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Loss of CXCR4 on non-classical monocytes in participants of the Women's Interagency HIV Study (WIHS) with subclinical atherosclerosis

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Aims To test whether human immunodeficiency virus (HIV) infection and subclinical cardiovascular disease (sCVD) are associated with expression of CXCR4 and other surface markers on classical, intermediate, and non-classical monocytes in women.

Methods and results sCVD was defined as presence of atherosclerotic lesions in the carotid artery in 92 participants of the Women's Interagency HIV Study (WIHS). Participants were stratified into four sets ($n = 23$ each) by HIV and sCVD status (HIV-/sCVD-, HIV-/sCVD+, HIV+/sCVD-, and HIV+/sCVD+) matched by age, race/ethnicity, and smoking status. Three subsets of monocytes were determined from archived peripheral blood mononuclear cells. Flow cytometry was used to count and phenotype surface markers. We tested for differences by HIV and sCVD status accounting for multiple comparisons. We found no differences in monocyte subset size among the four groups. Expression of seven surface markers differed significantly across the three monocyte subsets. CXCR4 expression [median fluorescence intensity (MFI)] in non-classical monocytes was highest among HIV-/CVD- [628, interquartile range (IQR) (295–1389)], followed by HIV+/CVD- [486, IQR (248–699)], HIV-/CVD+ (398, IQR (89–901)), and lowest in HIV+/CVD+ women [226, IQR (73–519)), $P = 0.006$ in ANOVA. After accounting for multiple comparison (Tukey) the difference between HIV-/CVD- vs. HIV+/CVD+ remained significant with $P = 0.005$ (HIV-/CVD- vs. HIV+/CVD- $P = 0.04$, HIV-/CVD- vs. HIV-/CVD+ $P = 0.06$, HIV+/CVD+ vs. HIV+/CVD- $P = 0.88$, HIV+/CVD+ vs. HIV-/CVD+ $P = 0.81$, HIV+/CVD- vs. HIV-/CVD+, $P = 0.99$). All pairwise comparisons with HIV-/CVD- were individually significant ($P = 0.050$ vs. HIV-/CVD+, $P = 0.028$ vs. HIV+/CVD-, $P = 0.009$ vs. HIV+/CVD+). CXCR4 expression on non-classical monocytes was significantly higher in CVD- (501.5, IQR (249.5–887.3)) vs. CVD+ (297, IQR (81.75–626.8) individuals ($P = 0.028$, $n = 46$ per group). CXCR4 expression on non-classical monocytes significantly correlated with cardiovascular and HIV-related risk factors including systolic blood pressure, platelet and T cell counts along with duration of antiretroviral therapy ($P < 0.05$). In regression analyses, adjusted for education level, study site, and injection drug use, presence of HIV infection and sCVD remained significantly associated with lower CXCR4 expression on non-classical monocytes ($P = 0.003$), but did not differ in classical or intermediate monocytes.

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Conclusion CXCR4 expression in non-classical monocytes was significantly lower among women with both HIV infection and sCVD, suggesting a potential atheroprotective role of CXCR4 in non-classical monocytes.

Keywords Non-classical monocytes • CXCR4 • Atherosclerosis • HIV • Cardiovascular risk assessment • Women

Introduction

Infection with the human immunodeficiency virus (HIV) is associated with an increased risk of early-onset and rapidly progressive cardiovascular disease (CVD), even among virally suppressed patients on effective antiretroviral therapy (ART).[1–3] Higher incidences of stroke, myocardial infarction, and advanced subclinical cardiovascular disease (sCVD) have been observed in HIV-infected compared to uninfected individuals.[4] The mechanism for increased CVD risk in HIV-infected individuals is likely multifactorial, involving both traditional CVD- and HIV-related risk factors. For example, HIV replication and exposure to certain antiretroviral medications like protease inhibitors may have unfavourable influences on the lipid profile.[5,6] ART has been associated with progression of subclinical atherosclerosis in some large-scale studies.[7] HIV-positive patients are also characterized by a higher frequency of concomitant traditional risk factors like smoking or hyperlipidaemia.[8]

It is especially important in women with HIV to further investigate risk factors for accelerating CVD to prevent adverse cardiovascular outcome,[9,10] as women are underdiagnosed regarding CVD.[11–13] There are significant gender differences in screening and diagnosis of CVD.[11,12] Even though sex-specific symptoms, traditional and novel risk factors along with expanded understanding of the sex-specific pathophysiology of CVD have been acknowledged in recent years, ischaemic heart disease continues to be the leading cause of morbidity and mortality in women in western countries, especially in women with HIV.[11] Traditional CVD risk factors are less frequent in women[11–14] resulting in unfavourable impact on diagnosis, prevention, and treatment strategies. Interestingly, there are also significant disparities in CVD burden among subgroups of women, who are socially disadvantaged because of race, ethnicity, income level, and education.[11–14]

Preventive strategies and early treatment of cardiovascular risk factors, e.g. statins or antiplatelet therapy, have been recommended in high-risk HIV-positive patients. Such treatments may also have pleiotropic effects and reduce circulating levels of pro-inflammatory proteins and cytokines, slowing down inflammatory processes in atherogenesis and inhibiting atheroprogression.[15] However, underlying mechanisms of endothelial cell dysregulation and atherosclerotic plaque formation remain elusive.[15]

Persistent innate immune activation mediated by platelets and monocytes may contribute to atherogenesis and atheroprogression in persons with HIV.[16,17] Monocytes are key players in atherogenesis, from the formation of the earliest asymptomatic atherosclerotic lesions to plaque rupture with potentially fatal outcomes.[18,19] Three distinct monocyte subpopulations have been defined based on their surface receptor expression of CD14 and CD16: classical (CD14++CD16-), intermediate (CD14+CD16+), and non-classical ($CD14^{dim}$CD16++) monocytes.[20,21] Specific monocyte subsets are believed to be differentially involved in the pathogenesis and outcome of acute coronary syndromes, heart failure, and stroke, amongst other conditions.[22–26] Although a subset-specific contribution of monocytes has been proposed in recent years, monocyte heterogeneity has not been analysed thoroughly in the context of HIV-related sCVD.[16,17,27] Experimental studies have suggested a causative role of monocytes in atherogenesis,[25] but several epidemiologic analyses have shown inconsistent associations between circulating monocyte counts, phenotypes and CVD in HIV infection.[7,8,16,28] Baker *et al.* showed in a prospective cohort of 436 patients with and without HIV infection that higher frequencies of CD16+ monocytes were associated with a greater likelihood of progression of coronary artery calcium (CAC) after adjusting for traditional and HIV-related risk factors.[29] Surface marker expression on monocytes was not associated with presence or progression of CAC.[29] Another study of 51 patients with HIV and 49 matched controls revealed that surface expression of the chemokine receptor CX3CR1 and the integrin CD11b can serve as independent predictors of carotid intima-media thickness (cIMT) progression in HIV infection.[16] In contrast, Longenecker *et al.*[30] found no association between cIMT of individuals with HIV and the proportions of monocyte subsets in peripheral blood. However, increased proportions of CD16+ monocytes have been associated with cardiovascular events and the occurrence of acute coronary syndromes in the general population[22,31] and therefore, could be markers of advanced rather than premature sCVD.

Clarifying the immunologic mechanisms and cell types that contribute to premature sCVD among individuals with HIV may help tailor CVD risk assessment in this population. We tested the associations of monocyte characteristics, including subset size, phenotype, and surface marker expression, with HIV serostatus and presence of sCVD in 92 women who participated in the Women's Interagency HIV Study (WIHS).

Methods

For a detailed description of the study design, patient collective, assessment of clinical parameters, flow cytometry and cell sorting, and statistical analysis, please see Supplementary material online.

Representative fluorescence-activated cell-sorting plots showing gating of monocytes to define subsets by CD14 and CD16 expression are illustrated in Supplementary material online, *Figure S1*.

Results

Median age of the 92 WIHS participants was 51.5 years [interquartile range (IQR 47–58)]. The majority (96%) was of either black race or Hispanic ethnicity, and 86% reported a history of smoking. Among the HIV-infected participants, 85% were on highly active antiretroviral therapy (HAART) and the median CD4+ T-cell count was 550.5 cells/μL (IQR 284–792). Clinical characteristics of the participants by matched group (HIV-/sCVD-, HIV-/sCVD+, HIV+/sCVD-, and HIV+/sCVD+) are summarized in *Table 1*. The numbers of classical, non-classical, and intermediate monocytes isolated from PBMCs were not significantly different among the groups (Supplementary material online, *Figure S2*).

Surface marker expression on monocyte subsets in all WIHS participants

The surface markers CXCR4, CCR5, CCR2, CD11b, CD163, CD36, and CX3CR1 were evaluated in classical, intermediate, and non-classical

Table I Demographic and clinical characteristics of study participants

	HIV−/sCVD−, *N* = 23	HIV+/sCVD−, *N* = 23	HIV−/sCVD+, *N* = 23	HIV+/sCVD+, *N* = 23	*P*-value
Demographic and behaviour-related characteristics					
Age at baseline vascular study visit (years) (median, IQR)	45 (40–50)	43 (40–51)	45 (43–52)	48 (43–53)	0.61
Black race or Hispanic ethnicity	22 (96)	22 (96)	22 (96)	22 (96)	1.00
Any history of smoking	20 (87)	19 (83)	20 (87)	20 (87)	1.00
Any current substance use[a]	10(43)	11 (48)	10 (43)	12 (52)	0.68
Hepatitis C virus infection status	6 (26)	12 (52)	9 (39)	13 (57)	0.15
Study site					<0.01
Bronx, NY	14 (61)	19 (83)	7 (30)	11 (48)	
Brooklyn, NY	6 (26)	2 (9)	2 (9)	10 (43)	
Washington, DC	1 (4)	0 (0)	1 (4)	0 (0)	
Los Angeles, CA	1 (4)	0 (0)	5 (22)	1 (4)	
San Francisco, CA	1 (4)	1 (4)	3 (13)	0 (0)	
Chicago, IL	0 (0)	1 (4)	5 (22)	1 (4)	
Education					0.94
Completed high school	15 (65)	13 (57)	14 (61)	13 (57)	
Did not finish high school	8 (35)	9 (39)	9 (39)	10 (43)	
HIV-related risk factors					
CD4+ count, cells/μL (median, IQR)	–	585 (382–816)	–	535 (265–792)	0.72
CD4/CD8 ratio (median, IQR)	–	0.8 (0.3–1.3)	–	0.6 (0.3–1.0)	0.46
Undetectable HIV-1 RNA level		14 (61)	–	13 (57)	0.76
Current ART use					1.00
HAART	–	20 (87)	–	19 (83)	
ART only	–	1 (4)	–	2 (9)	
No ART	–	2 (9)	–	2 (9)	
Cardiometabolic risk factors					
Body mass index (median, IQR)	30.5 (27–38)	29 (26–35)	28 (24–32)	29 (24–34)	0.36
Total cholesterol, mg/dL (median, IQR)	172 (148–183)	170 (138–200)	171.5 (145.5–204)	199.5 (170.5–221)	0.02
LDL cholesterol, mg/dL (median, IQR)	96 (72–112)	93.5 (67–123)	88.5 (65–116.5)	116.5 (94.5–131)	0.04
HDL cholesterol, mg/dL (median, IQR)	55 (44–60)	54 (42–59)	49.5 (43–59.5)	46 (40–55.5)	0.51
History of high cholesterol	16 (69.6)	17 (73.9)	21(91.3)	23 (100)	0.01
Current use of cholesterol medications	0 (0)	0 (0)	5 (22)	10 (43)	<0.0001
History of cholesterol medication use	1 (4)	0 (0)	7 (30)	11 (48)	<0.0001
Systolic blood pressure, mmHg (median, IQR)	125 (111–138)	122 (109–134)	128 (118–151)	127 (109–139)	0.38
History of hypertension	12 (52)	10 (43)	17 (74)	17 (74)	0.07
Current hypertensive medication use	10 (43)	11 (48)	14 (61)	15 (65)	0.39
History of diabetes	6 (26)	4 (17)	8 (35)	4 (17)	0.50
Creatinine, mg/dL (median, IQR)	0.8 (0.7–0.9)	0.9 (0. 8–1.0)	0.9 (0.7–1.0)	0.9 (0.7–1.2)	0.81
Current aspirin use	4 (17)	4 (17)	6 (26)	10 (43)	0.18
Self-reported menopause	10 (43)	13 (57)	9 (39)	15 (65)	0.22
Inflammatory biomarker levels					
sCD163, ng/mL (median, IQR)	630 (464–1253)	987 (700–1305)	772 (465–1118)	961 (700–1429)	0.06
sCD14, ng/mL (median, IQR)	1682 (1401–1943)	2123 (1842–2275)	1696 (1584–2219)	2027 (1779–2670)	<0.01
IL-6, pg/mL (median, IQR)	1.9 (0.8–2.5)	1.5 (1.0–2.8)	1.7 (1.1–2.4)	1.3 (1.1–2.7)	0.95
Galectin-3, ng/mL (median, IQR)	9.4 (8.1–11.1)	8.7 (5.9–12.8)	8.6 (7.2–10.4)	9.9 (7.7–12.8)	0.52
Galectin-3 binding protein, ng/mL (median, IQR)	9.2 (4.9–17.6)	15.4 (5.4–29.7)	10.5 (4.0–13.1)	14.4 (7.7–17.7)	0.15
hsCRP (median, IQR)	1.9 (0.7–4.8)	2.0 (0.9–7.7)	1.8 (0.9–4.3)	2.5 (1.3–7.4)	0.74

Characteristics of human immunodeficiency virus (HIV)-infected and HIV-uninfected women in the Women's Interagency HIV Study stratified by presence of subclinical cardiovascular disease.

Values are *n* (%) or median and interquartile range (IQR). Each group contains 23 participants, who were matched by age, race/ethnicity, smoking status, and age of specimen.

ART, antiretroviral therapy; HAART, highly active antiretroviral therapy; HDL, high-density lipoprotein; hsCRP, high sensitive C-reactive protein.

[a]Substance use includes intravenous drug, crack, and cocaine use.

monocytes (Supplementary material online, *Figure S3*). Based on ANOVA, there were differences in the expression of many of the analysed surface markers among the three subsets of monocytes (Supplementary material online, *Figure S3*). The differences in the expression of the chemokine receptors CX3CR1, CCR5 (CD195), CCR2 (CD192) and the scavenger receptor CD36 were expected based on published work.[27,32] However, the differences in CD163 (haemoglobin–haptoglobin receptor), CXCR4 and the integrin CD11b are new findings.

Surface marker expression on monocyte subsets stratified by HIV and sCVD status

We further assessed the expression of surface markers of interest by HIV and sCVD status within each of the three monocyte subsets. After accounting for multiple comparisons, only CXCR4 expression in non-classical monocytes remained significantly different across the four participant groups. *Figure 1A–C* shows the association of HIV and sCVD status with CXCR4 expression within classical (1A), intermediate (1B), and non-classical (1C) monocytes. The expression of CXCR4 on non-classical monocytes [given as median fluorescence intensity (MFI)] was highest among HIV-/sCVD- women [median 628, IQR (295–1389)], followed by HIV+/sCVD- [median 486, IQR (248–699)], HIV-/sCVD+ [median 398, IQR (89–901)], and lowest in HIV+/sCVD+ women [median 226, IQR (73–519)], ($P = 0.006$ for overall comparison in ANOVA analysis). After accounting for multiple comparison (Tukey) the comparison between HIV-/sCVD- vs. HIV+/sCVD+ remained significant with $P = 0.005$ (HIV-/sCVD- vs. HIV+/sCVD- $P = 0.04$, HIV-/sCVD- vs. HIV-/sCVD+ $P = 0.06$, HIV+/sCVD+ vs. HIV+/sCVD- $P = 0.88$, HIV+/sCVD+ vs. HIV-/sCVD+ $P = 0.81$, HIV+/sCVD- vs. HIV-/sCVD+, $P = 0.99$).

In pairwise comparisons, shown in *Figure 2A–F* as dot plots, CXCR4 expression on non-classical monocytes was significantly higher in HIV-/sCVD- women vs. (*Figure 2A*) HIV-/sCVD+ ($P = 0.050$), (*Figure 2B*) HIV+/sCVD- ($P = 0.028$), and (*Figure 2C*) HIV+/sCVD+ ($P = 0.009$). There were no differences in the comparison of (*Figure 2D*) HIV-/sCVD+ vs. HIV+/sCVD- ($P = 0.827$), (*Figure 2E*) HIV-/sCVD+ vs. HIV+/sCVD+ ($P = 0.266$), and (*Figure 2F*) HIV+/sCVD- vs. HIV+/sCVD+ ($P = 0.265$).

Interestingly, CXCR4 expression on non-classical monocytes was significantly higher in sCVD- [median 501.5, IQR (249.5–887.3)] compared to sCVD+ women [median 297, IQR (81.75–626.8), $n = 46$ per group, $P = 0.028$] illustrated in *Figure 3*.

CXCR4 expression on non-classical monocytes significantly correlated with several cardiovascular and HIV-related risk factors

Spearman's rank correlation analysis to evaluate correlations of CXCR4 expression on non-classical monocytes with cardiovascular and HIV-related risk factors among the whole cohort, given in *Figure 4A–P*, showed that CXCR4 expression on non-classical monocytes is

i. negatively correlated with systolic blood pressure ($r = -0.212$, $P = 0.042$) and platelet count ($r = -0.277$, $P = 0.022$), while positively correlated with left ventricular mass ($r = 0.268$, $P = 0.038$), all known CVD risk factors (*Figure 4A–C*),
ii. positively correlated with the duration of ART ($r = 0.338$, $P = 0.022$) and HAART ($r = 0.307$, $P = 0.038$ in HIV+ subjects, *Figure 4D,E*),
iii. positively correlated with CD4 T cell counts ($r = 0.270$, $P = 0.024$) and the CD4 to CD8 T cell ratio ($r = 0.299$, $P = 0.044$, *Figure 4F,G*),
iv. negatively correlated with the CD8 T cell count ($r = -0.295$, $P = 0.013$) and with apoptotic T cells (as detected by activated caspase-3, CD4 Casp3 $r = -0.547$, $P = 0.001$, CD8 Casp3 $r = -0.326$, $P = 0.006$, *Figure 4H–J*),

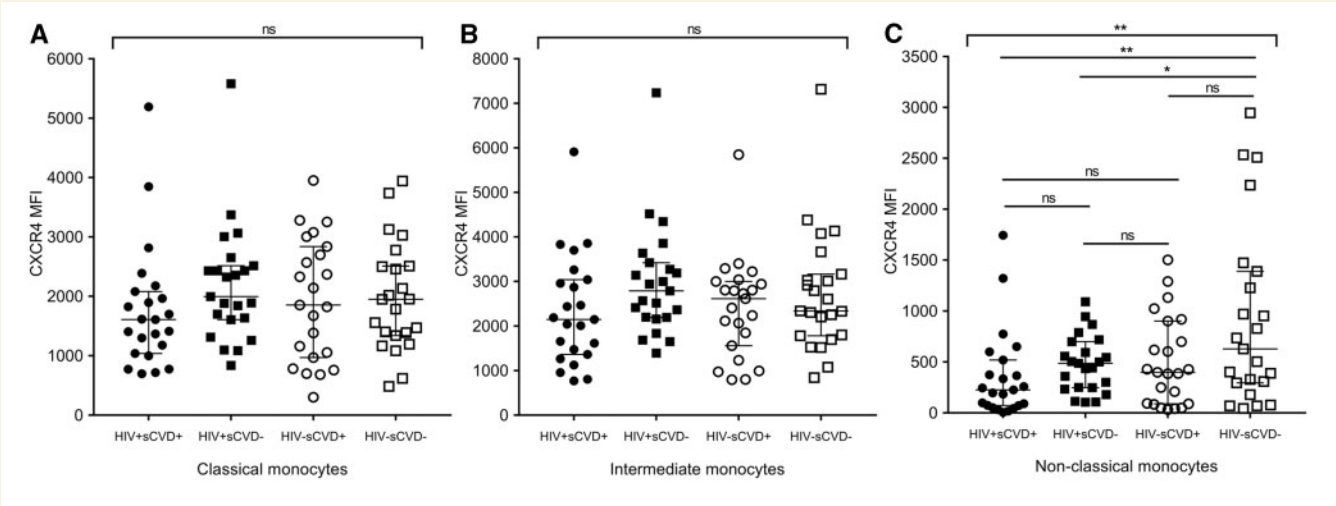

Figure 1 CXCR4 is differentially expressed on monocyte subsets in WIHS participants stratified by HIV and sCVD status. CXCR4 was evaluated by flow cytometry on classical (*A*), intermediate (*B*), and non-classical (*C*) monocytes isolated from frozen PBMCs from WIHS participants stratified by HIV and sCVD status ($n = 23$ per group). The graphs depict the differential distribution of CXCR4 surface marker expression measured by median fluorescence intensity (MFI) in the different subsets of monocytes and among the different groups of participants stratified by HIV and sCVD status as dot plots [median and interquartile range (IQR) are shown]. ANOVA analysis was performed. For this analysis, a *P*-value ≤0.050 was considered significant, indicated by *. For this analysis, a *P*-value ≤0.007 was considered significant after adjustment for multiple testing, indicated by ** (Tukey).

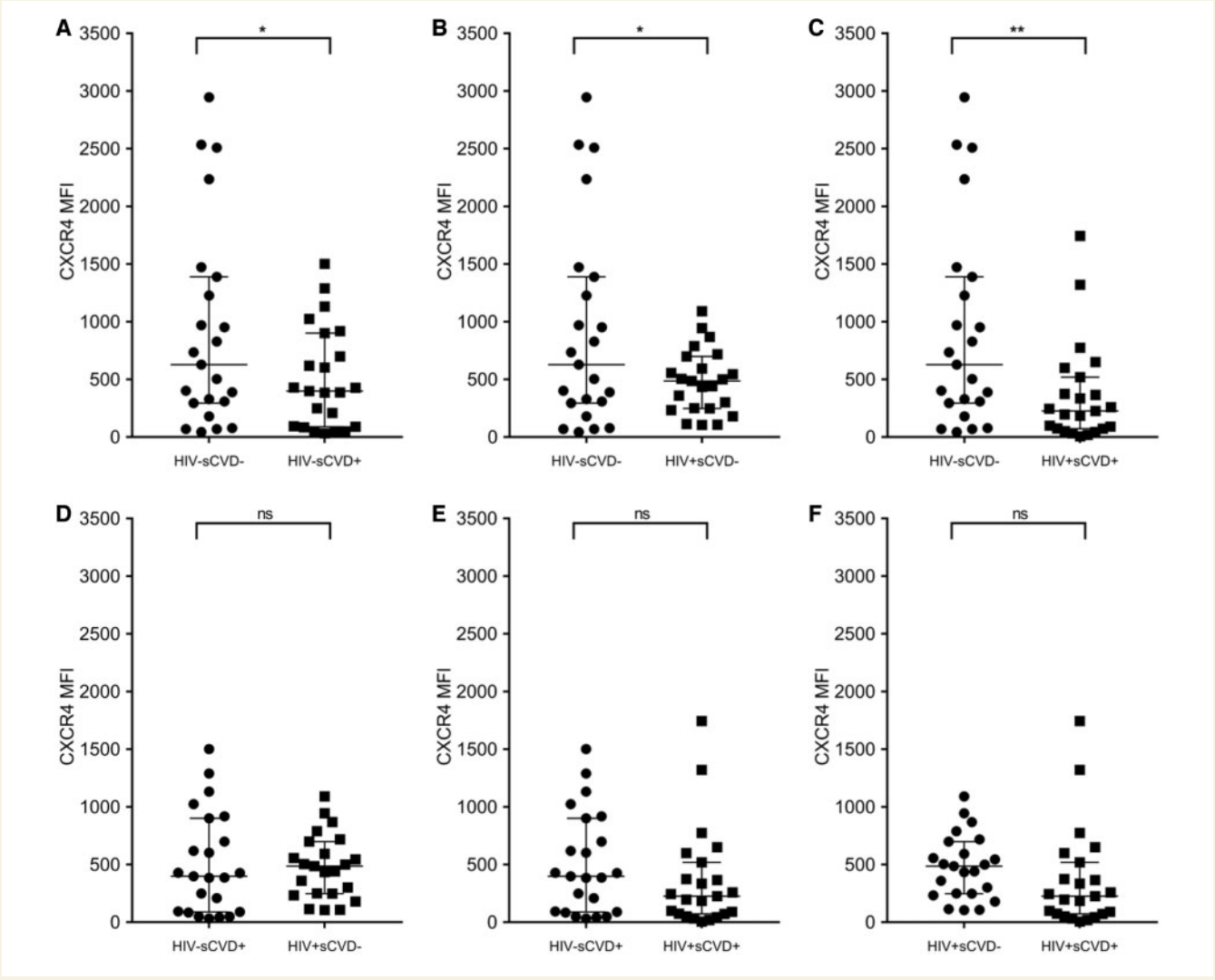

Figure 2 Pairwise comparison of CXCR4 expression on non-classical monocytes from WIHS participants stratified by their HIV and sCVD status. CXCR4 was evaluated on non-classical monocytes as in *Figure 1* ($n = 92$; 23 per group) and expressed as MFI. T-test was performed for paired group comparison. The graphs depict the different distribution of CXCR4 expression on non-classical monocytes between the groups as dot plots [median and interquartile range (IQR) are shown]. For this analysis, a *P*-value ≤0.050 was considered significant, indicated by *, *P*-value ≤0.010 indicated by **

v. not correlated with other cardiovascular risk parameters including (*Figure 4K*) high-sensitive C-reactive protein (hsCRP) ($r = -0.096$, $P = 0.361$), (*Figure 4L*) Framingham risk score ($r = -0.152$, $P = 0.148$), (*Figure 4M*) total cholesterol ($r = -0.105$, $P = 0.364$), (*Figure 4N*) LDL ($r = -0.171$, $P = 0.147$), (*Figure 4O*) HDL ($r = -0.009$, $P = 0.941$), and (*Figure 4P*) triglycerides ($r = -0.088$, $P = 0.447$).

Furthermore, we performed subgroup analysis of CXCR4 expression on non-classical monocytes and its correlation with traditional cardiovascular and HIV-related clinical risk factors of the four participant groups stratified by HIV and sCVD status. Data are shown in Supplementary material online, *Figures S4, S5, S6, and S7* for each group, respectively. Interestingly, we found a negative correlation of CXCR4 expression on non-classical monocytes with the Framingham risk score in HIV-/sCVD- participants of the WIHS (Supplementary material online, *Figure 4I*). CXCR4 expression correlated positively with the duration of ART ($r = 0.452$, $P = 0.030$) in HIV+/sCVD- participants (Supplementary material online, *Figure S6D*).

CXCR4 expression on non-classical monocytes was significantly associated with the presence of HIV infection and subclinical cardiovascular disease in women

In unadjusted regression analysis, lower CXCR4 expression on non-classical monocytes was significantly associated with the presence of sCVD (*Table 2*). Here, β is defined as mean difference in MFI of CXCR4 expression in each group compared with the reference group of HIV-/sCVD- participants. After accounting for education, study site, and history of IDU, lower CXCR4 expression on non-classical monocytes remained significantly associated with the presence of sCVD: HIV+/sCVD+ vs. HIV-/sCVD- [β, -472 U, 95% confidence interval (CI) (-782 to -161), $P = 0.003$]; HIV-/sCVD+ vs. HIV-/sCVD- [β, -308 U, 95% CI

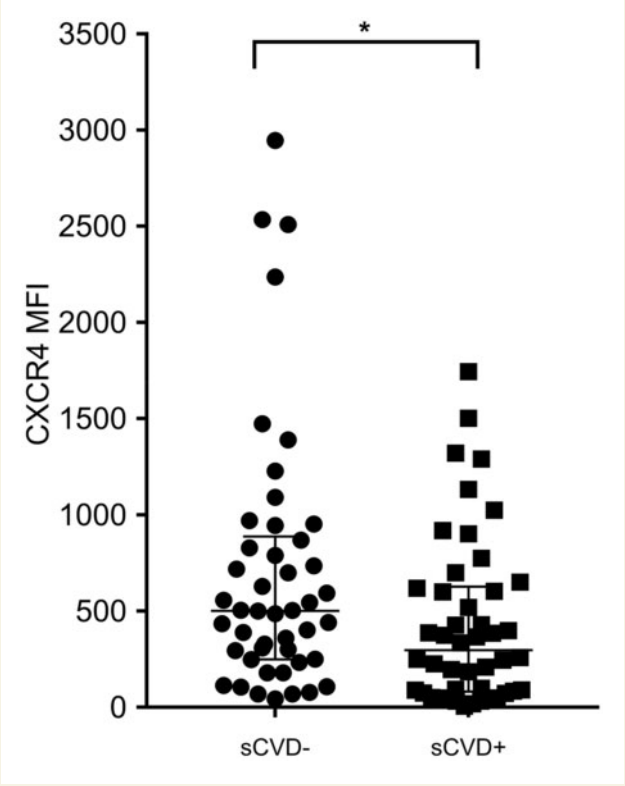

Figure 3 CXCR4 expression on non-classical monocytes is significantly different between sCVD+ and sCVD- WIHS participants regardless of HIV staus. CXCR4 was evaluated on non-classical monocytes among WIHS individuals stratified by sCVD status ($n = 46$ per group). The graphs depict the different distribution of CXCR4 expression on non-classical monocytes between the groups as dot plots [median and interquartile range (IQR) are shown]. T-test was performed for two groups' comparison. For this analysis, a *P*-value ≤0.050 was considered significant (*).

(-645 to 30), $P = 0.074$]; HIV+/sCVD- vs. HIV-/sCVD- [β, -552 U, 95% CI (-863 to -242), $P = 0.001$] (overall $P = 0.003$).

Discussion

In our study, we found differential surface marker expression on three subsets of monocytes in our sample of HIV-infected and uninfected women. Specifically, we showed that both HIV infection status and subclinical cardiovascular disease were associated with reduced CXCR4 expression on non-classical monocytes. Furthermore, Spearman's rank correlation analysis revealed correlations of CXCR4 expression on non-classical monocytes with traditional cardiovascular and HIV-related risk factors.

Non-classical monocytes play key roles in vascular homeostasis. In particular, non-classical monocytes show patrolling behaviour and actively patrol the vascular endothelium of arteries.[33,34] Although patrolling is seen under homeostatic conditions, it is influenced by triggers of the inflammatory response.[35,36] Human non-classical monocytes have been shown to patrol in mouse microvessels[35] and carotid arteries.[33,34] In mice, non-classical monocytes are atheroprotective,[36,37] but it is not known whether this translates to humans. Patrolling non-classical monocytes play an important role in several disease settings including atherosclerosis. They probably function to remove damaged cells and debris from the vascular endothelium.[33] These cells have also been associated with wound healing and the resolution of inflammation in damaged tissues.[35,36]

In our study, we described the association of reduced chemokine receptor CXCR4 expression on non-classical monocytes with presence of sCVD. The CXCL12/CXCR4 chemokine ligand/receptor axis plays a key role in cell trafficking during atherogenesis and atheroprogression. Several animal studies have suggested an atheroprotective role for CXCL12/CXCR4 interactions.[38–41] CXCR4 is the chemokine receptor for CXCL12 and macrophage migration inhibitory factor (MIF), a chemokine-like molecule with a known pro-atherogenic role.[39] CXCR4 is deeply involved in circadian changes in blood monocyte levels[42] and thought to be required for disposal of aged leucocytes.[43]

CXCR4 is also a co-receptor for HIV entry in the infection by T-tropic and M-tropic HIV-1 strains.[44,45] CXCR4 expression has been associated with susceptibility of monocytes to HIV infection and atherosclerosis.[46,47] Therefore, CXCR4 is a key target in the investigation of chemokine-dependent inflammatory response in HIV infection and sCVD.[47] CXCR4 in non-classical monocytes had not been previously investigated in HIV-infected individuals.

Similar to the findings of Longenecker *et al.*,[30] we did not find an association between HIV and the proportions of monocyte subsets in peripheral blood with subclinical atherosclerosis of carotid arteries. In contrast, Baker *et al.* showed that a higher percentage of CD16+ monocytes were associated with a greater likelihood of progression of CAC.[29] Increased proportions of CD16+ monocytes have been associated with cardiovascular events and the occurrence of acute coronary syndromes in non-HIV studies.[22,31,48] While expression of certain surface markers on monocytes was not associated with presence or progression of CAC in the Baker study, CXCR4 was not investigated.[36] Another study revealed that surface expression of CX3CR1 on CD16+ and CD11b on total monocytes can serve as independent predictors of cIMT progression in HIV infection,[16] which we did not find in our cohort. A recent study of treated individuals with HIV showed that cIMT correlated with the count of non-classical monocytes at baseline and with plasma levels of MCP-1 and TNF-α.[49]

$CD16^+$ monocyte (comprising non-classical and intermediate monocyte) numbers have also been positively correlated with vulnerable plaques in patients with coronary artery disease, and levels of $CD16^+$ monocytes have been found to be significantly decreased in patients receiving statin treatment.[26,31] These studies did not distinguish between $CD14^{dim}CD16^+$ (non-classical) and $CD14^+CD16^+$ (intermediate) subsets but grouped these two subsets into CD16-positive monocytes. A recent study of >900 patients suggested that it is mainly the $CD14^+CD16^+$ intermediate monocytes that are positive predictors for cardiovascular events, whereas the $CD14^{dim}CD16^+$ non-classical monocyte subset showed no correlation.[50]

Among women with HIV, reduced CXCR4 expression on non-classical monocytes was associated with presence of subclinical carotid artery disease even after adjustment for confounders. Monocyte migration requires signalling via chemokine receptors like CCR2, CX3CR1, and CXCR4 (in response to ligands CCL2, CX3CL1, CXCL12, and MIF, respectively).[16,20,38,46] These chemokine and adhesion receptors are differentially expressed on different subsets of monocytes.[40,51] Furthermore, chemokines and their receptors play a critical role in HIV infection, acquired immunodeficiency syndrome (AIDS), and atherosclerosis.[47] Non-classical monocytes have lower expression of CXCR4 than

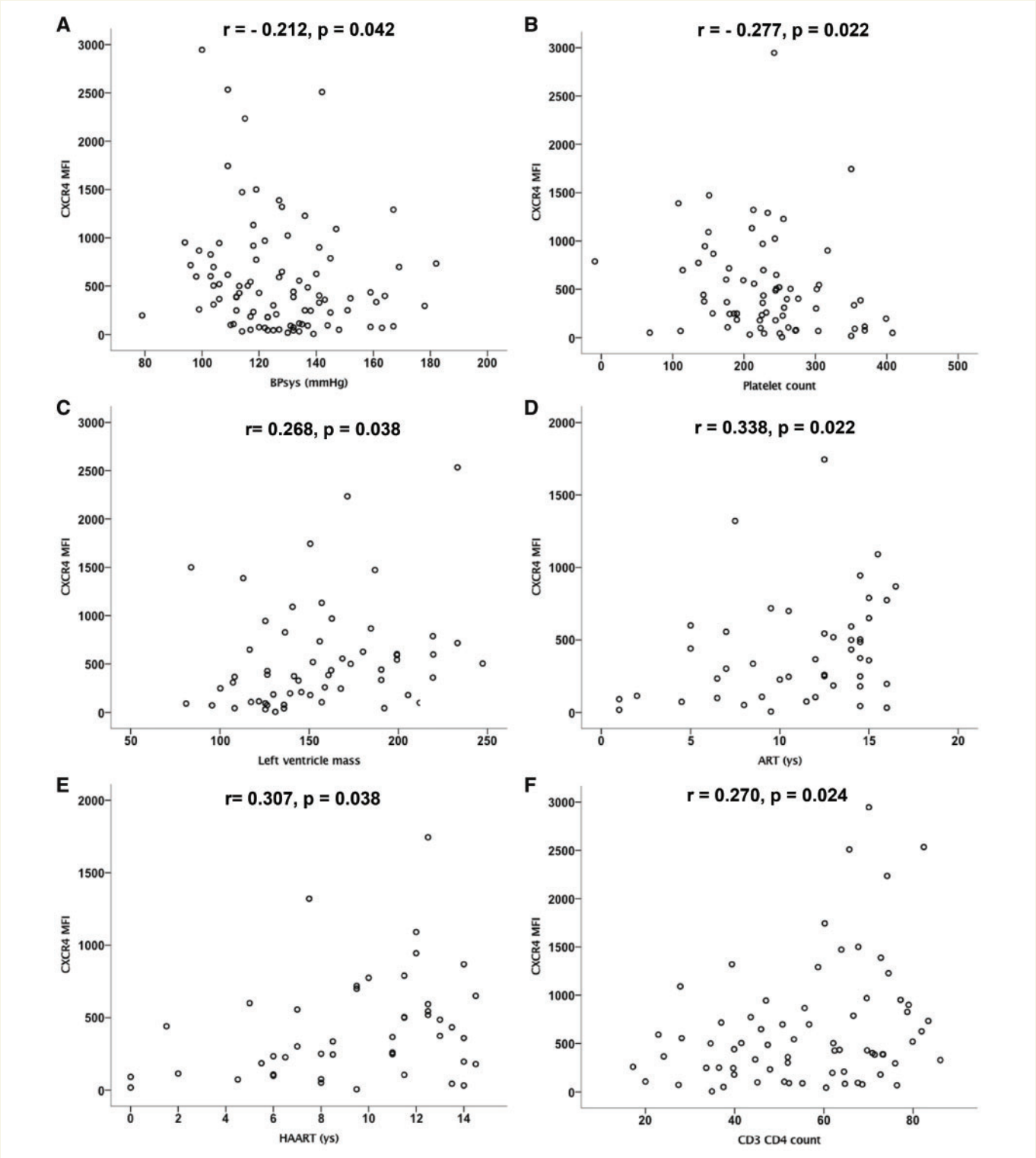

Figure 4 CXCR4 expression on non-classical monocytes correlates with traditional cardiovascular and HIV-related clinical risk factors. Spearman's rank correlation analysis was performed to evaluate correlations of CXCR4 expression on non-classical monocytes with cardiovascular and HIV-related risk factors among the whole cohort. Values are presented as Spearman's rank correlation coefficient *r*. CXCR4 expression on non-classical monocytes is (*A*) negatively correlated with systolic blood pressure (BPsys, $r = -0.212$, $P = 0.042$) and (*B*) blood platelet count ($r = -0.277$, $P = 0.022$) and (*C*) positively correlated with left ventricular mass ($r = 0.268$, $P = 0.038$), (*D*) the duration of ART ($r = 0.338$, $P = 0.022$) and (*E*) HAART ($r = 0.307$, $P = 0.038$ in HIV+ subjects) as well as with (*F*) blood CD4 T cell counts (CD3CD4 $r = 0.270$, $P = 0.024$) and (*G*) the CD4 to CD8 T cell ratio ($r = 0.299$, $P = 0.044$). CXCR4 on non-classical monocytes was negatively correlated with (*H*) the blood CD8 T cell count (CD3CD8, $r = -0.295$, $P = 0.013$) and with (*I, J*) apoptotic T cells [as detected by activated caspase-3, (*I*) CD4 Casp3 $r = -0.547$, $P = 0.001$, (*J*) CD8 Casp3 $r = -0.326$, $P = 0.006$]. There was no significant correlation of CXCR4 expression on non-classical monocytes with other cardiovascular risk factors as (*K*) hsCRP ($r = -0.096$, $P = 0.361$), (*L*) Framingham risk score ($r = -0.152$, $P = 0.148$), (*M*) total cholesterol ($r = -0.105$, $P = 0.364$), (*N*) LDL ($r = -0.171$, $P = 0.147$), (*O*) HDL ($r = -0.009$, $P = 0.941$),

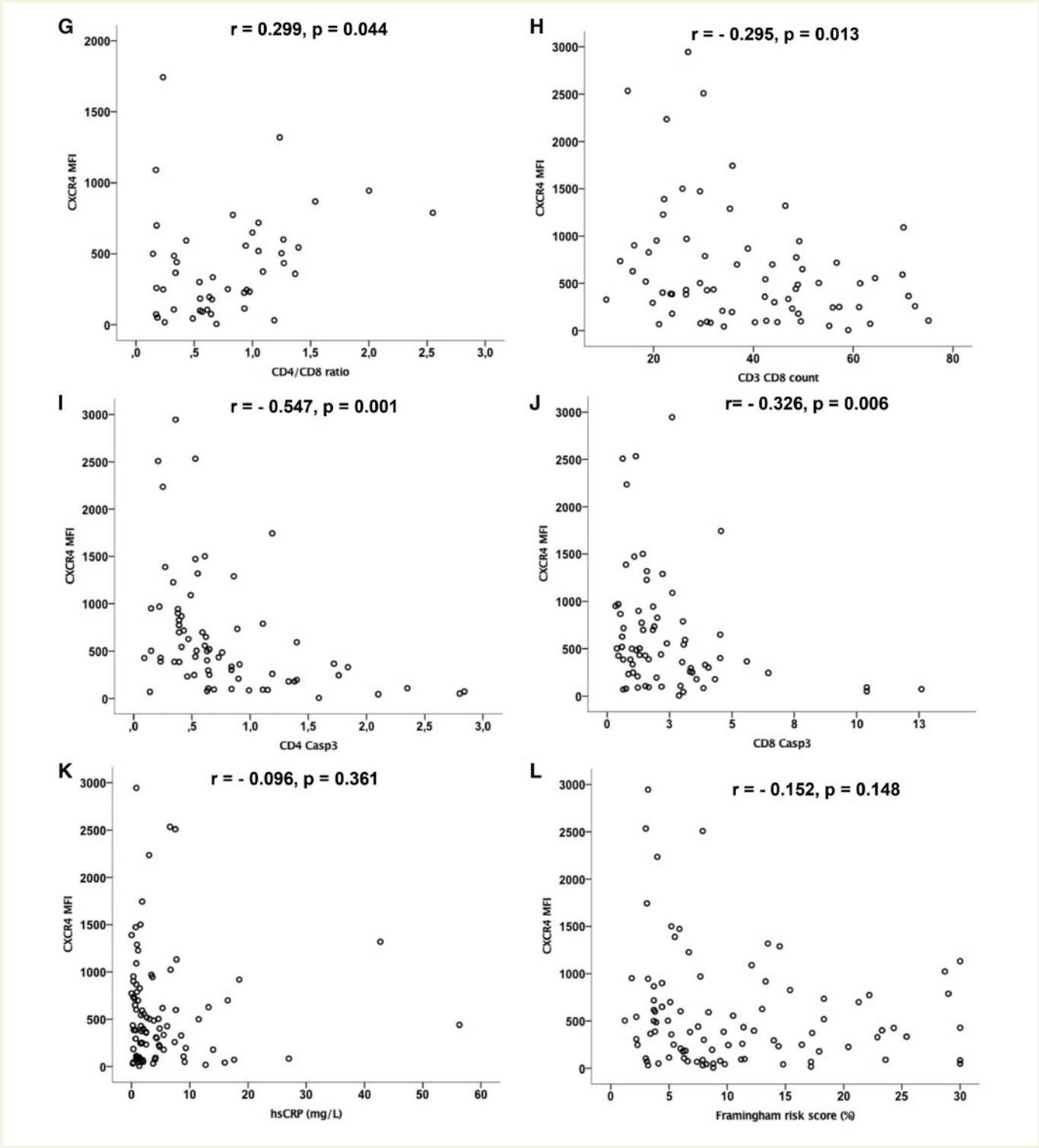

Figure 4 Continued.

classical monocytes. That CXCR4 expression on non-classical monocytes from HIV-infected individuals with CVD is even lower may therefore reflect an unfavourable monocyte phenotype or may impair their function. As regression analysis indicated that reduced expression of CXCR4 is associated with sCVD in our cohort and is an independent phenomenon. It would be of interest to extend these findings with a prospective longitudinal study determining whether the identified marker CXCR4 is predictive of clinical CVD. These results may contribute to future diagnostic and therapeutic approaches for the diagnosis and treatment of sCVD in HIV-positive individuals. Understanding the underlying pathogenesis of CVD in HIV patients, including the role of changes in monocyte phenotype, underpins the development of

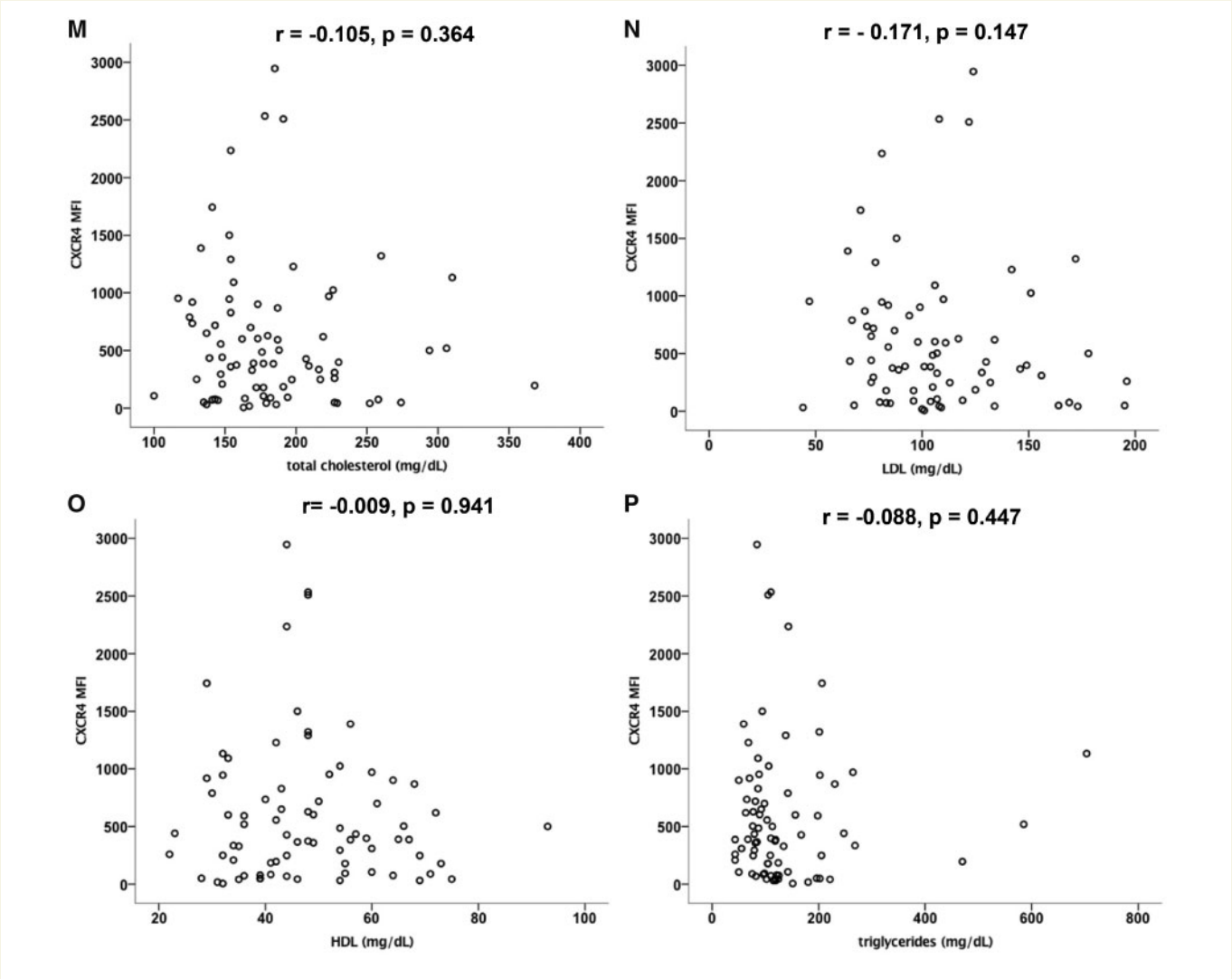

Figure 4 Continued.

predictive models that will be useful for disease management in chronic HIV infection.

Women, in particular, are underdiagnosed regarding cardiovascular diseases. There are significant gender differences in screening and diagnosis of CVD as it has been defined as a men's disease for decades.[11–14] Even though sex-specific symptoms, traditional and novel risk factors and expanded understanding of the sex-specific pathophysiology of CVD have been acknowledged in recent years, ischaemic heart disease continues to be the leading cause of morbidity and mortality in women in western countries, especially in women with HIV.[58–64] Therefore, novel biomarkers to detect high-risk patients at an early stage of the disease are of great clinical interest for an improved risk assessment, especially in women, who less frequently show traditional risk factors for advancing CVD.

Currently prediction of subclinical CVD such as early atherosclerosis in patients with HIV is limited to evaluation of risk factor profiles, which might underestimate the risk of the occurrence of adverse CV events in patients with HIV.[52]

Interestingly, there are also significant disparities in CVD burden among certain subgroups of women, who are socially disadvantaged, which relates to differences in risk factor prevalence, treatment strategies according to evidence-based guidelines, and other social and environmental factors.[58–66] As gender disparities are multifactorial, they reflect under-representation of women at risk of CVD in research, with the resultant unfavourable impact on women's cardiovascular outcome. For example, women with acute coronary syndrome undergo coronary angiography and revascularization less frequently than men.[58–66] Women with CVD are usually older than men when CVD is diagnosed and present more often with unspecific symptoms like dyspnoea, nausea, or vomiting than typical angina pectoris. Traditional risk factors (smoking, diabetes mellitus, and dyslipidaemia) are less frequent in women.[58–66] Furthermore, common disorders of pregnancy (gestational hypertension and diabetes) or endocrine disorders (polycystic ovary syndrome and early menopause) are associated with accelerated development of CVD. To further investigate potential, maybe even gender-specific risk factors for adverse cardiovascular outcome is especially important in women with HIV, who are at high risk to develop CVD.[9,53,54]

This study was based on the WIHS cohort. A similar cohort for HIV-infected men is the Multicenter AIDS Cohort Study (MACS). Our study generates the testable hypothesis that CXCR4 expression on non-

Table 2. Association between HIV/sCVD status and CXCR4 expression in non-classical monocytes in regression analysis

	Model 1: unadjusted		Model 2: adjusted for education, study site, history of injection drug use	
	Difference in mean MFI (95% CI)	*P*-value	Difference in mean MFI (95% CI)	*P*-value
HIV-/sCVD-	Ref.	–	Ref.	–
HIV+/sCVD-	-436 (-761 to -111)	**0.009**	-552 (-863 to -242)	**0.001**
HIV-/sCVD+	-412 (-737 to -87)	**0.014**	-308 (-645 to 30)	0.074
HIV+/sCVD+	-557 (-882 to -232)	**0.001**	-472 (-782 to -161)	**0.003**

The study was designed as a case-control observational study. Four groups of participants of the WIHS cohort were stratified by their HIV and sCVD status (HIV-sCVD- vs. HIV-sCVD+ vs. HIV+sCVD- vs. HIV+sCVD+) and were matched based on participant age, smoking status, race/ethnicity, and age of the specimen collection date of PBMC samples.
Regression analysis of these matched samples adjusted for education, study site, and history of injection drug use. Presence of HIV infection and sCVD remained significantly associated with lower CXCR4 expression on non-classical monocytes.
Test for overall difference by HIV/sCVD status: Model 1, $P = 0.006$; Model 2: $P = 0.003$.
Groups in each model were matched by age, race/ethnicity, smoking status, and specimen collection date. Test for overall difference by HIV/sCVD status: Model 1, $P = 0.006$; Model 2: $P = 0.003$.
MFI, median fluorescence intensity; sCVD, subclinical cardiovascular disease.

classical monocytes may be negatively correlated with CVD also in men which requires further investigations.[55]

It has been described that CXCR4 expression in $CD4^+$ and $CD8^+$ T cells as well as $CD14^+$ monocytes was significantly reduced in HIV-positive individuals when compared with uninfected controls.[46] Down-modulation of CXCR4 has been correlated with HIV disease progression, which further supports the reciprocal role that CXCR4 plays in cellular activation.[45,46] This down-regulation of the chemokine receptor expression is associated with elevated levels of the endogenously produced CXCR4 ligand chemokine CXCL12. CXCL12 governs differentiation of haematopoietic progenitors into either endothelial or macrophage-foam cells. CXCL12 ligates CXCR4 and CXCR7 and regulates monocyte/macrophage functions,[48–51] in particular cell migration, adhesion, and survival.[47,51]

Our data suggest that loss of CXCR4 expression on non-classical monocytes may increase the risk of atherogenesis. A potential mechanism for this protective role of CXCR4 might be the maintenance of arterial integrity and preservation of endothelial barrier function, a known function of non-classical monocytes.[33,36,56,57] It is possible that enhancing these potentially beneficial functions of CXCR4 by selective modulators could open novel therapeutic options, but more research is needed to corroborate this.

Given the cross-sectional nature and limited sample size of our study, one limitation of our findings is that they are hypothesis-generating, and therefore, the results should be replicated both longitudinally and in other study populations, including in men. Furthermore, our explanations for underlying pathomechanisms are speculative and will require further evidence. Finally, other unmeasured confounding parameters may be present; nonetheless, our careful matching of specimens and use of regression account for major known confounders including age, race, smoking status, and socioeconomic status.

Our findings show that subclinical atherosclerosis in women with HIV-related sCVD is associated with lower expression of CXCR4 on non-classical monocytes, and that surface markers are differentially expressed among monocyte subsets in women with and without HIV and with and without sCVD. Our study suggests that monocyte surface markers, including those on non-classical monocytes, may serve as novel biomarkers and predictors of sCVD in treated individuals with HIV. These findings highlight the important role for monocyte subsets in the progression of HIV-related cardiovascular pathology and need to be investigated in further large-scale studies.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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