Macrophage inflammatory markers are associated with subclinical carotid artery disease in women with HIV or HCV infection

Iftach Shaked, PhD[1,*], **David B Hanna, PhD**[2,*], **Christian Gleißner, MD**[9], **Brenda Marsh, MD**[10], **Jill Plants**[3], **Daniel Tracy, MPH**[2], **Kathryn Anastos, MD**[2], **Mardge Cohen, MD**[3], **Elizabeth T Golub, PhD**[4], **Roksana Karim, PhD, MD**[7], **Jason Lazar, MD**[6], **Vinayaka Prasad, PhD**[2], **Phyllis C. Tien, MD**[8], **Mary A Young, MD**[5], **Alan L Landay, PhD**[3], **Robert C Kaplan, PhD**[2], and **Klaus Ley, MD**[1]

[1]La Jolla Institute for Allergy and Immunology

[2]Albert Einstein College of Medicine

[3]Rush University

[4]Johns Hopkins Bloomberg School of Public Health

[5]Georgetown University Medical Center Division of Infectious Disease

[6]SUNY Downstate Medical Center Department of Cardiovascular Disease

[7]University of Southern California

[8]University of California, San Francisco

[9]University of Heidelberg, Germany

[10]University of California, San Diego

Abstract

Objective—Infection with hepatitis C (HCV) or human immunodeficiency virus (HIV) may be associated with atherosclerosis and vascular disease. Macrophages are a major component of atherosclerotic plaque, and classically activated (M1) macrophages contribute to plaque instability. Our goal was to identify plasma biomarkers that reflect macrophage inflammation and are associated with subclinical atherosclerosis.

Approach and results—We tested whether M1 macrophages produce galectin-3 binding protein (Gal-3BP) in-vitro. Then we measured Gal-3BP and the soluble macrophage biomarkers sCD163 and sCD14 in 264 participants in the Women's Interagency HIV Study. Women were positive for HIV, HCV, both, or neither (66 in each group, matched for age, race/ethnicity and

Correspondence to: Klaus Ley, MD, La Jolla Institute for Allergy & Immunology 9420 Athena Circle Drive La Jolla, CA 92037, Fax: (858) 752-6985 Phone: (858) 752-6661 klaus@liai.org.
[*]co-first author

smoking status). Carotid artery disease was assessed by ultrasound measurement of right distal common carotid artery intima-media thickness (cIMT), distensibility, and presence of atherosclerotic lesions (IMT>1.5 mm). Plasma Gal-3BP was higher in HCV+ than HCV− women (p<0.01), but did not differ by HIV status. The three inflammatory macrophage markers were significantly correlated with each other and negatively correlated with CD4+ counts in HIV-infected women. We defined a macrophage score as 1, 2 or 3 biomarkers elevated above the median. In models adjusted for traditional risk factors, higher macrophage scores were significantly associated with increased atherosclerotic lesions and lower carotid distensibility. Receiver-operator curve analysis of lesions revealed that the markers added predictive value beyond traditional risk factors and C-reactive protein.

Conclusions—The macrophage inflammatory markers Gal-3BP, sCD163 and sCD14 are significantly associated with carotid artery disease in the setting of HIV and HCV infection.

Key word list

atherosclerosis; women; AIDS; immune system; risk factors

INTRODUCTION

Many risk factors, including dyslipidemia, older age, high blood pressure, diabetes and smoking are established predictors of cardiovascular disease (CVD) [1]. The chronic inflammatory process in atherosclerosis is increasingly recognized as a contributing factor [2]. High sensitivity C-reactive protein (hsCRP) has shown some utility in atherosclerosis risk assessment[3] as a global marker of inflammation, but other markers may reflect more specific pathways.

Atherosclerosis is characterized by infiltration of monocytes into the wall of large and medium-sized arteries, where they form atherosclerotic plaque. These monocytes differentiate to macrophages and foam cells, some of which undergo apoptosis and secondary necrosis, forming a necrotic core that makes plaque vulnerable to rupture and clinical events like myocardial infarctions and stroke. Plaque macrophages show several different phenotypes that can be distinguished by surface markers using flow cytometry and immunofluorescence [4]. Atherosclerotic lesions contain macrophages with phenotypic characteristics of M1 and M2 polarization. In general, M1 macrophages are thought to be associated with inflamed, vulnerable plaques and M2 macrophages with a thick fibrous cap and less with risk of rupture [5].

In a study of aortas of $Ldlr^{−/−}$ mice, Leitinger et al. found that 40% of CD68+ macrophages showed an M1 phenotype and 25% an M2 phenotype, where M1 was identified by expression of CD86 and M2 by expression of mannose receptor (CD206) [6]. In human atherosclerotic lesions, macrophages with both M1 (expressing iNOS, HLA, CD86 and MARCO) and M2 (expressing mannose receptor, CD163 and dectin-1) characteristics have been found, often side-by-side in the same histological section [5]. M1 macrophages dominated in the rupture-prone shoulder regions of the plaque, M1 and M2 macrophages were equally represented in the fibrous caps, and M2 macrophages dominated in adventitial tissue. Mice lacking the nuclear receptor Nr4a1 showed enhanced atherosclerotic lesions in

spite of decreased numbers of circulating monocytes [7]. This was associated with increased expression of the M1 marker IL-12p70 and decreased expression of the M2 marker arginase-1 in peritoneal macrophages and increased mRNA expression for TNF, CD36 and SRA-1 in F4/80$^+$ macrophages sorted from aortae of *Apoe$^{-/-}$Nr4a1$^{-/-}$* mice [7]. Tissue macrophage polarization is a multifactorial cascade which may be driven by infective agents or endogenously formed TLR2 and TLR4 ligands, NOD like receptor activation of the NLRP3 inflammasome, and T helper cytokines [8,9]. In addition, the hematopoietic growth factor CSF2 (GM-CSF) promotes M1-like macrophages and CSF1 (M-CSF) promotes M2-like macrophages in atherosclerosis [10].

Specific serum or plasma proteins may act as biomarkers for macrophage activation. CD14, a GPI-linked co-receptor for lipopolysaccharide (LPS) expressed by both monocytes and macrophages, exists in a soluble form that can be detected in human plasma. M1 macrophages, which can be produced in the presence of interferon-γ and bacterial LPS, express CCR7, CD86 and MHCII and secrete IL-12p70 [11]. M2 macrophages, which can be activated *in vitro* through several pathways including addition of IL-4 [9], express many lectin-like cell surface receptors, among them the hemoglobin-haptoglobin receptor CD163 [12]. CD163 is shed from the cell surface by a proteolytic mechanism involving ADAM-17 [13], and this shed form is soluble and appears in the serum at detectable concentrations.

Since IL-12p70 is usually undetectable in plasma, we sought to find an alternative marker protein for M1 macrophages. Gal-3BP, a secreted 585-amino acid protein and member of the MSR cysteine-rich domain superfamily [14], is easily detected in plasma; it is a well-established plasma marker of viral infection and cancer [15–17]; however, its potential role in CVD has not been investigated. Correlating plasma Gal-3BP and CVD can be relevant in light of the pathogen burden hypothesis, which states that cumulative infection burden contributes to atherogenesis [18].

In the present study, we found that *in vitro* Gal-3BP is produced by M1, but not M2 or M0 (unpolarized) human macrophages. Then, we tested whether plasma markers of macrophage abundance and activation (sCD163, sCD14 and Gal-3BP) correlated with subclinical carotid atherosclerosis, using epidemiologic data from a subset of women participating in the Women's Interagency HIV Study (WIHS). Previous WIHS studies have established a relationship among known inflammatory markers, HIV infection, antiretroviral therapy, and cardiovascular risk factors with CVD[19, 20]. We selected four groups of women with HIV and/or HCV infection. Since both HIV and HCV infection have been associated with premature atherosclerosis, we hypothesized they would be also associated with macrophage activation as measured by sCD163, sCD14 and Gal3BP.

METHODS

Link to the online supplement.

RESULTS

In vitro study: Expression of Gal-3BP, CD14 and CD163 in M1 and M2 macrophages

Human monocytes were isolated from blood of healthy volunteers and differentiated to macrophages (M0) in M-CSF using standard methods under serum-free conditions[21]. These primary macrophages secreted IL-12p70, but not IL-10 into the cell culture supernatant when differentiated to M1 by incubation with LPS and IFN-γ for 24 hours (Figure 1A). M2 macrophages polarized by LPS and IL-4 for 24 hours expressed ample IL-10, but no IL-12 (p70) (Figure 1A). *LGALS3BP* (human gene encoding Gal-3BP) mRNA (Figure 1B) was expressed in M1, but not M2 macrophages, and Gal-3BP protein was secreted by M1, but not M2 macrophages (Figure 1C). The secreted material migrated at 90 kDa as expected and disappeared after knocking down *LGALS3BP* by antisense (Figure 1D).

As expected, CD14 was expressed on the surface of M0 and M1 macrophages and was reduced upon M2 differentiation (Figure 1E). Conversely, CD163 was expressed by M0 and M2 macrophages and disappeared upon M1 polarization (Figure 1E).

To confirm the relevance of these *in vitro* findings to human disease, we analyzed human postmortem coronary arteries from patients with coronary artery disease by immunofluorescence. We found expression of Gal-3BP in CD68+ plaque macrophages (Figure 1F).

Population-based study: Study population and inflammatory macrophage markers

We measured sCD14, sCD163 and Gal-3BP in plasma obtained from 264 women in the WIHS. Participants were selected based on their HIV and HCV status and stratified into four groups, each containing 66 women: HIV+/HCV+, HIV+/HCV−, HIV−/HCV+, and HIV−/HCV (Table 1). The median age was 45 (interquartile range: 41, 50); 62% of the study population were black, 26% were Hispanic, and 12% were white or of other race. At the time of assessment, 76% were current smokers, with the remaining 24% either past or never smokers. Mean BMI was 29.3 (SD 7.4), with HIV+ individuals on average having a lower BMI (mean 27.6 vs. 31.0, p=0.02). Among HIV+ women, 77% were on antiretroviral therapy at the time of their visit. Among HCV+ women, only 2% were currently on interferon treatment, with an additional 8% reporting past interferon use.

Table 1 shows the mean values of Gal-3BP, sCD163, and sCD14 levels in the study population in each of the four disease strata. Gal-3BP levels were significantly higher among HCV+ women than HCV− women (mean 12.4 μg/mL vs. 8.6 μg/mL, p<0.01), but did not differ based on HIV status (mean 10.3 μg/mL vs. 10.7 μg/mL, p=0.34). Dually-infected individuals had the highest mean levels of sCD163 and sCD14, whereas dually-uninfected individuals had the lowest levels. Women with either HIV infection only or HCV infection only had intermediate levels of sCD163 and sCD14. Correlations among the three macrophage markers were moderate and all positive, suggesting that the biological parameters measured by these biomarkers are partially overlapping (Figure 2). Gal-3BP levels were not significantly associated with traditional CVD risk factors including age, BMI, race/ethnicity or smoking status (data not shown). Regarding other CVD risk factors, both sCD163 and sCD14 levels were higher among older women, and sCD163 levels

increased with BMI. Inflammatory macrophage biomarker levels were not highly correlated with levels of hsCRP (Supplemental Figure I). Among HIV+ women, CD4+ count was moderately inversely correlated with inflammatory macrophage biomarkers (Supplemental Figure II).

Population-based study: Associations with subclinical carotid artery disease

Subclinical carotid artery disease was common in the study population, with 14% of women having at least one carotid artery lesion. Mean carotid distensibility was 17.1×10^{-6} m^2/N (SD 8.5), and the mean cIMT was 0.763 mm (SD 0.135).

To reflect the overall levels of macrophage-associated inflammation within an individual, we created a macrophage score which was defined as the number of biomarkers that were found to have levels above the population-wide median (range 0–3). In statistical models accounting for age, race/ethnicity, current smoking status, BMI, hsCRP, HIV, and HCV, higher macrophage score modeled as a continuous variable was significantly associated with both increased odds of lesion (OR 1.58 per elevated marker, 95% CI 1.15 to 2.18) and lower distensibility (β −1.44 units per marker, 95% CI −2.59 to −0.29) (Table 2). Having at least one elevated marker was associated with greater subclinical carotid artery disease, with the maximum number (i.e., 3) associated with the highest levels of disease.

Additional analyses examined individual markers for associations with carotid outcomes. Gal-3BP, sCD163, and sCD14 levels were each significantly associated with an increase in the odds of a carotid artery lesion (Table 2). We found that models that contained the three markers individually each increased the prediction of having a carotid artery lesion (C-statistic 0.735–0.743), compared with a model without these markers (C-statistic 0.714). Including all three markers simultaneously, either using the macrophage score or as individual covariates, further increased the predictive value (C-statistic 0.746 and 0.762, respectively).

Higher levels of the three markers were correlated with reduced distensibility in bivariate analyses (Supplemental Figure III), but only Gal3BP and sCD14 were significantly associated with distensibility after covariate adjustment (Table 2).

In stratified analyses by HIV and HCV infection status, we found an association between increasing macrophage score and the presence of carotid artery lesions among HIV- and HCV-infected participants, with the effect most pronounced and approaching statistical significance among dually-infected individuals (OR 2.81, 95% CI 0.96–8.23) (Table 2). In analyses of individual markers, none of the associations with carotid artery measurements differed significantly across subgroups defined by HIV and HCV after covariate adjustment, despite suggestions of bivariate correlations (e.g., Supplemental Figures IV and V). We observed a trend suggesting stronger associations of subclinical carotid artery disease with sCD163 in HIV-infected as compared with HIV-uninfected groups ($p_{interaction}$=0.09, Supplemental Table I). Findings did not differ appreciably after taking into account CD4+ count or current antiretroviral therapy use among HIV-infected women, or interferon therapy use among HCV-infected women (data not shown). In general, neither the macrophage biomarker score nor the three individual macrophage biomarkers were

associated with levels of cIMT, either overall or within subgroups defined by HIV and HCV status (Supplemental Tables I and II).

DISCUSSION

In our study, we found plasma markers of macrophage abundance, polarization and activation (Gal-3BP, sCD163 and sCD14) to be associated with two measures of subclinical carotid artery disease. Specifically, we found that higher macrophage scores, defined by elevations in multiple macrophage biomarkers, were significantly associated with both increased odds of carotid artery lesions and reduced carotid artery distensibility (inverse of stiffness). Additional analyses suggested that associations of the macrophage markers with carotid artery parameters may have been augmented in HIV− and/or HCV-infected women, as compared with women free of HIV and HCV. These associations remained after taking into account CD4+ count or adjusting for hsCRP, a known inflammation marker of CVD. We did not observe correlations between macrophage biomarkers and hsCRP levels. Thus, our data suggest that circulating macrophage biomarkers may reflect the inflammatory process contributing to atherosclerosis, above and beyond hsCRP, and therefore may provide additional predictive and/or diagnostic ability in the future with respect to subclinical CVD. We also found each of the macrophage markers to be more elevated in HIV-infected women with lower CD4+ counts, particularly sCD163 and sCD14, which is consistent with ongoing inflammation and immune activation in these women.

We identified Gal-3BP as a measurable and soluble M1 macrophage marker in our *in vitro* study, and then found that Gal-3BP was consistently up-regulated and positively correlated with subclinical carotid artery disease markers in a clinical study after adjustment for potential confounders. Accumulating data suggest that some plaque macrophages express M1 markers and others M2 markers [6]; although contribution to plaque instability and inflammation is mostly attributed to M1 markers [5]. Recent studies have given rise to interest in galectins as potential biomarkers of CVD [22]. In prior work, higher plasma levels of galectin-3 have been associated with increased heart failure risk [23] and higher mortality in those with heart failure [24, 25]. Human Gal-3BP binds specifically to human galectin-3 [14], which is interestingly an M2 marker [26]. To our knowledge, ours is the first study to examine associations between Gal-3BP and measures of subclinical CVD. Plasma Gal-3BP levels were increased in HCV-positive individuals, but did not differ by HIV infection status. The finding relating to HCV is consistent with prior data [15]. The lack of association between HIV and Gal-3BP levels differs from other studies [16, 17]. Underlying differences in study populations, calendar time effects, or availability of newer drugs in our study could have contributed to differences between studies.

Our study also provides further support of a role for sCD163 in CVD, as has been suggested by several recent studies [27–30]. While our study focused on women, a cross-sectional analysis of 102 HIV-infected men with undetectable viremia and 41 HIV-uninfected men without a history of CAD found sCD163 to be significantly associated with the presence of non-calcified atherosclerotic plaque, even after adjusting for HIV, traditional CVD risk factors, and age and race [28]. Another recent cross-sectional study among 81 HIV-infected individuals using positron emission tomography found sCD163 to be significantly

associated with arterial inflammation in the aorta, after adjusting for traditional CV risk factors [30]. However, other small cross-sectional or case-control studies in individuals with HIV have not found associations between sCD163 and plaque or myocardial infarction [31, 32], and therefore larger, prospective studies may help to further clarify the role of these markers in CVD pathogenesis. Although CD163 is expressed on M2 macrophages, sCD163 may not reflect this, because ADAM-17, the enzyme cleaving CD163 to become soluble, is expressed by M1 macrophages [13].

Higher sCD14 levels have been associated with all-cause mortality in studies of both the general population [33], as well as in those with HIV [34]. Here, we found that sCD14 level was associated with increased carotid artery lesions and decreased carotid artery distensibility after adjusting for HIV and HCV infection, corroborating other findings in the general population.[41, 42] Another HIV study showed an association between sCD14 levels and yearly rate of change in cIMT [35]. At least one other HIV study failed to find an association between sCD14 and cIMT or lesions, although the authors of that study acknowledged limited power (N=60) [32].

Our study is a first step in understanding the pathophysiology and potential role as risk predictors of these promising new biomarkers in our HIV/HCV population. However, it has limitations. Because the current study is cross-sectional, both macrophage markers and carotid artery outcomes were assessed at the same visit. Therefore, we cannot infer temporality or causality in our study, and thus longitudinal analyses will be important to further elucidate the roles of these markers in predicting the development of CVD, both with and without the presence of chronic viral infection. Indeed, before these markers can be considered for use as risk predictors in clinical practice, they will need to be validated in other populations as well as in the prediction of major adverse cardiac events. Second, this study was limited to women. Since it is well-recognized that the ability to accurately predict CVD risk differs in men compared with women [36], it would be important to replicate our findings to assess the role of these markers in men. Finally, we were limited by a relatively small sample size. However, our study is in fact larger than several other studies examining similar subclinical CVD outcomes in the HIV population. Furthermore, due to the excellent characterization of WIHS participants since 1993, we were able to include a substantial number of exceptionally well-matched women with documented HIV and HCV infection as well as those without infection, to be able to explore differences based on these factors and assess potential effect modification.

In conclusion, we demonstrated that three inflammatory biomarkers of macrophages that are measurable in human plasma are significantly associated with subclinical carotid artery lesions and distensibility in a subset of well-characterized women both with and without chronic viral infection, suggesting their potential for predictive or diagnostic value with respect to atherosclerosis. The association of plasma macrophage biomarkers with CVD may provide new insights pointing to possible new strategies for prevention or treatment of CVD in patients with and without HIV and HCV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

HCV	hepatitis C virus
HIV	human immunodeficiency virus
cIMT	carotid artery intima-media thickness
Gal-3BP	galectin-3 binding protein
sCD163	soluble cluster of differentiation 163
sCD14	soluble cluster of differentiation 14

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Significance

Macrophages are principal contributors to vascular inflammation and atherosclerosis pathology; they can adopt distinctive phenotypes, from inflammatory (M1) to wound healing (M2) phenotypes. While both present in the atherosclerotic plaque, M1 macrophages are more likely to contribute to inflammation and plaque rapture. There are limited tools to evaluate the inflammatory status of macrophages that populate the plaque. Virus infection can contribute to both CVD pathogenesis and macrophages polarization. Herein we measure plasma protein markers secreted from macrophages, in the blood collected from women positive for HIV, HCV, both or neither and correlate their levels to carotid artery disease, assessed by ultrasound measurement of right distal common carotid artery intima-media thickness (cIMT), distensibility, and presence of atherosclerotic lesions. We conclude that the macrophage activation status markers Gal-3BP (M1), sCD163 (M2) and sCD14 (M0) are significantly associated with carotid artery disease in the setting of HIV and HCV infection.
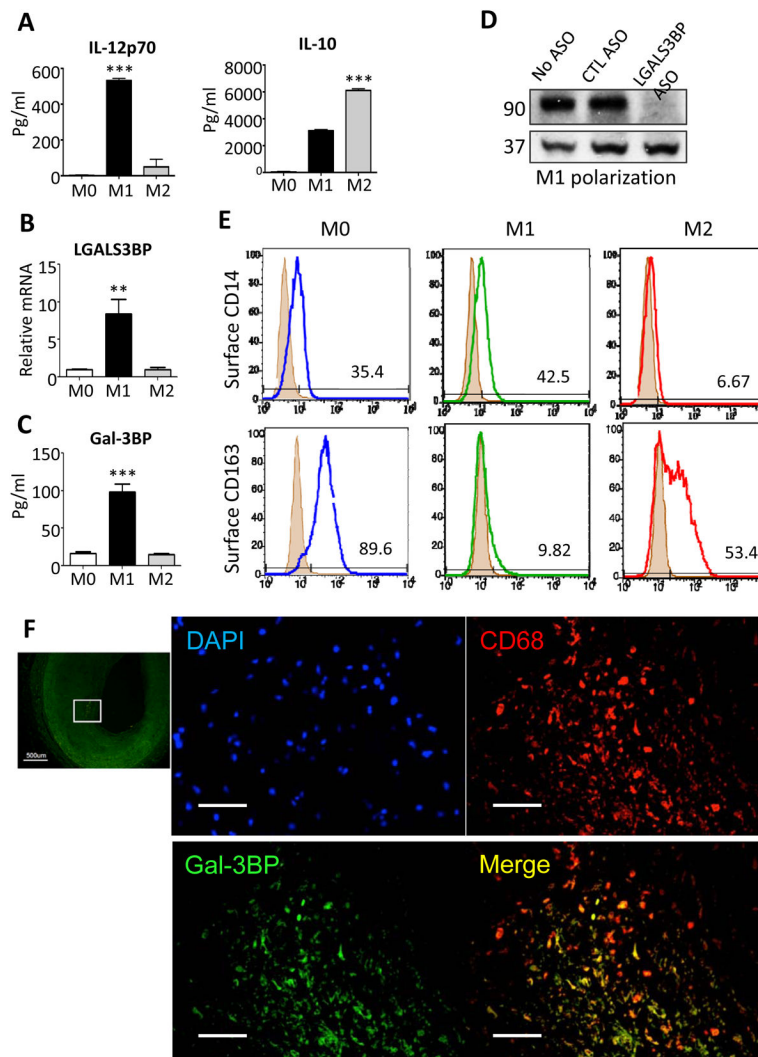
Figure 1. Gal-3BP, CD14 and CD163 expression in human macrophages
Human blood monocytes (all subsets) were incubated with M-CSF (100 ng/ml) for 6 days to produce monocyte-derived macrophages (M0). These macrophages were incubated with interferon-γ for 1 day to produce M1 or with IL-4 to produce M2 macrophages and then challenged with LPS (10 ng/ml). (A) Level of IL-12p70 and IL-10 in the conditioned media were measured by Elisa (B) Gal-3BP mRNA expression was measured by quantitative RT-PCR, expressed relative to H18S. ** P=0.0056 by paired t test, n=3 donors. (C) Gal-3BP protein production was measured using ELISA (P=0.0008). (D) M1 polarized macrophages were treated with cell-permeable antisense (ASO) against LGALS3BP or control sequence (CTL) for 48hr, conditioned media harvested for immunoblotting with anti-Gal-3BP and anti-ERK2 (loading control) antibodies. (E) CD-14 and CD-163 determined by flow cytometry in M0, M1 and M2 polarized macrophages (representative of 3 healthy blood donors). (F) Human postmortem coronary arteries from patients with coronary artery disease were stained with antibodies against Gal-3BP (FITC, green), CD68 (Texas red), and nuclear stain (DAPI, blue) to assess expression of Gal-3BP in plaque macrophages. Lowest panel

shows merge of Gal-3BP and CD68; right panels magnify boxed areas. Top: overview (scale bar = 500 μm), other scale bars = 100 μm
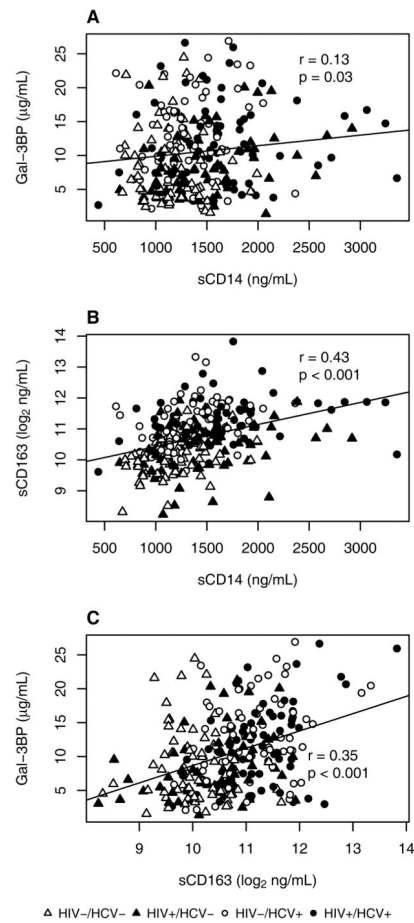
Figure 2. Spearman correlations comparing (A) Gal-3BP and sCD14, (B) sCD163 and sCD14, and (C) Gal-3BP and sCD163, across four HIV/HCV strata

White = HIV−/HCV−, red = HIV+/HCV−, blue = HIV−/HCV+, black = HIV+/HCV+.

Line represents regression fit to observed data.

Table 1

Characteristics of study population, by HIV/HCV strata.

	HIV−/HCV− (N=66) N (%)	HIV+/HCV− (N=66) N (%)	HIV−/HCV+ (N=66) N (%)	HIV+/HCV+ (N=66) N (%)
Age (median, IQR)	44 (40–48)	45 (41–49)	47 (43–51)	47 (43–52)
Race/ethnicity				
Black	41 (62)	41 (62)	41 (62)	41 (62)
Hispanic	17 (26)	17 (26)	17 (26)	17 (26)
Other	8 (12)	8 (12)	8 (12)	8 (12)
Current smoker	50 (76)	50 (76)	50 (76)	50 (76)
Body mass index (mean, SD)	29.9 (6.5)	28.4 (6.8)	32.0 (8.9)	26.8 (6.0)
C-reactive protein level (μg/mL) (median, IQR)	3.2 (1.1–6.8)	2.8 (1.2–5.6)	1.5 (0.4–4.3)	0.85 (0.3–2.45)
CD4+ count (cells/μL) (median, IQR)	N/A	410 (248–599)	N/A	392 (215–538)
HIV viral load (copies/mL) (median, IQR)	N/A	885 (80–21000)	N/A	98.5 (80–2200)
ART regimen at time of study				
Protease inhibitor-based	N/A	26 (39)	N/A	33 (50)
Non-protease inhibitor-based	N/A	21 (32)	N/A	22 (33)
Not on treatment	N/A	19 (29)	N/A	11 (17)
Inflammatory macrophage score (range: 0–3)				
0	21 (32)	15 (23)	8 (12)	4 (6)
1	35 (53)	24 (36)	11 (17)	9 (14)
2	8 (12)	14 (21)	28 (42)	21 (32)
3	2 (3)	13 (20)	19 (29)	32 (48)
Gal-3BP (μg/mL) (mean, SD) (range: 1.36–26.90)	8.75 (5.61)	8.43 (4.56)	12.69 (5.99)	12.14 (5.98)
sCD163 (ng/mL, log$_2$-transformed) (mean, SD) (range: 8.23–13.83)	10.07 (0.62)	10.43 (0.79)	11.13 (0.75)	11.25 (0.75)
sCD14 (ng/mL) (mean, SD) (range: 439–3360)	1,179 (287)	1,477 (464)	1,317 (330)	1,649 (560)

HCV = hepatitis C virus, HIV = human immunodeficiency virus, IQR = interquartile range, SD = standard deviation.

Table 2

Association between inflammatory macrophage markers and measures of subclinical carotid artery disease, Women's Interagency HIV Study.

	Any carotid artery lesion			Distensibility ($10^{-6} \times N^{-1} \times m^2$)		
	Odds ratio	95% CI	P	β	95% CI	P
Inflammatory macrophage score, per each elevated marker (range: 0–3)						
Among entire study population (N=256)						
Modeled as continuous	**1.58**	**1.15, 2.18**	**0.005**	**−1.44**	**−2.59, −0.29**	**0.01**
Modeled as categorical						
0 (reference group)	1.00			0.00		
1	4.18	0.84, 20.70	0.08	−1.57	−4.13, 1.00	0.23
2	4.97	0.78, 31.64	0.09	−1.47	−4.66, 1.72	0.37
3	**6.87**	**1.40, 33.71**	**0.02**	**−4.65**	**−8.06, −1.25**	**0.01**
Stratified analyses						
Modeled as continuous						
In HIV−/HCV− (N=65)	1.18	0.35, 3.98	0.78	0.59	−2.14, 3.33	0.67
In HIV−/HCV+ (N=63)	1.24	0.48, 3.21	0.66	**−2.03**	**−3.87, −0.18**	**0.03**
In HIV+/HCV− (N=64)	1.58	0.77, 3.24	0.21	**−2.36**	**−4.27, −0.45**	**0.02**
In HIV+/HCV+ (N=64)	2.81	0.96, 8.23	0.06	−1.43	−3.07, 0.21	0.09
Individual inflammatory macrophage markers, per each standard deviation						
Among entire study population (N=256)						
Gal-3BP	**1.48**	**1.01, 2.15**	**0.04**	**−1.44**	**−2.28, −0.61**	**<0.001**
sCD163	**1.85**	**1.21, 2.83**	**0.005**	−0.05	−1.26, 1.15	0.93
sCD14	**1.49**	**1.04, 2.12**	**0.03**	**−1.11**	**−2.10, −0.12**	**0.03**

CI = confidence interval, HCV = hepatitis C virus, HIV = human immunodeficiency virus, m = meters, N = Newtons.

All analyses adjusted for age, race/ethnicity, smoking status, body mass index, high-sensitivity C-reactive protein, HIV and HCV infection. Bold indicates significant at p<0.05.