

Enhanced Normalization of CD4/CD8 Ratio With Earlier Antiretroviral Therapy at Primary HIV Infection

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Background: Total CD4⁺ T-cell counts predict HIV disease progression but do not necessarily reflect normalization of immune function. CD4/CD8 ratio is a marker of immune dysfunction, a prognostic indicator for non-AIDS mortality, and reflects viral reservoir size. Despite antiretroviral therapy (ART), recovery of CD4/CD8 ratio in chronic HIV infection is incomplete; we hypothesize enhanced CD4/CD8 ratio recovery with earlier treatment initiation in recently infected individuals.

Methods: CD4⁺ count and CD4/CD8 ratio were analyzed using data from 2 cohorts: SPARTAC trial and the UK HIV Seroconverters Cohort where primary HIV infection (PHI) was defined as within 6 months from estimated date of infection. Using time-to-event methods and Cox proportional hazard models, we examined the effect of CD4/CD8 ratio at seroconversion on disease progression (CD4 <350 cells per cubic millimeter/ART initiation) and factors associated with time from ART initiation to CD4/CD8 normalization (ratio >1.0).

Findings: Of 573 seroconverters, 482 (84%) had abnormal CD4/CD8 ratios at HIV seroconversion. Individuals with higher CD4/CD8 ratio at seroconversion were significantly less likely to reach the disease progression endpoint [adjusted hazard ratio (aHR) (95% CI) = 0.52 (0.32 to 0.82), *P* = 0.005]. The longer the interval between seroconversion and ART initiation [HR (95% CI) = 0.98 per month increase (0.97, 0.99), *P* < 0.001], the less likely the CD4/CD8 ratio normalization. ART initiation within 6 months from seroconversion was significantly more likely to normalize [HR (95% CI) = 2.47 (1.67 to 3.67), *P* < 0.001] than those initiating later.

Interpretation: Most individuals presenting in PHI have abnormal CD4/CD8 ratios. The sooner the ART is initiated in PHI, the greater the probability of achieving normal CD4/CD8 ratio.

Key Words: primary HIV infection, seroconversion, acute HIV infection, CD4/CD8 ratio, CD4:CD8, early antiretroviral therapy

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BACKGROUND

Total CD4⁺ T-cell count is a validated surrogate marker of disease progression for HIV/AIDS.^{1–3} Although the risk of AIDS-related illness and opportunistic disease is significantly reduced once CD4 counts have recovered to levels >350 cells per cubic millimeter,^{4–6} the risk of non-AIDS morbidity persists.⁷ Despite recovery of total CD4⁺ T-cell counts with antiretroviral therapy (ART), the CD4/CD8 ratio often fails to normalize (defined as >1) when ART is initiated in chronic infection⁸ where reported normalization of CD4/CD8 ratio ranges from 6% to 26.3%.^{3,9–11} A recent study from a large Italian cohort reported a normalization rate of 29.4% despite 5 years of viral suppression on ART.¹²

Disruption of T-cell homeostasis by HIV induces CD4⁺ depletion and CD8⁺ T-cell expansion, resulting in an inverted

CD4/CD8 ratio.^{13,14} Low CD4/CD8 ratio in individuals on suppressive ART has been independently associated with persistently elevated markers of T-cell activation⁸ and measures of HIV viral reservoirs.^{15,16} In untreated HIV infection, CD4/CD8 ratio inversely correlates with marker of T-cell activation and exhaustion.¹⁷ In addition, low CD4/CD8 ratio independently predicts all-cause mortality and non-AIDS-related events.¹⁷ CD4/CD8 T-cell ratio may therefore better predict immune function than total CD4⁺ count alone^{17,18} and may contribute to the observed START trial result, identifying a significantly enhanced clinical outcome for individuals starting ART at CD4 counts >500 cells per cubic millimeter.¹⁹ Enhanced normalization of the CD4 T-cell count with ART initiated within 4 months of seroconversion has recently been demonstrated.²⁰ We hypothesized that CD4/CD8 ratio recovery would also be enhanced after immediate ART initiation initiated in primary HIV infection (PHI). We examined the dynamics of immune recovery by combining data from 2 cohorts of individuals with well-estimated dates of HIV seroconversion.

METHODS

Cohort Descriptions

The 2 cohorts have been described previously.^{21,22} Briefly, the UK HIV Seroconverters Cohort (UKHSC) is an observational cohort of routine clinical data from individuals with defined HIV seroconversion dates collected between 1994 and 2014. Data were restricted to 2 clinical centers able to provide CD8 T-cell data as these were not routinely collected within UKHSC. SPARTAC (Short Pulse Anti-Retroviral Therapy at Seroconversion) is an international randomized clinical trial of 2 intervention arms in PHI, 12 weeks or 48 weeks of transient therapy vs. deferred treatment (standard of care) across 8 countries.

Study Definitions

A normal CD4/CD8 T-cell ratio was defined as ≥ 1.0 .²³ PHI was defined as documented HIV infection within a maximum of 6 months from a previous negative HIV antibody test, p24 antigen positive in the absence of antibody, or an “incident” RITA, recent incidence test algorithm test.²¹ Disease progression was defined as CD4 <350 cells per cubic millimeter or long-term ART initiation.

Statistical Analysis

We examined the effect of CD4/CD8 ratio at PHI (baseline) on time to disease progression endpoint using time-to-event methods and Cox proportional hazards models, restricting to individuals with CD4⁺ >350 cells per cubic millimeter at the time of PHI diagnosis. Baseline CD4/CD8 ratio was included as a continuous variable and then as a categorical variable (<0.5, 0.5–1.0, 1.0). The multivariate analyses were adjusted for sex, age at PHI, risk group, ethnicity and enrollment from an African site (as proxy for ethnicity), baseline CD4 count, and HIV-1 viral load. Follow-up was censored at date of last recorded result. We checked for collinearity between baseline CD4 and baseline CD4/CD8 ratio.

We then examined the effect of the interval between PHI and ART initiation on time from ART initiation to normalized CD4/CD8 ratio, again using time-to-event methods and Cox proportional hazards models. All models were censored at the earlier of a break in ART treatment (>7 days) or last visit before April 24, 2014, whichever was earlier. Multivariate analyses were adjusted for sex, risk group, ethnicity, ART regimen, enrollment from an African site, year of seroconversion, interval between baseline ratio and ART initiation, and both CD4⁺ count, HIV-1 viral load, and age at ART initiation. For illustration purposes, we used an arbitrary cutoff of 6 months to examine the effect of a dichotomized duration of HIV infection at initiation of ART, ART started <6 months and ART initiated ≥ 6 months of estimated date of seroconversion. The cutoff for a normal CD4/CD8 ratio of 1.2 has been used by some groups^{3,9}; a sensitivity analysis was performed using this value. A further sensitivity analysis was performed using a cutoff of 0.5.

FINDINGS

Overall, 573 individuals contributed CD4/CD8 data; of whom, 482 (84%) had abnormal CD4/CD8 ratios (<1.0) at baseline in PHI. Information was not available on Fiebig staging although the vast majority of individuals were at stage V or VI. Of 84, 155, and 334 individuals presenting with CD4⁺ counts <350, 350–500, and >500 cells per cubic millimeter, 95% (n = 80), 91% (n = 141), and 78% (n = 261) had abnormal CD4/CD8 ratios, respectively. The median CD4/CD8 ratio (interquartile range) at baseline in PHI was 0.30 (0.21, 0.42) for those with CD4 count <350 cells per cubic millimeter, 0.46 (0.31, 0.66) for those with CD4 350–500 cells per cubic millimeter, and 0.55 (0.35, 0.80) if CD4 was >500 cells per cubic millimeter.

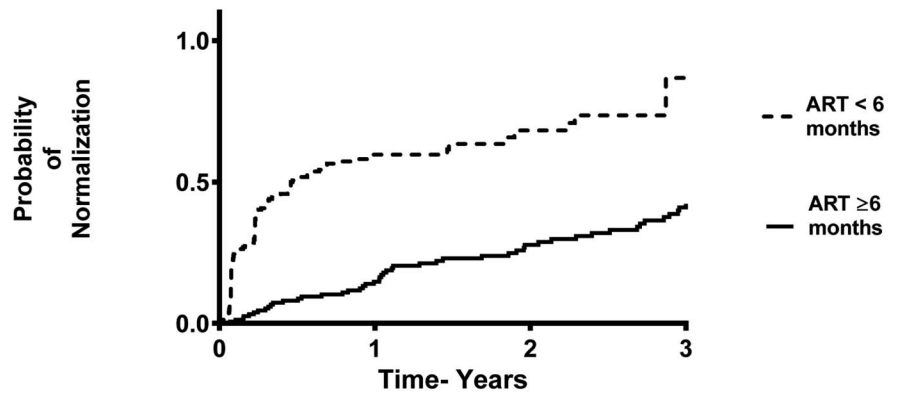
Of 286 ART-naive individuals with baseline CD4 counts >350 cells per cubic millimeter, the median time to the disease progression endpoint was 1.51 (95% CI: 1.32 to 2.37) years. Higher CD4/CD8 ratio at seroconversion was independently associated with lower risk of endpoint [adjusted hazard ratio (aHR) (95% CI) = 0.52 (0.32 to 0.82), $P = 0.005$], as was higher baseline CD4 count [aHR (95% CI) = 0.79 per 100 cell increase (0.71 to 0.86), $P < 0.001$].

Compared to individuals with CD4/CD8 ratio >1.0 at PHI, those with lower ratios had greater risk of disease progression [CD4/CD8 ratio <0.5: HR (95% CI) = 2.89 (1.74 to 4.79), $P < 0.001$; CD4/CD8 ratio >0.5 to ≤ 1.0 : HR (95% CI) = 1.97 (1.17 to 3.30), $P = 0.010$] (Fig. 1B).

We found strong evidence that those with longer time between PHI and ART initiation were less likely to achieve normal CD4/CD8 ratio [aHR (95% CI) = 0.98 per month increase (0.97 to 0.99), $P < 0.001$] after adjusting for confounding variables (including baseline HIV-1 Viral load and CD4 count). The only other significant covariate in the model was baseline CD4⁺ cell count with normalization more likely as count increased [HR = 1.12 (95% CI: 1.09 to 1.15) per 50 cells per cubic millimeter increase, $P < 0.001$]. Results from the sensitivity analysis (normal CD4/CD8 ratio >1.2 and >0.5) were qualitatively unchanged.

Of 468 individuals with abnormal CD4/CD8 ratio initiating ART, 309 commenced ART within 6 months and

Kaplan Meier: Time to CD4/CD8 Ratio Normalization

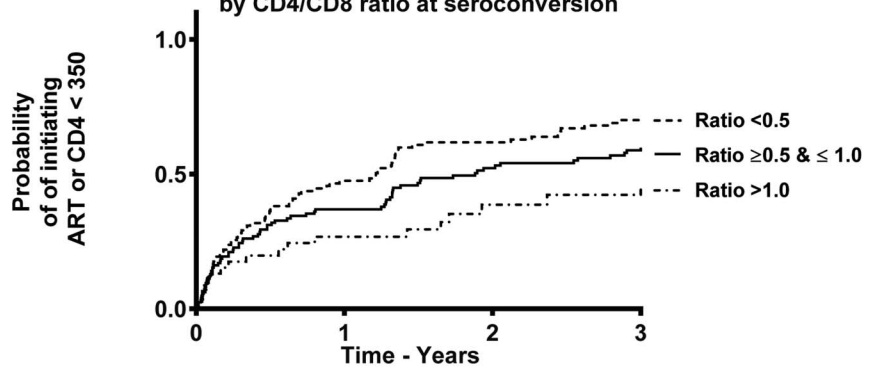


Number at Risk	0	1	2	3
ART <6/12	309	25	13	1
ART ≥6/12	159	108	73	49

A

FIGURE 1. A, Kaplan–Meier plots of the probability of time to normalization of the CD4/CD8 ratio for those initiated on ART within 6 months of estimated date of seroconversion and those who deferred ART to more than or equal to 6 months. B, Kaplan–Meier plots of the probability of initiating ART or CD4 count <350 according to CD4/CD8 ratio group at seroconversion: (1) ratio <0.5, (2) ≥0.5 and ≤1.0, or (3) >1.0. This analysis used data (n = 286) of those from the United Kingdom Register of Seroconverters and SPARTAC (including only those who were not randomized to starting treatment).

Kaplan Meier: Probability of Primary Endpoint* by CD4/CD8 ratio at seroconversion



Number at Risk	0	1	2	3
Ratio >1.0	46	29	18	14
Ratio ≥ 0.5, ≤ 1.0	125	74	51	42
Ratio < 0.5	114	56	37	29

*initiation of ART or CD4<350

B

159 initiated ≥6 months from seroconversion. Their baseline characteristics are shown in Table 1. Overall median (interquartile range) time to normalization of CD4/CD8 ratio was 715 (408, 988) days, 172 (117, 280) days for those in the <6-month group, and 1344 (1080, 1630) days for those in the ≥6-month group. Those initiating ART within 6 months from seroconversion were significantly more likely to normalize the CD4/CD8 ratio [HR (95% CI) = 2.47 (1.67 to 3.66), *P* < 0.001], see Figure 1A. In addition, within 1 year from the date of starting ART, 129/275 (46.9%) individuals in the <6-month group normalized the CD4/CD8 ratio, compared with 16/127 (12.6%) individuals in the ≥6-month group. A figure illustrating the dynamics of the CD4 and CD8 T-cells counts for each group is shown (see Fig. S2, Supplemental Digital Content, <http://links.lww.com/QAI/A810>).

INTERPRETATION

Our findings demonstrate that ART initiated within 6 months from PHI markedly increases the likelihood of normalization of the CD4/CD8 ratio compared with later initiation of therapy, irrespective of baseline CD4 count. This is in agreement with Serrano-Villar et al.²⁴ In addition, we found that the probability of achieving normalization of CD4/CD8 ratio is increased for each month closer to seroconversion ART is initiated. The normalization of CD4/CD8 ratio is relatively rapid with almost half of the individuals achieving this within 1 year of ART initiation. This finding is in contrast to the reported normalization rates of CD4/CD8 ratio in treated chronic HIV infection,^{3,10,11} where the chances of normalization within 1 year of ART initiation remain low and are comparable to those initiating ART ≥6 months in our study. Higher rates of CD4/CD8 ratio normalization have

TABLE 1. Baseline Characteristics of HIV Seroconverters Presenting During PHI by Time From Seroconversion to ART Initiation

	Initiated ART <6 mo After HIV SC, N = 309	Initiated ART ≥6 mo After HIV SC, N = 159	Total, N = 468
Male	219 (70.9%)	144 (90.6%)	363 (77.6%)
Ethnicity			
White	123 (39.8%)	101 (63.5%)	224 (47.9%)
Black	13 (4.0%)	7 (4.4%)	20 (4.3%)
Other/ unknown	173 (56.0%)	51 (32.1%)	224 (47.9%)
Risk group			
MSM	203 (65.9%)	138 (86.8%)	341 (73.0%)
MSW	103 (33.4%)	19 (12.0%)	122 (26.1%)
IDU	1 (0.3%)	0 (0.0%)	1 (0.2%)
Unknown	1 (0.3%)	2 (1.3%)	3 (0.6%)
Initial drug regimen			
PI-based regimen	251 (81.2%)	33 (20.8%)	284 (60.7%)
NNRTI-based regimen	49 (15.9%)	111 (69.8%)	160 (34.2%)
NNRTIs only	1 (0.3%)	11 (6.9%)	12 (2.6%)
Other	8 (2.6%)	4 (2.5%)	12 (2.6%)
Time in month from SC to ART initiation: median (IQR)	2.48 (1.33–3.35)	29.07 (15.2–51.1)	3.37 (1.93–16.07)
Age (yrs) at ART initiation: median (IQR)	33 (27–41)	38 (30–42)	34 (27–41)
CD4 count/mm ³ at ART initiation: median (IQR)	547 (408–687.0)	290 (220–400)	465 (307–622)
CD8 count/mm ³ at ART initiation: median (IQR)	980 (711–1370)	930 (700–1309)	965.5 (710–1350)
CD4:CD8 ratio at ART initiation: median (IQR)	0.55 (0.36–0.82)	0.32 (0.19–0.45)	0.45 (0.27–0.71)

IDU, injection drug user; IQR, interquartile range; MSM, men who have sex with men; MSW, men who have sex with women; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SC, seroconversion.

been reported in 2 studies of treated chronic infection, but only following up to a median of 10 years on ART.^{9,25}

Previously, international treatment guidelines have varied on the CD4 count threshold that ART initiation is recommended,^{26–28} with inconsistent guidelines on starting ART in PHI. However, in light of the recent findings reported by the START study,¹⁹ ART initiation regardless of CD4 T-cell count will be recommended by all international guidelines,²⁹ which will include immediate ART initiation in PHI. In this setting, PHI represents a specific scenario; despite

abnormal CD4/CD8 ratios in the majority of individuals at the time of seroconversion, irrespective of initial CD4 count, rapid ART initiation confers a significantly enhanced probability of immunological recovery, which has not been observed in later stage disease. Furthermore, given the high rate of serious non-AIDS events at relatively high CD4 counts seen in START, exploration of other biomarkers predictive of disease progression such as CD4/CD8 ratio is warranted.

There are limitations to our study. As with all observational studies, it is not possible to adjust for unmeasured confounders. For example, abnormal CD4/CD8 ratio has been associated with Cytomegalovirus (CMV) infection seropositivity.³⁰ As CMV data were not available from our cohorts, we were unable to adjust for CMV infection. Furthermore, abnormal CD4/CD8 is known to increase with age.³¹ Given the relatively young, median age of 34 years, of our cohort, we could not address the role of CD4/CD8 recovery in age groups >50 years, who are known to progress faster.³² In addition, the majority of those treated within 6 months received a boosted protease inhibitor–based regimen, reflecting the SPARTAC trial protocol of short-course ART, to avoid the risk of resistance to nonnucleoside reverse transcriptase inhibitors on stopping owing to their differential clearance.^{33,34} Those initiated beyond 6 months primarily received nonnucleoside reverse transcriptase inhibitor–based regimens in keeping with local guidelines. We, therefore, included ART class in the model and found no evidence of an effect on CD4/CD8 ratio normalization.

CD4/CD8 ratio may have additional potential uses as a biomarker in HIV cure research. Although there remains a lack of consensus as to the optimal measurement of viral reservoir,³⁵ quantification of reservoir size using total cellular HIV-1 DNA remains the best clinical biomarker to predict both disease progression and virological rebound among individuals interrupting ART.^{15,36} Although HIV-1 DNA was not measured for all individuals in this study, an analysis of a subset of individuals from the SPARTAC trial found an inverse correlation between HIV-1 DNA and CD4/CD8 ratio.³⁷ As such, normalization of CD4/CD8 ratio may be a future valuable biomarker, which better predicts those individuals with a smaller HIV reservoir. This could inform an algorithm to identify optimal candidates for cure interventions or treatment interruption. Furthermore, if CD4/CD8 ratio reflects HIV reservoir size, then our data support the finding by other groups that treatment with ART at PHI may limit HIV reservoir size.³⁸

In conclusion, CD4/CD8 ratio normalization, albeit rare when ART is started in chronic infection, is markedly enhanced with ART initiation in PHI, with a greater benefit the sooner ART is started.

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