MAJOR ARTICLE

Incidence, Clinical Presentation, and Outcome of Progressive Multifocal Leukoencephalopathy in HIV-Infected Patients during the Highly Active Antiretroviral Therapy Era: A Nationwide Cohort Study

Frederik Neess Engsig,[1] Ann-Brit Eg Hansen,[1] Lars Haukali Omland,[1] Gitte Kronborg,[3] Jan Gerstoft,[1] Alex Lund Laursen,[4] Court Pedersen,[2] Christian Backer Mogensen,[5] Lars Nielsen,[6] and Niels Obel[1]

Departments of Infectious Diseases at [1]Copenhagen University Hospital, Rigshospitalet, Copenhagen, [2]Odense University Hospital and University of Southern Denmark, Odense, [3]Copenhagen University Hospital, Hvidovre, [4]Aarhus University Hospital, Aarhus, [5]Kolding Hospital, Kolding, and [6]Helsingør Hospital, Helsingør, Denmark

Background. Human immunodeficiency virus (HIV) infection predisposes to progressive multifocal leukoencephalopathy (PML). Here, we describe the incidence, presentation, and prognosis of PML in HIV-1–infected patients during the period before highly active antiretroviral therapy (HAART) (1995–1996) and during the early HAART (1997–1999) and late HAART (2000–2006) periods.

Methods. Patients from a nationwide population-based cohort of adult HIV-1–infected individuals were included. We calculated incidence rates of PML and median survival times after diagnosis. We also described neurological symptoms at presentation and follow-up.

Results. Among 4649 patients, we identified 47 patients with PML. The incidence rates were 3.3, 1.8, and 1.3 cases per 1000 person-years at risk in 1995–1996, 1997–1999, and 2000–2006, respectively. The risk of PML was significantly associated with low $CD4^+$ cell count, and 47% of cases were diagnosed by means of brain biopsy or polymerase chain reaction analysis for JC virus. The predominant neurological symptoms at presentation were coordination disturbance, cognitive defects, and limb paresis. Thirty-five patients died; the median survival time was 0.4 years (95% confidence interval [CI], 0.0–0.7) in 1995–1996 and 1.8 years (95% CI, 0.6–3.0) in both 1997–1999 and 2000–2006. $CD4^+$ cell count >50 cells/μL at diagnosis of PML was significantly associated with reduced mortality.

Conclusions. The incidence of PML in HIV-infected patients decreased after the introduction of HAART. Survival after PML remains poor. In the management of PML, the main focus should be on prophylactic measures to avoid immunodeficiency.

Progressive multifocal leukoencephalopathy (PML) is a disease of the central nervous system and is caused by JC virus (JCV), a polyomavirus. It occurs almost exclusively in patients with severe defects in the cellular immune system. With the advent of the HIV pandemic, HIV infection has become the single most predisposing disorder for PML [1, 2].

Only a few studies have estimated the incidence of PML during the highly active antiretroviral therapy (HAART) era. The EuroSIDA study found that the incidence of PML has declined from 10 cases per 1000 person-years of follow-up during the pre-HAART era

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Reprints or correspondence: Dr. Frederik Neess Engsig, Dept. of Infectious Diseases, Rigshospitalet, Blegdamsvej 9, DK2100 Copenhagen Ø, Denmark (fren74@gmail.com).

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to 1 case per 1000 person-years at risk during the HAART era [3].

The clinical presentation of PML varies according to the localization of the disease, and the initial symptoms may be misdiagnosed as other HIV- or non–HIV-related cerebral lesions. To improve the initial diagnostic strategy, it is important to know the major presenting symptoms of PML.

Although HAART has clearly improved survival after the diagnosis of PML, there is no known causal treatment of the disease [4, 5]. During the pre-HAART area, the median survival time was only a few months after diagnosis [6]. Since the introduction of HAART, survival has improved significantly, with a 1-year probability of survival of ~50% or higher, compared with 5% or lower in patients not receiving HAART [7, 8]. However, PML still frequently induces irreversible neurological sequelae [5, 9–10].

To our knowledge, no nationwide population-based studies have been conducted on the incidence, presenting symptoms, and prognosis of HIV-associated PML during the HAART era. In the present study, we used a nationwide population-based Danish cohort to determine the incidence of PML in HIV-infected patients during the pre-HAART (1995–1996), early HAART (1997–1999), and late HAART (2000–2006) periods, along with the incidence of PML in patients with $CD4^+$ cell counts <200 or ⩾200 cells/μL. We also describe the presenting clinical and paraclinical characteristics of PML, as well as the mortality and neurological outcome of the disease.

METHODS

In a cohort of HIV-1–infected individuals, we estimated the incidence, clinical presentation, and prognosis of PML. In the first part of the study, the outcome was time to PML in a cohort of HIV-1–infected individuals. In the second part of the study, the population was all patients with PML, and the outcomes were neurological symptoms and mortality.

Setting. Denmark had a population of 5.4 million as of 31 December 2006 [11], with an estimated HIV prevalence of ~0.07% in the adult population [12]. Patients with HIV infection are treated in one of the country's 8 specialized medical centers, where they are seen on an outpatient basis at intended intervals of 12 weeks. Antiretroviral treatment is provided free of charge to all HIV-positive residents of Denmark. During our study's follow-up period (1 January 1995 through 31 December 2006), the national criteria for initiating HAART were HIV-related disease, acute HIV infection, pregnancy, $CD4^+$ cell count <300 cells/μL, and, until 2001, plasma HIV RNA level >100,000 copies/mL.

During the whole study period, polymerase chain reaction (PCR) analysis for JCV was performed at 2 laboratories. For details concerning PCR methods, see appendix A, which appears only in the electronic edition of the *Journal*.

Study population and data collection. The Danish HIV Cohort Study is a population-based prospective nationwide cohort study of all HIV-infected individuals ⩾16 years old treated at Danish HIV centers after 1 January 1995. The study has been described elsewhere [12]. Patients are consecutively enrolled, and multiple registrations are avoided through use of a unique 10-digit civil registration number assigned to all individuals in Denmark at birth or on immigration. Data are updated yearly and include demographics, date of HIV infection, AIDS-defining events, date and reason of death, antiretroviral treatment, and all $CD4^+$ cell counts and HIV RNA measurements. As of 31 December 2006, the cohort included 4660 Danish residents.

Outcomes. In the first part of the study, we estimated PML incidence rates in the entire Danish HIV cohort. PML was defined as either (1) demonstration of JCV by PCR analysis of cerebrospinal fluid (CSF) or by histopathological confirmation at brain biopsy or (2) a combination of characteristic clinical symptoms and neuroimaging findings highly suggestive of PML on magnetic resonance imaging (MRI) or computed tomography (CT) scans (for CT findings, hypodense lesions without contrast enhancement; for MRI findings, hyperintense white matter lesions on T2-weighted images or hypointense white matter lesions on T1-weighted images with no significant contrast enhancement and no mass effect).

In the second part of the study, the study cohort included the patients with a diagnosis of PML, and the outcomes were neurological symptoms and mortality. All patients with PML diagnosed between 1 January 1995 and 31 December 2006 were identified from the Danish HIV Cohort Study. Their patient files were examined by trained physicians, and information was extracted regarding neurological symptoms, laboratory results, and radiological findings at diagnosis of PML. The date of diagnosis of PML was defined as the date of initial clinical symptoms; clinical symptoms were counted as absent if they were not described in the patient files. If the temperature was not recorded in the files, it was assumed to be <37.5°C.

Neurological symptoms were estimated at 4 months and 3 years after the onset of symptoms (using the first available clinical evaluation after 4 months and 3 years, respectively). The neurological condition was registered as better, stable, or deteriorated compared with the initial presentation. In addition, a return to the pre-PML level of activity was noted, along with the need for help on a daily basis. If the patient files concluded that initiation of HAART had led to an immune reconstitution inflammatory syndrome (IRIS) related to PML, this was noted as well. Furthermore, we identified cases in which HAART had led to a rapid increase in $CD4^+$ cell counts and reviewed MRI scans for contrast enhancement as a sign of IRIS.

Baseline characteristics. Differences in characteristics be-

tween groups were evaluated by the χ^2 test and Fisher's exact test, as appropriate. Differences were considered significant at $P < .05$.

Incidence rates of PML. To estimate incidence rates, we calculated person-years at risk from the date HIV infection was diagnosed or from 1 January 1995 if the diagnosis preceded this date. Patients were censored at the date of last follow-up, the date of death, or 31 December 2006, whichever came first. PML was the outcome event. Incidence rates for diagnosis of PML and 95% confidence intervals (CIs) were calculated for 1995–1996, 1997–1999, and 2000–2006, corresponding to pre-HAART, early HAART, and late HAART periods. We also calculated the total periods of observation for HIV-infected patients with $CD4^+$ cell counts <200 or ⩾200 cells/mL. To determine $CD4^+$ cell counts at time points falling between measurements, the last measured value was carried forward.

Mortality in patients with PML. We computed person-years at risk from the diagnosis of PML to the date of death, date of last follow-up, or 31 December 2006, whichever came first. We used Kaplan-Meier analysis to construct survival curves and estimate median survival for the calendar periods 1995–1996, 1997–1999, and 2000–2006, corresponding to pre-HAART, early HAART, and late HAART periods. Causes of death were classified into 3 categories: (1) related to PML, (2) not related to PML but related to HIV infection, or (3) not related to HIV infection.

Multivariate models. We used Cox regression analysis to assess the influence of risk factors with regard to the incidence of PML in the HIV-infected population and prognostic factors for mortality in the patients with PML. The following covariates were included in crude and adjusted models: sex, race (white vs. nonwhite), route of infection (injection drug use vs. others), age at diagnosis of HIV infection (⩾40 vs. <40 years), and calendar year of diagnosis of HIV infection (before vs. after 1 January 1997). Furthermore, the $CD4^+$ cell count at the index date (<200 vs. ⩾200 cells/μL) was included in the Cox regression analysis of PML incidence. In the analysis of mortality, we further included AIDS-defining events before PML diagnosis, the $CD4^+$ cell count at the onset of PML (<50 vs. ⩾50 cells/μL), and antiretroviral treatment status at the diagnosis of PML. Confounding was evaluated by the change-in-estimate method, in which covariates changing the estimate by <10% were excluded from the model [13]. The study was approved by the Danish Data Protection Agency. SPSS statistical software (version 15.0; SPSS) was used for data analysis.

RESULTS

We identified 4660 patients with HIV-1 infection in the Danish HIV Cohort Study, 11 of whom had PML diagnosed before 1 January 1995, leaving 4649 patients in the study with a total of 27,693 person-years of follow-up. Of these, 1971 patients (42%) had HIV infection diagnosed before 1 January 1995, 1050 (23%) died during follow-up, 48 (1%) were unavailable for follow-up, and 182 (4%) emigrated. Of the patients, 3500 (75%) were male, 3669 (79%) were white, 2080 (44.7%) were men who have sex with men, and 534 (11%) were injection drug users. The median age at the index date was 33.9 years (interquartile range, 21.7–56.2 years), and 3327 (72%) of the patients had been exposed to HAART.

Incidence of PML. In the study period, 47 patients met the criteria for a diagnosis of PML. The incidence rates for diagnosis of PML decreased considerably from the pre-HAART period to the early and late HAART periods (table 1). Among all patients receiving HAART and those who had received HAART for >6 months, the PML incidence rates were 0.7 (95% CI, 0.4–1.3) and 0.8 (95% CI, 0.4–1.3) cases per 1000 person-years at risk.

The incidence rates were 0.2 (95% CI, 0.1–0.6) and 9.1 (95% CI, 6.7–12.3) cases per 1000 person-years at risk, respectively, for patients with $CD4^+$ cell counts ⩾200 versus <200 cells/μL. A $CD4^+$ cell count of ⩾200 versus <200 cells/μL at diagnosis of HIV infection was the only significant marker for a decreased risk of PML (incidence rate ratio, 0.2 [95% CI, 0.11–0.47]). Evaluation by the change-in-estimate method showed that age, sex, race, injection drug use, or diagnosis of HIV infection before 1 January 1997 did not confound the beneficial effect of a $CD4^+$ cell count ⩾200 cells/μL.

Twelve patients developed PML despite receiving >6 months of HAART. By review of patient files, 7 of the 12 patients were confirmed to have experienced treatment failure due to viral resistance and/or compliance problems.

Characteristics and presenting symptoms in patients with a diagnosis of PML. Characteristics of the 47 patients with a diagnosis of PML are shown in table 2. At the onset of PML symptoms, most of the patients had advanced HIV disease, with a low $CD4^+$ cell count (median, 50 cells/μL) and a high viral load (median, 4.9 $\log_{10}$ HIV RNA copies/mL). In 9 of the

Table 1. Incidence rates (IRs) for progressive multifocal leukoencephalopathy (PML).

Category	PYR	PML events	IR (95% CI), cases/1000 PYR
Observation period			
1995–1996	3903	13	3.3 (1.9–5.7)
1997–1999	6559	12	1.8 (1.0–3.2)
2000–2006	17,231	22	1.3 (0.8–1.9)
Receiving HAART	17,571	13	0.7 (0.4–1.3)
$CD4^+$ cell count			
⩾200 cells/μL	20,818	5	0.2 (0.1–0.6)
<200 cells/μL	4604	42	9.1 (6.7–12.3)

Note. CI, confidence interval; HAART, highly active antiretroviral therapy; PYR, person-years at risk.

Table 2. Demographics and HIV-related characteristics of patients with progressive multifocal leukoencephalopathy (PML) diagnosed during the study period.

Characteristic	All (n = 47)	PML diagnosis 1995–1997 (n = 13)	PML diagnosis 1998–2006 (n = 34)	P
HIV RNA level, median (IQR), $\log_{10}$ copies/mL	4.9 (3.7–5.6)	NA	4.9 (3.7–5.6)	
$CD4^+$ cell count at diagnosis, median (IQR), cells/μL	50 (27–160)	40 (18–123)	58 (25–162)	.51
$CD4^+$ cell count <200 cells/μL at PML diagnosis	6 (13)	2 (15)	4 (12)	>.99
AIDS diagnosis before PML diagnosis	18 (38)	6 (46)	12 (35)	.49
Male	35 (74)	13 (100)	22 (65)	.01[a]
Age, median (IQR), years	48.7 (43.3–53.0)	50.6 (45.2–54.2)	46.1 (44.2–51.5)	.48
Men who have sex with men	23 (49)	12 (92)	11 (32)	<.001[a]
Heterosexually infected	18 (38)	1 (8)	17 (50)	.01[a]
Injection drug user	4 (9)	0 (0)	4 (12)	.56
White	42 (89)	13 (100)	29 (85)	.14
Interval between HIV infection and PML,median (IQR), years	4.6 (1.2–10.9)	4.3 (2.5–10.7)	5.8 (0.1–12.0)	.91
Nadir $CD4^+$ cell count <200 cells/μL	46 (98)	13 (100)	33 (97)	.53
Died during study period	35 (74)	12 (92)	23 (68)	.08
IRIS reported	2 (4)	0 (0)	2 (6)	>.99

Note. Data are no. (%) of patients, unless otherwise indicated. IQR, interquartile range; IRIS, immune reconstitution inflammatory syndrome; NA, not available.

[a] $P < .05$.

34 patients whose PML was diagnosed after 1997, HIV infection was diagnosed within 3 months before the PML diagnosis. Almost all patients had a nadir $CD4^+$ cell count <200 cells/μL.

The predominant neurological symptoms at the presentation of PML were coordination disturbances, cognitive defects, and limb paresis (table 3). In accordance with the high frequency of coordination disturbances, 25 patients (53%) had lesions involving the cerebellum and/or brainstem that were visualized radiographically. Very few patients presented with common signs of infection, such as fever and leukocytosis, along with the neurological symptoms, but 18 (47%) of 38 had an increased sedimentation rate or C-reactive protein level (table 4). CSF pleocytosis was rarely seen (2%), but elevated levels of protein in the CSF were seen in 49% of patients. Abnormal decreases in CSF glucose level were not observed.

In 25 patients (53%), the diagnosis of PML was based exclusively on clinical symptoms combined with either MRI (18 patients) or CT (7 patients) findings. In 22 patients (47%), the PML diagnosis was confirmed by brain biopsy (14 patients) and/or PCR for JCV in the CSF (table 4). Thirteen of 31 patients tested had positive PCR results for JCV in the spinal fluid. Interestingly, 7 of the 14 patients whose PML was diagnosed by means of brain biopsy had negative PCR results for JCV. The negative PCR samples were equally distributed between the 2 laboratories and spread over the whole study period (data not shown).

Mortality. A total of 35 patients with PML died during the study period. According to the records, 28 deaths were related to PML, 5 were due to unknown causes, 1 was HIV related (lymphoma), and 1 was not related to HIV (end-stage liver disease). Eighteen patients died within 4 months after the onset of PML, and 12 were available for evaluation 3 years after the diagnosis of PML. Kaplan-Meier survival curves for the patients with a diagnosis of PML are shown in figure 1. The median survival time for all patients with PML diagnosed during the period 1995–2006 was 1.02 years (95% CI, 0.0–2.5 years). Of 13 patients with PML diagnosed before 1997, 12 died during the study period, with a median survival of 0.4 years (95% CI, 0.0–0.7 years). Of 34 patients whose PML was diagnosed from 1997 onward, 23 died before 31 December 2006; their median survival time was 1.8 years (95% CI, 0.9–2.6 years). We found no differences in survival among patients with PML diagnosed in 1997–1999 versus 2000–2006 (data not shown).

Of the 47 patients with PML, 36 were treated with HAART, and 24 of the 36 died during the study period. The median survival time in this group was 1.8 years (95% CI, 0.8–2.8 years). Of the 12 patients who had PML diagnosed >6 months after the start of HAART, 11 died; their median survival was 0.4 years (95% CI, 0.0–2.0 years). The median survival times did not differ between patients whose diagnosis was based on brain biopsy findings before death and those whose diagnosis was based on characteristic clinical and radiological findings (data not shown).

In unadjusted analyses, a $CD4^+$ cell count $\geq$50 cells/μL at the diagnosis of PML (mortality rate ratio [MRR], 0.47 [95% CI, 0.24–0.93]) and a diagnosis of PML after 1997 (MRR, 0.48 [95% CI, 0.24–0.97]) were associated with reduced mortality. Neither variable was confounded by age, $CD4^+$ cell count at

Table 3. Neurological symptoms at primary presentation of progressive multifocal leukoencephalopathy (PML), at first follow-up after 4 months, and after 3 years.

Category	Disease presentation (n = 47)	Follow-up	
		4 months (n = 29)	3 years (n = 11)
Neurological symptoms			
Cognitive defects	27 (57)	14 (48)	4 (36)
Coordination disturbance	32 (68)	16 (55)	4 (36)
Speech disturbance	20 (43)	20 (69)	10 (91)
Visual impairment	13 (28)	14 (48)	2 (18)
Facial palsy	3 (6)	3 (10)	0 (0)
Limb paresis	20 (43)	14 (48)	1 (9)
Sensory affection	8 (17)	3 (10)	1 (9)
Seizures	6 (13)	6 (21)	4 (36)
Need help in everyday life	27 (57)	20 (69)	6 (55)
Status at follow-up			
Progression of neurological symptoms	…	11 (38)	2 (18)
Unchanged neurological symptoms	…	4 (14)	1 (9)
Improvement of neurological symptoms	…	14 (48)	8 (73)[a]

Note. Data are no. (%) of patients.

[a] Five patients returned to their pre-PML level of activity.

index date, sex, race, injection drug use, or AIDS-defining diagnosis before PML. Viral load at the time of the onset of symptoms was not included in the analysis, because of a lack of data (43% missing).

Neurological outcome. The neurological symptoms were largely unchanged after 4 months of follow-up. The level of help needed to perform everyday activities was high during the follow-up period (table 3). Patients surviving >3 years fell into 2 groups: patients with progression of neurological symptoms or unchanged symptoms (27%), and those with a high degree of restitution or a return to their pre-PML level of life activity (73%). Rescanning was not done systematically. Of 15 patients who underwent MRI rescanning after the initiation of HAART, only 3 had a partial regression of lesions, but a wide time interval (varying from weeks to years) precludes systematic conclusions.

IRIS. Only 2 patients had IRIS reported in the patient files as a possible explanation for neurological deterioration after the initiation of HAART. Both patients died, at 40 and 59 days after the diagnosis of PML. Review of their MRI scans revealed no signs of contrast enhancement.

DISCUSSION

In this population-based cohort study, we found that the incidence of PML has steadily decreased after the introduction of HAART. Survival has improved, but the prognosis for patients with PML remains poor, with a high mortality rate and a high degree of neurological sequelae among survivors despite free access to HAART.

To our knowledge, this study is the first to present nationwide analysis of the incidence, presentation, and prognosis of PML in HIV-infected patients. A major strength of the study is the population-based design, with long and nearly complete follow-up. This allowed us to estimate the incidence rates of PML for pre-HAART, early HAART, and late HAART periods and the incidence of PML stratified by $CD4^+$ cell count.

Our study has some weaknesses. The diagnosis was confirmed by histology or PCR in only half of the patients. This may lead to the concern that some patients were misclassified. However, the group with confirmed PML did not differ in prognosis from the group whose diagnoses were based on clinical and radiological findings. Assessment of neurological deficits and performance by standardized scoring systems would have been preferable, because patient files are known to be incomplete at registration. Consequently, the retrospective design of this part of the study will probably tend to underestimate the degree of neurological symptoms and sequelae after the diagnosis of PML. The prognosis after diagnosis may therefore be even worse than what we have observed.

Our results show that the incidence of PML decreased after the introduction of HAART and that it continued to decline over time during the HAART period. This decrease is probably associated with increased effectiveness of HAART due to better and more simple drug regimens, as documented by increased survival [14] and decreased treatment failure [15]. The main

risk factor for the development of PML is low $CD4^+$ cell count, and we clearly demonstrated that the risk of PML is extremely low when the $CD4^+$ cell count is $\geqslant$200 cells/μL. We mainly observed PML in late presenters and in patients with failure of HAART due to compliance problems. Even though we expect the effectiveness of antiretroviral treatment to continue to increase in the near future, the incidence of PML will probably not decrease considerably in coming years unless more measures are taken to diagnose HIV infection earlier. Our data clearly demonstrate that the main focus in the management of PML should be on the prophylactic effect of maintaining a $CD4^+$ cell count $\geqslant$200 cells/μL.

The neurological symptoms we observed at the presentation of PML and at follow-up are in accordance with previous findings, in which the main presenting symptoms of PML were cognitive impairment, paresis, and cerebellar affection, with seizures in only a few patients [5, 9, 10, 16]. In accordance with a report from Albrecht et al. [10], half of the patients with PML had elevated protein levels but no pleocytosis in the CSF. These findings indicate that signs of universal or meningeal inflammation are not suggestive of PML.

PCR for JCV in CSF has high specificity (96%–99%), but reported sensitivities have varied from 57% to 90% [17, 18]. We also found a low sensitivity of PCR for JCV. It has been proposed that this low sensitivity is due to periventricular localization of PML lesions, which may result in low shedding of the virus in CSF. Therefore, ante- or postmortem biopsy, which has high sensitivity and specificity, is the reference standard [18]. However, biopsy may be contraindicated, the PML lesions may be difficult to access, and the patient may be unwilling to participate; for these reasons, most agree that the diagnosis is presumptive in patients with progressively deteriorating neurological function and characteristic findings on

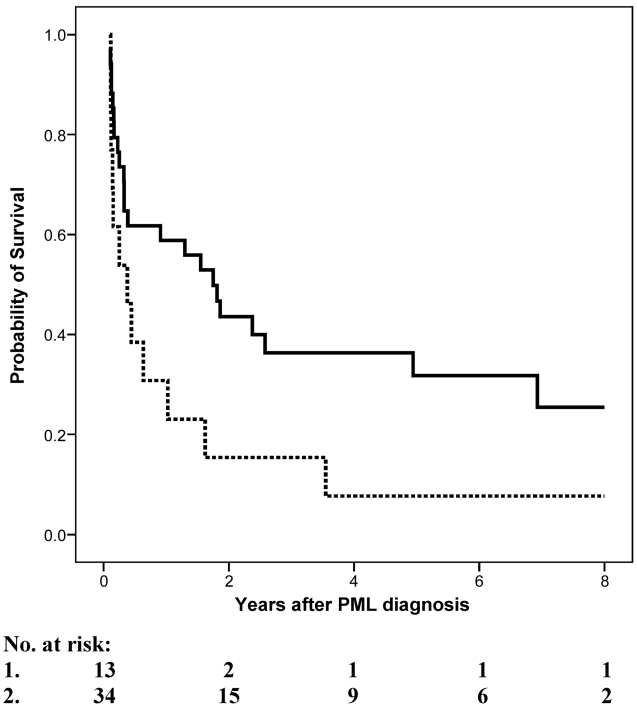

Figure 1. Kaplan-Meier curves for overall survival by time after progressive multifocal leukoencephalopathy (PML) diagnosis, before *(dotted line and group 1)* and after *(solid line and group 2)* 1997.

Table 4. Clinical and paraclinical data at presentation with progressive multifocal leukoencephalopathy (PML) and results of diagnostic tests.

Category, parameter	Proportion (%)
Clinical and paraclinical signs at presentation	
CSF pleocytosis (>10 cells/μL)	1/41 (2)
Elevated CSF protein level (>0.5 g/L)	19/39 (49)
Abnormal CSF glucose level compared with periphery	0/38 (0)
Fever >37.5°C	9/47 (19)
Fever >38.0°C	2/47 (4)
Leukocytosis in peripheral blood (>9 $\times$ 10^9 cells/L)	3/46 (7)
Elevated sedimentation rate or CRP level	18/38 (47)
Results of diagnostic tests for PML	
PML diagnosed by brain biopsy[a]	14/47 (30)
JCV detected by PCR in CSF	13/31 (42)
PML diagnosed by brain biopsy with PCR for JCV negativein CSF[b]	6/14 (43)
PML diagnosed by MRI/CT and clinical symptoms	25/47 (53)

Note. CRP, C-reactive protein; CSF, cerebrospinal fluid; CT, computed tomography; JCV, JC virus; MRI, magnetic resonance imaging; PCR, polymerase chain reaction.

[a] PML was diagnosed by means of in situ hybridization for JCV in 7 of the 14 patients and by means of JCV immunohistochemistry in 1 patient; 6 patients had characteristic histopathological features consistent with PML.

[b] One of the 14 patients whose PML was diagnosed by means of brain biopsy did not undergo PCR for JCV.

MRI or CT. This approach to diagnosis was exemplified in the present study; 53% of diagnoses were not based on brain biopsy findings or PCR for JCV. The difficulties in confirming the diagnosis are as likely to lead to the underdiagnosis of PML as to overdiagnosis.

We observed a beneficial effect of HAART on mortality in patients with a diagnosis of PML, with a median survival of 1.8 years. This finding is considerably improved compared with pre-HAART survival [19]; however, compared with other findings in HIV-infected patients with PML, the effect of HAART seemed to be lower in our study [6, 8, 9]. Previous studies were characterized by shorter observation periods. Furthermore, because they were not population based, they may have suffered from potential selection bias. In selected cohorts, there is a risk that the patients affected most by PML have not been included in the study population. A beneficial effect of cidofovir on PML has been proposed in some studies [7, 20]. Given that only 1 of our patients was treated with cidofovir, it could not have influenced our results.

The development of PML after 1997 was a considerable prognostic factor for survival, most likely because of the effect of HAART. This finding is supported by the identification of $CD4^+$ cell counts $\geqslant$50 cells/μL at PML diagnosis as another prognostic factor. Similar observations were made by Antinori et al. [8], who also found that both patients previously exposed to HAART who continued treatment after PML diagnosis and those starting HAART at the time of PML diagnosis had a significantly reduced risk of death compared with patients who did not receive HAART.

Although there is no specific treatment for PML, restoration of the immune defect is essential. Paradoxically, this may lead to PML as a part of IRIS. Of note, only 2 patients had a possible diagnosis of IRIS. The true incidence of IRIS among those with PML is unclear; the reported incidences vary from 6.5% to 18% among HIV-infected patients with PML [21, 22].

In conclusion, the present study shows that the incidence of PML has decreased and that its prognosis has improved in HIV-infected patients after the implementation of HAART, but the disease still has a high mortality rate, and patients who survive are often left with severe neurological sequelae. The main focus in the management of PML should be on prophylactic measures, on maintaining a high $CD4^+$ cell count through early diagnosis of HIV infection, and on initiating HAART before immunological deterioration.

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