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REVIEW

Diagnosis and Treatment of Progressive Multifocal Leukoencephalopathy Associated with Multiple Sclerosis Therapies

Eric M. L. Williamson[1] · Joseph R. Berger[1]

Abstract Progressive multifocal leukoencephalopathy (PML) is a rare, but serious, complication encountered in patients treated with a select number of disease-modifying therapies (DMTs) utilized in treating multiple sclerosis (MS). PML results from a viral infection in the brain for which the only demonstrated effective therapy is restoring the perturbed immune system—typically achieved in the patient with MS by removing the offending therapeutic agent or, in the case of HIV-associated PML, treatment with highly active antiretroviral therapies. Other therapies for PML remain either ineffective or experimental. Significant work to understand the virus and host interaction has been undertaken, but lack of an animal model for the disorder has significantly hindered progress, especially with respect to development of treatments. Strategies to limit risk of PML with natalizumab, a drug that carries a uniquely high risk for the development of the disorder, have been developed. Identifying factors such as positive JC virus antibody status that increase PML risk, at least in theory, should decrease the incidence rate of the disease. Whether other risk factors for PML can be identified and validated or unique strategies should be employed in association with other DMTs that predispose to PML and whether this has a salutary effect on outcome remains to be demonstrated. Identifying PML early, then promptly eliminating drug in the case of natalizumab-associated PML has demonstrated better outcomes, but the complication of PML continues to carry significant morbidity and mortality. While the scientific community has yet to identify targeted therapy with proven efficacy against JCV or PML there are several candidates being studied.

Keywords Multiple sclerosis · Progressive multifocal leukoencephalopathy · JC virus · Natalizumab · Immune suppression · Viral infections

Progressive Multifocal Leukoencephalopathy Incidence

No clinical disease has been associated with a primary infection by John Cunningham virus (JCV) and exposure is common, whereas the complication of progressive multifocal leukoencephalopathy (PML) is exceedingly rare and, in almost all instances, has been associated with significant underlying abnormality in cell-mediated immunity. PML was initially described in patients with underlying B-cell lymphoproliferative disorders [1]. Aside from patients with B-cell malignancies, PML has been observed in patients with other myeloproliferative diseases, carcinoma, congenital immune deficiencies, organ transplantation, and, less commonly, in granulomatous, inflammatory, or other immune-mediated conditions—noting many of these patients were treated with therapies that affect cell-mediated immune function before development of PML. Initially, the etiology of the disorder was unknown, but isolation of a polyoma virus from glial cell cultures of a patient with PML was accomplished in 1971, linking the initials of this patient (JC) with the causative virus [2]. In a large review spanning the 30 years from 1958 to 1984, only 230 cases were identified, with only 69 being confirmed pathologically [3]. Starting in the 1980s with the HIV/AIDS epidemic, AIDS rapidly

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became the most common underlying disorder predisposing to the development of PML, an illness that had been rather obscure to that point. In one study from southern Florida performed between the years of 1980 and 1994, Berger et al. [4] identified 154 patients with PML complicating AIDS. The prevalence of PML in the AIDS population led to further study and shifted the demographic so that PML became more common in the third to sixth decade of life rather than in elderly populations in which it had previously been seen. After the introduction of antiretroviral treatment for HIV, particularly, highly effective combined antiretroviral treatment, both the incidence and mortality rate of AIDS-related PML declined; however, this population remains the most affected and PML can be a significant complication and cause of mortality in HIV/AIDS populations.

In 2005 PML was first described in patients with multiple sclerosis (MS) and inflammatory bowel disease, conditions not previously associated with the illness. The first recognized cases of PML in patients with MS were attributed to use of natalizumab, an otherwise highly efficacious therapy for MS. Description of PML in patients on immune modulators sparked interest in biologic agents as potential risks and led to the observation that other, less specific immunosuppressive medications such as chemotherapies may have an associated increased risk of the disorder [5]. Natalizumab continues to be the drug most frequently associated with PML. While this drug, owing to the high incidence rate of PML with its use, has rightly garnered the most attention, PML has been associated with the use of other immunologic therapies, albeit in substantially smaller numbers. The incidence of PML with other immunologic agents used to treat MS pales in comparison to the number of cases thus far attributed to natalizumab use [6]. The development of PML in patients with MS and in those with other disorders that are not associated with severe long-standing immunosuppression has led to significant medical and scientific attention. Given the poor outcome for the majority of patients with PML, there is an unmet need to better understand not only how to prevent this disorder, but also how to treat it.

JCV

JCV is a primate polyomavirus related to BK virus, which also shares some similarities with simian virus 40 and Merkel cell virus [7]. It is composed of 5+ kilobases of double-stranded circular DNA enclosed in a capsid without a lipoprotein envelope that codes for approximately 6 to 9 proteins. The route of transmission is not well understood, but the virus is widespread among humans throughout the world based on antibody testing [8–11]. The presumed pathogenesis of PML is that an initial infection with JCV can result in

replication and establishment in sites of latency with subsequent viral genome mutations resulting in a neurotropic strain of the virus, dissemination to the brain, and active replication within oligodendrocytes in the absence of effective immune surveillance. The productive infection of oligodendrocytes results in their death, leading to areas of demyelination that give rise to the symptomatology and imaging abnormalities observed in PML. JCV replication only occurs in human cells. Animal models of PML are lacking, though a humanized mouse model in which the animals remain asymptomatic but demonstrate JCV in urine or blood periodically [12], as well as a polyoma-infected rhesus monkey model [13], are 2 examples that may provide a framework for future study. The full range of tissues in which virus may be latent remains to be determined, but sites of replication and dissemination of JCV (in tonsillar and parapharyngeal lymph nodes, gastrointestinal tissue, renal reservoirs, or peripheral blood mononuclear cells) [14], as well as periodic shedding (particularly in urine) or re-expression of the virus with circulation in B cells, have all been well described in humans. Infection with JCV in the central nervous system is primarily described in oligodendrocytes and PML does not occur in immunologically healthy people.

Pathologically, the neurotropic strain of JCV (or prototype virus) is associated with a genetic mutation in the noncoding control region of the archetype virus, which is the major form of the virus found in latent and persistent infections—the greatest concentrations of which are in the kidney and frequently shed in the urine of otherwise-normal individuals [15]. The archetype virus must undergo a genetic transformation in its control region to prototype virus in order to be able to grow efficiently in glial tissue [15]. Other mutations in JCV, such as in the viral capsid protein VP1, may also increase the risk of developing PML [16].

Importantly but somewhat controversially, JCV DNA has been detected in spinal fluid from unaffected patients with MS [17–21], albeit rarely and in very low copy numbers. It has additionally been detected in the cerebrospinal fluid (CSF) of HIV-infected patients in the absence of PML [22]. JCV DNA has also been identified by polymerase chain reaction (PCR) in as many as 18 to >50% of normal brains [14, 23–30]—if such studies truly demonstrate viral latency in the brain this may raise questions about the timing of viral entry into the brain, clinically silent brain infections, and whether the disorder arises from a latent brain infection rather than resulting from a viral mutation in the periphery proximate to glial infection then time of clinical and radiographic presentation.

Evidence for Prior Infection with JCV in PML

The route of transmission for an initial JCV infection is difficult to confirm given the lack of clinical disease, but a urine or

fecal–oral route seems likely [31]. In populations studied, seroprevalence increases with age and most individuals show evidence of exposure via antibody testing by the third or fourth decade [11, 32]. Despite the predominance of infection with JCV, or at least evidence of exposure, only a vanishingly small number of patients develop PML, meaning there may be high barriers to establishing a symptomatic brain infection. The nonpathogenic JCV archetype virus has been isolated from tissues of healthy patients and can be shed in urine [33] or identified in blood [34, 35]. The pathogenic JCV that causes PML differs from the archetype in the noncoding regulatory region of its DNA in that it typically consists of tandem repeats of 98 base pairs (bp) rather than the archetype virus, which has one 98-bp element and then 23- and 66-bp elements [36]. This regulatory region rearrangement seems unique in affected individuals, suggesting the pathogenic prototype is not acquired by transmission but instead develops within the host after acquisition of the commonly identified archetype JCV [37]. The pathologic transformation of JCV from archetype to prototype virus is not fully understood and it may take time, noting there has been correlation between duration of immune-suppression and PML incidence [38]. That said, there is evidence that immunosuppressed patients without PML have increased numbers of JCV prototype virus [27].

JCV Antibody Testing

The majority of adults are found to be seropositive for JCV antibodies; however, JCV antibodies do not protect against developing PML nor do they appear to be helpful in clearing the virus. JCV antibody positivity can be measured by a variety of mechanisms, but the test most commonly employed is an enzyme-linked immunosorbent assay for the detection of JCV antibodies in human serum and plasma. Individuals found to test negative for JCV antibodies carry less risk of developing PML in the setting of natalizumab [38]; however, the risk that a positive test for JCV antibodies carries is difficult to quantify—particularly when attempting to account for other established risk factors, such as prior immune suppression and duration of exposures [39, 40]. JCV antibody status is dynamic and can change—including not only serostatus changes from seronegative to positive. The cut-off value for a positive test and method of confirming indeterminate tests has also evolved over time [41]. Currently employed serum testing appears to be reproducible when run multiple times on the same sample, but the false-negative rate may be significant [42]. Other evidence of JCV infection, including evidence of viral shedding in the urine, might add further information [42–45].

The frequency of serum testing clearly influences the probability of a serostatus change when examining JCV antibody status—with higher frequency of seroconversion (changing from a negative to positive result) reported in more recent studies when patients are tested more often [46, 47]. The optimal frequency of testing is not well established. Currently employed testing provides not only a positive or negative result, but also a quantification of antibody levels—allowing for attempts to further stratify risk [48]. This, however, further complicates interpretation as index levels can fluctuate separately from serostatus change. Presumably, when quantifying a level we are measuring the magnitude of a mounted immune response, something that may be affected by a number of factors. Variability in testing is being studied, including the magnitude and frequency of changes in individuals and populations over time, and treatment with natalizumab itself may have effect on JCV indices [49–51].

Though we may not fully understand its utility or how to best use antibody testing, it is commonly employed in practice when caring for patients with MS—including in patients being treated with DMTs other than natalizumab, in which case the test has not been validated. At the present time, there are small numbers of patients with MS who developed PML associated with therapies other than natalizumab and even fewer who had JCV antibody testing prior to its development; thus, it is difficult to assess how this test may be used to assess risk in these populations. Use of antibody testing is complicated by the fact that we do not know if treatments themselves may influence test results, and particularly by the fact that treatment decisions are being influenced by results of any prior testing. There is inherent bias in not only who is tested but also how they are treated—for instance, JCV antibody levels may be different at baseline in patients who are selected to receive treatment with natalizumab *versus* other patients with MS on other treatments, who may be tested at different frequencies, if at all. The lack of mandatory reporting or testing, even in the case of natalizumab use where a risk evaluation and management strategy program is in place, may further hinder the ability to understand utility of testing [52]. With still small numbers of cases and even less from which we have data, it is difficult to confidently assess how reliable even a commonly employed tool such as JCV antibody testing and index values may be in assessing risk.

Despite attempts to utilize this tool to stratify risk and aid in decision-making to start or stop drug, the incidence of PML associated with natalizumab does not appear to be decreasing substantially [53]. This said, one must consider competing variables before assuming lack of efficacy, including more use of natalizumab over time—both number of exposures and duration that individual patients may be treated with drug. Another factor that could be at play is that we may be more vigilant about screening for and better at recognizing PML, which, though it could increase incidence, may, when combined with the ability to discontinue or reverse effect of therapy, contribute to better outcomes recognized in patients with

Fig. 1 JC Virus (JCV)–
progressive multifocal
leukoencephalopathy life cycle

JCV Replication and Spread
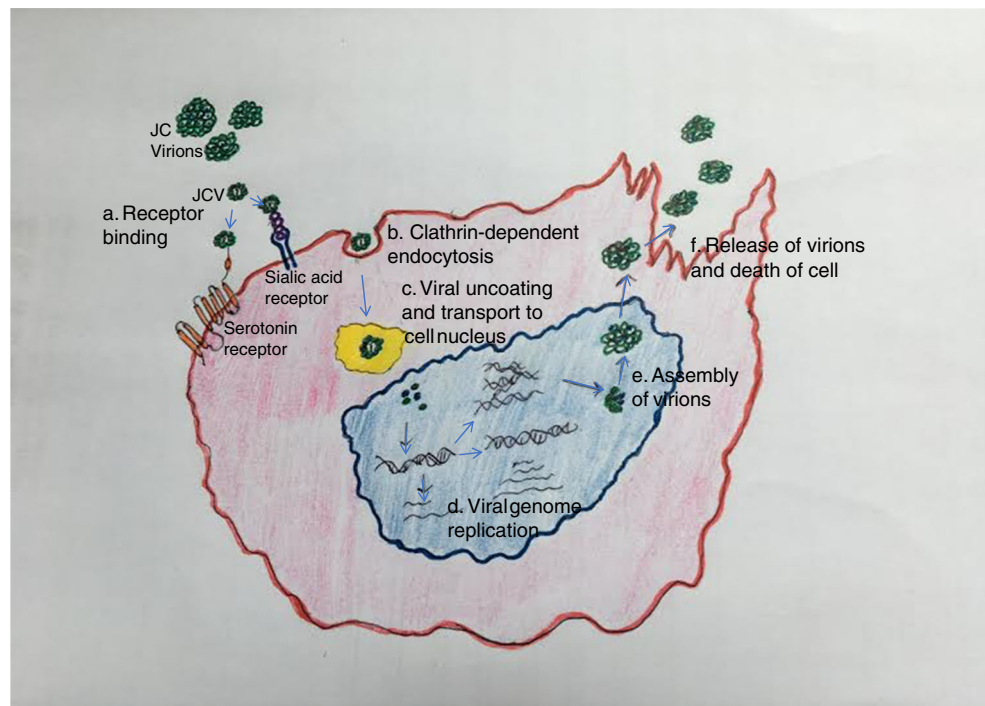
MS with PML than in other populations that encounter the disease. In fact, there have been a number of "asymptomatic" cases of PML published in the literature; presumably, there are additional unrecognized such cases—which could, again, complicate incidence estimations.

Despite limitations, it is plausible that JCV antibody testing can help us assess and decrease risk and incidence of PML if not improve outcomes. Only by collecting data prospectively and examining its value from incident PML cases might we be able to truly assess utility. Though applicability is debated in some instances [54], it is imaginable that other tools and techniques for assessing risk of PML, such as the use of urine PCR for JCV or other experimental predictive factors, such as L-selectin [55] or CSF lipid-specific IgM bands [56], may be used in conjunction with antibody testing in the future in cases where risk of PML appears to justify use.

JCV Replication and Spread

While many aspects of PML pathogenesis remain uncertain, it is unassailable that PML is extraordinarily rare in immunologically normal individuals. It chiefly occurs in persons with defects in cell-mediated immunity. Though its unclear if B cells are the primary source of latent infection, the need to rearrange the viral genome to make the prototype virus from the archetype seems to implicate that B cells must be involved in pathogenesis, as they are the one somatic cell that has the genetic machinery needed to accomplish this [34]. B cells have additionally been implicated in the transport of JCV

across the blood–brain barrier [57]. There are also hypotheses that release of immature B cells may lead to spread of latent JCV and there have been cases associated with the therapy rituximab, though the vast majority of patients developing PML while on this drug were being treated for a lymphoproliferative disorder that already put them at risk for the disease and had received other immunosuppressant therapies [58, 59]. There has been at least 1 case report of a patient with MS with natalizumab-associated PML who was subsequently treated with rituximab without adverse consequences [60]. Overall, it is unclear what effect lowering the circulating number of B cells, as may be accomplished by certain immunotherapies like the now approved for MS ocrelizumab, may have on JCV infections or risk of PML.

In order to infect an individual cell, JCV must attach to cell surface receptors. JCV binds sialic acid moieties on cell surface glycoproteins and glycolipids [61] and has been shown to use the 5-hydroxytryptamine 2A ($5\text{-}HT_{2A}$) serotonin receptor to accomplish this [7, 62]. This leads to clathrin-mediated endocytosis into the cell, delivery of JCV into the endoplasmic reticulum, virion uncoating, and retrograde transport to the cell nucleus. The viral genome subsequently replicates and assembles progeny virions, ultimately leading to necrosis and death of the cell with release of virions from the nucleus following membrane dissolution, allowing for propagation of infection [63] (Fig. 1).

The primary cells in the brain found to be infected with JCV are oligodendrocytes [64]. More rarely, cerebellar granule cells [65] and even cortical pyramidal neurons [66] may be infected and can result in distinct clinical entities, separate
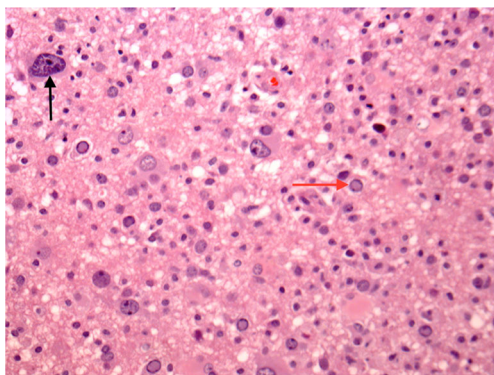
Fig. 2 Progressive multifocal leukoencephalopathy pathology. Black arrow highlights enlarged bizarre astrocyte; red arrow illustrates oligodendrocyte with intranuclear viral inclusion. Photo credit to MacLean Nasrallah, MD, PhD, Department of Pathology and Laboratory Medicine, University of Pennsylvania

from what we recognize as PML. The time to development of disease from infection of brain cells is unknown, particularly in light of described asymptomatic infections, but JCV propagation and PML is highly associated with immune suppression—presumed to allow activation of a latent virus. CD8 T cells are responsible for clearance of infections and CD4 T cells are responsible for antigen recognition then trafficking and signaling to CD8 cells so they can target and destroy infected cells. Low CD4 counts in the HIV/AIDS population precede the development of PML in this setting, whereas other forms of immune suppression may similarly limit the number of effective cells to handle infections. Fingolimod, also associated with risk of PML and used in the treatment of MS, decreases the number of circulating lymphocytes—which could effectively suppress the potential number available to fight infection of JCV; that said, there has not been a well-demonstrated correlation between fingolimod lymphopenia and PML risk. Despite a similarly small numbers of cases, association of lymphopenia in a few of the cases of dimethyl fumarate-associated PML, as well as cases of PML associated with dimethyl salts used in the treatment of psoriasis, contributed to the recommendation to screen for low absolute lymphocyte counts and to consider holding drug for potential risk that lymphopenia may carry [67]. Interestingly, there is a paucity of case reports of PML associated with the use of alemtuzumab in MS, despite the profound lymphocyte depletion that it can cause. One could question if there is something unique about this drug or some of the other disease-modifying therapies used in MS yet to be associated with PML, but one might also question if it is just too rare an occurrence to have been seen—keeping in mind that some of these or similar drugs have elsewhere been associated with PML [68].

Prevention of T cell entry into the central nervous system may preclude their function in the setting of natalizumab use, noting the leading theory for natalizumab-associated PML is that of decreased immunosurveillance—hypothesizing that

when T cells are sequestered in the periphery and unable to cross the blood–brain barrier to reach JCV-infected cells the virus can propagate in brain tissue [69, 70]. Following elimination of natalizumab, a robust response of CD4 and CD8 lymphocytes entering the brain can precipitate an exaggerated cytotoxic response in PML, which, in turn, mediates potentially severe bystander damage—consistent with an immune reconstitution inflammatory syndrome (IRIS) [71]. Outside of the context of IRIS, while there may be rare cases with contrast-enhancing lesions or signs of active inflammation, PML is typically characterized by a lack of inflammation. Propagation of JCV infection in the brain by the release of infected necrotic oligodendrocytes leads to coalescing islands of demyelination resulting from infection of adjacent cells that, if unchecked, become progressively larger resulting in the clinical disease recognized as PML.

Pathology of PML

JCV infection then replication leads to necrosis of oligodendrocytes, which not only releases virions to neighboring cells, but also causes death to the myelin sheath of the neurons that they protect, which, in turn, exposes and may predispose axons to damage. This described pathology and demyelination can impair conduction across axons in a similar fashion as in MS. Resultant clinical symptomatology depends on site and extent of damage. As viral particles spread to adjacent cells and infection progresses, clinical symptoms can accrue. Pathologically, PML is characterized by widespread multifocal demyelination. Histologically, infected oligodendrocytes display viral inclusions and nuclear enlargement, whereas reactive astrocytes of bizarre shapes and sizes are identified microscopically [72]. Astrocytes may harbor infection but are incapable of supporting the full cycle of viral replication [1]. The nuclei of infected oligodendrocytes stain strongly for JCV by immunohistochemistry. Histiocytes and macrophages enter the scene to phagocytose myelin and other debris (Fig. 2).

PML was named for the pathologic description of large plaques of myelin breakdown in subcortical white matter often involving U fibers [1]. Methods of virus isolation from infected cultures and extensive work on how the virus uses receptors has led to widespread use of hemagglutination to quantify viral particles [73]. Other clinical entities produced by JCV in the central nervous system, which, interestingly, appear to depend on different genomic mutations than the prototype JCV in PML, include granule cell neuronopathy and JCV encephalopathy. Both of these conditions are much more rare than PML and the pathology underlying these entities is distinct. In JCV granule cell neuronopathy, the white matter appears spared and oligodendrocytes are not infected; instead, immunohistochemistry reveals evidence of JCV infection

restricted to cerebellar granule cell neurons and there is selective depletion of cerebellar granule cells, Purkinje cell sparing, and enlarged, hyperchromatic, atypical nuclei in surviving granule cells [74]. JCV encephalopathy demonstrates laminar necrosis at the gray/white junction and bizarre, multinucleated astrocytes, whereas immunohistochemical staining reveals JCV infection of pyramidal neurons and astrocytes but overall sparing of oligodendrocytes [75].

PML IRIS is another pathological entity described in relationship to JCV; PML IRIS results from a reconstituting immune system causing an inflammatory parenchymal brain reaction in response to PML pathology. In the resultant lesions, there is accentuated PML pathology with massive demyelination and macrophage reaction, along with perivascular CD3$^+$ cell infiltrate predominated by CD8$^+$ cells [76].

Clinical Features

Clinical manifestations of PML can vary, but hemiparesis, ataxia, gait disturbance, visual deficits, and cognitive dysfunction are most commonly described [77]. Symptomatology results from white matter involvement in corresponding areas, such as frontal lobes for motor weakness, occipital lobes for visual field deficits, and cerebellum for ataxia. Limb weakness occurs in about 60% of PML presentations, gait disturbance in 65%, and visual field cuts in 20% [77]. The most common cognitive complaints are memory loss and behavioral disturbances, noting cognitive dysfunction may be the presenting symptoms in a third of cases. Focal seizures that can secondarily generalize occur in as many as 10% of patients.

JCV cerebellar granule cell neuronopathy spares supratentorial brain to preferentially infect cerebellar granule cells, producing cerebellar atrophy that manifests as ataxia, speech disturbance, and gait abnormalities. JCV encephalopathy is rare and characterized by altered mentation [74].

Neuroimaging

In the appropriate clinical context and often aided by CSF studies, characteristic neuroimaging can be used to diagnose PML [78]. Computed tomography of the head is not as sensitive as magnetic resonance imaging (MRI) of the brain but can reveal patchy hypodensities in the affected subcortical white matter. MRI can demonstrate JCV-mediated abnormalities in symptomatic patients and is the imaging modality of choice, noting that an unremarkable MRI argues strongly against the diagnosis of PML. Overall, findings on brain magnetic resonance neuroimaging in PML may mimic lesions seen in MS, which makes imaging surveillance a challenge in this setting [79]. Characteristic MRI findings in PML are bilateral, asymmetric, multifocal white matter plaque-like lesions that are T1 hypointense and T2/fluid-attenuating inversion recovery hyperintense, but unifocal abnormalities and lesions affecting only one hemisphere can be observed and gray matter involvement is more typical than in MS as deep gray structures are commonly affected in PML (Fig. 3). To additionally separate from clinical entities such as stroke, infarcts obey vascular boundaries, whereas PML does not. Diffusion restriction is common at the advancing edge of PML lesions [80–82]. Additionally, hypointense rims involving the U fibers on susceptibility-weighted imaging may be a helpful diagnostic clue [83, 84]. In the absence of PML IRIS, there is little to no edema or mass effect. Enhancement can be seen in natalizumab-associated PML or PML IRIS, though in less than a majority of cases [85, 86]; that said, contrast enhancement at the borders of PML lesions might be a helpful tool to distinguish IRIS [87].

In further differentiating from other demyelinating diseases, such as MS, the large size and confluence of plaques, predominantly subcortical location with involvement of subcortical U fibers, and sparing of spinal cord coupled with the appropriate clinical presentation argue in favor of PML. Good arguments in favor of screening patients felt to be at risk with serial imaging, such as patients with MS treated long-term with natalizumab, are substantiated by the fact that early diagnosis leads to improved survival [88].

Laboratory Studies

Historically, PML has been a pathological diagnosis. PCR detection of JCV DNA from CSF, first performed in 1992 [89], is now routine. Today, the combination of neuroimaging, clinical picture (which, in the case of MS, would necessitate use of a drug that has been associated with the illness), and CSF studies have supplanted the necessity of biopsy in the majority of cases.

In HIV-associated PML, advanced disease causing immune suppression to a CD4 count < 200 cells/ml typically precedes disease [4, 90, 91], but numbers can be higher. In MS, therapeutic agents that result in lymphopenia, particularly dimethyl fumarate, may similarly contribute to risk, although the risk attending the lymphopenia seen with fingolimod is unclear [68]. With natalizumab-associated PML, JCV antibody positivity is found in nearly all patients, but in cases of PML associated with HIV or drugs other than natalizumab, there have been no studies on JCV antibody positivity and risk.

CSF studies are helpful both in diagnosis of PML and in evaluation of other diseases. The typical profile is acellular, though mild elevations of white counts with lymphocytic predominance can be seen (up to 20 cells/ml), whereas normal to moderately elevated protein with glucose in the normal range should be expected [4]. Other viral etiologies or syphilis,
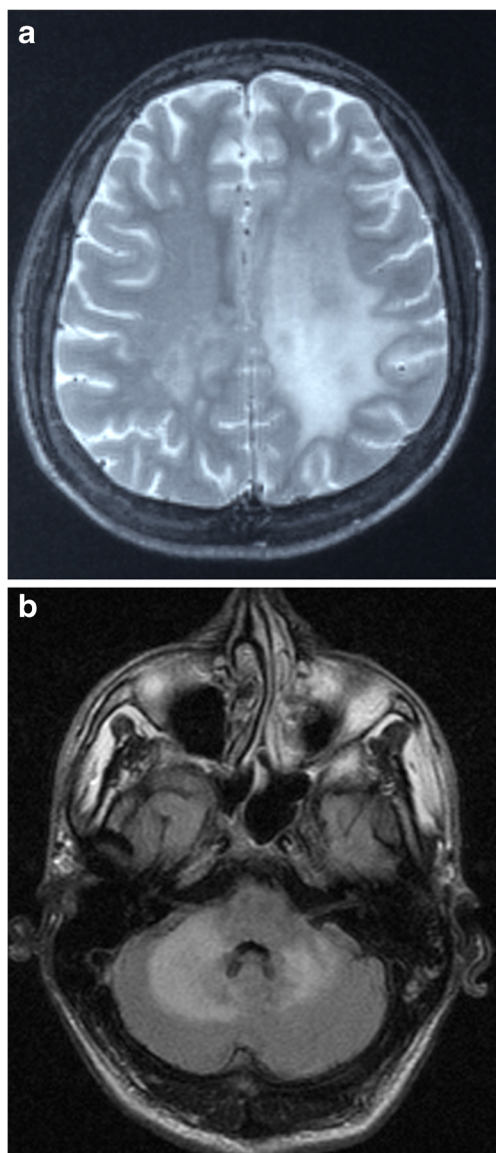
Fig. 3 Progressive multifocal leukoencephalopathy (PML) radiographically. (a) Typical lesion of PML as a T2 hyperintensity involving large portion of 1 hemisphere. (b) T2 fluid-attenuated inversion recovery image demonstrating hyperintensity in the bilateral cerebellum consistent with PML

especially in advanced immune suppression, may have a similar profile. Marked pleocytosis, especially if polymorphonuclear cell predominance and/or with hypoglycorrhachia, should raise suspicion of other diagnoses.

The sensitivity of PCR for JCV is estimated at 75% to 90% [92, 93], with the possibility that low copy number, specimen handling, or specimen quantity may impair the ability to detect virus. Specificity that JCV has been identified, however, is extremely high [93]. Current quantification assays have shown ability to detect JCV in CSF at 10 copies/ml [94] and continue to improve. This is important because the lower the copy number at diagnosis, the better the outcome.

Diagnosis

Whereas the gold standard for diagnosing PML was previously based on histopathology demonstration of the characteristic triad of demyelination, bizarre astrocytes, and oligodendroglial nuclear inclusions, diagnostics for PML have improved considerably such that detection of JCV DNA in CSF coupled with characteristic imaging in the correct clinical setting is now the norm [78]. Consensus from the American Academy of Neurology states that given the accuracy of characteristic neuroimaging and detection of JCV DNA in CSF, in a symptomatic patient with immune suppression or on immune modulatory therapy, these findings obviate the need for biopsy to diagnose PML [78]. If the clinical and neuroimaging pictures fit the diagnosis but the JCV PCR from CSF is negative, repeat testing should be performed along with work-up for other diagnoses. If diagnostic uncertainty remains, biopsy may be considered.

Prognosis

In PML associated with iatrogenic immune suppression, removal of the offending agent along with supportive care is the current recommendation, and survival rates are around 80% [77]. Reversal of natalizumab immune suppression by means of plasmapheresis has been used to restore immune function but is also not uncommonly associated with an IRIS phenomenon that can be severe, with both clinical and radiographic worsening [86].

In the patients who survive natalizumab-associated PML, significant morbidity is encountered in about two-thirds—including cognitive, visual, and motor deficits. We are desperately working to improve these outcomes and highlight that younger patients and those in whom PML is discovered earlier do better [95].

Treatment

Treatments for PML with proven efficacy are lacking. For the most part, the supportive evidence for the variety of strategies entertained to date is derived from case reports or small series evaluated without standardized outcome assessments, which limits generalization and clinical applicability. In future trials, not only blinding of treatment to examiners, but standardized inclusion and exclusion criteria, consensus imaging, viral DNA testing, and disability scales would be among some of the potential tools that may help us better understand the efficacy of treatments. That said, there have been several concepts tested that have advanced the experience with the disease and may be able to help guide future work in the arena of treating PML.

Since immune function restoration has been found to be the most effective approach to PML treatment, tangential approaches have been investigated. Interleukin (IL)-2, which stimulates T cells, and adoptive cytotoxic T-cell infusions for patients with T-cell deficiencies unrelated to HIV have reportedly been successful in battling PML [96], but owing to the anecdotal nature of such reports and concern for safety in the setting of MS, infusing T cells cannot be recommended for patients with MS who develop PML. IL-7 (CYT 107) stimulates proliferation of all cells in the lymphoid lineage and their development, survival, and homeostasis. It, too, has been claimed to have benefit in individual case reports, given as recombinant IL-7 with JCV capsid protein to boost JCV-specific T-cell responses [97]. Other cytokines, such as interferons (IFNs), have additionally been theorized to help, but the evidence is lacking. IFN-α stimulates innate and adaptive cell-mediated immune responses against viral infections and retrospective analysis suggested increased survival compared with historical controls [98], but a prospective pilot clinical study with patients with PML showed no benefit [99]. Similarly, IFN-β is unlikely to be beneficial as there was no effect on urinary JCV shedding in patients being treated for MS with any of the beta IFN *versus* glatiramer acetate [100]. Another potential trial would be a JCV vaccine with peptide antigens adapted to trigger a JCV-specific immune response in the host, with theory that this may be accomplished in the intestinal tract if given orally; however, we have the experience of JCV-antibody positivity that speaks against the effectiveness of mounting such an immune response at controlling infection.

In the case of natalizumab-associated PML, plasma exchange to negate effect of therapy is the standard of care, particularly in the case of recent drug infusion. In study of natalizumab removal, 3 plasma exchanges at 1.5× plasma volumes given over 5 to 8 days starting 10 to 14 days following natalizumab infusions reduced serum concentrations of drug by 92% and CCL2-induced leukocyte migration increased 2.2 times 18 days after plasma exchange [101]. Modeling suggested that 5 exchanges 2 days apart would yield serum natalizumab concentrations < 1 μg/ml in > 95% of patients. In the same study, α4-integrin saturations were reduced by > 50% in individuals with serum concentrations < 1 μg/ml.

Other strategies to more directly combat PML have included antiviral therapies, particularly targeting JCV replication and/or cell entry. Since JCV has been noted to use the 5-HT$_{2A}$ receptor for entry into cells, drugs that compete for binding to the receptor have been examined for ability to prevent viral spread. Therapies that reduce cell entry have been tried alone or in conjunction with viral replication inhibitors with varying results, but unfortunately no large-scale trials have been performed. Agents with effect at serotonin receptors, such as ziprasidone, olanzapine, and risperidone, have shown *in vitro* inhibition of JCV infection [62], though trials

have been less promising [102]. Chlorpromazine, another serotonin agonist, inhibits JCV infection and replication in glial tissue in culture [103, 104], but when combined with the replication inhibitor cidofovir, was unsuccessful in a single patient [105]. Despite case report or theoretical benefit in PML, risperidone also failed to prevent JCV infection of glial cells *in vitro* when studied [106]. Several reports on mirtazapine, another agent with serotonergic activity that may minimize cell entry of JCV, have demonstrated promising results—noting that early initiation of treatment has in some cases been identified as a positive prognosticator in PML [107, 108]. A report on the combination of mirtazapine with the nucleoside analog cytosine arabinoside also described a favorable outcome [109]. Still, case reports to date provide a small amount of anecdotal evidence and what has more recently been shown and what could explain the variable results of 5-HT$_{2A}$ blockade is that JCV may infect cells independently of the 5-HT$_{2A}$ receptor [110]. This fact may mean that successful treatment of PML may require medications that interrupt multiple sites along the pathway of progressive JCV infection.

Retrograde transport inhibitors have been studied, without report of clinical trial to date. Retro-2cycl inhibits retrograde transport of polyomaviruses to the endoplasmic reticulum and has been demonstrated to inhibit both initial virus infection and infectious spread of virus in cultured cells [111, 112]. Brefeldin A is an Arf1GTPase inhibitor that inhibits transport to the endoplasmic reticulum and virus disassembly, noting treatment of SVG-A cells with this agent reduced JCV infectivity by 50% in studies [113].

Inhibition of viral or DNA replication using additional nucleoside analogs has been attempted with varying results in the treatment of PML. The majority of data comes from treatment of PML in patients with AIDS and 1 trial by the AIDS Clinical Trial Group focused on this concept [114], but most of the data for use of viral replication inhibition comes from anecdotal evidence. Agents that have been tried include aciclovir, cidofovir, or brincidofovir (a lipid-ester derivative of cidofovir), cytarabine, ganciclovir, leflunomide (which inhibits DNA and RNA synthesis by inhibiting the mitochondrial enzyme dihydroorotate dehydrogenase involved in *de novo* pyrimidine synthesis and is similar to the agent teriflunomide used in MS), topotecan, vidarabine, and zidovudine. Case reports claiming benefit of ganciclovir or leflunomide have been cited [97, 115], as have inconclusive studies with topetecan [116]. Larger clinical study of cidofovir in patients with PML [117] and meta-analysis of cidofovir use showed no benefit [118], however. Mentioned clinical trial for treatment of PML used intrathecal or intravenous cytarabine added to antiretroviral therapy *versus* antiretrovirals alone but was a failure [114]. That said, the trial was conducted before widespread use of combination antiretroviral therapy and the underlying antiretroviral therapeutic strategies that were used are now considered antiquated. Additionally, as cytarabine is

not specific for JCV, further immune inhibition without adequate design to reconstitute the HIV-infected immune system may very well have contributed to the failure. Adding cytarabine and cidofovir was described to result in more favorable outcomes [119]. These agents are not frequently combined, as toxicities with each of these medications can be devastating. Still, additional reports have demonstrated efficacy in AIDS-associated PML [110]. How this should translate into treatment of PML associated with MS drugs such as natalizumab remains unclear.

Still other agents that reduce viral replication have been identified. A drug screening study to identify inhibitors of JCV replication yielded the candidates diclofenac, mafanamic acid, flunixin meglumin, isotretinoin, and mefloquine [110, 120]. Mefloquine, an antimalarial with unknown mechanism of action, demonstrated promise of clinical utility against PML [120]. Combining this with the fact that mefloquine is the only one of the mentioned drugs to have activity beyond the blood–brain barrier, as delivery of a therapeutic to the brain may be a key necessary feature of a therapeutic for PML, a clinical trial was initiated but then ended early when interim analysis showed no difference between treatment groups [121]. Poly ADP-ribose polymerase-1 inhibitors cause single-strand DNA breaks to inhibit DNA replication and repairs, and have shown activity *in vitro* by suppressing JCV replication and propagation in a neuroblastoma cell line [122], but otherwise have unknown clinical utility. Pharmacological cdk inhibitor R-roscovitine has been shown to suppress JCV replication in experimental models [123] and the same group previously showed potential benefit of silencing RNA Ag122, which targets JCV agnoprotein to effectively inhibit JCV infection *in vitro* in SVG-A cells when introduced into the brains of mice after injecting JCV-positive cells. This latter strategy significantly reduced the percentage of JCV-infected cells compared with control treatment [124].

The strategy of using combination therapy for cell entry plus viral replication inhibition or other more targeted ideas may prove more successful than use of solitary agents for fighting off JCV but awaits proof of concept and also must address the issue of effect on the host immune response.

Modulators of the immune response may also be necessary to combat PML IRIS. Glucocorticoids have been used in PML IRIS and are sometimes employed even prior to clear inflammatory response. Despite general immune suppression rather than targeted effect, benefit is claimed in a number of individual case reports [125]. Treatment of PML IRIS with administration of high-dose corticosteroids potentially followed by a prolonged taper has been most commonly described [71], noting this can pose a threat to the delicate immune system, especially if there is potential for other underlying infections [126]. The inflammation inhibitor maraviroc, a CCR5 antagonist, has also been advocated as a treatment of PML IRIS [127]. There have been no controlled trials for the treatment of PML IRIS nor is there an accepted standard definition of IRIS and the suggested therapies carry risk of suppressing the ability to control JCV infection in the brain. Still, in severe IRIS such as may be encountered and even demonstrated radiographically after restoration of immune function with PML, standard of care is to use immune suppression such as can be accomplished with intravenous steroids. Suggested therapy includes 1 g intravenous methylprednisolone for 3 to 5 days followed by taper and if symptoms worsen to retreat with the same dose of up to 2 g per day for 5 days.

Conclusion

Incidence of PML with MS drugs continues to rise, with natalizumab and other therapies. Debate about effectiveness of the use of prevention strategies such as JCV antibody testing is complicated, but these strategies will almost certainly improve with time. Concurrently, a number of targeted therapies to prevent JCV replication and spread are under study. Aside from anecdotal reports of agents to treat the primary infection, treatment of PML focuses on immune reconstitution at this time. Removing offending agents to restore immune function in the case of PML associated with MS and then managing the complications of any subsequent immune response, noting steroids are frequently employed in the setting of IRIS, is the current standard of care. At this stage, though continued work is being done to develop targeted treatment strategies, preventing PML, weighing the risk of therapy against risk of disease, and detecting the disorder early remain of paramount importance.

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Diagnosis and Treatment of Progressive Multifocal Leukoencephalopathy Associated With Multiple Sclerosis...

973

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