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BRIEF REPORT

Coronary Artery Plaque Composition and Severity Relate to the Inflammasome in People With Treated Human Immunodeficiency Virus

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Background. Inflammasome activation is increased in people with human immunodeficiency virus (PWH), but its relationship with coronary plaque is poorly understood in this setting.

Methods. In a large human immunodeficiency virus cardiovascular prevention cohort, relationships between caspase-1, interleukin (IL)-1β, and IL-18 and coronary plaque indices were assessed by multivariate logistic regression.

Results. Higher IL-18 and IL-1β were associated with Leaman score, an integrative measure of plaque burden and composition.

Conclusions. As Leaman score >5 is associated with cardiovascular events in the general population, future work is needed to determine how the inflammasome relates to events and whether strategies to reduce its activation affect events or plaque progression among PWH.

Keywords. atherosclerosis; HIV; inflammasome; Leaman score.

Persistent immune activation likely contributes to cardiovascular disease (CVD) risk in people with human immunodeficiency virus (PWH) despite antiretroviral therapy (ART) [1]. One plausible contributor to this heightened inflammation is the inflammasome, an innate immune complex that regulates the immunologic milieu through proinflammatory cytokines. Indeed, the inflammasome is a potential mediator of human immunodeficiency virus (HIV)-associated CVD risk, given its role in HIV pathogenesis and in CVD among the general population.

After priming and activation, the inflammasome complex is formed from a receptor protein (eg, NOD-like receptor protein family pyrin domain containing 3 [NLRP3]), an adaptor protein, and procaspase-1. Complex formation leads to procaspase-1 autocleavage into active caspase-1, which activates the cell membrane pore-forming gasdermin D and the proinflammatory cytokines interleukin (IL)-1β and IL-18 that are secreted for downstream immune and inflammatory signaling.

In the general population, inflammasome activation seems causally linked to incident CVD [2]. Among PWH, elevated inflammasome activity persists despite ART, and a potential linkage between markers of IL-1 activation and myocardial infarction was suggested in a small study [3, 4]. However, limited data exist relating inflammasome activation to coronary plaque indices among PWH [4]. To address this, we leveraged data from the mechanistic substudy of the Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE) to assess the association between inflammasome biomarkers and coronary plaque among ART-treated PWH.

METHODS

Study Design

REPRIEVE enrolled PWH 40–75 years on stable ART with low-to-moderate traditional CVD risk. A subset of United States-based participants was enrolled in a substudy involving coronary computed tomography angiography (CTA) and immune phenotyping [5]. Participants with a diagnostic baseline CTA and available inflammasome biomarkers were included. (Clinical Trials Registration NCT02344290; date of initial registration January 22, 2015.)

Computed Tomography Angiography Assessment

Coronary CTA acquisition and interpretation have been previously described [5]. The REPRIEVE CT Core laboratory

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reviewed the CTAs for coronary artery calcium (CAC) score, presence of coronary plaque, and presence of vulnerable plaque (defined as plaque positive remodeling, low attenuation, or napkin ring sign) blinded to clinical and laboratory data. The Leaman score was calculated as an overall index of plaque burden that combines localization of coronary lesions, plaque composition, and degree of stenosis. A Leaman score >5 has been associated with cardiovascular disease risk factors and incident cardiovascular events in the general population [6].

Biomarker Measurements

Ethylenediaminetetraacetic acid plasma samples were tested for inflammasome biomarkers IL-1β, IL-18, and caspase-1 (Temple University). Interleukin-1β was measured using a high-sensitivity assay (S-PLEX) by Meso Scale Diagnostics (K151ADSS-1). Interleukin-18 and caspase-1 were measured using Quantikine ELISAs by R&D Systems (DL180 and DCA100, respectively). All samples were run in duplicate with intraindividual variability <25%.

Statistical Analysis

Biomarker geometric means were compared between subgroups using analysis of variance. The relationships of biomarkers to the presence of plaque, vulnerable plaque, CAC >0, and Leaman score >5 were assessed via binary logistic regression, adjusted for age, natal sex, low-density lipoprotein (LDL) cholesterol, hypertension, current cigarette smoking, current and nadir CD4, and ART duration, per prior modeling [1]. Biomarkers were $\log_2$-transformed and divided by 0.32 to provide the odds of the presence of a plaque index per 25% higher biomarker level. Inference was guided by an alpha level of 0.05. Statistical analyses were performed using SAS, version 9.4 (SAS Institute, Inc., Cary, NC).

Patient Consent Statement

REPRIEVE was approved by the Mass General Brigham Human Research Committee and local institutional review boards at each site. Participants provided written informed consent for the parent trial.

RESULTS

Participant Demographics and Characteristics

Of the 752 participants, median age was 50 years, 18% were women, 47% were non-White, and 24% were Hispanic/Latino (Supplementary Table 1). Median atherosclerotic cardiovascular disease (ASCVD) risk was 4.5%. Median current CD4 was 601 (Q1–Q3 426–681) cells/mm^3, 51% had a nadir CD4 <200, and 98% had HIV VL <400 copies/mL.

Association of Inflammasome Biomarkers With Host Factors

Caspase-1, IL-18, and IL-1β were assessed in relationship to key host factors (Table 1). No statistically significant differences in biomarkers were seen by age, cigarette smoking, body mass index, or ASCVD risk score. Caspase-1 and IL-18 were 12.3% (95% confidence interval [CI], .3%–22.9%; $P = .045$) and 16.3% (95% CI, 8.2%–23.7%; $P < .001$) higher in men than women, respectively. No clear associations of biomarkers with current or nadir CD4 were observed. However, those with a CD4/CD8 ratio <1 had a 17.5% higher caspase-1 (95% CI, 8.4%–25.7%; $P < .001$), 6.5% higher IL-18 (95% CI, −.9% to 13.3%; $P = .08$), and 17.2% higher IL-1β (95% CI, 3.1–29.3; $P = .02$). Participants with detectable HIV VL demonstrated higher IL-18 (overall $P < .001$).

Association of Inflammasome Biomarkers With Plaque Indices

Logistic regression was used to assess the relationships between the 3 inflammasome biomarkers and the following coronary plaque indices: presence of plaque, vulnerable plaque, CAC, and Leaman score. In unadjusted analyses, higher IL-18 and IL-1β were associated with greater odds of having a Leaman score >5 versus ≤5, but not with other plaque endpoints (Table 2). After adjustment, each 25% higher IL-18 and IL-1β was associated with a 13% (95% CI, 1%–25%; $P = .026$) and 6% (95% CI, 1%–12%; $P = .03$) greater odds of having a Leaman score >5 (vs ≤5), respectively. In contrast, no statistically significant relationships were observed between caspase-1 and the plaque indices.

DISCUSSION

Increased inflammasome activation appears causally related to CVD in the general population and is seen in ART-treated PWH [2, 4]. However, whether this persistently increased inflammasome activation in PWH is associated with coronary atherosclerotic plaque or specific plaque phenotypes is unknown. We addressed this by leveraging the mechanistic substudy of REPRIEVE, making several key findings by assessing the relationship between caspase-1, IL-18, and IL-1β and plaque phenotypes by CTA among ART-treated PWH at low-to-moderate CVD risk. First, inflammasome pathway biomarkers tended to be higher in men, those who were viremic, and those with lower CD4/CD8 ratios. Most importantly, IL-18 and IL-1β were independently associated with Leaman score, a summative measure of plaque burden and composition related to cardiovascular events in the general population. These findings may provide a mechanistic link between innate immune activation and coronary atherosclerosis in PWH.

This study confirms and extends our knowledge of important relationships between inflammasome activity and key immune markers in PWH. In this study, we observed a relationship between greater inflammasome activity and viremia in a small subset of participants with a detectable viral load (VL) despite ART. Indeed, HIV itself is a signal for inflammasome activation [7]. Caspase-1 is a major contributor to

Table 1. Association of Host Factors With Inflammasome Pathway Biomarkers

Characteristic	Caspase-1 (pg/mL)			IL-18 (pg/mL)			IL-1β (fg/mL)		
	Geometric Mean (95% CI)	%Difference (95% CI)	*P* Value	Geometric Mean (95% CI)	%Difference (95% CI)	*P* Value	Geometric Mean (95% CI)	%Difference (95% CI)	*P* Value
Natal Sex									
Male	74.5 (70.6–78.7)	…	…	247.6 (238.2–257.5)	…	…	78.7 (72.7–85.3)	…	…
Female	65.3 (58.1–73.4)	−12.3 (−22.9 to −.3)	.045	207.2 (190.5–225.4)	−16.3 (−23.7 to −8.2)	<.001	83.9 (70.6–99.8)	6.6 (−11.9 to 29.0)	.51
Age (Years)									
<50	74.8 (69.4–80.5)	…	…	232.5 (220.4–245.3)	…	…	76.5 (68.6–85.4)	…	…
≥50	71.3 (66.8–76.2)	−4.6 (−13.7 to 5.3)	.35	246.0 (234.5–257.9)	5.8 (−1.5 to 13.7)	.12	82.2 (74.5–90.6)	7.4 (−7.3 to 24.3)	.34
Race									
White	72.6 (67.9–77.7)	…	.42[a]	267.9 (255.5–281.0)	…	<.001[a]	78.0 (70.6–86.2)	…	.081[a]
Black or African American	74.5 (68.6–80.8)	2.5 (−7.8 to 14.0)	.64	204.5 (193.2–216.5)	−23.7 (−29.1 to −17.8)	<.001	87.4 (77.5–98.6)	12.1 (−4.2 to 31.1)	.15
Asian	91.7 (58.5–143.9)	26.3 (−19.9 to 99.2)	.31	189.1 (137.9–259.3)	−29.4 (−48.7 to −2.9)	.032	49.8 (25.6 to 96.8)	−36.1 (−67.4 to 25.1)	.19
Other	65.9 (56.2–77.2)	−9.3 (−23.6 to 7.8)	.27	248.3 (222.2–277.4)	−7.3 (−17.9 to 4.5)	.22	66.4 (52.6–83.8)	−14.9 (−34.0 to 9.7)	.21
Ethnicity									
Not Hispanic or Latino	73.6 (69.5–77.9)	…	…	235.8 (226.3–245.8)	…	…	79.5 (73.0–86.5)	…	…
Hispanic or Latino	71.4 (64.5–79.1)	−2.9 (−13.6 to 9.1)	.61	255.2 (237.1–274.7)	8.2 (−.5 to 17.7)	.066	80.1 (69.0–93.1)	.9 (−15.1 to 19.8)	.92
Smoking Status									
Former/never smoker	71.5 (67.6–75.7)	…	…	238.4 (228.8–248.4)	…	…	77.2 (71.0–83.9)	…	…
Current smoker	77.0 (69.7–85.2)	7.7 (−4.0 to 20.9)	.21	244.0 (226.9–262.4)	2.3 (−5.8 to 11.2)	.59	87.6 (75.6–101.6)	13.6 (−4.2 to 34.6)	.14
BMI (kg/m²)									
<25	74.7 (68.7–81.3)	…	.28[a]	243.2 (228.7–258.6)	…	.38[a]	76.7 (67.6–86.9)	…	.39[a]
25–29.9	69.4 (64.1–75.0)	−7.2 (−17.3 to 4.1)	.20	243.9 (230.5–258.1)	.3 (−7.7 to 9.0)	.94	77.8 (69.3–87.3)	1.5 (−14.5 to 20.3)	.87
30+	75.8 (68.9–83.4)	1.4 (−10.8 to 15.2)	.83	230.1 (214.7–246.6)	−5.4 (−13.8 to 3.8)	.24	86.6 (75.2–99.8)	13.0 (−6.5 to 36.5)	.21
ASCVD Risk Score (%)									
0–<2.5	67.1 (60.6–74.4)	…	.29[a]	227.0 (210.8–244.4)	…	.34[a]	73.7 (63.3–85.6)	…	.10[a]
2.5–<5	76.4 (70.1–83.2)	13.7 (−.5 to 30.0)	.059	240.5 (226.0–256.0)	6.0 (−3.8 to 16.7)	.24	84.7 (74.6–96.2)	15.0 (−5.6 to 40.0)	.17
5–<7.5	72.4 (65.5–80.0)	7.9 (−6.5 to 24.4)	.30	244.4 (227.4–262.7)	7.7 (−2.9 to 19.4)	.16	71.5 (61.7–82.8)	−3.0 (−21.4 to 19.8)	.78
≥7.5	74.6 (66.7–83.3)	11.0 (−4.5 to 29.1)	.17	249.5 (230.2–270.3)	9.9 (−1.4 to 22.6)	.089	90.1 (76.5–106.2)	22.4 (−2.0 to 52.9)	.075
CD4 Count (cells/mm³)									
<350	74.9 (65.9–85.2)	…	.87[a]	256.4 (233.7–281.3)	…	.070[a]	86.4 (71.5–104.5)	…	.47[a]
350–499	71.7 (64.2–80.0)	−4.4 (−19.3 to 13.3)	.60	223.0 (205.9–241.5)	−13.0 (−23.0 to −1.7)	.025	73.9 (62.8–87.0)	−14.5 (−33.4 to 9.9)	.22
500+	72.7 (68.4–77.3)	−3.0 (−15.9 to 11.8)	.67	241.7 (231.3–252.6)	−5.7 (−14.9 to 4.4)	.26	80.0 (73.1–87.5)	−7.5 (−25.0 to 14.1)	.47
Nadir CD4 Count (cells/mm³)									
<50	77.3 (69.6–86.0)	…	.34[a]	231.3 (214.2–249.9)	…	.80[a]	90.4 (77.3–105.8)	…	.27[a]
50–199	75.3 (68.7–82.6)	−2.6 (−15.3 to 12.1)	.71	239.3 (223.8–256.0)	3.5 (−6.6 to 14.6)	.51	79.8 (69.6–91.5)	−11.7 (−28.3 to 8.7)	.24
200–349	68.8 (62.7–75.5)	−11.0 (−22.8 to 2.5)	.10	240.9 (225.0–257.9)	4.1 (−6.1 to 15.4)	.44	73.2 (63.8–84.1)	−19.0 (−34.3 to −.1)	.049
350+	71.0 (63.5–79.3)	−8.2 (−21.3 to 7.0)	.27	244.2 (225.2–264.9)	5.6 (−5.6 to 18.1)	.34	80.9 (68.6–95.4)	−10.5 (−28.7 to 12.4)	.34
CD4/CD8 Ratio	…	…	…	…	…	…	…	…	…
<1	78.6 (73.6–83.8)	…	…	250.2 (238.7–262.3)	…	…	86.3 (78.3–95.1)	…	…
≥1	64.8 (59.7–70.4)	−17.5 (−25.7 to −8.4)	<.001	234.0 (220.4–248.4)	−6.5 (−13.3 to .9)	.084	71.5 (63.1–80.9)	−17.2 (−29.3 to −3.1)	.019

Table 1. Continued

Characteristic	Caspase-1 (pg/mL)			IL-18 (pg/mL)			IL-1β (fg/mL)		
	Geometric Mean (95% CI)	%Difference (95% CI)	*P* Value	Geometric Mean (95% CI)	%Difference (95% CI)	*P* Value	Geometric Mean (95% CI)	%Difference (95% CI)	*P* Value
HIV-1 RNA (copies/mL)									
–<LLQ	71.0 (67.3–74.9)	…	.056[a]	234.4 (225.7–243.5)	…	<.001[a]	78.4 (72.5–84.8)	…	.49[a]
–LLQ–<400	84.0 (72.1–97.9)	18.3 (.6–39.1)	.042	270.5 (242.4–301.9)	15.4 (2.7–29.6)	.016	90.7 (72.3–113.7)	15.6 (–9.1 to 47.0)	.24
–400+	90.3 (64.4–126.5)	27.1 (–9.7 to 78.9)	.17	353.1 (277.1–449.8)	50.6 (17.9–92.4)	.001	77.8 (47.1–128.3)	–.8 (–40.3 to 64.6)	.97

Abbreviations: ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CI, confidence interval; HIV, human immunodeficiency virus; IL, interleukin; LLQ, lower limit of quantification; ref, reference; RNA, ribonucleic acid.

NOTES: Summary of analysis of variance model output for each inflammasome biomarker by host factor exposure. Geometric means are reported with associated percentage differences and *P* values.

[a]Denotes type III test of fixed effects was used to test equality of geometric means across 3 or more groups.

$CD4^+$ T-cell depletion via pyroptosis, an inflammatory form of cell death, which may in part account for the relationship with CD4/CD8 ratio, in addition to immune exhaustion [8].

We identified novel and potentially important sex-related differences in inflammasome activation among PWH. We are not aware of other data demonstrating higher IL-18 in men with HIV (vs women), which is particularly notable given that select markers of immune activation and inflammation tend to be higher in women with HIV compared to men [9]. This difference may be related to the differential regulation of androgen and estrogen response elements present in innate immunity gene promoters; Toll-like receptor 4-induced responses, for example, lead to tumor necrosis factor, IL-1, and IL-6 downregulation by estrogen through NF-kappaβ signaling [10]. However, the clinical implication of this sex-related difference is unknown. More importantly, we adjusted for sex in multivariate modeling, without a significant effect on the association of inflammasome biomarkers with coronary plaque indices.

It is notable that IL-18 and IL-1β were independently associated with Leaman score, a composite measure of plaque composition and severity. These data provide a link between a central innate immune component and clinically significant atherosclerosis. Leaman score quantifies the total obstructive and nonobstructive coronary plaque burden as a weighted, summative index that integrates the specific localization of plaque (accounting for coronary artery dominance), type of plaque (calcified vs noncalcified vs mixed), and degree of stenosis in each coronary segment [6]. Leaman score has been associated in several longitudinal studies with common predictors of CVD including age, male sex, hypertension, hyperlipidemia, and diabetes, but also with cardiovascular events [6, 11]. We did not detect an association between inflammasome biomarkers and individual plaque phenotypes but rather with a clinically relevant, integrated score. We previously assessed the association of plaque indices with other inflammatory biomarkers (C-reactive protein [CRP], soluble [s]CD14, sCD163, IL-6, monocyte chemoattractant protein-1, oxidized LDL, and lipoprotein-associated phospholipase A2) and observed that a 25% higher CRP was associated with 8% higher odds of Leaman score >5, whereas other biomarkers were not related to Leaman score. Although the effect size relating IL-18 and IL-1β to Leaman score in the current study was modest, it was comparable to or larger than that seen with CRP or other biomarkers in the study population [1]. Moreover, the relationship of heightened inflammasome activity to Leaman score was statistically significant compared with other pathways considered central contributors to atherosclerosis in PWH. Taken together, these data suggest a potentially relevant contribution of the inflammasome to CVD pathogenesis.

Our findings associating higher inflammasome pathway activity and atherosclerosis align with the few prior studies in this

Table 2. Association of Biomarkers of Inflammasome Activation With Coronary Plaque Phenotypes

Plaque Phenotype/ Biomarker	Unadjusted OR (95% CI)	Unadjusted *P* Value	Adjusted OR (95% CI)	Adjusted *P* Value
Leaman Score >5				
Caspase-1	1.04 (.97–1.11)	.29	1.04 (.96–1.12)	.32
IL-18	1.12 (1.02–1.23)	.013	1.13 (1.01–1.25)	.026
IL-1β	1.05 (1.00–1.10)	.049	1.06 (1.01–1.12)	.030
Plaque Present				
Caspase-1	1.01 (.96–1.06)	.76	1.01 (.95–1.06)	.84
IL-18	1.05 (.99–1.13)	.12	1.02 (.95–1.10)	.54
IL-1β	1.00 (.97–1.03)	.94	1.00 (.97–1.04)	.93
Plaque With Vulnerable Features				
Caspase-1	.99 (.93–1.05)	.72	.98 (.92–1.05)	.58
IL-18	1.04 (.96–1.13)	.31	1.02 (.93–1.11)	.72
IL-1β	1.01 (.97–1.05)	.67	1.01 (.97–1.05)	.63
Coronary Artery Calcium Score >0				
Caspase-1	1.01 (.96–1.06)	.81	1.01 (.95–1.07)	.79
IL-18	1.04 (.97–1.12)	.28	1.01 (.93–1.10)	.81
IL-1β	1.00 (.96–1.03)	.86	1.00 (.96–1.04)	.96

Abbreviation: CI, confidence interval; IL, interleukin; OR, odds ratio.

Notes: Odds ratios (95% CI) derived from binary logistic regression modeling. Odds ratios refer to odds of presence of plaque phenotype associated with a 25% higher biomarker. Adjusted models were adjusted for age, natal sex, race, low-density lipoprotein, hypertension, current smoking, antiretroviral therapy duration, current CD4, and nadir CD4.

setting. One prior study associated IL-18 (but not IL-1β) with coronary plaque presence [4]. This study included only men, had fewer participants with viral suppression, used a less sensitive assay for inflammasome biomarker evaluation, and had a higher prevalence of plaque compared with the present study. These features may explain some differences seen compared with our study. In clinical studies, limited evidence in PWH exists regarding the association between inflammasome activation and cardiovascular events. One study linked IL-1Ra to myocardial infarction with limited or no evidence for an association with IL-18 or IL-1β [3]. In the general population, Leaman score >5 is independently associated with a 2- to 5-fold increased hazard of myocardial infarction or cardiac death with effect sizes larger than common CVD predictors such as smoking or diabetes. Our observation linking IL-18 and IL-1β to Leaman score further reinforces the potentially important role of the inflammasome pathway in the development of CVD independent of traditional cardiovascular risk factors [6, 11].

Our data also suggest the possibility of differential regulation of the inflammasome. Because caspase-1 is more proximally involved in the inflammasome pathway, subtle transcriptional or translational changes that are difficult to detect may lead to larger, more apparent changes in downstream mediators such as IL-18 or IL-1β. We previously observed an effect of caspase-1 in the relationship of macrophage-specific arterial inflammation in PWH, but in this study we assessed coronary plaque, which may have a differential relationship to more downstream components of the inflammasome [12]. Moreover, despite IL-18 and IL-1β being byproducts of inflammasome activation, IL-18 had a relatively larger association with Leaman score than IL-1β (although with overlapping confidence intervals). Anti-IL-1β therapy has been shown to reduce CVD in the general population and arterial inflammation in PWH [2, 13]. However, IL-1β blockade does not impact IL-18 levels nor IL-18's independent association with CVD, suggesting distinct regulation of the inflammasome components [14]. Interleukin-18, not IL-1β, is constitutively expressed in mononuclear cells [15]. It is plausible that the higher degree of monocyte/macrophage activation in PWH may exacerbate this differential gene expression and the relative importance of these components, despite the canonical inflammasome model. Relatively decreased production of IL-18-binding protein, the natural IL-18 antagonist, has been described in HIV [16]. As such, differential regulation in decoy receptors, binding or accessory proteins, or feedback loops may also explain our findings.

Study strengths include detailed phenotyping in a large multicenter contemporary cohort of ART-treated PWH. The study was United States-based, but the cohort had relatively high proportions of women and non-White participants to support generalizability. The study was cross-sectional, which precludes the assessment of causality; future longitudinal analyses within REPRIEVE will assess the strength of each inflammasome biomarker to plaque development over time.

CONCLUSIONS

In summary, among ART-treated PWH at low-to-moderate traditional ASCVD risk but with excess plaque, higher IL-18 and IL-1β were associated with Leaman score, a measure of atherosclerotic burden linked to cardiovascular events in the general population [1]. Future longitudinal work is needed to determine how inflammasome activation relates to plaque progression and cardiovascular events and whether therapeutic strategies targeting the inflammasome mitigate these in PWH.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* 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.

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