Title

Immune Biomarkers in the Prediction of Future Myocardial Infarctions in People With Human Immunodeficiency Virus

Journal

Clinical Infectious Diseases, 70(8)

ISSN

1058-4838

Authors

Trevillyan, Janine M
Moser, Carlee
Currier, Judith S
et al.

Publication Date

2020-04-10

DOI

10.1093/cid/ciz765

Peer reviewed

Clinical Infectious Diseases

BRIEF REPORT

Immune Biomarkers in the Prediction of Future Myocardial Infarctions in People With Human Immunodeficiency Virus

Janine M. Trevillyan,[1,2] Carlee Moser,[3] Judith S. Currier,[1] and Tamer Sallam[4]

[1]Division of Infectious Diseases, David Geffen School of Medicine, University of California, Los Angeles; [2]Department of Infectious Diseases, Alfred Health and Monash University, Melbourne, Australia; [3]Harvard T. H. Chan School of Public Health, Boston, Massachusetts; and [4]Division of Cardiology, David Geffen School of Medicine, University of California, Los Angeles

In a retrospective case control analysis, following adjustments for high-sensitivity C-reactive protein (hsCRP), traditional cardiovascular risk factors, and the CD4/CD8 T-cell ratio, higher lipopolysaccharide-binding protein (LBP) was associated with future myocardial infarctions in hsCRP human immunodeficiency virus (HIV). LBP may be a marker of cardiovascular risk with utility in HIV.

Keywords. HIV; cardiovascular disease; acute myocardial infarction; inflammation; immune activation.

People living with human immunodeficiency virus (HIV; PWHIV) are at increased risk for cardiovascular disease (CVD), but attempts to predict acute myocardial infarctions (AMI), including the atherosclerotic cardiovascular disease Pooled Cohort Equations, lack sensitivity and specificity in this population [1]. HIV is characterized by unique perturbations of innate and adaptive immune responses that powerfully influence plaque stability, but are not well captured by traditional risk stratification approaches. The recently released American Heart Association statement on the characteristics, prevention, and management of CVD in PWHIV highlights this, identifying that "there is insufficient data to recommend routine measurement of inflammatory biomarkers as the additive value of these measurement for CVD risk stratification in HIV is unclear" [2]. Thus, research is needed to investigate the utility of such biomarkers to optimize risk prediction efforts.

This project aimed to determine whether changes in plasma biomarkers of inflammation, endothelial functions, and microbial translocation were associated with AMI in PWHIV and, specifically, to examine the correlations between levels of lipopolysaccharide-binding protein (LBP) and known markers of cardiovascular risk.

METHODS

A retrospective case control study was performed. Cases (n = 57) were adult PWHIV who had their first AMI while enrolled in an Acquired Immunodeficiency Syndrome Clinical Trials Group (ACTG) trial or while enrolled in the ACTG long-term follow-up cohort. AMIs are reported in all ACTG trials following a specific protocol, but were not externally adjudicated. Cases were 1:2 matched by age and sex to participants with HIV from the same study who had no history of CVD (controls; n = 114). Demographic data, current antiretroviral therapy (ART) regimens, and the most recent laboratory results for participants were collated from the parent study and cohort databases. As 3 HIV viral load (VL) assays were used across the parent studies (Roche Ultrasensitive HIV real-time polymerase chain reaction [threshold 50copies/ml], Roche COBAS AmpliPrep/Taqman HIV-1 [threshold 20c/ml], and Abbott RealTime HIV-1 [Threshold 40c/ml]), an undetectable HIV VL was defined as <50 copies/ml.

Stored plasma samples within the 3 months prior to the recorded AMI were utilized from the specimen repository. For controls, the AMI date in the matched case was used. Biomarkers of inflammation (high-sensitivity C-reactive protein, interleukin-6, and tissue necrosis factor alpha [TNFα]), coagulation (soluble platelet selectin [sP-selectin] and soluble CD40 ligand), and endothelial functions (soluble immunoglobulin- like cell adhesion molecules-1, soluble vascular cell adhesion molecules-1) were determined by Luminex magnetic bead technology, Milliplex Human Cytokine/Chemokine Magnetic Bead Panel (Billerica, MA). Levels of LBP, a lipopolysaccharide (LPS)-responsive glycoprotein involved in innate immune responses and atherogenesis, were measured by a commercial kit (Hycult Biotech Inc, Uden, The Netherlands, Edition 10–16) [3].

The primary outcome was the difference in LBPs between those with and without AMI.

Outcomes are described as medians (interquartile ranges [IQR]) or n (%), as appropriate.

Matched univariate conditional logistic regression was conducted to determine the participant characteristics and laboratory results associated with an increased risk of AMI. A P value < .05 was considered statistically significant. Multivariate conditional logistic regression examined associations between markers and risks of AMI, adjusting for the presence of traditional CVD risk factors (diabetes, hypertension, family history,

Received 11 June 2019; editorial decision 29 July 2019; accepted 12 August 2019; published online August 15, 2019.

Correspondence: J. M. Trevillyan, UCLA CARE Centre, 11075 Santa Monica Blvd, Suite 100, Los Angeles, CA 90025 (Jtrevillyan@mednet.ucla.edu).

Clinical Infectious Diseases® 2020;70(8):1764–7

DOI: 10.1093/cid/ciz765

current cigarette smoking, and total cholesterol-high density lipoprotein [HDL] ratio), the CD4/CD8 ratio, and high-sensitivity C-reactive protein (hsCRP). Associations between biomarkers, plasma lipids, and participant demographics were assessed using Spearman's correlations.

Assuming a mean difference in LBP of 3.5 pg/ml (± 8.7) between cases and controls, as demonstrated in the general population by Lepper et al [4], with 57 matched groups, this study had 85% power ($P < .05$) to detect a true difference in LBP.

All participants provided written informed consent for their samples and data to be used in future research. This sub-study was approved by the University of California, Los Angeles Institutional Review Board (16-001934).

RESULTS

We identified 57 participants with incident AMIs and matched them with 114 controls. The participants came from 14 ACTG studies (see Supplementary Table 1), with an earliest recruitment date of 1999 and latest recruitment date of 2012. There were 4 controls that were included twice, with different matched cases and, thus, different time points of data collection. As a result of the wide time frame of recruitment to the parent trials, participants overall had a lower CD4 nadir (median 149 cells/μl, IQR 39–309) and a higher percentage of detectable HIV VLs (60; 39%) than would be expected in an exclusively modern cohort.

Overall, participants were predominantly male (159; 93%) with a median age of 43 years (IQR 39–51). Most of the participants (101; 59%) identified as White, with 49 (29%) identifying as Black or African-American and 18 (11%) as Hispanic; there were no differences between cases and controls ($P = .380$; see Table 1).

In univariate analyses, having hypertension, having a family history of AMIs, being a current smoker, and having a higher total cholesterol-HDL ratio were each associated with an increased risk of an AMI. The HIV-specific variables of CD4%, CD8%, CD4/CD8 ratio, and nadir CD4+ T cell count were also associated with AMI, while a detectable HIV VL and currently taking ART were not (see Table 1).

Of the biomarkers of cardiovascular risk, only LBP (odds ratio [OR] 1.86, 95% confidence interval [CI] 1.26–2.73; $P = .001$) and TNFα (OR 1.42, 95% CI 1.00–2.03; $P = .048$) were significantly associated with future AMIs in a univariate analysis (see Table 1).

In the multivariate model adjusted for hsCRP, the presence of traditional cardiovascular risk factors and a lower CD4/CD8 T cell ratio, a higher LBP remained associated with the risk of a future AMI (OR 1.62, 95% CI 1.06–2.48 per 10 pg/ml increase; $P = .025$).

LBP correlated with a number of demographic and laboratory factors, including cigarette quantity (rho 0.32; $P = .038$), HIV VL (rho 0.33; $P < .001$), HDL cholesterol (rho −0.24; $P = .007$), and total cholesterol-HDL ratio (rho 0.24; $P = .0015$), CD4% (rho −0.34; $P = < .001$), CD8% (rho 0.29; $P = .001$), CD4/CD8 ratio (rho −0.37; $P < .001$), and body weight (rho −0.159; $P = .04$). LBP was correlated with hsCRP (rho 0.28; $P < .001$), TNFα (rho 0.25; $P < .001$), soluble immunoglobulin-like cell adhesion molecules-1 (rho 0.18; $P = .013$), and soluble vascular cell adhesion molecules-1 (rho 0.22; $P = .0025$), but not with interleukin-6, soluble CD40 ligand, or sP-selectin. There was a trend towards higher LBP levels in older samples, but LBP remained significantly associated with AMI when the age of the sample was included in the final model (OR 1.62, 95% CI 1.06–2.48; $P = .025$).

DISCUSSION

In this study, LBP was significantly associated with an increased risk of AMI in PWHIV. This remained true following adjustments for both traditional risk factors and hsCRP, suggesting that LBP may increase accuracy in predicting myocardial risks above those of more generalized inflammatory markers. While it's known that LBP is increased in untreated HIV infections [5], prior to this work, the association between LBP and coronary artery disease in PWHIV was unknown.

An acute phase protein with an important role in regulating microbial translocation–induced inflammation, LBP synthesis in the liver is highly inducible by LPS [6]. In contrast, LBP expression in macrophages is regulated by the liver X receptor, which regulates sterol metabolism and inflammation in response to oxidized cholesterol derivatives [7]. The liver X receptor–induced transcription of LBP has been shown to increase the survival of macrophages and promote atheroprogression [3]. In the general population, higher LBP has been associated with coronary artery disease and all-cause and cardiovascular mortality [4, 8]. Its levels also correlate with atherosclerotic severity (as estimated by the degree of stenosis or number of vessels involved on angiographic assessment) [8]. Because LBP plays an active role in the pathogenesis of atherosclerosis, rather than just being a nonspecific marker of systemic inflammation (as is hsCRP), it has the potential to show greater discrimination for coronary endpoints.

Following HIV acquisition, there is depletion of gut-associated lymphoid tissue, leading to the increased translocation of microbial products (most notably LPS) into the systemic circulation [9]. LPS is elevated in individuals with progressive HIV and correlates with markers of innate and adaptive immune activation [9]. This chronic immune activation and inflammation, in turn, promotes atherosclerosis [10]. The increase in microbial translocation seen with acute HIV infection is only minimally improved by ART, which may be why PWHIV remain at increased risk for CVD despite suppressive ART and why current antiretroviral exposure was not associated with

Table 1. Participant Demographics and Univariate Analysis

Variable	Cases, n (%) or median (IQR)	Controls	OR[a]	95% CI	*P* Value[b]
n	57	114	…	…	…
Age, years	43 (39, 51)	43 (40, 50)	1.47	0.92–2.34	.103
Gender, male	53 (93)	106 (93)	…	…	…
Race					
White	35 (61)	66 (58)	reference		…
Black	17 (30)	32 (28)	0.99	0.50–1.96	.997
Hispanic	5 (9)	13 (12)	0.61	0.17–2.15	.046
Cardiovascular risk factors					
Diabetes mellitus	1 (3)	8 (12)	0.28	0.03–2.32	.241
Hypertension	**11 (31)**	**6 (9)**	**5.08**	**1.25–19.14**	**.016**
Family history of AMI[c]	**11 (34)**	**9 (14)**	**7.56**	**1.63–34.92**	**.010**
Current smoker	**21 (58)**	**10 (14)**	**6.04**	**2.22–16.43**	**<.001**
Systolic BP, mmHg	127 (113–137)	120 (110–132)	1.01	0.98–1.03	.337
Body mass index, kg/m^2	25.5 (22.9–28.5)	26.0 (24.1–28.1)	1.03	0.95–1.12	.368
Lipids					
Total cholesterol, mg/dl	**196.5 (180–240)**	**183 (160–211)**	**1.13**	**1.02–1.25**	**.019**
HDL cholesterol, mg/dl	37 (30–49)	39 (33–53)	0.97	0.94–1.00	.166
LDL cholesterol, mg/dl	109 (89–153)	104 (81–143)	1.00	0.99–1.01	.180
Triglycerides, mg/dl	220 (148–337)	169 (107–237)	1.00	0.99–1.00	.087
Total cholesterol/HDL ratio	**5.25 (3.73–7.08)**	**4.5 (3.63–5.38)**	**1.45**	**1.12–1.86**	**.004**
HIV parameters					
CD4 %	**19 (10–29)**	**25 (15–33)**	**0.96**	**0.92–0.99**	**.034**
CD8 %	**53 (45, 61)**	**47 (37, 58)**	**1.03**	**1.00–1.06**	**.012**
CD4/CD8 ratio	**0.37 (0.15, 0.59)**	**0.53 (0.25, 0.85)**	**0.85**	**0.76–0.96**	**.009**
Nadir CD4+ count, cells/µl	**53 (16.5, 203.5)**	**193 (59, 345)**	**0.95**	**0.92–0.99**	**.016**
Detectable HIV VL[d]	26 (47)	34 (34)	1.54	0.73–3.33	.250
On ART	28 (49)	56 (29)	1.00	0.41–2.42	1.00
Duration of ART, years	5.1 (0.0, 9.2)	5.6 (0.0, 10.6)	0.94	0.85–1.05	.320
Plasma biomarkers					
LBP, pg/ml	**19.6 (10.0, 26.8)**	**12.1 (6.6, 19.6)**	**1.86**	**1.26–2.73**	**.001**
hsCRP, ng/ml	0.42 (0.11, 1.26)	0.16 (0.08, 0.44)	1.09	0.90–1.31	.356
IL-6, pg/ml	7.2 (2.5, 10.1)	3.77 (1.5, 8.6)	1.00	0.95–1.04	.950
sP-selectin, ng/ml	1.52 (1.10, 2.42)	1.46 (0.98, 2.06)	1.11	0.85–1.44	.415
sICAM-1, ng/ml	1.90 (1.35, 2.70)	1.29 (1.06, 1.83)	1.01	0.98–1.04	.406
sVCAM-1, ng/ml	12.2 (10.4, 15.3)	12.1 (10.0, 14.3)	1.01	0.92–1.12	.687
sCD40L, pg/ml	1019 (426, 1922)	859 (444, 1524)	1.00	0.99–1.00	.595
TNFα, pg/ml	**15.3 (10.7, 22.4)**	**13.1 (9.6, 19.0)**	**1.42**	**1.00–2.03**	**.048**

All results are of the last result available prior to the date of AMI, or the AMI date in matched cases for controls. Statistically significant results are highlighted in bold. Reference, This is to show that "white race" is the reference level and other odds ratios are in reference to the white (ie, in comparison to white participants, those of hispanic background were less likely to have a heart attack).

Abbreviations: AMI, acute myocardial infarction; ART, antiretroviral therapy; BP, blood pressure; CI, confidence interval; HDL, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; IQR, interquartile range; LBP, lipopolysaccharide binding protein; LDL, low-density lipoprotein cholesterol; OR, odds ratio; sCD40L, soluble CD40 ligand; sICAM-1, soluble immunoglobulin-like cell adhesion molecules-1; sP-selectin, soluble platelet selectin; sVCAM-1, soluble vascular cell adhesion molecules-1; TNFα, tissue necrosis factor alpha; VL, viral load.

[a]ORs for continuous variables are per 1 unit change, except total cholesterol (per 10 mg/dl), nadir CD4 (per 10 cells/ul), CD4/CD8 ratio (per 0.1 unit change), LBP, and TNFα (per 10pg/ml change).

[b]Calculated using univariate conditional logistic regression (taking into account matched status).

[c]Defined as a history of AMI in a first-degree male relative prior to the age of 55 or a first-degree female relative prior to the age of 65.

[d]Defined as ≥50 copies/ml, VL data at the time of AMI were missing in 19 (16%) controls and 4 (7%) cases.

AMI in this current study [11]. This study also provides evidence to support the proposed link between HIV and disruptions of the gastrointestinal barrier, by finding that a detectable HIV VL was associated with higher levels of LBP.

It remains to be seen whether interventions aimed at reducing microbial translocation, dyslipidaemia, or inflammation in PWHIV will impact LBP levels. A recent randomized controlled trial in a population with HIV found that statin therapy was not associated with changes in LBP [12].

This work has a number of limitations, including the small number of cases identified, despite the large pool of participants involved in ACTG trials over this period. Individuals recruited into these trials were likely at low risk for CVD, and those who did have events may thus, in some ways, not be representative

of PWHIV in general; a deficit of female and older participants limited the generalizability of the results to these populations also. Some of the data were from participants recruited into trials in the early 2000s, reflected in the lower rates of viral suppression, likely the consequence of older ART regimens in use. We were not able to comment on the impacts of specific ART regimens on AMI or LBP levels, given the wide variety of agents in use. Our ability to adjust for potential confounders was partially limited, as some data (such as hepatitis C status) were not collected in all trials. Missing data may also have affected our results.

CONCLUSION

LBP may be a novel marker of cardiovascular risk, and may have specific utility in PWHIV. This study supports further investigation into its role and predictive capacity in HIV-associated CVD.

Supplementary Data

Supplementary materials are available at *Clinical 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.

Notes

Author contributions. J. M. T. conceived the study design, performed the analysis, and prepared the manuscript. C. M. assisted with the statistical analysis and data extraction. J. S. C. conceived the study design with J. M. T. and assisted in drafting the manuscript. T. S. performed the laboratory analysis and assisted in the study design and drafting the manuscript.

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Financial support. This work was supported by the University of California, Los Angeles Center for AIDS Research (grant number 5P30 AI028697 for administrative support; grant numbers DK118086 and HL139549 for technical staff), the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (award numbers UM1 AI068634, UM1 AI068636, and UM1 AI106701), and the AIDS Clinical Trials Group Specimen Repository Pilot (grant number 116529:1).

Potential conflicts of interest. J. M. T. is supported by an Australian National Health and Medical Research Centre Early Career Fellowship (grant number APP1111626) and has received personal fees from Gilead Health Sciences, outside the submitted work. C. M. has received personal fees from Northwestern University's Division of Infectious Diseases, outside the submitted work. J. S. C. has received grants from Theratechnologies, outside the submitted work. T. S. reports no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Pirš M, Jug B, Eržen B, et al. Cardiovascular risk assessment in HIV-infected male patients: a comparison of Framingham, SCORE, PROCAM and DAD risk equations. Acta Dermatovenerol Alp Pannonica Adriat **2014**; 23:43–7.
2. Feinstein MJ, Hsue PY, Benjamin LA, et al. Characteristics, prevention, and management of cardiovascular disease in people living with HIV: a scientific statement from the American Heart Association. Circulation **2019**; 140:e98–e124.
3. Sallam T, Ito A, Rong X, et al. The macrophage LBP gene is an LXR target that promotes macrophage survival and atherosclerosis. J Lipid Res **2014**; 55:1120–30.
4. Lepper PM, Schumann C, Triantafilou K, et al. Association of lipopolysaccharide-binding protein and coronary artery disease in men. J Am Coll Cardiol **2007**; 50:25–31.
5. Lichtfuss GF, Hoy J, Rajasuriar R, Kramski M, Crowe SM, Lewin SR. Biomarkers of immune dysfunction following combination antiretroviral therapy for HIV infection. Biomark Med **2011**; 5:171–86.
6. Ding PH, Jin LJ. The role of lipopolysaccharide-binding protein in innate immunity: a revisit and its relevance to oral/periodontal health. J Periodontal Res **2014**; 49:1–9.
7. Zhao C, Dahlman-Wright K. Liver X receptor in cholesterol metabolism. J Endocrinol **2010**; 204:233–40.
8. Lepper PM, Kleber ME, Grammer TB, et al. Lipopolysaccharide-binding protein (LBP) is associated with total and cardiovascular mortality in individuals with or without stable coronary artery disease–results from the Ludwigshafen Risk and Cardiovascular Health Study (LURIC). Atherosclerosis **2011**; 219:291–7.
9. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med **2006**; 12:1365–71.
10. Ross R. Atherosclerosis–an inflammatory disease. N Engl J Med **1999**; 340:115–26.
11. Neuhaus J, Jacobs DR Jr, Baker JV, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J Infect Dis **2010**; 201:1788–95.
12. Longenecker CT, Sattar A, Gilkeson R, McComsey GA. Rosuvastatin slows progression of subclinical atherosclerosis in patients with treated HIV infection. AIDS **2016**; 30:2195–203.