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Impact of very early antiretroviral therapy during acute HIV infection on long-term immunovirological outcomes

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ABSTRACT

Objectives: We aimed to determine if starting antiretroviral therapy (ART) in the first 30 days after acquiring HIV infection has an impact on immunovirological response.
Methods: Observational, ambispective study including 147 patients with confirmed acute HIV infection (January/1995-August/2022). ART was defined as very early (≤30 days after the estimated date of infection), early (31-180 days), and late (>180 days). We compared time to viral suppression (viral load [VL] <50 copies/ml) and immune recovery (IR) (CD4+/CD8+ ratio ≥1) according to the timing and type of ART using survival analysis.
Results: ART was started in 140 (95.2%) patients. ART was very early in 24 (17.1%), early in 77 (55.0%), and late in 39 (27.9%) cases. Integrase strand transfer inhibitor (INSTI)-based regimens were the most used in both the overall population (65%) and the very early ART group (23/24, 95.8%). Median HIV VL and CD4+/CD8+ ratio pre-ART were higher in the very early ART group (P <0.05). Patients in the very early and early ART groups and treated with INSTI-based regimens achieved IR earlier (P <0.05). Factors associated with faster IR were the CD4+/CD8+ ratio pre-ART (hazard ratio: 9.3, 95% CI: 3.1-27.8, P <0.001) and INSTI-based regimens (hazard ratio: 2.4, 95% CI: 1.3-4.2, P = 0.003).
Conclusions: The strongest predictors of IR in patients who start ART during AHI are the CD4+/CD8+ ratio pre-ART and INSTI-based ART regimens.
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Introduction

Acute HIV infection (AHI) refers to the period immediately after acquiring HIV, when the virus spreads first through the mucosa and the local lymphoid tissue at the exposure site, and then disseminates systemically [1,2]. This initial phase of HIV infection can be divided into stages based on the sequential appearance of viral markers and antibodies in the blood [3]. It is during AHI when the latent HIV reservoir is established [4], although reseeding of the reservoir will continue in untreated chronic infection [5].

Early antiretroviral therapy (ART) during AHI rapidly decreases HIV viral load (VL) [6], reduces HIV transmission [7], and, while it does not prevent the establishment of the viral reservoir [4,8], it reduces its size [9,10]. Starting ART in the first 4-6 months after HIV infection has been linked to greater CD4+ T-lymphocytes (TL) counts and CD4+/CD8+ ratio increases [11–13] and shorter time to CD4+/CD8+ ratio normalization [14]. Since low CD4+/CD8+ ratios in people with HIV (PWH) correlate with immune activation, inflammation, and immune senescence [11,15,16], as well as increased morbidity and mortality [11,17], the normalization of the CD4+/CD8+ ratio serves as a marker for immune recovery. The privileged immunovirological recovery of PWH treated during early HIV infection has led to the belief that these patients might be optimal candidates for functional cure strategies. However, we still lack information on whether even earlier ART initiation could lead to further benefits.

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In this study, we aimed to describe a cohort of patients with AHI and to determine if starting ART in the first month after acquiring HIV infection has an impact on immunovirological response.

Patients and methods

Study design and participants

The Vall d'Hebron Acute HIV Infection (VHAHI) cohort is an ambispective cohort that includes patients 18 years of age or older with confirmed acute HIV-1 infection followed at the HIV outpatient clinic of Hospital Universitari Vall d'Hebron, a tertiary university hospital in Barcelona (Spain) that attends approximately 2200 PWH. In this study, patients enrolled in the VHAHI cohort between January 1995 and August 2022 were analyzed. Data were collected retrospectively from January 1995 until July 2019 and prospectively from August 2019 onward. We included follow-up data until July 2023.

Informed consent was obtained from all patients in the prospective cohort at the first clinic visit according to the recommendations given by the hospital ethics committee. Written informed consent was waived by the hospital ethics committee in the retrospective cohort.

Participants were classified into four 7-year groups according to the date of diagnosis: 1995-2001, 2002-2008, 2009-2015, and 2016-2022.

Study variables and data collection

Demographic, epidemiological, HIV-related variables (date of diagnosis, date of last previous negative HIV test, Fiebig stage, date of ART initiation, ART regimens), and CD4+ and CD8+ TL counts and HIV-1 VL at diagnosis, before starting ART and at months 1, 3, 6, 12, 18, 24, and 36 after starting ART were collected. Symptoms and signs of acute retroviral syndrome were confirmed by asking the participants and/or reviewing medical records. ART treatment selection was left to the treating physician's discretion.

Virological analyses

HIV-1 serology was determined using the techniques available at our center at each moment of the 28-year study period and confirmed by Western Blot or line blot immunoassay (LIA). HIV-1 VL was measured by real-time polymerase chain reaction assay with a lower limit of detection of 50 copies/ml.

Definitions

AHI infection was defined as (a) negative third or fourth generation assay for HIV-1 and positive HIV-1 plasma VL; (b) positive or negative third or fourth generation assay for HIV-1, negative or undetermined (<2 envelope bands) Western Blot or LIA, positive serum p24 antigen, and positive plasma HIV-1 VL; (c) positive third or fourth generation assay for HIV-1, and negative, undetermined or positive without p31 band Western Blot or LIA, and positive HIV-1 plasma VL; or (d) HIV-1 seroconversion in the last 6 months (evidence of a negative HIV-1 test in the last 180 days).

We calculated the estimated date of infection (EDI) using Fiebig staging [3] and previous HIV negative tests: 15 days before the first positive HIV test in Fiebig stage I, 20 days before the first positive HIV test in Fiebig stage II, 24 days before the first positive HIV test in Fiebig stage III, 29 days before the first positive HIV test in Fiebig stage IV, and 99 days before the first positive HIV test in Fiebig stage V. In Fiebig stage VI we defined the EDI as the midpoint between 99 days before the first positive HIV test and the date of the last HIV negative test. When Fiebig stage was unknown, we defined the EDI as the midpoint between the first positive HIV test and the last negative HIV test.

HIV-related hepatitis was defined as either aspartate aminotransferase or alanine aminotransferase values 10 times higher than the upper limit of normality in the absence of active hepatotropic virus infection. Thrombopenia was defined as platelet values below 150,000/mm^3 and severe thrombopenia was defined as platelet values below 100,000/mm^3 [18]. Lymphopenia was defined as less than 1,000 lymphocytes/mm3. Additional information can be found in the Supplementary Material section.

ART initiation was defined as very early (≤30 days after EDI), early (31-180 days), and late (>180 days). We defined viral suppression as HIV-1 VL <50 copies/mL and immune recovery as CD4+/CD8+ ratio ≥1.

End points

The primary end points were the time to viral suppression and immune recovery according to the timing of ART. Secondary end points were changes in epidemiological characteristics over time; the time to CD4+TL count ≥900 cells/µl [12] according to the timing of ART; the time to viral suppression, immune recovery, and CD4+TL count ≥900 cells/µl according to the type of ART; viral suppression and immune recovery at 12 months in the subgroup of patients who started ART before July 2022; and viral suppression and immune recovery at 36 months in the subgroup of patients who started ART before July 2020.

Statistical analysis

Categorical variables were expressed as absolute frequency and percentage, and continuous variables as median and interquartile range.

Fisher's exact test (for categorical variables) and Kruskal-Wallis test (for continuous variables) were used to perform comparisons between groups. We compared time to viral suppression, immune recovery, and CD4+TL count ≥900 cells/µl using the Kaplan-Meier method. We assessed for predictors of immune recovery and viral suppression using a multivariate Cox proportional hazards model to estimate hazard ratios (HR) and 95% CI. For additional information see the Supplementary Material section.

Statistical analyses were performed using IBM SPSS statistics software for Windows (Version 21; IBM, Armonk, NY, USA). All tests were two-tailed and a *P*-value of <0.05 was considered significant.

Results

Clinical and epidemiological characteristics and trends over time

One hundred and forty-seven patients met the criteria for confirmed AHI. Global demographic characteristics of these patients and temporal trends according to the period of the inclusion are shown in Table 1 and Supplementary Material (Table S1). The proportion of patients diagnosed in sexually transmitted infections (STI) and HIV clinics ($P = 0.001$) and the proportion of patients with concomitant STI at diagnosis ($P = 0.003$) increased significantly over time, with 38.6% of participants being diagnosed with a concomitant STI in the 2016-2022 period.

Clinical characteristics and Fiebig staging are shown in Table 2. In total, 115 (78.2%) patients had symptoms of acute retroviral syndrome. The proportion of symptomatic patients remained stable over the years ($P = 0.061$). A total of 75 (51%) patients had high aspartate aminotransferase or aminotransferase values, and eight

Table 1
Demographic characteristics at diagnosis and temporal trends of people with acute HIV infection.

	Total (n = 147)	1995-2001 (n = 4)	2002-2008 (n = 16)	2009-2015 (n = 39)	2016-2022 (n = 88)	*P*-value[a]
Age, years	32 (27-40)	36 (22-54)	31 (28-34)	32 (28-39)	32 (27-40)	0.903
Sex, male	134 (91.2)	2 (50)	13 (81.3)	37 (94.9)	82 (93.2)	0.022
Geographic origin						0.969
Spain	93 (63.2)	4 (100)	12 (75)	23 (59)	54 (61.4)	
Central/South America	32 (21.8)	0 (0)	3 (18.7)	9 (23)	20 (22.7)	
Europe (excluding Spain)	10 (6.8)	0 (0)	1 (6.3)	3 (7.7)	6 (6.8)	
Africa	6 (4.1)	0 (0)	0 (0)	3 (7.7)	3 (3.4)	
Other	6 (4.1)	0 (0)	0 (0)	1 (2.6)	5 (5.7)	
Symptoms (any)	115 (78.2)	4 (100)	11 (68.8)	32 (82.1)	68 (77.3)	0.061
Hospitalization	27 (18.4)	4 (100)	5 (31.3)	6 (15.4)	12 (13.6)	0.001
Place of diagnosis						0.001
Sexually transmitted infection/HIV clinics	54 (36.7)	0 (0)	3 (18.7)	7 (17.9)	44 (50)	
Emergency department	25 (17)	0 (0)	5 (31.3)	6 (15.4)	14 (15.9)	
Primary care	24 (16.3)	0 (0)	2 (12.5)	9 (23.1)	13 (14.8)	
Other	44 (29.9)	4 (100)	6 (37.5)	17 (43.6)	17 (19.3)	
Time from EDI to diagnosis, days	29 (24-99)	22 (20-28)	48 (29-99)	29 (24-90)	29 (22-99)	0.151
Time from EDI to first visit, days	49 (30-105)	33 (21-47)	104 (61-119)	75 (36-125)	43 (29-101)	0.006
Time from diagnosis to first visit, days	8 (2-20)	7 (0-25)	20 (4-69)	15 (6-32)	6 (2-14)	0.003
Time from EDI to ART, days	102 (34-203)	268 (94-1228)	516 (168-888)	209 (127-583)	43 (29-105)	<0.001
Time from diagnosis to ART, days	16 (5-107)	248 (68-1205)	452 (105-842)	179 (50-546)	8 (3-15)	<0.001
Time from first visit to ART, days	3 (0-56)	233 (58-1198)	325 (50-805)	67 (15-425)	0 (0-2)	<0.001
ART regimens						<0.001
INSTI-based	91 (61.9)	0 (0)	0 (0)	15 (38.5)	76 (86.4)	
NNRTI-based	24 (16.3)	1 (25)	9 (56.2)	13 (33.3)	1 (1.1)	
PI-based	21 (14.3)	1 (25)	5 (31.3)	7 (17.9)	8 (9.1)	
Other	4 (2.7)	2 (50)	0 (0)	0 (0)	2 (2.3)	
Lost before ART	7 (4.8)	0 (0)	2 (12.5)	4 (10.3)	1 (1.1)	

Results are expressed as number (%) or median (interquartile range). ART, antiretroviral therapy; EDI, estimated date of infection; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
[a] *P*-values are the result of Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables.

Table 2
Clinical presentation of patients with acute HIV infection.

Patient characteristics (n = 147)	n (%)
Route of transmission	
Men who have sex with men	107 (72.8)
Heterosexual	29 (19.7)
Injecting drug user	8 (5.4)
Unknown	2 (1.4)
Other	1 (0.7)
Drug use	51 (34.7)
Chemsex	15 (10.2)
Previous STI	66 (44.9)
STI at diagnosis	41 (27.9)
Previous pre-exposure prophylaxis use	4 (2.7)
Fiebig stage	
I	1 (0.7)
II	24 (16.3)
III	19 (12.9)
IV	40 (27.2)
V	40 (27.2)
VI	18 (12.2)
Unknown	5 (3.4)
Acute retroviral syndrome	115 (78.2)
Fever	107 (72.8)
Odynophagia	57 (38.8)
Lymphadenopathy	57 (38.8)
Myalgia/arthralgia	45 (30.6)
Rash	43 (29.3)
Diarrhea	40 (27.2)
Headache	34 (23.1)
Nausea/vomiting	29 (19.7)
Cough	21 (14.3)

STI, sexually transmitted infection.

(5.4%) fulfilled the criteria for HIV-related hepatitis. Thrombopenia was present in 51 (34.7%) cases, and severe thrombopenia was present in 21 (14.2%) cases. Lymphopenia was present in 36 (24.5%) cases. Thirty-two (21.8%) individuals had less than 350 CD4+TL/µl at diagnosis, and 11 (7.5%) patients had less than 200 CD4+TL/µl. Five (3.4%) patients presented meningoencephalitis. Seven (4.7%) patients developed a B or C event of the Centers for Disease Control and Prevention classification [19] during AHI: three oropharyngeal candidiasis, three esophageal candidiasis, and one herpes simplex esophagitis. Twenty-seven (18.2%) patients were hospitalized during a median of 6 days (4-9), none were admitted to the intensive care unit, and none died during AHI. The proportion of patients who were hospitalized decreased over time ($P = 0.001$).

Treatment was started in 140 (95.2%) patients, a median of 102 days (34-203) after the EDI. Six (4.1%) individuals were lost to follow-up before starting ART, and one participant died before starting ART (but not during the period of AHI). Seventeen (11.6%) participants were lost to follow-up within 36 months of ART initiation. Timing of ART was very early in 24 (17.1%) cases, early in 77 (55.0%) and late in 39 (27.9%) cases. Integrase strand transfer inhibitor (INSTI)-based regimens were the most frequently used (65%), followed by non-nucleoside reverse transcriptase inhibitor (NNRTI)-based (17.1%) and protease inhibitor (PI)-based (15%) regimens. Up to 2014 NNRTI-based and PI-based therapies were the most commonly used regimens, as compared to the most recent time periods, when INSTI-based regimens were more common ($P < 0.001$). In total 134 (95.7%) participants started ART with a 3-drug regimen.

Time from the EDI or confirmed diagnosis to ART initiation, and from first HIV clinic visit to ART initiation decreased significantly over time ($P < 0.001$). Time from the EDI to diagnosis remained stable over time ($P = 0.151$) (Table 1).

Immunovirological evolution

At diagnosis median CD4+TL count and HIV VL were 455 cells/µl (330-580) and 1,100,000 copies/ml (108,000-8,750,000), respectively, without significant changes over time ($P = 0.460$ and $P = 0.896$). Immunovirological baseline characteristics and evolution according to ART initiation timing are shown in Table 3. Me-

Table 3
Immunovirological response according to ART initiation timing.

	Very early ART (n = 24)	Early ART (n = 77)	Late ART (n = 39)	*P*-value[a]
Age, years	33 (26-40)	31 (27-41)	32 (28-36)	0.817
Sex, male	23 (95.8)	70 (90.9)	34 (87.2)	0.573
Time from EDI to ART, days	24 (22-26)	93 (41-109)	475 (209-861)	<0.001
ART regimens				<0.001
INSTI-based	23 (95.8)	59 (76.6)	9 (23.1)	
NNRTI-based	0 (0)	8 (10.4)	16 (41.0)	
PI-based	0 (0)	9 (11.7)	12 (30.8)	
Other	1 (4.2)	1 (1.3)	2 (5.1)	
Pre-ART				
HIV VL (copies/mL)	3,300,000 (873,500-10,000,000)	531,000 (103,500-2,640,000)	115,500 (40,750-207,500)	<0.001
CD4+TL (cells/ µL)	425 (311-500)	480 (360-600)	315 (252-454)	<0.001
CD8+ TL (cells/ µL)	580 (320-1,040)	1,023 (713-1,355)	938 (648-1,292)	0.003
CD4+/CD8+ ratio	0.71 (0.50-1.33)	0.49 (0.25-0.74)	0.39 (0.20-0.62)	<0.001
CD4+/CD8+ ratio ≥1	8 (33.3)	10 (13.0)	1 (2.6)	0.003
1 year after ART[b]	n=22	n=77	n=39	
HIV VL <50 copies/mL	16 (72.7)	63 (81.8)	32 (82.1)	0.159
CD4+TL (cells/ µL)	930 (720-1,035)	775 (606-1,045)	690 (505-835)	0.030
CD8+ TL (cells/ µL)	635 (472-903)	760 (550-1,050)	995 (713-1,187)	0.010
CD4+/CD8+ ratio	1.32 (1.00-2.04)	1.08 (0.81-1.43)	0.67 (0.53-0.93)	<0.001
CD4+/CD8+ ratio ≥1	15 (68.2)	41 (53.2)	5 (12.8)	<0.001
3 years after ART[c]	n=10	n=54	n=38	
HIV VL <50 copies/mL	8 (80)	45 (83.3)	29 (76.3)	0.360
CD4+TL (cells/ µL)	840 (578-1,015)	880 (560-1,070)	680 (572-954)	0.439
CD8+ TL (cells/ µL)	670 (640-1,060)	759 (520-870)	692 (566-982)	0.935
CD4+/CD8+ ratio	0.96 (0.90-2.13)	1.34 (0.88-1.58)	0.98 (0.75-1.29)	0.160
CD4+/CD8+ ratio ≥1	3 (30.0)	33 (61.1)	15 (39.5)	0.094

Results are expressed as number (%) or median (interquartile range). ART, antiretroviral treatment; EDI, estimated date of infection; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TL, T-lymphocytes; VL, viral load.
[a] *P*-values are the result of Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables.
[b] Participants who started ART until July 2022 (1 year before the analysis).
[c] Participants who started ART until July 2020 (3 years before the analysis).

dian HIV VL and CD4+/CD8+ ratio pre-ART were higher in the very early ART group (P <0.001). INSTI-based regimens were more frequent in the very early ART group (P <0.001). The proportion of patients with immune recovery was higher in the very early ART group at 1 year (P <0.001), but there were no differences in immune recovery at 3 years (P >0.05). Viral suppression was similar between very early, early, and late ART groups at 1 and 3 years (P >0.05 for all comparisons). If we exclude participants who were lost to follow-up or were not taking ART, rates of viral suppression at 3 years were 100% (8/8) in the very early ART group vs 97.8% (45/46) in the early ART group vs 93.5% (29/31) in the late ART group (P = 0.675). Baseline characteristics and immunovirological evolution at 1 and 3 years according to the type of ART are shown in Table S2 in the Supplementary Material section.

Results of the survival analysis are shown in Figure 1. Median time to viral suppression was 17 weeks (95% CI 15.00-19.00), without differences according to timing of ART (P = 0.741) or according to the ART regimen (P = 0.245).

Regarding immunological outcomes, patients in the very early and early ART groups achieved immune recovery earlier (21 weeks in the very early ART group [95% CI 0.00-55.84] vs 29 weeks in the early ART group [95% CI 10.61-47.39] vs 155 weeks in the late ART group [95% CI 130.15-179.85]; P = 0.001). Time to immune recovery was similar between the very early and early ART groups (P = 0.687). Patients in the very early ART group achieved CD4+TL counts ≥900 cells/µl significantly earlier (P = 0.029). Patients treated with INSTI-based regimens achieved immune recovery and CD4+TL counts ≥900 cells/µl significantly earlier (P <0.001 and P = 0.005, respectively).

In the adjusted multivariate Cox proportional hazards model, the variables associated with earlier immune recovery were the pre-ART CD4+/CD8+ ratio (HR 9.3, 95% CI 3.1-27.8, P <0.001) and the use of INSTI-based ART regimens (HR 2.4, 95% CI 1.3-4.2, P = 0.003) (Table 4). The only factor associated with earlier viral suppression using the adjusted multivariate Cox proportional hazards model was the pre-ART CD4+/CD8+ ratio (HR 1.5, 95% CI 1.1-2.1, P = 0.025) (Table S3 in the Supplementary Material section).

Discussion

In our study, we hypothesized that starting ART in the first 30 days after HIV infection could lead to immune and virological benefits compared to a later start during AHI.

Previous studies have described higher rates of immune recovery in patients treated within 4-6 months of acquiring HIV infection [11–14,20]. Furthermore, some cohorts (which mostly included participants with chronic HIV infection) have linked INSTI-based ART regimens to greater CD4+/CD8+ ratio gain and faster CD4+/CD8+ ratio restoration [21,22]. In our study, which only included participants with confirmed AHI, the initial Kaplan-Meier survival analysis suggested that very early and early ART was associated with earlier CD4+/CD8+ ratio normalization. While we did not find significant differences in time to immune recovery between very early and early ART, it should be noted that 33.3% of participants in the very early ART group were excluded from the survival analysis because they had a CD4+/CD8+ ratio ≥1 before the start of ART (compared to only 10% in the early ART group). Thus, even if the rate of CD4+/CD8+ ratio normalization was similar between the two groups, very early treated individuals were more likely to have and, therefore, maintain a normal CD4+/CD8+ ratio. In our study, there were no long-term (36 months) differences in median CD4+TL counts, CD8+TL counts, and CD4+/CD8+ ratio according to the timing of ART. Although we

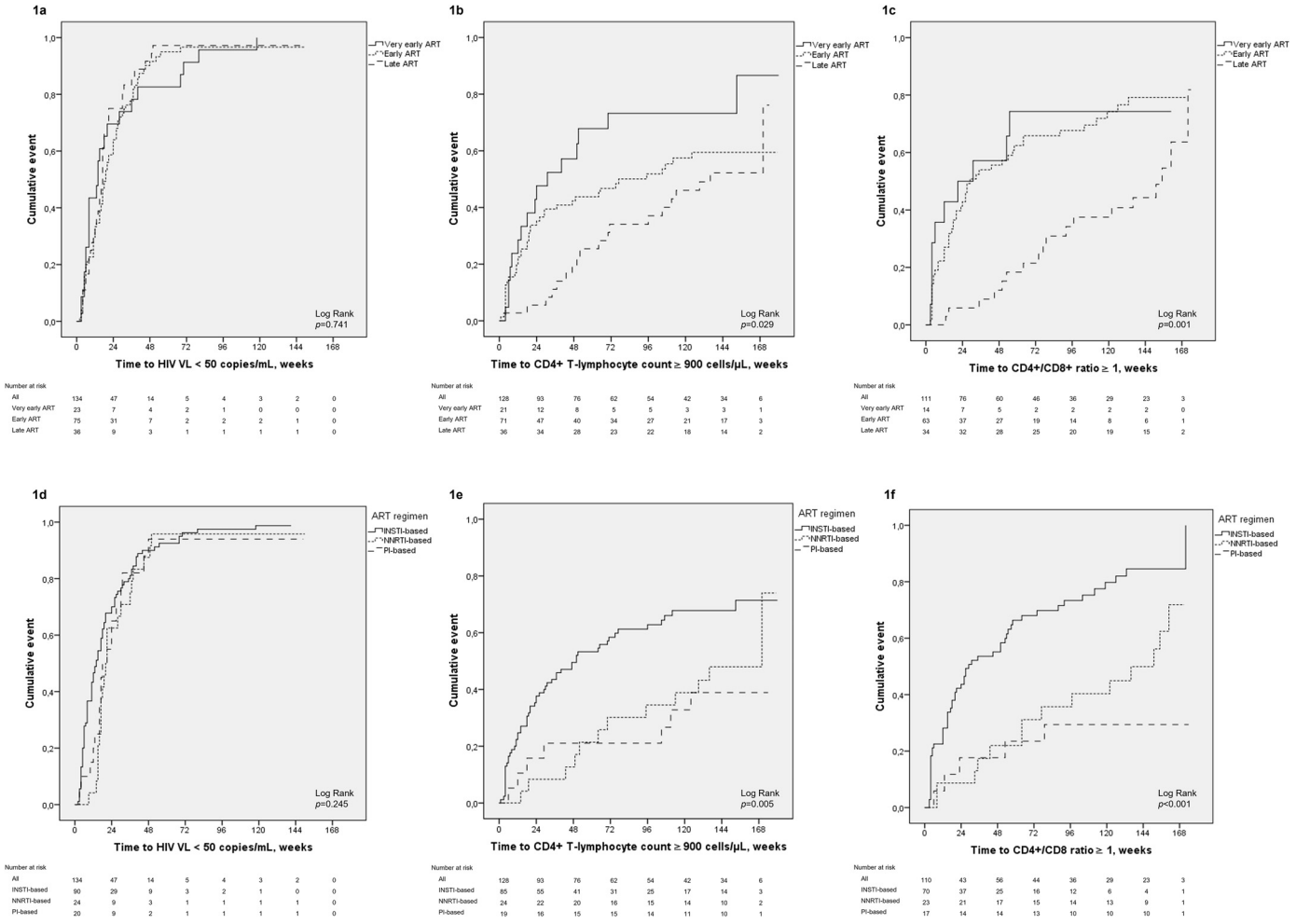

Figure 1. Kaplan-Meier survival analysis. Time since ART initiation to (1a) undetectable VL (HIV VL <50 copies/ml), (1b) CD4+ TL count ≥900 cells/µl and (1c) immune recovery (CD4+/CD8+ ratio ≥1) according to ART timing. Time since ART initiation to (1d) undetectable VL, (1e) CD4+ TL count ≥900 cells/µl and (1f) immune recovery according to ART regimen.
ART, antiretroviral treatment; TL, T-lymphocytes; VL, viral load.

have not assessed if there are long-term differences in the function of immune effector cells in our cohort, Takata et al. [23] have recently described preserved functional HIV-specific CD8+ T cells in individuals treated during AHI compared to those who started ART during chronic HIV infection.

After performing Cox proportional hazards model, we found that early or very early ART were not predictors of immune recovery. Indeed, the best predictors of immune recovery were a higher CD4+/CD8+ ratio pre-ART and INSTI-based ART regimens. A recent retrospective cohort comparing INSTI, NNRTI, and PI-based ART regimens started during AHI found similar proportions of patients achieving CD4+TL counts ≥900 cells/µl and CD4+/CD8+ ratio ≥1 [24]. Conversely, in our cohort, a higher proportion of patients treated with INSTI-based therapies achieved immune recovery

Table 4
Cox Proportional Hazards Model for predictors of immune recovery (CD4+/CD8 ratio ≥ 1).

Predictive factor	Univariate		Multivariate	
	HR (95% CI)	*P-value*	HR (95% CI)	*P*-value[a]
Very early ART (<30 days from EDI) (reference: late ART, >180 days from EDI)	2.984 (1.357-6.564)	0.007		
Early ART (31-180 days from EDI) (reference: late ART, >180 days from EDI)	2.601 (1.483-4.563)	0.001		
INSTI-based ART regimen	3.203 (1.873-5,478)	<0.001	2.371 (1.344-4.184)	0.003
CD4+/CD8+ ratio pre-ART	17.000 (5.848-49.420)	<0.001	9.261 (3.084-27.808)	<0.001
CD4+ TL pre-ART, cells/µL	1.002 (1.001-1.003)	<0.001		
HIV VL pre-ART, copies/mL	1.000 (1.000-1.000)	0.390		
STI at diagnosis	1.476 (0.894-2.438)	0.128		
Age, years	1.023 (0.996-1.050)	0.097		

ART, antiretroviral treatment; EDI, estimated date of infection; CI, confidence interval; HR, hazard ratio; INSTI, integrase strand transfer inhibitor; STI, sexually transmitted infection; TL, T-lymphocytes; VL, viral load.
[a] Adjusted for age (at diagnosis), timing of ART (very early or early compared to late), CD4+TL count pre-ART, CD4+/CD8+ ratio pre-ART, HIV VL pre-ART, STI at diagnosis, and INSTI-based ART regimen (vs other regimens).

at 12 and 36 months after starting ART compared to those who initiated other regimens. Although these results support the association between INSTI and CD4+/CD8+ ratio restoration [21,22], we could not find a link between timing of ART initiation and earlier immune recovery. Pre-ART median CD4+TL values and CD4+/CD8+ ratio were higher both in the very early ART group and in patients treated with INSTI-based therapy. Our findings could be partially explained by the widespread use of INSTI-based regimens in the very early ART group, which also had the highest pre-ART median CD4+/CD8+ ratio.

We did not find differences in the proportion of participants achieving viral suppression at 12 or 36 months according to the timing or type of ART. In a previous study [25], participants who started ART during Fiebig I stage achieved earlier viral suppression but had lower pre-ART HIV VL than those treated in later Fiebig stages. In our cohort, the HIV VL was significantly higher in the very early treatment group, but most individuals in this group were in Fiebig stages II-IV (only one participant was in Fiebig stage I). These disparities may explain the faster viral suppression found by Crowell et al. [25]. In any case, in our study, the only factor associated with earlier viral suppression was the pre-ART CD4+/CD8+ ratio, probably because it indicates an overall better-preserved immune function.

Reasons for hospitalization in people with AHI may vary depending on the treating physician's discretion and/or the year in which AHI occurred. Our hospitalization rate was slightly lower (18.4% vs 25.4%) than previously reported in a similar cohort [18]. Although we observed similar rates of meningoencephalitis (3.4% vs 4.9%) and CD4+TL counts <350 cells/µl (21.8% vs 25%), and a higher proportion of participants with severe thrombocytopenia (14.2% vs 2.4%), less than 5% suffered from an opportunistic infection. This could explain this lower percentage of hospitalization in our cohort. It is worth noting that, despite the need for hospital admission, there were no deaths related to HIV during AHI in either cohort.

We found no changes in the time from EDI to diagnosis of HIV infection over time. These observations emphasize the need to strengthen screening strategies. If we could diagnose HIV infection in earlier stages, we might decrease the burden of hidden HIV infection and, therefore, the risk of new infections caused by people who are unaware of their HIV status. Nevertheless, time between EDI and first clinic visit, between first clinic visit and ART initiation, and between EDI and ART initiation decreased significantly over time. Since 2016 the median time from first clinic visit to ART initiation went down to less than 1 day, which is effectively a same-day treatment. The earlier we start ART after acquiring HIV infection, the more preserved the CD4+/CD8+ ratio will be, which is the most important predictor of immune recovery according to our findings. These results are consistent with updates in HIV and ART clinical guidelines over the last 15 years, shifting from a delayed start after CD4+TL counts fell under a threshold to immediate ART initiation once the diagnosis is made and the patient is ready to start it [26,27]. Besides the benefits of early ART on immune recovery, as it is well known that AHI involves a greater probability of HIV transmission due to the high VL [28], this rapid ART initiation could hamper HIV transmission in our environment by swiftly suppressing viral replication.

There are some limitations that should be considered when interpreting these results. First, men who have sex with men were the main population included in our cohort, especially from Spain and Central/South America, and, thus, our observations might not be extensive to other populations. Second, as the cohort encompasses more than 20 years, recommendations on which regimen and when to start ART have changed over time. Biases in ART prescription, as well as confounding factors, cannot be excluded. In our cohort more than 95% of participants who started ART during the first 30 days after the EDI received an INSTI-based regimen. Although we have tried to adjust for potential confounders, our results should be interpreted considering a possible prescription bias. Even if this were the case, as current worldwide guidelines recommend starting INSTI-based regimens, we believe our results are relevant to people with AHI starting ART nowadays. Third, as not all participants in the cohort have completed a 36-month follow-up, the sample size 3 years after the start of ART is relatively small. Finally, this being an ambispective cohort, it may be subject to biases inherent in all study cohorts. To avoid this issue, we applied strict inclusion criteria and analyzed only confirmed cases of AHI. Patient clinical data were exhaustively reviewed one by one to assess the presence of symptoms, and to try to reduce any biases.

Despite these limitations, we believe that our work expands the knowledge on PWH diagnosed and treated during AHI, which is a very specific population that deserves special attention due to its clinical and immunological peculiarities.

Our findings have potential clinical implications. In our study, very early ART was not associated with better or faster immune recovery compared to early ART. However, since higher pre-ART CD4+/CD8+ ratios are linked to faster immune recovery and the CD4+/CD8+ ratio is more likely to be preserved in the earliest stages of AHI, our data support the recommendation that ART should be started as soon as possible during AHI.

In conclusion, our results suggest that, in patients with AHI, the pre-ART CD4+/CD8+ ratio is the strongest predictor of immune recovery. A first-line INSTI-based ART regimen could be more effective than other regimens in achieving earlier immune recovery. Still, further analyses are needed to know if this faster immune restoration has some impact on the burden of comorbidities in PWH, immune activation, or HIV-cure strategies.

Declarations of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

AC and JN, have received honoraria and/or speaking fees and/or financial support for attending conferences from Abbvie, Gilead, Janssen-Cilag, Merck Sharp & Dome, and ViiV Healthcare outside of the submitted work. JB, JG, PAL, VD, and VF have received honoraria and/or speaking fees and/or financial support for attending conferences from Gilead, Janssen-Cilag, Merck Sharp & Dome, and ViiV Healthcare outside of the submitted work. PS has received honoraria and/or speaking fees and/or financial support for attending conferences from Gilead, Janssen-Cilag, Merck Sharp & Dome, Pfizer, and ViiV Healthcare outside of the submitted work. MJB has received speaking fees from Gilead outside of the submitted work. AM has received speaking fees from ViiV Healthcare outside of the submitted work. The remaining authors declare no conflicts of interest.

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Ethical approval

This study was approved by the hospital ethics committee (PR(AG)377/2019 and PR(AG)358/2021) and followed the ethical principles based on the latest version of the Declaration of Helsinki. The processing of the patients' personal data collected in the studies complies with the Data Protection Act 1998 and with the European Directive on the Privacy of Data.

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Author contributions

PS, JN, and VF designed the study. PS was responsible for data collection. PS, JN, ARS, PAL, JG, VD, AM, MA, LR, BP, JB, AC, MJB, and VF contributed to data analysis and interpretation. PS and JN drafted the manuscript, which was critically revised and amended by ARS, PAL, JG, VD, AM, MA, LR, BP, JB, AC, MJB, and VF. PS and JN contributed equally to this work. All authors read and approved the final version of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.09.009.

References

[1] Cohen MS, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 infection. *N Engl J Med* 2011;**364**:1943–54. doi:10.1056/NEJMra1011874.
[2] Robb ML, Eller LA, Kibuuka H, Rono K, Maganga L, Nitayaphan S, et al. Prospective study of acute HIV-1 infection in adults in East Africa and Thailand. *N Engl J Med* 2016;**374**:2120–30. doi:10.1056/NEJMoa1508952.
[3] Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS* 2003;**17**:1871–9. doi:10.1097/00002030-200309050-00005.
[4] Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. *Proc Natl Acad Sci U S A* 1998;**95**:8869–73. doi:10.1073/pnas.95.15.8869.
[5] Pankau MD, Reeves DB, Harkins E, Ronen K, Jaoko W, Mandaliya K, et al. Dynamics of HIV DNA reservoir seeding in a cohort of superinfected Kenyan women. *PLOS Pathog* 2020;**16**:e1008286. doi:10.1371/journal.ppat.1008286.
[6] Ananworanich J, Eller LA, Pinyakorn S, Kroon E, Sriplenchan S, Fletcher JL, et al. Viral kinetics in untreated versus treated acute HIV infection in prospective cohort studies in Thailand. *J Int AIDS Soc* 2017;**20**:21652. doi:10.7448/IAS.20.1.21652.
[7] Kroon EDMB, Phanuphak N, Shattock AJ, Fletcher JLK, Pinyakorn S, Chomchey N, et al. Acute HIV infection detection and immediate treatment estimated to reduce transmission by 89% among men who have sex with men in Bangkok. *J Int AIDS Soc* 2017;**20**:21708. doi:10.7448/IAS.20.1.21708.
[8] Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997;**278**:1295–300. doi:10.1126/science.278.5341.1295.
[9] Buzon MJ, Martin-Gayo E, Pereyra F, Ouyang Z, Sun H, Li JZ, et al. Long-term antiretroviral treatment initiated at primary HIV-1 infection affects the size, composition, and decay kinetics of the reservoir of HIV-1-infected CD4 T cells. *J Virol* 2014;**88**:10056–65. doi:10.1128/JVI.01046-14.
[10] Massanella M, Bender Ignacio RA, Lama JR, Pagliuzza A, Dasgupta S, Alfaro R, et al. Long-term effects of early antiretroviral initiation on HIV reservoir markers: a longitudinal analysis of the MERLIN clinical study. *The Lancet Microbe* 2021;**2**:e198–209. doi:10.1016/S2666-5247(21)00010-0.
[11] Serrano-Villar S, Sainz T, Lee SA, Hunt PW, Sinclair E, Shacklett BL, et al. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. *PLOS Pathog* 2014;**10**:e1004078. doi:10.1371/journal.ppat.1004078.
[12] Le T, Wright EJ, Smith DM, He W, Catano G, Okulicz JF, et al. Enhanced CD4+ T-cell recovery with earlier HIV-1 antiretroviral therapy. *N Engl J Med* 2013;**368**:218–30. doi:10.1056/NEJMoa1110187.
[13] Hocqueloux L, Avettand-fènoël V, Jacquot S, Prazuck T, Legac E, Mélard A, et al. Long-term antiretroviral therapy initiated during primary HIV-1 infection is key to achieving both low HIV reservoirs and normal T cell counts. *J Antimicrob Chemother* 2013;**68**:1169–78. doi:10.1093/jac/dks533.
[14] Davy-Mendez T, Napravnik S, Zakharova O, Kuruc J, Gay C, Hicks CB, et al. Acute HIV infection and CD4/CD8 ratio normalization after antiretroviral therapy initiation. *J Acquir Immune Defic Syndr* 2018;**79**:510–18. doi:10.1097/QAI.0000000000001843.
[15] Sainz T, Serrano-Villar S, Díaz L, González Tomé MI, Gurbindo MD, de José MI, et al. The CD4/CD8 ratio as a marker T-cell activation, senescence and activation/exhaustion in treated HIV-infected children and young adults. *AIDS* 2013;**27**:1513–16. doi:10.1097/QAD.0b013e32835faa72.
[16] Lu W, Mehraj V, Vyboh K, Cao W, Li T, Routy JP. CD4:CD8 ratio as a frontier marker for clinical outcome, immune dysfunction and viral reservoir size in virologically suppressed HIV-positive patients. *J Int AIDS Soc* 2015;**18**:20052. doi:10.7448/IAS.18.1.20052.
[17] Domínguez-Domínguez L, Rava M, Bisbal O, Lopez-Cortés L, Portilla J, Podzamczer D, et al. Low CD4/CD8 ratio is associated with increased morbidity and mortality in late and non-late presenters: results from a multicentre cohort study, 2004–2018. *BMC Infect Dis* 2022;**22**:379. doi:10.1186/s12879-022-07352-z.
[18] Nicolás D, Suárez A, Ambrosioni J, Manzardo C, Ligero C, Costa J, et al. Prevalence, clinical characteristics and outcome of severe primary HIV-1 infection: a prospective cohort study. *Int J Infect Dis* 2019;**88**:73–9. doi:10.1016/j.ijid.2019.08.001.
[19] 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep* 1992;**41**:1–19.
[20] Thornhill J, Inshaw J, Kaleebu P, Cooper D, Ramjee G, Schechter M, et al. Brief report: enhanced normalization of CD4/CD8 ratio with earlier antiretroviral therapy at primary HIV infection. *J Acquir Immune Defic Syndr* 2016;**73**:69–73. doi:10.1097/QAI.0000000000001013.
[21] Serrano-Villar S, Martínez-Sanz J, Ron R, Talavera-Rodríguez A, Fernández-Felix BM, Herrera S, et al. Effects of first-line antiretroviral therapy on the CD4/CD8 ratio and CD8 cell counts in CoRIS: a prospective multicentre cohort study. *Lancet HIV* 2020;**7**:e565–73. doi:10.1016/S2352-3018(20)30202-2.
[22] De Salvador-Guillouët F, Sakarovitch C, Durant J, Risso K, Demonchy E, Roger PM, et al. Antiretroviral regimens and CD4/CD8 ratio normalization in HIV-infected patients during the initial year of treatment: a cohort study. *PLoS One* 2015;**10**:e0140519. doi:10.1371/journal.pone.0140519.
[23] Takata H, Kakazu JC, Mitchell JL, Kroon E, Colby DJ, Sacdalan C, et al. Long-term antiretroviral therapy initiated in acute HIV infection prevents residual dysfunction of HIV-specific CD8+ T cells. *EBiomedicine* 2022;**84**:104253. doi:10.1016/j.ebiom.2022.104253.
[24] Ambrosioni J, Farrera J, de Lazzari E, Nicolás D, Manzardo C, Hernández-Meneses MM, et al. Immunological and virological efficacy of different antiretroviral regimens initiated during acute/recent HIV infection. *AIDS* 2020;**34**:2269–74. doi:10.1097/QAD.0000000000002685.
[25] Crowell TA, Phanuphak N, Pinyakorn S, Kroon E, Fletcher JLK, Colby D, et al. Virologic failure is uncommon after treatment initiation during acute HIV infection. *AIDS* 2016;**30**:1943–50. doi:10.1097/QAD.0000000000001148.
[26] Lundgren JD, Babiker AG, Gordin F, Emery S, Grund B, et al., INSIGHT START Study Group Initiation of antiretroviral therapy in early asymptomatic HIV infection. *N Engl J Med* 2015;**373**:795–807. doi:10.1056/NEJMoa1506816.
[27] *EACS guidelines. Version 11.1.* Belgium: European AIDS Clinical Society; 2022.
[28] Pinkerton SD. Probability of HIV transmission during acute infection in Rakai, Uganda. *AIDS Behav* 2008;**12**:677–84. doi:10.1007/s10461-007-9329-1.