CD4:CD8 Ratio and CD8 Cell Count and Their Prognostic Relevance for Coronary Heart Disease Events and Stroke in Antiretroviral Treated Individuals: The Swiss HIV Cohort Study

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Introduction: HIV infection leads to a persistent expansion of terminally CD8 T cells and CD8 T suppressor cells, a marker of chronic immune activation leading to a low CD4:CD8 ratio that may persist in the presence of potent antiretroviral therapy and regained CD4 helper cells. It remains unclear whether a low CD4:CD8 ratio is associated with cardiovascular diseases.

Methods: We conducted an observational cohort study to investigate the association of immune depression and activation as characterized by the proxy of the CD4:CD8 ratio on the hazard of coronary heart disease (CHD) and stroke among treated individuals living with HIV, while accounting for viral load and known risk factors for cardiovascular diseases and exposure to abacavir or protease inhibitors. We used Cox proportional hazard models with time-dependent cumulative and lagged exposures to account for time-evolving risk factors and avoid reverse causality.

Results: CD4, CD8, and CD4:CD8 immunological markers were not associated with an increased hazard for CHD. CD8 cell count lagged at 12 months above 1000 cells per µL increased the hazard of stroke, after adjusting for sociodemographics, cardiovascular risk factors, and exposure to specific types of antiretroviral drugs.

Conclusions: This analysis of treated HIV-positive individuals within a large cohort with long-term follow-up does not provide evidence for a prognostic role of immune dysregulation regarding CHD. However, increased CD8 cell count may be a moderate risk factor for stroke. Early detection and treatment of HIV-positive individuals are crucial for an optimal immune restoration and a limited CD8 cells expansion.

Key Words: HIV infection, cardiovascular diseases, immunosenescence, chronic inflammation markers

INTRODUCTION

Cardiovascular diseases (CVDs) represent the second most important reason of death in antiretroviral therapy (ART)-treated people living with HIV (PLWHIV).1,2 Reasons relate to increasing age and exposure to common risk factors for CVD,
such as hypertension, dyslipidemia, diabetes, smoking, and—of minor relevance—exposure to certain antiretroviral drugs such as protease inhibitors (PIs) and abacavir.1,4

In HIV infection, several factors are contributing to chronic immune activation, in particular, continuous viral replication and CD4 cell depletion that are associated with higher risk of CVD.5-7 HIV infection leads to a persistent expansion of terminally differentiated effector memory CD8 T cells that is accompanied by a progressive decline of naive and central memory CD8 T cells and associated with a lower CD4:CD8 cell ratio.8 Quantitative and functional defects in CD8 T cells remain even after long-term effective ART.9 Persistent elevation of CD8 T-cell count after long-term ART was found in several case–control studies to be associated with overall mortality and mortality from non–AIDS-defining events.10,11

Expansion of CD8 T cells with the consequence of a low CD4:CD8 ratio in particular in well-treated and virologically suppressed individuals may characterize a subpopulation with distinct immunological abnormalities11 and may constitute a population at risk of “immunosenescence.” In elderly uninfected individuals, this immune phenotype is characterized by a low naive/memory T-cell ratio, expansion of cytomegalovirus–specific CD8+ T cells, enrichment of CD28– and PD-1+ T cells, increased CRP and IL-6 levels, reduced T-cell telomere lengths, and lower CD4:CD8 ratio.11,12 Low CD4:CD8 cell ratio in the presence of viral suppression is associated with invariant natural killer T-cell activation and pro-inflammatory marker production, which may promote a state of chronic immune activation and increased risk for CVD and malignancies.13,14 Evidence from case–control15,16 and cohort studies17,18 for an association of CD8 T cells and CD4:CD8 ratio as proxies for immunosenescence and immune activation and risk of CVD is conflicting because many studies were too small and could not correct for important confounders. We investigated the independent association of CD8 T cells and CD4:CD8 ratio and the risk of coronary heart disease (CHD) and stroke in the Swiss HIV Cohort Study (SHCS).

METHODS

The SHCS is a collaborative study between various health partners and provide a research platform that promotes HIV-related research in Switzerland.19-21 Since 1988, this observational study prospectively and routinely collects a host of data on HIV-positive individuals aged above 16 years, every 6 months. Sociodemographic data, behavioral data, laboratory results (including CD4 and CD8 cell counts and HIV-1 RNA viral load), ART regimen, comedication, and clinical events are collected at enrollment and/or at each follow-up visits. Cardiovascular events and risk factors, including cholesterol, blood glucose, weight, and smoking, are also stored in a routine basis in the database since April 2000.

Baseline and Time-Updated Exposure Variables

We assessed the association of baseline and time-updated exposure variables on the risk of CHD and ischemic stroke. CHD was defined as myocardial infarction, coronary angioplasty/stenting, or coronary artery by-pass grafting, whereas stroke includes cerebral infarction and carotid endarterectomy. Baseline patient characteristics included sex (male/female), education (compulsory school, vocational training, higher education, and other/unknown), HIV transmission group (men having sex with men, injecting drug user, and other/unknown), and calendar year (<2010 and 2010+). Time-updated variables were as follows: (1) age; (2) CD4 cells counts (square root transformed and categorized as <200, 200–350, 350–500, and 500+ cells per μL); (3) CD8 cells counts (square root transformed and categorized as <1000 and 1000+ cells per μL); (4) CD4:CD8 ratio (log transformed and categorized as <0.5, 0.5–1.0, and 1.0+); (5) HIV RNA viral load (log10 transformed and categorized as <50, 50–500, and 500+ copies/mL); (6) dyslipidemia [total cholesterol >6.2 mmol/L or high-density lipoprotein <1.03 mmol/L or (triglycerides >2 mmol/L and fasting)]; (7) diabetes [glucose >11.1 or glucose ≥7 mmol/L if fasting or on antidiabetic drugs (oral or insulin)]; (8) hypertension [systolic >140 mm Hg or diastolic >90 mm Hg or on antihypertensive drugs (if diabetes: systolic >135 mmHg or diastolic >85 mmHg)]; (8) obesity (body mass index >30 kg/m²); (9) metabolic syndrome [having any 3 of the following conditions: abdominal obesity (waist circumference >102 cm in men, >88 cm in women), triglycerides ≥1.69 mmol/L, low high-density lipoprotein cholesterol (<1.03 mmol/L in men and <1.29 mmol/L in women), blood pressure ≥130/≥85 mm Hg, or diabetic]; (10) smoking (no/yes); (11) PI exposure (no/yes); (12) integrase inhibitor exposure (no/yes); (13) didanosine exposure (no/yes); and (14) abacavir exposure (no/yes). Time-updated variables are updated at the end of each follow-up month and missing information is imputed using the last observation carried forward (LOCF).

Statistical Analysis

We used time-dependent covariates Cox models to model time from enrollment to a first cardiovascular event, 1 year after the last clinical visit, death, or cohort administrative censoring, whatever came first. The choice of the Cox models was justified by the prospective nature of our study and by the ability of such models to encompass covariates that change over time. So each follow-up month, subjects who have experienced an event are compared with those currently at risk. We included in our analysis all individuals in the SHCS followed between April 2000 (when routine collection of CVD started) and March 2021, with a minimum of 1 follow-up visit and 1 measurement of CD4, CD8, and RNA viral load at enrollment or in a time window of 3 months. We excluded individuals with a cardiovascular event recorded before April 2000, and individuals who had started ART before 2000. Follow-up times before April 2000 were left-truncated at April 1, 2000.

Risk factors for CHD and stroke were selected in 2 steps. First, we selected cardiovascular risk factors such as dyslipidemia, hypertension, diabetes, obesity, metabolic syndrome, smoking, positive cytomegalovirus serology, PI,
integrase inhibitor,22 and didanosine and abacavir exposures by modeling their association to the hazard of CHD and stroke, adjusted for patient characteristics, including age, sex, educational status, transmission group, and calendar year. Cardiovascular risk factors were considered at baseline and at 3 different lags (12, 24, and 36 months) to avoid the risk of detecting associations that reflect reverse causality. By lag, we understand an exposure observed at a defined number of months before a given follow-up. Among cardiovascular risk factors for which we considered having enough evidence of correlation with our outcome (P < 0.05), we selected the representation that presented the better fit model (lowest Akaike criteria). Second, we selected immunological and virological factors based on their significant association adjusted for patient characteristics and cardiovascular risk factors identified in the previous step. We considered lagged variables at 12, 24, and 36 months, and cumulative exposure using simple moving average (SMA) over the past 12 and 24 months. Twelve-month SMAs were also lagged at 12 and 24 months, whereas 24-month SMAs were lagged at 12 months. Nadir and lagged 12-month nadir were additionally considered for CD4 and CD4:CD8 ratio. Variables selected at the 2 aforementioned steps were carried into the final multivariable Cox models for CHD and stroke.

Proportional assumption of the Cox models were assessed by testing the nonsignificance of the relationship between Schoenfeld residuals and time, and log-linearity of the continuous covariates was assessed by visual inspection of smooth plots of the Martingale residuals. For the final models, missing baseline cardiovascular risk factors were imputed using multivariate imputation by chained equations and we pooled results over 5 imputed dataset. In sensitivity analyses, we compared results obtained with multiple imputation of baseline missing information with results from complete cases without imputation, and results with time-varying covariates updated with LOCF were compared with results where LOCF is restricted to a maximum period of 12 months. All analyses were done in R Project for Statistical Computing (version 4.0.3) software23 using packages “survival” (version 3.2–7), “mice” (version 3.13.0), and “mitools” (version 2.4).

RESULTS

A total of 15,303 HIV-infected individuals without record of CVD before April 2000 have been followed by the SHCS between April 2000 and March 2021 [median follow-up 11.1 years; interquartile range (IQR): 5.2–18.1 years]. CHD was diagnosed in 563 HIV-infected individuals over 174,857 person-years (PY) [incidence rate (IR) 3.22 per 1000 PY; 95% CI: 2.96 to 3.50], and stroke was diagnosed in 275 HIV-positive individuals over 174,947 PY (IR 1.57 per 1000 PY; 95% CI: 1.40 to 1.77). For the current analysis, 9257 HIV-positive individuals had immunological and virological measurements 3 months from baseline and were selected (median follow-up 9.9 years; IQR: 5.1–15.6 years). Individuals who had started ART before April 2000 or never started ART were excluded (Fig. 1 for flow chart for HIV-infected individuals’ selection). Baseline sociodemographic characteristics of HIV-positive individuals included in our analysis were similar to the characteristics of HIV-positive individuals followed overall during the study period with regard to age, sex, and education (Table 1). However, the proportion of injecting drug users in the sample selected for analysis was half of the proportion of injecting drug users among the HIV-positive individuals followed-up, reflecting the challenges in following up for this particular population.

Important CVD risk factors identified in preliminary analyses were dyslipidemia, hypertension, metabolic syndrome, smoking, PI exposure, and abacavir exposure, all lagged at 36 months for CHD and hypertension lagged at 36 months and smoking lagged at 36 months for stroke (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/B962). Because metabolic syndrome is a cluster of conditions that include hypertension and abnormal lipid levels, the variable was not carried forward for further analyses of CHD. The associations between the hazard of CHD and stroke and each considered functional form of time-updated CD4 and CD8 cell counts, CD4:CD8 ratio, and HIV RNA viral load, after adjusting for sociodemographics and important cardiovascular risk factors, are given in Tables S2–S7, Supplemental Digital Content, http://links.lww.com/QAI/B962. None of the immunological and virological factors, neither as continuous nor as categorized variable, was associated with the hazard of CHD. CD8 lagged at 12 months and categorized was the only immunological factor associated in preliminary analyses and was therefore carried forward into a final model for stroke.

Results of mutually adjusted hazards for CHD and stroke, after imputation of missing baseline cardiovascular risk factors, are shown in Table 2. Age, male, dyslipidemia, hypertension, smoking, and PI and abacavir exposures, all lagged at 36 months, were all positively associated to the hazard of CHD. For stroke, age, hypertension, and smoking, all lagged at 36 months, and CD8 cell count above 1000 cells per μL lagged at 12 months, were positively associated to the hazard of stroke. In HIV individuals with a 12-month lagged CD8 cell count above 1000 cells per μL, the risk of stroke after adjusting for sociodemographic and known cardiovascular risk factors was increased by more than 60% compared with individuals with CD8 below 1000 cells per μL (adjusted hazard ratio: 1.61, 95% CI: 1.06 to 2.45).

Results from HIV individuals with complete baseline covariates, without imputation of missing cardiovascular risk factors such as dyslipidemia, hypertension, and smoking, were similar (see Table S8, Supplemental Digital Content, http://links.lww.com/QAI/B962). The selection of CVD risk factors was robust with regard to the imputation of missing laboratory measurements; the effect estimates with missing baseline information imputed using multiple imputation on 5 datasets and with time-varying variables updated using LOCF restricted to a maximum of 1 year are presented in supplementary appendix (see Table S9 and Table S10, Supplemental Digital Content, http://links.lww.com/QAI/B962). Both final models for CHD and stroke satisfied the proportional assumption of the Cox regression model according to the test of temporal independence of the Schoenfeld residuals. Linearity of the relationships between the
| Sociodemographic Characteristics | Overall (n = 15,303) | Selected for Analysis (n = 9257) | Coronary Heart Disease Patients Analyzed (n = 199) | Stroke Patients Analyzed (n = 124) |
|---------------------------------|----------------------|----------------------------------|-----------------------------------------------|----------------------------------|
| **Age (yr)**                    |                      |                                  |                                               |                                  |
| <50                             | 13,073 (85.4%)       | 7837 (84.7%)                    | 109 (54.8%)                                   | 68 (54.8%)                      |
| 50-65                           | 1919 (12.5%)         | 1223 (13.2%)                    | 76 (38.2%)                                    | 41 (33.1%)                      |
| ≥65                             | 311 (2.0%)           | 197 (2.1%)                      | 14 (7.0%)                                     | 15 (12.1%)                      |
| Female                          | 4301 (28.1%)         | 2470 (26.7%)                    | 18 (9.0%)                                     | 29 (23.4%)                      |
| **Education**                   |                      |                                  |                                               |                                  |
| Compulsory school               | 3074 (20.1%)         | 1631 (17.6%)                    | 26 (13.1%)                                    | 18 (14.5%)                      |
| Vocational training             | 6170 (40.3%)         | 3537 (38.2%)                    | 98 (49.2%)                                    | 69 (55.6%)                      |
| Higher education                | 4780 (31.2%)         | 3339 (36.1%)                    | 70 (35.2%)                                    | 31 (25.0%)                      |
| Other/unknown                   | 1279 (8.4%)          | 750 (8.1%)                      | 5 (2.5%)                                      | 6 (4.8%)                        |
| **Transmission group**          |                      |                                  |                                               |                                  |
| Men having sex with men         | 6446 (42.1%)         | 4333 (46.8%)                    | 98 (49.2%)                                    | 46 (37.1%)                      |
| Injecting drug user             | 2436 (15.9%)         | 723 (7.8%)                      | 15 (7.5%)                                     | 11 (8.9%)                       |
| Other/unknown                   | 6421 (42.0%)         | 4201 (45.4%)                    | 86 (43.2%)                                    | 67 (54.0%)                      |
| **Calendar year**               |                      |                                  |                                               |                                  |
| ≥2010                           | 4118 (26.9%)         | 3876 (41.9%)                    | 35 (17.6%)                                    | 20 (16.1%)                      |
| **Cardiovascular risk factors** |                      |                                  |                                               |                                  |
| Dyslipidemia                    |                      |                                  |                                               |                                  |
| No                              | 6375 (41.7%)         | 3911 (42.2%)                    | 62 (31.2%)                                    | 46 (37.1%)                      |
| Yes                             | 6181 (40.4%)         | 3827 (41.3%)                    | 110 (55.3%)                                   | 56 (45.2%)                      |
| Missing                         | 2747 (18.0%)         | 1519 (16.4%)                    | 27 (13.6%)                                    | 22 (17.7%)                      |
| Hypertension                    |                      |                                  |                                               |                                  |
| No                              | 8817 (57.6%)         | 5539 (59.8%)                    | 97 (48.7%)                                    | 50 (40.3%)                      |
| Yes                             | 3843 (25.1%)         | 2200 (23.8%)                    | 81 (407%)                                     | 57 (46.0%)                      |
| Missing                         | 2643 (17.3%)         | 1518 (16.4%)                    | 21 (10.6%)                                    | 17 (13.7%)                      |
| Diabetes                        |                      |                                  |                                               |                                  |
| No                              | 12,333 (80.6%)       | 7316 (82.2%)                    | 162 (81.4%)                                   | 97 (78.2%)                      |
| Yes                             | 259 (1.7%)           | 165 (1.8%)                      | 10 (5.0%)                                     | 5 (4.0%)                        |
| Missing                         | 2711 (17.7%)         | 1479 (16.0%)                    | 27 (13.6%)                                    | 22 (17.7%)                      |
| Obesity                         |                      |                                  |                                               |                                  |
| No                              | 12,055 (78.8%)       | 7289 (78.7%)                    | 163 (81.9%)                                   | 100 (80.6%)                     |
| Yes                             | 637 (4.2%)           | 454 (4.9%)                      | 11 (5.5%)                                     | 7 (5.6%)                        |
| Missing                         | 2611 (17.1%)         | 1514 (16.4%)                    | 25 (12.6%)                                    | 17 (13.7%)                      |
| Metabolic syndrome              |                      |                                  |                                               |                                  |
| No                              | 10,526 (68.8%)       | 6604 (71.3%)                    | 130 (65.3%)                                   | 79 (63.7%)                      |
| Yes                             | 1976 (12.9%)         | 1123 (12.1%)                    | 41 (20.6%)                                    | 23 (18.5%)                      |
| Missing                         | 2801 (18.3%)         | 1530 (16.5%)                    | 28 (14.1%)                                    | 22 (17.7%)                      |
| Smoking                         |                      |                                  |                                               |                                  |
| No                              | 6588 (43.1%)         | 4428 (47.8%)                    | 86 (43.2%)                                    | 54 (43.5%)                      |
| Yes                             | 6440 (42.1%)         | 3261 (35.2%)                    | 86 (43.2%)                                    | 52 (41.9%)                      |
| Missing                         | 2275 (14.9%)         | 1568 (16.9%)                    | 27 (13.6%)                                    | 18 (14.5%)                      |
| Cytomegalovirus status          |                      |                                  |                                               |                                  |
| No                              | 945 (6.2%)           | 914 (9.9%)                      | 21 (10.6%)                                    | 17 (13.7%)                      |
| Yes                             | 6607 (43.2%)         | 6503 (70.2%)                    | 133 (66.8%)                                   | 79 (63.7%)                      |
| Missing                         | 7751 (50.7%)         | 1840 (19.9%)                    | 45 (22.6%)                                    | 28 (22.6%)                      |
| **Immunological and viral factors** |                    |                                  |                                               |                                  |
| CD4 cell counts (cells/μL)      |                      |                                  |                                               |                                  |
| <200                            | 3332 (21.8%)         | 2421 (26.2%)                    | 58 (29.1%)                                    | 43 (34.7%)                      |
| 200–350                         | 3298 (21.6%)         | 2069 (22.4%)                    | 45 (22.6%)                                    | 31 (25.0%)                      |
| 350–500                         | 3181 (20.8%)         | 1991 (21.5%)                    | 38 (19.1%)                                    | 18 (14.5%)                      |

(continued on next page)
continuous covariates and estimated log hazard were satisfied according to a visual inspection of the Martingale residuals. CD4 and CD8 cell counts, CD4:CD8 ratio, and HIV viral load were, however, still explored as variables categorized according to cut-off that are commonly used in other studies 

### DISCUSSION

Whether CD8 cell count and CD4:CD8 ratio as proxy variables for chronic immune stimulation by HIV play a role in the elevated cardiovascular risk has been subject to contradicting results in the literature and motivated our work. Several cohort studies have investigated the association between CD4:CD8 ratio and risk of different non-AIDS-defining events that were combined. Results are conflicting because of the chosen approaches, model definitions, and different examined endpoints. In the large ART-CC cohort including 49,865 patients, CD4:CD8 cell ratio was not prognostic for overall mortality or non-AIDS-defining mortality after adjustment for other factors (in particular CD4 cell count). Information on smoking was not collected in ART-CC. CD8 cell count had a U-shaped association with non-AIDS mortality but was not prognostic in adjusted analysis.\(^{24}\) In the Italian ICONA cohort, a CD4:CD8 ratio <0.3 was independently associated with increased risk of non-AIDS-defining events, but in the time-updated analysis, this association was no longer statistically significant. There were 71 non-AIDS-defining events in 3236 individuals.\(^{25}\) Current CD4:CD8 ratio <0.3 was associated with increased risk of the composite endpoint of non-AIDS-defining events (including cardiovascular events and chronic kidney disease) in a Thai cohort with a median period of viral load suppression of 6.1 years.\(^{26}\) In the French APROCO/COPILOTE cohort study of 1227 patients who were followed over a median of 9.2 years, CD4:CD8 ratio was in the analysis with adjustment for CD4 cell count not associated with increased risk of non-AIDS-defining events (which included bacterial infections, CVD, and malignancies). Only few cohorts were able to look more specifically into these associations with an explicit focus on CVD and non-AIDS-defining malignancies. In the US Vanderbilt Cohort of 2006 PLWHIV, CD4:CD8 was inversely related to the risk of CHD once accounting for other non-AIDS-defining events (including cardiovascular events and chronic kidney disease) in a Thai cohort with a median period of viral load suppression of 6.1 years.\(^{26}\) In the French APROCO/COPILOTE cohort study that included 1206 PLWHIV, CD4:CD8 ratio was no longer independently associated with CHD once accounting for CD4 cell count.\(^{18}\) None of these cohorts including the present study collected data on additional inflammation markers, which

### TABLE 1. (Continued) Sociodemographic Characteristics and Baseline Cardiovascular, Immunological, and Virological Risk Factors of HIV-positive Individuals Followed up Between April 2000 and March 2021, Included in the Study and With CHD and Stroke

|       | Overall (n = 15,303) | Selected for Analysis (n = 9257) | Coronary Heart Disease Patients Analyzed (n = 199) | Stroke Patients Analyzed (n = 124) |
|-------|---------------------|---------------------------------|-----------------------------------------------|----------------------------------|
| CD8 cell counts (cells/\(\mu L\)) |                     |                                 |                                               |                                  |
| <1000 | 8912 (58.2%)        | 5574 (60.2%)                   | 108 (54.3%)                                   | 70 (56.5%)                       |
| 1000+ | 5068 (33.1%)        | 3002 (32.4%)                   | 76 (38.2%)                                    | 45 (36.3%)                       |
| Missing | 1323 (8.6%)        | 681 (7.4%)                     | 15 (7.5%)                                     | 9 (7.3%)                         |
| CD4:CD8 ratio |                     |                                 |                                               |                                  |
| <0.5  | 7985 (52.2%)        | 5150 (55.6%)                   | 129 (64.8%)                                   | 79 (63.7%)                       |
| 0.5–1 | 4447 (29.1%)        | 2533 (27.4%)                   | 39 (19.6%)                                    | 25 (20.2%)                       |
| 1+     | 1548 (10.1%)        | 893 (9.6%)                     | 16 (8.0%)                                     | 11 (8.9%)                        |
| Missing | 1323 (8.6%)        | 681 (7.4%)                     | 15 (7.5%)                                     | 9 (7.3%)                         |
| Viral load of HIV (copies/mL) |                     |                                 |                                               |                                  |
| <50   | 3760 (24.6%)        | 1039 (11.2%)                   | 17 (8.5%)                                     | 9 (7.3%)                         |
| 50–500 | 1089 (7.1%)        | 455 (4.9%)                     | 11 (5.5%)                                     | 7 (5.6%)                         |
| 500+  | 9879 (64.6%)        | 7763 (83.9%)                   | 171 (85.9%)                                   | 108 (87.1%)                      |
| Missing | 575 (3.8%)         |                                 |                                               |                                  |

Dyslipidemia: total cholesterol >6.2 mmol/L or high-density lipoprotein <1.03 mmol/L or (triglycerides >2 mmol/L and fasting), diabetes: glucose >11.1 mmol/L or glucose ≥7 mmol/L if fasting or on antidiabetic drugs (oral or insulin), hypertension: systolic >140 mm Hg or diastolic >90 mm Hg or on antihypertensive drugs (systolic >135 mm Hg if diabetic or diastolic >85 mm Hg if diabetic), obesity: body mass index >30 kg/m², metabolic syndrome: having any 3 of the conditions abdominal obesity (waist circumference >102 cm in men, >88 cm in women), triglycerides ≥1.69 mmol/L/low high-density lipoprotein cholesterol (<1.03 mmol/L in men, <1.29 mmol/L in women), blood pressure ≥130/≥85 mm Hg/ diabetes.
is a limitation. Several nested case–control studies, most of them recruiting patients from existing cohorts, also found an association between CD4:CD8 cell count and CHD, but the design of these studies has known limits.15,16 Others found CD4:CD8 ratio to be inversely associated surrogate markers for CHD through carotid intima thickening.27,28 These inconclusive findings may relate to low event rates from small cohorts and the use of composite endpoints including a large proportion of non-AIDS-defining events other than CVD. Several studies did not use cumulative exposure models for studying the independent contribution of CD8 T cells and CD4:CD8 ratio for CVD prediction. Most studies limited their analysis to the time points after full virological suppression and did not model pre-ART virological exposure or immunosuppression or lagged single or cumulative time exposures to minimize reverse causality bias.

High CD8 counts or low CD4:CD8 ratio among virologically suppressed PLWHIV reflect immunoactivation and immunosenesence.29 Association between inflammation, chronic infections, and atherosclerosis or stroke has been established, with multiple pathways of action.30 However, it is difficult to say if an increased CD8 cell count contributes directly to the occurrence of stroke or whether it is a consequence of other events that drive this clinical outcome. We note that neither CD4 nor viral load showed to be strongly associated to the hazard of stroke and CHD. Importantly, exposure to PI and abacavir (lagged by 36 months) were both associated to the risk of CHD in the mutually adjusted model. This is an important result that confirms previous studies31–33 and stresses the necessity to balance risks and benefits while introducing an abacavir- and PI-based regimen, in particular, to patients with a high CHD risk.

Our results are important for ART-treated individuals and their treating clinicians in an era where HIV has become a chronic condition and CVD an important cause of morbidity. Evidence from our large cohort with long-term follow-up indicates that in well-treated individuals, traditionally monitored immune markers do not seem to have relevant prognostic value for CHD and stroke. Management of known risk factors for CHD and stroke and the switch from PIs and abacavir to alternative regimens if possible are the likely most efficient strategies to prevent CHD and stroke. Whether additional treatment of inflammation markers will further benefit PLWHIV and risk for CHD has to be shown in ongoing trials.34

The choice of all time-dependent covariates was done among several functional forms that include lagged and cumulative lagged exposures captured through single moving average. Lagging covariates avoid bias because of reverse causality that might happen when covariate is more a marker of an event rather than a predictor. Lagging a covariate can be seen as the length of the delay until the recorded covariate starts to affect the hazard. This might imply a loss of power because observations in the first months of follow-up, where past information is not available, are discarded. However, observed times to event in this study were mostly larger than 36 months for both CHD (median time 105 months; IQR: 52–156 months) and stroke (median time 99 months; IQR: 42–155 months).

With the exception of baseline immunological and virological variables for which the availability was part of the inclusion criteria into our study, missing baseline covariates were imputed using multivariate imputation by chained equations. Time-varying covariates were updated at the beginning of each follow-up month using LOCF, a widely used popular imputation method in longitudinal data analysis. Despite this method is often seen as conservative and has
been criticized, we preferred it to a linear interpolation or a carry back and forward method between the follow-up visits that makes use of the future to predict the present. Psychoactive substances are known to be an important driver of cardiovascular damages, and it has been reported that consumption of those drugs is more important within injecting drug user and men having sex with men transmission groups.35 Because SHCS started to collect data on recreational drugs only from 2007 onwards, we were not able to further control for addiction in the current analysis and this can be seen as a limitation. Also, CD4 and CD8 cell counts are the 2 markers used to routinely monitor immune system in SHCS. Data on T-cell activation or exhaustion and CVD biomarkers, such as the high-sensitivity C-reactive protein, were not available. The well-established SHCS provides a high-quality dataset with regular biannual follow-up visits, a vast amount of information on associated risk factors over more than 20 years and a substantial amount of cardiovascular events. Despite our study population was relatively young with a median age of 37 years, we had considerable statistical power to assess the association of the identified most important time-evolving immunological, viral, and cardiovascular risk factors to the hazard of both CHD and stroke. Also, SHCS enabled to estimate IRs of stroke and CHD within the HIV-positive population of Switzerland. In United States, HIV infection has been associated with an increased risk of acute myocardial infarction36 and stroke.37 The global burden disease estimated an ischemic stroke incidence below 0.413/1000 for 2019, suggesting that the risk of stroke might be at least 3 times higher in the HIV population in Switzerland. However, our IRs remain difficult to compare with the overall Swiss population because stroke and CHD data are lacking at Swiss population level. The shortage of population-based information together with the lack of personal identifier that makes difficult to link different databases are reported as the main obstacles to get those statistics.39

**CONCLUSIONS**

Immune dysregulation as indexed by CD8 cell count and the CD4:CD8 cell ratio does not seem to be prognostic markers for CHD. An increased CD8 cell count may be a moderate risk factor for stroke. If confirmed by other studies, this adds to our knowledge on the importance of early HIV diagnosis and treatment for optimal immune restoration and limitation of terminally differentiated memory CD8 T cells expansion as induced by unopposed HIV replication.

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**TABLE 2. Results From Multivariate Cox Regression Models for CHD and Stroke**

| Coronary Heart Disease | Stroke |
|------------------------|--------|
| **aHR (95% CI)**       | **aHR (95% CI)** |
| Age (time-varying) [yr]|        |
| <50                    | 1.00 (0.32 to 3.15) |
| 50-65                  | 1.10 (0.32 to 3.15) |
| ≥65                    | 1.19 (0.32 to 3.15) |
| Sex                    |        |
| Male                   | 1.00 (0.32 to 3.15) |
| Female                 | 0.98 (0.32 to 3.15) |
| Education              |        |
| Compulsory school      | 1.00 (0.32 to 3.15) |
| Vocational training    | 0.98 (0.32 to 3.15) |
| Higher education       | 0.98 (0.32 to 3.15) |
| Other/unknown          | 0.98 (0.32 to 3.15) |
| Transmission group     |        |
| Men having sex with men| 1.00 (0.32 to 3.15) |
| Injecting drug user    | 0.98 (0.32 to 3.15) |
| Other/unknown          | 0.98 (0.32 to 3.15) |
| Calendar year          |        |
| <2010                  | 0.80 (0.32 to 3.15) |
| ≥2010                  | 0.98 (0.32 to 3.15) |
| Hypertension lagged 36 months |  |
| No                     | 1.00 (0.32 to 3.15) |
| Yes                    | 1.19 (0.32 to 3.15) |
| Smoking lagged 36 months|        |
| No                     | 1.00 (0.32 to 3.15) |
| Yes                    | 1.19 (0.32 to 3.15) |
| Dyslipidemia lagged 36 months |  |
| No                     | 1.00 (0.32 to 3.15) |
| Yes                    | 1.19 (0.32 to 3.15) |
| PI exposure lagged 36 mo |        |
| No                     | 1.00 (0.32 to 3.15) |
| Yes                    | 1.19 (0.32 to 3.15) |
| Abacavir exposure lagged 36 mo |  |
| No                     | 1.00 (0.32 to 3.15) |
| Yes                    | 1.19 (0.32 to 3.15) |
| CD8 lagged 12 months [cells/μL] |  |
| <1000                  | 1.00 (0.32 to 3.15) |
| 1000+                  | 1.00 (0.32 to 3.15) |

**Dyslipidemia**: total cholesterol >6.2 mmol/L or high-density lipoprotein <1.03 mmol/L or triglycerides >2 mmol/L fasting, hypertension: systolic >140 mm Hg or diastolic >90 mm Hg or on antihypertensive drugs or (systolic >135 mm Hg if diabetic or diastolic >85 mm Hg if diabetic). Parameter estimates are pooled estimates from models fitted to 5 imputed datasets. aHR, adjusted hazard ratio.
