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Advances in the management of PML: Focus on natalizumab

■ ABSTRACT

Progressive multifocal leukoencephalopathy (PML), a rare opportunistic infection of the central nervous system, occurs mainly in the setting of broad-based and selective immunosuppression. The immunomodulatory agent most often implicated in the development of PML is the monoclonal antibody natalizumab. Management of PML begins with risk stratification. Factors that predict the risk of PML are JC virus (JCV) antibody status, history of chemotherapy use, and cumulative exposure to natalizumab. The risk of natalizumab-related PML increases up to a duration of 36 months of therapy, after which the risk appears to level off. If suspicious for PML, the use of a sensitive JCV polymerase chain reaction assay permits early diagnosis. Immune reconstitution represents the mainstay of treatment for PML. With rapid reversal of immunosuppression followed by immunologic recovery, almost all patients suffer clinical deterioration termed immune reconstitution inflammatory syndrome (IRIS). High-dose corticosteroids are often recommended if a clinical and imaging syndrome resembling IRIS develops after immune restoration.

Progressive multifocal leukoencephalopathy (PML) is a rare opportunistic infection of the central nervous system (CNS). Although originally associated with broad-based immunosuppression (human immunodeficiency virus infection, lymphoproliferative disorders, and immunosuppressive medications), recognition of PML in patients with selective immunosuppression is growing. This restricted immunodeviation can arise from autoimmune disorders such as systemic lupus erythematosus, selective immunosuppressive therapies (eg, rituximab,

leflunomide, and efalizumab), or immunosuppression limited to the CNS (eg, treatment with natalizumab).

This article reviews approaches to the management of PML, with specific recommendations regarding PML associated with natalizumab therapy.

■ APPROACH TO PML TREATMENT

The ideal approach to PML treatment is generally two-pronged: antiviral therapies to directly reduce viral replication and immune reconstitution that empowers the immune system to attack the JC virus (JCV). Challenges to treatment are the difficulty in culturing JCV for in vitro studies, lack of an animal model of PML, and infrequency of PML cases.

Antiviral therapies

At present, no antiviral agent has confirmed efficacy in PML. Nucleoside analogues, serotonin 5-hydroxytryptamine receptor antagonists (to block the JCV receptor), and several cytokines provided exciting prospects in preclinical studies for treatment of PML in humans. Unfortunately, subsequent clinical studies of cytarabine, cidofovir, and interferon alfa all yielded disappointing results. A derivative of cidofovir, CMX001, is also being evaluated for efficacy in PML. Mefloquine was identified through a broad pharmaceutical screening study to have strong antiviral effects in vitro, but a clinical trial to assess its effects was stopped. It remains unclear whether the failure of clinical studies after successful in vitro studies is secondary to low drug penetration into the CNS, treatment initiation too late in the course of PML, or other differences not yet fully understood.

Immune reconstitution

Given the widespread failure of antiviral regimens, the mainstay of PML treatment is immune reconstitution. When immunosuppression is secondary to a medical disorder, efforts are pursued to reverse the primary disorder. For example, highly active antiretroviral therapy significantly prolongs survival in antiretroviral-naïve acquired immunodeficiency syndrome patients.^{1,2} Decreasing the

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intensity of immunosuppressive therapy in solid organ transplant may improve survival with PML. When PML is associated with biologic therapies for autoimmune diseases, early diagnosis and immediate suspension of therapy is thought to improve outcomes.

■ EXPERIENCE WITH NATALIZUMAB

PML in the setting of natalizumab therapy is related to cumulative exposure to natalizumab. As of August 4, 2011, there had been 150 cases of natalizumab-related PML documented in more than 88,000 patients exposed to natalizumab worldwide³ (see page S18, "Multiple sclerosis, natalizumab, and PML: Helping patients decide"). The incidence of PML in natalizumab-treated patients varies according to the number of infusions received, but the incidence of PML by each epoch of treatment exposure (1 to 24 infusions, 25 to 36 infusions, 37 to 48 infusions) appears to have remained stable over time.³

The mortality associated with natalizumab-related PML was 19% (29 deaths among the 150 confirmed cases) as of August 4, 2011.³ In cases with at least 6 months of follow-up, mortality has remained at about 20%. Many who survived were left with serious morbidity and permanent disability, although interpretation of disability is difficult because functional impairment is a hallmark of multiple sclerosis (MS) irrespective of PML. Survival in patients with natalizumab-associated PML appears to be better than with PML associated with other conditions, possibly because of early diagnosis achieved through clinical vigilance and swift immune reconstitution through natalizumab discontinuation and either plasmapheresis or immunoabsorption. Predictors of survival include younger age at diagnosis, less disability prior to onset of PML, more localized disease on magnetic resonance imaging (MRI) of the brain, and shorter time from symptom onset to PML diagnosis.

Clinical characteristics of natalizumab-associated PML

Several clinical observations should increase suspicion of natalizumab-associated PML.³⁻⁵ For example, the most common presenting symptoms are cognitive, motor, language, and visual impairment. Gadolinium-enhancing lesions are observed at presentation in about one-half of patients. Seizures and paroxysmal events can occur at presentation, which helps to differentiate PML from an MS relapse.

Approximately one-half of patients with natalizumab-associated PML have an initial viral load of less than 500 copies/mL, underscoring the need for ultrasensitive polymerase chain reaction (PCR) testing. An ultrasensitive JCV assay (Focus Diagnostics, Cypress, California) is available that can detect less than 50 copies/mL of JCV DNA. Because the viral copy numbers in the cerebrospinal fluid (CSF) may be low in patients treated with natalizumab, the CSF PCR may be falsely negative. In several cases of

PML, JCV was undetectable in the CSF by PCR, identified only later by repeat PCR or brain biopsy.⁴ Serum JCV PCR is not useful in the screening or diagnosis of PML.

Natalizumab-associated PML has not been observed with therapy of 6 months' or less duration. After 6 months of natalizumab therapy, new MRI lesions are rare in patients who are negative for neutralizing antibodies. A new MRI lesion in such a patient should be considered suspicious for PML. Our standard protocol is to check for neutralizing antibodies at 6 months in all patients treated with natalizumab. Symptoms of PML develop in affected patients whose duration of therapy ranges from 6 to 81 infusions. Symptoms often develop well before PML is diagnosed.^{4,5}

Forty-six percent of patients treated with natalizumab who develop PML have received previous autologous bone marrow transplantation or chemotherapy, including mitoxantrone, azathioprine, methotrexate, and mycophenolate mofetil. In comparison, up to 25% of MS patients who were treated with natalizumab (13% in the United States, 24% in Europe) have had prior chemotherapy treatment. Prior immunosuppressive therapy increases the risk of PML by two- to fourfold, which may explain the higher rate of PML in Europe compared with that of the United States.^{4,5}

Testing for immune response to JCV

A JCV enzyme-linked immunosorbent assay (ELISA) test has been developed that identifies patients with an immune response to JCV. Among MS patients, 55% test positive for JCV through this assay.⁶ The false-negative rate of the test is 5%, and the overall annual seroconversion rate is estimated to be about 2%, necessitating repeat testing.

Based on results of this assay, the estimated risk of PML in seropositive patients is about 1 in 500.⁶ The test was positive in 28 of 28 patients who developed PML. The probability of this relationship occurring by chance is 0.5528, which suggests that this assay is useful to stratify risk for development of PML. Although the rate of false negatives makes the test an imperfect predictor, it is still useful in clinical practice. The test became available clinically in late summer 2011. Further longitudinal observation studies (STRATIFY-1 and STRATIFY-2) on the use of the JCV ELISA to detect anti-JCV antibodies in the blood of natalizumab-treated patients with MS are under way.

Stratifying risk for natalizumab-related PML

Three factors may predict the risk of PML: JCV antibody status, history of chemotherapy use, and duration of natalizumab treatment. Estimates of risk of PML have been derived from these factors,^{1,6} with differences in patient profiles producing risk estimates that range from approximately 1 in 40,000 to 1 in 100. Overall, the estimated risk of a JCV-negative person who is chemotherapy-naïve is approximately 1 in 40,000. With prior chemotherapy, this risk increases to approximately 1 in 15,000. Among

TABLE**Risk and JCV antibody status^{6,7}**

JCV antibody status	Risk stratification		Risk after 24 mo therapy	Comparison
	Prior chemotherapy?	Overall risk		Lifetime risk of death associated with selected causes
Negative	No	~1:28,397*	~1:15,046*	Airplane crash, 1:20,000
	Yes	~1:7,099*	~1:3,761*	Drowning, 1:8,942
Positive	No	~1:710	~1:376	Fire/smoke, 1:1,116
	Yes	~1:177	~1:94	Car accident, 1:100

*Estimate; no cases reported

JCV = JC virus

patients who are JCV antibody-positive, the overall risk of PML is 1 in 500 for chemotherapy-naïve patients and 1 in 200 for those previously exposed to chemotherapy. To give these ratios some perspective, the lifetime risk of dying in a car accident is 1 in 100 (Table).^{6,7}

Natalizumab holidays and PML risk

The possibility of reducing the risk of PML in natalizumab-treated patients through natalizumab holidays is attractive. When exploring this option, one must consider whether the risk of recurrent disease activity with treatment interruption outweighs the potentially decreased risk of PML.⁸ A randomized controlled multicenter clinical trial of natalizumab interruption is ongoing, with the recruitment phase complete after enrollment of 175 patients. Patients taking natalizumab at study entry have been randomized to one of three arms: continuation of monthly natalizumab for 6 months, placebo for 6 months, or an alternate treatment (interferon beta-1a, glatiramer acetate, or monthly intravenous steroids) for 6 months administered open-label by clinician and patient choice.

The primary outcome measures are markers of immune function and overall disease activity during treatment interruption and after resumption. Patients are monitored monthly using MRI to measure disease activity. Those who experience relapse will have the option of returning to natalizumab therapy or switching to an alternate treatment. The results of this prospective, randomized, controlled trial will provide a greater understanding of the safety issues surrounding natalizumab holidays.

Management of natalizumab-related PML

Management of patients taking natalizumab starts with risk stratification in an attempt to prevent the development of PML. If suspicion for PML is raised based on symptoms, early diagnosis can be accomplished through the use of a sensitive JCV PCR assay, with a repeat PCR if negative. Natalizumab treatment should be withheld during the workup for PML.

In the setting of natalizumab therapy, where the immu-

nosuppression is compartmentalized to the CNS, functional leukocytes are only millimeters away from where they are needed to fight JCV infection. Plasmapheresis has been shown to accelerate removal of natalizumab, accelerate desaturation of the targeted alpha-4-integrin receptor, and restore leukocyte transmigration in vivo (Figure).⁹ Desaturation of the integrin receptor occurs at natalizumab serum drug levels less than 1 µg/mL. Statistical modeling from pharmacokinetic measurements during a plasmapheresis study projected that clinically relevant integrin receptor desaturation is accelerated by 82 days through the use of five plasmapheresis treatments.⁹ Accordingly, plasmapheresis (or immunoabsorption) is recommended in natalizumab-treated patients who develop PML. Putative antiviral therapies can be considered but have so far yielded disappointing results in clinical trials. Additional trials are under way.

When immunosuppression is rapidly reversed in cases of natalizumab-associated PML, an overly exuberant immune response targeting JCV in the CNS is observed 2 to 6 weeks later. The response, termed immune reconstitution inflammatory syndrome (IRIS), is not always easy to differentiate from progression of PML. Nonetheless, most clinicians recommend high-dose corticosteroids if a clinical and imaging syndrome resembling IRIS develops several weeks after immune restoration.¹⁰ The objective is to achieve the immune reconstitution needed to control JCV infection while limiting the collateral damage of inflammation on the remaining brain tissue.

SUMMARY

Risk factors for natalizumab-associated PML include duration of treatment with natalizumab, previous chemotherapy, and JCV antibody serology. Early diagnosis requires the use of an ultrasensitive JCV PCR assay. Treatment is focused on early diagnosis, immediate cessation of pharmacologic causes of immunosuppression, and active efforts to accelerate immune restoration.

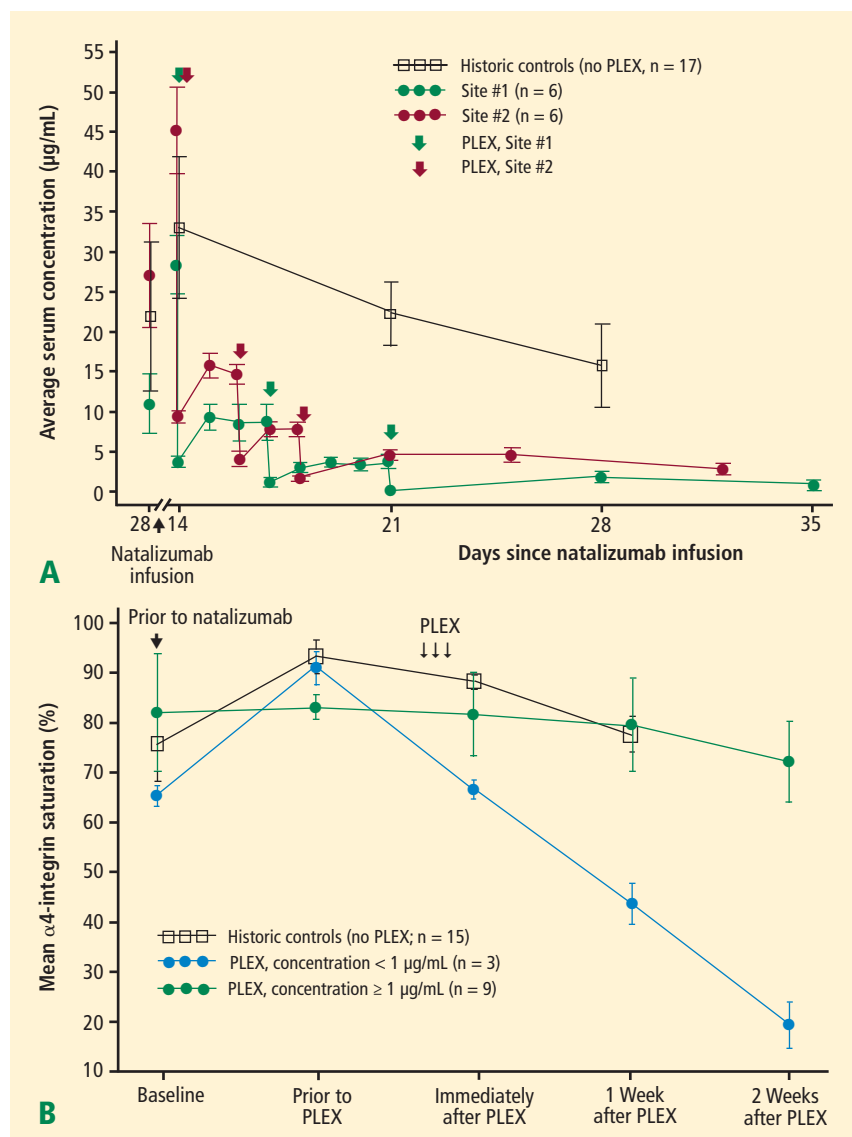


FIGURE. Effects of plasma exchange (PLEX), or plasmapheresis, on serum concentration of natalizumab (**A**) and alpha-4-integrin saturation (**B**). Historic data were obtained from a separate group of patients with multiple sclerosis after six monthly doses of natalizumab, with no PLEX. For alpha-4-integrin saturation (**B**), PLEX patients were divided into two groups: those with sustained natalizumab concentration of less than 1 µg/mL after PLEX and those with natalizumab concentration of 1 µg/mL or greater after PLEX.⁸

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DISCUSSION

Dr. Calabrese: What are your thoughts about plasmapheresis for rituximab-related cases of PML?

Dr. Fox: It's probably not going to be as helpful as with natalizumab. Rituximab has pharmacokinetics that are similar to those of other monoclonal antibodies, with a half-life in the range of 14 to 20 days. So it's pretty much absent from the body within 1 to 2 months of infusion. The

enduring benefit from rituximab comes not from the persistent presence of the monoclonal antibody, but the persistent absence of CD19 B cells. Plasmapheresis is unlikely to be effective because it won't accelerate return of CD19 B cells to the peripheral circulation. In rituximab-related PML, stimulating the bone marrow to produce more B cells in order to restore the immune system is more likely to be effective. In contrast, I did recommend plasmapheresis in a case of efalizumab-related PML. Because efalizumab is a binding antibody to the CD11a receptor, we wanted to accelerate its removal.

Dr. Molloy: In an MS patient who responds well to natalizumab, do you ever explore a strategy of dose reduction or extending the dosing interval of natalizumab?

Dr. Fox: Let me put that into a clinical context. A 35-year old man has had relapsing-remitting MS for 3 years. Two years ago, after disease activity occurred while he was using an injectable therapy, he started natalizumab and has been clinically and radiologically stable on natalizumab. Then, he gets the JCV assay, it's positive, and he asks if it's time to get off natalizumab "because of the risk of that brain virus."

What do I tell him? Should I change the dosing interval? At this point, we are not doing either. One reason is the unpredictable pharmacokinetics of the drug. The dose and dosing regimen were chosen to have 85% or greater receptor saturation in 95% or more of patients over the course of the recommended 4-week dosing interval. If you increase the interval to 6 weeks or 8 weeks, you can't predict in individual patients whether or not meaningful desaturation occurs and thus allows some immune cells to enter the brain to protect against PML (but not too many, or MS disease activity will return).

Dr. Simpson: Do you have an algorithm for working up patients?

Dr. Fox: It depends on the level of suspicion given the patient's symptoms. It's difficult to find a single MS

patient who does not have some fluctuation of symptoms over time and some worsening of symptoms such as stiffness, fatigue, and cognitive difficulties. They all have changes in mood, so if one took any symptom change—any change in their report of mood and cognition—as the cutoff for a workup, we wouldn't be giving natalizumab at all. But if a patient or family says, "I am worried," then we need to work it up. Also, if there are clearcut new or worsening neurologic symptoms, we pursue a workup. Often, the change in symptoms is revealed when the patient comes in for his or her monthly infusion and the nurse asks the four questions from the preinfusion checklist (as part of the mandatory Tysabri Outreach: Unified Commitment to Health [TOUCH] prescribing program for natalizumab).¹¹

If there are new symptoms, we hold infusions and do a two-stage evaluation. The first stage is a brain MRI to evaluate for change from baseline (the US Food and Drug Administration requires a brain MRI at baseline before starting natalizumab therapy). Most patients undergo a brain MRI every 6 to 12 months while on natalizumab therapy, with instructions to the neuroradiologist to evaluate carefully for new lesions. In our institution, the PML MRI evaluation is a fine-tooth-comb assessment of lesions from the most recent MRI compared with the current MRI. Depending on the results of the current MRI and on our level of suspicion, we may proceed to a spinal tap, even if the MRI findings are stable. We have done 8 to 10 spinal taps in patients taking natalizumab when we were suspicious enough to evaluate for PML. Occasional patients continue to have active disease, relapses, and new lesions even without developing antibodies while taking natalizumab.

Dr. Rudick: We need a quick, quantitative analysis method to compare one MRI with another. It is easy to say, "Consider PML if there are new lesions." It's not so easy to know if the lesions are new. We are participating in a National Institutes of Health study regarding identification of biomarkers of interferon's effects, and the study requires obtaining MRI scans at baseline and 6 months. We have state-of-the-art subtraction MRI to quantify new lesions on the followup MRI. However, there is significant disagreement on the number of new lesions determined by clinical raters, and disagreement between the clinical raters and the numbers generated by the computer program.

Dr. Major: Is the incidence of natalizumab-related PML based on the number of months or on the number of infusions?

Dr. Fox: It is based on the number of infusions. You bring up a good point because these patients may interrupt treatment when they go on vacation, for example,

or have a lapse in insurance coverage. Most patients follow the every-4-weeks protocol and receive 13 infusions in a year. Perhaps 10% to 15% do not follow it precisely.

Dr. Molloy: Is everyone who takes natalizumab being followed for PML even if they discontinue natalizumab? Have any differences emerged in the factors that predispose to PML among those who continue therapy compared with those who discontinue? I ask because I'm wondering why the incidence appears to stabilize, or even go down, after 36 infusions.

Dr. Fox: PML has not been reported beyond several months after stopping natalizumab; concern about PML can decrease fairly quickly after stopping the drug. Many of us expected the risk of PML to continue rising with cumulative treatment, so were pleasantly surprised to see a plateau in the risk of PML after about 36 months. We don't understand what leads to this plateau.

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