

JCV detection in multiple sclerosis patients treated with natalizumab

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Received: 3 August 2009 / Revised: 7 December 2009 / Accepted: 21 December 2009
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Abstract Natalizumab therapy is associated with an increased risk of progressive multifocal leukoencephalopathy (PML). Because the prognosis of established PML is uniformly dismal, identification of highly susceptible patients to the disease may improve outcomes. We wanted to investigate whether serial plasma and cerebrospinal fluid (CSF) screening for polyomavirus would identify patients with laboratory evidence of viral infection prior to the development of clinical PML. Two hundred MS patients had pre-treatment CSF/plasma screening for JC virus (JCV) and BK virus (BKV) DNA, and thereafter every six treatments of natalizumab. In all positive patients treatment is stopped (due to potential risk of PML), they have follow-up clinical examinations and plasma/CSF JCV/BKV tests until all evaluations are normal. No patient developed clinical evidence of PML. Eight of the 200 patients had detectable JCV or BKV DNA. Five patients were positive for BKV DNA in the CSF and three patients were positive for JCV DNA (one in plasma, two in CSF). After cessation of natalizumab treatment, all patients converted to undetectable viral DNA. Screening for JCV in CSF in natalizumab-treated patients could help identify those at heightened risk for developing PML and discontinuing treatment in these patients may abort development of the clinical illness.

Keywords JC virus · Natalizumab · Progressive multifocal leukoencephalopathy · Cerebrospinal fluid · Multiple sclerosis

Introduction

Natalizumab was FDA-approved for the treatment of relapsing-remitting multiple sclerosis (MS) in November 2004 based on the results of two multi-center phase III trials [13, 16]. However, 3 months later, therapeutic use of natalizumab was suspended after progressive multifocal leukoencephalopathy (PML) was detected in three patients who participated in the phase III trials [18]. After retrospective determination that the occurrence of PML was restricted to three out of the 3,417 trial patients, the FDA re-approved use of natalizumab as monotherapy for MS with a black-box warning about PML. In an attempt to closely detect any further cases of PML, natalizumab treatment was confined to infusion centers participating in the TOUCHTM program (in the USA) with mandatory monitoring and reporting requirements [14].

The TOUCHTM program, as detailed below (methods), is based on clinical and radiological evaluations to detect PML [17]. However, when PML is clinically detectable by symptomatology or brain imaging it reflects established disease with poor prognosis as there is no effective treatment. To enhance the screening requirements of the TOUCHTM program we tested plasma and cerebrospinal fluid (CSF) of all patients for JCV (JC virus) DNA prior to commencing and after every 6 months' of treatment with natalizumab. We also screened for BKV (BK virus), a closely related member of the *Polyomaviridae* family.

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Materials and methods

Patient selection

Patients receiving natalizumab were prospectively included in the analysis ($n = 200$). Patients were considered for natalizumab if they had clinically definite MS and were unresponsive or intolerant to first line MS treatments. As advised by the FDA, all the patients were also included in the TOUCHTM Program, and received their natalizumab infusion in a center enrolled in the TOUCHTM Program [17]. This program is designed to detect any change in neurological status that may indicate evidence of PML in natalizumab-treated patients. Prior to the initial infusion all patients must have a complete neurological evaluation and brain magnetic resonance imaging (MRI) with and without gadolinium. Before each subsequent infusion, patients are required to respond to a series of questions that ensure clinical stability. If any change in neurological status occurs, the underlying mechanism must be ascertained and PML ruled out before proceeding with natalizumab treatment. In addition, the patient is evaluated by a neurologist 3 and 6 months after the first infusion, and every 6 months thereafter. Reauthorization to receive natalizumab is renewed every 6 months based on an absence of clinical evidence of PML. Furthermore, prescribing physicians are required to complete questionnaires on all patients discontinuing treatment confirming that PML is not evident or under investigation. According to the TOUCHTM Program, if there is any clinical or radiological suspicion of PML, CSF will be analyzed for JCV DNA [17].

Clinical design

This study is designed to enhance the surveillance of the TOUCHTM Program by introducing screening laboratory methods to detect JCV replication before the development of clinical PML. Thus, all patients receiving natalizumab are screened for JCV (and BKV) DNA in plasma and CSF at baseline, and then after six monthly treatments. Ninety-three patients have received 18 treatments (and thus 4 JCV/BKV screenings), and 104 patients discontinued treatment before completing 18 months (2–4 screenings depending on which treatment number they discontinued). In addition, three patients had original JCV/BKV screenings but then did not start natalizumab treatment. Including repeat positive testing, there have been 630 JCV/BKV screenings, 200 baseline and 430 screenings otherwise. There is no control group in the study but the baseline values of each patient provide an objective comparison to post-treatment results. It is anticipated that this screening will continue until all patients have their screening after 18 months on treatment or a screening if treatment is discontinued.

Natalizumab is discontinued in any patient who has a positive JCV test. All positive patients undergo an immediate neurological evaluation and a brain MRI to detect clinical PML. Subsequently, all positive patients are maintained treatment-free and have follow up clinical examinations and plasma and CSF JCV (and BKV) tests until all evaluations are normal. Patients discontinuing natalizumab treatment at any point other than at a 6-month interval have plasma and CSF viral testing as well as a complete clinical assessment. Informed consent was obtained from all patients prior to obtaining blood and CSF samples with an IRB-approved protocol and the study was performed in accordance with ethical standards.

Laboratory testing for JCV and BKV DNA

DNA extraction and qualitative real-time PCR for detection of JCV from patient plasma and CSF was done commercially. DNA was extracted from 0.2 mL clinical specimen using the automated MagNA Pure Total Nucleic Acid kit (Roche). qPCR reactions were done using TaqMan real-time chemistry (Roche). The primers and probes are proprietary (Focus Diagnostics) but are directed to highly conserved regions of the large T antigen specific to each virus. According to Focus Diagnostics, there is no cross-reactivity of the primers and probes between JCV and BKV, or with 20 viral and non-viral pathogens. Each reaction has an internal control to show that the PCR reaction has not been inhibited. The lower limit for detection of viral DNA of this assay is 500 copies/mL. In other studies on patients with PML, it has been reported that there is a false-negative detection rate for JC virus of 15–25% with virtually no false-positive results [1, 4, 7]. Testing for BKV was performed in a similar manner.

Results

All patients were viral DNA free in CSF and plasma at baseline. No patients have developed clinical or brain MRI evidence of PML. However, post-treatment, we detected viral DNA in either plasma or CSF in eight patients. It is noteworthy that no differences could be discerned in age range, sex, past treatment record, or current medical history between patients who developed detectable JCV/BKV and those who did not (Table 1).

Three of the 200 patients had detectable JCV DNA. Two of the patients had JCV DNA detected in the CSF (plasma remained negative) while the other patient was positive for JCV only in plasma. The JCV DNA detection in plasma occurred at the 6-month test. One case of JCV DNA in CSF was detected at 4 months of treatment when they stopped natalizumab treatment due to a patient

Table 1 Demographics of positive JCV or BKV patients

Patient ID	Age	Sex	Disease duration	Disease type	EDSS score	Previous MS treatments
1	36	F	12	RRMS	3.5	Weekly IFN- β 1a, GA, IVIG, IFN- β 1b, MP
2	65	F	28	SPMS-R	6.5	GA, Mito, MTX
3	44	F	20	SPMS-R	5.0	Solumedrol, weekly IFN- β 1a, mycophenolate, IVIG, Mito, MTX
4	32	F	12	SPMS-R	6.0	Weekly IFN- β 1a, MP, IVIG
5	57	F	13	SPMS-R	5.0	IFN- β 1b, IVIG, Mito, MTX, MP
6	38	F	30	RRMS	3.0	IFN- β 1b, weekly IFN- β 1a, high dose IFN- β 1a
7	46	M	26	RRMS	3.5	IFN- β 1b, azathioprine, GA, weekly IFN- β 1a, IVIG, high dose IFN- β 1a, MP, MTX
8	54	F	12	RRMS	4.0	Weekly IFN- β 1a, MP, MTX

RRMS relapse remitting multiple sclerosis, SPMS-R secondary progressive multiple sclerosis with relapses, EDSS expanded disability status score [10], IFN- β interferon- β , GA glatiramer acetate, IVIG intravenous immunoglobulin, MP methylprednisolone, Mito mitoxantrone, MTX methotrexate

Table 2 Characteristics of JCV or BKV positive patients

Patient ID	Immediate pre-natalizumab treatment and drug-free washout period in weeks	Positive virus detection and time detected at	Follow up test results
1 (36 F)	IFN- β 1b 104	JCV in plasma at 6 months	Negative at 8 weeks
2 (65 F)	MTX 16	JCV in CSF at 4 months	Negative at 4 weeks
3 (44 F)	Weekly IFN- β 1a 5	JCV in CSF at 18 months	Negative at 6 weeks
4 (32 F)	Weekly IFN- β 1a, MP and IVIG 16	BKV in CSF at 6 months	Negative at 5 weeks
5 (57 F)	MP 16	BKV in CSF at 6 months	Positive at 4, 9 weeks; negative at 13 weeks
6 (38 F)	Weekly IFN- β 1a 8	BKV in CSF at 6 months	Positive at 8 weeks; negative at 22 weeks
7 (46 M)	MTX 16	BKV in CSF at 6 months	Negative at 6 weeks
8 (54 F)	MTX 12	BKV in CSF at 6 months	Negative at 12 weeks

perception of lack of efficacy. The other JCV DNA case occurred after 18 months of natalizumab treatment. After cessation of treatment, the patients reverted to negative CSF/plasma values at follow up testing. All three of the patients with JCV positive samples had stable and unchanged brain MRI findings on repeat testing. Five patients were positive for BKV DNA in the CSF (plasma remained negative) at 6 months of treatment, all of whom converted to undetectable viral DNA within 5–22 weeks (Table 2). All five BKV positive patients are neurologically stable.

Discussion

Natalizumab treatment is highly effective in MS as it reduces relapse rates by over 60%, but is not considered a first-line therapy because of its association with the development of PML in the initial phase III trials [13, 18].

Since the reintroduction of natalizumab under the TOUCHTM Program in July 2006, over 60,000 patients (Biogen IdecTM) have received this treatment worldwide.

Current reporting indicates that 27 cases of PML have been found in patients taking natalizumab (direct communication, Biogen IdecTM). None of the 27 patients who developed PML had prior or serial CSF analysis to determine the presence of JCV. Retrospectively, however, in a recent patient diagnosed with PML in Europe, quantitative PCR confirmed the presence of 53 copies/mL of JCV DNA in the CSF prior to initiation of plasma exchange, although no virus had been detected previously using less sensitive PCR methods [11]. In this regard, it should be noted that the TOUCHTM Program is not followed in Europe, although by report these patients were closely monitored and there does not appear to have been an undue delay in diagnosis of PML. Our most notable findings are that in three of 200 patients who at baseline were negative for JCV DNA, converted after natalizumab

treatment to having detectable JCV DNA in their plasma or CSF. It is important to emphasize that in all three patients these findings occurred in the absence of radiological or clinical evidence of PML and discontinuation of natalizumab resulted in reversal of their JCV DNA findings.

PML is caused by JCV infection and lysis of oligodendrocytes which leads to widespread white matter demyelination. Infection with JCV, a double-stranded DNA virus of the *Polyomaviridae* family, occurs in childhood and is estimated to be present in up to 70% of asymptomatic adults [14]. In these asymptomatic carriers, plasma or CSF JCV DNA is not detectable [9]. In profoundly immunosuppressed patients, and in conditions such as AIDS, a proportion of these asymptomatic JCV carriers develop PML reflecting activation of the virus. However, not all patients who have detectable JCV DNA, presumably indicative of viral activation, develop PML. Plasma JCV DNA appears to correlate with a state of immunosuppression whereas CSF JCV DNA is strongly associated with the development of PML [9]. However, two recent reports in patients with MS have suggested that JCV may occasionally be detected in the CSF without PML [2, 8]. Alvarez-Lafuente et al. [2] reported that two of 34 patients had detectable JCV DNA in the CSF but the result was not reproducible in one of the two patients. No untoward effects were seen in the patients who had CSF JCV, probably because the viral DNA copy load was less than 10 copies/mL in both patients. Iacobaeus et al. [8] similarly found low copy CSF JCV DNA in two of 252 MS patients. In both these studies, CSF JCV DNA was detected in MS patients who had never received natalizumab therapy. In our assay, the lower limit of detection for JCV DNA is 500 copies/mL and therefore a positive result is likely to be of greater consequence. There is the possibility that a patient could develop PML at a lower copy load than our assay would detect. However, this screening test does allow detection of patients who have JCV in their CSF and have not yet developed clinical PML. Irrespective of the viral copy load, it is clinically difficult to ignore our findings because the positive patients in our study were negative for JCV prior to natalizumab treatment. Although a separate control group was not studied, it is noteworthy that all 200 patients had been on single or combination immuno-modulatory therapy for their multiple sclerosis at baseline and these treatments did not result in any patient with a positive JCV/BKV test at pre-screening.

We also tested for BKV, a closely related virus to JCV. BKV is the causative agent of polyomavirus-associated nephropathy (PVN) of the kidney following renal transplant leading to a gradual loss of graft function [15]. BKV

activation has not been linked to PML but it is present with JCV in some brain samples of PML patients [6]. In patients without PML, BKV DNA is occasionally detected by PCR in non-neurological and neurological diseases including MS [3, 5]. CSF detection of BKV DNA is not seen in asymptomatic or immunosuppressed patients [12]. Thus, detection of the JCV-related BKV could indicate a state of immunosuppression, and also act as a control against non-specific detection of polyomavirus DNA. This is supported by our pretreatment data as all 200 patients had undetectable BKV DNA by PCR in plasma and CSF. However, following natalizumab treatment, five patients had detectable CSF, but not plasma, BKV DNA. None of the five patients had any discernable clinical consequences of this CSF BKV conversion and with discontinuation of natalizumab treatment all patients have reverted to their baseline CSF status. Although BKV is not the causative agent of PML, BKV DNA found in the CSF potentially reflects infection or activation in the CNS of natalizumab-treated patients. None of the positive tests for JCV or BKV DNA in this study were associated with changes in inflammatory cells or blood-brain barrier dysfunction (data not shown). These findings are somewhat unsettling given the propensity of natalizumab to be associated with PML. Thus, it may be prudent to have a high index of suspicion to detect opportunistic infections in general, in patients treated with natalizumab.

Infection of the brain by JCV when associated with immunosuppressive therapy is likely a result of impaired CNS immune surveillance for periods of greater than 6 months [1]. In this study, after 4–18 months of natalizumab therapy, the detection rate of JCV in plasma/CSF of the 200 patients was 1.5%. This is greater than the reported rate for PML in patients with MS on natalizumab (direct communication, Biogen IdecTM) but would explain the viral replication stage before PML occurs. Detection in the CSF is possibly a consequence of impaired lymphocyte trafficking across the blood–brain barrier. Whatever the mechanism underlying the induction of JCV in positive patients, it should be stressed that at no time has there been any clinical evidence of PML in our patients. It is not known whether discontinuation of natalizumab aborted the development of impending PML in our patients and whether such testing would have prevented PML in the four recent cases. At present, however, it may be prudent to test for plasma and CSF JCV DNA in all patients undergoing natalizumab treatment at 6-month intervals as an early indicator for potential PML development.

Acknowledgment The funding for this study was supported with MSRCNY funds and was approved by the MSRCNY Board of Directors.

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