (AZA), 2 (1.7%) to Daclizumab (DAC), 2 (1.7%) to Ocrelimab (OCRE) and 1 (0.9%) to Rituximab (RTX), respectively]. For the remaining 21 (18.3%) patients, neurologists decided to stop every therapy due to progression course of the disease.

At T1 JCV index data were available for 107 patients (93% of 115). Out of 115 subjects, 69 (60% of 115) were JCV-positive and 38 (33.0% of 115) were JCV negative. In the subgroup of JCV positive, 48 (69.6% of 69) were already positive at baseline and remained positive (18 patients in low positive JCV index group switched in the high positive JCV index group), 19 (27.5%) seroconverted from a negative to a positive status during the treatment period and no patients seroconverted from a positive to a negative status. In the subgroup of JCV negative, 27 (71.1% of 38) were already negative at baseline, 7 (18.4%) seroconverted from a positive to a negative status during the treatment period and no patients seroconverted from a negative to a positive status. For 6 subjects (2 in JCV positive group and 4 in JCV negative group, respectively) it was not possible to match JCV data between T0 and T1 due to not available index values in one of the timepoints.

After a mean time of NTZ suspension of 35.8 ± 23.6 months (T2), 80 (69.6%) of 115 patients underwent to a new JCV index determination (remaining 35 patients were already followed in other centres). Out of 80, 63 (78.8%) patients were discontinuers due to high JCV index, while 17 patients (21.3%) interrupted treatment for other reasons (disease progression, adverse events).

From T1 to T2, 72 (90% of 80) patients did not change their respective JC status, while 5 (6.3% of 80) seroconverted [2 patients (2.5% of 80) to a positive status and 3 patients (3.8% of 80) to a negative status, respectively]. For the remaining 3 patients matching data were not available.

Table 2 also shows the variations of JCV status during the follow-up in all NTZ patients divided into subgroups (negative JCV index, low positive JCV index and high positive JCV index).

Table 3 shows JCV index changes among different time-points. In the whole cohort of NTZ patients, there was a statistically significant difference in JCV index values between T0 and T1 (0.60 ± 0.98 vs 0.88 ± 1.39, p = 0.007). Dividing into subgroups, there was not an increment of JCV index value in the group of continuers (0.14 ± 0.39 vs 0.19 ± 0.52, p = 0.1), while the variation of JCV index remained statistically significant in the group of patients who stopped treatment (1.29 ± 1.20 vs 2.35 ± 1.51, p = 0.008). Moreover, evaluating the JCV index changes at each time-point, in the group of discontinuers the JCV index increase was greater during NTZ treatment period (T0 vs T1, p < 0.0009) and remained statistically significant also during the period of discontinuation (T1 vs T2, p = 0.04).

As showed in Fig. 2, the analysis of variance (ANOVA) confirmed that the JCV index increase was statistically significant between T0 and T1 and T0 and T2 (p=0.004 and
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While the variation of JCV index between T1 and T2 was not statistically significant (p = 0.09). Considering the rate of seroconversion of JCV index during the follow-up period, the percentage of patients who seroconverted to a positive status during NTZ treatment was greater than who seroconverted after NTZ discontinuation and it was statistically significant (p = 0.008) (Table 4). There was no difference in the percentage of patients who seroconverted to a negative status during the follow-up. There were no correlations between seroconversion to a positive status and years of disease, duration of NTZ treatment, previous immunosuppressors or number of previous DMTs.

Considering the type of exit strategy used after NTZ discontinuation, patients who did not shift to another drug remained with stable high level of JCV index, in line with our results previously reported, while patients already JCV positive who switched to another DMTs had a progressive increase of JCV index. This increase was statistically significant in the group who shifted to FTY, with a gain of 0.5 point of index in 11 patients (p < 0.05) and of 1 point of index in 5 patients (p < 0.05) compared with other DMTs (Fig. 3).

### 4 Discussion

We retrospectively investigated anti-JCV antibody status changes in patients with MS during NTZ treatment. Moreover, our study also evaluated the JCV index longitudinal modification after NTZ discontinuation.

According to our results, during NTZ treatment, patients showed a statistically significant increase in their JCV index (1.20 vs 1.90, p = 0.0009) and NTZ treatment was associated with a significant percentage of patients (16.5%) seroconverted to a positive status. Similar results have been reported from other study in literature in which the annualized seroconversion rate has been described as 6–7% in the first year of NTZ therapy, progressing to 10–25% after 4 years of continuous treatment with this drug [15–19].

The median worldwide prevalence of JCV [20] and the seropositivity rates [21] among adults with MS has been found to be of 58.0% and between 50.0% and 90.0%, respectively. Seropositivity for JCV may be subject to a variety of influences and the values reported by different authors may therefore be somewhat skewed. For example, some large

### Table 2 The variations of JCV status during the follow-up

| JCV index at T0 (mean ± SD) | JCV index at T1 (mean ± SD) | JCV index at T2 (mean ± SD) |
|----------------------------|-----------------------------|-----------------------------|
| n = 285                    | n = 208 (73%)               | n = 33 (11.6%)              |
| JCV index at T1 (mean ± SD)| n = 34 (11.9%)              | n = 10 (3.5%)               |
| n = 115                    | n = 38 (33.0%)              | n = 7 (6.1%)                |
| JCV index at T1 (mean ± SD)| n = 62 (53.9%)              | n = 8 (7.0%)                |
| n = 80                     | n = 19 (23.8%)              | n = 9 (11.3%)               |
| JCV index at T2 (mean ± SD)| n = 52 (65%)                | n = 0                       |
| n = 2.35 ± 1.51            | 0.28 ± 1.52                 | 1.23 ± 1.52                 |
| JCV index at T2 (mean ± SD)| 3.31 ± 1.51                 |                            |

### Table 3 Outcome 1: JCV index changes among different time-points

| JCV index at T0 (mean ± SD) | JCV index at T1 (mean ± SD) | JCV index at T2 (mean ± SD) | p value |
|-----------------------------|-----------------------------|-----------------------------|---------|
| Whole cohort                |                            |                            | 0.007   |
| 0.60 ± 0.98                 | n.a.                       | 0.68 ± 1.39                 |         |
| Continuers                  |                            |                            | 0.1     |
| 0.14 ± 0.39                 | n.a.                       | 0.19 ± 0.52                 |         |
| Discontinuers               |                            |                            | 0.009   |
| 1.29 ± 1.20                 | 1.90 ± 1.45                 | 2.45 ± 1.45                 | T0 vs T1|
| JCV > 1.5                   | Patients at T0             | Patients at T1              | T1 vs T2|
| n = 56                      | n = 62                     | n = 52                      |         |
| JCV < 1.5                   | Patients at T1             | Patients at T2              | T0 vs T2|
| n = 10                      | n = 7                      | n = 9                      |
| JCV < 0.9                   | Patients at T1             | Patients at T2              | T2: 0.008|
| n = 43                      | n = 36                     | n = 19                      |         |
| n.a. patients               | n = 6                      | n = 10                     |         |

JCV index – John Cunningham (JC) virus index, T0 before natalizumab treatment initiation, T1 at the time of the natalizumab discontinuation, T2 last follow-up, SD standard deviation, n.a. not available.
studies did not find any association between JCV positivity and the previous use of NTZ or other immunosuppressive drugs [15]. Conversely, other authors reported that seroconversion rates increased by more than 8.0% per year of use of NTZ [22]. A recent meta-analysis of JCV seroconversion during treatment with NTZ established that the rate of change of serological status was 10.8% per year [23].

In our study cohort, patients with a negative JCV serology at baseline (170 of 209, 81.3%) remained negative during the follow-up, confirming data in other studies showing that

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**Table 4** Rates of seroconversion

|                        | From T0 to T1 | From T1 to T2 | p value |
|------------------------|--------------|--------------|---------|
| Patients sero-         |              |              |         |
| converted to a positive status | 16.5% (19 of 115) | 2.5% (2 of 80) | 0.008   |
| Patients sero-         |              |              |         |
| converted to a negative status | 6.1% (7 of 115) | 3.8% (3 of 80) | 0.1     |

T0 before natalizumab treatment initiation, T1 at the time of the natalizumab discontinuation, T2 last follow-up

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**Fig. 2** JCV index status among the three time-points. *JCV index* – John Cunningham (JC) virus index, T0 before natalizumab treatment initiation, T1 at the time of the natalizumab discontinuation, T2 last follow-up, na not available

**Fig. 3** Differences in JCV index status in discontinuers group according to the different exit strategies. *JCV index* John Cunningham (JC) virus index, *IFN* Interferon, *COP* Copolymer/Glatiramer Acetate, *ATZ* Alemtuzumab, *AZA* Azathioprine, *DAC* Daclizumab, *DMF* dimethyl fumarate, *FTY* Fingolimod, *OCRE* Ocrelizumab, *RTX* Rituximab, *TER* Teriflunomide, *DMT* disease modifying treatment
JCV-negative patients or patients with antibody levels below or equal to 0.9 both have a low risk of seroconversion [24].

During NTZ treatment, in our cohort of patients 7 subjects seroconverted to a negative status. Analyzing the data in detail, for 3 patients the presence of JCV antibody was tested before 2011 when Stratify II Test was not available and only qualitative test (negative/positive) was performed, so we could speculate that these patients had a JCV value slightly higher than 0.9 before starting NTZ (and for this reason they had been screened as positive) and their seroconversion to a negative status could be considered as a false positive value. The remaining 4 patients had a JCV positive value only in the first determination of index and subsequent values were negative, however it not possible to define if a laboratory error could be considered or not.

In those patients who were already JCV index positive before starting NTZ, JCV index values progressively increased during treatment (doubling in about 40% of patients after a mean time of NTZ treatment of 32 ± 14 months and an average of 26 NTZ administrations), so much to be the main reason of discontinuation. It could seem that this group of patients would be more willing to stop therapy than patients who seroconverted to positive status during treatment. Possible explanations is that they had a longer time of NTZ exposure and a greater number of NTZ infusions, so Clinicians recommend the shift to a more safer DMT based on recently approved guidelines, on their professional experience and on patients’ clinical features and concerns [25].

The JCV index increase or the seroconversion rate to a positive status remained consistently high also after NTZ discontinuation in about 80% of patients. Our results suggest that NTZ could act as an indelible signature on the therapeutic history of patients, influencing not only the disease course of MS due to improvement of the clinical condition, but also the longitudinal JCV antibody serology. Patients tend to maintain high JCV index values in the long period after NTZ discontinuation, therefore reducing the possibility of restarting treatment.

In patients with MS treated with NTZ for more than 24 months who are positive for anti-JCV antibodies, at least six-monthly follow-up tests are recommended by consensus in order to detect seroconverters and to discuss treatment continuation in patients with increased risk of PML [2, 10, 26]. Usually clinicians prefer to stop therapy and to shift to a safer DMT [25].

Since NTZ is considered one of the most effective DMTs in MS, during the post-NTZ follow-up period it is believed that testing JCV index at frequent intervals could be a good strategy to identify possible JCV index reduction, to better stratify patient’s risk and to adopt different therapeutic strategies (alternative therapies or more frequent MRI scanner to detect early PML development). Results obtained in this study seem to demonstrate that PML risk imprinting related to NTZ cannot be mitigated with treatment discontinuation. However further studies are needed to uphold our data.

Furthermore, analyzing in detail the group of JCV index patients after NTZ suspension and correlating the variations of their JCV values with the type of DMTs chosen as exit strategy post NTZ treatment, our result demonstrated that there was a statistically significant progressive increase of JCV index in the group of patients who shifted to FTY. This result is in line with another study published in 2018 by Aoyama e coll. [27] in which an association between increased JCV index and therapy with FTY is reported, especially related to duration of treatment. FTY act a functional antagonist of sphingosine 1-phosphate (S1P), which regulates lymphocyte egress from secondary lymphoid organs to the circulation. As result, FTY reduces the amount of circulating lymphocytes and thereby the transmigration of pathogenic immune cells into the central nervous system (CNS) [28]. FTY has also a role on modification of B cell subsets, reducing circulating memory B cells, while increasing the proportions of transitional B cells and regulatory B cells (B-reg) [29]. Despite severe lymphopenia, this is the reason because FTY treated patients have only a mild elevated risk of infectious complications and maintain their immunocompetence. Based on this immune profile, in our study the increase of JCV index in patients who stopped NTZ and shifted to FTY could be explained by the presence of new circulating transitional B cells and B-regs induced by FTY. These series of cells, created after NTZ discontinuation, has not yet come into contact with JCV, so it would react to virus as a new infectious agent, thereby expanding B cell population and promoting the role of antibody-producing B cells. It could be the reason because the titers of JCV antibody are increased with the use of FTY.

The main limitation for this study is the retrospective design. Firstly, in our study the cut-off of 0.9 instead of 0.4 was used as the threshold for positive JCV index status. However, this choice has been justified by the fact that patients in treatment with NTZ were stratified for PML risk based on the previous study [7] in which it was demonstrated that JCV index value equal or higher of 0.9 was associated with a higher risk of PML. Recently, it was suggested that the risk-stratification algorithm used by clinicians in their clinical practice could be inadequate, because according to recent estimates PML seems occur more frequently than expected [30]. For this reason, there is a growing interest regarding new measures to potentially improve the PML risk stratification, in particular the L-selectin blood-test [31] and lipid-specific Immunoglobulin M (IgM) bands in cerebrospinal fluid (CSF) [32]. Furthermore, a better management of NTZ treatment through the extension of the time interval between infusions [33] or customizing the dosage based on the patient’s weight [34] could further reduce the PML
incidence. Further prospective studies should apply a cut-off of 0.4 in order to improve the knowledge about JCV index variations during MS treatments.

Secondly, our study did not include a control group. As it is a retrospective study, when data were collected in 2010, NTZ was the unique second-line therapy used with an estimated risk of PML. In our MS centre, for clinical practice, only patients who would have started NTZ or who stopped NTZ underwent to Stratify test. Thus, no data about JCV index variations were available in patients untreated or treated with different DMTs. More recently, with the development of new MS treatments and the finding of PML cases in patients treated with other therapies than NTZ, the determination of JCV status has been performed in mostly patients with MS.

As a further consequence of the retrospective design of the study, for some patients data about JCV index were available only as a qualitative result (negative/positive) because they collected before 2011 when Stratify II Test was not used. For this reason, the seroconversion to a negative status observed during NTZ treatment for a very small number of patients could be considered as a false positive data.

Finally, a more frequent rate of NTZ discontinuation in JCV positive than in JCV negative patients was found. This is in line with the current literature and confirmed by common clinical practice that suggest to consider NTZ discontinuation in JCV positive patients after 24 NTZ doses, as the growing risk of PML is related to high number of NTZ administrations. However, we cannot excluded that the high frequency of JCV positive found in NTZ discontinues group could have biased our results, influencing treatment duration and size of discontinuers subgroup.

5 Conclusion

Our data confirmed that a high percentage of patients shift to or remain in a positive JCV status during NTZ treatment, while no patients seroconvert to a negative status after suspension.

Discontinuers patients who stopped treatment because of high level of JCV index tend to remain in the same JCV status during the follow-up period, independently from the time interval between discontinuation and start of new DMTs. The finding of an increase of JCV index in those patients subsequently treated with FTY may rise the question if the use of this kind of treatment as exit strategy after NTZ discontinuation would represent the safer choice. Moreover, because an increase of JCV index could expose patients to a higher risk of PML, one could speculate that after NTZ discontinuation the use of treatment acting selectively on B cells could be considered a valid therapeutic approach.

NTZ discontinuation seems not to be able to interfere on JCV status modification on long time period. It makes non-sense to repeat a JCV determination every six months to expect a seroconversion to a negative value in order to restart treatment.

Compliance with Ethical Standards

Funding This study was not fonded.

Conflict of interest Eleonora Sgarlata declares there is no conflict of interest. Clara G. Chisari declares there is no conflict of interest. Emmanuele D’Amico declares there is no conflict of interest. Enrico Mileffiorini received grants from Biogen, Merck Serono, Novartis Genzyme and Roche, consulting fees from Biogen, Merck Serono, Novartis Genzyme and Roche, travel support for congress participations from Biogen, Merck Serono, Novartis Genzyme and Roche; he also served as advisory board member for Bayer, Biogen, Merck Serono, Novartis Genzyme and Roche, travel support for congress participations from Almirall, Bayer, Biogen, Merck Serono, Novartis Genzyme, Teva and Roche, consulting fees from Biogen, Merck Serono, Novartis Genzyme and Roche, travel support for congress participations from Almirall, Biogen, Merck Serono, Novartis Genzyme and Roche, travel support for congress participations from Almirall, Bayer, Biogen, Merck Serono, Novartis Genzyme, Teva and Roche; he also served as advisory board member for Almirall, Bayer, Biogen, Merck Serono, Novartis Genzyme, Teva and Roche. He was also fonded by Pfizer and FISM for epidemiological studies.

Ethical approval This study protocol was approved by the local Ethical Committee of the University of Catania (Catania 1).

Informed consent Each patient participating to the study signed an Informed Consent specifically designed for the study.

References

1. Polman CH, O’Connor PW, Havrdova E, Hutchinson M, Kapos L, Miller DH, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med. 2006;354:899–910.
2. Major EO, Yousry TA, Clifford DB. Pathogenesis of progressive multifocal leukoencephalopathy and risks associated with treatments for multiple sclerosis: a decade of lessons learned. Lancet Neurol. 2018;17:467–80.
3. Bloomgren G, Richman S, Hotermans C, Subramanyam M, Goelz S, Natarajan A, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. N Engl J Med. 2012;367:1870–80.
4. Ho P-R, Koendgen H, Campbell N, Haddock B, Richman S, Chang I. Risk of natalizumab-associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: a retrospective analysis of data from four clinical studies. Lancet Neurol. 2017;16:925–33.
5. Gorelik L, Lerner M, Bixler S, Crossman M, Schlain B, Simon K, et al. Anti-JC virus antibodies: implications for PML risk stratification. Ann Neurol. 2010;68:295–303.
6. Lee P, Plavina T, Castro A, Berman M, Jaiswal D, Rivas S, et al. A second-generation ELISA (STRATIFY JCV™ DxSelect™) for detection of JC virus antibodies in human serum and plasma to support progressive multifocal leukoencephalopathy
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risk stratification. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2013;57:141–6.
7. Plavina T, Subramanyam M, Bloomgren G, Richman S, Pace A, Lee S, et al. Anti-JC virus antibody levels in serum or plasma further define risk of natalizumab-associated progressive multifocal leukoencephalopathy. Ann Neurol. 2014;76:802–12.
8. Sørensen PS, Bertolotto A, Edan G, Giovannoni G, Gold R, Havrdova E, et al. Risk stratification for progressive multifocal leukoencephalopathy in patients treated with natalizumab. Mult Scler Houndmills Basingstoke Engl. 2012;18:143–52.
9. Schwab N, Schneider-Hohendorf T, Pignolet B, Breuer J, Gross CC, Göbel K, et al. Therapy with natalizumab is associated with high JC virus seroconversion and rising JCV index values. Neurol Neuroimmununoid Neuinflammation. 2016;3:e195.
10. McGuigan C, Crainer M, Guadagno J, Kapoor R, Mazibrada G, Molyneux P, et al. Stratification and monitoring of natalizumab-associated progressive multifocal leukoencephalopathy risk: recommendations from an expert group. J Neurol Neurosurg Psychiatry. 2016;87:117–25.
11. Sangalli F, Moiola L, Ferré L, Radaelli M, Barcella V, Rodegher M, et al. Long-term management of natalizumab discontinuation in a large monocentric cohort of multiple sclerosis patients. Mult Scler Relat Disord. 2014;3:520–6.
12. Scarpazza C, De Rossi N, Tabiadon G, Turrini MV, Gerevini S, Capra R. Four cases of natalizumab-related PML: a less severe course in extended interval dosing? Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol. 2019;
13. Impact of Disease-Modifying Treatments on the Longitudinal Evolution of Anti-JCV Antibody Index in Multiple Sclerosis. - PubMed - NCBI [Internet]. [cited 2019 Mar 6]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30410866.
14. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. 2011;69:292–302.
15. van Kempen ZLE, Leurs CE, de Vries A, Vennegoor A, Rispens T, Wattjes MP, et al. John Cunningham virus conversion in relation to natalizumab concentration in multiple sclerosis patients. Eur J Neurol. 2017;24:1196–9.
16. Peters J, Williamson E. Natalizumab therapy is associated with changes in serum JC virus antibody indices over time. J Neurol. 2017;264:2409–12.
17. Outtreyck O, Zéphir H, Salleron J, Onagna J-C, Etcheberria A, Collongues N, et al. JC-virus seroconversion in multiple sclerosis patients receiving natalizumab. Mult Scler Houndmills Basingstoke Engl. 2014;20:822–9.
18. Raffel J, Gafson AR, Malik O, Nicholas R. Anti-JC virus antibody titres increase over time with natalizumab treatment. Mult Scler Houndmills Basingstoke Engl. 2015;21:1833–8.
19. Correia I, Jesus-Ribeiro J, Batista S, Martins AI, Nunes C, Macário MC, et al. Anti-JCV antibody serostatus and longitudinal evaluation in a Portuguese Multiple Sclerosis population. J Clin Neurosci Off J Neurosurg Soc Australas. 2017;45:257–60.
20. Paz SPC, Branco L, Pereira MA de C, Spessotto C, Fragoso YD. Systematic review of the published data on the worldwide prevalence of John Cunningham virus in patients with multiple sclerosis and neuromyelitis optica. Epidemiol Health. 2018;40:e2018001.
21. Bellizzi A, Anzivino E, Rodio DM, Palamara AT, Nencioni L, Pietropaolo V. New insights on human polyomavirus JC and pathogenesis of progressive multifocal leukoencephalopathy. Clin Dev Immunol. 2013;2013:839719.
22. Vennegoor A, van Rossum JA, Leurs C, Wattjes MP, Rispens T, Murk JL a. N, et al. High cumulative JC virus seroconversion rate during long-term use of natalizumab. Eur J Neurol. 2016;23:1079–85.
23. Schwab N, Schneider-Hohendorf T, Hoyt T, Gross CC, Meuth SG, Klotz L, et al. Anti-JCV serology during natalizumab treatment: review and meta-analysis of 17 independent patient cohorts analyzing anti-John Cunningham polyoma virus sero-conversion rates under natalizumab treatment and differences between technical and biological sero-converters. Mult Scler Houndmills Basingstoke Engl. 2018;24:563–73.
24. JCV serology in time: 3 years of follow-up. - PubMed - NCBI [Internet]. [cited 2019 Aug 8]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/?term=CV+serology+in+time%3A+3+years+of+follow-up.
25. Montalban X, Gold R, Thompson AJ, Otero-Romero S, Amato MP, Chandraratna D, et al. ECTRIMS/EAN Guideline on the pharmacological treatment of people with multiple sclerosis. Mult Scler Houndmills Basingstoke Engl. 2018;24:96–120.
26. Clerico M, Artusi CA, Di Liberto A, Rolla S, Bardina V, Barbero P, et al. Natalizumab in Multiple Sclerosis: Long-Term Management. Int J Mol Sci [Internet]. 2017 [cited 2019 Jan 16];18. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5454853/.
27. Aoyama S, Mori M, Uzawa A, Uchida T, Masuda H, Ohtani R, et al. Serum anti-JCV antibody indexes in Japanese patients with multiple sclerosis: elevations along with fingolimod treatment duration. J Neurol. 2018;265:1145–50.
28. Brinkmann V, Billich A, Baumruker T, Heining P, Schmouder R, Francis G, et al. Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. Nat Rev Drug Discov. 2010:9;883–97.
29. Blumenfeld-Kan S, Staun-Ram E, Miller A. Fingolimod reduces CXCR4-mediated B cell migration and induces regulatory B cells-mediated anti-inflammatory immune repertoire. Mult Scler Relat Disord. 2019;34:29–37.
30. Borchardt J, Berger JR. Re-evaluating the incidence of natalizumab-associated progressive multifocal leukoencephalopathy. Mult Scler Relat Disord. 2016;8:145–50.
31. Schwab N, Schneider-Hohendorf T, Pignolet B, Spadaro M, Görlich D, Meinl I, et al. PML risk stratification using anti-JCV antibody index and L-selectin. Mult Scler Houndmills Basingstoke Engl. 2016;22:1048–60.
32. Delgado-García M, Mateosz P, Alcina A, Fedetz M, García- Sánchez MI, Ruiz-Peña JL, et al. A new risk variant for multiple sclerosis at the immunoglobulin heavy chain locus associates with intrathecal IgG, IgM index and oligoclonal bands. Mult Scler Houndmills Basingstoke Engl. 2015;21:1104–11.
33. Ryerson LZ, Foley J, Chang I, Kister I, Cutter G, Metzger RR, et al. Risk of natalizumab-associated PML in patients with MS is reduced with extended interval dosing. Neurology. 2013;80:1452–62.
34. Foley J, Gudesblatt M, Zarif M, Lathi E. Low Body Weight as a Potential Surrogate Risk Factor for Progressive Multifocal Leukoencephalopathy (P2.244). Neurology. 2014;82:P2.244.