CD4:CD8 Ratio and CD8 Count as Prognostic Markers for Mortality in Human Immunodeficiency Virus–Infected Patients on Antiretroviral Therapy: The Antiretroviral Therapy Cohort Collaboration (ART-CC)

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Background. We investigated whether CD4:CD8 ratio and CD8 count were prognostic for all-cause, AIDS, and non-AIDS mortality in virologically suppressed patients with high CD4 count.

Methods. We used data from 13 European and North American cohorts of human immunodeficiency virus–infected, antiretroviral therapy (ART)–naive adults who started ART during 1996–2010, who were followed from the date they had CD4 count ≥350 cells/µL and were virologically suppressed (baseline). We used stratified Cox models to estimate unadjusted and adjusted (for sex, people who inject drugs, ART initiation year, and baseline age, CD4 count, AIDS, duration of ART) all-cause and cause-specific mortality hazard ratios for tertiles of CD4:CD8 ratio (0–0.40, 0.41–0.64 [reference], >0.64) and CD8 count (0–760, 761–1138 [reference], >1138 cells/µL) and examined the shape of associations using cubic splines.

Results. During 276 526 person-years, 1834 of 49 865 patients died (249 AIDS-related; 1076 non-AIDS-defining; 509 unknown/unclassifiable deaths). There was little evidence that CD4:CD8 ratio was prognostic for all-cause mortality after adjustment for other factors: the adjusted hazard ratio (aHR) for lower vs middle tertile was 1.11 (95% confidence interval [CI], 1.00–1.25). The association of CD8 count with all-cause mortality was U-shaped: aHR for higher vs middle tertile was 1.13 (95% CI, 1.01–1.26). AIDS-related mortality declined with increasing CD4:CD8 ratio and decreasing CD8 count. There was little evidence that CD4:CD8 ratio or CD8 count was prognostic for non-AIDS mortality.

Conclusions. In this large cohort collaboration, the magnitude of adjusted associations of CD4:CD8 ratio or CD8 count with mortality was too small for them to be useful as independent prognostic markers in virally suppressed patients on ART.

Keywords. CD8 count; CD4:CD8 ratio; mortality; HIV; antiretroviral therapy.

CD4^{+} T-lymphocyte (CD4) counts and human immunodeficiency virus type 1 (HIV-1) RNA measurements (viral load [VL]) are the most important prognostic markers for disease progression and recovery after starting combination antiretroviral therapy (ART) in people living with HIV (PLWH) [1–4]; most AIDS-related deaths occur in patients with CD4 count <350 cells/µL [5]. However, PLWH on ART with CD4 count >350 cells/µL and suppressed VL experience higher mortality than the general population, mainly due to non-AIDS causes [6, 7]. The CD4:CD8 ratio and CD8^{+} T-lymphocyte (CD8) counts have been suggested as prognostic markers for mortality, in addition to VL and CD4 count, in such patients [8–12]. The Insight Strategic Timing of Antiretroviral Treatment (START) study group showed that in PLWH diagnosed with a CD4 count >500 cells/µL, those with a VL >50 000 copies/mL or with a CD4:CD8 ratio <0.5 benefited the most from early treatment compared with deferring treatment to when CD4 count reached 350 cells/µL [13]. Moreover, they suggested that the best surrogate markers for the effect of early treatment on clinical outcomes were controlling the VL and increasing the CD4:CD8 ratio.

CD8 counts increase in response to acute infection, but also remain raised in chronic infections such as HIV. CD8 counts respond to ART more slowly than CD4 counts. In the general population, CD4:CD8 ratio declines with age and is associated with mortality [14]. The ratio is considered to be a marker of cumulative inflammation and immunological changes associated with aging [15]. Results of previous studies that examined associations of CD4:CD8 ratio or CD8 count with mortality in HIV-infected patients on ART were inconsistent [16–21].

We investigated whether the CD4:CD8 ratio or CD8 counts were independently associated with all-cause, AIDS, and non-AIDS mortality in patients treated with ART with suppressed VL and CD4 count >350 cells/μL using data from the Antiretroviral Therapy Cohort Collaboration (ART-CC).

METHODS

Eligibility

We combined data from 13 European and North American cohorts (see Appendix) participating in ART-CC (www.art-cohort-collaboration.org) [22]. Participation of cohorts has been approved by their ethics committees or institutional review boards according to local regulations. Cohorts use standardized methods of data collection, and schedule follow-up visits at least every 6 months.

Eligible patients were ART naive and aged ≥16 years when they started a combination ART regimen containing at least 3 antiretroviral drugs between 1996 and 2010. Follow-up began on the date after starting ART (baseline) when the patient's CD4 count first exceeded ≥350 cells/μL, a CD8 count measurement was recorded, and VL was lower than the limit of detection of the assay or <200 copies/μL. In a sensitivity analysis, baseline was defined as the date of the second successive CD4 count ≥350 cells/μL with viral suppression. Patients were considered lost to follow-up if there was a gap of >1 year between the date the patient was last known to be alive and the cohort-specific database close date and were censored 6 months after the date they were last known to be alive. Database close date varied among cohorts between 31 May 2012 and 31 July 2013.

Cause of Death Information

Information on mortality was obtained either through linkage with vital statistics agencies and hospitals or through physician report and active follow-up of patients. We adapted the Cause of Death (CoDe) project protocol (www.cphiv.dk/CoDe.aspx) [23] to classify causes of death. We used a computer algorithm developed by the Mortalité 2000–2005 Study Group [24] to classify deaths where *International Classification of Diseases, Tenth Revision* (ICD-10) codes were available, which were also coded by a clinician and compared. For deaths described by free text, 2 clinicians independently classified each death. Disagreements between clinicians and/or computer-assigned codes were resolved via panel discussion, as described previously [25, 26].

If information from ICD-10 codes or free text was insufficient to determine the cause, deaths were labeled "unclassifiable" and in these analyses were combined with deaths with no information and labeled "unknown." Deaths were coded as AIDS-related if there was an AIDS-defining condition(s) close to death and/or a low CD4 count (<100 cells/μL) prior to death and a diagnosis compatible with AIDS as cause of death. All other deaths (excluding "unknown" deaths which were not included in analyses of cause-specific mortality) were assumed to be non–AIDS related.

Statistical Methods

Covariates included in multivariable models were chosen a priori from literature [27]. We categorized age (16–29, 30–39, 40–49, 50–59, 60–69, and ≥70 years), year of ART initiation (1996–1999, 2000–2003, 2004–2007, and 2008–2010), and baseline CD4 count (350–499, 500–749, and ≥750 cells/μL). We analyzed CD4:CD8 ratio and CD8 count both as continuous variables using spline transformations to capture nonlinear associations flexibly and in tertiles of their distribution at baseline, to compare the magnitude of associations in the same metric. The knots for the cubic splines were positioned by default using Harrell recommended percentiles [28] at 0.3, 0.53, and 1.02 for CD4:CD8 ratio and at 510, 909, and 1528 cells/μL for CD8 count. The tertile groups were 0–0.40, 0.41–0.64, and >0.64 for CD4:CD8 ratio and 0–760, 761–1138, and >1138 cells/μL for CD8.

We plotted smoothed (using a moving average over 3 years) mean CD4 count, CD4:CD8 ratio, and CD8 count by time since baseline. We used Cox models stratified by cohort to estimate unadjusted, adjusted (for sex, age, people who inject drugs transmission group, calendar period of ART initiation, AIDS status, time from ART initiation to baseline) and additionally adjusted (for baseline CD4 count) mortality hazard ratios (HRs) for the lowest and highest tertiles compared to the middle (reference) tertile for baseline CD4:CD8 ratio and CD8 count. Separate models were estimated for all-cause, AIDS-related, and non-AIDS-related mortality. In the analysis of AIDS-related mortality, those experiencing non-AIDS-related death were censored at date of death (and vice versa). In a sensitivity analysis we modeled AIDS and non-AIDS mortality using competing risks regression.

We modeled and plotted adjusted HRs (compared to the median) across the range of CD4:CD8 ratios and CD8 counts using spline transformations of these variables. Spline plots were truncated at 0.1 and 1.25 for CD4:CD8 ratio (97.5% of sample) and 10 and 2000 cells/μL for CD8 count (95% of sample). We used 2 degrees of freedom likelihood ratio (LR) tests to assess the evidence that CD4:CD8 ratio and CD8 count (modeled using splines and as tertiles) were prognostic.

RESULTS

Characteristics

Of 66 610 patients with both CD4 and CD8 counts recorded, 49 865 (75%) reached a CD4 count ≥350 cells/μL with suppressed

VL and therefore were included in analyses. Of these, 1834 died during 276 526 person-years (6.7 deaths/1000 person-years). Two hundred forty-nine deaths (14%) were AIDS-related, 1076 (59%) non-AIDS-related, and 509 (28%) classified as unknown. Duration of ART before reaching CD4 ≥350 cells/µL was longer in those who started ART with a lower CD4 count. However, nearly half of patients in our analysis had undetectable VL and reached CD4 ≥350 cells/µL within 6 months of ART initiation (Table 1). Patient demographics and clinical characteristics at baseline are shown in Table 1. The median age was 37 (interquartile range [IQR], 31–45) years. The median baseline CD4 count, CD8 count, and CD4:CD8 ratio were 446 (IQR, 386–566) cells/µL, 930 (IQR, 679–1271) cells/µL, and 0.52 (IQR,

Table 1. Characteristics (Within 3 Months) of Patients at Start of Follow-up (Baseline) (N = 49 865)

Variable	Patients, No. (%)
Female sex	13 724 (28)
PWID transmission	3638 (7)
AIDS	9283 (19)
Age, y	
16–29	7425 (15)
30–39	18 487 (37)
40–49	15 204 (30)
50–59	6296 (13)
60–69	2060 (4)
≥70	393 (0.8)
Years from ART start to reaching CD4 count ≥350 cells/µL (rounded to nearest year)[a]	
0	23 693 (48)
1	11 440 (23)
2	4939 (10)
3	2953 (6)
4	1893 (4)
≥5	4947 (10)
Calendar period of ART initiation	
1996–1999	11 739 (24)
2000–2003	11 763 (24)
2004–2007	12 369 (25)
2008–2012	13 994 (28)
CD4 count, cells/µL	
350–499	31 779 (64)
500–749	13 367 (27)
≥750	4719 (9)
CD4:CD8 ratio	
0–0.40	16 276 (33)
0.41–0.64	17 011 (34)
>0.64	16 578 (33)
CD8 count, cells/µL	
0–760	16 671 (33)
761–1138	16 583 (33)
>1138	16 611 (33)

CD8 and CD4:CD8 have been split into tertiles calculated when the patients reach baseline. Baseline was defined as date of reaching a CD4 count ≥350 cells/µL with an undetectable viral load (lower than lower limit of viral assay or <200 copies/mL).

Abbreviations: ART, antiretroviral therapy; PWID, people who inject drugs.

[a]Year 0 contains those who reached a CD4 count ≥350 cells/µL ≤6 months after ART start.

0.37–0.74), respectively. Baseline CD4 count was less correlated with CD4:CD8 ratio ($r = 0.075$) than with CD8 count ($r = 0.128$) (difference in correlations $P < .0001$).

Trajectories of Biomarkers Over Time

Smoothed mean CD4 count, CD8 count, and CD4:CD8 ratio up to 10 years after baseline are shown in Figure 1. Mean CD4 count increased steadily, from 507 to 678 cells/µL at 10 years. Mean CD8 count declined from 1040 at baseline to 942 cells/µL at 1 year, then plateaued at around 930 cells/µL. Mean CD4:CD8 ratio increased sharply from 0.49 at baseline to 0.58 at 1 year, then less rapidly to 0.73 at 10 years.

All-Cause Mortality

Rates of all-cause mortality declined with increasing CD4:CD8 ratio (Figure 2), but confidence intervals (CIs) were wide and there was little evidence (LR $P = .17$) that CD4:CD8 ratio was prognostic after adjusting for other variables. In contrast, CD8 count showed a U-shaped association with all-cause mortality after adjusting for other variables (LR $P = .007$), with lowest mortality rates near the median value and highest rates for those with high CD8 counts (Figure 2). Before adjustment, lower tertile of baseline CD4:CD8 ratio (hazard ratio [HR], 1.20 [95% CI, 1.08–1.34]) and higher tertile of CD8 count (HR, 1.26 [95% CI, 1.13–1.41]) were each associated with higher all-cause mortality compared with the middle (reference) tertile (Table 2), but associations were attenuated after adjustment to 1.11 (95% CI, 1.00–1.25) and 1.13 (95% CI, 1.01–1.26), respectively. Neither tertiles of CD4:CD8 ratio nor of CD8 count were prognostic for all-cause mortality (LR $P = .16$ and $P = .11$, respectively).

AIDS-Related Mortality

AIDS-related mortality declined with increasing CD4:CD8 ratio (LR $P = .005$) and decreasing CD8 count (LR $P = .009$) (Figure 3). The adjusted hazard ratio (aHR) for AIDS-related
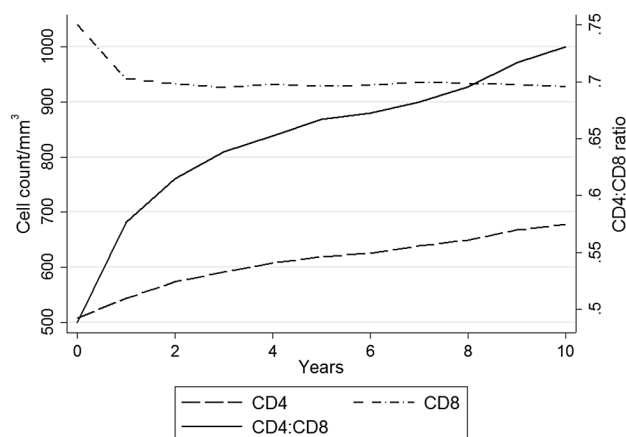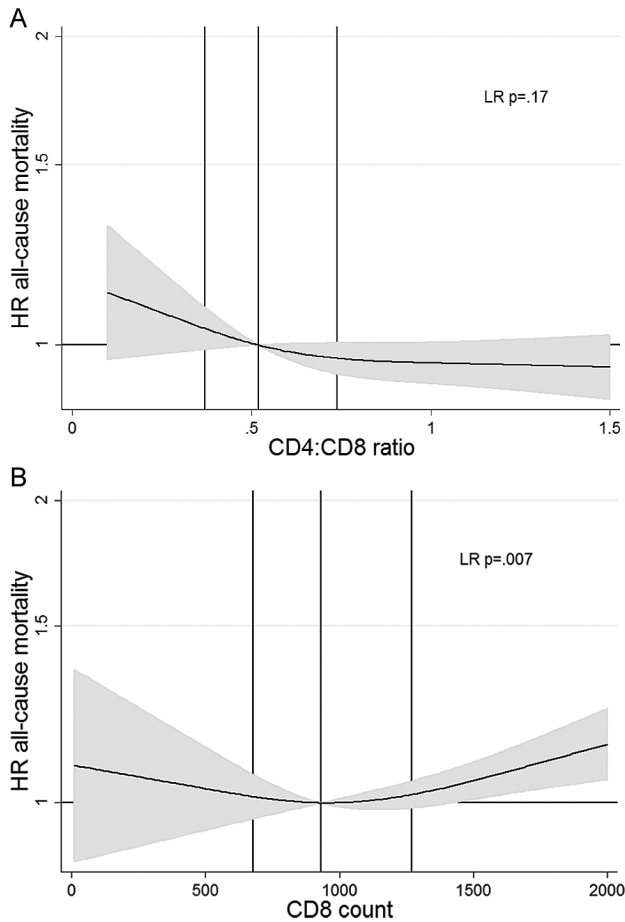

Figure 1. Smoothed mean CD4 count, CD8 count, and CD4:CD8 ratio, by years after baseline (reaching a CD4 count ≥350 cells/µL and viral suppression).

Figure 2. Adjusted hazard ratios (HRs) for all-cause mortality with median as comparator for CD4:CD8 ratio (upper panel) and CD8 count (lower panel), based on cubic spline models and adjusted for other prognostic variables. Gray-shaded area indicates the 95% confidence interval and the vertical lines indicate the median and interquartile range. Abbreviation: LR, likelihood ratio.

mortality for the lowest (vs middle) tertile of CD4:CD8 ratio was 1.28 (95% CI, .95–1.73) and for the highest (vs middle) tertile of CD8 was 1.36 (95% CI, 1.01–1.84) (Table 2). Tertiles of both CD4:CD8 ratio and CD8 count were prognostic for AIDS-related deaths (LR P = .016 and P = .0037, respectively).

Non-AIDS-Related Mortality

When CD4:CD8 ratio was modeled as a continuous variable, there was no evidence (LR P = .79) that it was prognostic for non-AIDS-related mortality (Figure 3). However, there was weak evidence (LR P = .1) that mortality was higher in the lower and upper tertiles compared with the middle tertile of CD4:CD8 ratio (aHR, 1.10 [95% CI, .95–1.28] and 1.19 [95% CI, 1.01–1.39], respectively). CD8 count had a u-shaped association with non-AIDS mortality (Figure 3), but there was little evidence that it was prognostic after adjusting for other variables (LR P = .14 for continuous and P = .38 for tertiles).

In sensitivity analyses (1) using competing risk regression and (2) starting follow-up from second CD4 count >350 cells/μL (Supplementary Table 1), estimates were similar to those in the main analysis.

DISCUSSION

Based on a large dataset combined from European and North American clinical cohorts, we compared the utility of CD4:CD8 ratio and CD8 count as prognostic markers for mortality in PLWH on ART with suppressed VL and CD4 counts >350 cells/μL. Although mean CD8 counts decreased immediately after baseline, they plateaued at a level considered higher than normal in the general population. Mean CD4 count continued to increase over 10 years of follow-up. Mean CD4:CD8 ratio

Table 2. Mortality Hazard Ratios for All-Cause, AIDS-Related, and Non-AIDS-Related Deaths Across Tertiles of CD4:CD8 Ratio and CD8 Count (N = 49 865)

Mortality	CD4:CD8 Ratio			LR P Value	CD8 Count, Cells/μL			LR P Value
	0–0.40	0.41–0.64	>0.64		0–760	761–1138	>1138	
All-cause mortality								
Unadjusted	1.20 (1.08–1.34)	1	1.05 (.93–1.18)	.003	1.04 (.92–1.17)	1	1.26 (1.13–1.41)	<.001
Adjusted, no CD4[a]	1.10 (.98–1.23)	1	1.12 (1.00–1.27)	.11	1.04 (.92–1.17)	1	1.14 (1.02–1.27)	.06
Fully adjusted[b]	1.11 (1.00–1.25)	1	1.07 (.95–1.21)	.16	1.05 (.93–1.18)	1	1.13 (1.01–1.26)	.11
AIDS mortality								
Unadjusted	1.39 (1.04–1.86)	1	0.82 (.59–1.15)	.003	0.94 (.66–1.32)	1	1.55 (1.15–2.09)	.001
Adjusted, no CD4[a]	1.23 (.92–1.65)	1	0.87 (.62–1.22)	.095	0.93 (.66–1.32)	1	1.38 (1.02–1.86)	.024
Fully adjusted[b]	1.28 (.95–1.73)	1	0.77 (.54–1.10)	.016	0.94 (.67–1.33)	1	1.36 (1.01–1.84)	.037
Non-AIDS mortality								
Unadjusted	1.20 (1.04–1.39)	1	1.15 (.99–1.34)	.039	1.08 (.92–1.25)	1	1.23 (1.06–1.42)	.016
Adjusted, no CD4[a]	1.10 (.95–1.27)	1	1.24 (1.06–1.44)	.026	1.08 (.93–1.26)	1	1.11 (.96–1.29)	.35
Fully adjusted[b]	1.10 (.95–1.28)	1	1.19 (1.01–1.39)	.097	1.09 (.93–1.27)	1	1.10 (.95–1.27)	.38

Data are presented as hazard ratio (95% confidence interval) unless otherwise indicated. Deaths: all-cause = 1834; AIDS-related = 249; non-AIDS-related = 1076. Likelihood ratio P value is a test with 2 degrees of freedom for models containing CD4:CD8 ratio or CD8 ratio term against models without these terms.

Abbreviation: LR, likelihood ratio.

[a]The same as the fully adjusted analysis but without adjustment for CD4.

[b]Adjusted for sex, AIDS status, CD4 count, age, and viral load at baseline; time from antiretroviral therapy (ART) initiation to baseline; calendar year of ART initiation; injection drug use transmission; and stratified by cohort.
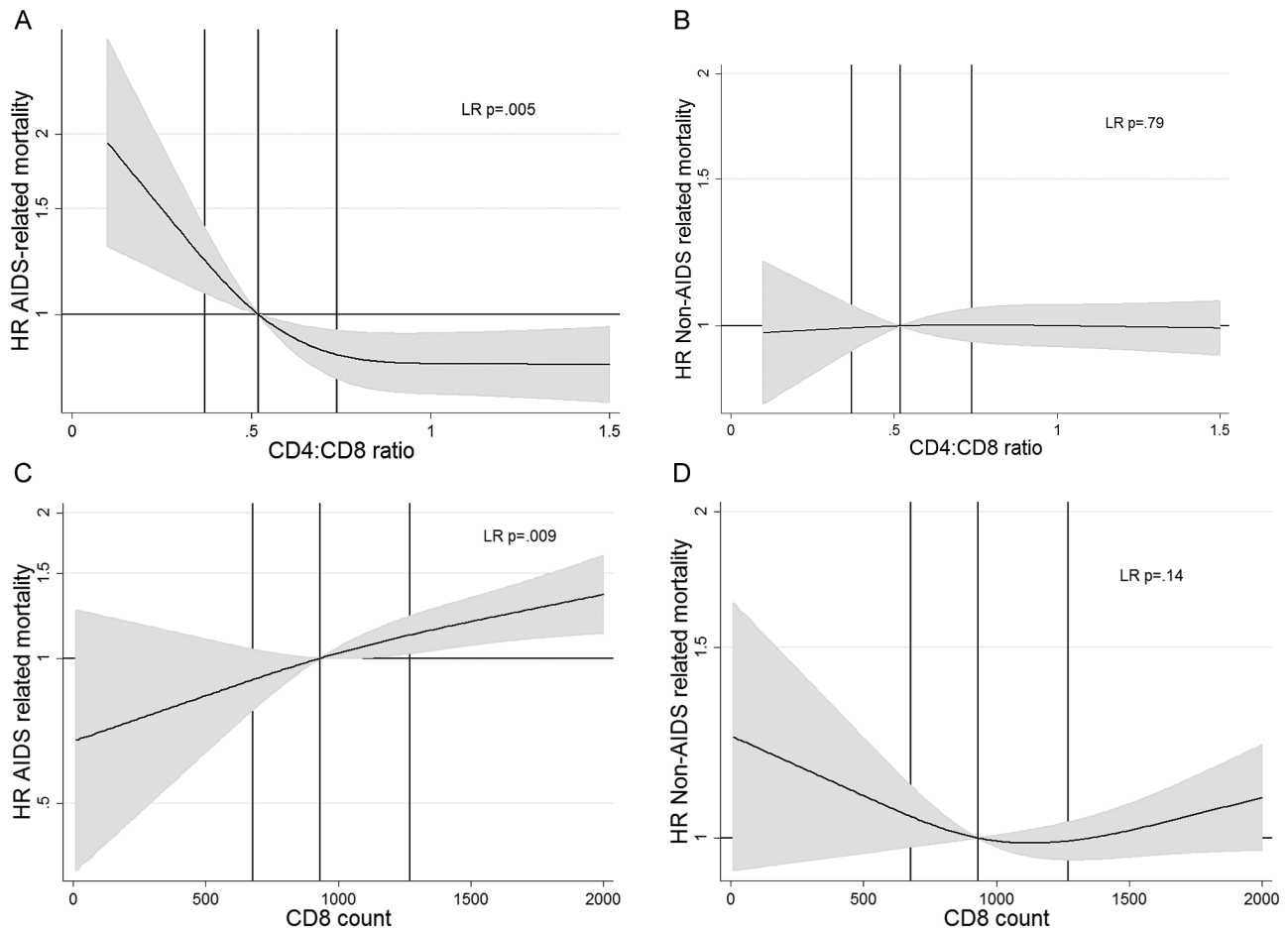
Figure 3. Plots of adjusted hazard ratio (HR) of AIDS-related (left panels) and non-AIDS-related (right panels) mortality with median as comparator for CD4:CD8 ratio (upper panels) and CD8 count (lower panels) with 95% confidence intervals. Modeled using cubic splines. The vertical lines indicate the median and interquartile range. Abbreviation: LR, likelihood ratio test.

also increased during follow-up, mostly due to increases in CD4 rather than decreases in CD8 counts, but remained short of the mean in the general population: 1.8 [29]. Associations of CD4:CD8 ratio and CD8 count with all-cause mortality were attenuated after adjustment for other prognostic factors at baseline; there was little evidence that CD4:CD8 ratio was prognostic independent of CD4 count. The association of CD8 count with all-cause mortality appeared U-shaped, with higher mortality for those with higher and lower values compared with those with values near the median. AIDS-related mortality declined with increasing CD4:CD8 ratio and decreasing CD8 count. There was little evidence that CD4:CD8 ratio or CD8 count was independently prognostic for non-AIDS mortality.

Immune Dysregulation and Inflammatory Processes
Viral infections such as HIV cause a high turnover of T cells and an accumulation of CD8 [15]. Elevated CD8 count is a marker of immune dysregulation and ongoing inflammatory processes, which may lead to increased non-AIDS-related morbidity and mortality in those treated long term for HIV infection [20].

In the general population, CD4:CD8 ratios decrease with age; many T-cell abnormalities related to aging have been observed at younger ages in HIV-infected individuals [15]. Inflammation is associated with untreated HIV infection, but ART has differential effects on markers of inflammation with some declining more than others: Interleukin 6, C-reactive protein, cystatin C, and D-dimer remain elevated with successful ART [15, 30, 31].

All-Cause Mortality
Studies that followed patients for mortality from ART start have found that the dominant prognostic marker is CD4 count and the majority of deaths are AIDS related [9]. If a patient's CD4 count is very low at ART start, then their CD8 count will also be low. This correlation between CD4 and CD8 count at ART start in immunosuppressed patients implies that the ratio is unlikely to be a useful predictor of mortality in the first year of ART [32]. However, in the START trial, which included only patients who were not immunosuppressed, low CD4:CD8 ratio was associated with the primary endpoint, which included serious AIDS and non-AIDS morbidity as well as all-cause mortality [13].

A study from the Danish HIV cohort illustrates the difficulty of interpreting CD8 count as a prognostic marker. Mortality was predicted by low CD8 count (<500 cells/μL) at ART start, but by high CD8 count 1 year afterward (>2000 cells/μL) and 10 years afterward (>1500 cells/μL) [20]. The latter 2 results are in concordance with our findings, which applied only to those with a relatively high CD4 count. Two other studies that included patients with no CD4 count restriction from ART start found that low CD8 count predicted AIDS events or all-cause mortality [3, 19]. In contrast, a large French study did not find that CD4:CD8 ratio or CD8 count added prognostic value for all-cause mortality independent of CD4 in patients with CD4 count >200 cells/μL [21], similar to our findings. These studies used a range of cutoff values for CD4:CD8 ratio or CD8 count, and did not always control for CD4 count, making comparisons difficult.

AIDS-Related Mortality

In contrast to our study, which found that high CD8 count was associated with AIDS-related mortality, the Danish study found that low CD8 count (<500 cells/μL) 1 year after ART start was associated with an increased risk [20]. Again the different results could be due to the difference in patient populations and sample size. Interestingly, CD8 count <400 cells/μL specifically predicted occurrence of pneumonia and death in a study based in the United States [33]. In agreement with our finding of low CD4:CD8 ratio associated with AIDS-related mortality, albeit with wide CIs due to few deaths, an Italian study also found an association of low CD4:CD8 ratio with higher risk of AIDS events and AIDS-related deaths among virally suppressed patients [18].

Non-AIDS-Related Mortality

The CD4 count measured at ART start loses its predictive value as duration of ART increases, whereas CD4:CD8 ratio has, at least in some studies, been found to predict non-AIDS morbidity and mortality after long-term ART [34]. A small case-control study reported an association between low CD4:CD8 ratio and serious non-AIDS-related events [16, 17], but in a study that included data from 4 cohorts and 3 trials, this result failed to replicate in those with high CD4 count [16]. A larger Italian study of patients with suppressed VLs found an elevated risk of non-AIDS mortality in those with CD4:CD8 <0.3 compared with between 0.3 and 0.8 [18], whereas we found little evidence that CD4:CD8 ratio or CD8 count was independently prognostic for non-AIDS mortality. Studies have also found associations between low CD4:CD8 ratio and coronary artery disease, but were underpowered to consider mortality [35].

Strengths and Weaknesses

Our study is observational and therefore there may be unmeasured confounding, by factors such as, for example, smoking. Although there is uncertainty about the outcome of patients lost to follow-up, we would have known if they had died, as most cohorts link to death registries. Strengths of this study include prospective study design and the large sample size and numbers of deaths which were coded by a standard procedure. Our results are likely generalizable across high-resource settings as contributing cohorts came from a wide range of countries and the sample size was much larger than any previous studies of CD8 as a prognostic marker in the treated HIV-infected population [34]. Estimating associations of CD4:CD8 ratio and CD8 count using tertiles allowed us to compare HRs based on the same metric and, together with models based on cubic splines, allowed us to examine the shape of associations across the range of each measure.

Implications

Our data show that CD8 count recovers less well than CD4 count and remains high for a lengthy period, as has been observed in other studies [20], which may indicate ongoing immune dysregulation. This implies a need for increased monitoring and early start of treatment before the CD8 count is elevated to levels it will not recover from. The consequences of this may be seen in a longer period of follow-up than we have been able to study here as our results show little evidence that CD4:CD8 is associated with all-cause mortality in a HIV population with high CD4 counts and suppressed VLs. There is slightly more evidence of an association with CD8 count and all-cause mortality in this population but the pattern was not strong despite the large size of our dataset, indicating little usefulness as a prognostic factor for all-cause mortality. The same could be said of the association of both CD4:CD8 ratio and CD8 count with non-AIDS mortality, although it is possible that associations may only be with specific causes of morbidity and death that we were unable to look at here. Our finding that both low CD4:CD8 ratio and high CD8 count were associated with AIDS mortality in this population with high CD4 counts and suppressed VLs implies that both CD4:CD8 ratio and CD8 count could account for some excess AIDS mortality in a HIV population that was otherwise healthy.

CONCLUSIONS

Our study of patients treated with ART who are virally suppressed and have CD4 count >350 cells/μL, which is the largest to date investigating the associations of CD4:CD8 ratio and CD8 count with cause-specific mortality, does not lend strong support to using either measurement as a prognostic marker for non-AIDS related mortality. However, the failure of many patients in this long-term treated HIV-infected population to reach the levels of CD4:CD8 ratio or CD8 count considered to be normal in the general population may indicate ongoing immune dysregulation. This may have longer-term consequences than we have been able to study here, or associations may be only with specific causes of death. Therefore, these markers might contribute to the immunological evaluation of

patients in long-term follow-up. Nevertheless, our results showing poor CD4:CD8 ratio recovery support treatment earlier in the course of HIV infection to better preserve immune function in PLWH as is now recommended by treatment guidelines.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

References

1. Hulgan T, Shepherd BE, Raffanti SP, et al. Absolute count and percentage of CD4+ lymphocytes are independent predictors of disease progression in HIV-infected persons initiating highly active antiretroviral therapy. J Infect Dis 2007; 195:425–31.
2. Hulgan T, Raffanti S, Kheshti A, et al. CD4 lymphocyte percentage predicts disease progression in HIV-infected patients initiating highly active antiretroviral therapy with CD4 lymphocyte counts >350 lymphocytes/mm3. J Infect Dis 2005; 192:950–7.
3. Bonnet F, Thiebaut R, Chene G, et al. Determinants of clinical progression in antiretroviral-naive HIV-infected patients starting highly active antiretroviral therapy. Aquitaine Cohort, France, 1996–2002. HIV Med 2005; 6:198–205.
4. Fregonese F, Collins IJ, Jourdain G, et al; Program for HIV Prevention and Treatment Study Group. Predictors of 5-year mortality in HIV-infected adults starting highly active antiretroviral therapy in Thailand. J Acquir Immune Defic Syndr 2012; 60:91–8.
5. Young J, Psichogiou M, Meyer L, et al; Opportunistic Infections Project Team of COHERE. CD4 cell count and the risk of AIDS or death in HIV-infected adults on combination antiretroviral therapy with a suppressed viral load: a longitudinal cohort study from COHERE. PLoS Med 2012; 9:e1001194.
6. Alejos B, Hernando V, López-Aldeguer J, et al. Overall and cause-specific mortality in HIV-positive subjects compared to the general population. J Int AIDS Soc 2014; 17(4 suppl 3):19711.
7. Lewden C, Bouteloup V, De Wit S, et al; Collaboration of Observational HIV Epidemiological Research Europe (COHERE) in EuroCoord. All-cause mortality in treated HIV-infected adults with CD4 >/=500/mm3 compared with the general population: evidence from a large European observational cohort collaboration. Int J Epidemiol 2012; 41:433–45.
8. Taylor JM, Fahey JL, Detels R, Giorgi JV. CD4 percentage, CD4 number, and CD4:CD8 ratio in HIV infection: which to choose and how to use. J Acquir Immune Defic Syndr 1989; 2:114–24.
9. Burcham J, Marmor M, Dubin N, et al. CD4% is the best predictor of development of AIDS in a cohort of HIV-infected homosexual men. AIDS 1991; 5:365–72.
10. Kvale D, Aukrust P, Osnes K, Müller F, Frøland SS. CD4+ and CD8+ lymphocytes and HIV RNA in HIV infection: high baseline counts and in particular rapid decrease of CD8+ lymphocytes predict AIDS. AIDS 1999; 13:195–201.
11. Pirzada Y, Khuder S, Donabedian H. Predicting AIDS-related events using CD4 percentage or CD4 absolute counts. AIDS Res Ther 2006; 3:20.
12. Gebo KA, Gallant JE, Keruly JC, Moore RD. Absolute CD4 vs. CD4 percentage for predicting the risk of opportunistic illness in HIV infection. J Acquir Immune Defic Syndr 2004; 36:1028–33.
13. Babiker A, Grund B, Sharma S, et al; INSIGHT START Study Group. The role of plasma HIV RNA and T cell subset counts/percent and ratio in explaining the benefit of immediate antiretroviral therapy (ART) initiation in HIV+ individuals with high CD4+ counts [abstract 7083]. In: AIDS Conference, Durban, South Africa. 2016.
14. Czesnikiewicz-Guzik M, Lee WW, Cui D, et al. T cell subset-specific susceptibility to aging. Clin Immunol 2008; 127:107–18.
15. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. Annu Rev Med 2011; 62:141–55.
16. Serrano-Villar S, Sainz T, Lee SA, 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.
17. Serrano-Villar S, Pérez-Elías MJ, Dronda F, et al. Increased risk of serious non-AIDS-related events in HIV-infected subjects on antiretroviral therapy associated with a low CD4/CD8 ratio. PLoS One 2014; 9:e85798.
18. Mussini C, Lorenzini P, Cozzi-Lepri A, et al. CD4/CD8 ratio normalisation and non-AIDS-related events in individuals with HIV who achieve viral load suppression with antiretroviral therapy: an observational cohort study. Lancet HIV 2015; 2:98–106.
19. Leung V, Gillis J, Raboud J, et al; CANOC Collaboration. Predictors of CD4:CD8 ratio normalization and its effect on health outcomes in the era of combination antiretroviral therapy. PLoS One 2013; 8:e77665.
20. Helleberg M, Kronborg G, Ullum H, Ryder LP, Obel N, Gerstoft J. Course and clinical significance of CD8+ T-cell counts in a large cohort of HIV-infected individuals. J Infect Dis 2015; 211:1726–34.
21. Guiguet M, Kendjo E, Carcelain G, et al; FHDH-ANRS CO4 Epidemiology Group. CD4+ T-cell percentage is an independent predictor of clinical progression in AIDS-free antiretroviral-naive patients with CD4+ T-cell counts >200 cells/mm3. Antivir Ther 2009; 14:451–7.
22. May MT, Ingle SM, Costagliola D, Justice AC, de Wolf F, Cavassini M. Cohort profile: Antiretroviral Therapy Cohort Collaboration (ART-CC). Int J Epidemiol 2014; 43:691–702.
23. Kowalska JD, Mocroft A, Ledergerber B, et al; Eurosida Study Group. A standardized algorithm for determining the underlying cause of death in HIV infection as AIDS or non-AIDS related: results from the EuroSIDA study. HIV Clin Trials 2011; 12:109–17.
24. Lewden C, May MT, Rosenthal E, et al. Changes in causes of death among adults infected by HIV between 2000 and 2005: The "Mortalite 2000 and 2005" surveys (ANRS EN19 and Mortavic). J Acquir Immune Defic Syndr 2008; 48:590–8.
25. Antiretroviral Therapy Cohort Collaboration. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996–2006: collaborative analysis of 13 HIV cohort studies. Clin Infect Dis 2010; 50:1387–96.

26. Ingle SM, May MT, Gill MJ, et al; Antiretroviral Therapy Cohort Collaboration. Impact of risk factors for specific causes of death in the first and subsequent years of antiretroviral therapy among HIV-infected patients. Clin Infect Dis **2014**; 59:287–97.

27. May M, Porter K, Sterne JA, Royston P, Egger M. Prognostic model for HIV-1 disease progression in patients starting antiretroviral therapy was validated using independent data. J Clin Epidemiol **2005**; 58:1033–41.

28. Harrell FE. Regression modeling strategies: with applications to linear models, logistic regression, and survival analysis. Springer-Verlag: New York, **2001**.

29. Valiathan R, Deeb K, Diamante M, Ashman M, Sachdeva N, Asthana D. Reference ranges of lymphocyte subsets in healthy adults and adolescents with special mention of T cell maturation subsets in adults of south Florida. Immunobiology **2014**; 219:487–96.

30. McComsey GA, Kitch D, Sax PE, et al. Associations of inflammatory markers with AIDS and non-AIDS clinical events after initiation of antiretroviral therapy: AIDS clinical trials group A5224s, a substudy of ACTG A5202. J Acquir Immune Defic Syndr **2014**; 65:167–74.

31. Hattab S, Guiguet M, Carcelain G, et al. Soluble biomarkers of immune activation and inflammation in HIV infection: impact of 2 years of effective first-line combination antiretroviral therapy. HIV Med **2015**; 16:553–62.

32. Dianzani U, Omedè P, Marmont F, et al. Expansion of T cells expressing low CD4 or CD8 levels in B-cell chronic lymphocytic leukemia: correlation with disease status and neoplastic phenotype. Blood **1994**; 83:2198–205.

33. Gohil SK, Heo M, Schoenbaum EE, Celentano D, Pirofski LA. CD8+ T cells and risk for bacterial pneumonia and all-cause mortality among HIV-infected women. J Acquir Immune Defic Syndr **2012**; 60:191–8.

34. 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.

35. Serrano-Villar S, Moreno S, Fuentes-Ferrer M, et al. The CD4:CD8 ratio is associated with markers of age-associated disease in virally suppressed HIV-infected patients with immunological recovery. HIV Med **2014**; 15:40–9.

APPENDIX

Contributing cohorts. Cohorts included in this article were the French Hospital Database on HIV (FHDH); the Italian Cohort of Antiretroviral-Naive patients (ICONA); the Swiss HIV Cohort Study (SHCS); the AIDS Therapy Evaluation project, The Netherlands (ATHENA); the Aquitaine Cohort; the Royal Free Hospital Cohort; the South Alberta Clinic Cohort; ART Observational Medical Evaluation and Research (HOMER), Canada; HIV Atlanta Veterans Affairs Cohort Study (HAVACS), United States; Osterreichische HIV-Kohortenstudie (OEHIVKOS), Austria; Vanderbilt, United States; 1917 Clinic Cohort, University of Alabama, Birmingham, United States (UAB); and the Koln/Bonn Cohort.

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