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MAJOR ARTICLE

Leukocyte Count and Coronary Artery Disease Events in People with HIV: A Longitudinal Study

Emma F. Avery[1*], Julia N. Kleynhans[1*], Bruno Ledergerber[2], Isabella C. Schoepf[1,3,4], Christian W. Thorball[5], Neeltje A. Kootstra[6], Peter Reiss[7], Lene Ryom[8], Dominique L. Braun[2,9], Maria C. Thurnheer[10], Catia Marzolini[11], Marco Seneghini[12], Enos Bernasconi[13], Matthias Cavassini[14], Hélène Buvelot[15], Roger D. Kouyos[2,9], Jacques Fellay[5,16], Huldrych F. Günthard[2,9], and Philip E. Tarr[1], for the Swiss HIV Cohort Study

[1] University Department of Medicine and Infectious Diseases Service, Kantonsspital Baselland, University of Basel, 4101 Bruderholz, Switzerland; [2] Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, 8091 Zurich, Switzerland; [3] Department of Infectious Diseases, Bern University Hospital, University of Bern, 3010 Bern, Switzerland; [4] Hepatology, Department for Visceral Surgery and Medicine, Bern University Hospital, University of Bern, 3010 Bern, Switzerland; [5] Precision Medicine Unit, Lausanne University Hospital and University of Lausanne, 1011 Lausanne, Switzerland; [6] Department of Experimental Immunology, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, Netherlands; [7] Department of Global Health and Division of Infectious Disease, Amsterdam University Medical Centers, University of Amsterdam, and Amsterdam Institute for Global Health and Development, Amsterdam, The Netherlands; [8] Centre of Excellence for Health, Immunity, and Infections, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [9] Institute of Medical Virology, University of Zurich, Zurich, 8091 Zurich, Switzerland; [10] Department of Infectious Diseases, Bern University Hospital, University of Bern, 3010 Bern, Switzerland; [11] Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, 4031 Basel, Switzerland; [12] Division of Infectious Diseases,

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* these authors contributed equally to the manuscript

Corresponding author: Philip E. Tarr, MD, University Dept. of Medicine and Infectious Diseases Service, Kantonsspital Baselland, University of Basel, 4101 Bruderholz, Switzerland; philip.tarr@unibas.ch

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Kantonsspital St Gallen, 9007 St. Gallen, Switzerland; [13] Division of Infectious Diseases, Ospedale Regionale Lugano, University of Geneva and Università della Svizzera italiana, 6900 Lugano, Switzerland; [14] Infectious Diseases Service, Lausanne University Hospital, University of Lausanne, 1011 Lausanne, Switzerland; [15] Division of Infectious Disease, Geneva University Hospital, 1205 Geneva, Switzerland; [16] School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

Background. People with HIV (PWH) have increased cardiovascular risk. Higher leukocyte count has been associated with coronary artery disease (CAD) events in the general population. It is unknown whether the leukocyte-CAD association also applies to PWH.

Methods. In a case-control study nested within the Swiss HIV Cohort Study, we obtained uni- and multivariable odds ratios (OR) for CAD events, based on traditional and HIV-related CAD risk factors, leukocyte count, and confounders previously associated with leukocyte count.

Results. We included 536 cases with a first CAD event (2000-2021; median age 56 years, 87% male, 84% with suppressed HIV-RNA) and 1464 event-free controls. Cases had higher latest leukocyte count prior to CAD event than controls (median [interquartile range], 6495 [5300-7995] vs. 5900 [4910-7200]; p<0.01), but leukocytosis (>11000/uL) was uncommon (4.3% vs. 2.1%; p=0.01). In the highest vs. lowest leukocyte quintile at latest time point prior to CAD event, participants had univariable CAD-OR=2.27 (95% confidence interval, 1.63-3.15) and multivariable adjusted CAD-OR=1.59 (1.09-2.30). For comparison, univariable CAD-OR for dyslipidemia, diabetes, and recent abacavir exposure were 1.58 (1.29-1.93), 2.19 (1.59-3.03), and 1.73 (1.37-2.17), respectively. Smoking and, to a lesser degree, alcohol and ethnicity attenuated the leukocyte-CAD association. Leukocytes measured up to 8 years pre-event were significantly associated with CAD events.

Conclusions. PWH in Switzerland with higher leukocyte counts have an independently increased risk of CAD events, to a degree similar to traditional and HIV-related risk factors.

Keywords. HIV infection, coronary artery disease, leukocytes, multivariable analysis, white blood cells.

INTRODUCTION

People with HIV (PWH), have an increased risk for coronary artery disease (CAD) events compared to the general population. [1, 2] CAD risk in PWH is related to traditional CAD risk factors, HIV-related factors including chronic inflammation [3, 4], immunosuppression [5, 6], potential deleterious effects of certain antiretroviral therapy (ART) agents [7, 8], and individual genetic background [9]. An increased CAD risk may persist in PWH with suppressed HIV viremia [1, 2]. This suggests a role for low level inflammation and immune activation in the

pathogenesis of CAD in PWH and has generated considerable interest in inflammatory biomarkers for CAD event prediction in PWH [4, 10, 11].

Leukocytes are implicated in the pathogenesis of atherosclerosis, and ever since the 1980ies, studies in the general population have shown leukocyte count in the peripheral blood to be an independent risk factor for CAD events. [12-15] Whether blood leukocytes are associated with CAD events in PWH has not been verified. Therefore, the aim of this report is to assess an independent association of leukocyte count with CAD events in participants of the Swiss HIV Cohort Study (SHCS), analyzed in the context of traditional and HIV-related CAD risk factors. We also considered multiple factors that may influence leukocytes, including ethnicity [16], smoking [17], infections, and alcohol intake [18].

METHODS

Study population.

We included PWH enrolled in the SHCS (www.shcs.ch, [19]), an observational study that has prospectively enrolled PWH since 1988, and has captured rich cardiovascular, metabolic, genetic, and other data since 1999. Participants provided written informed consent. The study was approved by the local ethics committees. Cases had a first CAD event and controls were CAD event-free during the study period (01.01.2000-31.10.2021).

CAD events.

CAD events were defined as per the Data Collection on Adverse events of Anti-HIV Drugs (D:A:D) study and the World Health Organization's MONICA Project [20], as we have previously published [9, 21]. CAD events included myocardial infarction, coronary angioplasty/stenting, coronary artery bypass grafting and fatal cases (confirmed at autopsy or ascertained by the treating HIV physician as sudden death with no other likely cause *plus* evidence of CAD before death).

Case-control matching.

As in our previous CAD case-control studies [9, 21], we used incidence density sampling, [22] aiming to select 1-3 event-free controls for each case. We used risk-set sampling [23], i.e. we matched controls at the CAD event date of the corresponding cases (matching date) on similar observation *duration*, and their observation *period* was during similar calendar periods, in order to account for differences in ART (with different CAD risk associations [8, 24]) in use during different time periods and other differences. Matching criteria were sex, age +/-4 years, and date of SHCS registration +/-4 years. Observation time started at SHCS registration; observation ended for cases at the matching date (CAD event date) and for controls ended at the first regular SHCS follow-up visit after the matching date, respectively.

Power calculation.

To capture odds ratios of ≥1.6 would need 255 cases and 2 controls per case [30], assuming an exposure correlation between pairs in the case-control set of 0.2 [30].

Leukocyte count.

The SHCS database routinely includes total leukocytes, total lymphocytes, CD4, and CD8 counts. For the main analysis, we compared latest leukocyte count before the matching date in cases and controls and. In addition, we considered leukocyte count at increasing intervals before the matching date. In exploratory analyses, we obtained neutrophils, eosinophils, and the neutrophil-lymphocyte ratio retrospectively for University Hospital Zurich participants where approximately 20% of SHCS participants are followed.

Clinical CAD risk factors.

Co-variables were defined a priori, based on their CAD association in the general population, as reported previously [9, 21, 25], and were ascertained at the latest SHCS visit prior to the matching date except for CD4 nadir (lowest CD4 value during the study period). Co-variables included age (per 10 years older, added in order to detect any residual effect of suboptimal matching, as we have done previously [21]), family history of CAD, smoking, diabetes mellitus, hypertension, and dyslipidemia (total cholesterol >6.2 mmol/L or HDL<1 mmol/L [men] and <1.2 mmol/L [women] or use of lipid-lowering drugs [25]). HIV-related co-variables included viral load (HIV RNA < or ≥50 copies/mL), CD4 nadir, and ART exposures until the matching date, based on their CAD-association in the D:A:D study [8, 24], including recent (past 6 months) abacavir, didanosine, and integrase inhibitors; and cumulative (>1 year) exposure to lopinavir, indinavir, boosted darunavir, and stavudine [9]; hepatitis C [26] and cytomegalovirus (CMV) seropositivity [27].

Potential confounding variables associated with leukocyte count.

These were defined *a priori*, based on reported associations in the general population. We considered both current smoking (vs. past/never [28]), and daily cigarettes smoked (never, not currently, ≤5/day, 6-20/day, >20/day, unknown[29]); ethnicity (White/Black/Hispanic/Asian) [16]; and alcohol (none/mild vs. moderate/heavy; defined in the SHCS until 2012 as </≥40g (men), </≥20g (women), and using the AUDIT-C questionnaire beginning in 2013 (</≥4 points [men], </≥2 points [women]; see: hepatitis.va.gov/alcohol/treatment/audit-c.asp#S1X) [18]; we also tested each variable in the CAD event model for a potential interaction with leukocytes (**Supplementary Methods, Supplementary Table 1**). We did not analyze corticosteroid use and non-HIV inflammatory conditions because these were recorded pre-event in only 8 cases/36 controls, and in 3 cases/5 controls, respectively, and because of insufficient available details (e.g. specific diagnoses, date, corticosteroid duration/dose).

Infection episodes.

Because infections may influence leukocytes, we assessed non-opportunistic infections (recorded in the SHCS since 2017, defined as leading to hospitalization or antibiotic use for ≥5 days) and opportunistic infections in the year prior to matching date in cases and controls.

Sensitivity analyses.

To test the robustness of the leukocyte-CAD association; i) we replaced all risk factors by the 10-year Framingham risk score (FRS) for CAD, or ii) by FRS risk category (<10% vs. ≥10% risk); iii) analysis restricted to participants with suppressed HIV RNA at matching date; and iv) after adding the latest estimated glomerular filtration rate (eGFR) before CAD event to the model (note that kidney function is available in the SHCS after 1.1.2002).

Statistical analyses.

Characteristics of cases and controls were compared using Fisher's exact test (categorical variables) and Wilcoxon rank-sum test (continuous variables). Univariable, bivariable and multivariable conditional logistic regression analyses were used to estimate associations of the different risk factors with CAD and their interactions. We decided a priori to stratify leukocyte counts into quintiles for better visualization of potentially non-linear associations with CAD events. Variables were entered into the *multivariable* model if their association in the univariable model had $p<0.2$. Model fit and interactions were analyzed using Akaike and Bayesian information criteria and likelihood ratio tests. The effect of potential confounders on the leukocyte-CAD association was tested on a 1:1 basis (bivariable models including interaction terms). Trajectories of total leukocytes, leukocyte subtypes, and smoking over the past 15 years were created using local polynomial smoothing with the epanechnikov kernel. We used Stata/SE 17.0 (StataCorp, College Station, TX, USA).

RESULTS

Participants, CAD events.

Participant disposition is shown in **Figure 1** and participants' characteristics in **Table 1**. The final study population included 2000 participants, i.e., 536 cases with a first CAD event and 1464 matched CAD event-free controls. Registration of participants started in January 1985, and CAD events are considered until August 2021. The median (IQR) date of CAD events was 03.05.2013 (05.10.2007-18.09.2017), and the median (IQR) duration of observation was 13.1 (8.1-19.2) years. CAD events included myocardial infarction (n=274), coronary angioplasty/stenting (n=211), coronary artery bypass grafting (n=39), and fatal CAD cases (n=12). [21] Cases were more likely to be smokers, diabetic, dyslipidemic, hypertensive, or have a CAD family history (**Table 1**).

Latest Leukocyte Count, Observed data.

Median time from the latest leukocyte measurement to CAD event (matching date) was 56 (IQR 30-94) days in cases, and 60 (IQR, 29-91) days in controls. Latest median leukocyte count prior to matching date was higher in cases than controls (p<0.01; **Table 1**). Leukocytosis (>11000/uL) was uncommon but more frequent in cases than controls (4.3% vs. 2.1%; p=0.01). **Figure 2** shows the range of leukocytes in each leukocyte quintile and how the number of cases increases and the number of controls decreases in the higher leukocyte quintiles. **Supplementary Table 2** shows leukocytes for cases and controls in each quintile.

Longitudinal Leukocyte Values, Observed data.

Median (IQR) leukocyte count was higher in cases than controls at 1 year (p<0.01), 2 years (p<0.01), 3 years (p<0.01), 5 years (p=0.04) prior to CAD event, but not at 8, 9, and 10 years prior to CAD event (p=0.06, p=0.69, and p=0.72, respectively). Longitudinal observed leukocyte trajectories are shown in **Figure 3A**. Longitudinal trajectories of observed total lymphocytes (**Figure 3B**), CD4 cells (**Figure 3C**), CD8 cells (**Figure 3D**), and observed HIV-RNA trajectories (**Figure 3E**) were similar in cases and controls. Longitudinal leukocyte trajectories in smokers vs. non-smokers showed an apparent dose-relation regarding cigarettes smoked per day (**Figure 3F**).

Leukocyte Count and CAD Events, Univariable Model.

In the latest sample prior to CAD event, leukocyte count was associated with CAD events (per 1000 leukocytes higher, CAD-OR=1.11, 95% CI, 1.05-1.16). Compared to participants in the first (lowest) leukocyte quintile, participants in the 2nd, 3rd, 4th, and 5th (highest) quintile had univariable CAD-OR=1.13 (95% CI, 0.80-1.59), 1.44 (1.02-2.03), 1.69 (1.22-2.35), and 2.27 (1.64-3.15) respectively. For comparison, univariable CAD-OR for hypertension, dyslipidemia, diabetes and recent abacavir exposure were 1.40 (1.12-1.73), 1.58 (1.29-1.93), 2.19 (1.59-3.03), and 1.73 (1.37-2.17), respectively. Univariable associations of all individual risk factors with CAD are shown in **Figure 4** and **Supplementary Table 3**.

Longitudinal Leukocyte Counts and CAD Events, Univariable Model.

Leukocyte count (5th vs. 1st quintile) remained significantly associated with CAD events when measured at year -1 (CAD-OR=1.81; 95% CI, 1.30-2.53; n=1896 participants), year -2 (1.66; 1.18-2.33; n=1749), year -3 (1.56; 1.09-2.22; n=1617), year -5 (1.74; 1.14-2.64; n=1231), year -8 (2.18; 1.24-3.84; n=657), but not at year -9 (1.12; 0.59-2.13; n=499), or year -10 (0.66; 0.33-1.35; n=369) prior to the CAD event (**Supplementary Table 4**).

Leukocyte Count and CAD Events, Multivariable Model.

In the final model, participants had increased adjusted CAD risk in the 5^{th} (highest) leukocyte quintile, i.e. participants in the 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} vs. 1^{st} (lowest) quintile had adjusted CAD-

OR=0.96 (95% CI, 0.66–1.38), 1.30 (0.90–1.90), 1.29 (0.90–1.85), and 1.59 (1.09-2.30), respectively (**Figure 4, Supplementary Table 3**). For comparison, multivariable CAD-OR for hypertension, dyslipidemia, diabetes, and recent abacavir exposure were 1.54 (1.21-1.97), 1.44 (1.15-1.81), 2.15 (1.5-3.07), and 1.81 (1.41-2.33), respectively.

Leukocyte Count and CAD Events, Potential Confounders.

Median leukocyte count was higher in cases than controls in most confounder categories (**Figure 4**). In individual 1:1 bivariable analyses (**Table 2, Supplementary Table 1)**, last leukocyte count remained associated with CAD events but the association was attenuated when we added smoking status (5^{th} vs. 1^{st} leukocyte quintile, CAD-OR=1.85 [1.31-2.61]) or the latest number of cigarettes smoked per day (5^{th} vs. 1^{st} leukocyte quintile, CAD-OR=1.82 (1.30–2.56); p<0.01), suggesting that smoking in part explains the leukocyte-CAD association. The leukocyte-CAD event association was attenuated to a lesser degree by alcohol or when considering ethnicity. Attenuation was minimal for the other individual confounders. All interactions were discarded due to lack of significance (all *P* >0.05; **Table 2, Supplementary Table 1**).

Leukocyte Count and Infection Episodes.

There were 12 cases and 26 controls with an opportunistic infection (OI) in the year prior to CAD event (p=0.47; **Supplementary Table 5**). Serious non-OI (SNOI) were documented in 15/163 (9.2%) cases vs. 21/447 (4.7%) controls (p=0.05). Participants with/without OI (p=0.64) and with/without SNOI (p=0.59) had similar median latest leukocytes (**Supplementary Table 5**).

Sensitivity Analyses with Framingham Risk Score (FRS).

After adjustment for FRS, leukocyte count remained associated with CAD events. Participants in the 5th vs. 1st leukocyte quintile had CAD-OR=1.64 (1.16–2.32) when adjusting for FRS, and CAD-OR=1.82 (1.30–2.56) when adjusting for FRS category (≥10% vs <10%; **Supplementary Tables 6A and 6B**).

Sensitivity Analysis, participants with suppressed HIV RNA.

In multivariable analysis restricted to participants with suppressed viremia at the latest pre-event time point (n=1559 participants), results remained essentially unchanged; participants in the 5^{th} vs. 1st leukocyte quintile had CAD-OR=1.63 (1.06-2.50) **(Supplementary Table 7)**.

Leukocyte Subsets and CAD Events, Univariable Models.

Leukocyte subsets were available for the 517/2000 participants followed at University Hospital Zurich. Latest median neutrophil count was higher in 132 cases vs. 385 controls (p<0.01), i.e. 3835/uL (IQR, 2800-4925) vs. 3220/uL (IQR, 2470-4230). Longitudinal observed neutrophil count showed divergent trajectories in cases and controls up to 12 years prior to CAD event

(**Figure 3G)**. Zurich participants in the 5th vs. 1st *leukocyte* quintile had CAD-OR=4.78 (2.31-9.87), and in the 5th vs. 1st *neutrophil* quintile had CAD-OR=2.19 (1.13-4.26; **Supplementary Table 8**). Due to the high correlation between leukocytes and neutrophils (Spearman's rho=0.85, p<0.01), results from *simultaneous* modelling (leukocytes and neutrophils in the same model) cannot be interpreted. We found no evidence of an association of eosinophil count or neutrophil:lymphocyte ratio with CAD events (data not shown).

Sensitivity Analysis including estimated glomerular filtration rate (egfr).

eGFR (available pre-CAD event in 1546/2000 participants) was associated with CAD events (univariable CAD-OR=1.15 [1.08-1.23] per 10 ml/min/1.73m2 lower eGFR; **Supplementary Table 9**). When we included latest eGFR in the final model, participants in the 5th vs. 1st leukocyte quintile had CAD-OR=1.52 (1.00-2.31), i.e. CAD-OR was essentially unchanged but the 95% CI was wider.

DISCUSSION

Multiple studies have recorded associations of CAD with biomarkers of inflammation and coagulation in PWH [4, 10, 11] and multiple studies document associations of CAD with leukocyte count in the general population [12-15]. To our knowledge, this is the first report of an independent association of leukocyte count with CAD events in PWH. Our study has three main findings: First, participants with the highest leukocytes (top quintile, >7810/uL) had a 1.59-fold increased CAD event risk in the final multivariable model. This effect size of high leukocytes was similar to the effect of established CAD risk factors, including hypertension, diabetes, dyslipidemia, or recent abacavir exposure. Second, as in the general population, leukocyte count within normal range values was a predictor of CAD events and overt leukocytosis was infrequent. Third, the leukocyte-CAD association was in part explained by smoking, a well recorded CAD risk factor known to increase leukocytes [17]. While the association of black ethnicity or alcohol with lower leukocytes is well established [16, 18], these factors only minimally modified the leukocyte-CAD association in our study. The contribution of leukocyte count to CAD events in PWH may demonstrate the potential clinical value of monitoring leukocytes, a cheap, routinely available biomarker with short turnaround time. While this was beyond the scope of our study (this will require prospective trials), our findings suggest how knowledge of chronically elevated leukocytes increasing CAD event risk by >50% in the 20% PWH in the top leukocyte quintile may motivate clinicians to place even more emphasis on the optimization of cardiovascular risk factors, and, perhaps, primary prevention of CAD with statins in such persons.

Our result of an independent association of leukocyte count with CAD events in PWH appears robust, because it persisted after consideration of traditional and HIV-associated CAD risk factors, and in sensitivity analyses adjusting for Framingham risk score. Additional strengths of

our study are the inclusion of only leukocyte values taken until the day before the CAD event, in order to address the issue of reverse causation (i.e. leukocytes being elevated *because* of a CAD event). In addition, we included all CAD events that occurred in the well-established SHCS over a >21-year time period, and all CAD events were validated using internationally standardized procedures [20, 24].

Additional support for a true leukocyte-CAD event association in PWH is provided by the increase in leukocyte count in CAD cases vs. controls that can already be shown 8 years before the CAD event. This suggests the association of high leucocytes with CAD event risk is not attributable to short term inflammatory/infectious illness immediately before the CAD event that might cause bursts of inflammation and thereby contribute to plaque rupture and CAD events. Our results stand in contrast mechanistically to the association in the general population of acute pneumonia or influenza with increased short-term CAD event risk [31]. Indirect support for the relevance of high leukocytes to CAD risk is afforded by data showing that adding leukocyte count to the Veterans Aging Cohort Study Index improved prediction of mortality [32].

In our Zurich subpopulation, high leukocytes had a larger CAD-odds ratio than high neutrophils. Leukocytes may provide a pathogenetic link between atherosclerosis and activation of pro-coagulatory mechanisms, and some general population literature [33, 34] points to a stronger neutrophil-CAD than leukocyte-CAD association [35]. However, the precise role of different leukocyte subtypes in predicting CAD events remains unresolved.

The leukocyte-CAD association was in part attenuated by smoking, a factor that is well-recorded to increase leukocytes, but less so by alcohol and black ethnicity, both of which may decrease leukocytes, or other factors with an established inflammatory link such as detectable HIV viremia or abdominal obesity.

Our study has limitations. Our population was 87% male, 94% white, and relatively young; therefore, results should only cautiously be extrapolated to other PWH. Leukocyte subtypes were available only in the Zurich participants, and insufficient information was available to analyze possible associations of leukocytes with chronic inflammatory conditions or corticosteroid therapy. Inflammatory markers such as high sensitivity CRP and IL-6 are not routinely measured in the SHCS. A potential link between inflammatory biomarkers and leukocytes would therefore be an important avenue for future investigation. Finally, we did not compare the leukocyte-CAD association in our PWH with a control population without HIV. However, the effect size of leukocytes on CAD risk that we report in PWH is very similar to effect sizes reported in the general population[14, 15].

In conclusion, we show how a high leukocyte count, most often in the normal range, may identify PWH at independently increased risk for CAD events. This increased risk persists after adjustment for traditional and HIV-related risk factors. Our findings expand on how

inflammation (that may not yet be captured by current CAD risk assessment methods) may contribute to high leukocytes and CAD events in PWH.

NOTES

Author Contributions. Study design: EFA, JNK, BL, RDK, HFG, PET. Data management, participant selection, case-control matching: BL. Data acquisition: BL, DLB, MCT, CM, MS, EB, MC, HB, PET. Data analysis: EFA, JNK, BL, PET. Drafting of the manuscript: EFA, BL, PET. Critical review and revision of the manuscript: All authors.

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Swiss HIV Cohort Study (SHCS) members. Anagnostopoulos A, Battegay M, Bernasconi E, Boni J, Braun DL, Bucher HC, Calmy A, Cavassini M, Ciuffi A, Dollenmaier G, Egger M, Elzi L, Fehr J, Fellay J, Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gunthard HF (President of the SHCS), Haerry D (deputy of "Positive Council"), Hasse B, Hirsch HH, Hoffmann M, Hosli I, Huber M, Kahlert CR, Kaiser L, Keiser O, Klimkait T, Kouyos RD, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Marzolini C, Metzner KJ, Muller N, Nicca D, Paioni P, Pantaleo G, Perreau M, Rauch A (Chairman of the Scientific Board), Rudin C (Chairman of the Mother & Child Substudy), Scherrer AU (Head of Data Centre), Schmid P, Speck R, Stockle M, Tarr P, Trkola A, Vernazza P, Wandeler G, Weber R, Yerly S.

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Table 1: Characteristics of Cases and Controls

		All Participants (n=2000)	Cases (n=536)	Controls (n=1464)	P-Values
Male sex, n (%)*		1734 (86.7)	464 (86.6)	1270 (86.8)	0.94[a]
Age at CAD event (years), median (IQR)*		56 (49-63)	56 (49-63)	56 (49-62)	0.54[b]
Date of CAD event, median (IQR)*		03.05.2013 (05.10.2007-18.09.2017)	21.04.2013 (10.09.2007-15.09.2017)	15.05.2013 (30.10.2007-18.09.2017)	0.83[b]
Duration of observation (years), median (IQR)*		13.1 (8.1-19.2)	13.2 (8.2-19.2)	13.1 (8.0-19.2)	0.97[b]
Ethnicity, n (%)	White	1876 (93.8)	514 (95.9)	1362 (93.0)	0.07[a]

	Black	78 (3.9)	14 (2.6)	64 (4.4)	
	Hispanic	19 (1.0)	5 (0.9)	14 (0.9)	
	Asian	27 (1.4)	3 (0.5)	24 (1.6)	
HIV acquisition mode, n (%)	heterosexual	589 (29.5)	162 (30.2)	427 (29.2)	0.14[a]
	MSM	1008 (50.4)	258 (48.1)	750 (51.2)	
	IDU	335 (16.8)	103 (19.2)	232 (15.9)	
	other	68 (3.4)	13 (2.4)	55 (3.8)	
Smoking status, n (%)	current	847 (42.4)	285 (53.2)	562 (38.4)	<0.01[a]
	past	599 (30)	146 (27.2)	453 (30.9)	
	never	554 (27.7)	105 (19.6)	449 (30.7)	
Cigarettes smoked per day, number of smokers, n (%)	≤5 cig/day	113 (5.7)	28 (5.2)	85 (5.8)	<0.01[a]
	6-20 cig/day	527 (26.4)	194 (36.2)	333 (22.8)	
	>20 cig/day	183 (9.2)	55 (10.3)	128 (8.7)	
	Unknown	24 (1.2)	8 (1.5)	16 (1.1)	
Cocaine use i.v. and not i.v. , n (%)	Recent**	77 (3.9)	23 (4.3)	54 (3.7)	0.86[a]
	Ever	(8.5)	45 (8.4)	124 (8.5)	
Alcohol use, last recorded prior to endpoint, n (%)	none/mild	1492 (88)	404 (89.6)	1088 (87.5)	0.27[a]
	moderate/heavy	203 (12)	47 (10.4)	156 (12.5)	
Education Level, n (%)	Mandatory school	331 (16.6)	96 (17.9)	235 (16.1)	0.26[a]
	Apprenticeship	947 (47.4)	264 (49.3)	683 (46.7)	
	Higher Education	619 (47.4)	154 (28.7)	465 (31.8)	
	Other/ missing	103 (5.2)	22 (4.1)	81 (5.5)	

Family History of CAD, n (%)		226 (11.3)	87 (16.2)	139 (9.5)	<0.01[a]
Diabetes mellitus, n (%)		199 (10)	80 (14.9)	119 (8.1)	<0.01[a]
Hypertension, n (%)		613 (30.7)	190 (35.5)	423 (28.9)	0.01[a]
Dyslipidemia, n (%)		1026 (51.3)	319 (59.5)	707 (48.3)	<0.01[a]
CMV seropositivity, n (%)		1712 (85.6)	1239 (84.6)	473 (88.3)	0.04[a]
Hepatitis C seropositivity, n (%)		457 (22.9)	135 (25.2)	322 (22.0)	0.13[a]
Framingham risk score (10-year risk), n (%)	<10%	803 (40.2)	142 (26.5)	661 (45.2)	<0.01[a]
	10-20%	815 (40.8)	240 (44.8)	575 (39.3)	
	>20%	382 (19.1)	154 (28.7)	228 (15.6)	
Leukocytes/uL, median (IQR)	Latest before CAD event	6020 (5000-7460)	6495 (5300-7995)	5900 (4910-7200)	<0.01[b]
	One year before CAD event	5900 (4900-7200)	6200 (5040-7700)	5800 (4810-7100)	<0.01[b]
	Two years before CAD event	5900 (4820-7300)	6145 (5000-7600)	5800 (4800-7200)	<0.01[b]
	Three years before CAD event	5800 (4770-7100)	5920 (4900-7400)	5780 (4700-7000)	0.01[b]
	Five years before CAD event	5755 (4600-7080)	5880 (4685-7605)	5700 (4525-6900)	0.04[b]
	Eight years before CAD event	5600 (4500-6860)	5800 (4590-7400)	5500 (4500-6690)	0.06[b]
	Nine years before CAD event	5500 (4500-6990)	5600 (4410-7100)	5500 (4535-6920)	0.69[b]
	Ten years	5495 (4500-7000)	5500 (4400-7000)	5450 (4500-6910)	0.72[b]

	before CAD event				
CD4 at matching date, median (IQR)		545 (389-762)	546 (384-769)	545 (390-760)	0.86[b]
CD4 nadir (cells/µL), median (IQR)		166 (72-261)	158 (64-254)	170 (78-265)	0.8[b]
CD4 nadir <50 cells/µL, n (%)		356 (17.8)	107 (20.0)	249 (17.0)	0.13[a]
Previous AIDS, n (%)		566 (28.3)	161 (30.0)	405 (27.7)	0.31[a]
On ART, n (%)		1893 (94.7)	519 (96.8)	1374 (93.9)	<0.01[a]
On ART, HIV RNA <50 copies/mL (undetectable), n (%)		1702 (85.1)	452 (84.3)	1250 (85.4)	<0.01[a]
Total years on ART prior to CAD event, median (IQR)		11.7 (6.5-17.7)	12 (7.5-18.5)	11.5 (6.2-17.5)	<0.01[b]
Received Abacavir in 6 months prior to CAD event, n (%)		500 (25)	173 (32.3)	327 (22.3)	<0.01[a]
Received Didanosine in 6 months prior to CAD event, n (%)		66 (3.3)	26 (4.9)	40 (2.7)	0.02[a]
Received an integrase inhibitor in 6 months prior to CAD event, n (%)		477 (23.9)	142 (26.5)	335 (22.9)	0.10[a]
Lopinavir, exposure ≥1 year, n (%)		514 (25.7)	151 (28.2)	363 (24.8)	0.13[a]
Indinavir, exposure ≥1 year, n (%)		402 (20.1)	114 (21.3)	288 (19.7)	0.45[a]
Darunavir, exposure ≥1 year, n (%)		320 (16.0)	91 (17.0)	229 (15.6)	0.49[a]

Stavudine, exposure ≥1 year, n (%)		727 (36.4)	225 (42.0)	502 (34.3)	<0.01[a]

Note. All data shown apply to the matching date and are number (%) of participants, unless otherwise indicated.
* Age, sex, date of CAD event, and observation duration were matching criteria. Due to residual imbalance, median age of cases was 0.27 years older than controls (p<0.01).
** in 6 months prior to matching date
[a] Fisher's exact test
[b] Wilcoxon rank-sum test
Abbreviations. ART, antiretroviral therapy; CAD, coronary artery disease; CMV, cytomegalovirus; IDU, intravenous drug use; IQR, interquartile range; MSM, men who have sex with men.

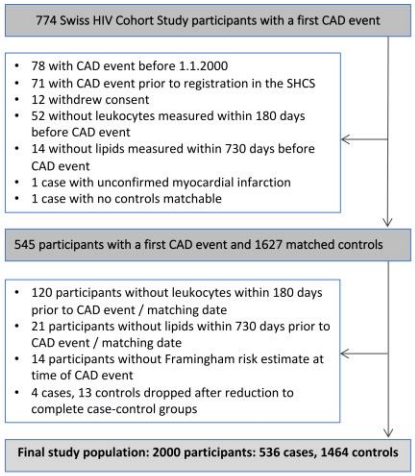
Table 2: Bivariable analyses showing CAD odds ratio (95% confidence interval) for 5th (highest) vs. 1st (lowest) leukocyte quintile, with 1:1 addition of individual variables that may influence leukocyte count

Variable	CAD odds ratio (95% confidence interval) for 5th (highest) vs. 1st (lowest) leukocyte quintile	Likelihood ratio test for Interaction
Univariable Analysis		
Leukocytes, 5th (highest) vs. 1st (lowest) quintile	2.27 (1.63-3.15)	--
Individual Bivariable Analyses (Leukocyte quintiles plus individual variables added 1 :1)		
+ Smoking status (current vs. previous vs. never)	1.82 (1.30–2.56)	0.130
+ Number of cigarettes smoked daily	1.85 (1.31–2.61)	0.589
+ Ethnicity	2.21 (1.59–3.07)	0.861
+ Last alcohol intake: moderate/heavy	1.98 (1.39–2.81)	0.351

All variables were associated with the CAD-Odds Ratio and had p<0.01.

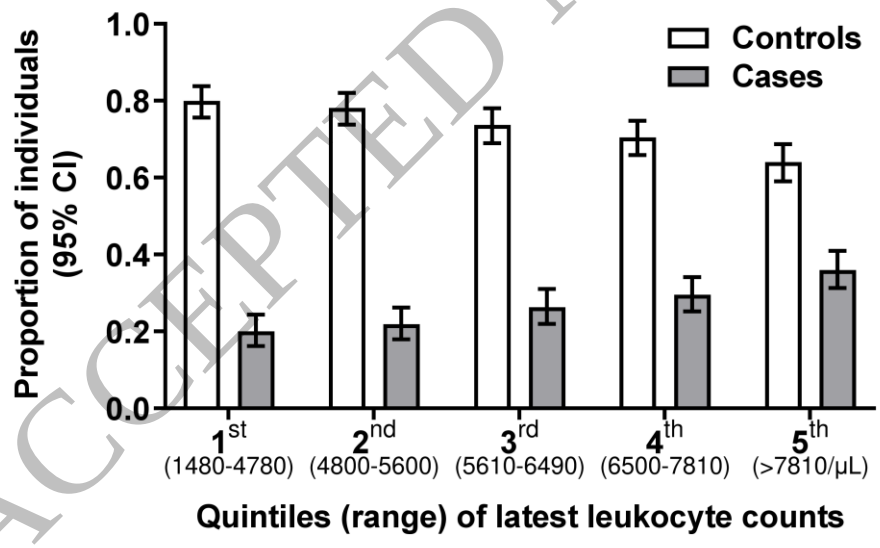
FIGURE LEGENDS

Figure 1: Study Flowchart



Abbreviations: CAD, coronary artery disease; MI: myocardial Infarct

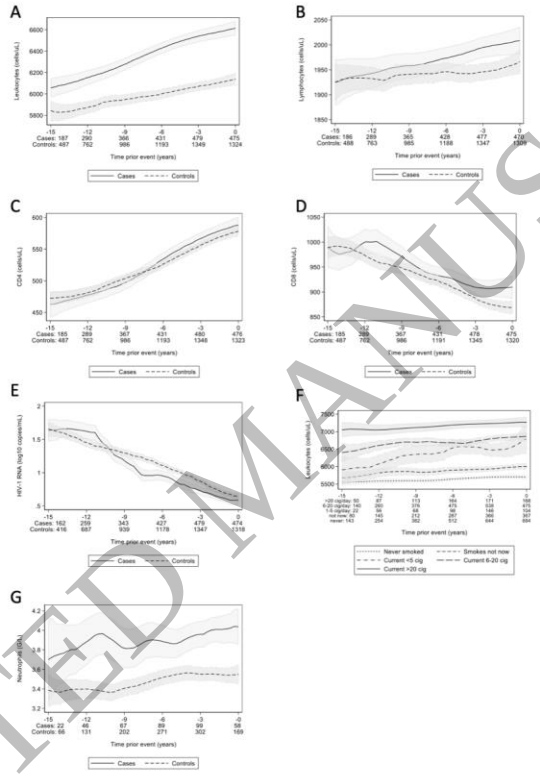
Figure 2: Distribution of Leukocyte Count in 1464 Controls Without Coronary Artery Events (white bars) and in 536 Cases With Coronary Artery Events (gray bars).



We divided CAD cases and CAD-event free controls into 5 quintiles according to their clinical CAD risk and their latest leukocyte count prior to the matching date. We show here the number, percentage and 95% confidence intervals of participants in each quintile, plus the range of leukocyte counts in each quintile.

Distribution of cases and controls according to latest leukocyte count prior to matching date. There were 78 (20.1%) cases vs. 311 (79.9%) controls in the 1st quintile, 90 (21.9%) vs. 321 (78.1%) in the 2nd quintile, 100 (26.3%) vs. 280 (73.7%) in the 3rd quintile, 124 (29.5%) vs. 296 (70.5%) in the 4th quintile, and 144 (36%) vs. 256 (64%) in the 5th quintile. **Abbreviations**: CAD, coronary artery disease; CI, confidence interval.

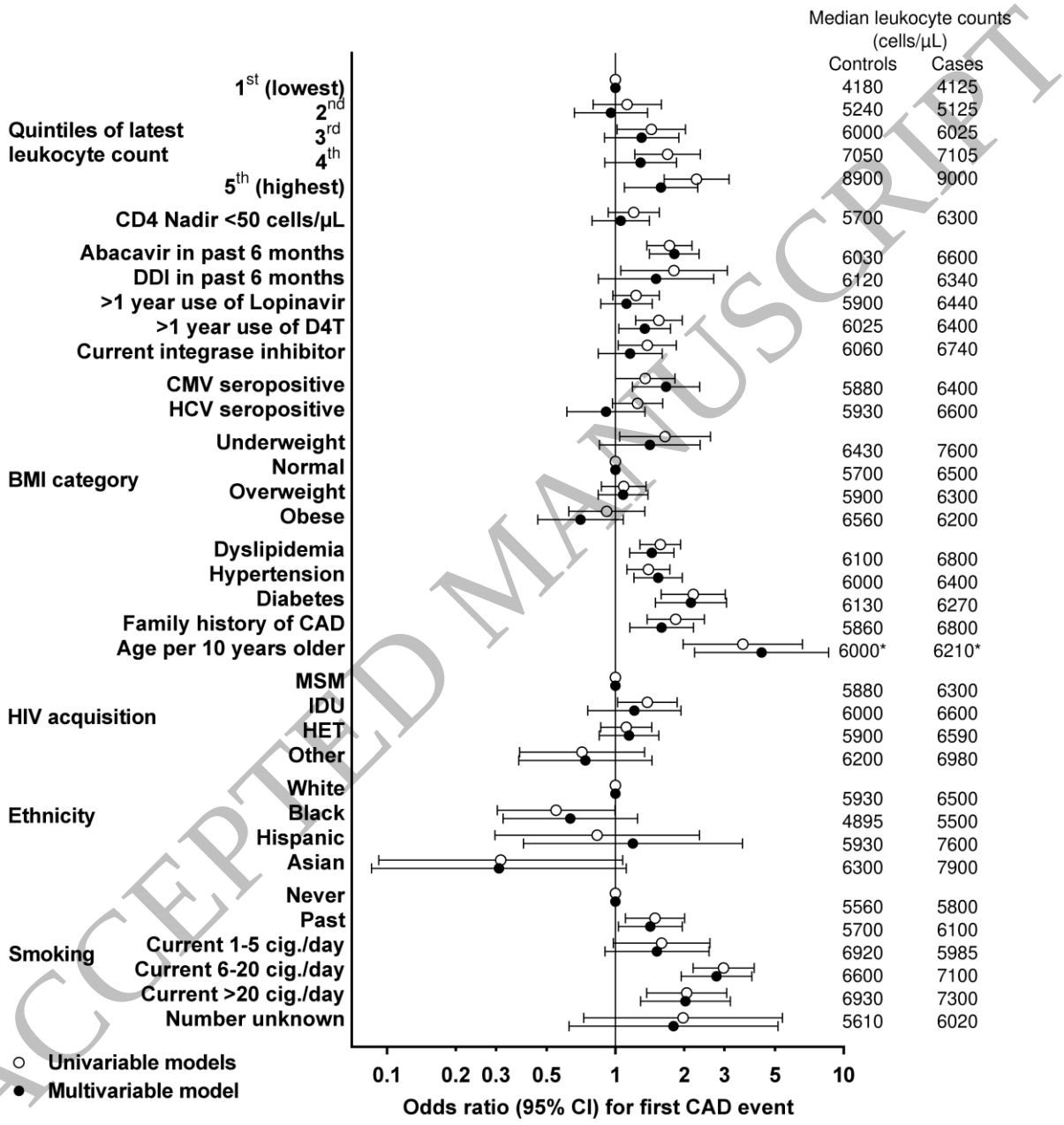
Figure 3A-G: Descriptive Longitudinal Trends for Leukocyte Count, Leukocyte subsets, HIV-RNA and Neutrophil Count in Cases and Controls.



Descriptive (observed) trajectories of total leukocytes and different leukocyte subtypes (Figures 3A-3D) and HIV RNA (Figure 3E) over time for controls vs. cases. The lines show the cell counts and the shaded areas denote the 95% confidence intervals created with local polynomial smoothing. We considered only parameters that were from regular (per protocol) 6-monthly follow-up SHCS visits up until 1 day prior to the CAD event (cases) and matching date (controls). Figure 3F depicts the leukocyte count stratified by different smoking amount categories. Figure 3G shows the observed trajectories of total neutrophils for the University of Zurich subpopulation over time for controls vs. cases. The graphs portray an open cohort design (all participants are included, irrespective of observation duration). The graphs portraying a closed cohort (where only participants with ≥15 years observation time are included) can be found in the **Supplementary Figure 1**.

Abbreviations: CAD, coronary artery disease; CIG, cigarettes.

Figure 4: Odds ratios (ORs) for coronary artery disease (CAD) events (with 95% confidence intervals [CIs]), according to individual clinical risk factors and latest leukocyte quintiles.



* Median leukocyte counts are for persons aged >56 years (median)

Results show univariable and bivariable conditional logistic regression of associations of latest leukocyte count with CAD events, for 536 cases and 1464 controls. Latest leukocyte count (5th [highest] vs. 1st [lowest] was significantly associated with CAD events in univariable analysis and in multivariable analysis, i.e. adjusted for all variables shown. Note: All odds ratios and 95% confidence intervals shown in **Figure 4** are also tabulated in **Supplementary Table 3.** The

righthand panel shows median leukocyte counts (cells/uL) in cases and controls in the different categories.

Abbreviations: CAD, coronary artery disease; CI, confidence interval.