Predictive value of CD8+ T cell and CD4/CD8 ratio at two years of successful ART in the risk of AIDS and non-AIDS events

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Summary

Background While increased CD8 counts and low CD4/CD8 ratio during treated HIV correlate with immunosenescence, their additional predictive values to identify individuals with HIV at higher risk of clinical events remain controversial.

Methods We selected treatment-naive individuals initiating ART from ACTG studies 384, 388, A5095, A5142, A5202, and A5257 who had achieved viral suppression at year 2. We examined the effect of CD8+ T cell counts and CD4/CD8 at year 2 on the probability of AIDS and serious non-AIDS events in years 3–7. We used inverse probability weighting methods to address informative censoring, combined with multivariable logistic regression models.

Findings We analyzed 5133 participants with a median age of 38 years; 959 (19%) were female, pre-ART median CD4 counts were 249 (Q1-Q3 91–372) cell/μL. Compared to participants with CD8 counts between 500/μL and 1499/μL, those with >1500/μL had a higher risk of clinical events during years 3–7 (aOR 1.75; 95%CI 1.33–2.32). CD4/CD8 ratio was not predictive of greater risk of events through year 7. Additional analyses revealed consistent CD8 count effect sizes for the risk of AIDS events and noninfectious non-AIDS events, but opposite effects for the risk of severe infections, which were more frequent among individuals with CD8 counts <500/μL (aOR 1.70; 95%CI 1.09–2.65).

Interpretation The results of this analysis with pooled data from clinical trials support the value of the CD8 count as a predictor of clinical progression. People with very high CD8 counts during suppressive ART might benefit from closer monitoring and may be a target population for novel interventions.

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Introduction

People with HIV (PWH) on antiretroviral therapy (ART) with undetectable HIV RNA and adequate CD4+ T cell counts have a higher risk of multiple non-AIDS complications compared with people without HIV,[1,2] and may have a shortened lifespan.[3,4] This excess risk is predicted in part by the CD4+ T cell counts.[3] The prevention and management of these non-AIDS

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Research in context

Evidence before this study

We searched Google Scholar without language restrictions with the terms "HIV", "ART or antiretroviral", "CD8 T cell", "CD4/CD8 ratio", "severe non-AIDS events", "non-AIDS conditions", and "mortality" for articles published up to June 6, 2021. We selected studies in which the association between CD4/CD8 ratio and/or CD8 counts were reported and severe non-AIDS events or mortality were reported. Despite multiple studies suggesting an independent effect of the CD4/CD8 ratio or CD8+ T cell counts in predicting disease progression, controversy persists as to their real value in clinical medicine. Most of the evidence linking these biomarkers with clinical outcomes was generated in retrospective cohort studies and limited by the lack of systematic CD8+ T cell count monitoring, the inability to adjudicate clinical events, significant lost-to-follow-up rates, and different time interval between measurement and the assessed outcomes.

Added value of this study

Our study provides evidence that patients with high CD8+ T cell counts (> 1500 cells/μL) measured at year 2 after ART initiation were more likely to develop a subsequent clinical event during the follow-up that those with lower counts. This effect was independent of the CD4+ T cell count. Because this association is not detected with the CD4/CD8 ratio, our findings suggest that the CD8+ T cell count is more informative than the CD4/CD8 ratio when measured after 2 years of ART. To our knowledge, no other studies have analyzed the associations between the CD8+ T cell counts and the CD4/CD8 ratio with infectious and noninfectious events. We found that participants with increased CD8+ T cell counts were protected against infectious non-AIDS events.

Implications of all the available evidence

The CD4/CD8 ratio has been repeatedly shown to be an indicator of immunosenescence and a predictor of long-term mortality. The CD8+ T cell count might be a useful biomarker of clinical endpoint to be used in evaluating novel therapies for ongoing immune dysfunction during treated infection.

complications, including heart disease and cancer, would likely be improved if better prognostic biomarkers become available.

AIDS-associated diseases have traditionally been the ultimate clinical consequence of HIV infection. The use of antiretroviral therapy to prevent AIDS has successfully been monitored by the CD4+ T-cell counts. However, during the past years, the CD4/CD8 ratio and the CD8+ T cell counts have been increasingly recognized as new markers, given their correlations with the putative mechanisms contributing to the development of AIDS and non-AIDS-related comorbidities, including cumulative toxicities of antiretroviral drugs,[5] immunosenescence,[6–10] CMV serostatus[11–13] HCV coinfection,[14] loss of thymic function,[15] bacterial translocation,[16,17] persistent inflammation[18] and markers of HIV persistence.[19] Also, many studies have found independent associations between the CD4/CD8 ratio or the CD8+ T cell counts and the risk of all-cause mortality and non-AIDS events.[7,20–23]

Despite multiple studies suggesting an independent effect of the CD4/CD8 ratio or CD8+ T cell counts in predicting disease progression, controversy persists as to their real value in clinical medicine.[7,21,23–25] Most of the evidence linking these biomarkers with clinical outcomes was generated in retrospective cohort studies and limited by the lack of systematic CD8+ T cell count monitoring, the inability to adjudicate clinical events, and significant lost-to-follow-up rates. Even across studies that have found associations between the CD4/CD8 ratio or the CD8+ T cell counts and a higher risk of adverse clinical outcomes, the cut-offs that more accurately predict the risk and the type of events that can be predicted remain unclear.[21,22,26,27] Finally, it has often proved difficult to define with precision the impact of the CD8+ T cell count independent of the CD4+ T cell count, as high CD8+ T cell counts may be a homeostatic response to low CD4+ T cell counts.[28]

Here, we used data from the AIDS Clinical Trials Group (ACTG) clinical trials 384, 388, A5095, A5142, A5202 and A5257 to more carefully define the role of CD8+ T cell counts in predicting future events. Participants in these antiretroviral therapy studies were carefully characterized during the randomized clinical studies and then referred for long-term observation in the ACTG Longitudinal Linked Randomized Trials (ALLRT) study.[29] Clinical events were carefully defined in these studies, and the CD8+ T cell count was routinely measured over time. We examined whether the CD8+ T cell counts and CD4/CD8 ratio after 2 years of suppressive ART predict the probability of subsequent clinical events during years 3 to 7, independently of the CD4 count, and investigated different cut-offs and their correlation with T cell activation. We specifically modelled our results to define the role of CD8+ T cell counts in predicting disease progression independent of the CD4+ T cell count.

Methods

Study population

The ACTG parent studies 384, 388, A5095, A5142, A5202, and A5257 were randomized treatment-naïve clinical trials where participants received their initial antiretroviral therapies (ART) and were evaluated every 4 to 16 weeks. The calendar year of ART initiation was

1998–1999 for 384 and 388, 2001–2002 for A5095, 2003–2004 for A5142, 2005–2007 for A5202, and 2009–2011 for A5257 (summarized in **Table S1**). The majority of participants (67%) chose to continue long-term follow-up in the ALLRT study after their parent study ended.[29] ALLRT was a prospective cohort study of HIV+ participants from ACTG randomized clinical parent studies and conducted clinical evaluations every 16 weeks.[30]

In this analysis, we included participants who were on ART and virologically suppressed (<200 copies/ml) at year 2 of ART and who had CD4+ and CD8+ T cell counts available at year 2.

Clinical outcomes

Clinical outcome was defined as the first clinical event after year 2, and through year 7 of ART while remaining on suppressive ART. Clinical events of interest were AIDS-defining events (ADEs) according to the standard definition,[31] and non-AIDS-defining events (NADEs) (liver, cardiovascular, renal disease, non-AIDS cancers, fractures, diabetes and serious bacterial infection, and non-accidental death). Among NADE, infectious NADE included peritonitis, hepatic failure, ascites, pneumonia and urosepsis, and neoplasias associated with viral infections (Hodgkin's disease, vulval cancer, oropharyngeal squamous cell carcinoma, anal cancer, oropharyngeal cancer).

Covariates

Our primary prognostic variables were CD8+ T cell and CD4/CD8 ratio at year 2. CD8+ T cell counts at year 2 were categorized as <500, 500–1499 and ≥1500; CD4/CD8 ratio were categorized as <0.4, 0.4 – 1, >1 and quartiles. These thresholds were selected prior to the analysis and were based on thresholds previously defined.[7,20,21] Other year 2-variables considered were CD8+ T cell changes from ART initiation, CD4/CD8 changes from ART initiation, CD4+ T cell count at year 2 (continuous or categorical: ≤200, >200), CD4+ T cell changes from ART initiation, and history of a clinical event before year 2. We also included age at ART initiation, sex, race/ethnicity (White, Black and Hispanic/Other), current or previous injection drug use, and the type of ART regimen used initially (defined based on the use of a boosted protease inhibitor regimen, a non-nucleoside reverse transcriptase inhibitor, or an integrase inhibitor). Gender (i.e., reporting on transgender women and men) was not available.

Measurement of T cell activation

Whole blood was obtained in tubes containing ethylenediaminetetraacetic acid. Specimens were spun at 400 g for 10 min, and plasma was pipetted and spun again at 800 g for 10 min. Plasma was aliquoted, frozen, and stored at −70°C until assayed. We also performed flow cytometric evaluation of immune activation (HLA-DR+CD38+) on CD4+ and CD8+ T-cells. The T-cell phenotype was characterized by polychromatic flow cytometry on fresh PBMCs.[32]

Statistics

We investigated how the probability of experiencing a clinical event in years 3–7 depends on CD8+ T cell counts or CD4/CD8 ratio at year 2, had everyone remained on suppressive ART. Participants were censored at the time of ART interruption ≥ 21 days, viral failure (2 consecutive HIV RNA ≥200 copies/ml), end of follow-up (including loss to follow-up) before year 7, whichever came first. Demographics, variables at ART initiation and year 2 were summarized, as well as the incident clinical events.

To address informative censoring, we applied inverse probability of censoring weighting (IPCW) for participants without clinical events who did not remain in follow-up through to year 7 after ART initiation. To illustrate the concept behind IPCW, suppose that a study population at a given analysis year includes a subset of 10 identical event-free participants of whom 5 are lost to follow-up or are analytically censored in the next year. IPCW then assigns the 5 who remained on suppressive ART double the statistical weight. In general, IPCW assigns weights to participants remaining on suppressive ART: the inverse of their estimated probability of being in follow-up and on suppressive ART based on their (time-updated) characteristics. IPCW was applied yearly in our analysis; statistical weights accumulate through to year 7. Then a weighted logistic regression is applied. This approach has been proven to lead to estimators with desirable statistical properties.[33] The use of IPCW in this longitudinal analysis means that the "operative" sample size at each of the years since ART initiation in Figure 1 remains the original (the n = 5133 at ART year 2). But through statistical reweighting, the statistical weight of a participant lost to follow-up or analytically censored (due to ART interruption or viral failure) is transferred to a participant remaining in follow-up and on suppressive ART.

Pooled logistic regression models for remaining in follow-up with suppressive ARTs, were fitted with sex, age, race/ethnicity, injection drug use, time since year 2 and time-updated CD4 and CD8 cell counts as covariates. The IPCW weight of each non-censored participant was the reciprocal of the cumulative estimated probability of remaining in follow-up with suppressive ART from the pooled logistic regression.[33,34] The cumulative probability of clinical events subsequent to year 2 was estimated using IPCW weights and plotted by CD8+ T cells and CD4/CD8 ratio at year 2. Logistic regression models with IPCW weights estimated the association between CD8+ T cell counts and CD4/CD8 ratios at year 2 and

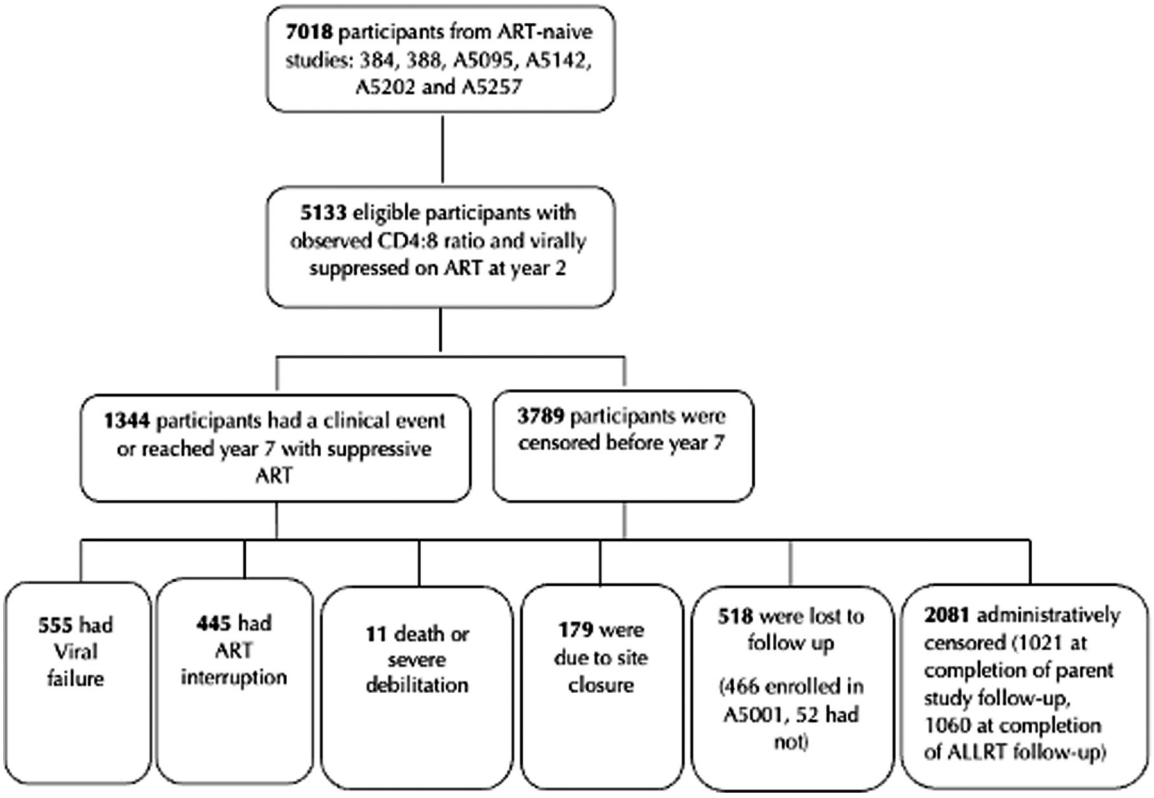

Figure 1. Parent ACTG studies.

clinical events during years 3–7. We performed unadjusted logistic regression models with IPCW weights for each covariate variable to identify prognostic factors for clinical events. Statistical significance throughout was based on Wald tests. We also evaluated whether findings were changed if we additionally adjusted for year 2 CD4+ T cell counts. In the final adjusted IPCW weighted regression to assess the association between CD8+ T cell counts at year 2 and clinical events during years 3–7, we included covariates with unadjusted p-value <0.05 (excepting CD4/CD8 ratio and CD4+ T cells at year 2). Similarly, a final IPCW weighted regression to assess CD4/CD8 ratio at year 2 included additional covariates with unadjusted p-value <0.05 (excepting CD8+ T cells and CD4+ T cells at year 2).

To assess if the prediction of CD8+ T cell counts and CD4/CD8 ratio at year 2 varied for different types of clinical outcome, we separately conducted the same analyses on ADE events, infectious NADE and noninfectious NADE. In the analysis of ADE, a participant with NADE (only) was analyzed as not having a clinical event. The same approach was applied for the infectious NADE and noninfectious NADE analyses.

No formal sample size or power calculations were performed for this investigation. A previous, similar analysis with a sample size of $n = 1025$ subjects identified a significant effect for year 1 CD8 activation,[35] and we anticipated a sample size for this analysis would be more than 3 times as large as that evaluation of CD8 activation. There was no randomization involved in this analysis. The data sources (parent ACTG studies) had randomized initial ART, whereas our analyses used year 2 as the 'baseline' timepoint. Similarly, some of the data sources (parent ACTG studies 384, 388, A5095, A5142, A5202, and A5257) had blinded initial ART, which was not relevant for the aims of this study.

We used Spearman's correlation coefficients to assess the relationships between absolute T cell numbers, CD4/CD8 ratio and T cell activation markers.

Ethics

This data analysis project was approved by the Antiretroviral Therapy Strategies Subcommittee of the ACTG (study approval DACS322). The contributing data sources were ACTG studies, each of which was approved by the institutional review board at each of the participating clinical sites. Every participant provided written informed consent.

Data sharing statement

Study data are available upon request from the Statistical and Data Management Center (SDAC) of the AIDS

Clinical Trials Group; the request may be made by emailing: sdac.data@sdac.harvard.edu. The reason for the restriction on public data deposition is because of ethical restrictions.

Role of funders
The study funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding and senior authors had full access to all the data and the statistical reports.

Results

Demographics and clinical characteristics
Among 7018 participants from ACTG parent randomized trials, 5133 participants had CD4/CD8 ratio and were on suppressive ART at year 2, therefore were included in the analysis. Of these participants, 3839 were enrolled into ALLRT and had a longer follow up, 379 had a clinical event, 965 reached year 7 while remaining on suppressive ART, and 3789 (74%) were censored due to viral failure, stopping ART or loss to follow-up (Figure 1).

Demographic and clinical characteristics are shown in Table 1. Among 5133 participants, 959 (19%) were female, 2168 (42%) were White, 1731 (34%) were Black, and 407 (8%) reported current or previous injection drug use. The median age was 38 years.

At ART initiation, 1881 (37%) were HIV RNA >100,000 copies/ml and 2143 (42%) had CD4+ T cell counts ≤200 cells/μL. The median CD8+ T cell was 778 cells/μL and the median CD4/CD8 ratio was 0.3.

At year 2, the median CD4+ T cell was 503 cells/μL, CD8+ T cell was 772 cells/uL, and CD4/CD8 ratio was 0.7. From ART initiation to year 2, 1682 participants (33%) had had a previous clinical event between ART initiation and the year 2 timepoint. Between ART initiation and two years later, the median (Q1, Q3) changes of CD4+ T cell were 253 cells/μL (164, 359), the median (Q1, Q3) changes of CD8+ T cell were -25 cells/μL (-275, 223), and the median (Q1, Q3) changes of CD4/CD8 ratio were 0.4 (0.2, 0.5). At year 2, 798 (16%) had a CD8+ T cell count below 500 cell/μL, 4051 (79%) between 500 and 1500 cell/μL and 284 (6%) greater than 1500 cell/μL. At year 2, 1209 (24%) had a CD4/CD8 ratio below 0.4 cell/μL, 2801 (55%) of 0.4 to 1.0, and 1123 (22%) greater than 1.0.

Cumulative incidence of clinical events in years 3–7
During years 3–7, 32 (8.4%) had cardiovascular disease, 88 (23.2%) had renal failure, 110 (29.0%) had diabetes, 38 (10.0%) had non-AIDS related cancer, 26 (6.9%) had ADE and 32 (8.4%) had non-accidental death as shown in **Supplementary Table 2.** Of the 358 subjects experiencing NADE events, 64 cases were infections.

Cumulative probability of clinical events by CD8+ T cell and CD4/CD8 ratio at year 2
As shown in Figure 2, the cumulative probability of subsequent clinical events over time was estimated to be higher among participants with CD8+ T cell ≥1500 cell/μL, especially after year 6. Participants with CD4/CD8 ratio <0.4 had a slightly higher probability of subsequent clinical events during years 3 to 4 (Figure 3).

Association between CD8+ T cell at year 2 and subsequent clinical events
A total of 284 (6%) of participants had a high CD8+ T cell count ≥1500 cell/μL at year 2. These patients were more likely to develop a subsequent clinical event during years 3–7 compared to those with 500–1499 cell/μL (unadjusted odds ratio (OR): 1.93 (1.49, 2.52); CD4-adjusted OR: 1.91 (1.47, 2.49); adjusted OR: 1.75 (1.33, 2.32)) (Table 2). High CD8 + T cell (≥1500 cell/μL) also predicted subsequent ADE (CD4-adjusted OR: 3.49 (1.69, 7.21); adjusted OR: 2.78 (1.34, 5.76)) and non-infectious NADE (CD4-adjusted OR: 2.18 (1.65, 2.88); adjusted OR: 2.04 (1.52, 2.75)), but not infectious NADE (CD4-adjusted OR 0.26 (0.07, 1.00), as shown in Table 3.

Association between CD4/CD8 ratio at year 2 and subsequent clinical events
Participants with a low CD4/CD8 ratio <0.4 at year 2 were not more likely to develop a subsequent clinical event from unadjusted and CD4-adjusted analyses compared to those with CD4/CD8 ratio >1 (unadjusted OR: 0.96 (0.76, 1.21); CD4-adjusted OR 1.09 (0.82, 1.46)) (Table 2). Low CD4/CD8 ratio <0.4 also did not predict subsequent ADEs, infectious NADEs or non-infectious NADEs (Table 3).

High CD8+ T cells and low CD4/CD8 ratio at year 2 correlate with higher immune activation
The CD4+ T cell counts, CD8+ T cell counts and CD4/CD8 ratio showed significant correlations with the T cell activation parameters measured cross-sectionally at year 2, with the CD4/CD8 ratio the parameter more strongly associated with the %CD4+ HLA-DR+CD38+ T cells (N = 444, Rho -0.47, p < 0.0001) and with the %CD8+ HLA-DR+CD38+ T cells (N = 780, Rho -0.27, p < 0.0001) (**Supplementary Table 3** and **Supplementary Fig. 1**).

Discussion
In this large prospective cohort of treatment-naïve individuals initiating ART from multiple ACTG clinical trials we demonstrate that participants with high CD8+ T cell counts (> 1500 cells/μL) measured at year 2 after ART initiation were more likely to develop a subsequent

Characteristic		Total (*N* = 5133)	Clinical event in years 2−7	
			Yes (*N* = 379)	No (*N* = 965)
Female Sex	*N* (%)	959 (19%)	79 (21%)	174 (18%)
Race/ethnicity	White	2168 (42%)	186 (49%)	453 (47%)
	Black	1731 (34%)	133 (35%)	262 (27%)
	Hispanic/other	1234 (24%)	60 (16%)	250 (26%)
Age (years)	Median (Q1, Q3)	38 (31, 45)	44 (38, 51)	39 (33, 45)
Injection drug use (currently/previously)	N (%)	407 (8%)	41 (11%)	54 (6%)
Initial ART regimen	Boosted PI + NRTIs	1855 (36%)	120 (32%)	214 (22%)
	NNRTI + NRTIs	1970 (38%)	146 (39%)	532 (55%)
	INSTI + NRTIs	523 (10%)	28 (7%)	0 (0%)
ART regimen at year 2	Boosted PI + NRTIs	1939 (38%)	126 (33%)	241 (25%)
	NNRTI + NRTIs	1982 (39%)	149 (39%)	548 (57%)
	INSTI + NRTIs	545 (11%)	29 (8%)	0 (0%)
Pre-ART HIV RNA (copies/ml)	>100000	1881 (37%)	167 (44%)	387 (40%)
Pre-ART CD4 count	Median (Q1, Q3)	249 (91, 372)	208 (63, 350)	203 (55, 325)
	<=50	933 (18%)	87 (23%)	226 (23%)
	51-200	1210 (24%)	95 (25%)	253 (26%)
	201-350	1500 (29%)	103 (27%)	285 (30%)
	351-500	983 (19%)	59 (16%)	122 (13%)
	>500	504 (10%)	35 (9%)	77 (8%)
CD4 count at year 2	Median (Q1, Q3)	503 (348, 668)	476 (311, 656)	463 (321, 609)
CD4 change from pre-ART to year 2	Median (Q1, Q3)	253 (164, 359)	251 (151, 377)	251 (165, 350)
Pre-ART CD8 count	Median (Q1, Q3)	778 (527, 1093)	763 (496, 1084)	744 (493, 1,073)
CD8 count at year 2	Median (Q1, Q3)	772 (578, 1022)	818 (584, 1061)	770 (588, 1014)
	<500	798 (16%)	61 (16%)	146 (15%)
	500-1499	4,051 (79%)	287 (76%)	770 (80%)
	>=1500	284 (6%)	31 (8%)	49 (5%)
CD8 change from pre-ART to year 2	Median (Q1, Q3)	-25 (-275, 223)	-30 (-300, 296)	6 (-248, 262)
Pre-ART CD4:CD8 ratio	Median (Q1, Q3)	0.3 (0.1, 0.4)	0.2 (0.1, 0.4)	0.2 (0.1, 0.4)
	<0.15	1468 (29%)	130 (34%)	338 (35%)
	0.15-0.3	1313 (26%)	103 (27%)	246 (25%)
	>0.3	2349 (46%)	146 (39%)	379 (39%)
CD4:CD8 ratio at year 2	Median (Q1, Q3)	0.7 (0.4, 1.0)	0.6 (0.4, 0.9)	0.6 (0.4, 0.9)
	<0.4	1209 (24%)	103 (27%)	267 (28%)
	0.4-1	2801 (55%)	202 (53%)	523 (54%)
	>1	1123 (22%)	74 (20%)	175 (18%)
CD4:CD8 change from pre-ART to year 2	Median (Q1, Q3)	0.4 (0.2, 0.5)	0.4 (0.2, 0.5)	0.3 (0.2, 0.5)
History of a clinical event on or before year 2	N (%)	1681 (33%)	186 (49%)	321 (33%)

***Table 1*: Demographics pre-ART variables and variables at year 2 of study participants.**

clinical event during the follow-up that those with lower counts. This effect was independent of the CD4+ T cell count. While both increased CD8+ T cell counts and decreased CD4/CD8 ratios correlate with T cell activation, only the presence of >1500 CD8+ T cell counts was clearly predictive of both AIDS and noninfectious non-AIDS events through year 7 of ART, although this phenotype was uncommon (6%). Because this association was not detected with the CD4/CD8 ratio, our findings suggest that the CD8+ T cell count is more informative than the CD4/CD8 ratio when measured after 2 years of ART.

Persistently high CD8+ T cell counts or low CD4/CD8 ratios during ART have been linked with AIDS and non-AIDS morbidity and mortality in some,[7,21,23,36] but not all[24,25] studies. Expansion of CD8+ T cells, typically resulting in inversion of the CD4/CD8 ratio, is a classical hallmark of immunosenescence. T cell 'immunosenescence' is generally defined as a low naive/memory T cell ratio due to impaired thymic function,[37] enrichment for CD28-T cells, increased CRP and IL-6 levels, reduced T cell telomere lengths, expansion of CMV-specific CD8+ T cells, and a low CD4/CD8 ratio.[11–13,38] This senescent immunologic profile has been linked with mortality in

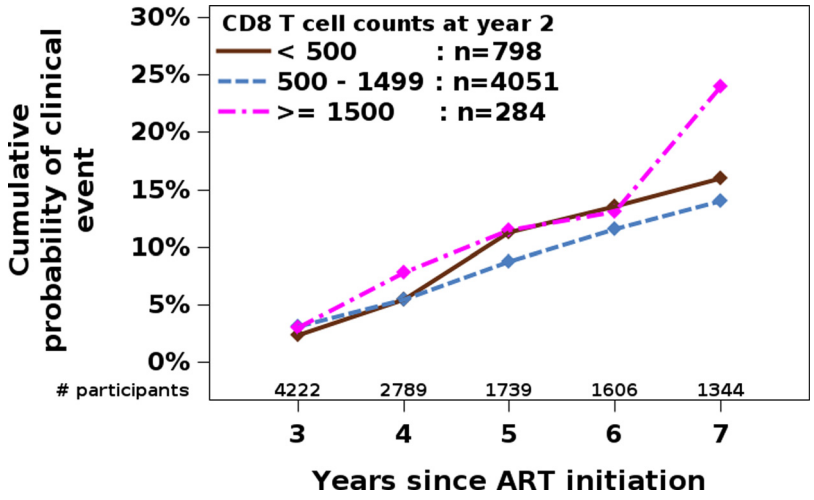

Figure 2. *Estimated cumulative probability of clinical events subsequent to year 2 by CD8+ T cell at year 2*. Also presented are the number of participants at each year who either had an observed clinical event previously, or who remained in follow-up on suppressive ART (i.e., non-censored).

the general population,[39] and also among PWH.[7,21,23,36] Because untreated HIV infection is associated with each of these immunologic traits, it has been proposed that HIV accelerates the aging of human immune system.[40] The extent to which successful ART reverses these HIV-induced immune changes and, consequently, restores health, is currently the subject of intense investigation. Among the current research gaps, it is unclear whether the CD8+ T cell counts and the CD4/CD8 ratio are overlapping prognostic predictors, which cut-offs to use, and what type of clinical events they can predict.

Previous studies focused on CD8+ T cells have reported that increased values are associated with a variety of conditions in PWH, including myocardial infarction,[41] restenosis after coronary stenting,[18] cancer,[42] and non-AIDS mortality.[22] The CD4/CD8 ratio has been correlated with a wide range of clinical events, including impaired vaccine response,[43] bacterial infections,[27] myocardial infarction,[23] cancer,[20,44] frailty,[45] and non-AIDS mortality.[7,21] Other studies have found associations between either the CD8+ T cell counts, the CD4/CD8 ratio or both and all-cause mortality, but not additional predictive value was found,[24] or the magnitude of adjusted associations of these biomarkers with mortality was too small to be useful as independent prognostic markers.[25]

A number of reasons could explain the differences in the reported associations across studies. Because we felt

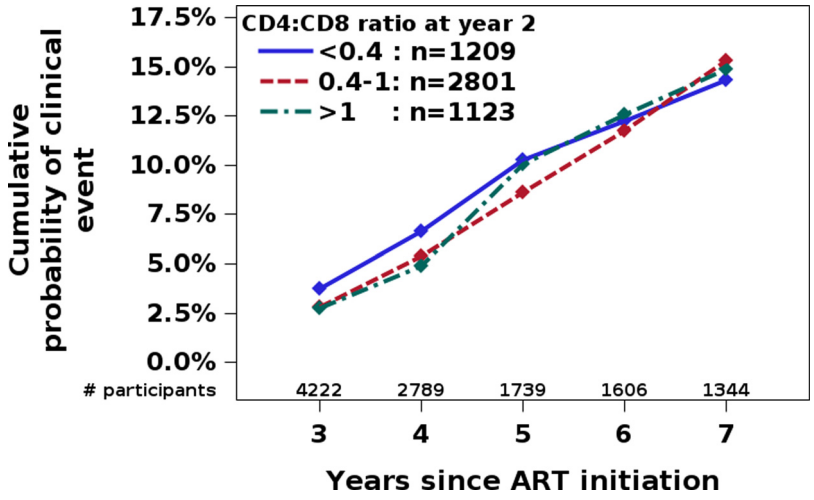

Figure 3. *Estimated cumulative probability of clinical events subsequent to year 2 by CD4/CD8 ratio at year 2*. Also presented are the number of participants at each year who either had an observed clinical event previously, or who remained in follow-up on suppressive ART (i.e., non-censored).

Variables		Unadjusted		CD4-adjusted		Multivariable adjusted*	
		Odds Ratio (95% CI)	*P*-value	Odds Ratio (95% CI)	*P*-value	Odds Ratio (95% CI)	*P*-value
CD4 count at year 2	≤200 vs >200	1.34 (1.01, 1.77)	0.04			0.95 (0.71, 1.28)	0.75
CD4 count at year 2	every 100 cells increase	1.02 (0.99, 1.06)	0.13				
CD8 count at year 2	<500 vs 500-1499	1.19 (0.96, 1.48)	0.12	1.21 (0.97, 1.51)	0.08	1.19 (0.94, 1.49)	0.15
	≥1500 vs 500-1499	**1.93 (1.49, 2.52)**	**<0.001**	**1.91 (1.47, 2.49)**	**<0.001**	**1.75 (1.33, 2.32)**	**<0.001**
	Overall Test:		<0.001		<0.001		<0.001
CD4/CD8 ratio at year 2	<0.4 vs >1	0.96 (0.76, 1.21)	0.72	1.09 (0.82, 1.46)	0.56	0.73 (0.56, 0.94)	0.02
	0.4-1 vs >1	1.04 (0.84, 1.28)	0.73	1.11 (0.88, 1.39)	0.38	0.97 (0.78, 1.21)	0.79
	Overall Test:		0.68		0.68		0.009
CD4/CD8 ratio at year 2	Q1 vs Q4	0.89 (0.71, 1.11)	0.3	1.01 (0.76, 1.34)	>0.90	0.70 (0.55, 0.89)	0.004
	Q2 vs Q4	1.05 (0.85, 1.31)	0.63	1.14 (0.90, 1.46)	0.29	0.91 (0.72, 1.15)	0.42
	Q3 vs Q4	0.86 (0.68, 1.09)	0.22	0.91 (0.71, 1.15)	0.42	0.94 (0.73, 1.20)	0.61
	Overall Test:		0.21		0.22		0.01

Table 2: **Odds ratios and 95% confidence intervals for clinical events at years 3–7.**

* Each row adjusted for race/ethnicity, injection drug use, age, initial ARV regimen, pre-ARV HIV viral load and history of clinical events. Not adjusted for CD4 (see CD4-adjusted results). Q1 represents the lowest 25% of the population for CD4/CD8 ratio at year 2, Q2 represents the next 25%, Q3 represents the next 25% and Q4 represents the highest 25% of the population. Statistical significance was calculated using Wald tests.

that the relevant point is how to predict the risk of clinical progression in patients on stable ART, we used the CD8+ T cell counts and CD4/CD8 ratio achieved at year 2 of ART, in contrast with previous studies in which the timing of CD4+ and CD8+ T cell count evaluation from ART evaluation was variable.[7,21,23–25,46] For example, in our previous studies linking the CD4/CD8 ratio with severe non-AIDS events and mortality, the measurements were those more proximal to the events.[7,20] The different definitions of non-AIDS events across studies could also explain the differences. While many previous studies have a retrospective design, this study was performed in a prospective cohort in which the clinical events were previously formally specified in the protocol and were prospectively adjudicated. It is plausible that the CD8+ T cell counts and the CD4/CD8 ratio are mechanically linked with some but not all the different types of non-AIDS events. Hence, the distribution of non-AIDS events in each cohort could also play a role in the observed associations, and the socio-demographic characteristics of the study population likely play an influence. The time at risk evaluated following the CD8 + T cell counts and CD4/CD8 ratio measurement is an important factor that could explain the results. As shown in Figure 2, participants with a CD4/CD8 ratio <0.4 had a slightly higher probability of subsequent clinical events during years 3 to 4, but not during years 4 to 7.

In contrast, the CD8+ T cell counts remained predictive of clinical events during the entire period. We think that these observations suggest that the CD4/CD8 ratio predicts clinical events in the short-term, but not in the long-term, indicating that the excess risk of events captured by the CD4/CD8 ratio is attenuated as it slowly recovers during long-term ART.[47] The CD8+ T cell counts, which show smaller changes over time,[48] more robustly predict disease.

The major strengths of the study include the prospective design, the large sample size, the large number of observations, the long follow-up, and the analytical strategy using IPW to address informative censoring. Several limitations must be taken into account when interpreting our results. First, the main limitation is related to the observational design, which could have resulted in residual confounding interfering in the results and in missing information on potential confounders, including unnoticed viral failures, and HCV or CMV serostatus. We had the opportunity to study the prevalence of CMV serostatus in a subset of this population and found that 94% of this population was CMV seropositive.[49] Hence, we do not think that the lack of information on this covariate represents a significant source of bias in our cohort. In addition, as is always a possibility in a study of clinical events, there could be some degree of underreporting, but if any, it should not have occurred differentially in the study groups. Second, this cohort is representative of a middle-aged population

Variables		AIDS		Infectious Non-AIDS		Non-infectious Non-AIDS	
		Odds Ratio (95% CI)	*P*-value	Odds Ratio (95% CI)	*P*-value	Odds Ratio (95% CI)	*P*-value
CD4 Adjusted							
CD8 count at year 2	<500 vs 500−1499	1.47 (0.72, 3.02)	0.29	1.61 (1.04, 2.50)	0.03	1.26 (0.99, 1.60)	0.06
	≥1500 vs 500−1499	**3.49 (1.69, 7.21)**	**<0.001**	0.26 (0.07, 1.00)	0.05	**2.18 (1.65, 2.88)**	**<0.001**
	Overall Test:		0.003		0.01		<0.001
CD4:CD8 ratio at year 2	<0.4 vs >1	1.30 (0.40, 4.19)	0.66	0.73 (0.37, 1.43)	0.36	1.12 (0.82, 1.54)	0.49
	0.4−1 vs >1	1.75 (0.65, 4.68)	0.26	0.96 (0.58, 1.61)	0.89	1.09 (0.85, 1.40)	0.49
	Overall Test:		0.38		0.49		0.76
CD4:CD8 ratio at year 2	Q1 vs Q4	1.88 (0.58, 6.07)	0.29	0.69 (0.37, 1.31)	0.26	1.08 (0.79, 1.47)	0.63
	Q2 vs Q4	2.26 (0.78, 6.52)	0.13	0.96 (0.56, 1.65)	0.87	1.14 (0.87, 1.48)	0.35
	Q3 vs Q4	2.05 (0.72, 5.89)	0.18	0.73 (0.42, 1.28)	0.27	0.87 (0.67, 1.14)	0.32
	Overall Test:		0.47		0.44		0.21
Multivariable Adjusted*							
CD4 count at year 2	≤200 vs >200	1.79 (0.82, 3.94)	0.15	0.83 (0.42, 1.67)	0.61	1.03 (0.75, 1.40)	0.87
CD8 count at year 2	<500 vs 500−1499	1.86 (0.90, 3.85)	0.09	**1.70 (1.09, 2.65)**	**0.02**	1.21 (0.94, 1.56)	0.13
	≥1500 vs 500−1499	**2.78 (1.34, 5.76)**	**0.006**	**0.20 (0.05, 0.78)**	**0.02**	**2.04 (1.52, 2.75)**	**<0.001**
	Overall Test:		0.01		0.003		<0.001
CD4/CD8 ratio at year 2	<0.4 vs >1	1.24 (0.44, 3.50)	0.68	0.69 (0.39, 1.23)	0.21	0.74 (0.56, 0.98)	0.04
	0.4−1 vs >1	1.71 (0.66, 4.42)	0.27	1.05 (0.64, 1.71)	0.85	0.96 (0.75, 1.22)	0.72
	Overall Test:		0.38		0.18		0.04
CD4/CD8 ratio at year 2	Q1 vs Q4	1.57 (0.56, 4.36)	0.39	0.68 (0.40, 1.16)	0.16	0.73 (0.56, 0.95)	0.02
	Q2 vs Q4	1.91 (0.70, 5.23)	0.21	1.01 (0.61, 1.68)	>0.90	0.89 (0.69, 1.15)	0.38
	Q3 vs Q4	2.10 (0.75, 5.90)	0.16	0.85 (0.49, 1.48)	0.58	0.90 (0.69, 1.19)	0.47
	Overall Test:		0.51		0.37		0.11

***Table 3*: Odds ratios and 95% confidence intervals for AIDS events, Infectious non-AIDS and noninfectious non-AIDS events at years 3−7 separately.**

* Each row adjusted for race/ethnicity, injection drug use, age, initial ARV regimens, pre-ARV HIV viral load and history of clinical events, and not adjusted for CD_4 counts.

Q1 represents the lowest 25% of the population for CD_4/CD_8 ratio at year 2, Q2 represents the next 25%, Q3 represents the next 25% and Q4 represents the highest 25% of the population.

Statistical significance was calculated using Wald tests.

with a predominance of men in the United States, and from 385 registered non-AIDS events, the most frequent was diabetes mellitus (28.6%), followed by renal failure (22.9%) and bacterial infections (14.5%) during a maximum follow-up of 7 years. Hence, we cannot extrapolate our results to women or to individuals with longer follow-up. Third, the expected higher prevalence of diabetes mellitus and obesity in the United States compared to other countries[50] could also influence the results. Last, our findings might be affected by the antiretroviral drugs used in the parent studies (Table S1). Because integrase inhibitors have been associated to greater CD8+ T cell decrease and CD_4/CD_8 ratio normalization,[51,52] it would be necessary to reproduce our findings in cohorts included more modern ART regimens and earlier ART initiation than those represented in our study.

To our knowledge, no other studies have analyzed the associations between the CD8+ T cell counts and the CD_4/CD_8 ratio with infectious and noninfectious events. Because immunosenescence is expected to result in an increased risk of infections,[50] we expected that high CD8+ T cell counts and low CD_4/CD_8 ratios would be linked with a greater risk of infectious events. Intriguingly, we found that participants with increased CD8+ T cell counts were protected against infectious non-AIDS events. This finding is consistent with the results reported in a cohort study of 885 PWH, where increased risk of bacterial pneumonia was associated with low (<400/uL) CD8+ T cells.[53] We think that a future direction for this field is to understand further whether these altered immune profiles (i.e., high CD8+ T cell counts or low CD_4/CD_8 ratios despite effective ART) confer protection against certain type of non-AIDS events such as the risk of bacterial infections.

In summary, the results of this analysis in a large prospective cohort support the value of the CD8+ T cell count on suppressive ART as a predictor of clinical progression. High absolute levels of CD8+ T cell counts at year 2 (≥ 1500 cells/mm^3) were predictive of overall clinical events through year 7. In separate modeling, these year 2 measures were also predictive of AIDS-defining events and noninfectious non-AIDS-defining events through year 7. Conversely, participants with high CD8+ T cell counts showed lower risk of severe infections. The CD8+ T cell count might be a useful

biomarker of clinical endpoint to be used in evaluating novel therapies for ongoing immune dysfunction during treated infection.

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Declaration of interests

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Supplementary materials

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