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The effect of highly active antiretroviral therapy–induced immune reconstitution on development and outcome of progressive multifocal leukoencephalopathy: Study of 43 cases with review of the literature

Paola Cinque,[1] Simona Bossolasco,[1] Anna Maria Brambilla,[1] Antonio Boschini,[2] Cristina Mussini,[3] Chiara Pierotti,[1] Adriana Campi,[4] Salvatore Casari,[5] Davide Bertelli,[6] Maurizio Mena,[7] and Adriano Lazzarin[1]

[1]Clinic of Infectious Diseases, San Raffaele Hospital, Milano, Italy; [2]San Patrignano Medical Center, Rimini, Italy; [3]Infectious Diseases Clinic, University of Modena, and Reggio Emilia School of Medicine, Modena, Italy; [4]Department of Neuroradiology, San Raffele Hospital, Milano, Italy; [5]Clinic of Infectious and Tropical Diseases, University of Brescia, Brescia, Italy; [6]Ist Division of Infectious Diseases, Spedali Civili, Brescia, Italy; and [7]Division of Internal Medicine and Infectious Diseases, Cuggiono Hospital, Cuggiono, Italy

The authors investigated the effect of highly active antiretroviral therapy (HAART) on the onset and outcome of progressive multifocal leukoencephalopathy (PML) in a group of 43 patients with histological or clinicovirological diagnosis of PML. In eight of these cases (19%), PML symptoms presented 21 to 55 days after the start of HAART, concomitantly with a CD4 cell-count increase and plasma human immunodeficiency virus type 1 (HIV-1) RNA load (VL) decrease. Four of these patients died of PML. Apart from baseline VL, we did not identify any other variable that could distinguish these forms of *immune reconstitution* PML from those occurring in patients either untreated or failing to respond to therapy. To compare the viroimmunological response to HAART with PML outcome, we evaluated a subgroup of 23 patients untreated at the time of PML onset. No different pattern of response to HAART was observed between patients who died or survived to PML. However, start of HAART was delayed of $\geq$3 months after onset of PML in half of the latter patients. In conclusion, HAART-associated immune reconstitution seems to play a role on development of a substantial number of PML cases. Although the authors could not demonstrate a directly deleterious effect of HAART on PML progression, prompt initiation of HAART after diagnosis of PML and subsequent successful response were often associated with bad PML outcome. *Journal of NeuroVirology* (2003) **9(suppl. 1),** 73–80.

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Introduction

Most human immunodeficiency virus (HIV)-infected patients receiving highly active antiretroviral therapy (HAART) experience a gradual restoration of their immune defenses, in parallel with a decrease of plasma HIV-1 RNA levels. During the first weeks of HAART, it is not infrequent to observe inflammatory reactions that, in some cases, may be associated with clinical deterioration. These manifestations are the expression of a paradoxical response of the host's immune system to a variety of infectious or noninfectious antigens and have been termed as *immune reconstitution disease* (IRD) (De Simone *et al*, 2000; Shelburne *et al*, 2002).

Address correspondence to Dr. Paola Cinque, Clinic of Infectious Diseases, San Raffaele Hospital, Via Stamira d'Ancona, 20, 20127 Milano, Italy. E-mail: paola.cinque@hsr.it
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JC virus (JCV)-induced progressive multifocal leukoencephalopathy (PML) has been suggested to be, in some cases, an IRD. Cases of PML can be observed that develop soon after the start of HAART (Mayo *et al*, 1998). Furthermore, inflammatory reactions, e.g., contrast enhancement at magnetic resonance imaging (MRI) or perivascular infiltrates in brain biopsies, have been described in HAART-treated patients during the period of immune reconstitution (Miralles *et al*, 2001).

The outcome of PML is unpredictable at the time of disease onset. Although approximately half of HAART-treated patients will improve or stabilize, and eventually survive to PML, the other half of patients will inevitably deteriorate and die. We recently hypothesized that HAART-associated immune reconstitution could be deleterious in certain cases of HIV-associated PML (Cinque *et al*, 2001). This hypothesis was based on the observation that PML patients who start HAART frequently progress despite an efficient viroimmunological response to therapy. On the contrary, we noticed that the rate of patients showing PML stabilization was higher among patients switching from a two–nucleoside reverse transcriptase inhibitor (NRTI) regimen to HAART or in patients taking HAART irregularly because of drug toxicity. Taken together, these observations suggest that a gradual, rather than drastic, immune reconstitution might be associated with better PML prognosis.

The objectives of the present study were to verify this hypothesis on an enlarged number of patients and in particular, (1) to describe frequency and main features of PML presenting in patients successfully treated with HAART; (2) to describe frequency and main features of PML cases that progress despite immunological and virological response to HAART; (3) to review the literature for PML cases that developed during succesful HAART or progressed with evidence of inflammatory reactions; (4) to discuss hypotheses regarding mechanisms of disease onset and progression.

Results

Out of 43 PML patients, 27 (63%) were receiving no antiretroviral therapy at onset of the disease. Twenty of these 27 patients were naïve for anti-HIV drugs, whereas 7 had previously been treated with HAART ($n = 4$) or zidovudine alone ($n = 3$). All of the latter had discontinued treatment at least 3 months before development of PML. Sixteen of 43 patients (37%) were either receiving HAART ($n = 13$) or a 2-NRTI combination ($n = 3$) at the onset of PML: 5 had failed to respond and 11 were showing a virological and immunological response. Based on type of HAART and treatment response, patients were thus divided into three groups: *untreated* ($n = 27$), *treated-responders* ($n = 11$), and *treated-failure* ($n = 5$) (Table 1).

Onset of PML during HAART

In 9 of the 11 treatment-responders, PML symptoms presented 21 to 55 days from the start of HAART (8 patients, median 31 days) or of a 2-NRTI combination (1 patient, 91 days). In the other two patients, PML developed after 9 and 13 months, respectively (Table 2). As expected, both viral load (VL) and the time interval between start of treatment and PML onset were significantly lower in the treated-responders than in the treated-failure or untreated patients and the rate of patients with a previous diagnosis of AIDS was higher in the treated-failure group. Both median CD4 values and proportion of patients with CD4 greater than 200 were higher in the treated-responders, although not significantly. None of the other variables differed significantly between treated-responders and the other two groups, either examined separately or as a whole (Table 1).

Progression of PML during HAART

Overall, the disease progressed to death in 23 patients (53%) and turned to inactive in the remaining 20, who are all still alive after up to 75 months from PML onset. Significant difference between the three patient groups was neither observed in the rate of progression, nor in the duration of disease in patients who died of PML (Table 1).

The group of 27 patients who were untreated at PML onset was chosen for comparing the response to HAART with the evolution of PML. In 23 of these patients, sufficient follow-up data were available for comparison with baseline values. A significant VL decrease was observed within the first 8 weeks of treatment in both patients with progressive disease ($n = 12$, $P = .004$; signed rank test) and in those who showed stabilization ($n = 11$, $P = .01$). After 8 weeks of therapy, the VL decrease was of less than 1 log in two of the former patients and in three of the latter. The number of CD4 cells increased in 8 of 11 patients with disease progression and in 5 of 6 who survived. Contrast-enhancing MRI lesions were observed in two patients, 23 and 25 weeks, respectively, after the start of HAART. Clinical improvement preceded this finding. Because one of these patients had sequential MRI evaluations and cerebrospinal fluid (CSF) specimens, it was possible to document that clearance of JCV DNA from CSF was antecedent of at least 7 weeks contrast enhancement appearance at MRI.

Other variables were also compared to outcome in the whole group of 27 patients. These included the interval between PML onset and start of HAART, PML as first acquired immunodeficiency syndrome (AIDS)-related event, presence of concomitant central nervous system (CNS) diseases, baseline CD4 and CD8 cell counts, CD4/CD8 ratio, plasma and CSF

Table 1 Distribution of patient and treatment variables at PML onset in 43 patients with HIV-associated PML divided according to previous treatment

	A. Untreated (n = *27*)	*B. Treated-responders*[1] (n = *11*)	*C. Treated-failure*[1] (n = *5*)	P
Age (median years, range)	36 (28–55)	37 (32–42)	37 (32–42)	n.s.
Sex (F:M)	10:17	3:8	0:5	n.s.
Previous AIDS	7 (26%)	3 (27%)	(100%)	.002[2]
				.007[3]
Concomitant CNS OIs	6 (22%)	0	2 (40%)	n.s.
Days from anti-HIV therapy		35 (21–386)	500 (249–1236)	.003[4]
Type of anti-HIV therapy		2NRTI = 1	2NRTI = 2	
		NRTI + PI = 8	NRTI + PI = 2	
		NRTI + NNRTI = 1	NRTI + NNRTI = 1	
		3NRTI = 1		
CD4 (cells/μl)	68 (6–725)	70 (23–419)	42 (21–104)	n.s.
CD8 (cells/μl)	583 (185–1686)	563 (207–1127)	469 (309–733)	n.s.
CD4/CD8 ratio	0.14 (0.01–0.53)	0.26 (0.02–0.61)	0.19 (0.06–0.34)	n.s.
Plasma HIV-1 RNA (log copies/ml)	5.20 (3.13–>6.00)	3.31 (1.90–4.11)	5.38 (4.69–>6.00)	.004[5]
				.0005[6]
CSF HIV-1 RNA (log copies/ml)[7]	2.88 (2.60–5.74)	2.60 (2.60–3.55)	2.99 (2.60–4.54)	n.s.
No. patients examined	9	6	4	
CSF JCV DNA load (log copies/ml)[7]	3.44 (2.00–7.71)	3.24 (2.74–5.60)	3.12 (2.88–5.93)	n.s.
No. patients examined	15	7	4	
MRI enhancement[8]	0 (1)	0 (2)	0	
Infratentorial MRI lesions	15 (58%)	9 (82%)	4 (80%)	n.s.
PML progression	14 (52%)	6 (55%)	3 (60%)	n.s.
Survival (days)[9]	121 (61–547)	123.5 (59–233)	98 (71–100)	n.s.

Note. The number indicates the number of patients if not otherwise specified. Similarly, nonsignificant results were obtained by comparing only the eight patients who developed PML within 55 days of HAART to the other groups.
[1]Group B includes one patient who responded and group C two patients who did not respond to a combination of 2 NRTIs.
[2]B versus A.
[3]B versus C.
[4]Regimen containing versus not containing protease inhibitors (PI).
[5]B versus A.
[6]B versus C.
[7]Only patients whose CSF was sampled within 40 months from PML onset were considered.
[8]A total of three patients developed contrast enhancement 12 (one patient in group A), 23, and 25 weeks (two patients in group B) after initiation of HAART.
[9]Only patients who died of PML were considered.

HIV-1 RNA load, type of HAART, treatment with cidofovir, and year of PML onset. None of these variables differed significantly between the groups of patients with bad ($n = 14$) or good outcome ($n = 13$) (Mann-Whitney or chi-square test). Ten patients with bad outcome received protease inhibitors (PIs) + 2 NRTIs and four patients PIs + a non-nucleoside RTI (NNRTI). Twelve of the 13 patients who survived were treated with PI + 2 NRTIs and one with three NRTIs.

A first remarkable observation, however, was that the interval between PML and HAART was uniformly between 13 and 59 days in patients with progressive disease (median 38 days) and either less than 26 days (six patients) or more than 71 (seven patients, range 71–239) in patients who would stabilize. Three of the latter had received 2 NRTIs for 61 to 239 days before initiating HAART. Secondly, an increasing trend was observed with calendar year in the rate of patients in whom PML progressed. Considering the whole initial group of 43 PML patients, this finding became significant: patients who survived to PML were 16 of 26 (62%) in the years 1996–1998, but only 4 of 17 (24%) in the period 1999–2001 ($P = .015$, chi-square test).

Review of the literature

Onset of PML during HAART

Only a few PML cases have been retrieved developing a few to several weeks or months after the start of HAART in patients who responded to treatment in terms of increasing CD4 cells and decreasing viral load (Table 2).

Progression of PML during HAART and possible effects of immune reconstitution

There are recent case reports of PML patients whose conditions worsened either transiently or progressively following the start of HAART. Miralles *et al* (2001) described three patients who worsened clinically and radiologically. Because of this worsening, brain biopsy was performed at week 7, 14, and 15, respectively, after HAART. Histological examination showed in all the cases a marked perivascular infiltrate with lymphoplasmacytic cells. Eventually, one of these patients died, one improved later in time,

Table 2 Main characteristics of patients who developed PML while receiving successful anti-HIV therapy

Case no.	*Sex, age*	*Means of diagnosis*	*Days after HAART*	*Type of anti-HIV therapy*	*Inflammatory reactions*	*Pretherapy CD4 (cells/μl)*	*PML CD4 (cells/μl)*	*Pretherapy VL (copies/ml)*	*PML VL (copies/ml)*	*Outcome (days of survival)*	*Reference*
1	M, 37	CSF JCV+	21	3TC, d4T, RTV	CE MRI lesions at week 12	8	53	150000	2170	Survived (1895)	This article
2	M, 37	CSF JCV+	25	AZT, 3TC, IDV		40	na	104000	<400[1]	Survived (1766)	This article
3	M, 39	CSF JCV+	26	3TC, d4T, IDV		11	23	440000	430	Died (59)	This article
4	F, 32	CSF JCV+	30	3TC, d4T, RTV		162	387	132000	1900	Survived (1825)	This article
5	F, 37	CSF JCV+	32	3TC, d4T, ABV		3	38	1300000	5800	Died (146)	This article
6	M, 42	CSF JCV+	35	AZT, 3TC, IDV		62	70	86000	<80	Survived (1558)	This article
7	F, 45	CSF JCV+	38	AZT, 3TC, NFV		154	419	42000	<400	Died (98)	This article
8	M, 33	CSF JCV+	55	3TC, d4T, IDV		na	241	66700	5100	Died (216)	This article
9	M, 36	Biopsy	91	ddI, d4T	CE MRI lesions at week 23	na	397	na	12800	Survived (1825)	This article
10	M, 36	CSF JCV+	279	AZT, 3TC, SQV		57	67	na	<300	Died (101)	This article
11	M, 35	CSF JCV+	386	3TC, d4T, NVP		140	418	na	<500	Died (233)	This article
12	M, 36	Clinicoradiological	2 weeks	AZT, 3TC, IDV		22	57	na	na	Improved	Mayo *et al*, 1998 Collazos *et al*, 1999
13	M, 40	Clinicoradiological	2 months	AZT, 3TC, IDV	CE MRI lesions at PML onset	166	300	114000	460	Improved	Mayo *et al*, 1998
14	M, 37	Clinicoradiological	5 weeks	3TC, d4T, RTV	CE MRI lesions at PML onset	26	332	22000	1200	Improved	Mayo *et al*, 1998 Collazos *et al*, 1999
15	M, 51	CSF JCV+	1 year	3TC, d4T, SQV-SG		na	656[2]	na	4095[2]	Died (60)	Tantisiriwat *et al*, 1999
16	M, 45	Biopsy	9 months	AZT, 3TC, IDV	CE MRI lesions at PML onset	na	36	27522	4047	Died (90)	Tantisiriwat *et al*, 1999
17	M, 37	CSF JCV+	3 months	3TC, d4T, RTV, SQV		na	na	na	2590	Improved	Tantisiriwat *et al*, 1999

Note. CD4 cell counts and VL (plasma viral load) were obtained within 1 month from start of treatment or PML onset, unless otherwise indicated.
[1]Obtained 10 weeks after PML onset.
[2]Obtained 2 months after PML onset.
AZT: zidovudine; 3TC: lamivudine; ddI: didanosine; d4T: stavudine; ABV: abacavir; RTV: ritonavir; IDV: indinavir; NFV: nelfinavir; SQV: saquinavir; SQV-SG: saquinavir soft gel; CE MRI: contrast enhancement at magnetic resonance imaging.

and one was lost to follow-up. This report also illuminates on the frequency of HAART-induced inflammatory reactions in HAART-treated patients. A review of 28 brain biopsies performed between 1995 and 2001 showed a marked perivascular lymphomonocytic infiltrate in 5 cases (18%), 4 of whom were receiving HAART, and 1 2 NRTIs. Overall, such inflammatory aspects were seen in 4 of 9 HAART-treated patients and 1 of 19 HAART-untreated, resulting in a borderline significant distribution.

Kotecha *et al* (1998) reported of another patient who worsened clinically and radiologically but subsequently stabilized. Brain biopsy performed at the time of worsening showed a perivascular inflitrate, that like in the cases described by Miralles *et al*, consisted mainly of T lymphocytes, with B lymphocytes and plasma cells present in a lower proportion. Interestingly, this infiltrate was more prominent in a brain area that appeared as enhanced at MRI.

Contrast-enhancing MRI lesions were also described by Collazos *et al* (1999) in four patients during the phase of HAART-induced immune reconstitution. PML lesions turned to nonenhancing after 3 to 6 months in parallel with symptom improvement. Thurner *et al* (2001) presented a case in whom enhancement was observed after 4 weeks of HAART. It was intense, diffuse, distributed in the gray matter, and accompanied by mass effect. Also in this patient, both enhancement and mass effects disappeared with clinical improvement.

Finally, we reported of a transient worsening of MRI lesions in concomitance with a clinical improvement, i.e., onset of new lesions and/or extension of preexistent lesions, in four of five HAART-treated patients (Giudici *et al*, 2000). Such finding was observed after the first 3 months of HAART and was followed by radiological improvement or stabilization following another 3 to 6 months. A similar findings was also observed in two patients reported by Collazos *et al* (1999) who showed a contrast enhancement before or concomitantly with MRI worsening.

Discussion

Successful therapy with anti-HIV drugs can improve CD4 counts and function. In some cases, however, tissue injury may occur as a result of an aberrant response of a newly reconstituted immune system to a previously quiescent infection. IRD is defined by the onset of clinical symtpoms and/or pathological evidence of inflammation not explained by other causes, in concomitance with an increase in CD4 and a decrease in VL induced by anti-HIV drugs. Usually, these pictures are observed within the first weeks or months from the start of an antiretroviral treatment (Shelburne *et al*, 2002). We identified eight patients (19% of our cases) in whom PML developed within 55 days from the start of HAART. A fall in VL was constantly observed, together with a slight to marked increase in CD4 cells in most of the patients, which is consistent with an IRD. Although only few descriptions of immune reconstitution–associated PML were retrieved in the literature, this event was not uncommon in our series. Actually, its frequency might partly account for the relatively high prevalence of PML still observed in the HAART era and the higher proportion of PML cases observed in HAART-treated than in HAART-naïve patients (Antinori *et al*, 2001).

Two main scenarios have been hypotesized for IRDs. In the first one, the presence of latent or incubating infection and an only partially restored host's response are required. Typically, these forms present soon after initiation of HAART, i.e., 1 to 4 weeks. The second possibility is based on the presence of residual antigen in the presence of an adequate or aberrant immune response. These manifestations occur at a later time after the start of HAART, i.e., 2 to 4 months (Cooney, 2002). According to our observations, the common demonstration of JCV in CSF in cases of immune reconstitution PML, as well as the short interval between start of HAART and disease onset, suggests the presence of underlying mechanisms of the first type.

Different from the majority of IRD, however, our cases of immune reconstitution PML were often associated with poor outcome. Indeed, these forms behaved similarly to the classical PML cases, apart from the significantly lower VL and the higher CD4 cell counts at disease onset. In particular, the presence and amount of JCV DNA in CSF did not differ between patients with or without immune reconstitution PML. These observations make us speculate that HAART-induced improvement of the immune conditions might have triggered an early event in the pathogenesis of PML. Theoretically, latent or productive JCV infection might have been present in the brain at the start of therapy. However, there is so far no convincing evidence supporting the possibility of JCV latency of brain oligodendrocytes in HIV-infected patients. On the other hand, the hypothesis of a productive, yet subclinical, JCV infection of these cells would argue against the relatively high frequency of immune reconstitution PML cases, unless hypothesizing the possibility of a "transient" infection or of a long "incubation" period of the disease. Alternatively, HAART might have contributed to development of PML by favoring trafficking of either latently or persistently JCV-infected peripheral lymphocytes into the brain.

The second part of our study evaluated the possibility that HAART could be harmful on disease progression. To this purpose, we examined in detail the group of patients who were not taking any anti-HIV medication at the time of PML onset, which was regarded as the most appropriate to compare the evolution of PML with CD4 and VL changes in response to HAART. In general, most of these patients responded to HAART in terms of VL decrease and also showed a substantial increase in the CD4 cell counts.

Neither the rate nor the extent of viroimmunological response to HAART differed between patients in whom PML progressed or not, clearly indicating that a substantial number of patients progressed to death despite optimal response to HAART.

In our previous evaluation of a smaller group of HAART-treated patients with HIV-related PML, we not only observed that the disease can progress despite response to HAART, but also collected clues supporting the idea that a more gradual response to HAART may be associated with better PML prognosis. We found a higher rate of PML stabilization in patients switching from a 2-NRTI regimen to HAART or in those with a low adherence to HAART. In the present study, we additionally showed that the proportion of patients who survived PML was significantly higher in the first years of HAART than in the more recent years. This observation might support the hypothesis that an important immune reconstitution may be harmful on PML outcome, because more recent HAART regimens are less toxic and more potent than in earlier times and both adherence to therapy and patient management have substantially improved.

Notably, we also noticed that more than half of the patients with a favorable outcome had started HAART 3 months or more after development of PML. This finding may result only from selection of patients with a less aggressive disease; however, it is also possible that delaying of therapy might have avoided a possible bad effect of HAART on the first phases of disease evolution. The above observations in general, and this latter in particular, induce us to hypothesize that the first weeks of HAART might be crucial for subsequent evolution of PML. In other words, the overlapping of early PML and immune reconstitution might render the patient more vulnerable to deleterious effects of inflammation.

No factors have so far been clearly found that may predict PML outcome. Low JCV DNA levels in CSF have been reported to be associated with long survival (Yiannoutsos *et al*, 1999; Garcia de Viedma *et al*, 2002). However, because CSF samples are frequently drawn weeks after PML onset, it is possible that low CSF levels are a consequence, rather than the cause, of disease stabilization. In this regard, we have recently observed that CSF JCV DNA levels decrease in patients with favorable PML evolution, although their levels at PML onset are similar to those found in patients with poorer outcome (data not shown). On the other hand, other factors, e.g., CD4 cell counts or use of cidofovir, have not consistently been demonstrated to influence PML prognosis (De Luca *et al*, 2000; Marra *et al*, 2002). Interestingly, a cytotoxic T-lymphocyte activity against JCV antigen has been observed in patients who survived to PML (Koralnik *et al*, 2002). Comparison of this response with the extent of immune reconstitution will likely help elucidate the complex interactions between the virus and the host's immune response to HAART.

Finally, an important finding in the context of HAART-induced immune reconstitution in PML patients is the presence of inflammatory reactions, such as perivascular infiltrates at brain biopsy or contrast-enhancing MRI lesions. The histological demonstration of inflammation at the time of PML worsening supports the hypothesis that HAART-induced immune reactions can exert a bad influence on PML evolution (Miralles *et al*, 2001; Kotecha *et al*, 1998). On the other hand, MRI signs of inflammation, primarily contrast enhancement, have most commonly been observed in patients with favorable disease evolution.

Most likely, contrast enhancement is the expression of an altered permeability and integrity of brain vessels induced by inflammation, and comparison of radiological with histological findings has shown that the contrast-enhancing regions correspond to areas containing a perivascular infiltrate (Kotecha *et al*, 1998; Woo *et al*, 1996). Already in the pre-HAART era, contrast-enhancing lesions were more frequently observed in patients with a prolonged course of disease (Berger *et al*, 1998). According to our own and others' experience, contrast enhancement is usually associated with good PML outcome also in patients receiving HAART. However, there is no uniformity as for the time of appearance of enhancement, which can be observed after a variable interval of only a few to several weeks (up to 25 in our cases) after starting HAART. In addition, contrast-enhancing lesions have been described either before (Collazos *et al*, 1999; Kotecha *et al*, 1998) or following (our cases) clinical or radiological PML improvement. Interestingly, in one patient here described, JCV clearance from CSF preceded the appearance of MRI enhancement. This observation, as well as the finding that enhancement often follows clinical improvement, suggest that, in certain cases, inflammation might have no influence on disease evolution, and simply be an epiphenomenon of immune reconstitution.

In this regard, transient worsening of MRI lesions is common in patients in whom PML improves clinically (Giudici *et al*, 2000; Collazos *et al*, 1999). Furthermore, extensive white matter lesions unrelated to PML have occasionally been observed after a few weeks of HAART in patients otherwise asymptomatic (personal observation). These MRI alterations—contrast-enhancing, paradoxical MRI worsening and PML-unrelated leukoencephalopathy—have all in common of being observed soon after the start of HAART, being related to immune reconstitution and associated with good patient outcome. Although the underlying phenomena are not known, they appear as manifestations of a transient nonharmful brain inflammation, possibly resulting from recovered immune competence and consequent entry of inflammatory cells in the brain.

In conclusion, HAART-induced immune reconstitution was associated with PML development in approximately one fifth of our cases. These forms could

be defined as "immune reconstitution PML," although, phenotypically, they appear nearly identical to those observed in patients untreated with anti-HIV drugs. The unfavorable evolution of PML in HAART-treated patients was not associated with inadequate response to therapy, as indicated by the evidence of CD4 cells and VL improvement. On the contrary, there are suggestions that potent and early treatment regimens might even be harmful for PML evolution. Whether PML progression in successfully HAART-treated patients depends on a paradoxical rather than insufficient, inadequate, or untimely host's response to JCV infection, it remains an open question.

Patients and methods

We studied 43 patients with HIV-related PML, who were observed in six Italian hospitals in the period 1996–2001. All patients had a history of HAART, either before, after, or both before and after PML onset. PML was diagnosed by histology on tissues obtained by brain biopsy ($n = 2$) or by polymerase chain reaction (PCR) JCV DNA detection in CSF ($n = 41$) (Cinque *et al*, 1996). Diagnosis was confirmed at postmortem examination in four of the latter patients.

The following variables were recorded for each patient: demographic characteristics, risk factors, baseline and follow-up MRI and laboratory findings, survival, and treatment with antiretroviral drugs or cidofovir. HAART was defined as a combination of at least three drugs belonging to one or more of the PI, NRTI, or non-NRTI (NNRTI) classes. The term HAART has been used throughout this article, although a combination of 2 NRTIs was administered in three patients before developing PML and in four additional patients after PML onset HAART treated patients were defined as "treatment-responders" or "treatment-failure" in the presence or absence, respectively, of a plasma VL decrease from baseline of at least 1 $\log_{10}$.

JCV DNA load in CSF was measured by means of a newly developed real-time PCR assay using primers amplifying a fragment from the large T–antigen region (forward primer: 5′-GAGTGTTGGGATCCTGTG TTTTC-3′; reverse: 5′-GAGAAGTGGGATGAAGAC CTGTTT-3′; probe: 5′-(FAM)-TCATCACTGGCAAAC ATTTCTTCATGGC-(TAMRA)-3′). The sensitivity limit of the assay was of 100 genome equivalents/ml.

The review of the literature was performed through the National Library of Medicine (Medline). Articles were selected using variable combinations of the following terms: HIV, HAART, progressive multifocal leukoencephalopathy, immune reconstitution, immune reconstitution syndrome or disease, and immune restoration syndrome or disease.

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