Peripheral lymphocyte subsets and JCV antibody index in MS patients treated with natalizumab: a longitudinal analysis.

A. Carotenuto[1] , R. Lanzillo[1], L. Del Vecchio[2], G. Scalia[2], F. Ausiello[1], M. Moccia[1], C.V. Russo[1], V. Brescia Morra[1]

1.Department of Neurosciences, Reproductive Science and Odontostomatology, Federico II University, Naples, Italy
2.CEINGE Advances biotechnology, Naples 80131, Italy

Background

Natalizumab (NAT) is a monoclonal antibody directed against the α4 subunit of the α4β1 (very late antigen-4, VLA-4) integrin on the lymphocytes. It acts mainly preventing the binding between VLA-4 and the vascular endothelium cell adhesion molecule 1 (VCAM-1). The result consists of a reduced extravasation of inflammatory immune cells across the blood–brain barrier into the central nervous system (CNS), consequently increasing the number of immune cells in the peripheral blood. The risk of progressive multifocal leukoencephalopathy (PML) in course of natalizumab treatment can be stratified, according to anti–JC virus antibodies presence, prior use of immunosuppressants, and the number of natalizumab administrations [1]. Even if, quantification of anti-JCV antibody index has increased individual predictive value, with higher titers related to increased risk a large proportion of MS patients treated with natalizumab have an high anti-JCV antibody index without developing PML.

Aims

To evaluate lymphocyte subsets modification during NAT, their correlation with anti-JCV antibody index and to identify its presumptive immunological predictive role of PML.

Patients & Methods

This is a 24 months prospective longitudinal study on RR MS patients treated with NAT. Demographic and clinical data were collected. The assessment of immune cell subsets in peripheral blood was performed by flow cytometry at month 0, 1, 3, 12 and 24. The ratio between CD4+ and CD8+ lymphocytes was calculated. Anti JCV antibodies index was evaluated at month 0, 12 and 24. Linear regression and Pearson chi2 tests were used for statistical analysis.

Results

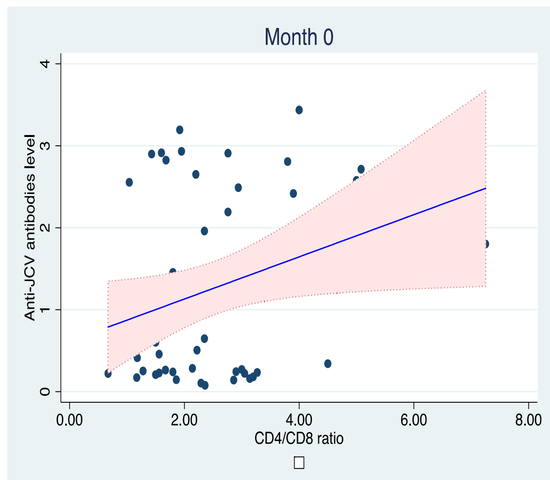
Fifty-one MS patients were enrolled (33 F) with a mean age of 39 y (SD 1,41) and a median disease duration of 10 y (range). Median EDSS was 3,5 (range 2 – 7). Nineteen patients were naïve to any disease modifying treatment. Clinical and demographic features are summarized in table 1.

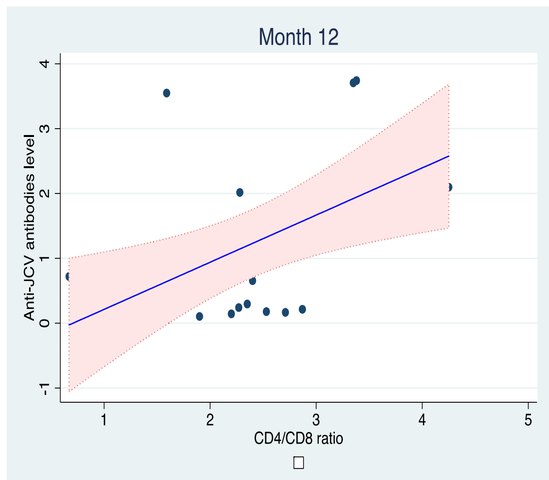
After 2 years, natalizumab induced an increase of white blood cell count (p=0,001), total lymphocytes (p<0,001), C19+ lymphocytes (p=0,008) and a decrease of CD3+ lymphocytes (p=0,005) and CD4+ T lymphocytes (p=0,01), as such as CD4/CD8 ratio (p=0,01). At month 24 CD3+ and CD8+lymphocytes count inversely related to anti-JCV antibodies index (p=0,01 and p=0,001 respectively), while CD4+lymphocytes levels were directly related (p=0,02). CD4/CD8 ratio was positively correlated to anti-JCV antibodies index at month 0 (p=0,04), 12 (p=0,007) and 24 (p=0,001) (Figure 1).

One patient developed PML 26 months after NAT introduction. Her CD4/CD8 ratio at time of PML diagnosis was 1,35, lower then mean CD4/CD8 mean ratio (2,02 ± 0,8).

Table 1

Characteristic	
Subjects	52
Female sex, N (%)	33 (63,46)
Age, mean ± SD (years)	38,61 ± 1,41
Disease duration, median (months)	126,5
EDSS, median (Range) *	3,5 (2 - 7)
Pre-treatment ARR , median (Range) *	0,69 (0,22 - 6,60)
Naive to DMT, N (%)	19 (36,54)
Patients treated with immunosuppressant prior to Natalizumab, N (%)	2 (3,85)
JCV + patients, N (%)	31 (59,62)





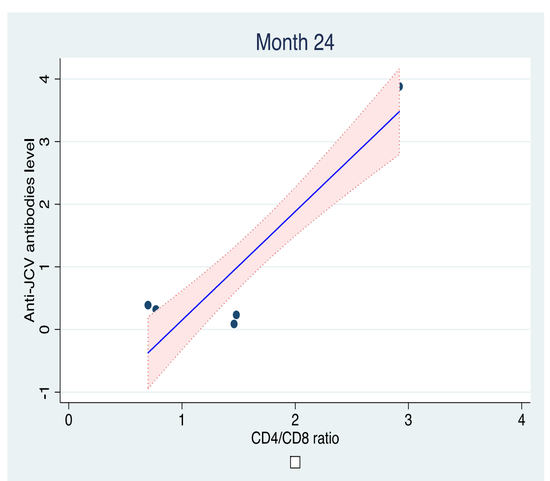


Figure 1

Scatter plot showing the correlation between Anti JCV antibody level and CD4/CD8 ratio at month 0, 12, 24

Conclusion

In the last few years, several studies evaluating specific biomarkers for MS patients under natalizumab therapy have been carried out to improve the safety of the therapy [2-4]. Our study confirms that lymphocytes subsets modify on NAT therapy and suggests that CD4/CD8 ratio could be an important red flag in estimating PML risk. Implications of lymphocytes subset alterations in the pathogenesis of PML are under analyses.

References

•1. Bloomgren, G., et al., *Risk of natalizumab-associated progressive multifocal leukoencephalopathy.* N Engl J Med, 2012. **366**(20): p. 1870-80.

•2. Schwab, N., et al., *L-selectin is a possible biomarker for individual PML risk in natalizumab-treated MS patients.* Neurology, 2013. **81**(10): p. 865-71.

•3. Munoz-Culla, M., et al., *Blood miRNA expression pattern is a possible risk marker for natalizumab-associated progressive multifocal leukoencephalopathy in multiple sclerosis patients.* Mult Scler, 2014. **20**(14): p. 1851-9.

•4. Villar, L.M., et al., *Lipid-specific immunoglobulin M bands in cerebrospinal fluid are associated with a reduced risk of developing progressive multifocal leukoencephalopathy during treatment with natalizumab.* Ann Neurol, 2015. **77**(3): p. 447-57.