

Associations Between B-Cell Subsets and Subclinical Coronary Artery Disease in Ugandans With and Without HIV

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Background. People living with HIV-1 (PLWH) have an increased risk of cardiovascular disease (CVD), influenced by chronic inflammation, immune dysregulation, and antiretroviral therapy (ART). B cells regulate immune responses, but their contribution to HIV-associated atherosclerosis remains poorly defined.

Methods. In a cross-sectional study, we enrolled 40 PLWH and 60 people without HIV (PWoH) in Uganda, matched 1:1.5 for age and CVD risk. Peripheral blood mononuclear cells were profiled by mass cytometry to define immune cell subsets. Coronary computed tomography angiography quantified coronary artery disease using the segment stenosis score (SSS). We used multivariable hurdle regression to estimate the effect sizes of immune clusters, atherosclerotic cardiovascular disease (ASCVD) risk score, HIV status, and gender.

Results. Median age was 60 years, with no difference by HIV status. People living with HIV had a lower proportion of CCR7⁺ naïve B cells than PWoH (median 1.5% vs 1.8%; *P*-value adjusted [padj] = .03). Across all participants, higher CCR7⁺ naïve B cells (ratio = 0.55, *P* = .02), CXCR3⁺CX3CR1⁺ B cells (ratio = 0.54, *P* = .03), and plasmablasts (ratio = 0.57, *P* = .003) were associated with lower SSS. HIV-positive status was linked to nearly 3-fold higher SSS (*P* < .01). In stratified analyses, classical monocytes (CD14⁺CD16⁺) correlated with higher SSS among PLWH. When classical monocytes were held at the median, higher CCR7⁺ naïve B cells were protective in PLWH (ratio = 0.55, *P* = .02).

Conclusions. This exploratory study suggests that lower frequencies of naïve B cells in PLWH are associated with differences in subclinical atherosclerosis. However, the mechanisms cannot be inferred from this study.

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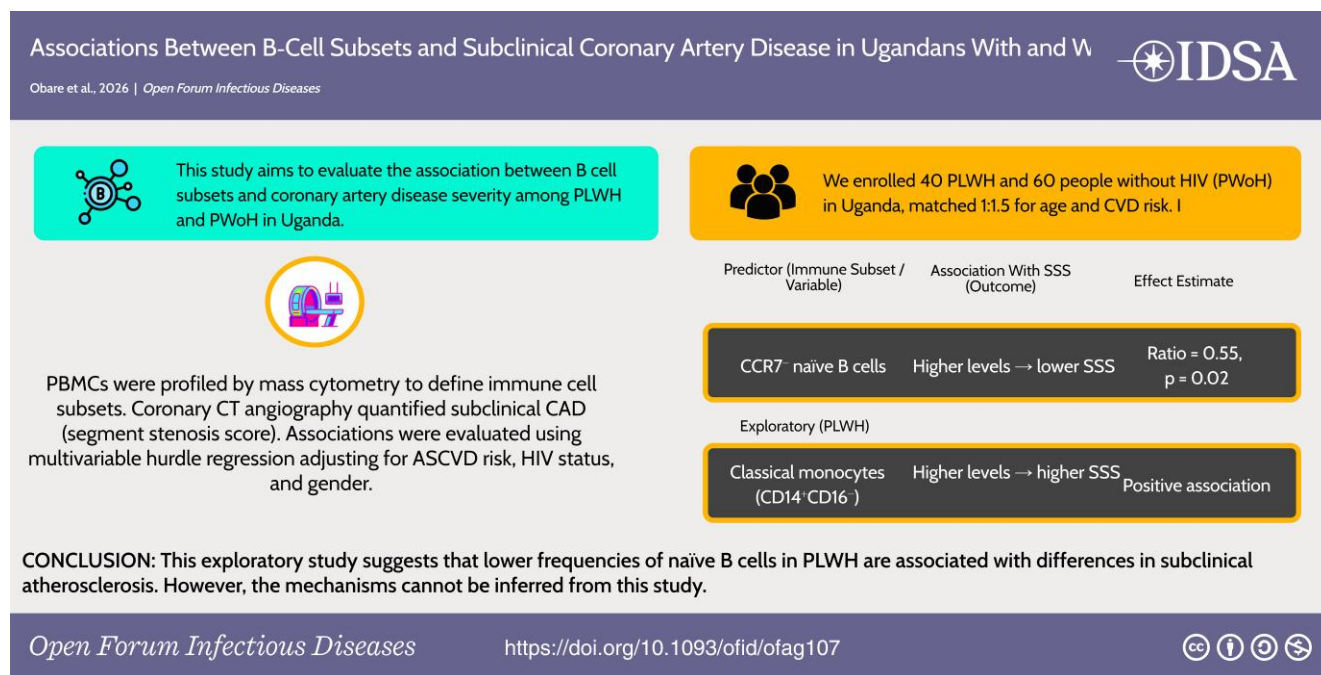
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This graphical abstract is also available at Tidbit: <https://tidbitapp.io/tidbits/associations-between-b-cell-subsets-and-subclinical-coronary-artery-disease-in-ugandans-with-and-without-hiv>

Keywords. B cells; cardiovascular disease; chronic inflammation; HIV; segment stenosis score.

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality worldwide, with an elevated burden among people living with HIV-1 (PLWH) [1]. This increased risk is driven by a combination of traditional CVD risk factors, ART-related metabolic effects, and persistent immune activation [2]. Despite effective ART, PLWH exhibit residual inflammation and immune dysregulation that contribute to atherosclerosis [3].

While the role of T cells and monocytes in atherosclerosis among PLWH has been extensively studied, B cells are increasingly recognized as key regulators of vascular inflammation [4, 5]. Naïve B cells, which decline during chronic HIV infection [6–8], have been shown to secrete atheroprotective IgM antibodies that clear oxidized lipids and reduce foam cell formation [9]. Memory B cells and B cell–monocyte interactions may also contribute to pro-inflammatory pathways that accelerate plaque formation [10].

Most studies that have investigated the role of B cells in atherosclerosis have been conducted in murine models [9, 11, 12], with limited human data available. This study aims to evaluate the association between B-cell subsets and coronary artery disease severity among PLWH and PWoH in Uganda. We hypothesize that immune dysregulation in PLWH, particularly a reduction in naïve B cells, is associated with an increased risk of CVD.

METHODS

Study Design and Participants

This is a cross-sectional secondary analysis of data from the mUTIMA (Ugandan sTudy of HIV effects on the Myocardium and Atherosclerosis) study, a prospective cohort study conducted between 2017 and 2019. Participants for this sub-analysis comprised 100 individuals who underwent completed coronary computed tomography angiography (CCTA), including 40 PLWH on antiretroviral therapy (ART), matched 1:1.5 with 60 PWoH of similar age and with shared cardiometabolic risk factors [13, 14]. Methods and findings from the initial baseline examination of the original cohort have been previously published [13–15].

Patient Consent Statement

The mUTIMA study was approved by the University Hospitals of Cleveland Institutional Review Board, the Joint Clinical Research Centre Research Ethics Committee, and the Uganda National Council for Science and Technology. All participants in this study provided informed consent, including consent for future analyses of stored samples. The Vanderbilt Institutional Review Board approved the mass cytometry analyses of de-identified samples.

Data Collection

Demographic data, clinical parameters, and cardiovascular risk markers were gathered. Diabetes and hypertension were

defined as self-reported or medical chart diagnosis or medication use for the condition. The 10-year ASCVD risk was estimated using the 2013 pooled-cohort equations specific to the “other” race category [16].

Coronary Computed Tomography Angiography

Coronary computed tomography angiographies were performed as previously published [13]. The segment involvement score (SIS) was determined by counting the number of coronary artery segments with detectable atherosclerosis, irrespective of the degree of luminal narrowing. Using an 18-segment model, non-evaluable segments, due to technical limitations, were assumed to be normal when their number was modest. The segment severity score (SSS) accounts for both the extent and severity of CAD by assigning a weight to each diseased segment based on the degree of luminal obstruction. The weights range from 1 for <25% obstruction to 5 for total occlusion. The SSS was calculated by summing these weights across all affected segments, with a maximum possible score of 90. These scores together provide a comprehensive assessment of the burden and severity of CAD [13]. There were 34 participants with non-zero SSS, ¹¹/₄₀ PLWH and ²³/₆₀ PWOH (see [Supplementary Figure 1](#), for distribution).

Cytometry by Time-of-Flight

We assessed immune cell types using mass cytometry ([Supplementary Table 1](#)). Cryopreserved peripheral blood mononuclear cells were thawed, washed, and resuspended in staining medium, then labeled with a metal-conjugated antibody panel ([Supplementary Table 1](#), Standard BioTools Inc) as previously published [17]. Data were acquired on a Helios CyTOF (Fluidigm), tuned according to the manufacturer’s instructions, and normalized with EQ beads.

Raw CyTOF FCS files were exported with the sample run date appended. Five-channel bright beads (mass channels 140, 151, 153, 165, and 175) were used to normalize marker intensities over the set of raw FCS files without concatenation using the *premassa* R package’s normalizer tool. Low-quality events were removed using CyTOF Gaussian waveform parameters. Doublets, including B-cell complexes, were not excluded. Files were gated on TIME to remove fluidic clogs and trimmed to exclude unstable start/end periods. CD4 versus CD8 tailing events from isotope mass overlap (145 vs 146 amu) were removed, while rare +16 oxidation artifacts were ignored. DNA (193/191) gating excluded debris, and bead exclusion ensured complete removal of calibration beads. Live CD45+ cells were gated in FlowJo ([Supplementary Figure 2](#)).

Each subset was exported for independent analysis. Non-informative markers were excluded. Files were concatenated, transformed ($\text{asinh}[x/5]$), and batch-corrected (25 PCs, Harmony R package, $\lambda=4$, batch = run date). Uniform manifold approximation and projection (UMAP) was used for visualization, and Leiden clustering (kNN = 30, resolution = 3) defined populations.

Cluster abundances were calculated and compared between groups stratified by HIV status ([Supplementary Figure 3](#)).

Statistical Analysis

Baseline characteristics between PLWH and PWOH were compared using the Wilcoxon Rank-Sum test for continuous variables and Pearson’s Chi-squared test for categorical variables. Continuous variables are reported as medians with interquartile ranges, and categorical variables as frequencies and percentages.

The outcome, SSS, ranged from 0 to 21, with 66% of participants scoring zero. The excess of zeros and the skewness toward positive values necessitated the use of a hurdle regression model. The hurdle model separates the binary process of having any stenosis (zero vs non-zero) from the conditional distribution of positive values. This allows more accurate and interpretable inference than standard models. This approach explicitly addresses zero inflation and heterogeneity in effect sizes, making it well-suited to this data structure.

Multivariable hurdle regression was used to estimate the associations between immune clusters, atherosclerotic cardiovascular disease (ASCVD) risk score, HIV status, and gender with SSS. Immune clusters were square root transformed to improve normality and model fit. To assess whether associations varied by HIV status or ASCVD risk, we included interaction terms (*clusterASCVD*, *clusterHIV-status*, and *ASCVD*HIV-status*). We also tested whether naïve B cells modified the effects of other clusters.

Effect estimates for continuous predictors are reported as the ratio of SSS comparing the upper (Q3) quartile with the lower (Q1) quartile, along with 95% confidence intervals (CIs) and *P*-values derived from the count component of the hurdle model. Forest plots were used to visualize effect sizes, and predicted SSS values were plotted against ASCVD risk or immune clusters, overall and stratified by HIV status.

All *P* values were 2-sided, with multiple-comparison adjustments when comparing immune clusters by HIV status. Analyses (heat maps, line graphs, and forest plots) were performed in R (v4.3.2), with bar plots generated in GraphPad Prism (v10).

RESULTS

Demographics and Clinical Parameters

The median age was 60 years, and no significant differences were observed based on HIV status. Females constituted 62% of PLWH and 57% of PWOH. Diabetes was more prevalent among PLWH (82%) than among PWOH (63%; *P* = .04). Statin use was low (6%), with comparable rates across all groups. The median nadir CD4⁺ cell count was 146 cells/mm³ among PLWH, and the median ART duration was 13 years. A significant proportion (86%) achieved complete viral suppression (<20 copies/mL), and no PLWH had a viral load >254 copies/mL. Approximately 25% of participants were on protease inhibitors, 7% were on integrase strand transfer

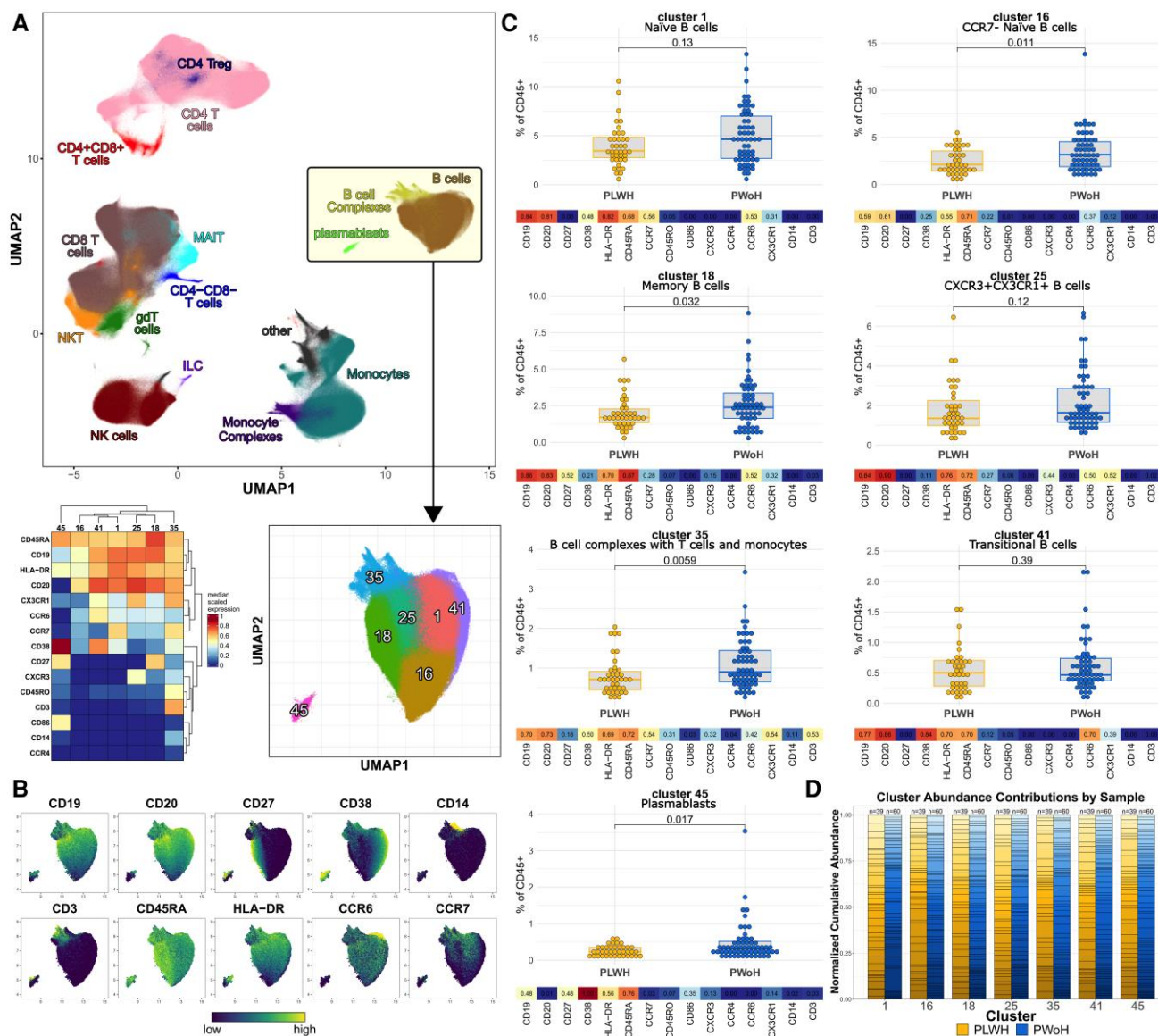


Figure 1. B cell phenotyping using mass cytometry. UMAP shows immune cell clusters concatenated from all participants ($n = 100$). The B cell cluster is highlighted, and markers expressed on the B cells are shown on the heatmap and UMAPs below (B). Box plots show individual proportions of B cell clusters by HIV status (C). Contribution of individual participants from each group (PLWH, $n = 39$, and PWOH, $n = 60$) to each of the B cell clusters (1, 16, 18, 25, 35, 41, and 45). Statistical analysis by the Mann–Whitney U test.

inhibitors, and 7% received abacavir. The median (interquartile range [IQR]) $CD4^+$ cell count among PLWH was 508 (380–668 cells/ mm^3). The groups showed no clinical or statistically significant differences in BMI ($P = .18$) and waist circumference ($P = .39$). Median total cholesterol levels were higher but not statistically significant in PLWH (224 mg/dL) than in PWOH (195 mg/dL, $P = .10$; [Supplementary Table 2](#)).

Naive B Cells are Significantly Higher in Proportion in PWOH Compared With PLWH

To determine differences in immune cells, we visualized high-dimensional data using UMAP ([Figure 1A and B](#)). With $CD45^+$

cells as the denominator, we distinguished $CD8^+$ T cells, monocytes, $CD4^+$ T cells, NK cells, gd T cells, MAIT cells, ILCs, NKT cells, and B cells in blood samples from all participants ([Figure 1A and B](#)). For this study, we focused on B-cell subsets ([Figure 1A](#)). The heatmap shows differential expression of B-cell markers. Cluster 35 consists of B-cell–T-cell–monocyte complexes. The remaining clusters are defined by additional markers they express. Plasmablasts have high expression of CD38 (Cluster 45). Cluster 18 consists of $CD27^+$ memory B cells, cluster 41 consists of transitional B cells, cluster 25 consists of $CXCR3^+ CXCR1^+$ B cells, and the rest are naive B cells (Clusters 1, 16). When stratified by HIV status, the median percentage of $CCR7^-$ naive B cells (Cluster 16, $P = .011$), memory

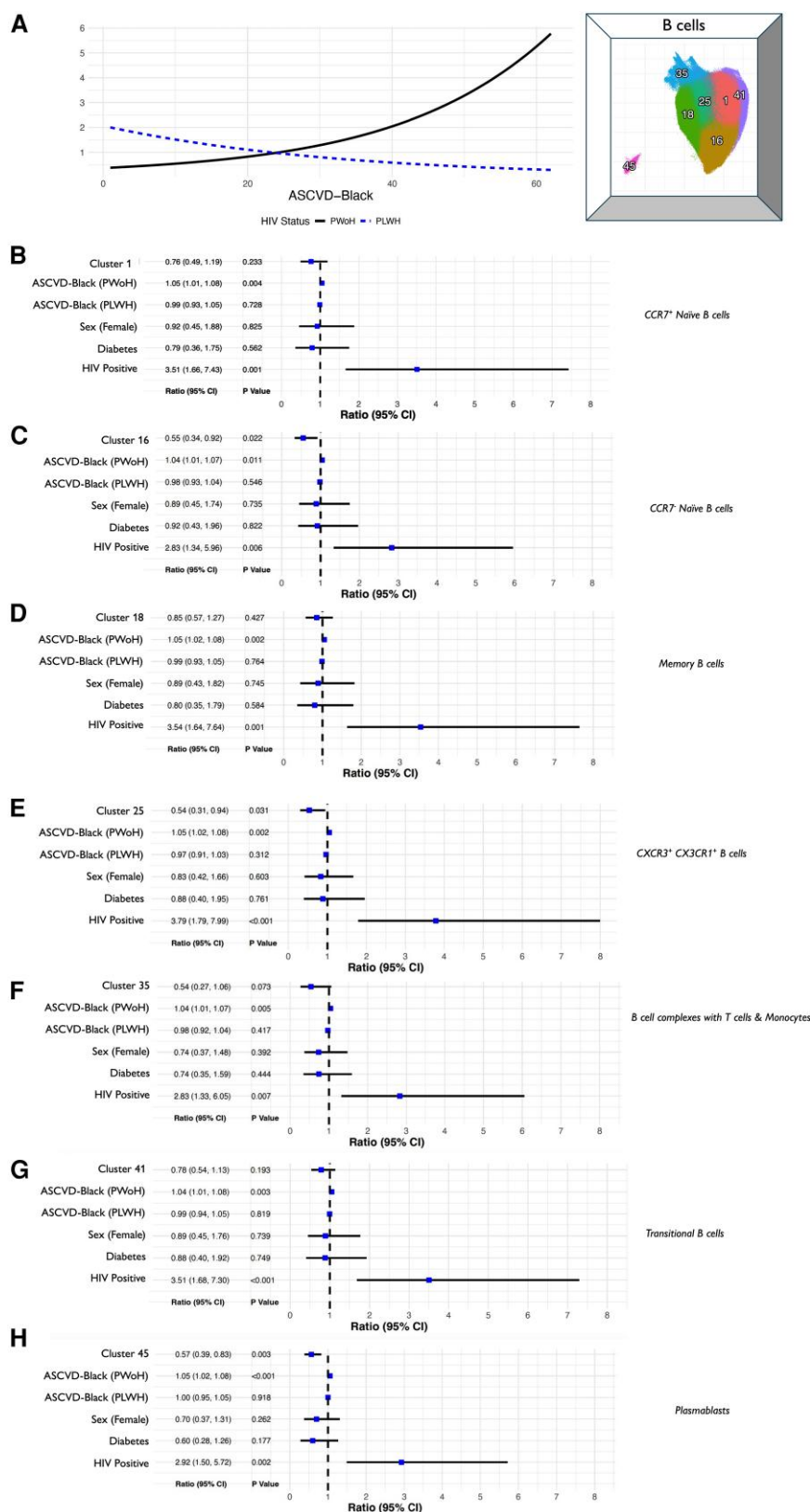


Figure 2. Associations of B cell subsets with SSS by HIV and ASCVD Risk. *A*, Shows the Hurdle model-predicted SSS against ASCVD, stratified by HIV status, with CCR7⁺ naïve B cells set at the median value and sex (female) and diabetes (yes) at their mode. *B-H*, Present the ratios in SSS comparing Q₃ to Q₁ for each cluster, while keeping ASCVD at the median value, or comparing Q₃ to Q₁ of ASCVD among PWoH or PLWH, or female versus male (sex), yes versus no (diabetes), or PLWH versus PWoH (HIV-positive). The *P* values for the interaction term between ASCVD and HIV status are 0.098 (*B*), 0.080 (*C*), 0.098 (*D*), 0.026 (*E*), 0.042 (*F*), 0.114 (*G*), and 0.062 (*H*). Q₃: upper quartile; Q₁: lower quartile.

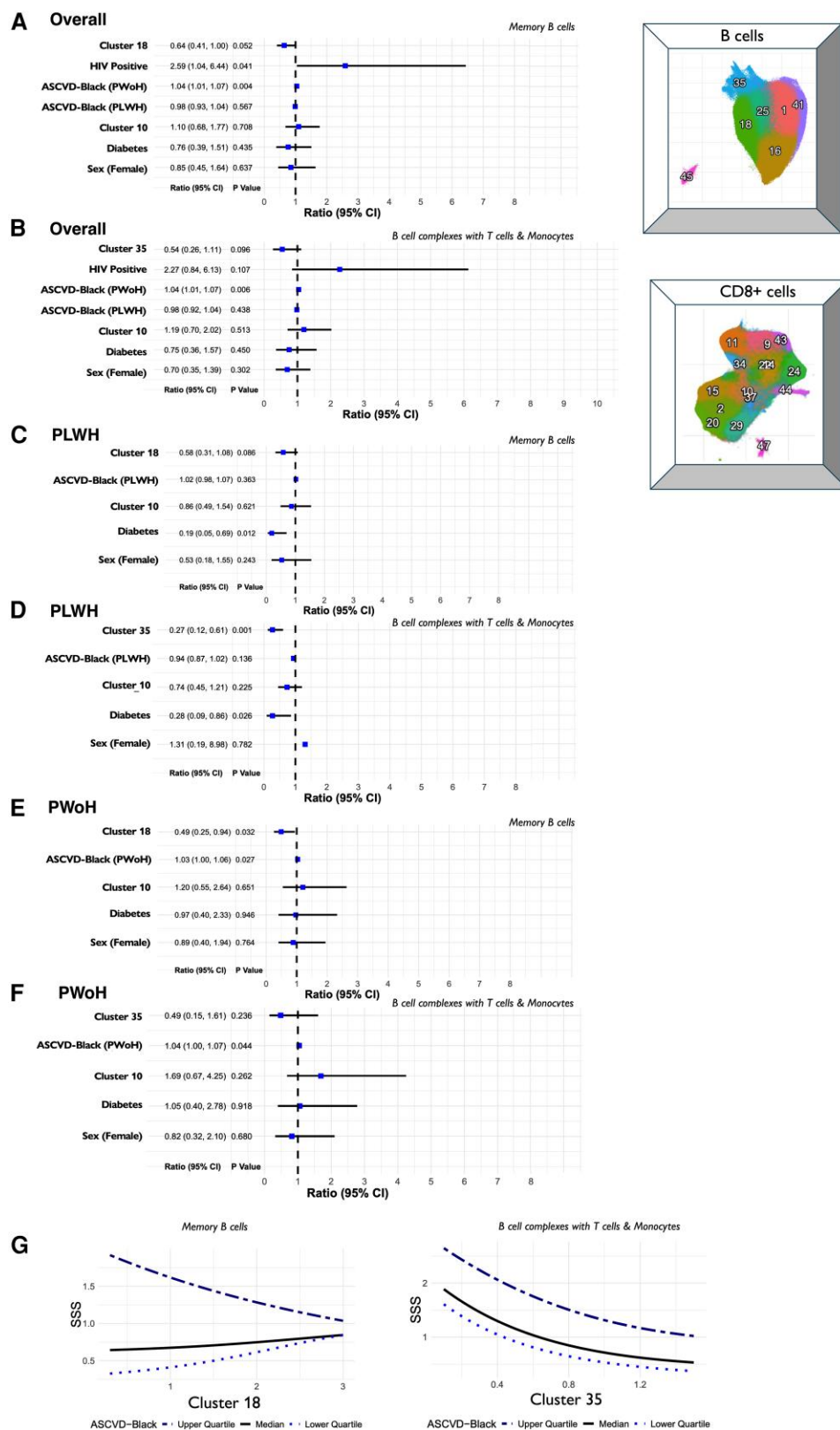


Figure 3. CD57⁺GPR56⁺CX3CR1⁺CD8⁺ memory T cell subset modifies the relationship between memory B cells and SSS. A-F, Present the ratios in SSS comparing Q₃ to Q₁ for each cluster while keeping ASCVD risk at the median value, or comparing Q₃ to Q₁ of ASCVD among PLWH or PWoH while keeping each cluster at the median value, female versus male (sex), yes versus no (diabetes). The *P* values for the interaction term between ASCVD and HIV status are 0.069 (A) and 0.05 (B). The *P* values for the interaction term between ASCVD, cluster 18, and cluster 35 are 0.028 (A) and 0.997 (B), respectively. G, Shows the Hurdle model-predicted SSS against Clusters 18 and 35, with ASCVD fixed at the Q₃ median or Q₁. Q₃: upper quartile; Q₁: lower quartile.

B cells (Cluster 18, $P=.032$), B-T-monocyte complexes (Cluster 35, $P=.0059$), and plasmablasts (Cluster 45, $P=.017$) was lower among PLWH (Figure 1C). Differences of all immune clusters by HIV status were performed (Supplementary Table 3).

HIV Status and ASCVD Risk Independently Shape B-Cell Associations With SSS

To investigate the role of B-cell subsets in the severity of coronary artery disease, we evaluated their associations with the SSS using a hurdle regression model. For all the subsets, we evaluated whether there was an interaction between HIV status and ASCVD risk score. We included sex and diabetes status in the model because there was a statistical difference in diabetes prevalence between PLWH and PWOH. The association of CCR7⁺ naïve B cells (Cluster 1) with SSS was dependent on the level of ASCVD risk but not statistically significant ($P=.098$), as shown in Figure 2A. The shapes of the ASCVD-SSS curves were similar for all other B-cell subsets, with a significant association between SSS and CXCR3⁺ CX3CR1⁺ B cells (Cluster 25, $P=.026$) and B cell complexed with T cells and monocytes (Cluster 35, $P=.042$). However, HIV-positive status and the ASCVD risk score among PWOH were strong independent risk factors for SSS (Figure 2B-H).

Naïve B-cell clusters and plasmablasts were negatively associated with SSS, while HIV status and ASCVD risk score among PWOH were positively associated with SSS. Specifically, there was a negative association between CCR7⁺ naïve B cells (Cluster 16, ratio 0.55; $P=.022$) but not CCR7⁺ naïve B cells (Cluster 1, ratio 0.76; $P=.23$). Interestingly, plasmablasts (Cluster 45) were also negatively associated with SSS (Cluster 45, ratio 0.57; $P=.003$). There was a small cluster of B cells that was between CCR7⁺ naïve B cells and memory B cells, CXCR3⁺ CX3CR1⁺ B cells (Cluster 25). These cells were also associated with a significantly lower SSS (ratio, 0.54; $P=.031$) and exhibited an ASCVD-dependent relationship, with the ASCVD-SSS association differing by HIV status (P -interaction = .04). Memory B cells (Cluster 18, ratio, 0.85; $P=.43$) were not associated with SSS. The other subsets, including transitional B cells and T-B cell complexes, were also not significant.

In summary, HIV-positive status and the ASCVD risk score among PWOH were strong independent risk factors for SSS. Significant negative associations with SSS were observed for CCR7⁺-naïve B cells (Cluster 16), plasmablasts (Cluster 45), independent of HIV status or ASCVD risk among PWOH. CXCR3⁺ CX3CR1⁺ B cells (Cluster 25) exhibited an ASCVD-dependent relationship with SSS.

CD57⁺ GPR56⁺ CX3CR1⁺ CD8⁺ Memory T Cell Adjustment Strengthens the Negative Relationship Between Memory B Cells and SSS

Cluster 10, a CD57⁺ GPR56⁺ CX3CR1⁺ CD8⁺ memory T cell subset, among other subsets, was significantly higher in PLWH than PWOH (median 2.1 vs 1.4, adjusted $P<.01$,

Supplementary Table 2). Given the relationships between antigen-presenting cells, such as B cells and monocytes, and their interactions with T cells, we assessed whether cluster 10 modulates the association between a memory B cell subset (Cluster 18), CD14⁺ monocyte-B cell complexes (Cluster 35), and SSS. Among all participants, memory B cells (Cluster 18) were associated with lower SSS (ratio = 0.64; $P=.052$), while HIV-positive status was associated with higher SSS (ratio = 2.59; $P=.041$). ASCVD score among PWOH was also significantly associated with SSS (ratio = 1.04; $P=.004$) (Figure 3A). There was also a negative association between CD14⁺ monocyte-B cell complexes (Cluster 35) and SSS among all participants, but it was not significant (ratio = 0.54; $P=.096$) (Figure 3B).

Among PLWH, the association between memory B cell Cluster 18 and SSS was not significant (Figure 3C; ratio = 0.58; $P=.086$). However, in PWOH, Cluster 18 was associated with lower SSS (Figure 3E; ratio = 0.49; $P=.032$). Paradoxically, diabetes was associated with lower SSS in PLWH but not in PWOH (Figure 3C, D). The T cell-monocyte-B cell (Cluster 35) was associated with lower SSS (ratio = 0.27; $P=.001$) in PLWH (Figure 3D) but not in PWOH (ratio = 0.49; $P=.236$) (Figure 3F). Increasing proportions of Clusters 18 were associated with decreased SSS in individuals with higher ASCVD risk (upper quartile, $P=.028$) but not Cluster 35 (upper quartile, $P=.997$) (Figure 3G). The inclusion of CD57⁺ GPR56⁺ CX3CR1⁺ CD8⁺ memory T cells (Cluster 10) in the model unmasked a significant negative association between memory B cells and SSS, especially in PWOH (Figure 3E). This suggests that the contribution of memory B cells to the vascular disease burden may be obscured by the influence of CX3CR1⁺ CD8⁺ T cells and that the effects of memory B cells on SSS are context dependent.

CCR7⁺ Naïve B Cells (Cluster 16) are Associated With Lower SSS in PWOH.

In the overall cohort (Figure 4A), SSS decreases as Cluster 16 increases, a trend consistent across all levels of ASCVD risk, suggesting no meaningful interaction between ASCVD and Cluster 16 (ratio = 0.42; $P=.84$). The forest plot shows a negative association between Cluster 16 and SSS (ratio = 0.35; $P=.031$) and a positive association between HIV-positive status and SSS (ratio = 2.91; $P=.038$).

Among PLWH (Figure 4B), CCR7⁺ naïve B cells (Cluster 16) were not significantly associated with decreased SSS (ratio = 0.54; $P=.15$). There was no interaction between the ASCVD risk score and Cluster 16 (ratio = 0.92; $P=.12$). In PWOH (Figure 4C), naïve B cells were significantly associated with decreased SSS (ratio = 0.22; $P=.003$). Including cluster 10 in the model did not change the relationship between Cluster 16 and SSS (Figure 4A-C). Increasing proportions of CCR7⁺ naïve B cells were significantly associated with reduced SSS in PWOH, where the association was modified by ASCVD (P -interaction = .02).

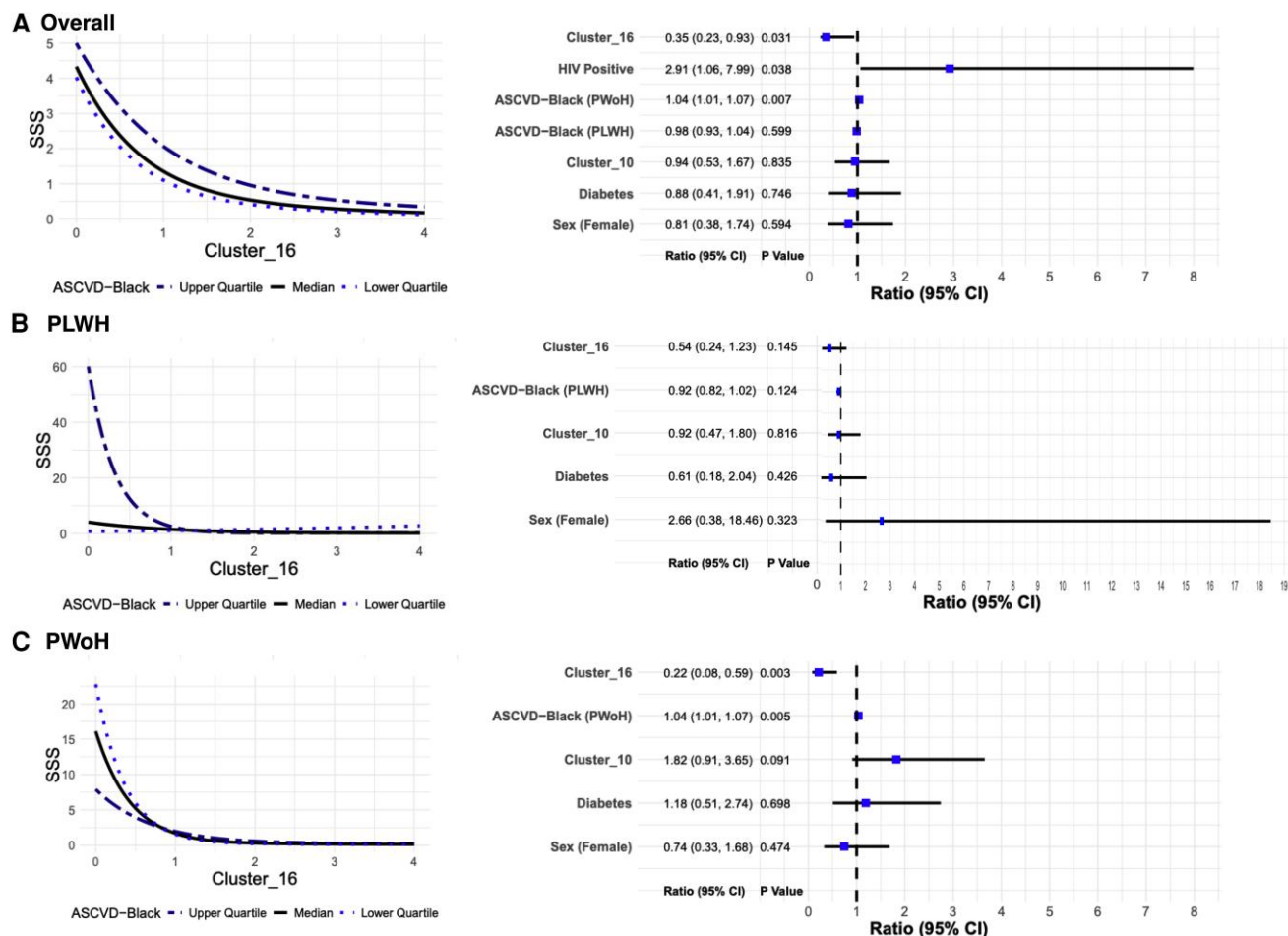


Figure 4. The effect of Cluster 16 on SSS varies the ASCVD risk score in PWoH. Panels to the left show the Hurdle model–predicted SSS against Cluster 16, while the ASCVD is fixed at Q₃, median, or Q₁, among the entire cohort (A), PLWH (B), or PWoH (C). Panels to the right present the ratios in SSS comparing Q₃ to Q₁ for each cluster while keeping ASCVD risk at the median value, or comparing Q₃ to Q₁ of ASCVD among PWoH or PLWH while keeping each cluster at the median value, female versus male (sex), yes versus no (diabetes), or PLWH versus PWoH while keeping ASCVD at the median value. The *P*-value for the interaction term between ASCVD and HIV status is 0.081 (A). The *P* values for the interaction term between ASCVD and cluster 16 are 0.41 (A), 0.07 (B), and 0.02 (C). Q₃: Upper quartile; Q₁: Lower quartile.

In contrast, no significant interaction was observed in PLWH (*P*-interaction = .07) or the overall cohort (*P*-interaction = .41), suggesting that the protective effect of naïve B cells may be attenuated in the context of HIV.

Protective Association of Naïve B Cells Against SSS Emerges in the Context of Classical Monocytes in PLWH

Both monocytes and CD8 T cells have been implicated in the pathogenesis of atherosclerotic cardiovascular disease. The majority of the CD8⁺ T cell clusters in this cohort, including clusters 9 (CD8 TCM) and 11 (CD8 Naïve), were significantly higher in PLWH than PWoH (Figure 1B, Supplementary Table 2). A higher CD4:CD8 ratio is associated with decreased risk of CAD among PLWH [18]. We investigated whether there was any interaction between CD8⁺ TCM cells (Cluster 9), phenotyped as CXCR3⁺ NKG2D⁺ CD27⁺ CD28⁺ CD127⁺ CD8⁺ T cells, and naïve B cells (Cluster 16) across different quartile

comparisons. The interactions were compared at higher frequencies (Quartile 3, Q3) versus lower frequencies (Quartile 1, Q1) for each immune cell cluster. Additionally, we assessed the modifying effect of naïve B cells (Cluster 16) categorized into quartiles (Q1–Q3). Thus, for 3 clusters (CD8 T cells—9, and 11; and monocyte—7), each row represents a specific combination of high/low frequencies of the T cells and monocyte clusters and naïve B cell Cluster 16.

Among all participants, a higher proportion of naïve B cells (Cluster 16) showed a significant inverse relationship with SSS. When Cluster 9 (CD8 T cells) was set at the median, increased naïve B cells (Q3 vs Q1) were associated with 45% lower SSS (ratio = 0.55, 95% CI: 0.32–0.94, *P* = .028), suggesting better clinical outcomes (Figure 5A). When Cluster 7, classical monocytes, were held at the median, Cluster 16 levels did not associate with SSS (ratio = 0.64, 95% CI: 0.35–1.16, *P* = .139). Lastly, when Cluster 11 (Naïve CD8⁺ T cells) were held at the median,

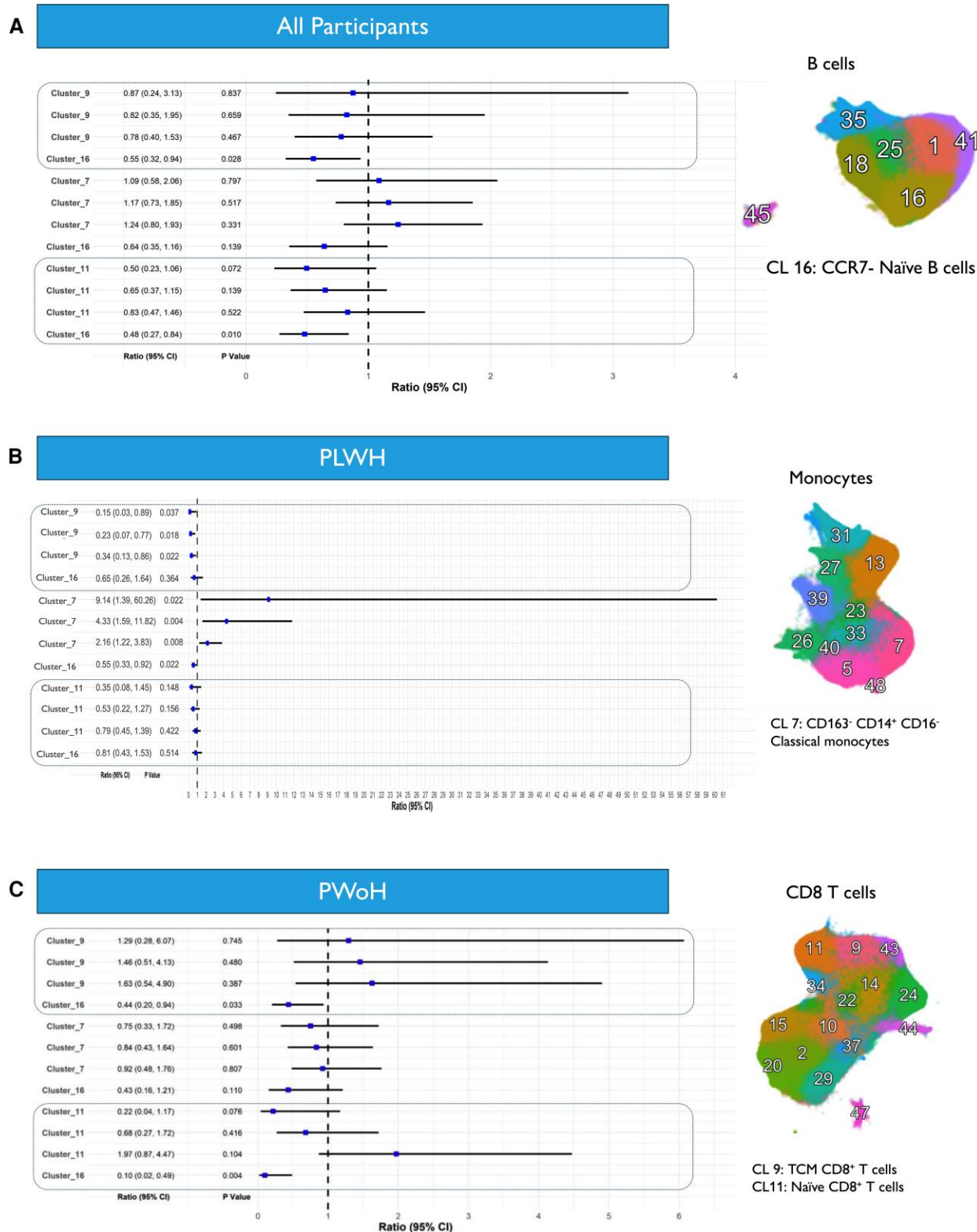


Figure 5. Divergent associations of monocytes and naïve B cells with SSS in PLWH. Forest plots show the effect estimates and 95% CIs for 3 models assessing the impact of CD8⁺ T cells (Clusters 9-top and 11-bottom), monocytes (Cluster 7-middle), and their interactions with naïve B cells (Cluster 16). A-C, Present the ratios in SSS comparing Q₃ to Q₁ for each of clusters 9, 7, and 11, while keeping cluster 16 at Q₁, median or Q₃, ASCVD risk at the median value, among all patients (A) or within PWoH (B) or PLWH (C) subgroups. The *P* values for the interaction terms between Cluster 9 and Cluster 16 are 0.787 (A), 0.261 (B), and 0.261 (C). The *P* values for the interaction terms between Cluster 7 and Cluster 16 are 0.644 (A), 0.510 (B), and 0.132 (C). The *P* values for the interaction terms between Cluster 11 and Cluster 16 are 0.138 (A), 0.020 (B), and 0.199 (C). UMAPs on the right show each cluster in the context of total B cells, monocytes, and CD8⁺ T cells. Q₃: upper quartile; Q₁: lower quartile.

higher Cluster 16 levels were associated with a 52% lower SSS (ratio = 0.48, 95% CI: 0.27–0.84, $P = .010$), again showing a consistent protective effect.

Among PLWH, Cluster 9 (CD8 TCM) cells were negatively associated with SSS in all quartiles. When they were set at the median, the relationship between naïve B cells and SSS was not significant (ratio = 0.65, 95% CI: 0.26–1.64, $P = .36$) (Figure 5B). On the other hand, all quartiles of Cluster 7 (CD163– classical monocytes) were positively associated with SSS. When the classical monocytes were held at the median, higher levels of Cluster 16 were associated with lower SSS (ratio = 0.55, 95% CI: 0.33–0.92, $P = .022$). When naïve CD8⁺ T cells (Cluster 11) were held at the median, naïve B cells were not significantly associated with SSS (ratio = 0.81, 95% CI: 0.43–1.53, $P = .51$). However, the interaction between naïve B cells and naïve CD8⁺ T cells was significant in PLWH (P -interaction = .020), indicating that the association of naïve B cells with SSS varied according to the frequency of naïve CD8⁺ T cells, even though no effect was evident at the median.

Among PWOH, high naïve B cells were significantly associated with lower SSS, except when classical monocytes were included in the model (Figure 5C). Overall, the data suggest that higher frequencies of naïve B cells are associated with favorable outcomes (lower SSS) predominantly among PWOH. In PLWH, this protective association was only evident when classical monocytes were accounted for and was not observed in the context of CD8⁺ T cells. In contrast, classical monocytes themselves were consistently associated with higher SSS.

DISCUSSION

In this exploratory study, we observed differences in circulating B-cell subsets between PLWH and PWOH, and several immune populations showed associations with measures of subclinical CAD. PLWH had lower frequencies of naïve, memory, and CXCR3⁺CX3CR1⁺ B-cell subsets, as well as plasmablasts, compared with PWOH, despite long-term antiretroviral therapy. While these findings align with prior descriptions of HIV-associated B-cell alterations [6, 19], the implications for cardiovascular disease among PLWH had not yet been investigated. Several B-cell subsets showed divergent associations with SSS. Naïve B cells and plasmablasts may have a protective role, as indicated by the negative association with SSS. In PLWH, the inverse association between naïve B cells and SSS was clearer when classical monocytes were included, suggesting that monocyte-driven inflammation may obscure B-cell protection. Together, we observed that the negative association between B-cell subsets and SSS was strongest in PWOH, whereas in PLWH, the relationship was detectable primarily when classical monocytes were included in the model.

IgM expressed by naïve B cells has been implicated in the clearance of oxidized lipids and suppression of inflammatory

cascades [12, 20]. We found that naïve B cell frequencies were inversely associated with SSS, particularly among individuals with higher ASCVD risk scores. These findings are hypothesis-generating and motivate future studies to determine whether naïve B cell–related immune features interact with established cardiovascular risk factors.

CCR7 directs lymphocyte homing to secondary lymphoid tissues through its ligands CCL19/CCL21, on high endothelial venules and supports B-cell entry into lymph nodes, together with CXCR4/CXCR5 [21]. CCR7⁺ naïve B cells likely reflect a lymphoid-homing, GC-competent phenotype, whereas CCR7[−] naïve B cells may be more circulation-prone and shaped by chronic inflammation. Beyond classic lymphoid homing, CCR7 expression on human B cells varies, and chronic HIV infection perturbs this axis; reports describe reduced CCR7 and broader disruption of trafficking receptors in exhausted/tissue-like memory B cells [22]. In this context, the inverse association between CCR7[−] naïve B cells and coronary disease measures in PLWH may reflect broader disruptions in B-cell trafficking during chronic HIV infection, but this relationship remains exploratory and requires validation in larger studies.

Cell-to-cell complexes are dynamic cellular interactions that may contribute to inflammation and have been reported to be increased in PLWH with diabetes, possibly contributing to the pathogenesis of cardiometabolic conditions [17]. The role of monocytes was notable in this study: classical monocytes were positively associated with SSS among PLWH, consistent with established links between monocyte activation and vascular dysfunction in HIV [23, 24]. In contrast, cell-to-cell complexes involving B cells were inversely associated with SSS in PLWH; this observation is intriguing but its biological relevance remains unclear.

Traditional ASCVD risk scores remain valuable but do not capture immune dysregulation [25]. Prior research shows these models can underestimate risk in chronic inflammatory states [26, 27]. Taken together, these findings indicate that immune profiles differ between PLWH and PWOH, with distinct patterns of association with measures of subclinical coronary artery disease. However, given the modest sample size, subgroup design, and the heterogeneous clinical characteristics of the comparator group, these observations cannot establish causality or directionality. Rather, they highlight areas warranting further investigation, particularly the relationship among naïve B cells, monocyte-driven inflammation, and vascular disease in HIV.

CONCLUSION

Naïve B cells were inversely associated with CAD burden, especially in PWOH with high ASCVD risk. On the other hand, classical monocytes correlated with higher SSS in PLWH. These results suggest that immune profiling could strengthen prediction models. Larger longitudinal studies with detailed

mechanistic assessments are needed to clarify these relationships and evaluate whether immune phenotypes could complement existing cardiovascular risk stratification approaches in HIV.

LIMITATIONS

While these findings provide valuable insights into B cell subset changes and their potential contributions to cardiovascular risk in PLWH, several limitations should be considered. First, ASCVD risk scores, though widely used, may not capture the full spectrum of risk factors, particularly in HIV, where traditional risk prediction models may underestimate cardiovascular events. Although we adjusted for key confounders, unmeasured factors, such as inflammatory biomarkers, antiretroviral therapy duration, and lifestyle influences, may still contribute to the observed associations. The relatively small sample size and the inclusion of PWoH with existing cardiometabolic risk factors limit the extent to which we can isolate the effect of HIV status from shared comorbidities. As such, findings should be interpreted as hypothesis-generating. Additionally, this study is observational; causal inferences cannot be made. Future studies using mechanistic approaches and longitudinal designs are needed to further explore the role of immune cell subsets in HIV-associated cardiovascular risk.

Supplementary Data

Supplementary materials are available at [Open Forum Infectious Diseases](https://openforum.infectiousdiseases.org) 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.

Notes

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References

- Shah ASV, Stelzel D, Lee KK, et al. Global burden of Atherosclerotic cardiovascular disease in people living with HIV: systematic review and meta-analysis. *Circulation* **2018**; 138:1100–12.
- Obare LM, Temu T, Mallal SA, Wanjalla CN. Inflammation in HIV and its impact on Atherosclerotic cardiovascular disease. *Circ Res* **2024**; 134:1515–45.
- Zicari S, Sessa L, Cotugno N, et al. Immune activation, inflammation, and Non-AIDS Co-Morbidities in HIV-infected patients under long-term ART. *Viruses* **2019**; 11:200.
- Obare LM, Bonami RH, Doran AC, Wanjalla CN. B cells and atherosclerosis: a HIV perspective. *J Cell Physiol* **2024**; 239:e31270.
- Aranguren M, Doyon-Laliberté K, El-Far M, et al. Subclinical atherosclerosis is associated with discrepancies in BAFF and APRIL levels and altered Breg potential of precursor-like marginal zone B-cells in long-term HIV treated individuals. *Vaccines (Basel)* **2022**; 11:81.
- Boliar S, Murphy MK, Tran TC, et al. B-Lymphocyte Dysfunction in chronic HIV-1 infection does not prevent cross-clade neutralization breadth. *J Virol* **2012**; 86:8031–40.
- Moir S, Fauci AS. B-cell responses to HIV infection. *Immunol Rev* **2017**; 275: 33–48.
- Moir S, Buckner CM, Ho J, et al. B cells in early and chronic HIV infection: evidence for preservation of immune function associated with early initiation of antiretroviral therapy. *Blood* **2010**; 116:5571–9.
- Ebrahimian T, Dierick F, Ta V, et al. B cell-specific knockout of AID protects against atherosclerosis. *Sci Rep* **2023**; 13:8723.
- Moir S, Ho J, Malaspina A, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J Exp Med* **2008**; 205:1797–805.
- Kyaw T, Tay C, Hosseini H, et al. Depletion of B2 but not B1a B cells in BAFF receptor-deficient ApoE mice attenuates atherosclerosis by potentially ameliorating arterial inflammation. *PLoS One* **2012**; 7:e29371.
- Kyaw T, Tay C, Krishnamurthi S, et al. B1a B lymphocytes are atheroprotective by secreting natural IgM that increases IgM deposits and reduces necrotic cores in atherosclerotic lesions. *Circ Res* **2011**; 109:830–40.
- Longenecker CT, Bogorodskaya M, Margevicius S, et al. Sex modifies the association between HIV and coronary artery disease among older adults in Uganda. *J Int AIDS Soc* **2022**; 25:e25868.
- Alencherry B, Erem G, Mirembe G, et al. Coronary artery calcium, HIV and inflammation in Uganda compared with the USA. *Open Heart* **2019**; 6:e001046.
- Peters M, Margevicius S, Kityo C, et al. Association of kidney disease with abnormal cardiac structure and function among Ugandans with HIV infection. *J Acquir Immune Defic Syndr* **2021**; 86:104–9.
- Goff DC, Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association task force on practice guidelines. *Circulation* **2014**; 129:S49–73.
- Obare LM, Simmons J, Oakes J, et al. CD3+ T-cell: CD14+ monocyte complexes are dynamic and increased with HIV and glucose intolerance. *J Immunol* **2025**; 214:516–31.
- Castilho JL, Shepherd BE, Koethe J, et al. CD4+/CD8+ ratio, age, and risk of serious noncommunicable diseases in HIV-infected adults on antiretroviral therapy. *AIDS* **2016**; 30:899–908.
- Moir S, Malaspina A, Ho J, et al. Normalization of B cell counts and subpopulations after antiretroviral therapy in chronic HIV disease. *J Infect Dis* **2008**; 197: 572–9.
- Binder CJ. Naturally occurring IgM antibodies to oxidation-specific epitopes. *Adv Exp Med Biol* **2012**; 750:2–13.
- Okada T, Ngo VN, Ekland EH, et al. Chemokine requirements for B cell entry to lymph nodes and Peyer's patches. *J Exp Med* **2002**; 196:65–75.
- Yan Y, Chen R, Wang X, et al. CCL19 and CCR7 expression, signaling pathways, and adjuvant functions in viral infection and prevention. *Front Cell Dev Biol* **2019**; 7:212.
- Libby P, Lichtman AH, Hansson GK. Immune effector mechanisms implicated in atherosclerosis: from mice to humans. *Immunity* **2013**; 38:1092–104.
- Temu TM, Polyak SJ, Zifodya JS, et al. Endothelial dysfunction is related to monocyte activation in antiretroviral-treated people with HIV and HIV-negative adults in Kenya. *Open Forum Infect Dis* **2020**; 7:ofaa425.
- Wong ND, Budoff MJ, Ferdinand K, et al. Atherosclerotic cardiovascular disease risk assessment: an American society for preventive cardiology clinical practice statement. *Am J Prev Cardiol* **2022**; 10:100335.
- Vasan RS, van den Heuvel E. Differences in estimates for 10-year risk of cardiovascular disease in black versus white individuals with identical risk factor profiles using pooled cohort equations: an in silico cohort study. *Lancet Digit Health* **2022**; 4:e55–63.
- Triant VA, Perez J, Regan S, et al. Cardiovascular risk prediction functions underestimate risk in HIV infection. *Circulation* **2018**; 137:2203–14.