Natalizumab treatment for multiple sclerosis: updates and considerations for safer treatment in JCV positive patients

Tratamento com Natalizumabe para esclerose múltipla: atualizações e considerações para um tratamento mais seguro em pacientes positivos para o VJC

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ABSTRACT

Natalizumab is currently one of the best options for treatment of patients with Multiple Sclerosis who have failed traditional prior therapies. However, prolonged use, prior immunosuppressive therapy and anti-JCV antibody status have been associated with increased risk of developing progressive multifocal leukoencephalopathy (PML). The evaluation of these conditions has been used to estimate risks of PML in these patients, and distinct (sometimes extreme) approaches are used to avoid the PML onset. At this time, the biggest issue facing the use of Natalizumab is how to get a balance between the risks and the benefits of the treatment. Hence, strategies for monitor JCV-positive patients undergoing Natalizumab treatment are deeply necessary. To illustrate it, we monitored JCV/DNA in blood and urine of a patient receiving Natalizumab for 12 months. We also bring to discussion the effectiveness of the current methods used for risk evaluation, and the real implications of viral reactivation.

Keywords: multiple sclerosis, Natalizumab, JCV, risk factors, progressive multifocal leukoencephalopathy, viruria.

RESUMO

Natalizumabe é atualmente uma das melhores opções para o tratamento de pacientes com Esclerose Múltipla que não respondem aos tratamentos tradicionais. No entanto, o seu uso prolongado, o uso de terapia imunossupressora prévia e o status sorológico antivírus JC têm sido associados com o risco aumentado de desenvolvimento de Leucoencefalopatia Multifocal Progressiva (LEMP). A avaliação destas condições tem sido utilizada para estimar os riscos do desenvolvimento de LEMP nestes pacientes, e abordagens distintas (por vezes extremas) são empregadas para evitar o aparecimento dessa patologia. Atualmente, o grande desafio está em obter um equilíbrio entre os riscos e os benefícios do tratamento com Natalizumabe. Assim, é crucial desenvolver estratégias para monitorar pacientes portadores do vírus JC sob tratamento com Natalizumabe. A título de ilustração, pesquisamos o vírus no sangue e na urina de um paciente sob tratamento durante 12 meses. Também discutimos a eficácia dos métodos atualmente utilizados para avaliação de riscos e as implicações reais de reativação viral.

Palavras-chave: esclerose múltipla, Natalizumabe, vírus JC, leucoencefalopatia multifocal progressiva, viruria.

Natalizumab (Tysabri), used for treatment of relapsing-remitting multiple sclerosis (MS), is a monoclonal antibody directed to the a4β1 integrin, a subunit of an adhesion molecule expressed on the surface of T lymphocytes. The antibodies act by blocking the migration of T Lymphocytes from blood to the CNS through the blood brain barrier (BBB) and attenuate the inflammatory effects¹. The AFFIRM study showed that monotherapy with Natalizumab (NTZ) for 2 years decreased the relapse rate by 68% and the disability progression rate by 42% compared with placebo². NTZ is well tolerated and the overall incidence of serious adverse events is low. Although the efficacy of NTZ is up to

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Progressive multifocal leukoencephalopathy is a demyelinating disease of the central nervous system that usually leads to death or severe disability. PML is characterized by destruction of myelin-producing oligodendrocytes and astrocytes and has almost exclusively been reported in immunocompromised patients. Particularly, it manifests in individuals with reduced cellular immunity, including patients with HIV, hematological diseases, or receiving immunosuppressive therapies. Since the late 1980s until 2008 PML was responsible to be the cause of death of 4-5% of HIV-infected patients. Since 2005 it was announced the withdrawal of NTZ from the market and approved as monotherapy for the treatment of relapsing forms of MS. However, even with a careful administration protocol, PML cases in MS patients have been growing, with more than 400 cases reported so far (http://multiple-sclerosis-research.blogspot.com.br/2013/10/natalizumab-pml-update-september-2013.html).

A SHORT BACKGROUND OF PML

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JC virus is the PML etiological agent. It is a non-enveloped double-stranded circular DNA virus of 5,130 base pairs. The viral genome codes for six genes: Large and small T antigen, capsid genes VP1, VP2 and VP3, agnoprotein and the regulatory region (RR), which can be classified as the archetype or rearranged according to their structural features.

The genetic structure of RR directly affects viral transcription and replication by change the level of DNA/protein and cofactors binding sites, thus leading to distinct cellular tropism. The urinary shedding of JCV with archetype structure is frequent among healthy and immunocompromised individuals, but rearranged forms with deletions, insertions and duplications are usually found in viruses from blood and brain of patients with PML. Much less frequently, the rearranged form of JCV can be found in the urine of patients experiencing asymptomatic reactivation. In addition to RR, variability in VP1 sequences found in CSF and brain of PML patients, but not in urine, also reinforces the relationship between the variants or genetic changes and viral tropism.

Currently, the most accepted idea is that PML arises as a consequence of reactivation of latent JCV in the kidneys, leading to viremia and as a consequence, viruses present in blood and/or B-lymphocytes enter the brain and cause disease. However, viral reactivation in kidneys or blood preceding its migration to the brain is controversial since different groups have found viruses in brain of healthy individuals as well as no viremia at all in some affected patients. It was also found latent JCV in lymph, spleen, bone marrow and tonsil demonstrating that the virus may establishes latency in many tissues.

NATALIZUMAB AND PML

PML in patients receiving Natalizumab was first reported in 2005 in three individuals during clinical studies. By the middle of 2011, all reported cases of PML in people receiving NTZ arose in patients who were under treatment for more than 1 year. In the same year, based on post-marketing reports, the overall risk of PML was estimated around 1.51 per 1,000 patients (95%CI 1.27-1.79). More detailed historic investigation of these patients revealed that particular conditions could work as risk factors for PML development. For example, patients who developed PML were more likely to have been treated with immunosuppressant before receiving Natalizumab and the incidence of PML over time tended to be lower in the first 12 months of treatment but increased through time. Therefore, risk management strategies have been developed based on increased risk in patients with (i) anti-JCV antibodies, (ii) longer duration of Natalizumab treatment and (iii) prior immunosuppressive therapy (Figure 1). The risk of PML among patients with none of these conditions is very low and almost unchangeable through the treatment since the annual seroconversion rate is low.

Although the outcomes of natalizumab-treated patients with PML are generally better than those reported in HIV infected individuals, the clinical vigilance, early PML diagnosis, and cessation of Natalizumab treatment on suspicion of PML has been used to avoid the onset of the disease.

JCV is ubiquitous in human population and can be as prevalent as 80% according to some studies. It is important to remind that JCV establishes latency in urinary tract and may be excreted during life without any consequence. In other words, JCV antibody detection does not provide all the necessary information regarding viral replication in MS patients undergoing NTZ treatment.

Given the risk to PML development increases according to the treatment extent, some MS centers employ few months suspension of Natalizumab after one year of treatment (drug holiday) in order to restore the immune surveillance in JCV-positive patients. However, this approach remarkably increases the risk of rebound of MS activity and is not sufficient to extinguish the risk of developing the disease. It is now evident that PML is a complex and not fully understood disease, in which viral and host factors might play a role in disease onset.

For this reason, the development of clinical and laboratory markers that assure the treatment safety for extended time in JC serologically positive individuals are pivotal.
To illustrate it, we describe the JCV replication dynamics in the urine and blood of a JCV positive MS patient receiving NTZ over 12 months and the detailed molecular investigation of the complete VP1 gene and RR.

THE FOLLOW UP

Urine and blood samples from a 38 year-old female patient, with MS first diagnosed as relapsing-remitting form in 1990 and receiving NTZ were monthly monitored through Real Time PCR for one year for the presence and viral load of JCV. From all positive samples, we sequenced the VP1 and RR (see Table for primers used in both reactions).

The patient was previously treated with Glatiramer acetate and interferon β-1A. She responded well to the NTZ and no relapses were reported during the follow-up. The average expanded disability status scale (EDSS) score was evaluated before NTZ introduction (EDSS=6) and remained stable throughout the treatment.

JCV DNA was not detected in blood samples in any time-point evaluated. However, all the urine samples were positive, including the one collected before NTZ introduction. The viral load, which was about 770,000 copies/ml at the first sampling, experienced a slight decrease in the first three months of treatment but significantly increased after the fourth month, reaching to $1.10^9$ copies/ml during the 8th, 9th and 10th months of treatment (Figure 2). This observation is in keeping with the Laroni et al., that found that viruria could occur before the Natalizumab introduction, but gradually increase during the treatment.

The JCV/VP1 gene and RR were successfully sequenced from viruses sampled at all time-points (the RR from the last three months was also cloned to deep investigate putative mutants present as minor population) and no nucleotide change was detected in the consensus sequences during the follow-up. Nevertheless, a careful inspection of the electropherograms revealed the emergence of a non-synonymous change at nucleotide 86 of the VP1 gene, at the very end of the N’ terminal region (aminoacid site 29) in viruses

Table. Sets of primers used for RR and VP1 amplification for sequencing.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Viral region</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>JCRS*</td>
<td>RR</td>
<td>ATTAGTGCAAAAAAGGAAAAAACAGGG</td>
</tr>
<tr>
<td>JCRAS*</td>
<td>RR</td>
<td>CTGGAATCCAGCTGGTGACAAGGCCAAACAG</td>
</tr>
<tr>
<td>70_F</td>
<td>VP1</td>
<td>CTAATGGATGTTGCCTTTAC</td>
</tr>
<tr>
<td>991_R</td>
<td>VP1</td>
<td>CCTCAAAAACACTTAACCTTCCTC</td>
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Figure 1. Risk for PML in MS patients under NTZ therapy. The risk is based on the time of treatment with NTZ, anti-JCV antibodies status and previous Immunosuppressive therapy. IS=Immunosuppressive (adapted from http://multiple-sclerosis-research.blogspot.com.br/)

Figure 2. Viral load/ml in urine samples. The graph shows the number of JC viruses/ml detected through Real Time PCR in urine at each time-point. Time 0 corresponds to the sample collected immediately before the first Natalizumab infusion.
from the sixth, seventh, eighth and ninth months of treatment (Figure 3). This change (Adenine to Guanine) would lead to the substitution of a glutamate to glycine (E29G). However, these mutants never reached to the majority population, and were no longer observed in the following months. This mutation was not related to any change already described as associated to PML.

Intra-host JCV variability, especially in the RR region is almost a consensus for PML patients, but the origin of the mutants has been subject of investigation. Some argue that the high rate of viral replication in urine or blood of an infected patient allows the emergence of mutant viruses with different tropism31,32. In contrast, it has also been suggested that variants with different tropism circulates among the population, and the infection by a more or less pathogenic strain happens by chance33. There is also the possibility that JCV establish latency in other tissues besides the urinary tract, as indicated by findings of JCV in brain of healthy individuals16,17,18. It is in accordance to the usual presence of archetype virus in urine but rearranged forms in blood and brain of PML individuals.

Here we showed that despite no JCV rearranged forms came out in the urine and no viremia occurred after 12 months of treatment, VP1 mutant viruses emerged in the urine concomitant to the increase of the viral load (see Figures 2 and 3). The emergence of variants during extensive viral replication is not surprising for viruses that experience high evolutionary rates31,34,35. Nevertheless, JCV, similarly to other DNA viruses, is genetically stable through time since its substitution rate ranges between $10^{-7}$ to $10^{-8}$ s/s/y36. Thus, it is less likely (although not impossible) that JCV variant, observed in this study, results from a within-patient mutation emergence.

We then envisage a scenario where the patient analyzed was infected during its lifetime by distinct variants, and the virus predominantly detected during the whole study was probably the one with the best fitness (variant A). Through the follow-up, the variant that emerged at the sixth month (variant B) was reactivated, possibly as a consequence of the Natalizumab treatment or any other unknown cause.

In situations where high-load persistent viral infection is already established and viral replication is constant, functional impairments or low frequencies of virus-specific T cells is not uncommon37. Therefore, it is possible that the emergence of the variant B concomitant to the already established variant A caused both, increased viral load, and stimulated the cellular immune response. As a consequence, the variant B was controlled, the viral load decreased and only the original (and possibly less immunogenic) viruses remained detectable.

Furthermore, it is also possible that the inefficient viral control is consequence of the effects of NTZ in the immune system. NTZ was showed to disturb the balance between cytokines, up regulating some pro-inflammatory cytokines38 and decreasing the expression of the co-stimulatory molecule CD134 on CD4(+)CD26(HIGH) T-cells39. Also, Perkins and coworkers reported that patients receiving Natalizumab who developed PML do not present JCV-specific T cell response or had JCV-specific CD4 T cell responses uniquely dominated by IL-10 production40. Unfortunately, no immunological test was performed in the present study to confirm if this would be the case here. Altogether, the above-discussed data reinforce that PML in NTZ patients is a combination of altered cellular and cytokine expression and viral factors.

In summary, the availability of NTZ represents a real gain in terms of better quality of life for MS patients, but it also
resulted in a new group of risk for PML. While the minimal risk of PML among these patients is as low as 0.00006% the maximal risk can reach to 1.17% in a JCV carrier with previous exposure to other chemotherapies and receiving NTZ for more than 24 months.

The putative JCV reactivation, associated to an inefficient viral control caused by Natalizumab support that both viral replication and immunological status of the patients should be monitored through the treatment in order to identify patients at imminent risk of PML without the need to suspend arbitrarily the therapy.

References


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