# Progressive multifocal leukoencephalopathy in patients living with HIV

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# PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY IN PATIENTS LIVING WITH HIV

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## Abbreviations

AIDS - Acquired Immunodeficiency Syndrome

ART - Antiretroviral Therapy

cART - Combination Antiretroviral Therapy

Cho/Cr - Choline/Creatine ratio

CMV - Cytomegalovirus

CNS - Central Nervous System

CSF - Cerebrospinal Fluid

CT - Computed Tomography

DNA - Deoxyribonucleic Acid

DWI - Diffusion-Weighted Imaging

EBV - Epstein-Barr Virus

EU - European Union

FLAIR - Fluid-Attenuated Inversion Recovery

GCS - Glasgow Coma Scale

ART - Highly Active Antiretroviral Therapy

HAP - Hospital Acquired Pneumonia

HIV - Human Immunodeficiency Virus

HSV-1 - Herpes Simplex Virus Type 1

HSV-2 - Herpes Simplex Virus Type 2

ICU - Intensive Care Unit

JCV - John Cunningham Virus

MSM - Men who have Sex with Men

MRI - Magnetic Resonance Imaging

MRS - Magnetic Resonance Spectroscopy

MSCT - Multislice Computed Tomography

PCR - Polymerase Chain Reaction

PCP - Pneumocystis Pneumonia

PLWH - People Living with HIV

PML - Progressive Multifocal Leukoencephalopathy

RNA - Ribonucleic Acid

TB - Tuberculosis

TMP-SMX - Trimethoprim-Sulfamethoxazole

WHO - World Health Organization

HHV8 - Human Herpesvirus 8

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## 1. Abstract

**Title:** Progressive multifocal leukoencephalopathy in patients living with HIV

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HIV infection is a healthcare problem worldwide, leading to opportunistic infections such as candidiasis, Kaposi sarcoma, and PML. There are several prevention programs and campaigns that aim to reduce the burden of HIV infection and AIDS. However, they remain prevalent and are associated with complications, especially in late presenters.

PML, an AIDS-defining disease caused by the JC virus, presents with neurological symptoms due to demyelination of the CNS. Although in most cases, diagnosis is done by a combination of clinical features, imaging, and CSF analysis, sometimes false negative results can arise, which can delay treating those patients. Therefore, an interdisciplinary team is essential for the correct management of patients with non-classical diagnostic results. In those cases, PML cannot be ruled out, and further tests that include brain biopsy are required.

Those points will be presented in a case report and supported by similar cases from literature, emphasizing the importance of comprehensive evaluation in late presenters with neurological manifestations of PML.

## 2. Sažetak

**Naslov:** Progresivna multifokalna leukoencefalopatija u bolesnika koji žive s HIV-om

**Autor:** Keren Tova Goldstein

Infekcija HIV-om je zdravstveni problem širom svijeta, što dovodi do oportunističkih infekcija poput kandidijaze, Kaposijevog sarkoma i PML-a. Postoji nekoliko programa prevencije i kampanja koje imaju za cilj smanjenje opterećenja infekcijom HIV-om i AIDS-om. Međutim, i dalje su prisutni i povezani su s komplikacijama, posebno kod kasno otkrivenih pacijenata.

PML, bolest koja definira AIDS i uzrokovana je JC virusom, manifestira se neurološkim simptomima zbog demijelinizacije CNS-a. Iako se u većini slučajeva dijagnoza postavlja kombinacijom kliničkih obilježja, snimanja i analize likvora, ponekad se mogu pojaviti lažno negativni rezultati, što može odgoditi liječenje tih pacijenata. Stoga je interdisciplinarni tim ključan za ispravno upravljanje pacijentima s neklasičnim dijagnostičkim rezultatima. U tim slučajevima, PML se ne može isključiti, a potrebna su daljnja ispitivanja koja uključuju biopsiju mozga.

Ti će se aspekti predstaviti u prikazu slučaja, kao i potkrijepiti sličnim slučajevima iz literature, čime se naglašava važnost sveobuhvatne procjene kod kasno otkrivenih pacijenata s neurološkim manifestacijama PML-a.

# 3. Introduction

## 3.1 Human Immunodeficiency Virus Infection

The retrovirus, known as the human immunodeficiency virus (HIV), is known to target CD4+ T cells. HIV-1 is the more common subtype that is found throughout the world, but HIV-2 is limited to West Africa and is hence less common (4).

Human immunodeficiency virus infection is primarily transmitted through sexual contact, sharing needles, blood and blood products, organ transplantation, or from mother to child during birth, pregnancy, and breastfeeding. It targets CD4+ cells, such as macrophages and T cells, which are essential for immunological defense. patients gradually lose their immune function as the virus destroys these cells.

Acute infection, clinical latency, and acquired immunodeficiency syndrome (AIDS) are the three main stages of HIV infection progression (2). The virus multiplies quickly during the acute phase, and approximately half of the infected individuals may experience flu-like symptoms. This is known as an acute retroviral syndrome, as it occurs occurring within 2-4 weeks of infection. However, many infected patients may remain with no symptoms. The clinical latency stage, which can last several years after the acute phase, occurs when the virus is still active but reproduces slowly. Some persons stay asymptomatic throughout this phase, while others may experience non-AIDS-defining illnesses such as oral hairy leukoplakia. At the last stage, known as AIDS or advanced HIV infection, an individual's immune system is severely weakened, making them vulnerable to opportunistic infections and malignancies like Kaposi sarcoma. The CD4+ count is less than 200 cells/ μl at this point (1).

Antigen/antibody-based tests that identify several indicators of infection, such as HIV-1 RNA and p24 antigen, which manifest soon after infection, are among the diagnostic techniques for HIV (3). HIV continues to be a serious threat to public health despite improvements in diagnosis and treatment, particularly for young individuals in their 20s and 30s. Millions of people get HIV worldwide.

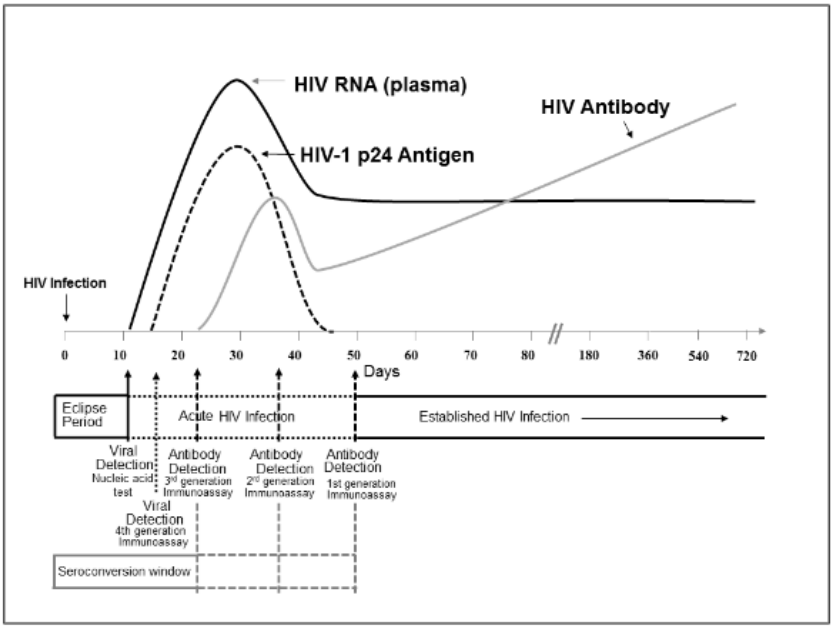


Figure 1 (3)

### 3.2 Epidemiology

HIV infection is prevalent in every continent, but the rate varies between each continent and country.

The global prevalence of HIV is 36.9 million cases worldwide, according to the global burden of HIV/AIDS in 2019, which is 0.5% of the global population. Sub-Saharan Africa continues to bear the heaviest burden, accounting for one-third of the global HIV cases despite comprising only 2% of the global population. Other countries that are especially noted with HIV are South Africa, Portugal, Brazil, Mexico, Peru, Spain, Germany, and the United States. Globally, HIV incidence shows a bimodal distribution, peaking in infancy due to perinatal transmission and among young adults aged 20-39, largely through sexual contact and needle sharing. Despite global efforts and success in reducing AIDS mortality and HIV incidence thanks to prevention programs, widespread availability of condoms, circumcision, pre-exposure prophylaxis, and treatment for mother-to-child transmission, the incidence has started to rise in certain countries since 2010. A concerning trend is the increasing incidence of HIV in various parts of Europe, North America, and South America, especially among key populations such as men who have sex with men (MSM).

The transition of HIV to a chronic condition due to successful antiretroviral therapy has led to a higher number of people living with HIV (PLWH), adding to the health system burden. However, significant challenges estimated 17.2 million people living with HIV are not on ART, and among those who are, only 44% achieve viral suppression (5).

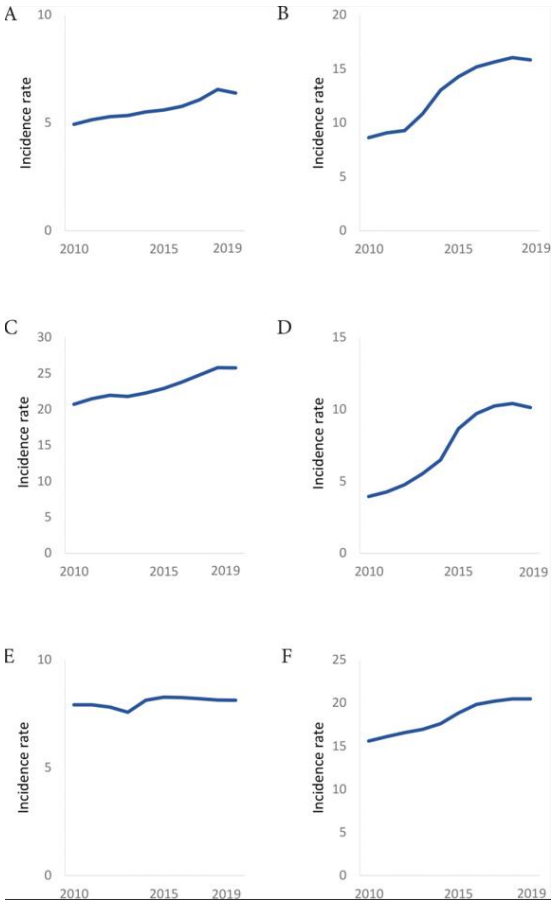


Figure 2. Rising HIV incidence rates (per 100,000 population) over the last decade in selected countries. (A) Italy. (B) Portugal. (C) Argentina. (D) Spain. (E) United Kingdom. (F) United States.

When compared to the worldwide HIV rates, HIV in Europe has similar but also different epidemiological distribution.

First, the population in Europe with HIV is MSM, people who inject drugs, prisoners, sex workers, and transgender individuals. These groups face a higher risk due to various factors including social, economic, and legal challenges. Data from national HIV case

surveillance systems in Europe generally lack detailed information on these populations, particularly regarding sex work, gender identity, or imprisonment histories. MSM and drug users have been the most affected groups, and in 2019, 39% of new HIV diagnoses in the EU were attributed to MSM. In comparison, transmission via injecting drug use accounted for about 4% of new cases.

Second, HIV rates across different European countries are different. Higher rates are observed in the western and southern parts. Although HIV incidence among MSM is declining in several Western European countries due to effective prevention measures and early treatment, it remains high or is increasing in some Eastern European countries.

To reduce the high prevalence of HIV, several interventions were suggested. These include condoms, pre- and post-exposure prophylaxis, higher prevalence of HIV testing, proper initiation of antiretroviral therapy, and access to clean injecting equipment (6).

Another definition of the stage during the initial presentation is defined a ‘late presentation.’ This definition's importance is in assessing prevention programs' effectiveness. Recent guidelines recommend ART for all untreated patients with a CD4 count <350 cells/μL. Across Europe, almost one-third of individuals infected with HIV are diagnosed late with their disease progression, leading to delayed initiation of treatment (7). Because AIDS-defining diseases occur more commonly as CD4 cell count decreases, especially when CD4 <200 cells/μL, it is important to identify HIV infection as early as possible to improve prognosis. When patients present for care with a CD4 count <350 cells/μL, or when there are AIDS-defining diseases regardless of CD4 counts, it is considered a late presentation. When patients present with CD4 <200 cells/μL, it is considered a presentation with advanced HIV disease (8). In Europe, 47% of those diagnosed with HIV infection had a CD4 count <350 cells/μL at the time of diagnosis, making them under the late presentation definition. However, the clinical stage is also considered to adjust the estimation. For example, for the year 2012, only 33% of the new cases are considered as late presenters instead of 42.4% when adjusting clinical stage. Similarly, among MSM, 18.2% of patients would have been considered late presenters instead of 30.9%.

Regarding the suggested initiatives that aim to decrease HIV prevalence, the goal of the '95-95-95 objectives' established by the United Nations Programme on HIV/AIDS (UNAIDS) can be an example. In 2021, the suggestion was made to end the AIDS epidemic by 2030. By 2025, 86% of all persons living with HIV should have viral suppression according to these targets, which call for 95% of all chronic HIV patients to be diagnosed, 95% of those diagnosed to receive antiretroviral medication, and 95% of those treated to have viral suppression. Progress toward these goals has been recorded by the WHO European Region, which includes 55 countries in Europe and Central Asia, as of 2023, with 84% diagnosed, the same number receiving treatment, and 93% of those receiving viral suppression. However, the region's overall virus suppression rate is only 65%, which is still below the goal of 86%. The results of this initiative also vary across different sub-regions. The West sub-region has shown the best results, nearly meeting the targets, whereas the Centre and East sub-regions do not, especially in treatment coverage to achieve viral suppression. Among the 20 EU/EEA countries providing full data, 77% of PLHIV are virally suppressed, with 91% diagnosed and 93% of those treated reaching viral suppression. An estimated 791,531 PLHIV in the region still have transmissible virus levels. Of these, nearly half are undiagnosed, about a third are diagnosed but not on treatment, and 15% are on treatment but not virally suppressed (9).

### 3.3 Opportunistic infection in HIV and AIDS

The term "opportunistic infections" refers to the wide range of infections that may occur in an individual with a chronic HIV infection who is not receiving antiretroviral therapy and experiences a decline in CD4+ cells. These infections are caused by usually benign pathogens that can cause disease in an immunocompromised host (e.g., patients with AIDS). To diagnose AIDS, a patient must have an AIDS-defining disease or have CD4+ less than 200 cells/ μl. There are many potentially lethal opportunistic infections in these patients, which are frequently difficult to treat (10).

Compared to the general population, HIV-positive individuals are more likely to acquire common opportunistic infections, even with high CD4+ cell counts. However, in HIV patients with CD4+ cell counts less than 200 cells/µl, the risk of developing opportunistic infections and subsequent death remains the highest. *Toxoplasma gondii, Pneumocystis jiroveci, Cryptococcus neoformans, Mycobacterium avium, Mycobacterium tuberculosis, Cytomegalovirus, Herpes simplex* viruses, and *Histoplasma capsulatum* are among the many bacterial, viral, fungal, and protozoal infections that these individuals are susceptible to (11,12).

### 3.3.1 All CD4+ counts

***Mycobacterium tuberculosis:***

The acid-fast bacillus *Mycobacterium tuberculosis* is responsible for the greatest death rate among HIV/AIDS patients. It is extremely common (33%) in individuals with AIDS. Although tuberculosis (TB) is a lung infection, it can also have extrapulmonary manifestations and spread during the latter stages of HIV/AIDS. TB can attack any CD4+ T stage, and any organ system may be affected by this bacterium. Fatigue, weakness, weight loss, and fever are some of the symptoms. Hemoptysis and a persistent cough are symptoms of pulmonary TB. Urinary tract involvement and meningitis are possible outcomes. Widespread bloodstream infections are linked to a high death rate and cause lesions in numerous organs (miliary TB) (12).

### 3.3.2 CD4+ count < 200 cells/µl

***Pneumocystis jirovecii pneumonia (PCP):***

One of the main causes of death for HIV patients is *Pneumocystis jirovecii*, which is spread by air. PCP is 4.9 times more likely to develop in immunocompromised people. Characteristics include a history of fever, dyspnea, and cough. A ground glass pattern is seen in the diffuse, bilateral, symmetrical interstitial infiltrates shown on the chest x-ray (13).

***Mucocutaneous candidiasis:***

Mucocutaneous candidiasis, caused by *Candida albicans,* is an HIV infection that manifests as oropharyngeal, esophageal, or vulvovaginal illness. Painless, white, plaque-like lesions appear in the oral cavity, often known as thrush. This is the first sign of immunosuppression that most HIV patients experience. These lesions are easily scraped off. Dysphagia, or burning discomfort retrosternal, is a possible symptom of esophageal candidiasis. Mucosal burning and itching are the symptoms of vulvovaginal candidiasis in women living with HIV. The prevalence of candidiasis in HIV patients has dramatically decreased after combined antiretroviral therapy was introduced (14).

**Kaposi sarcoma:**

*Human herpesvirus 8*, A double-stranded DNA *herpesvirus*, is the cause of Kaposi sarcoma. The HHV 8 virus spreads through contaminated saliva and blood. HHV-8 affects individuals with advanced-stage HIV with a CD4+ T cell count <200 cells/µl (15). Patients usually present with several rapidly growing raised tumors either viscerally or cutaneous. Mostly affects the skin, but it can also spread to the mucosa, viscera, and lymph nodes. When the gastrointestinal tract is involved, it can lead to diarrhea and abdominal pain and, in extreme situations, bleeding and bowel blockage. When the pulmonary system is involved, patients may present with hemoptysis, coughing, and/or dyspnea (16).

### 3.3.3 CD4+ count < 100 cells/µl

***Cryptococcus neoformans:***

About 5–8% of HIV-positive individuals develop cryptococcosis, fungal meningitis spread by the respiratory system. Most cases of cryptococcosis are seen in advanced-stage HIV patients with a CD4+ T cell count of less than 50 cells/µl. This pathogen is distinguished by a thick capsule made of polysaccharides. may show up as meningoencephalitis or subacute meningitis. Headache, fever, and malaise are the

typical initial symptoms. The symptoms of pulmonary cryptococcosis can include fever, dyspnea, coughing, chest discomfort, and positive blood cultures (17).

***Herpes simplex viruses (HSV):***

Two possibilities of the Herpesviridae family that can cause infection in persons who are immunocompetent or immunocompromised are *human herpes simplex viruses 1 and 2.* Nearly all HIV-positive patients (around 95%) have either HSV-1 or HSV-2 infection (18). The virus is dormant in the nerve root ganglia, and sores in the region of the lips, mouth, and genitalia occasionally appear as a sign of reactivation. Sexual contact with HSV-2 can result in anogenital ulcers, often known as genital herpes. Frequent and severe HSV infections, such as encephalitis, keratoconjunctivitis, genital herpes, and newborn herpes transmitted during labor, are common in patients with AIDS (19).

***JC virus infection:***

John Cunningham virus (JCV) is a double-stranded DNA polyomavirus. Patients usually acquire the primary infection during childhood and are asymptomatic. The virus spreads hematogenously from the primary site of infection to secondary sites, including the brain, and establishes latent infection (20). During the state of immunosuppression, the virus undergoes molecular changes that allow it to replicate in glial tissues. This results in PML, an illness affecting the white matter of the brain that is characterized by many sites of demyelination. Symptoms might range from hemiparesis and aphasia to weakness in one extremity. When lesions are near the cortex, seizures may occur (21).

### 3.3.4 CD4+ count < 50 cells/µl

**Cytomegalovirus:**

A member of the herpesvirus family, *cytomegalovirus* is a double-stranded DNA virus. It is a highly prevalent opportunistic infection that can lead to localized or disseminated end-organ disease. Contact with infected blood, saliva, semen, vaginal fluids, and breast milk can spread the infection. Retinitis is the most prevalent complaint, usually

unilateral, but if left untreated, it can lead to blindness. Other manifestations include encephalitis, esophagitis, and colitis. Pneumonitis caused by CMV is rare (22).

***Toxoplasma gondii encephalitis:***

A major CNS opportunistic illness in AIDS patients, presenting with focal encephalitis mostly. Fever, disorientation, headaches, or motor weakness can also occur. Individuals with impaired immune systems, especially those with AIDS who have a CD4+ T cell count of less than 100 cells/µl, may reactivate an asymptomatic, latent infection. However, patients with a CD4+ T cell count of less than 50 cells/µl are the most susceptible. Contaminated vegetables, raw meat, and touch with cat feces can all spread the disease. HIV individuals can receive effective treatment with pyrimethamine, sulfadiazine, and TMP-SMX. The prevalence of toxoplasmosis is decreased by ART treatment (23).

***Bartonellosis:***

*Bartonella henselae* and *Bartonella quintana* cause bacillary angiomatosis, an illness characterized by skin lesions that can mimic Kaposi sarcoma in HIV patients. Usually, Bacillary angiomatosis affects patients with a CD4+ T cell count of less than 50 cells/µl in the later stages of HIV infection. While *Bartonella henselae* transmission is cat scratch dependent, Bartonella quintana is spread by the body louse. HIV patients' illnesses are linked to *Bartonella henselae* and *Bartonella quintana* infections. Since bacillary angiomatosis spreads hematogenously, lesions can be found in numerous organ systems; however, cutaneous symptoms are the most easily diagnosed and are the most common. Night sweats, weight loss, and fever are typical (24).

### 3.4 Progressive multifocal leukoencephalopathy – History

Progressive multifocal leukoencephalopathy (PML) was first described by Aström, Mancall, and Richardson in 1958. PML had been mostly diagnosed in individuals with lymphoproliferative diseases, especially B cell abnormalities, up until 1981, when the AIDS epidemic began (25).

One year later, based on the shape of the inclusion bodies, Cavanaugh and Greenbaum hypothesized a viral etiology for PML. Electron microscopy investigations indicated that the most likely causal agent was the JC virus. Six years later, the JC virus was isolated from the PML patient's brain (26).
The mechanism of the viral infection remains unknown, but the presence of the virus in tonsillar tissue suggests respiratory or oropharyngeal transmission. On the other hand, saliva and oropharyngeal secretions hardly ever contain the virus. Still, the most likely transmission route may be oral exposure through contaminated water or other sources (27).

### 3.5 Progressive multifocal leukoencephalopathy – Epidemiology

PML is an illness that primarily affects individuals with weak immune systems. The incidence and mortality of PML in HIV patients decreased with the use of antiretroviral therapy; however, even with highly active antiretroviral therapy (ART), HIV-associated PML is a serious clinical problem (28) (29).
Before the introduction of combination antiretroviral therapy, 3-7% of HIV patients experienced PML, which was the cause of 18% of central nervous system cases that resulted in death. Nowadays, the incidence of PML cases decreased due to the current treatment, although not as much as the other opportunistic infections. Even with patients who have CD4+ T cells greater than 200 cells/µl, those starting combination antiretroviral therapy and those with persistent viral suppression are still susceptible to developing PML (30).
According to current estimates, up to 5% of all AIDS patients eventually acquired PML. The median survival time for PML in AIDS patients is approximately 6 months. However, up to 10% of patients will experience a partial recovery and a prolonged survival (>12 months), this may be linked to the antiretroviral therapy which improves survival (31).

### 3.6 PML - Pathophysiology

The JC virus is a small DNA virus that belongs to the human polyomavirus family. This virus can reactivate and cause PML, a demyelinating disease (32). Both inhalation and ingestion of contaminated water or food have been suggested as major modes of human transmission (33). A British survey found that the level of the pathogen in the population, as measured in blood serum (seroprevalence), increases with age (34).
The urinary tract and bone marrow were found to be a possible site of latency for the JC virus. About 30% of healthy individuals eliminate the virus, and its DNA has been found in the bone marrow of both HIV-positive and HIV-negative patients who do not have PML (35).
Another location that was found to be a potential site of JV virus persistence is the CNS. The virus can reach the CNS as a latent, low-level benign infection during primary infection. To support this hypothesis, JC virus DNA has been discovered in the brain of patients without PML by polymerase chain reaction investigations, including in the oligodendrocytes and astrocytes (36).
In PML patients, hematogenous spread to the CNS via bone marrow or another peripheral source is suggested. Because there is no animal model for PML, the exact mechanism of the condition is still unknown, but there is strong evidence that JC reactivation rather than primary infection is the cause of PML. Patients with PML typically have IgG antibodies against structural proteins, while the presence of IgM is rare. The reactivation theory is further supported by the fact that PML is hardly observed in children (37).

According to the current theories, PML pathogenesis involves multiple stages, including initial JC virus infection, latent or persistent infection, neurotropic strain rearrangement and reactivation, brain entry, infection of oligodendrocytes, and an inefficient immune response (38).
B cells are assumed to play a crucial role in pathogenesis by enabling viral entry into the central nervous system. However, as of right now, the exact mechanism is still unknown, and the function of infected B cells is still hypothetical (39).

Immunity specific to the JC virus is essential for preventing and controlling chronic infections. When the JC virus is eliminated in the urine, healthy individuals respond with CD4+ T cells, and 73% of immunocompetent individuals have cytotoxic T lymphocytes specific to the JC virus. On the other hand, loss of immune control promotes viral reactivation and replication in the brain, resulting in oligodendrocyte lytic infection and brain damage. JC virus-specific CD4, CD8, and B-cell responses in blood and CSF are frequently restored after immune restoration with combination antiretroviral treatment, leading to remission (40).

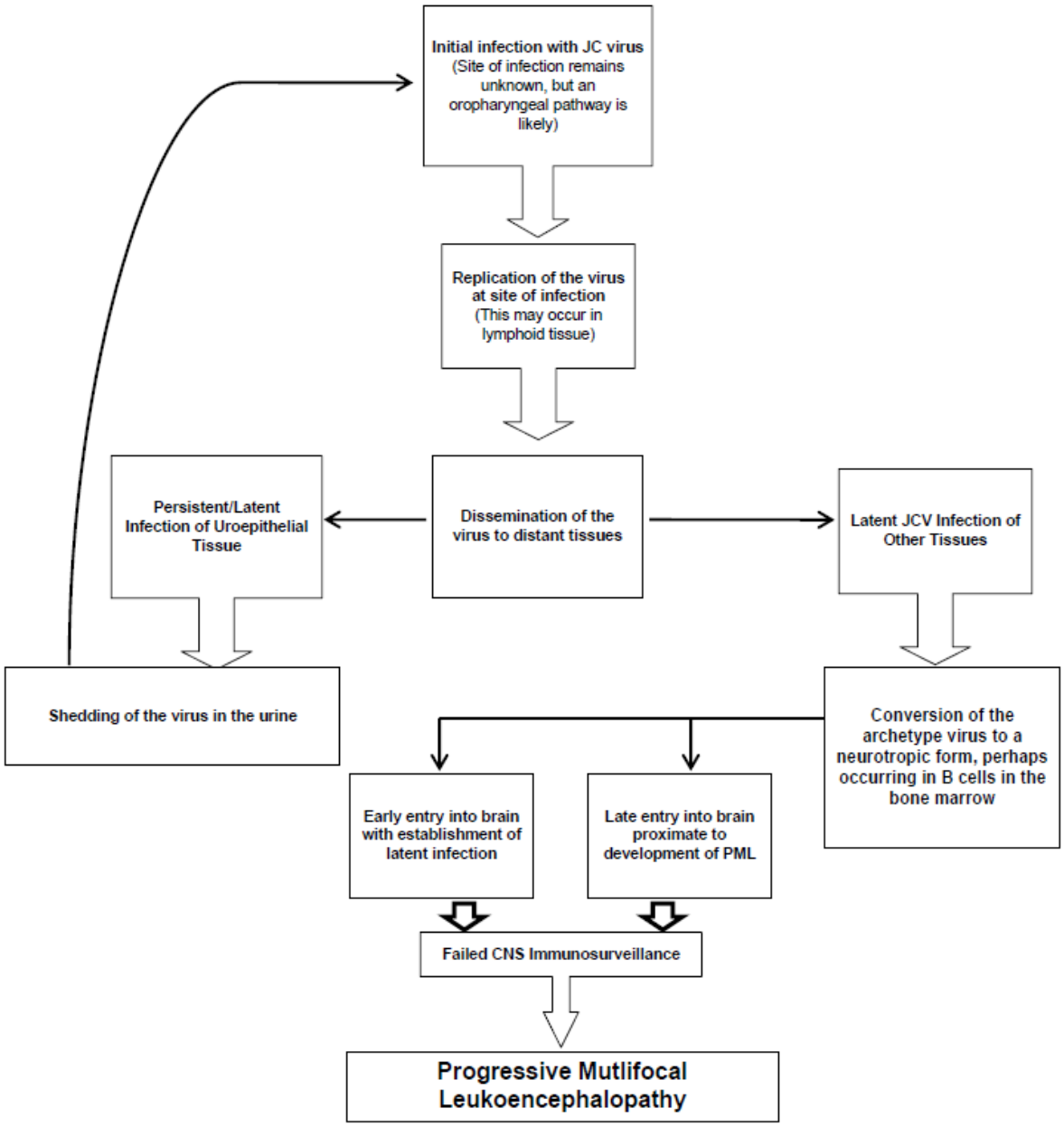


Figure 3. Proposed pathways from JC virus to the development of PML (39)

### 3.7 Histopathology of PML

Progressive multifocal leukoencephalopathy is characterized by histopathology by larger oligodendroglia nuclei with nuclear inclusions, atypical astrocytes, and multifocal areas of demyelination (41) (figure 4). Lesions are often located in the parieto-occipital and frontal regions of the white matter. The size of the lesions ranges from 1 mm to several centimeters. However, the lesions can develop anywhere and frequently involve gray matter. While brainstem involvement is more prevalent, the spinal cord may also be damaged, but it is much less common (42).

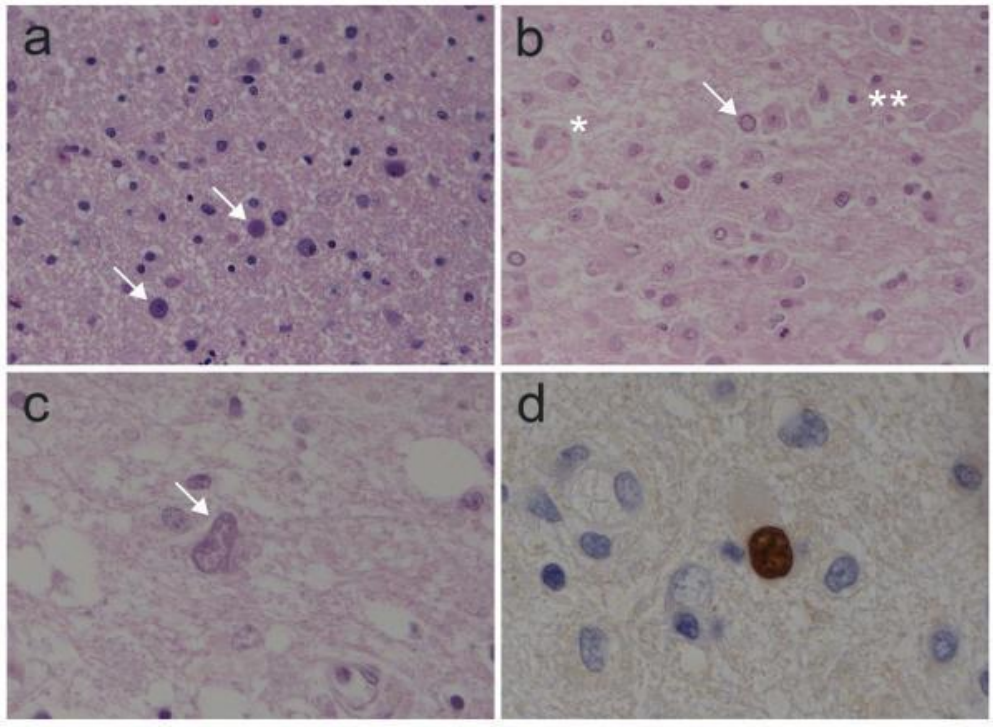


Figure 4. Histopathological findings of PML. a. Early PML: white matter vacuolization, infected oligodendrocytes (arrows) with enlarged and basophilic nuclei. b. Demyelinating PML: foamy macrophages engulfing myelin debris (*), scattered inflammatory cells (**) and infected oligodendrocytes (arrow) with enlarged basophilic nuclei. c. An enlarged, bizarre-appearing astrocyte (arrow) with atypical multilobate nuclei and altered chromatin. d. In situ hybridization with JCV-specific probe, showing JCV DNA as brown nuclear staining of an infected oligodendrocyte (42).

### 3.8 PML – clinical manifestation

PML is a severe demyelinating disease of the central nervous system, typically associated with immunosuppression, especially in HIV patients. The symptoms present in PML-affected individuals depend on the site of the lesion. PML typically presents with a variety of neurological abnormalities, including sensory impairments, hemiparesis, aphasia, ataxia, memory loss, confusion, and dysmetria. Headaches, behavioral changes, and visual disturbances such as diplopia or blindness may occur (44). About 20% of cases result in seizures (43). About one-third of the patients have cognitive abnormalities, but isolated dementia without other deficits is rare (45).

Before cART, PML had a few months median survival rate and was almost always deadly. However, in almost half of patients, illness stability is seen with cART (46). Up to 80% of individuals have weakness at the time of diagnosis, which can range from hemiparesis to quadriparesis. Meningitis, meningoencephalitis (47), progressive myoclonic ataxia, pure cerebellar syndrome (48), and muscle wasting are other examples of atypical presentations of PML.

### 3.9 PML – Diagnosis

Determining a diagnosis is important for patient care and clinical research. There are a few steps involved in the diagnosis of PML. CSF analysis, radiological detection of brain lesions using neuroimaging, laboratory evaluation, and clinical suspicion based on localized neurological impairments are some examples used for diagnosing PML (49).

#### 3.9.1 CSF analysis

CSF analysis is useful for evaluating various disorders as well as for diagnosing PML (50). Although minor increases in white counts with a lymphocytic predominance can be observed (up to 200 cells/ml), the usual profile is acellular. Normal to moderate increased protein and glucose can be predicted, and mild mononuclear pleocytosis. Normal glucose levels are maintained, and while oligoclonal bands are occasionally seen, they are not helpful for the diagnosis (51).

### 3.9.2 JC virus PCR

One of the main diagnostic methods is the polymerase chain reaction (PCR), which finds JC virus DNA in CSF (52). To diagnose PML, it is sufficient to have a positive JC virus PCR result on the CSF of a patient with appropriate history and imaging, which removes the need for a tissue biopsy (53).

Certain PML cases remain PCR-negative, be attributed to low viral concentration or inconsistent virus shedding. However, a negative PCR test does not rule out PML. If the first PCR findings are negative and the suspicious level is still high, it is advised to repeat the CSF testing (54).

### 3.9.3 Neuroimaging

To diagnose PML, neuroimaging - ideally an MRI, is essential since it reveals distinctive brain abnormalities. White matter damage is often indicated by these lesions appearing hypointense on T1-weighted sequences and hyperintense on T2-weighted and FLAIR sequences (Figure 5). MRI is typically more sensitive than CT at identifying lesions, which can detect lesions undetected by CT. Lesions can occur anywhere in the brain, including the gray matter, although they are most frequently seen in the brainstem, cerebellar peduncles, and subcortical white matter (55), (58) (Figure 5).

More diagnostic information is provided by more recent imaging methods. Based on water diffusion, diffusion-weighted imaging (DWI) may distinguish between necrosis and gliosis and active infection and cell swelling (56). (figure 6)

To differentiate PML from HIV-1 encephalopathy, proton MR spectroscopy (MRS) frequently reveals reduced N-acetyl-aspartate ratios due to neuronal damage and higher choline/creatine ratios due to demyelination (57).

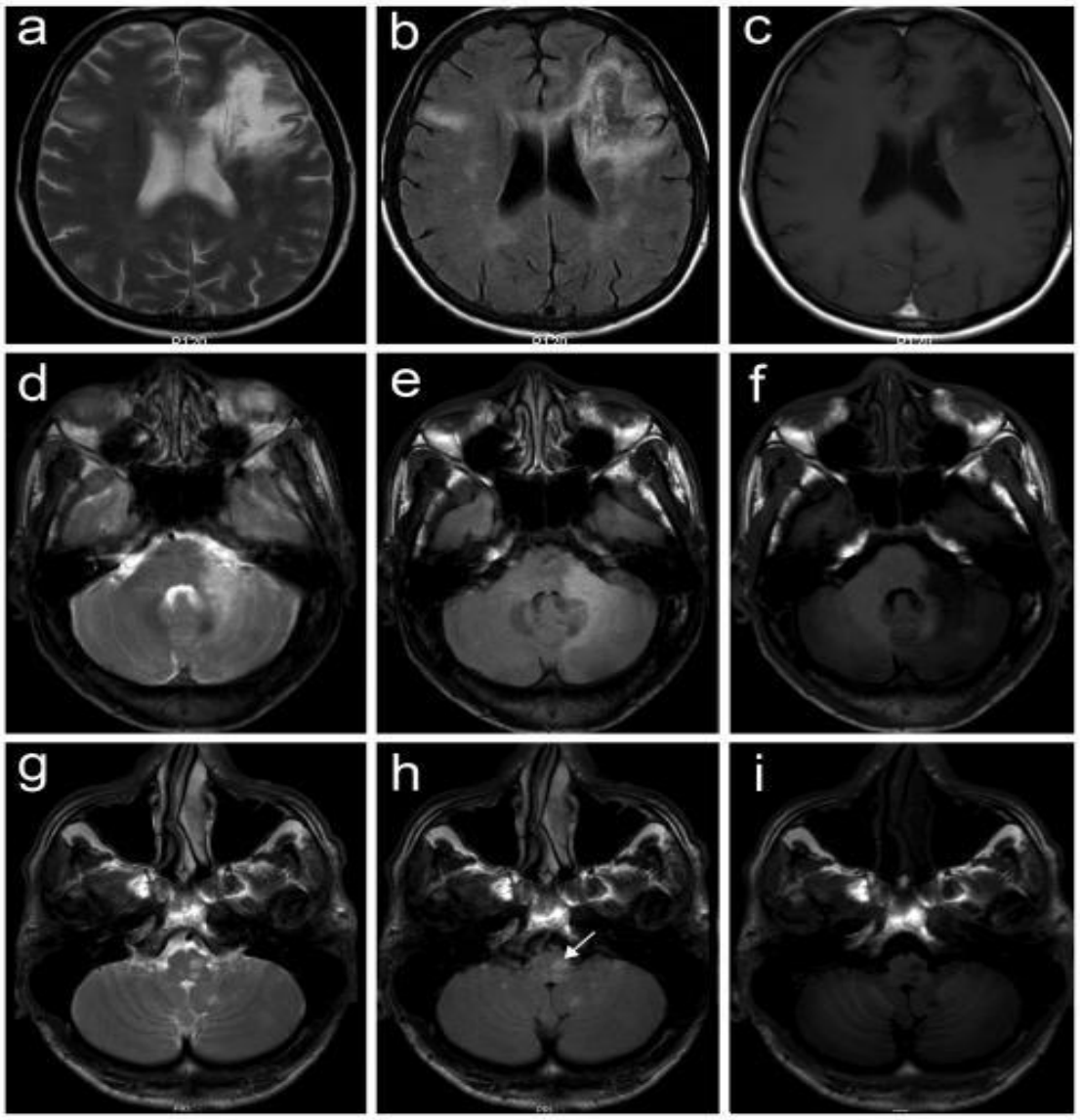


Figure 5. MRI appearance of active PML lesions (a,d,g, T2; b,e,h, FLAIR; c,f,,i, Gd-T1 axial sequences). In all cases, the lesions are hyperintense (white) in T2 and FLAIR sequences and hypointense (dark) in T1 sequences, showing no enhancement after Gd administration. **a-c.** Hemispheric localization: large white matter signal alteration in the left frontal lobe extending to the corpus callosum and left deep white matter, with additional FLAIR/T2 high-intensity signal alterations in the right frontal and temporal lobes. **d-f.** Cerebellar localization: large signal alterations of the left middle cerebellar peduncle and hemisphere. **g-i.** Brain stem localization: focal signal alterations of the left bulb (arrow) and cerebellar hemispheres. (58)

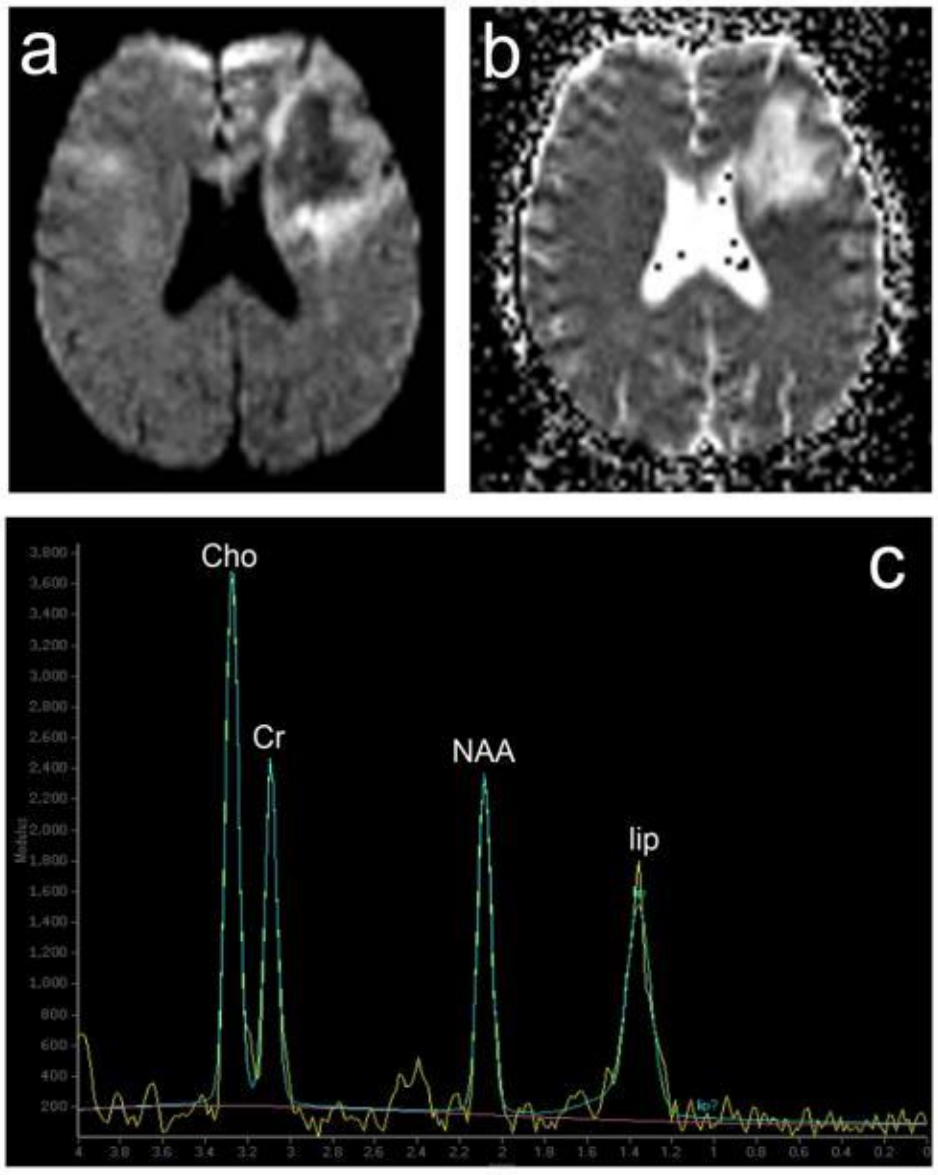


Figure 6. Active PML lesions at Diffusion Weighted Imaging (DWI), Apparent Diffusion Coefficient (ADC) map and Magnetic Resonance Spectroscopy (MRS). **a**. DWI, hyperintense signal (white) of the periphery and low intensity signal (dark) of the center of the lesion. **b.** ADC map, hyperintense signal (white) of the center and hypointensity (dark) of the lesion periphery. **c.** MRS, in correspondence of the center of the lesion, increased choline (Cho) and creatine (Cr) and relative ratio (Cho/Cr), marked reduction of N-acetyl-aspartate (NAA) and lipid (lip) peak.

(58)

### 3.9.4 Brain biopsy

In cases where attempts to detect JCV DNA in the CSF are unsuccessful, negative, or imaging characteristics are unusual, a brain biopsy is necessary to get an etiological diagnosis of PML (59).
Tissue histology previously mentioned helps to identify PML. Viral confirmation can be confirmed by immunohistochemistry, in situ nucleic acid hybridization, electron microscopy, or the discovery of JCV proteins, JCV DNA, or JC virions (60), (61).

Although PML is usually diagnosed based on the combination of clinical features, radiological findings, and a positive CSF PCR to detect JC virus DNA, sometimes there are false negative results that may require further evaluation with a brain biopsy. A detailed explanation and approach for those patients will be discussed later in this article under the case report and its discussion.

## 3.10 PML - therapeutic approach

PML has no specific treatment. Thus, the primary strategy is to restore the host's adaptive immune response, which seems to extend survival (62), (63).
The recommended course of treatment for HIV-positive individuals who develop PML is to optimize or start effective antiretroviral medication (ART) (64). Prolonged survival can result from ART's ability to stabilize or improve PML. Those on antiretroviral therapy (ART) have a one-year survival rate of about 50%, whereas those off ART have a rate of about 10%.

When treating individuals who do not have HIV, the main approach is to decrease or stop using immunosuppressive drugs such as glucocorticoid or calcineurin inhibitors, when practical. Immunosuppressant medications should not be reduced for example, if there is a risk that the reduction or discontinuation in transplant recipients may raise the risk of organ rejection (65).
Case studies, however, show the potential advantages because some patients with liver, kidney, and heart transplants have survived PML after lowering or stopping immunosuppression (66).

Several pharmacologic treatments have been studied for the treatment of JC virus. None, however, have demonstrated a significant therapeutic advantage. An example can be checkpoint inhibitors (67): pembrolizumab (68) and nivolumab (69) both target PD-1, which boosts T-cell activation.

Clinical trials have demonstrated varying degrees of effectiveness for medications such as cidofovir (70), mefloquine (71), mirtazapine, and cytarabine. The serotonin receptor antagonist mirtazapine showed modest improvement in reported situations; however, there is no proof from controlled trials to support this claim (72).

While there is some hope for experimental therapies, the key to successful management is to reduce immunosuppression in non-HIV patients and optimize ART in HIV patients.

### 3.11 PML - prognosis, morbidity, and mortality

The clinical history of PML is generally progressive and often fatal disease, with treatment options and underlying diseases influencing the degree of morbidity and mortality. In a cohort of 584 PML patients from a French national healthcare database, the one-year mortality rate was 38%, with a median time of 63 days from diagnosis to death (73).

For patients with positive HIV, before the development of efficient antiretroviral medication, only 10% of HIV patients with PML lived longer than a year (74).

The one-year survival rate has increased to 50% or more with the introduction of ART. According to Danish cohort research (75), the median survival rose from 0.4 years in the pre-ART period to 1.8 years in the ART era.

In patients without HIV infection, the total median survival is estimated at around three months (76). Even with improvements in therapy, PML is still a fatal disease with high morbidity and variable outcomes (77).

## 4. Methodology

I have chosen to present a case report of a patient with advanced HIV infection and PML that was confirmed through brain biopsy and tissue DNA analysis. The reason for this decision is due to the difficulties in diagnosis, including the frequent failure of CSF examination to produce positive results. The goal of this case is to highlight the importance of invasive diagnostic techniques. These techniques may play important role in verifying the diagnosis of PML, particularly in situations when CSF analysis is insufficient. Thus, this case report emphasizes the value of a thorough diagnostic strategy in the management of advanced HIV-associated PML and the need for physicians to consider more invasive methods when non-invasive techniques are inconclusive.

## 5. Case presentation

A 49-year-old male patient was admitted to the hospital in March 2023 for the evaluation of weakness in his arms and progressive malaise, which had been ongoing for several months.

The illness began in November 2022 when the patient first observed bluish skin changes on his body and face. Additionally, he noticed restricted finger movement and sporadic cramps in his left hand, accompanied by localized tingling sensations. Over time, stiffness gradually developed in his left arm, specifically in the elbow and shoulder areas. In December 2022, the patient also began experiencing tingling sensations and weakness in his right hand. Since the onset of these symptoms, the patient has been experiencing daily headaches, often accompanied by nausea and a general feeling of weakness. The patient also had neck pain for the past few months, with no other reported complaints.

His past medical history includes herpes zoster on the right hemithorax in December

2021, resulting in residual herpetic neuralgia. Other than that, the patient has no significant medical history and does not use medications for chronic diseases.

The patient was admitted to the hospital in March 2023. During the initial examination, the patient presented with the following vital signs: a blood pressure reading of 140/80 mmHg, a heart rate of 100 beats per minute, a respiratory rate of 22 breaths per minute, an oxygen saturation level of 98% in room air, a body temperature of 36.2°C, and a Glasgow Coma Scale (GCS) score of 15. The left arm was observed to be held in flexion. In terms of skin condition, there was evidence of seborrheic dermatitis. Additionally, numerous bluish lesions, some up to 1cm in diameter, were noted on the face, trunk, legs, and even the glans penis, which raised suspicion of Kaposi's sarcoma. During the neurological examination, the patient exhibited normal gait and posture, but specific findings included the absence of left biceps and triceps reflexes and a positive Babinski sign on the left side. The patient's left patellar and Achilles reflexes were reinforced (graded as 3+). Additionally, the sensory examination revealed impaired superficial sensation, predominantly on the left side, affecting fine touch, pinprick, and temperature perceptions.

The laboratory findings upon admission revealed several significant results, including an accelerated erythrocyte sedimentation rate of 46 mm/1st hour, elevated C-reactive protein level of 49.7 mg/L, and pancytopenia characterized by a hemoglobin level of 130 g/L, leukocyte count of 2.6 x 10^9/L, and thrombocyte count of 130 x 10^9/L. However, the patient's serum electrolytes, creatinine, urea, and liver enzyme levels were all within the normal range.

The patient tested positive for HIV infection. The evaluation of HIV infection indicated a significant impairment of immunity, with an absolute CD4+ lymphocyte count of 97 cells/μL of blood and an HIV-1 RNA PCR of 1624000 copies/mL of plasma.

Several diagnostic tests were conducted during the patient's hospitalization. Microbiological tests that were performed on March 13, 2023, included a urine test,

blood culture, and various serology markers. The patient tested positive for anti-HBc total, indicating previous exposure to hepatitis B, but other hepatitis markers were negative. Serology markers also revealed TPHA (80) and negative RPR. In addition, Toxoplasma and CMV serology tests showed positive IgG results. The patient's HBV DNA in serum was negative, and CMV DNA in plasma was detected at a level of 3273 copies/mL.

Cerebrospinal fluid (CSF) examination revealed 1 mononuclear cell/µl, protein level 0.65 g/l, glucose 3.3 mmol/l (glucose in blood 5.5 mmol/l), and Cl 128 mmol/l. The blood-brain barrier evaluation indicated dysfunction (zone 2). Microbiology CSF evaluation yielded no bacterial or viral pathogen (including JCV, EBV, Toxoplasma, and CMV), besides HIV-1, 10160 copies/mL.

Furthermore, a biopsy of the palate conducted on March 30, 2023, revealed a histological diagnosis of Kaposi sarcoma; with tumor cells showing positive immunohistochemical reactions for CD34 and HHV8.

In radiological assessments, a chest X-ray on March 13, 2023, showed certain abnormalities in lung hila and interstitial lesions but no infiltrates. Abdominal ultrasound (March 14, 2023) indicated liver enlargement with a granular echo structure but no focal lesions. Other organs appeared normal. Abdominal MSCT scans on March 15, 2023, revealed various findings, including a meteoritic colon (the presence of excessive gas in the colon, which can result in abdominal distension or bloating), and a 5 mm lesion on the left adrenal gland (adenoma). A gastroscopy on March 27, 2023, identified antral gastritis. March 31, 2023, a colonoscopy revealed normal findings with minor hemorrhoidal nodes.

A brain MRI on March 29, 2023, revealed changes in the brain parenchyma, predominantly involving cerebral white matter, raising suspicion of progressive multifocal leukoencephalopathy (PML), HIV encephalopathy, and chronic microbleeds.

Antiretroviral therapy was initiated on March 13, 2023, leading to a decrease in HIV-1

viremia and an increase in the number of CD4 lymphocytes. The treatment includes emtricitabine/tenofovir and dolutegravir and primary prophylaxis for *Pneumocystis jirovecii* pneumonia (trimethoprim-sulfamethoxazole 1x960 mg every other day).
During the hospital stay, the patient received symptomatic therapy (parenteral rehydration, correction of electrolyte imbalance, analgesics, gastroprotection, and anticoagulant prophylaxis with low-molecular-weight heparin). Regular physical therapy was provided, resulting in the gradual regression of right-hand paresis, while left-hand paresis and stiffness of the left hand persisted.
Treatment with acyclovir was also initiated for proven HSV-2 infection from a perianal lesion.

The patient was discharged for home care but later, in mid-April, he experienced a significant deterioration marked by weakness, speech and swallowing difficulties, and motor deficits. Pain and cramping in both arms were also reported and almost daily diffuse headaches, which were relieved with medications.

The patient was readmitted to the department on May 3, 2023. During this stay, he continued to receive antiretroviral therapy (emtricitabine/tenofovir and dolutegravir), primary prevention for pneumocystis pneumonia with cotrimoxazole, esomeprazole for gastroprotection, enoxaparin for thromboprophylaxis, pain management, and symptom relief treatments.
An extensive diagnostic workup (microbiological, radiological, immunological) was performed. The cerebrospinal fluid analysis showed no signs of pleocytosis, and once more, the JC virus was not detected. Additionally, HIV-1 viremia levels were declining.

Due to worsening neurological deficits, an urgent brain MRI was carried out on May 4, 2023, which indicated an increase in pathological infiltration in the brain. A stereotactic biopsy was scheduled in response. Additionally, due to the progression of Kaposi's sarcoma with mucosal involvement, pegylated liposomal doxorubicin (40 mg IV) was administered on May 5. During hospitalization, the patient developed hospital-acquired pneumonia, which was treated with meropenem and linezolid starting May 7.

The patient's condition deteriorated further, leading to his transfer to the intensive care unit (ICU) on May 8, 2023, where he was intubated and mechanically ventilated. A tracheotomy was performed on May 19 due to the extended need for airway management. The patient also developed *Clostridioides difficile* enterocolitis, for which oral vancomycin was prescribed. During the later course of hospitalization, there was improvement in the patient's overall condition, regression of diarrhea, and improvement in laboratory findings. On May 24, 2023, the patient was transferred from the intensive care unit to the Department for the infections in the immunocompromised patients.

A stereotactic brain biopsy was performed on May 10, 2023, at the University Hospital Dubrava. The histopathological findings indicated necrotizing leukoencephalopathy linked to HIV. During the further course of the evaluation, a report received on June 2, 2023, indicated the detection of JCV DNA and EBV DNA from the brain biopsy sample. The final diagnosis of progressive multifocal leukoencephalopathy was established.

On November 14, 2023, the patient was discharged to the rehabilitation medical center with the recommended daily physical therapy.

## 6. Discussion

The case report describes a 49-year-old male HIV patient with a low CD4 count (97 cells/μL), who experienced opportunistic infections. He presented with neurological symptoms as well as skin changes indicating Kaposi sarcoma. His condition required further evaluation that included blood and CSF laboratory tests, and brain imaging. The brain changes that were seen on MRI and involved the cerebral white matter, raised suspicion of PML but also for HIV encephalopathy, and chronic microbleeds. Making a definitive diagnosis was influenced by a sterile CSF analysis that was negative for the JC virus. The patient experienced a recurrence of neurological symptoms after initiating ART, requiring extensive workups that were once again negative for the JC virus in the CSF analysis. During this evaluation, results were positive for PML after performing a brain biopsy. Those results supported the clinical presentation and the brain MRI findings. During this workup, the patient’s condition deteriorated, and some complications arose, including severe HAP.

Our case report focuses on the importance of a negative initial workup for PML, with negative CSF analysis but a positive biopsy. It shows that there are potential false-negative results when performing less sensitive and specific diagnostic tests. It should remind the medical professionals who care for the patient that negative results do not rule out PML when there are clinical and radiological manifestations. Similar to our case, studies have suggested that before effective ART, JCV PCR of CSF for PML diagnosis had a sensitivity of 72-92% and specificity of 92-100%. Modern ultrasensitive PCR techniques have a sensitivity of >95% or higher (28). Those studies emphasize, similarly to our case, that a negative PCR test of CSF does not rule out PML. Therefore, the current guidelines in case of a "possible PML" should be treated accordingly after the appropriate exclusion of other neurologic conditions such as primary central nervous system lymphoma and HIV encephalopathy.

When reviewing several PML cases, it can be seen that there are other reported cases of PML with negative CSF analysis. One similar case showed negative results that were false and were proven by an autopsy (78). Another case reported a negative CSF PCR with the presence of JCV-specific antibodies indicating intrathecal production. In the latter case, as in our case and other cases, brain biopsy tissue was positive. However, that biopsy was performed after natalizumab discontinuation, and the diagnosis was further supported by the rise of JCV-specific IgG antibodies in the CSF (79). This result raises a theory that immunosuppressive therapy can affect CSF PCR analysis. It is also seen in other studies suggesting that ART-induced recovery of the immune system leads to decreased viral replication and clearance of JCV DNA from the cerebrospinal fluid (28). This supports our case, in which stereotactic biopsy was the definitive diagnostic test to prove the presence of PML.

Another important point made in our case report and also seen in other cases found in the literature, is that a brain biopsy is more sensitive for diagnosis of PML than CSF PCR. It is correct especially when other conditions such as primary CNS lymphoma and HIV encephalopathy are part of the differential diagnosis, or when there is inconclusive workup for PML. The treatment of PML includes the use of ART but there are some other anti-PML drugs. Delayed diagnosis of PML, especially when there is a negative initial workup, usually causes late initiation of this therapy and can affect a patient's mortality (80). To reduce those delays, a brain biopsy should be considered. However, because it is an invasive test with many possible complications, it is sometimes not performed. Therefore, the physicians caring for the patient should always balance those diagnostic tests with the understanding that a negative CSF analysis does not rule out the disease but should be performed before the brain biopsy.

However, some limitations exist, challenging possible conclusions regarding a proper PML diagnosis. They focus on the fact that a single case report does not have enough statistical power to confirm the association between negative CSF results and the actual presence of the diseases. To answer those limitations, publications of similar case reports are needed, as more patients increase the power of a study. Similarly,

performing meta-analyses and clinical trials answer the same limitations of lack of a proper number of participants. In addition, utilization of better diagnostic methods and collaboration between interdisciplinary teams can also help in diagnosis of PML, as those aim to make diagnosis more precise and thorough.

## 7. Conclusion

HIV is a retrovirus that is transmitted through sexual contact, needle sharing, and perinatal transfer. Therefore, the vulnerable populations are MSM, sex workers, drug users, and infants born to women in these population groups. HIV affects CD4+ T cells, causing immunosuppression. When the CD4 count drops significantly, some opportunistic infections occur, making HIV a public health concern.

Despite campaigns and health programs that exist to reduce transmission, and although several treatment options are available, HIV is still prevalent worldwide. It is especially true in a common phenomenon that occurs when patients enter healthcare later in the progression of the disease, called late presenters. Those patients have a worse prognosis and require more healthcare attention and resources. It emphasizes the fact that early and correct diagnosis of HIV, and its associated AIDS-defining diseases should be made to rapidly initiate ART and prevent deterioration.

Opportunistic infections occur when patients develop diseases due to severe immunosuppression, including candidiasis, Kaposi sarcoma, cryptococcal meningitis, PCP, and others. Those conditions also raise a public health concern due to morbidity, mortality, and challenges in diagnosis. Their management is important to further reduce the burden of HIV and AIDS. PML is caused by one of those opportunistic infections seen in HIV patients, making it an AIDS-defining disease. It is caused by the activation of the JC virus and manifests with neurological signs and symptoms due to progressive demyelination of the CNS. To treat it, a proper diagnosis should be made, which can sometimes be challenging.

PML in most cases, is diagnosed based on clinical features, brain imaging, and CSF analysis for viral DNA. A positive combination of those tests confirms the diagnosis and is an indication for treatment initiation. However, sometimes those tests are falsely negative which delays diagnosis and therefore the initiation of treatment. It is the case especially if the healthcare providers use those false negatives to rule out PML and fail

to order a brain biopsy. It was seen in our patient, who had repeated negative CSF PCR results, as well as in other cases in the literature.

Therefore, when approaching complex patients with HIV who are late presenters and have manifestations of the disease, an interdisciplinary team should manage the patient. This team, including infectious diseases specialists, radiologists, neurologists, nurses, molecular biologist, and clinical microbiologist, ensures extensive evaluation even in case of negative test results. As in our case report, the coordination between all those healthcare professionals allowed for acquiring a correct diagnosis under challenging conditions.

In summary, physicians should always suspect complex diseases in late presenters, calling for an interdisciplinary team to manage those patients. It should also be kept in mind that negative tests do not always rule out disease and further steps need to be done, as seen in our patient with negative CSF PCR but positive brain biopsy. Those are essential for initiating proper treatment and reducing the burden of the disease, a significant public health concern.

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## 10. Biography

Keren Tova Goldstein was born in Israel on the 6 of February 1994. She was volunteering in Magen David Adom in Israel for 2 years and realized she wanted to be a doctor. She finished her two years of required reserve service in the Israeli Defense Force (IDF). During 2018-2024, Keren studied general medicine at the University of Zagreb, School of Medicine, Croatia, and finished her degree in the MSE program.