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CD4/CD8 ratio during natalizumab treatment in multiple sclerosis patients

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ABSTRACT

Although improved, the risk of progressive multifocal leukoencephalopathy is a constant threat for patient affected by multiple sclerosis treated with natalizumab.

We performed a 24 months longitudinal study aimed to evaluate the total WBC and lymphocytes subsets modifications and their correlations with anti-JCV antibody index after 1 and 2 years of natalizumab treatment.

Natalizumab induced an increase of WBC, total and C19 + lymphocytes together with a decrease of CD3 +, CD4 + T lymphocytes and CD4/CD8 ratio, which was positively related to anti-JCV antibodies index at month 0, 12 and 24.

Our study confirms that lymphocytes subsets are modified under NAT therapy. Implications of lymphocyte subsets alterations in the pathogenesis of PML are under analyses.

1. Introduction

Natalizumab (NAT) is a humanized monoclonal antibody targeting the very late activating antigen-4 (VLA-4), a molecule involved in the migration of the lymphocytes through the blood–brain barrier (BBB) into the central nervous system (CNS). NAT has shown a high efficacy in patients affected by relapsing-remitting multiple sclerosis (RR-MS) based on a significant reduction in annualized relapse rate (ARR) and the risk of sustained disability progression (Polman et al., 2006; O'connor et al., 2014; Butzkueven et al., 2014). Its mechanism of action is based on the prevention of lymphocytes migration in the CNS, increasing, consequently, the number of immune cells in the peripheral blood (Engelhardt and Kappos, 2008). However, NAT treatment can affect lymphocyte subsets in different ways, as the α4β1integrins are expressed differently on lymphocyte subtypes, with higher levels on B, CD8 +, CD4 + T cells and on memory cells than on T or naive cells. Unfortunately, NAT treatment has been associated with an increased risk of progressive multifocal leukoencephalopathy (PML), a rare brain opportunistic infection, caused by the JC virus (JCV), a common polyomavirus (Agostini et al., 1996). Primary infection by JCV usually occurs early life and the benign form of JCV remains asymptomatic in the kidneys throughout life (Chesters et al., 1983; Heritage et al., 1981), without clinical implications in the immune competent subjects.

The reasons why some patients develop PML and most others do not may be related to different factors such as rare bone marrow or other sites of viral latency, effects of drugs immobilizing specific progenitors into the periphery, host factors that predispose to viral replication, and trafficking of virus into the brain, where it may escape from a depleted or dysfunctional immune system (Major, 2010). This serious rare disease generally occurs in severely immune-compromised individuals, especially in AIDS patients, as well as in some patients receiving immunosuppressive (mycophenolate mofetil) or biological (e.g., efalizumab, natalizumab, alemtuzumab, and rituximab) therapies (Martin et al., 2006; Neff et al., 2008).

Estimation of PML risk is an important step for informed risk-benefit evaluation and treatment decision in MS patients. Since JCV infection is a prerequisite for PML development, a two-step enzyme-linked immunosorbent assay (ELISA) to detect anti-JCV antibodies was developed in order to stratify PML risk (Stratify test) (Gorelik et al., 2010). Actually, positive status with respect to anti–JC virus antibodies, prior use of immunosuppressant drugs, and increased duration of NAT treatment are associated with distinct degrees of PML risk in NAT-treated patients (Bloomgren et al., 2012). Moreover, anti-JCV antibody index, measured in serum/plasma, can improve the estimation of PML

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risk. It has been shown that anti-JCV index was significantly higher in NAT-treated patients with multiple sclerosis who developed PML without prior immunosuppressant use (Plavina et al., 2014). However, ELISA to detect anti-JCV antibodies (Stratify test) and the evaluation of anti-JCV index is an expensive, time-consuming and not straightforward accessible assay, therefore other biological markers of PML risk have been sought (Lanzillo et al., 2014).

Several studies evaluated the peripheral lymphocyte pattern during NAT treatment (Stuve et al., 2006; Koudriavtseva et al., 2014; Warnke et al., 2015; Iannetta et al., 2016), showing a disproportional increase in peripheral subsets of lymphocytes, and of CD4/CD8 ratio both in CNS and to a lesser extent in peripheral blood. Recently increased peripheral blood CD8 + effector subsets percentages were also associated with JCV-DNA positivity (Iannetta et al., 2016), but the antibody index was not evaluated. The aim of our study was to evaluate the lymphocyte pattern modification during NAT treatment in MS patient and its relationship with anti-JCV antibody index, in order to identify alternative, reliable immunological markers able to improve the safety of therapy, and to guide tailored clinical decisions.

2. Patients and methods

This is a longitudinal study on NAT treated patients, who began treatment between May 2011 and July 2013, followed for 24 months.

Inclusion criteria were age older than 18 and diagnosis of RR MS according to 2010 McDonalds criteria (Polman et al., 2011). Exclusion criteria were presence of other autoimmune diseases other than MS, systemic steroids within 30 days before blood sampling.

No informed consent was required since assessments and blood sampling were performed as part of safety program for clinical practice.

Patients received natalizumab 300 mg i.v. every 28 days. They were assessed monthly for 24 month.

JCV antibody test (Stratify) was performed centrally (Unilabs, Denmark) at baseline (T0) after 12 (T1) and 24 (T2) months, through a two-step assay consisting of an enzyme-linked immunosorbent assay, (L'vov et al., 2010). The result of this test was a dichotomous (negative/positive) result and in the case of borderline results (nOD between 0.1 and 0.25), the percentage of inhibition at a confirmatory test was performed. Only successively, normalized OD (nOD450) values were provided for scientific purposes.

We used index cut-off to determine high or low risk of PML, as previously described (Plavina et al., 2014).

3. Flow cytometry analysis

The assessment of immune cell subsets in peripheral blood was performed by flow cytometry at T0, T1 and T2. The number of total white blood cells (WBC) and the different immunological subtypes, including CD3 + lymphocytes, CD4 + T helper lymphocytes, CD8 + T suppressor lymphocytes, and CD19 + B lymphocytes, were obtained. EDTA whole blood was used for multi-color flow cytometry immunophenotyping. This was performed by flow cytometer FACS Canto II (Becton Dickinson). The ratio between T and B-lymphocytes was also calculated.

4. Statistical analysis

In order to evaluate variations of WBC and peripheral lymphocytes subsets during natalizumab treatment between T0, T1 and T2, a one-way ANOVA for repeated measures with a Huynh-Feldt correction when data violated the assumption of sphericity. A Bonferroni post hoc test was used when applicable for a between-time pairwise means comparison. The relationship between WBC, lymphocytes, CD3, CD19/20/22, CD4, CD8, CD4/CD8 ratio and JCV index for each time point has been tested through a Spearman's rank-order correlation. The Spearman's correlation coefficients (r_s) and p-values for each analysis have been reported. Stata 12.0 and Microsoft Excel have been used for data processing and analysis. Results are considered statistically significant if $p < 0.05$.

Table 1
Demographic and clinical characteristics of MS patients.

Characteristic	
Subjects	52
Female sex, N (%)	33 (63.5)
Age, mean ± SD (years)	38.6 ± 1.4
Disease duration, median (months)	126.5
EDSS, median (range)[a]	3.5 (2–7)
Pre-treatment ARR, median (range)[a]	0.7 (0.2–6.6)
Naive to DMT, N (%)	19 (36.5)
Patients treated with immunosuppressant prior to natalizumab, N (%)	2 (3.8)
JCV + patients, N (%)	31 (59.6)

[a] We miss data from 1 patient for this feature.

5. Results

Fifty-two MS (33 Female) patients, were enrolled and followed for 24 months on NAT therapy. Demographic and clinical features of patients are summarized in Table 1.

Nineteen patients (36.5%) were naïve to any DMT, and 2 patients (3.8%) had been treated with immunosuppressant drug before starting NAT.

Baseline JCV index, lymphocytes count, WBC count, CD3, CD4, CD8, CD19/20/22 lymphocytes proportions and CD4/CD8 ratio are summarized in Table 2.

WBC and lymphocytes count significantly increased during NAT treatment ($p < 0.001$) with a significant increase between T0 and T1 ($p = 0.003$ and $p < 0.001$ respectively) without any further increase during the second year (Table 2, Fig. 1a, b).

CD3 + lymphocytes proportion decreased during NAT treatment ($p < 0.001$) with a significant drop between T0 and T1 ($p = 0.003$) without any further modification during the second year (Fig. 1c, e). The same trend has been reported for the CD4 + lymphocytes proportion which decreased from T0 to T1 ($p = 0.01$) and then remained stable.

CD8 + lymphocytes proportion was not modified by NAT treatment ($p = 0.12$). (Fig. 1f).

CD19/20/22 + lymphocytes proportion grew during NAT treatment ($p = 0.006$) with a significant rise between T0 and T1 ($p = 0.03$) without any further increase during the second year (Fig. 1d).

CD4 +/CD8 + lymphocytes ratio significantly decreased during NAT treatment ($p = 0.04$) with a marked decrease between T0 and T2 ($p = 0.04$). (Fig. 1g).

JCV seroconversion took place in 5 patients (13%), from negative to positive and in 2 patients (5%), from positive to negative.

A total of 17 patients (33%) had at baseline a JCV index higher than 1.5 and were, therefore, considered at higher risk of PML. Index was not related to demographic or clinical characteristics.

WBC and Lymphocytes total counts were not related to JCV index at any time-point as well as the CD19/20/22 lymphocyte proportions.

Table 2
JCV antibodies index and peripheral blood immune cell subsets at baseline.

JCV index, median (range)	0.54 (0.08–3.44)
CD3 + %, mean ± SD	70.83 ± 1.17
CD4, mean ± SD	46.69 ± 1.55
CD8, mean ± SD	20.56 ± 1.38
CD 19/20/22, mean ± SD	14.75 ± 1.07
CD4/CD8, mean ± SD	2.72 ± 0.24
WBC count, median (range) × 1000	7.01 (2.9–13.9)
Lymphocytes count, median (range) × 1000	1.98 (0.89–5.61)