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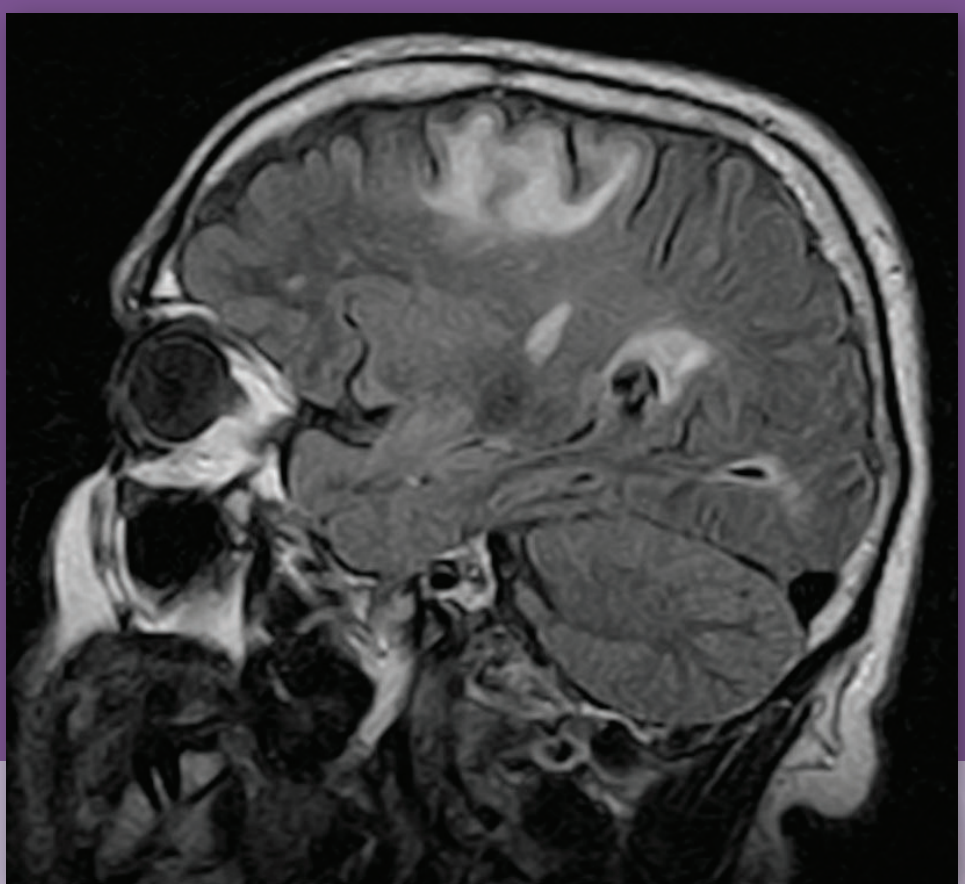
Identifying Patients at Risk for PML



PML in Immunocompromised Patient Populations: Historical Context

PML Risk Factors, Risk Reduction, and Improved Outcomes

Emerging Data in Treatment-Associated PML

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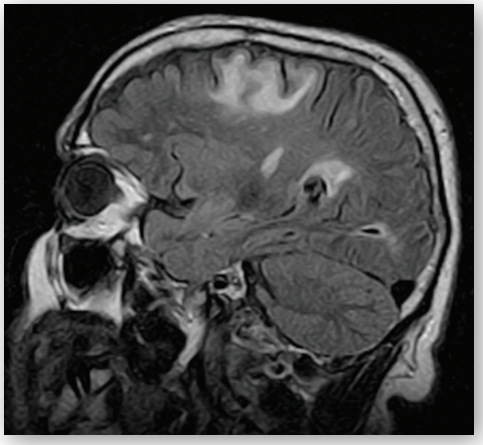
Identifying Patients at Risk for PML



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PML in Immunocompromised Patient Populations: Historical Context

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Introduction

Progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the central nervous system (CNS), has been recognized for more than 50 years. The disease was initially thought to be a complication of hematologic malignancies but is now understood to develop under a broad range of circumstances related to immunologic dysfunction. Impairment in immune defenses allows an otherwise benign and common virus to proliferate and trigger the events that define this disorder. The incidence of PML increased rapidly during the AIDS epidemic before the introduction of antiretroviral therapies. If well controlled, HIV is no longer a significant risk factor for PML. Rather, although this disease has multiple etiologies, it is now most commonly seen as a sporadic complication of immunosuppressive therapies. In these cases, the natural history differs from the historical experience. In particular, current data demonstrate that survival is now a reasonable expectation if PML is detected promptly and managed appropriately. Developing a perspective on the origins of PML may be useful for clinicians who treat patients with diseases or treatments that alter immune function.

History of PML

The name PML was bestowed in 1958 by investigators who also hypothesized, although they could not prove, that the disease had an underlying viral pathogenesis.[1] A description of the same condition, for which no formal name was given, was published in Germany in 1930,[2] suggesting PML may have a long established if unrecognized place among human diseases. In the 1958 publication,

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PML was linked to lymphoproliferative malignancies, particularly Hodgkin's and non-Hodgkin's lymphomas. Subsequent publications, including one a year later,[3] expanded the evidence that a compromised immunoregulatory system permitted some pathogen, such as a virus, to become active in the CNS and trigger a demyelinating process.

The viral pathogen was not definitively isolated until 1971.[4] Named after the initials of the patient from which the pathogen was first cultured, the John Cunningham virus (JCV) is a polyomavirus of the genus Papovaviridae.[5] It is among several polyomaviruses, such as the BK virus, which appear to be commonly acquired in childhood and can reside indefinitely in the healthy human host with few or no clinical consequences.[6] The JCV infection rate has been reported to be in the range of 33% to 91% from antibody studies conducted in healthy individuals and patients with a variety of conditions.[7-10] Infection rates are generally age-related. For example, a seroprevalence study in England and Wales found the overall figure to be 35%, but it reached 50% among those between the ages of 60 and 70 years.[11] Although JCV may reside in a latent state in many host tissues, particularly the kidney, it does not appear to circulate readily based on the infrequency with which it can be detected in the blood of immunocompetent and otherwise healthy adults.[12]

Like other diseases produced by opportunistic infections, overt PML manifests when altered immune function eliminates the usual barriers to the proliferation of the latent virus with which it is associated.[13] These barriers, only partially understood, are likely to be complex and not readily breached based on the infrequency of PML even in those individuals with prolonged and severe immunosuppression. Pre-existing conditions associated with risk of PML include, in addition to the hematologic malignancies with which it was first associated, AIDS, solid tumors, congenital immunodeficiencies, granulomatous diseases such as tuberculosis, and a relatively long list of immunosuppressive drugs, including those used for control of cancer, autoimmune disorders, and inflammation.[14]

Prior to the AIDS epidemic, PML was perceived as an exceedingly rare disease. Although some type of immune dysfunction appeared to be a prerequisite for PML, no additional set of characteristics could be identified that further isolated those at risk. The increased incidence of PML secondary to AIDS-related immune dysfunction stimulated many of the studies that have helped better characterize this disease. With the introduction of antiretroviral therapies, the rates of AIDS-related PML have fallen, but they may not have receded to pre-AIDS rates because of the increasing use of immunosuppressive therapies used in the treatment of cancer and a host of autoimmune conditions that are also associated with PML.[15]

Epidemiology

Prior to the AIDS epidemic, the population-based incidence of PML in the United States was an estimated 0.15 per million. This incidence increased by approximately four-fold to 0.6 per million with the onset of AIDS.[16] In the era of uncontrolled HIV infection prior to the introduction of effective antiretroviral therapies, approximately 80% of PML cases were attributed to AIDS with up to 4% of patients with AIDS developing PML prior to death.[17] In a recently published analysis from a health insurer database, which excluded AIDS patients, hematologic malignancies increased the risk of PML by approximately eight-fold and bone marrow transplantation increased the risk by approximately 35-fold relative to a general population without these risk factors.[18]

PML typically occurs in patients with some form of immunocompromised function,[19] but the literature regarding specific risk factors other than AIDS is dominated by case reports, underscoring broadly disseminated etiologies. A small number of cases of idiopathic PML have been reported that include individuals who are immunocompetent.[20] In those who are immunosuppressed, such as those with lymphoproliferative disorders, it remains unclear whether the disease or the treatments represent the greater threat for PML, although both may be important.[21] In one series, the risk of PML in chronic lymphocytic leukemia (CLL)

patients increased with disease progression as well as with the longer duration of the illness.[22] Susceptibilities involving specific types of immune function may be identifiable. In hematologic malignancies, such as non-Hodgkin's lymphoma and CLL, the rates of PML calculated over 100,000 person years of vulnerability have ranged from 8.3 to 11.1, but the rate increases by more than three-fold to 35.4 in the same patients undergoing bone marrow transplantation.[18] Immunosuppression may not be a prerequisite for PML, but the rise and fall in the incidence of PML that followed the dissemination and then control of AIDS suggests it is important. Even in the absence of HIV, reported cases of PML in both idiopathic CD4+ depletion as well as in myelodyspasia without treatment support the premise that it is immune function rather than the infectious process which is most important to the risk of this disorder.[23,24]

However, the relative importance of the particular immune dysfunction or to what degree the specific dysfunction correlates with specific immune mediators is unclear. In PML associated with immunosuppressive therapies, cases have been observed after treatment with agents that exert very different effects on immune function. These include but are not limited to rituximab,[25] an inhibitor of B cell function; natalizumab,[26] an antibody against α4-integrins; and mycophenolate mofetil, an inhibitor of lymphocyte function.[27] The importance of the specific immunomodulator relative to the diseases at which it is targeted is also unclear. One study of all U.S. hospital discharges over a 7-year period reported an incidence of 4 cases of PML per 100,000 discharges coded for systemic lupus erythematosus and 0.4 cases for the same number of discharges for rheumatoid arthritis (RA).[28] This study indicated that the background rate of PML when excluding all other known causes of PML, such as HIV infection, bone marrow transplant, and malignancy, is 0.2 per 100,000 discharges. In patients with autoimmune disorders, the difficulty of isolating a cause of PML is increased by complicated histories that may include sequential or simultaneous exposure to multiple therapies that affect immune function.[29] In one series of PML cases in RA patients that was derived from a population sample of 129,000, one of four PML cases had no exposure to a biologic therapy, rendering the risk estimates for those whose PML was presumably related to an immunosuppressive to a level only slightly greater than previous estimates of the background rate.[30]

Status of PML: Current Trends

Initial cases of PML indicated that this was a uniformly fatal disease, but the prognosis of this infectious leukoencephalopathy has changed substantially over the last 15 years. While the development of antiretroviral therapy increased the 1-year survival of AIDS-related PML from 10% to 50%,[31] even higher rates of survival have been recently reported for PML linked to therapies that affect immune function. In a status summary of cases of PML associated with natalizumab that was presented at the 2011 joint meeting of the European and Americas Committees for Treatment and Research in Multiple Sclerosis (ECTRIMS and ACTRIMS), the current survival rate was reported as 82% (130 patients remain alive of the 159 who have developed PML).[32] In a much smaller series of PML associated with rituximab, survival was 50%,[33] which, although lower, remains substantially higher than survival rates for etiologies, such as AIDS, that were once more common.

The potential for further improvements in PML treatment outcome based on better characterization of risk factors and better therapeutic strategies is substantial. In a study of the relationship between immune reconstitution inflammatory syndrome (IRIS) and the onset of natalizumab-associated PML, 17 patients in a series of 40 were found to have already developed IRIS at the time of diagnosis (early IRIS), while 23 developed IRIS after withdrawal of therapy (late IRIS).[34] JCV load increased more than 10-fold on average in those with early IRIS versus less than two-fold in those with late IRIS. Although survival was only slightly lower in the early IRIS group (70.6% vs. 78.3%) corticosteroid therapy during IRIS was associated with

significantly lower risk of significant disability. Such data provide a potential direction for further studies to guide intervention in the disease process. One set of specific recommendations for reducing the risk and consequences of PML advocates clinical vigilance and prompt drug discontinuation as the best approach to limiting clinical sequelae.[35]

The potential therapeutic benefits of the immunoregulatory agents associated with PML are large in many target populations, producing an acceptable benefit-to-risk ratio in the context of the low risk of PML. While it may no longer be appropriate to characterize PML as a rare condition, it is appropriate to recognize that the prognosis has evolved as well. For patients who are candidates to receive therapies associated with PML, the favorable benefit-to-risk ratio of treatment can be substantially enhanced by considering risks, such as previous exposure to immunosuppressants, and vigorously applying practical algorithms of vigilance.

Conclusion

PML is associated with a broad range of factors that affect immune function, including AIDS and immunomodulatory therapies. While control of PML requires early intervention, including discontinuation of therapies associated with this disease, the clinical consequences of this disorder vary and are not as dismal as historically presented. The majority of patients who develop PML survive. Vigilance for initial symptoms is essential. While PML should not deter use of effective therapies in appropriate candidates, sensitivity to the potential for this complication can guide strategies to minimize risk. ■

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PML Risk Factors, Risk Reduction, and Improved Outcomes

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Introduction

Currently, a substantial majority of patients who develop progressive multifocal leukoencephalopathy (PML) in association with immunoregulatory therapies survive their disease.[1] This departure from the nearly uniform fatal outcome in the cases of PML initially associated with lymphoproliferative disorders and subsequently with AIDS encourages the effort to identify which characteristics of the host or the virus determine risk for an adverse outcome. Currently, the molecular steps that permit an otherwise latent JC virus (JCV) to proliferate in the central nervous system (CNS) to produce PML are unknown, but disparities in outcome suggest that there is heterogeneity in the process that may allow risk factors to be defined. In patients who are candidates for immunosuppressive therapies, both the ability to identify risks of developing PML and the ability to identify risks of an adverse outcome have major importance for improving the benefit-to-risk ratio of the immunomodulating therapies with which this disease has been associated. While it is likely that greater awareness of PML, leading to early initiation of supportive care, has already contributed to the improving rates of survival, the ongoing and intensive effort to isolate specific risks may eventually produce strategies to avoid PML entirely or to intervene when PML is reversible.[2]

PML Background: Altered Immune Function

PML is a consequence of reactivation of JCV, a lifetime asymptomatic infection that persists in a latent state.[3] Once thought to infect most of the human population, more recent estimates suggest that infection rates in a healthy population may be less than 40%.[4] While JCV can periodically reactivate within local reservoirs, such as the kidney, producing viral shedding detectable in the urine,[5] some as yet unidentified set of events permits the virus to proliferate in the CNS and induce

Dr. Chapagain has no conflicts of interest to disclose.

PML.[6] Although PML and the underlying role of JCV have been recognized for more than 40 years,[7] the steep increase in the incidence of PML driven by the AIDS epidemic greatly advanced the description of its features. The incidence of PML has been higher with the state of immunodeficiency associated with AIDS relative to any other known cause,[8] but attention is now turning to other etiologies because HIV control provides an effective means to avoid AIDS-related PML.

While a few PML cases in the absence of immune deficiency[9] have been reported, the current effort to better understand this disease is primarily driven by its association with an array of immunoregulatory therapies. The list of implicated therapies is long, but the most common associations have been with monoclonal antibodies. Even though the specific targets of these antibodies, such as lymphocyte signaling or B cell activation, differ, it is suspected that there is some common sequence of events that removes barriers to JCV proliferation. The therapeutic antibodies most closely associated with PML to date are natalizumab and rituximab,[10,11] which demonstrate efficacy in a variety of autoimmune disorders, including multiple sclerosis (MS), rheumatoid arthritis, and Crohn's disease.[12] For both agents, the risk of PML has been considered sufficiently low for regulatory agencies to guard their availability, but risk stratification may provide an opportunity to further improve their benefit-to-risk ratio.

Prior to the AIDS epidemic, less than 250 cases of PML had been reported worldwide from the time that this entity was first described in 1958.[13] The incidence of PML increased precipitously during the AIDS epidemic, but it is useful to recognize that only 4% of HIV-infected patients who developed AIDS and died of their disease had developed PML prior to death.[14] This contributes to evidence that impaired immune function is not the sole risk of PML. Due to the infrequency with which PML occurs in relationship to any risk factor, including AIDS or treatment with an immunoregulatory therapy, it appears likely that complex events must take place in sequence or in concert to precipitate disease development. Identifying these events and the factors that generate these events presents an opportunity to develop strategies to lower PML risk.

However, the specific events that lead to PML may differ by etiology, a possibility suggested by differences in outcome based on associated cause. Prior to the AIDS epidemic, when PML was most closely associated with lymphoproliferative disorders and bone marrow transplant, survival was uncommon.[15,16] Survival remained poor during the AIDS epidemic until the development of effective antiretroviral therapies, when PML mortality fell from 90% to 50%.[17] Mortality rates also appear to have fallen in transplant-associated PML, in which survival rates recently approached 30%,[18] but the best rates of survival have been seen in patients developing PML in association with monoclonal antibodies. While 2 of 4 patients (50%) survived in one recent assessment of rituximab-associated PML,[19] the survival rate was 81% in the most recent data reported for natalizumab-associated PML (October 2011).[20] The speed of immune reconstitution may explain this discrepency in outcome. Natalizumab can be removed with relatively quick immune reconstitution, whereas in AIDS cases, even with HAART, immune reconstitution takes longer, leading to worse outcomes.

Host Factors: Identifying Determinants of Risk

JCV infection is a prerequisite for PML development. However, screening for JCV infection using DNA as a biomarker prior to initiation of immunomodulatory therapy does not appear to be useful given the currently available methodologies. While Chen and colleagues[21] suggested that surveillance of JCV DNA in the blood or urine may be useful in identifying patients potentially at risk of developing PML, a larger study found no difference in the prevalence of JCV DNA in the plasma and urine specimens before and several weeks after the initiation of natalizumab therapy.[22] In this series, five patients who were JCV DNA negative in the blood developed PML, suggesting that JCV DNA detection in peripheral body fluids may be of limited use in isolating those patients who are or who are not likely to develop PML on a therapeutic antibody. Ultimately, the relationship between the presence or absence of viremia and later development of PML is not well understood.

Furthermore, there is uncertainty about the relationship between latent JCV infection in the

periphery relative to active infection in the CNS. Whether PML occurs subsequent to primary entry of JCV into the CNS via infected lymphocytes or as cell-free virus or whether it is a result of secondary reactivation of latent CNS virus upon immunosuppression is unclear. White et al[23] demonstrated JCV DNA in brains of 68% non-PML patients and argued that PML may occur from reactivation of latent JCV infection in the brain. However, JCV DNA was found in the brain of only 11% of non-PML patients in a recent study.[24] It is most likely that JCV replication in the CNS is a completely separate event and has very little or no relation with JCV detection in the blood or excretion in urine. While screening JCV in the CNS or CSF may be of some value as a risk discriminator, this approach is not routinely feasible in most settings and thus is of limited use. Moreover, extensive variation in JCV DNA detection rates among non-PML individuals may reflect disparity in the quality of JCV DNA analysis.

While the relationship between systemic reactivation of JCV and reactivation within the CNS is unclear, a recent study suggested that immunosuppression may increase risk of JCV latency both in the periphery and the CNS and may facilitate JCV reactivation.[25] In this study, a highly sensitive polymerase chain reaction methodology was used to analyze postmortem brain and kidney tissues from 78 immunocompetent and 60 immunosuppressed individuals, including patients with HIV. JCV DNA was significantly more common in the brain ($P < 0.001$) and in the kidney ($P = 0.009$) of patients who were immunosuppressed. Moreover, HIV patients having JCV DNA in their brains had lower CD4 counts, on average, than those without JCV DNA in their brains. These findings suggest that prior immunosuppression may increase latent JCV infection of the brain and kidney and these individuals may be at higher risk of developing PML after immunomodulatory drug therapy.

Similarly, in a series of four patients who developed PML in association with rituximab treatment, three had received a prior biologic regimen or were exposed to extensive immunosuppression.[19] In post-marketing surveillance of patients treated with natalizumab, prior exposure to immunosuppressants was a PML risk factor,[26] even though neither exposure to natalizumab nor prior use of immunosuppressants increased the risk of being positive for anti-JCV antibodies.[27] PML rates have been low with a low number of infusions, averaging 0.04 per 1,000 patients in the first year of treatment.[26] Both longer periods of natalizumab exposure and immunosuppression prior to the initiation of natalizumab increase risk of PML. Specifically, an analysis as of March 2011 showed that in patients exposed to 25-48 months of natalizumab in the post-marketing setting without prior immunosuppressant use, PML risk was 1.37 per 1,000. In those with this length of exposure and prior immunosuppressant therapy, the incidence increased to 4.3 cases per 1,000 patients.[26]

Besides immunosuppression, many other host factors may also play a role in PML development. Of these, elevated pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), have recently been shown to act as JCV promoters.[28] While the exact role of blood TNF-α level in JCV activation and PML development among MS patients is unclear, many MS patients may have increased TNF-α in the blood, and there is interest in determining whether higher TNF-α is associated with the higher PML development after immunotherapy. Other efforts to employ specific markers of immune function as a predictor of PML risk have not been successful. Most recently, an assay of intracellular adenosine triphosphate (iATP) production, which is a marker of T cell function, was evaluated in a relatively large series of natalizumab-treated patients. Based on the fact that patients who subsequently developed PML had iATP levels in the normal range and not different than those who did not develop PML, the study was considered negative.[29]

Viral Factors: Looking for Pathogen Characteristics

Viral characteristics also hold promise for assessing PML risk. While archetype JCV infection is common, only rearranged JCV causes PML. Moreover, point mutations in capsid viral protein 1 (VP1), the protein that mediates binding to sialic acid receptor on cell surfaces, has recently been shown to be associated with PML.[30,31] In a recent study, more than 95% of CSF sequences from PML patients contained mutations on JCV VP1 proteins.[30] Interestingly, VP1-derived virus-like particles carrying these mutations had different ganglioside speci-

ficity and failed to bind to peripheral cell types but still bound to brain-derived cells, suggesting that VP1 mutations may favor JCV neuroinvasion.[30]

In another study, 81% of natalizumab-associated PML patients had VP1 point mutations in their blood or CSF sequences. Almost 50% of the mutations were at residues known to be important for sialic acid binding by JCV.[32] Similarly, noncoding control region (NCCR) rearrangements were observed in 100% of the blood or CSF sequences from patients with PML. While NCCR rearrangements were observed in the absence of VP1 mutations, VP1 mutations were not observed in the absence of NCCR rearrangements, suggesting that VP1 mutations might have developed after the NCCR rearrangement.[32] It is not known whether the DNA sequence variations in the natalizumab-associated PML patients will have a practical clinical value for prognosis or guiding treatment.

Host-Viral Interplay

Risk of PML may also be mediated by as yet undetermined host genetic susceptibility. While ability to generate and maintain JCV-specific cytotoxic T cells provides important protection against PML development,[13] specific mutations that influence T cell development may increase PML susceptibility. Similarly, human leukocyte antigen (HLA) type, which has been found to influence the risk and the prognosis of other infectious diseases, may play a role in PML development. A recent study exploring the possible association between HLA variation and PML development or progression suggested that HLA-B18 was present in significantly higher proportion of Caucasians having HIV-associated PML.[33] The study did not find a similar association in African-Americans. Similarly, HLA-A68 was less frequent in PML survivor Caucasians than PML progressors, while HLA-Cw4 was less frequent in African Americans survivors. Although sample size was small and none of these differences reached statistical significance, the authors argued that genetic markers of PML susceptibility have substantial potential for screening candidates for immunomodulatory therapies.

Survival Factors in Patients with PML

The variability in survival based on specific patient characteristics supports the probability that PML is a treatable if not preventable disease. In AIDS patients, the early use of a 5-drug antiretroviral drug regimen designed to rapidly restore immune response has been associated with a reduction in PML-associated mortality.[34] Although recovery of immunologic function might be expected to improve defenses against opportunistic infections, the improvement in survival after introduction of the 5-drug regimen was specifically associated with recovery of anti-JCV T-cell responses and JCV clearance from CSF. Conversely, a high viral burden in the CSF as measured with JCV DNA copies was associated with a reduction in survival.

Several studies have shown that a strong JCV-specific cytotoxic T cell response is associated with longer survival among AIDS-associated PML patients.[2,35-37] A recent study characterized the cellular immune response against JCV by using enzyme-linked immunosorbent spot (ELISpot) and intracellular cytokine staining (ICS) assays in PML patients with various clinical outcomes.[2] When 18 PML progressors were compared to 42 PML survivors, an early CD8+ response was found in 100% of the survivors versus 27% of the progressors ($P = 0.001$). There was also a trend for early CD4+ cell response and survival (80% vs. 45.5%; $P = 0.18$), although the trend was not significant. This study suggests that JCV-specific CD8+ T-cells may be important in PML survival. In turn, monitoring these cells by intracellular cytokine staining may be useful as a PML prognostic marker. Interestingly, even though immune reconstitution inflammatory syndrome (IRIS) was more frequent among PML survivors, interferon gamma producing CD4+ and CD8+ T cells were similar between IRIS and non-IRIS PML patients.

In a study of natalizumab-associated PML, survivors were younger (median of 40 vs. 54 years), had a lower average Expanded Disability Status Scale (EDSS) score (3.5 vs. 5.5) and were diagnosed sooner after the onset of symptoms (44 vs. 63 days) than the patients who did not survive.[38] Moreover, patients who did not survive PML were likely to have diffuse disease on MRI (70% vs. 14%). However, gender, duration of MS, and JCV viral load in the CSF were similar in the two groups. Essentially all the patients, most of whom developed IRIS, were managed with rapid

TABLE 1 Characteristics of AIDS-Associated PML Patients

Characteristics	Berenguer et al[41] (2003)	Wyen et al[42] (2004)	Falcó et al[43] (2008)	Engsig et al[44] (2009)
Total PML cases (confirmed/probable)	118 (42/76)	35 (25/10)	61 (8/53)	47 (22/25)
Median age at PML onset (range)	36 (33-40)	38 (21-55)	42 (38-47)	48.7 (43-53)
Male/Female cases	98/20	34/1	54/7	35/12
Median CD4 counts (cells/μL) (range)	85 (40-160)	90 (8–812)	90 (27-177)	50 (27-160)
Plasma HIV load (Log/mL) Mean (SD or range)	4.85 (±1.21)	4.8 (1.7-5.4)	5 (3.86-5.45)	4.9 (3.7-5.6)
PML after HAART: no. (%)	39 (33.1)	20 (57)	21 (34.4)	13 (27.6)
Mean time of PML onset after HIV diagnosis (range)	NR	4.4 y (0-18)	NR	4.3 y (2.5-10.7)
Prognostic factor (↑ survival)	CD4 ≥100/μL	No change	CD4>200/μL* HIV<400/mL*	CD4 ≥50/μL
Probable IRIS: no. (%)	10 (8.5%)	4 (11.4%)	16 (23%)	2 (4%)
Mean/Median survival time	209 wks	>553 d	15 mo	1.8 y

*non-significant; NR, Not reported; IRIS, Immune reconstitution inflammatory syndrome.

withdrawal of natalizumab and treated with corticosteroids, so these were not discriminatory survival variables.

However, due to the infrequency of PML in non-AIDS patients, the ability to test treatment strategies is limited. Controlled trials are impractical. In case studies, a variety of strategies have been associated with good outcomes. In one recently published case, a patient on natalizumab with recently diagnosed PML was treated with plasma exchange and mefloquine.[39] When IRIS developed two months later, high-dose methylprednisolone controlled symptoms except during a brief period when the dose was tapered. When the steroids were eventually discontinued after 10 months, there was no PML progression or recurrence of IRIS. Although the authors of this case suggested a concomitant type 2 herpes simplex virus (HSV-2) infection may have precipitated the PML, they reported that aggressive therapy can control progression. While mefloquine was used empirically in this case, a randomized study of mefloquine in mostly HIV-positive patients did not provide any evidence of benefit against PML.[40] Over the course of the 24-week study, there were only 8 deaths (22%) in the 27 evaluable patients, but the mortality rate was numerically, although not statistically, greater in the melfoquine group.

Risks, Outcomes, and Treatments

The importance of a complex interaction between various host- and JCV-related factors for the development of PML is apparent in the infrequency of this disease with any etiology and the difficulty in isolating risk factors. The evidence that outcome is also influenced by multiple factors has been best demonstrated in the extensive studies of AIDS-associated PML. According to these studies, AIDS-related PML is primarily a disease of young adults, with median age at the onset of PML ranging from 36 to 48 years (Table 1).[41-44] A vast majority of AIDS-associated PML patients are male and PML is usually associated with high HIV-1 viral load and low CD4 counts (Table 1).

For AIDS-associated PML, the key factor in regard to both risk of developing PML and prognosis is immunologic function. In a study of 35 consecutive cases of AIDS-related PML, 15 had never received antiretroviral therapy, and 9 had been treated for less than 6 months.[42] Initiation of antiretroviral therapy was associated with an improvement in outcome. Treatment with cidofovir, which has shown efficacy against JCV in vitro, did not affect relative outcome. Although the overall mortality in this series was 40%, the median survival time after 553 days of follow-up has not been reached. In a separate study that evaluated sur-

vival in AIDS-associated PML, CD4+ cell count was the only variable with prognostic significance.[41] In those with a CD4+ count less than 100 cells mm^3, the odds ratio of death was increased by 2.71-fold (95% CI, 1.19 to 6.15) relative to those with a higher CD4+ count. In a cohort analysis of 47 cases of PML in 4,649 patients with HIV, the incidence was 1.3 per 1,000 patient years at risk in the HAART era versus 3.3 prior to HAART.[44]

In natalizumab-associated PML, which is the most widely studied PML linked to an immunoregulatory therapy, a study of 102 PML patients suggested that prior use of immunosuppressants, presence of anti-JCV antibody, and duration of natalizumab use are the key risk factors for PML development.[45] Patients who were anti-JCV antibody negative were at lowest risk (≤ 0.11 cases per 1,000 patients treated). Patients who had all 3 risk factors (anti-JCV antibody positive, prior immunosuppressant use, and natalizumab treatment duration 25 to 48 months) were at greatest risk (≈ 8 cases per 1,000 patients treated).[45] Perhaps the most important prognostic markers for survival in patients with natalizumab-associated PML are a shorter time from symptom onset to diagnosis and localized disease on MRI at diagnosis.[38]

Cancer-associated PML, although the oldest known form of this encephalopathy, is perhaps the least studied. Sporadic case reports, including those recently published,[46,47] acknowledge that the relative role of therapy versus the underlying disease for developing PML is difficult to ascertain. Due to the infrequency of cancer-associated PML, risk factors other than immunodeficiency are unknown. However, early recognition of PML, as in PML associated with immunoregulatory therapies, may be critical in taking appropriate steps to improve PML outcome and survival.

Overall, the data collected so far suggest that there are identifiable risks for PML as well as for a poor outcome when PML develops. For AIDS-related PML, higher CD4 counts, presence of JCV specific CD8+ T cells, and lower CSF JCV DNA levels appear to be survival factors. For immunomodulatory agents like natalizumab, 3 risk factors (JCV seropositivity, prior immunosuppressant therapy, and duration of treatment) have demonstrated good discriminatory value in risk assessment.

Conclusion

Survival in individuals with PML is improving. While this appears to be true even among patients with AIDS- or lymphoid malignancy–associated PML, the highest rates of survival so far have been observed in patients who develop PML in association with an immunomodulatory therapy. Survival rates are likely to improve further with vigilance for early signs and symptoms in those individuals at risk. Early detection permits interventions, such as immune restoration, discontinuation of the precipitating therapy, and initiation of anti-inflammatory therapies, to modify risk of progression. Strategies to identify patients at risk of developing PML, including genetic markers, may permit meaningful methods of PML prophylaxis. It is reasonable to anticipate new prognostic tools and targeted therapies from the intensive and ongoing effort to better understand the pathophysiology of PML. ■

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Emerging Data in Treatment-Associated PML

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Introduction

Natalizumab, a highly specific α4-integrin antagonist, is effective in the treatment of autoimmune inflammatory diseases.[1,2] In the placebo-controlled trials that led to regulatory approval of this agent for multiple sclerosis (MS), an improvement in clinical outcomes was accompanied by diminished disease activity in the central nervous system (CNS).[3-6] Progressive multifocal leukoencephalopathy (PML) has been diagnosed in association with the use of multiple pharmacologic agents, most typically immune suppressants or immunomodulators. It has also been seen in various disease states, most typically AIDS. Mortality and morbidity statistics are highly variable depending on the associated agent or disease state. The most recent post-marketing data (October 2011) identify a survival rate of 81% in patients who develop natalizumab-associated PML,[7] a higher rate than observed historically.[1,8] An active effort is under way to further define risk stratification factors that may reduce the risk of PML. Enhanced clinical vigilance and the identification of factors associated with improved prognosis may improve outcomes.

Natalizumab: Efficacy and Safety

The disease-modifying therapies (DMTs) employed in MS and other autoimmune disorders alter events in the inflammatory cascade that drive pathogenicity. The full spectrum of activity of the mainstays of DMT in MS, such as interferon-beta (IFN-β) and glatiramer acetate (GA), as well as newer compounds, such as natalizumab and other monoclonal antibodies, is not fully understood, but the reduction in risk of relapse associated with these drugs is typically accompanied by a reduction in markers of disease activity. In the case of natalizumab for relapsing-remitting MS, this includes a reduction in lesion progression in the CNS as measured by magnetic resonance imaging (MRI).[9]

Dr. Foley has received consulting fees from Biogen Idec, Genzyme, and Teva and has received honoraria from Biogen Idec and Teva.

In the clinical trials, natalizumab was tested both as a monotherapy and as an adjunctive agent to IFN-β 1a and GA. In the placebo-controlled AFFIRM study, natalizumab monotherapy was associated with a 68% reduction ($P < 0.001$) in the risk of relapse at 1 year, a 42% reduction ($P < 0.001$) in risk of sustained disability progression over 2 years, and an 83% reduction ($P < 0.001$) in the risk of new or enlarging hyperintense lesions, as detected by T2-weighted MRI, over 2 years.[10] In the SENTINEL study, the combination of natalizumab and IFN-β reduced the probability of confirmed disability progression by 24% ($P = 0.02$) at 12 weeks and reduced relapse rates by 55% ($P < 0.001$) at 1 year relative to IFN-β alone.[11] In the GLANCE study, natalizumab plus GA produced a 61% decline ($P = 0.029$) in new or enlarging T2-hyperintensive lesions ($P = 0.029$) and a 74% decline ($P = 0.02$) in new gadolinium-enhancing lesions relative to GA alone.[12]

These trials led to the approval of natalizumab for the treatment of relapsing-remitting MS in 2004. The drug was voluntarily withdrawn from the market in 2005 on the basis of three cases of PML, two of which developed in MS patients also taking IFN-β 1a.[13] After further clinical safety analyses, when the incidence of natalizumab-associated PML was an estimated 1 per 1,000 patients treated,[14] the drug was reintroduced. The first post-marketing case of PML was reported in 2008. Since that time, cases have continued to accrue but at rates that reflect the large increase in exposures.[15] According to data updated from the manufacturer in October 2011, nearly 100,000 patients have received natalizumab in the post-marketing setting. Of these, about two-thirds were on natalizumab for at least 12 months.[7] The 170 cases of PML translate into an overall incidence of 1.82 per 1,000 patients.

Of the 170 confirmed cases of PML, 33 patients (19%) died and 137 (81%) survived.[7] In those who died, the median time from the onset of symptoms to death was 2.2 months. In survivors, the median duration of follow-up is 11 months. In an analysis of disability in a consecutive series of survivors with at least nine months of follow-up, disability as assessed by Karnofsky scores was mild in 17%, moderate in 50%, and severe in 33%.

PML Risk Stratification: Recent Reports

Several factors appear to be required for latent JC virus (JCV) to become an opportunistic pathogen. These include changes in immune function and viral mutations that facilitate infection of oligodendroglial cells in brain parenchyma.[16] The mechanisms that allow archetypal (benign) JCV to mutate and become pathogenic remain unclear and likely have to do with a complex interplay of host and viral factors.

While research efforts are ongoing to improve PML risk assessment, current data support the use of three factors. In addition to JCV infection, a prerequisite for all cases of PML, these are immunosuppression prior to initiation of natalizumab and more than 24 months of natalizumab exposure.[17] These independent but additive risk factors derived from the natalizumab-related PML cases are providing a basis for risk stratification applicable to clinical management.

The pivotal role of latent JCV is well supported by more than 40 years of research into the causes of PML, but data to support JCV as a prerequisite for PML are also available from natalizumab-associated disease specifically. For example, JCV antibodies were detected prior to diagnosis in 100% of the natalizumab-treated MS patients who subsequently developed PML in one series.[18] This differs from the expected 54% anti-JCV antibody prevalence observed in natalizumab-treated MS patients in the STRATA study.[19] It is not clear whether higher titers of antibodies represent an increased risk of PML, but these analyses are planned.

In an analysis performed in consecutive patients, prior exposure to immunosuppressants was associated with three- to four-fold increased risk of natalizumab-associated PML.[20] The relative increase in risk of PML was most pronounced in those individuals with the longest exposure to natalizumab. While cases of PML have been reported prior to 12 infusions of natalizumab, these remain uncommon. Data as of October 2011 estimate an incidence of only

0.04 per 1,000 patients in the first year of treatment. The incidence climbs modestly to 0.55 in patients treated for more than 12 but less than 25 infusions, but it rises at a steeper rate for those treated for at least 25 months. However, while the incidence rate reaches 2.01 in those treated for 25 to 36 infusions, greater exposure does not appear to generate a further increase. The incidence rate among those treated for 37 to 48 infusions is approximately 1.5. However, there is an additive increase for those who remain on natalizumab for a long duration and have had prior exposure to immunosuppressants. In these, the incidence rate climbs to 4.3 per 1,000 patients.

Other factors with the potential for risk stratification are being pursued. For example, an initial analysis demonstrating increasing serum concentrations of natalizumab over extended periods of treatment has generated the hypothesis that relative inhibition of the integrin receptor may influence risk of PML.[20] In a study of 270 patients, trough pharmacokinetic measures were obtained cross-sectionally to gauge kinetic stability. Between 24 and 196 weeks of therapy, serum concentrations increased from 17.3 to 31 μg/mL.

Another set of experiments to isolate modifiable risks for PML are exploring the relationship between long-term natalizumab therapy and unblocked α4-integrin expression.[21] This series of studies is based on speculation that the sustained and progressive decrease in α4-integrin expression explains both the therapeutic effect of natalizumab and its mechanism for producing PML. The ability to develop adhesion molecule expression profiles might be of use as a biomarker to identify a therapeutic window that could separate favorable clinical activity from risk of an opportunistic CNS infection.

Studies to identify patient susceptibility of PML are being conducted parallel to research designed to isolate characteristics of JCV that might confer risk. In a study of JCV genotypes, mutations in the coding regions of the VP1 capsid and in the non-coding control region (NCCR) have been associated with PML.[22] If these are confirmed as conferring increased pathogenicity, it is possible that screening or monitoring of JCV for specific genetic mutations could be a tool for risk stratification. For example, an independent study of genetic mutations in the coding region VP1 protein expression has suggested changes in viral activity that might permit increased penetration of JCV through the blood-brain barrier.[23]

Ultimately, the greatest likelihood of isolating patients at risk of PML may be derived from a more complete understanding of the interaction of natalizumab with immunoregulatory function. The efficacy of natalizumab appears to be attributable to its ability to inhibit trafficking of immune cells across the blood brain barrier, but new evidence outlining other secondary effects may be relevant to both benefit and risk. In a flow cytometry study conducted in 18 MS patients, the predicted inhibition of natalizumab on the 4 subunit of the very late activation (VLA) antigen was accompanied by inhibition of the β-1 subunit on T cells, B cells, natural killer (NK) cells, and NK T cells but not on monocytes.[24] If the favorable effects of natalizumab on MS inflammatory activity can be distinguished from the events leading to PML, a variety of potential risk reduction strategies might be possible.

PML Outcomes: Recent Reports

Like factors associated with increased risk of acquiring PML, risk factors for adverse outcome might also be used in selecting patients who are expected to derive the most favorable benefit-to-risk ratio from treatment. In a recent study that evaluated survival and functional outcome in 133 postmarketing natalizumab-associated PML cases, the factors associated with improved survival were younger age at PML diagnosis (median 43 vs. 51.5 years), less disability at diagnosis (median EDSS score 4 vs. 5.5), shorter time from symptom onset to diagnosis (median 28 vs. 38 days), and more localized disease on MRI.[25] Survival in this series of patients was 82%.[25] The majority of cases developed immune reconstitution inflammatory syndrome (IRIS) and were treated with corticosteroids. Most cases were managed with rapid removal of natalizumab from the circulation (eg, plasma exchange).

The potential for IRIS to serve as a signal for aggressive therapy with the potential to improve outcome remains one of speculation, but there are other markers being pursued for their potential to be employed in outcome stratification. One of these, intracellular CD4+-ATP-concentration (iATP) functionality, may be useful for evaluating immune cell competence and, in turn, risk of opportunistic infections, including JCV/PML, during immunosuppressive therapy.[26] In a study with iATP based on assays of cell-mediated immunity in immunosuppressed patients with PML, including those taking natalizumab, rituximab, or efalizumab, levels of this marker were below the third percentile versus healthy controls in 14 of the 16 patients.[26] However, a recent study with iATP in a natalizumab-treated MS patient population did not find this a useful tool for predicting PML risk.[27]

One important obstacle to the identification of prognostic variables in patients who develop PML on natalizumab is that the small number of cases limits confidence about the typical natural history of this disease. Although it is presumed that symptoms of PML, which include weakness or paralysis, vision loss, and cognitive deficits, occur within weeks of the JCV activation in the brain, an unrecognized spectrum of manifestations, including relatively slowly progressing disease, is possible. In one case report, MRI evidence of PML was retrospectively identified 3 months before the onset of symptoms.[28] Even after symptoms developed, the course was characterized as indolent. Nine natalizumab infusions were administered after the MRI evidence of PML first developed. While PML was initially treated with intravenous immunoglobulin (IVIG), the course remained mild even after development of IRIS, which was effectively treated with corticosteroids.

PML Therapy: Reversing Adverse Outcome

The most important initial step in the management of natalizumab-associated PML is the discontinuation of therapy, but optimal management is still being defined.[29] Plasmapheresis to accelerate natalizumab clearance has become the standard of care as the initial intervention followed by steroid therapy to minimize IRIS, but various regimens are employed. These include strategies to prevent JCV intracellular invasion with such agents as the serotonin receptor–inhibitor mirtazapine or to prevent viral replication with the antimalarial agent mefloquine or the antiviral cidofovir. IVIG and corticosteroids have been employed, generally to control IRIS, but controlled studies of these approaches are also lacking.[30]

There remain a number of agents that deserve clinical trials. For example, an investigational oral lipophilic nucleotide analogue of cidofovir (CMX001) showed promise in a recently reported case study of natalizumab-associated PML.[31] Although data from a single case can only be considered anecdotal, biweekly administration of CMX001 for 3 weeks reduced JCV in the CSF. Consistent with this steady decline in viral load, the patient, who had also been treated with plasmapheresis, IVIG, and multidisciplinary neurorehabilitation, stabilized and then recovered without ever developing IRIS.

Several guidelines for the use of natalizumab in clinical practice have been published or are in the process of being developed. In guidelines recently released by the Italian Neurological Society, natalizumab use is recommended for those who have failed to respond to the commonly used front-line DMTs.[29] These guidelines cite current indications for natalizumab issued by the European Medicines Agency. The potential benefits are reviewed in the context of current morbidity and mortality data. In another recently published set of consensus recommendations, which included participants from Europe and North America, similar conclusions were drawn.[1] In the European labeling for natalizumab, called the Summary of Product Characteristics, stratification for risk factors, including screening for JCV and evaluating prior exposure to immunosuppressants, is recommended. Again, the clinical utility of natalizumab in patients not controlled or unable to tolerate first-line MS therapies is acknowledged.

While discontinuation of natalizumab in patients who develop PML is prudent, it is im-

portant to recognize that patients not initiated on an alternative face a resurgence of MS. In a study that evaluated disease course after the voluntary suspension of natalizumab, a consistent return of disease activity as measured with MRI was observed regardless of overall natalizumab exposure.[32] Although there was no rebound of activity above the placebo-treated levels, the return of the activity was consistent with the known pharmacokinetic and pharmacodynamic properties of natalizumab. The authors emphasized that suitable alternatives should be considered when interruption of natalizumab is undertaken. However, some research suggests that risk of return of disease activity may be evident even with these alternative therapies.[33]

Anti-JCV antibody testing, particularly in individuals with prior exposure to immunosuppressants, is an emerging concept for screening candidates for natalizumab. In a study of a two-step assay, all 17 of the samples taken from PML patients were positive for JCV.[19] Although the impact of the measurable false-negative rate with this assay is still being evaluated in the context of improving the benefit-to-risk ratio of natalizumab, an effective and simple tool for detecting the presence of JCV would be expected to minimize PML risk.

Conclusion

PML is a serious potential complication of natalizumab treatment, but the absolute risk remains manageable, particularly when risk factors are recognized. Further research into stratification of risk is now ongoing, but 3 factors are well established. These are the presence of JCV, prior exposure to immunosuppressants, and more than 24 infusions of natalizumab. These risk factors may be useful to the neurologist and the patient when weighing treatment decisions. In patients who develop PML, survival rates have been increasing and far exceed those associated with other causes of PML, such as AIDS. Progress in isolating the mechanisms of PML has the potential to improve risk assessment, allowing a more personalized approach to natalizumab therapeutics. Promising ongoing studies to understand the relationship between immunologic function and risk of PML may also yield new strategies to diminish the impact of this disease. ■

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