CD49d expression on T Lymphocytes and CD8 effector percentage as predictors of JC virus reactivation in Mulptiple Sclerosis patients on natalizumab treatment

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Background CD49d is an α4-integrin that associates with integrin-β1 to form the very late antigen-4 (VLA-4)**1**. Blockade of CD49d with the humanized monoclonal antibody natalizumab strongly suppresses trafficking of inflammatory leukocytes into the central nervous system (CNS) and leads to a significant decrease in the clinical relapse rate of relapsing-remitting form of multiple sclerosis (RRMS)**2**. Natalizumab is currently the most effective therapy against RRMS. However, potential reactivation of the John Cunningham virus (JCV) and development of progressive multifocal leukoencephalopathy (PML) are major concerns**3**. The STRATIFY JCV® assay is a validated test to measure anti-JCV antibodies in human serum and is used to stratify MS patients for higher or lower risk of developing PML. However, because of the occurrence of JCV infection without the development of a detectable antibody response, additional markers are required in order to appropriately stratify patients . The aim of this study was to longitudinally assess CD49d expression and CD4 + and CD8 + T-lymphocyte phenotype alterations in peripheral blood of RRMS patients treated with Natalizumab in order to provide an effective method to predict JCV reactivation and development of PML.

Methods 26 subjects diagnosed with RMS eligible to be treated with natalizumab were enrolled in this study.
Blood and urine samples were collected before the first natalizumab infusion (T0), 12 and 24 months post-treatment initiation. JCV serology at baseline, 12 and 24 months post-natalizumab treatment was assessed using the STRATIFY JCV® assay. JCV-DNA detection was performed using a quantitative polymerase chain reaction (qPCR) in blood and urine. CD49d expression and T-lymphocyte subsets were assessed using flow cytometry.

Results16 patients were followed for all 3 timepoints, while 10 patients were included only at T1 and T2 . In order to identify JCV-infected individuals, JCV-DNA PCR and anti-JCV antibody detection were compared.

A non-significant increase of JCV-DNA positivity was found from T0 to T2 in 16 patients (Figure 1A) and from T1 to T2 in all 26 patients (Figure 1B) in either blood, urine or blood and/or urine.

We found that 3/16 JCV-DNA+ subjects (2 in blood and 1 in both blood and urine) at T0, 3/26 JCV-DNA+ subjects (1 in blood and 2 in urine) at T1 and 5/26 JCV-DNA+ subjects (3 in blood, 2 in both blood and urine) at T2 were negative for JCV serology according to the STRATIFY JCV® assay. Natalizumab reduced CD49d expression on memory and effector subsets of peripheral blood CD4+ and CD8+ T-lymphocytes. (fig 2)
Moreover, accumulation of peripheral blood CD8+ memory and effector cells was observed after 12 and 24 months of treatment. In particular, **higher percentages of CD8+ effectors were observed in subjects with detectable JCV-DNA**.

At T0 CD49d Median Fluorescence Intensity was higher in CD8+ than CD4+ (p=0.011). No statistical differences after 12 and 24 months of natalizumab treatment (T1 and T2, respectively) were observed (Figure 3). On overall CD4+ T-lymphocytes CD49d expression remained unchanged, while it decreased in overall CD8+ T-lymphocytes

CD8+ and CD4+ T-lymphocyte percentages increased from T0 to T2. The percentages of CD4+ N, CM, EM, E and CD8+ N remained constant, while the percentages of CD8+ CM, EM and E increased from T0 to T2 (**Table 2).**

At T1, CD8+ and CD8+ E percentages were found to be statistically significant predictors of JCV-DNA positivity. At T2 CD8+, CD4+ E, CD8+ E, CD4+HLA-DR+CD38+ percentages were associated with the presence of JCV-DNA. CD8+ E percentage was the best predictor of JCV-DNA positivity in blood and/or urine after both 12 and 24 months of natalizumab treatment

Conclusions JCV is detectable in urine and blood of natalizumab treated RMS patients. Some subjects showed a negative results to the STRATIFY JCV® assay, despite a positivity for JCV-DNA detection by Q-PCR. In treated patients, natalizumab reduced CD49d expression in leukocytes and caused an increase of peripheral blood T-lymphocyte percentages after 12 and 24 months of treatment. Taken together these elements suggest an **impairment of CNS immune surveillance mechanisms, with the occurrence of JCV reactivation and dissemination from latency sites**.

Natalizumab treatment reduces CD49d expression on leukocytes, facilitating the mobilization of latently JCV-infected cells and reducing central nervous system immune surveillance. The longitudinal evaluation of T-lymphocyte subsets and CD49 dexpression by flow cytometry could be a promising and effective method toassess the JCV reactivation risk. .

Specifically, monitoring of CD8+ E percentage in peripheral blood could be performed in order to identify subjects with a higher risk of JCV reactivation, requiring the evaluation of JCV-DNA and NCCR sequencing by molecular biology methods.

	Baseline (T0)	12 months (T1)	24 months (T2)
F/M	7/9	15/11	15/11
Median age in years (IQR)	30.5 (25.2-37)	34 (28.7-40.2)	35 (29.7-41.2)
Median years of disease (IQR)	5.5 (1.25-9.5)	7 (2-12.75)	8(3-13.75)
Median EDSS (IQR)	2 (1.25-2.75)	2(1-3)	2(1.75-3)
No Therapy* (/N)	5/16	8/26	8/26
Interferon β *(/N)	9/16	13/26	13/26
Mitoxantrone and Interferon β *(/N)	1/16	1/26	1/26
Glatiramer Acetate *	1/16	4/26	4/26
Stratify JCV™ (+/-)	4/12	11/15	9/17

Table 1. Demograpphic data and clinical features of paitients. F: female; M: male; N: totsl number of patients; IQR: interquartile range; EDSS: Expanded Disability Status Scale; * therapy before strting natalizumab

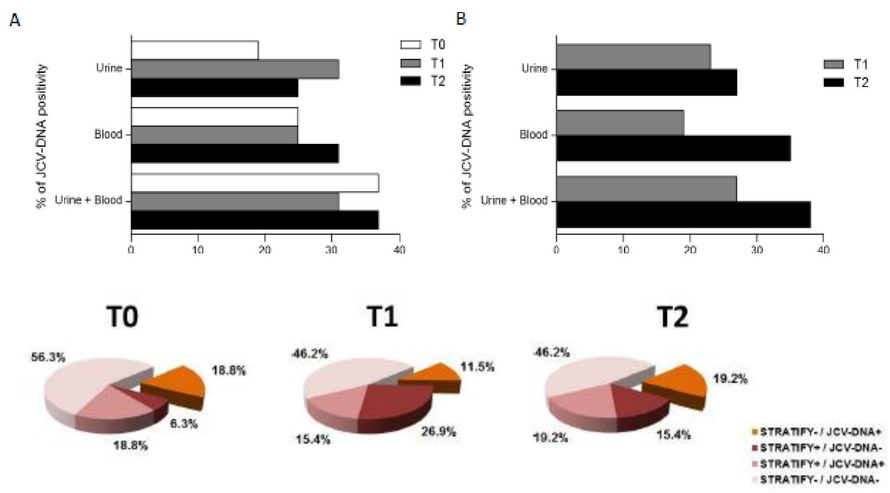

Figure 1. Percentage of JCV-DNA positivity in urine and blood of 16 (A) and 26 RRMS patients (B)

Figure 2. Concordance and discordance of JCV-DNA PCR detection with stratify JCV test in natalizumab treated patients

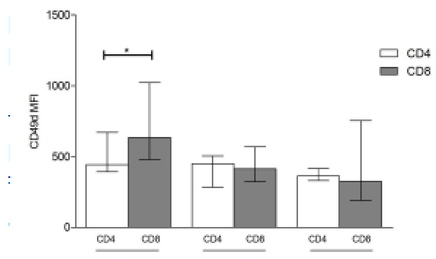

Figure 3. CD49d median fluorescence intensity (MFI) in CD4* and CD8*. *:0.05<p<0.01

	T0 (median [IQR])	T1(median [IQR])	T2(median [IQR])	*p* (Friedman test)
CD4⁺	445 [395-673]	453 [286-505]	366 [333-416]	*p=0.1738*
CD4⁺ N	338 [216-435]	351 [202-595]	371 [237-432]	*p=0.4046*
CD4⁺ CM	693 [568-1096]	483 [412-674]	269 [132-487]	***p≤0.0001***
CD4⁺ EM	833 [630-1195]	577 [434-827]	355 [194-776]	***p=0.0388***
CD4⁺ E	1030 [728-1269]	525 [372-731]	366 [215-447]	***p=0.0003***
CD8⁺	635 [479-1022]	414 [326-570]	327 [193-755]	***p=0.0468***
CD8⁺ N	474 [310-574]	360 [266-612]	424 [207-670]	*p=0.6456*
CD8⁺ CM	830 [553-1087]	488 [349-621]	425 [260-631]	***p=0.0087***
CD8⁺ EM	983 [668-1194]	635 [537-792]	361 [262-714]	***p=0.0002***
CD8⁺ I	911 [654-1002]	514 [426-689]	372 [208-654]	***p=0.0006***
CD8⁺ E	1006 [603-1256]	687 [444-732]	425 [220-505]	***p=0.0001***

Table 2. CD49d median fluorescence intensity in CD4⁺ and CD8⁺ populations and subpopulations. N: naïve, CM: central memory, EM: effector memory, E: effectors, I: intermediate.

Table 2. CD49d median fluorescence intesity in CD4+ and CD8+ populations and subpopulations.
N: naive; CM:central memory; EM:effector memory; E:effectors; I: intermediate

1Yaldizli O, Putzki N. Natalizumab in the treatment of multiple sclerosis. Ther Adv Neurol Disord. 2009 Mar;2(2):115-28. 2Nali LH, Moraes L, Fink MC, Callegaro D, Romano CM, Oliveira AC. Natalizumab treatment for multiple sclerosis: updates and considerations for safer treatment in JCV positive patients. Arq Neuropsiquiatr. 2014 Dec;72(12):960-5 3Khalili K, White MK, Lublin F, Ferrante P, Berger JR. Reactivation of JC virus and development of PML in patients with multiple sclerosis. Neurology. 2007 Mar 27;68(13):985–90. 4Gorelik 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 Sep;68(3):295–303.

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