CORONARY HEART DISEASE RISK AND HIV INFECTION: THE ROLES OF INFLAMMATION AND CO-MORBID DISEASE

by

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ABSTRACT

My research interest is the intersection of communicable and non-communicable (chronic) disease. Human immunodeficiency virus (HIV) infection and coronary heart disease (CHD) provide an interesting model to study this intersection because 1) treated HIV infected people are living long enough to die from non-AIDS causes 2) ischemic heart disease is the leading cause of death worldwide and 3) HIV/AIDS is among the top 10 causes of death. It is therefore of public health importance to examine the mechanisms by which HIV contributes to CHD. This dissertation focuses on the roles that co-morbdity and inflammation play within this intersection.

The first intersection, lipid and lipoprotein dysregulation, is a great place to start because it 1) co-occurs with HIV (and other) infections, 2) is an important risk factor for atherosclerotic CHD and 3) is modifiable with existing, inexpensive therapy. We review lipid and lipoprotein metabolism alterations in untreated HIV infection.

The second intersection is inflammation, an immune response process and an integral component of atherosclerosis. Prior work was limited by comparisons of inflammatory biomarkers in HIV infected and (typically healthier) uninfected populations from different cohorts and limited adjustment for comorbid conditions that also influence these biomarkers.

Paper 1 compares inflammatory biomarkers between HIV infected and behaviorally and demographically similar uninfected Veterans with similar burdens of comorbid disease.

The third intersection focuses on other diseases co-morbid with HIV that also contribute to CHD risk. Specifically, Paper 2 compares inflammatory biomarkers between HIV infected people with or without hepatitis C co-infection as an example of HIV-related comorbidity. Paper 3 compares risk for acute myocardial infarction by blood pressure and HIV status as an example of comorbidity that is not directly related to HIV infection.

In summary, this dissertation contributes insights into the pathogenesis of CHD in the setting of HIV infection. It emphasizes the importance of studying HIV as a disease with pleiotropic effects (on biomarkers and organ systems) that can interact with each other, modifying their independent contributions to CHD risk.

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PREFACE

I would not have been able to start, continue or complete this dissertation without the support of my wife, my family, my housemates, my friends, my co-workers, the VACS Project team, the VACS participants, Lori Smith, Gwendolyn O'Brien, Lindsay Flynn, Carol Rogina, Amy Justice, and Matthew Freiberg. Thank you.

1.0 LITERATURE REVIEW: HIV INFECTION AND DYSREGULATION OF LIPID & LIPOPROTEIN METABOLISM

People with human immunodeficiency virus (HIV) may be at increased risk for coronary heart disease (CHD) due at least in part to pro-atherogenic lipid and lipoprotein dysregulation associated with the virus and its treatment. This lipid and lipoprotein dysregulation occurs as part of the host's normal immune response [1]. There is a net beneficial effect of *acute* lipid dysregulation to the host. However, with *chronic* infections such as HIV, comes chronic dysregulation of lipids and lipoproteins, some of which are associated with atherosclerotic heart disease. To understand the contribution of HIV to CHD via an atherosclerotic mechanism, it is important to understand exactly how HIV infection alters lipid and lipoprotein metabolism independently of antiretroviral therapy (ART). We hypothesize that a difference in etiology of lipid dysregulation in HIV infected persons compared to uninfected persons may result in different effects of lipid dysregulation on CHD risk and may require different approaches to treating lipid dysregulation in HIV infection.

1.1 AIMS

We conducted a literature review with two aims: 1) to describe HIV-related changes in the rates of synthesis and clearance of serum lipids and lipoproteins independently of ART, 2) to describe how HIV-related changes in serum lipid/lipoprotein metabolism alter the content and distribution of cellular lipids. We propose a mechanism wherein HIV may benefit from the chronic dysregulation in lipid and lipoprotein metabolism.

1.2 METHODS

We searched PubMed on 08/07/2012. We created searches to define the following concepts (Table A1): lipids/lipoproteins, HIV, kinetic studies, HIV uninfected, antiretroviral therapy naïve, title pertaining to lipids or lipoproteins, hypertriglyceridemia, elevated free fatty acids (FFAs), high density lipoprotein (HDL), HDL- triglycerides, low or very low density lipoprotein (LDL, VLDL), LDL- or VLDL- cholesterol reduction, LDL or VLDL triglycerides, cell membrane structure.

For Aim 1, we combined the search concepts for lipids and lipoproteins, HIV, kinetic studies with those for 1) HIV uninfected or 2) antiretroviral therapy naïve or 3) title pertaining to lipids or lipoproteins. We then discarded abstracts that were either irrelevant to lipid/lipoprotein kinetics in HIV, described lipid/lipoprotein responses to drug therapy, or did not have an HIV uninfected or antiretroviral therapy naïve control group. We selected articles that investigated the independent effect of HIV on lipid/lipoprotein kinetics.

For Aim 2, we combined the search concepts for hypertriglyceridemia, elevated FFAs, HDL/LDL/VLDL cholesterol reduction, and HDL/LDL/VLDL triglycerides with the concepts for cell membrane structure. We selected articles that described how changes in serum lipids concentrations affect cellular lipid concentrations and distribution.

1.3 RESULTS

1.3.1 The bigger picture – infection and changes in metabolism

The literature on changes in protein and energy metabolism following infection is vast. A consistent pattern of negative protein and energy balance has been associated with localized and systemic infections by different pathogens [2-5]. This is relevant to this literature review because lipoproteins, as their names suggest, are proteins, and lipids, such as triglycerides, are energy sources.

AIDS patients receiving total parenteral nutrition have been shown not to gain lean body mass when an active secondary infection was present [6]. This highlights an important difference between the metabolic effects of infection versus malnutrition. Macallan et al demonstrated an increase in whole body protein turnover in HIV infected versus uninfected persons [7]. Asymptomatic ART-naïve HIV infection has been associated with 7-15% increase in resting energy expenditure [8,9], with higher levels reported amongst AIDS patients [10,11]. Other work has reported reduced energy intake, rather than elevated total daily energy expenditure, as the driving factor behind weight loss in HIV-associated wasting [12]. Additionally, infections co-morbid with HIV, such as hepatitis C and tuberculosis also have effects of on energy metabolism [13,14].

This context of metabolic alterations is necessary to understand that the alterations in lipids and lipoproteins presented below are occurring as part of a larger set of metabolic changes that accompany infection.

1.3.2 A brief primer on lipid and lipoprotein metabolism

Cells need lipids to maintain their structure and as an alternate source of energy to glucose. Lipids are obtained exogenously (from diet) or endogenously (primarily synthesized in the liver). Lipids are transported to and from cells by lipoproteins. Lipoproteins are often distinguished by the apolipoproteins with which they are associated.

The endogenous and exogenous pathways of lipid and lipoprotein metabolism are summarized in Figure 1[15]. In the exogenous pathway, dietary cholesterol and esterified triglycerides are combined with apolipoprotein B-48 (apoB-48), phospholipid, and free cholesterol to create chylomicrons in intestinal cells. Chylomicrons acquire apoliporotein E (apoE) and apolipoprotein C (apoC) from other lipoproteins and lose triglycerides (delipidation via the action of lipoprotein lipase (LPL)) to form chylomicron remnants. Chylomicron remnants are removed from circulation by the liver via the interaction between LDL-related protein, LDL-receptors, and hepatic apoE and apoB receptors.

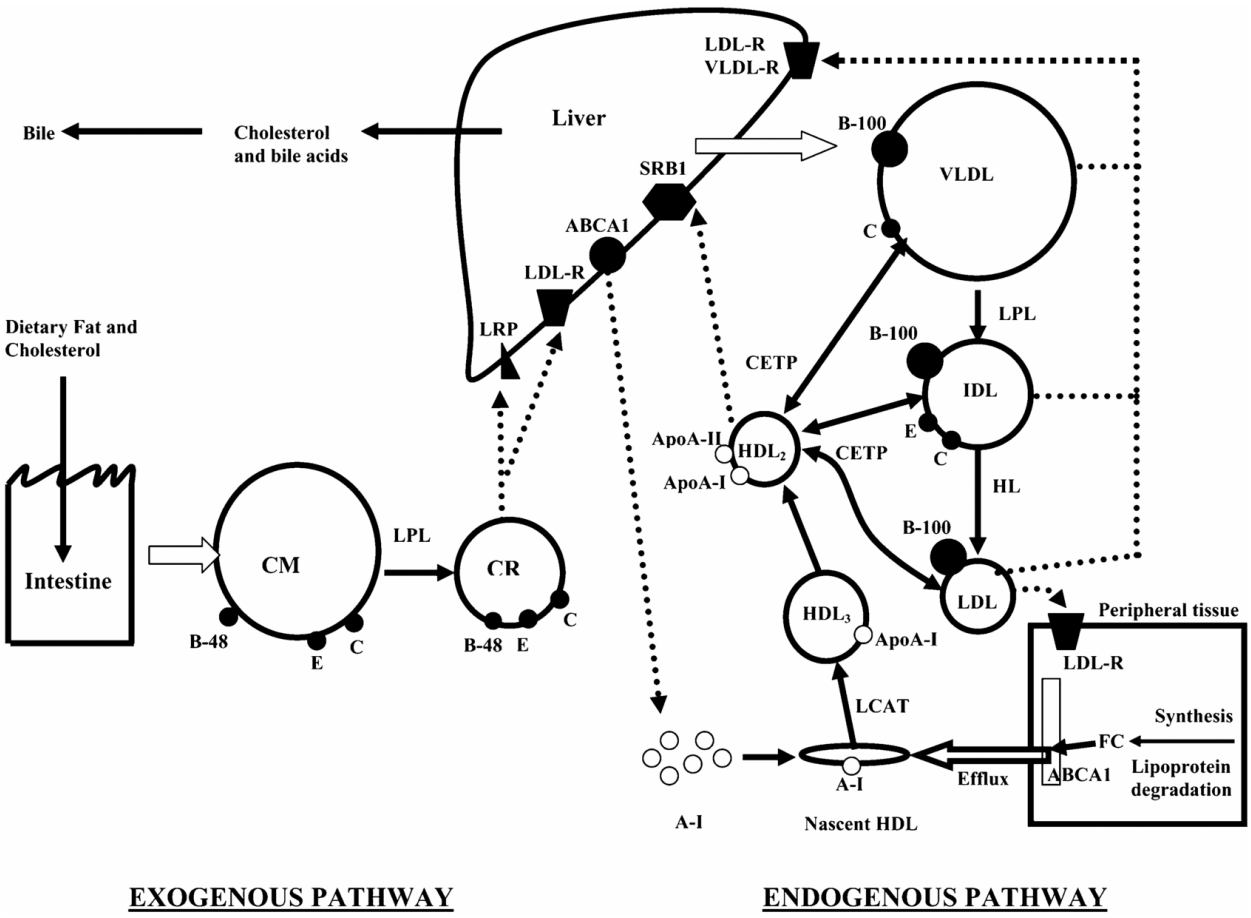

Figure 1. Exogenous and endogenous pathways of lipid and lipoprotein metabolism. Copied with permission from Chang et al [15]

Triglycerides and cholesterol synthesized endogenously are transported into circulation by VLDL. VLDL is broken down to form VLDL remnants, intermediate density lipoprotein (IDL) (transiently) or LDL via LPL or hepatic lipase action. LDL cholesterol is taken up into extrahepatic tissue via the interaction between apoB-100 and LDL receptors on plasma membranes. LDL cholesterol, VLDL remnants and IDL-cholesterol that are not taken up by extrahepatic cells are returned to the liver via interaction with VLDL receptors, LDL-related protein and apoB-100 or apoE (for IDL). Unused cholesterol from within extrahepatic cells is effluxed by HDL through the action of reverse cholesterol transport mediated by apolipoprotein A-I (apoA-I) and apolipoprotein A-I (apoA-II). Free cholesterol in HDL is taken up by the liver via scavenger receptor BI (SR-BI) while esterified HDL-cholesterol can be transferred to VLDL, IDL or LDL via cholesterol ester transfer protein (CETP) in exchange for triglycerides.

1.3.3 A brief primer on stable isotope kinetic studies

The information in this primer is mainly summarized from a review by Chan et al [15]. Stable isotope kinetic studies allow us to quantify lipid and lipoprotein metabolism. For them studies to describe the synthesis and breakdown of these particles, a tracer and a trace are needed. A tracer is a moiety involved in the kinetics of the system under investigation. A tracee is used to track the tracer. For example, Apo-B100 remains associated with VLDL, IDL, and LDL and so is a suitable tracee for these lipoproteins. Apo-AI and apo-AII, though not permanently associated with HDL, still make for suitable tracees with the help of isotopically labeled amino acids (tracers) and compartment modeling.

Compartment models partition a biological system into a set of interconnected compartments through which tracees and their associated tracers move and are tracked. Stable

isotope tracers like ^{2}H and ^{13}C labeled leucine are injected into a subject and endogenously incorporated into apolipoproteins (and subsequently lipoproteins), or other tracees of interest. Mathematical (differential) equations are used to model the movement of labeled lipoproteins through the compartments of the system enabling description of the kinetics of the metabolic pathways involved in the system. The concepts of endogenous labeling and compartment models are illustrated in Figure 2 and Figure 3[15].

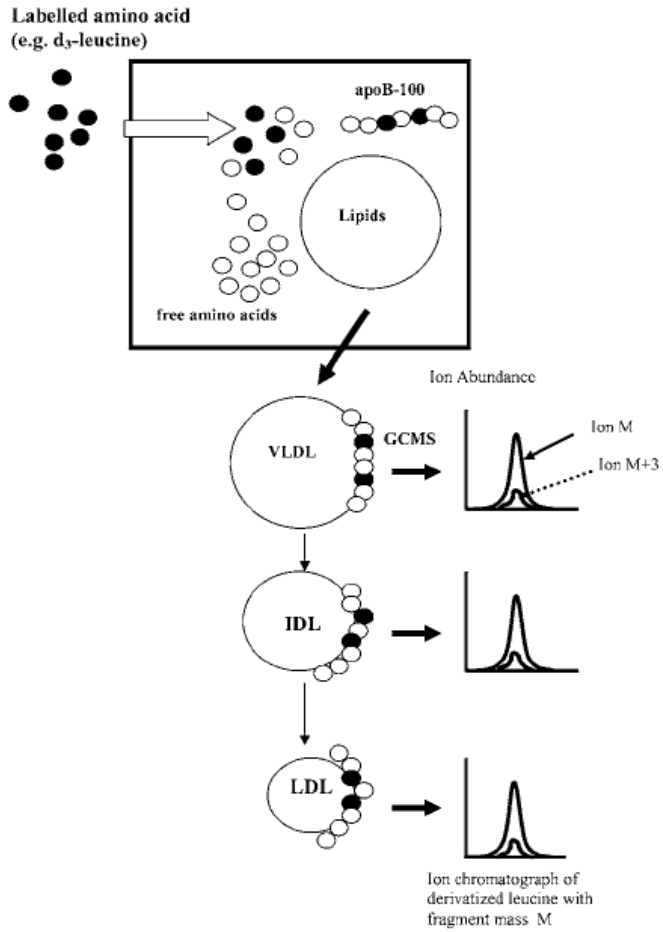

Figure 2 **Principle of endogenous labelling of apoB-100 with an amino acid labelled with a stable isotope and detection by GC–MS**

Newly synthesized apoB-100 is incorporated with labelled (●) and unlabelled (○) amino acids. d_3-leucine, [$^2H_3$]leucine.

Figure 2. Endogenous isotopic labeling. Copied with permission from Chang et al. [15]

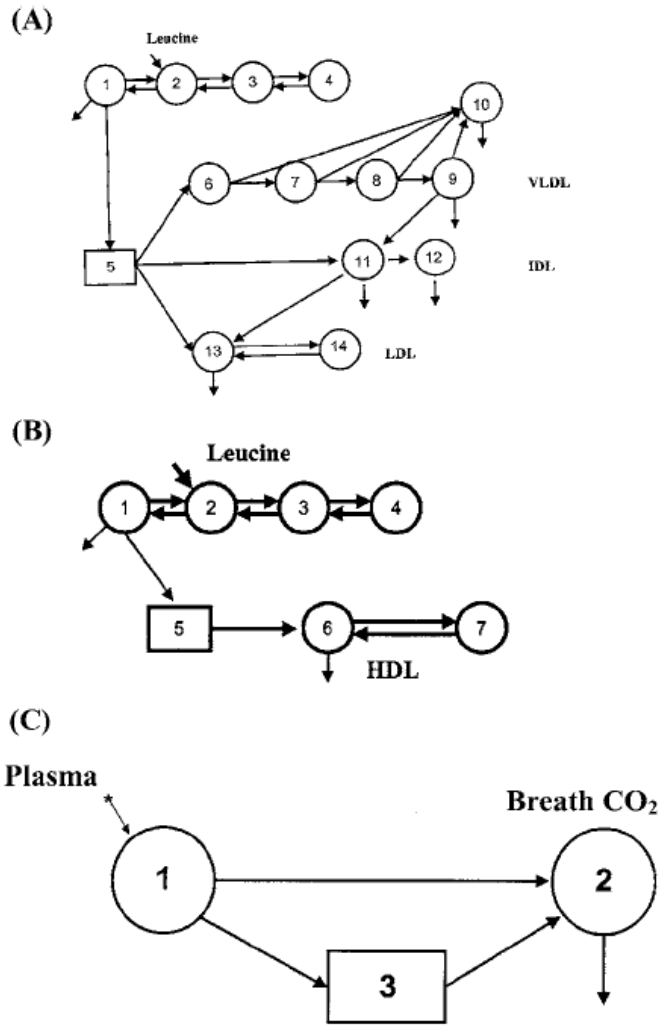

Figure 3 Compartment models describing apoB-100 (A), apoA-I (or apoA-II) (B) and chylomicron remnant-like emulsion (C)

Figure 3. Compartment models. Copied with permission from Chang et al[15]

Outcomes from kinetic studies include absolute or fractional synthetic rates (ASR, FSR), fractional catabolic rate (FCR), clearance rate, secretion rate residence time, percentage uptake, and counts per minute (defined in Table 1).

Table 1: Definitions of outcomes from stable isotope kinetic studies

Outcome	**Definition**
Pool	Compartment
Pool size	The mass of tracee in a specific compartment determined as the product of plasma volume and trace concentration
Fractional synthetic rate (FSR)	The rate of incorporation of a precursor into a product per unit of product mass. Calculated as quotient of initial rate of change of in product enrichment and initial precursor enrichment
Absolute synthetic rate (ASR)	Product (mathematical) of FSR and mass of product
Fractional catabolic rate (FCR)	The fraction of tracee irreversibly removed from a compartment per unit of time.
Clearance rate	The rate at which the tracee leaves the pool.
Secretion rate	The rate at which the tracee enters the pool. Derived from the product of FCR and tracee pool size.
Residence time	Inverse of FCR

1.3.4 Effects of HIV infection on blood lipid and lipoprotein kinetics.

Aim 1 was to describe HIV-related changes in the rates of synthesis and clearance of blood lipids and lipoproteins independently of antiretroviral therapy (ART). We found six studies that met criteria for Aim 1 and included 2 additional studies due to the scarcity of data on HDL kinetics in this population (Table 2). All selected studies had HIV-uninfected control groups. Three studies explicitly included HIV treatment naïve participants [16-18], one study was conducted before widespread availability of antiretroviral therapy [19], and two studies were conducted *in vitro* [20,21]. The two studies that examined HDL kinetics were included though they did not include ART-naïve subjects [22,23]. Sample sizes ranged from 5 – 40 participants and matching, if performed, was often based primarily on age and gender.

Grunfeld et al published early data indicating slower triglyceride clearance in (presumably untreated) AIDS participants compared to age and gender matched uninfected participants [19]. Umpleby et al showed that HIV infection decreased synthesis of LDL in untreated HIV infected people compared to uninfected people, although treated individuals experienced decreased breakdown of LDL allowing it to remain in circulation for a longer time [17]. In this study, controls were younger (~33 vs. 40 years), less likely to be male, and had slightly lower mean BMI (~23 vs. 24 kg/m^2). Participants were predominantly Caucasian.

In response to graded glucose infusion, ART treatment-naïve individuals had higher plasma FFA levels and plasma triglyceride levels than did uninfected individuals. This difference was maintained after ART initiation and eight weeks of treatment [18]. This group also showed that secretion rates for apolipoprotein-B associated with very low-density lipoproteins (VLDL-apoB) and VLDL-triglyceride were lower in treatment-naïve HIV-infected versus uninfected

participants though the rates were similar after ART initiation among infected participants [18]. The fractional catabolic rate (FCR) and clearance of VLDL-apoB and VLDL-TG were also lower in treatment-naïve HIV infected versus uninfected participants, a finding that persisted eight weeks after initiation of ART [18]. Again, controls were younger (31 vs. 41 years) with slightly lower mean BMI (22.5 vs. 24.5 kg/m$^{2;}$ p=0.12).

Shahmanesh et al reported no significant differences in lipid kinetics between treatment-naïve infected individuals and uninfected individuals [16]. However, in treated individuals, VLDL-apoB and intermediated-density lipoprotein-apoB (IDL-apoB) FCRs were lower, while absolute secretion rates (ASRs) were similar and consequently residence times were higher compared to uninfected individuals [16]. Participants were predominantly white and controls were again younger (~33 vs. 40 years) and more likely to be female.

In two separate studies [22] [23], Jahoor et al showed higher FSR of HDL-apoA1 in treated HIV-infected males and children compared to uninfected controls. In both studies, there was no difference in ASR of HDL-apoA1 by HIV status. In the adult study, controls were younger (35 vs. 40 years) and had lower mean BMI (21 vs. 24 kg/m^2), whereas in the pediatric study, the controls were less likely to be malnourished.

In-vitro data showed decreased synthesis of lipid precursors (phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine) and major lipid synthetic intermediates (phosphatidic acid, diacylglycerol (DAG)) in human T-lymphotrophic virus type III (HTLVIII) infected versus uninfected ERIC cells (CD4+ T-cell line susceptible to HIV cytopathology). There was, however, increased synthesis of neutral lipids (triglycerides, cholesterol esters) in these cells [21]. A separate in-vitro study found increased synthesis of DAG, 1-alkyl-2-acyl-sn-

glycerol (AAG) [both diglycerides], and ceramide (a glycosphingolipid) after eight days of HTLV-III infection of CEM cells (human leukemia cell line).

With regard to serum/plasma lipids, these studies described lower or similar total cholesterol, HDL-cholesterol, LDL-cholesterol and apo-B100, and higher or similar triglycerides in infected versus uninfected individuals.

Table 2: Summary of articles selected for literature review describing effects of HIV infection on lipid and lipoprotein metabolism

Year [Ref]	Relevant study procedures	Study groups	HIV/ART Duration (years)	Lipid(s)/ lipoprotein(s)/ measured	Relevant result(s)
2005 [16]	Overnight fast, baseline blood sample, 1-^{13}C Leucine infusion (9 hours), blood samples during infusion every 30 minutes Exclusions include Fasting glucose >6mmol/L, lipid altering drugs, elevated liver enzymes, recent weight loss ≥10%	40 HIV+ ART-treated 15 PI+, 25 (PI-) Current CD4: 435±187 (PI+), 469±265 (PI-)) Pre-ART HIV RNA (log copies/ml): 5.12±0.54 (PI+), 4.83±0.74 (PI-) 15 HIV+ treatment naïve (TN). Current CD4: 367±222) HIV RNA (log copies/ml): 4.39±0.76 12 presumed uninfected(high HIV risk individuals required negative test within prior 3 months)	HIV duration: not reported ART duration PI+: 4.1±1.2 PI-: 2.6±0.2	VLDL-apoB100, IDL-apoB100, Plasma total VLDL-c, IDL-c, VLDL-TG, IDL-TG, HDL-c, LDL-c Nonesterified fatty acids (NEFA)	Lower plasma HDL-c and higher IDL-TG in TN HIV+ vs. HIV- No difference in other plasma lipoprotein-cholesterol in TN HIV+ vs. HIV- Lower plasma HDL-c and higher TG, IDL-TG and IDL-c in PI group vs. HIV- Lower plasma HDL-c and higher TG in (PI-) group vs. HIV- No difference in NEFA between any HIV+ vs. HIV- No significant differences in lipid kinetics between TN HIV+ and HIV- VLDL-apoB and IDL-apoB FCRs were lower in the treated groups vs. HIV- No significant differences in VLDL-apoB and IDL-apoB ASRs in treated groups vs. HIV- VLDL-apoB and IDL-apoB residence times were higher in the treated groups vs. HIV-

Table 2 continued

Year [Ref]	Relevant study procedures	Study groups	HIV/ART Duration (years)	Lipid(s)/ lipoprotein(s)/ measured	Relevant result(s)
1992 [19]	Standardized diet, overnight fast, plasma sampling, heparin administration, plasma sampling (7 samples over 50 minutes) Exclusions include Diabetes, renal failure, nephrotic syndrome, active hepatitis, cirrhosis, lipid altering drugs	29 HIV+ including 14 HIV+ without history of opportunistic infection or malignancy, and 15 HIV+ with AIDS 16 HIV antibody negative age- and gender-matched uninfected	HIV duration not reported ART duration not reported; Study likely completed before widespread availability of ART	TG, HDL-c, HDL_2-c, HDL_3-c, apo-A1, apo-B100, calculated LDL-c and VLDL-c, postheparin lipase, FFA TG, cholesterol, phospholipid and protein content of HDL LDL and VLDL	Higher plasma TG in AIDS vs. HIV+, AIDS vs. HIV- but not HIV+ vs. HIV- Lower serum cholesterol in AIDS and HIV+ vs. HIV- Lower HDL-c, HDL_2-c, HDL_3-c, and apo-A1 in AIDS and HIV+ vs. HIV- Lower LDL in AIDS and lower apo-B100 in AIDS and HIV+ vs. HIV- Higher calculated VLDL in AIDS vs. HIV+, AIDS vs. HIV- but not HIV+ vs. HIV- Higher FFA in AIDS vs. HIV+, AIDS vs. HIV- but not HIV+ vs. HIV- Slower clearance of TG-rich particles in AIDS vs. HIV+, AIDS vs. HIV-, and HIV+ vs. HIV- Significant correlation between TG clearance time and TG levels Higher LDL-TG and lower LDL-phospholipid content but similar LDL-c and LDL-protein content in AIDS vs. HIV- Higher HDL-TG and lower HDL-c content but similar HDL-protein and HDL-phospholipid content in AIDS vs. HIV- No difference in VLDL composition in AIDS vs. HIV- No difference in LDL-TG or HDL-TG content in HIV+ vs. HIV- (data not shown) Lower concentrations of total, hepatic (AIDS only) and lipoprotein lipase in AIDS and HIV+ vs. HIV-

Table 2 continued

Year [Ref]	Relevant study procedures	Study groups	HIV/ART Duration (years)	Lipid(s)/ lipoprotein(s)/ measured	Relevant result(s)
2005 [17]	Overnight fast, baseline blood sample, 1-^{13}C leucine infusion (9 hours), blood samples during infusion every 60 minutes Exclusions included Fasting glucose >6mmol/L, lipid altering drugs, elevated liver enzymes, recent weight loss ≥10%	39 HIV+ ART treated 15 PI+, 24 (PI-): Current CD4: 435±187(PI+), 479±266 (PI-); Pre ART HIV RNA (log copies/ml): 5.1±0.5 (PI+), 4.8±0,8 (PI-) 13 HIV+ treatment naïve (TN). Current CD4: 377±238; HIV RNA (log copies/ml): 4.3±0.8	HIV duration not reported ART (PI+): 4.1±1.2, ART(PI-): 2.6±1.2	Plasma total LDL-c, LDL-TG, HDL-c, LDL-apoB100, oxidized LDL (oxLDL) Nonesterified fatty acids (NEFA)	No difference in plasma TG in TN vs. HIV- Higher plasma TG in PI+ but not PI- vs. HIV- Lower HDL-c in all HIV+ groups vs. HIV- No difference in LDL-c, LDL-c/apoB ratio, LDL-TG, LDL-TG/apoB ratio, oxLDL/apoB ratio or NEFA by HIV status Lower LDL-ASR in TN and treated HIV+ vs. HIV- Lower LDL-FCR and higher LDL-RT in treated HIV+ vs. HIV- No difference in LDL-FCR or LDL-RT in TN HIV+ vs. HIV-
		12 presumed uninfected (high HIV risk individuals required negative test within prior 3 months)			

Table 2 continued

Year [Ref]	Relevant study procedures	Study groups	HIV/ART Duration (years)	Lipid(s)/ lipoprotein(s)/ measured	Relevant result(s)
2005 [18]	Procedures done before and 8 weeks after ART inititiation: Standard diet, overnight fast, baseline blood sample, glucose infusion study(day 1), 1-^{13}C Leucine infusion and 1,1,2,3,3-$^{2}H_5$ glycerol injection over 12 hours with blood sampling ~every 30 minutes for 3 hours, then every 60 minutes for 2 hours, then after 120, 180, 60 and 60 minutes (day 2) Exclusions included: Drugs affecting glucose or lipoprotein metabolism, diabetes, lipid disorders, unstable weight	13 treatment naïve (TN) males who subsequently started ARTs (regimens chosen independent of current study) 14 healthy, younger, normolipemic subjects with similar BMI	HIV duration: not reported ART: 8 weeks	FFA, VLDL-apoB, VLDL-TG Fasting plasma VLDL-TG, VLDL-c, VLDL-apoB, IDL-TG, IDL-c, IDL-apoB, HDL-TG, HDL-c, HDL-apoA1, glucose	Lower plasma glucose levels in pre-ART vs. HIV- but no difference in plasma glucose in post-ART vs. HIV-. Higher plasma FFA (in response to glucose infusion) in both pre-ART and post-ART phase in HIV+ vs. HIV- Higher total plasma TG (in response to glucose infusion) in both pre-ART and post-ART phase in HIV+ vs. HIV- No difference in VLDL-apoB and VLDL-TG levels between TN HIV+ and HIV- Higher VLDL-apoB and VLDL-TG in treated HIV+ vs. HIV- Lower VLDL-apoB FCR (and clearance) and VLDL-TG FCR (and clearance) in TN HIV+ vs. HIV- Lower VLDL-apoB secretion rate and VLDL-TG secretion rate in TN HIV+ vs. HIV- but similar rates in treated HIV+ vs. HIV-
1999 [22]*	10-hour overnight fast, weight/height measurement, baseline blood sampling, continuous $^{2}H_5$ phenylalanine infusion for 6 hours, blood sampling every hour.	5 males without secondary infections on ART 5 healthy, uninfected (3 male, 2 female)	4±1	HDL-apoA-I	Lower plasma concentration of HDL-apoA1 in HIV+ vs. HIV- Higher FSR of HDL-apoA1 in HIV+ vs. HIV- No difference in ASR of HDL-apoA1 in HIV+ vs. HIV-

Table 2 continued

Year [Ref]	Relevant study procedures	Study groups	HIV/ART Duration (years)	Lipid(s)/ lipoprotein(s)/ measured	Relevant result(s)
2003 [23]*	Standardized meal, baseline blood sampling, primed, intermittent 1-^{13}C leucine and $^{2}H_3$-leucine infusion for 4 hours, blood sampling at 5.25, 5.5, 5.75 hours from baseline	5 infants (3 males, 2 females) and 1 young male without secondary infection 4 uninfected infants (1 male, 3 females) born to HIV infected mothers from similar socio-economic background as HIV+	~0.5-2.5	HDL-apoA-I	Lower plasma concentration of HDL-apoA1 in HIV+ vs. HIV- Higher FSR of HDL-apoA1 in HIV+ vs. HIV- No difference in ASR of HDL-apoA1 in HIV+ vs. HIV-
1992 [20]	Infection of CEM cell line with HTLVIII-b, quantitation of cellular lipids after 4 and 8 days	CEM cells		Diacylglycerol (DAG), alkylacylglycerol (AAG), ceramide	No difference in phospholipid concentration between infected and uninfected cells Small increase in DAG after 4 days Higher AAG (2.1 fold) and ceramide (2.8 fold) in infected vs. uninfected cells after 4 days Higher DAG (1.8-fold), AAG (2.6-fold) and ceramide (4.1-fold) in infected vs. uninfected cells after 8 days.

Table 2 continued

Year [Ref]	Relevant study procedures	Study groups	HIV/ART Duration (years)	Lipid(s)/ lipoprotein(s)/ measured	Relevant result(s)
[21]	Infection of ERIC cell line with HTLVIII-b, addition of radiolabelled lipid precursor (oleate), lipid kinetics after 4 days	ERIC cells			Lower percentage uptake of radiolabelled tracer in infected vs. uninfected cells Lower counts per minute for radiolabelled tracer present in the following lipid classes in infected vs uninfected cells: phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidic acid, DAG. Higher counts per minute for radiolabelled tracer present in the following lipid classes in infected vs uninfected cells: triacylglycerol, cholesterol esters.

*Despite the lack of ART-naïve subjects, these studies were included in this review as they were the only ones that met other criteria for AIM 1 and examined HDL kinetics.
HIV RNA, CD4 counts (cells/dl), HIV duration and ART duration presented as mean ± standard deviation.
PI±: ART regimen including or not including a protease inhibitor

1.3.5 Effects of HIV infection on cellular lipids.

Aim 2 was to describe how HIV-related changes in lipid/lipoprotein metabolism (in serum) altered the content and distribution of *cellular* lipids and lipoproteins. The results from Aim 2 may be better understood in the context of the dependence of HIV replication on host cell lipids [24,25] (reviewed in [26,27]). However, it is important to remember that many of the alterations in lipid metabolism described in Aim1 are not specific to HIV infection [1].

HIV entry and budding of new virions have been shown to occur preferentially at lipid-enriched plasma membrane domains [24,28-31]. Lipid rafts have been described as dynamic, highly-ordered plasma membrane microdomains enriched in cholesterol, sphingolipids, and glycophosphatidylinositol-linked proteins [29,32] though their exact nature *in vivo* is debated [33,34]. Postulated reasons for this site preference include the importance of cholesterol as a structural element in the viral envelope [35] and proximity-mediated activation of signaling proteins that occurs when these microdomains coalesce into larger domains [27,36-38].

Specifically one structural hypothesis suggests that viral fusion to host cells may be optimized when the fluidity – determined by cholesterol and sphingolipid content – of the viral envelope is similar to that of the part of the host cell membrane where fusion occurs [35].

The signaling hypothesis suggests that viral entry at lipid rafts simultaneously impairs the immune response to infection and alters signaling events promoting the spread of the virus to uninfected cells. For example, resting T-lymphocytes are made susceptible to HIV infection by stimulation through CD40 receptor binding [39] and lipid rafts are associated with CD40 clustering [40].

1.3.6 Effects of HIV-related alterations in lipid and lipoprotein metabolism on cellular lipid content and distribution.

Given the potential importance of cellular lipids in viral replication, Aim 2 was to describe how serum lipids altered the content and distribution of cellular lipids and lipoproteins. We found 12 studies that met criteria for Aim 2. Most of these studies were performed in vitro or in animal models.

The results from Aim 1 suggest increased triglyceride levels associated with HIV due to increased VLDL triglyceride content and reduced triglyceride clearance. Wang et al [41] reported that triglyceride–rich lipoproteins congregate (colocalize) with lipid rafts on endothelial cells. Triglyceride-rich lipoprotein lipolysis and colocalization of triglyceride-rich lipoprotein remnants was shown to cause aggregation of lipid rafts and movement of membrane components from raft to non-raft membrane regions.

Results from Aim 1 also indicate an association between HIV infection and lower HDL-cholesterol. Compared to people with low HDL-cholesterol, those with higher levels had higher cell membrane phospholipids and lower cholesterol to phospholipid ratios [42], which is less conducive to lipid raft formation. In fact, HDL has been shown to interact with and deplete lipid raft domains [43]. The HDL receptor, SR-BI, however, is not strongly associated with lipid raft domains [44]. Oxidized HDL interacting with macrophages decreases macrophage membrane fluidity, which was associated with decreased free cholesterol efflux from the macrophage [45]. Compared to swine smooth muscle cells exposed to HDL, cells exposed to LDL had higher cholesterol to protein ratios, cholesterol to phospholipid ratios, and higher microviscosity (when cholesterol content of medium was ≥40 micrograms/ml) [46].

In Aim 1 we reported lower or similar LDL-cholesterol in HIV infected versus uninfected people. Prior work also suggests a reduction and subsequent increase in LDL-cholesterol following HIV seroconversion and ART initiation respectively [47]. Lovastatin, an LDL-cholesterol reducing agent [48] has been shown to reduce sterol synthesis and inhibit HIV replication [31]. Incubation of endothelial cells with LDL in vitro resulted in increased cellular cholesterol content, increased cholesterol to phospholipid ratio, decreased cell membrane fluidity, and enhanced attachment of monocytes [49]. Additionally, the surface area of lipid rafts on endothelial cells has been shown to decrease with exposure to oxidized LDL [50]. Oxidized LDL induced the appearance of ceramide-enriched surface membrane microdomains on macrophage cell surfaces [51]. Ceramide displaces cholesterol from lipid rafts [52], which may alter the composition and physical properties of the raft. Oxidized LDL also facilitates cholesterol efflux and disrupts the structure and order of cholesterol rich membrane domains in endothelial cells [41,53].

1.4 DISCUSSION

This review has linked several important concepts: 1) HIV infection alters serum/plasma lipid metabolism, 2) serum/plasma lipid alterations typically observed in HIV are associated with alterations in cellular lipids that may favor lipid raft formation, and 3) cellular lipid distribution, particularly lipid raft formation, may play an important role in multiple aspects of the HIV life cycle.

The studies reviewed suggest that some features of HIV infection (for example increased triglyceride and decreased HDL concentrations, longer LDL residence times, decreased

phosphatidylcholine (a phospholipid) synthesis) may create conditions conducive to lipid raft formation. Given the dependence of HIV replication on these rafts, we propose that infection-mediated alterations in lipid metabolism, intended as a beneficial pro-inflammatory immune process, may also create conditions that are more conducive to viral replication. Whether HIV actively "hijacks" this process or passively benefits from it is unclear and would be an interesting area for future study. This is particularly relevant to future HIV treatment given that studies in Aim 1 suggest an interaction between ART and HIV infection wherein ART exacerbates some of the lipid dysregulation observed in HIV [16-18].

The associations between oxidized lipoproteins and lipid rafts raise interesting questions. Oxidized HDL was associated with decreased cholesterol efflux from plasma macrophage plasma membranes, while oxidized LDL was associated with decreased lipid raft area and cholesterol displacement from rafts. Whether HIV infection is associated with HDL oxidation is unclear. However, HIV infection may be associated with increased prevalence of oxidized LDL [54], possibly due to longer LDL residence times [55]. While oxidized LDL may be detrimental to lipid raft maintenance – and consequently, HIV replication – the pro-inflammatory state associated with LDL oxidation may offset this by recruiting more HIV-susceptible immune cells into circulation. A reasonable hypothesis may suggest that HIV infection manipulates LDL-oxidation and LDL metabolism to minimize the detrimental effects on lipid rafts while maximizing the immune activation associated with lipoprotein oxidation.

As the principal site of cholesterol degradation, endogenous lipid metabolism, and acute phase protein synthesis, the liver is strongly implicated in infection-mediated changes in lipid metabolism. Whether and how HIV alters liver synthetic function, and whether altered hepatic

function provides net benefit to the host or virus over time are also important areas for future research.

1.5 CONCLUSION

In summary, we have described specific alterations in lipid and lipoprotein kinetics associated with HIV and how these alterations affect cellular lipids. Based on the dependence of successful HIV replication on host cellular lipids, we have proposed that HIV may “hijack” other components of the immune response (lipid dysregulation) in addition to “hijacking” T-lymphocyte activation to amplify viral replication.

2.0 PAPER 1: HIV STATUS, BURDEN OF COMORBID DISEASE AND BIOMARKERS OF INFLAMMATION, ALTERED COAGULATION AND MONOCYTE ACTIVATION

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2.1 ABSTRACT

Background: Biomarkers of inflammation, altered coagulation, and monocyte activation are associated with mortality and cardiovascular disease (CVD) in the general population and among human immunodeficiency virus infected people (HIV+). We compared biomarkers for inflammation, altered coagulation, and monocyte activation between HIV+ and uninfected people in the Veterans Aging Cohort Study (VACS).

Methods: Biomarkers of inflammation (interleukin-6 (IL-6)), altered coagulation (D-dimer), and monocyte activation (soluble CD4 (sCD14)) were measured in blood samples from 1525 HIV+ and 843 uninfected VACS participants. Logistic regression was used to determine the association between HIV infection and prevalence of elevated (>75th percentile) biomarkers adjusting for confounding comorbidities.

Results: HIV+ Veterans had less prevalent CVD, hypertension, diabetes, obesity, hazardous drinking and renal disease, but more dyslipidemia, hepatitis C, and current smoking than uninfected Veterans. Compared to uninfected Veterans, HIV+ Veterans with HIV RNA ≥500 copies/ml or CD4 count <200 cells/mm^3 had a significantly higher prevalence of elevated IL-6 (OR:1.54; 95% CI=1.14-2.09, OR:2.25; 95% CI:1.60-3.16 respectively) and D-dimer (OR:1.97; 95% CI:1.44-2.71, OR:1.68; 95% CI:1.22-2.32 respectively) after adjusting for comorbidities. HIV+ Veterans with a CD4 cell count <200 cells/mm^3 had significantly higher prevalence of elevated sCD14 compared to uninfected Veterans (OR:2.60; 95% CI:1.64-4.14). These associations still persisted after restricting the analysis to Veterans without known confounding comorbid conditions.

Conclusion: These data suggest that ongoing HIV replication and immune depletion significantly contribute to increased prevalence of elevated biomarkers of inflammation, altered coagulation, and monocyte activation. This contribution is independent of and in addition to the substantial contribution from comorbid conditions.

2.2 INTRODUCTION

Untreated HIV infection and resulting immune depletion are associated with mortality and cardiovascular diseases (CVD) [56]. Biomarkers of inflammation and altered coagulation are associated with mortality and CVD within the general population [57-60] and among HIV infected people [61-64]. Further, soluble CD14 (sCD14), a biomarker of monocyte activation is linked with increased mortality risk among HIV infected individuals [65] and the general population [66]. However, it is unknown whether inflammation, altered coagulation, and monocyte activation are primary mechanisms driving the association between HIV infection and mortality and CVD.

Prior studies comparing HIV infected to uninfected individuals suggest that HIV infection is associated with inflammation, altered coagulation, and monocyte activation [65,67-69]. However, these studies were limited by the comparison of HIV infected and uninfected populations from different cohorts [65,67,69] and by their inability to consider degree of viral RNA suppression, immune depletion [68], or comorbid conditions that also influence levels of these biomarkers [69]. The latter limitation is particularly problematic in the study of HIV because those with HIV likely have a greater burden of disease related to behaviors like smoking, alcohol, and drug use. Comparing HIV infected people to uninfected people who have a lower burden of non HIV-related disease makes it difficult to determine whether elevated biomarkers in those with HIV are driven by HIV infection or by the increased comorbidity burden among the HIV infected.

The objective of this study was to investigate the effect of HIV status on biomarkers of inflammation, altered coagulation, and monocyte activation in the Veterans Aging Cohort Study (VACS). VACS contains detailed clinical data on HIV measures, substance use, and comorbidity, and an uninfected population with a comparable comorbidity burden.

2.3 METHODS

2.3.1 Subject selection

Subjects were selected from the VACS [70]. Briefly, the VACS is an observational, prospective longitudinal study of HIV infected and age, race/ethnicity, sex, and site matched uninfected Veterans in care from eight United States Department of Veterans Affairs (VA) medical centers across the United States. In 2005-2006, the VACS collected and banked blood and DNA specimens on enrolled subjects from these sites. A total of 1,525 HIV infected and 843 uninfected VACS participants consented to provide blood specimens for future studies. These specimens were collected using serum separator and EDTA blood collection tubes and shipped to a central repository at the Massachusetts Veterans Epidemiology Research and Information Center in Boston, Massachusetts.

2.3.2 Independent variables

HIV status was our primary independent variable. To understand the effects of poor viral suppression and immune depletion, we stratified HIV by viral RNA (uninfected, HIV infected with RNA <500 or ≥500 copies/mL) and by CD4 count (uninfected, HIV infected with CD4 count <200, 200-499, or ≥500 cells/mm^3). We further stratified these variables by antiretroviral therapy (ART) to understand the impact of ART among those with poor viral suppression or immune depletion. Current ART status was defined as taking ART within 90 days before or up to seven days after blood specimen collection.

2.3.3 Dependent variables

Biomarkers of inflammation (interleukin 6 (IL-6)), altered coagulation (D-dimer), and monocyte activation (soluble CD14 (sCD14)) were our dependent variables (Laboratory for Clinical Biochemistry Research, University of Vermont). IL-6 was measured using a chemiluminescent immunoassay (QuantiGlo IL-6 immunoassay, R&D Systems, Minneapolis, MN). Calibration was done by the manufacturer and is traceable to National Institute for Biological Standards and Control 89/548 (IU/ml). Four levels of controls were run per sample, with the inter-assay coefficients of variability (CVs) ranging from 7.68% to 12.29%. D-dimer was measured using the STAR automated coagulation analyzer, (Diagnostica Stago) using an immuno-turbidometric assay (Liatest D-DI; Diagnostica Stago, Parsippany, NJ). Four controls, with inter-assay CVs ranging from 2.77% to 14.78%, were used. The lab measured sCD14 with an ELISA (Quantikine sCD14 Immunoassay, R&D Systems Inc) with a detectable range of 40 – 3200 ng/mL, using a standard 200-fold sample dilution. Again, four controls were used, with inter-assay CVs ranging from 7.19% to 8.11%.

2.3.4 Covariates

Sociodemographic data included age, sex, and race/ethnicity. We defined prevalent cardiovascular disease (CVD) as any of the following occurring prior to the date of blood specimen collection: 1) A myocardial infarction as defined by the VA Ischemic Heart Disease Quality Enhancement Research Initiative (IHD-QUERI) [71] or the Cardiovascular Health Study [72]; 2) International Classification of Disease (ICD-9) or Current Procedural Terminology (CPT)

codes for congestive heart failure, coronary artery bypass graft, percutaneous coronary intervention, and ischemic stroke.

Blood pressure was averaged over the three routine outpatient blood pressure measurements performed closest to the date of blood specimen collection. Hypertension was categorized based on Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure thresholds as no hypertension (blood pressure <120/80 mmHg and no documentation of antihypertensive medication); pre-hypertension (120-139/80-89 mmHg and no antihypertensive medication); controlled hypertension (<140/90 mmHg and documentation of antihypertensive medication); or uncontrolled hypertension (blood pressure ≥140/90 mmHg) [73].

Diabetes was diagnosed using glucose measurements, use of insulin or oral hypoglycemic agents, and/or ≥1 inpatient and/or 2 outpatient ICD-9 codes [74]. Current, past, and never smoking and body mass index were determined from the VA Health Factors dataset [75].

Cholesterol lowering medication use was assessed using patient pharmacy data. Cholesterol measurements (i.e., high-density lipoprotein (HDL); low-density lipoprotein (LDL) and serum triglycerides) were obtained from the VA Decision Support System (DSS). HDL was categorized as high, medium or low (≥60, 40-59, <40 mg/dL), LDL as optimal, near optimal, borderline high, or high/very high (<100, 100-129, 130-159, ≥160 mg/dL), and serum triglycerides as normal, borderline high, high/very high (<150, 150-199, ≥200 mg/dL) per National Cholesterol Education Program Adult Treatment Panel III criteria [76].

Cocaine and alcohol use were determined by self-report. We categorized alcohol use with data from the Alcohol Use Disorders Identification Test (AUDIT-C) and alcohol abuse and dependence diagnoses using ICD-9 codes based on prior work in VACS [77]: 1) current

infrequent/moderate drinking without alcohol abuse/dependence diagnosis, 2) current infrequent/moderate drinking with abuse/dependence diagnosis, 3) current hazardous drinking with or without abuse/dependence diagnosis, 4) past drinking. HCV virus (HCV) infection was defined as a positive HCV antibody test or at least 1 inpatient and/or 2 outpatient ICD-9 codes [78]. Renal disease was defined as an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73m^2 per National Kidney Foundation Kidney Disease Outcomes Quality Initiative thresholds for chronic kidney disease [79].

We collected data on HIV-1 RNA, CD4 count, and current antiretroviral therapy. We used CD4 counts and HIV-1 RNA measurements obtained as part of clinical care at the closest time point within 180 days before or after the date of the blood specimen collection.

2.3.5 Statistical analyses

Variables that were normally distributed, continuous but not normally distributed, or categorical were compared by HIV status using *t* tests, Wilcoxon rank sum tests, or χ^2 tests respectively. Mean age and race/ethnicity adjusted biomarker levels were compared by linear regression after transforming biomarker variables to approximate normality. Median biomarker levels were compared by comorbid disease status using Kruskall-Wallis tests. Spearman rank analysis with a Bonferroni correction for multiple comparisons was used to test correlations between biomarkers and continuous variables.

We used multivariable logistic regression to estimate the association between HIV infection and prevalence of elevated (i.e., >75th percentile) IL-6, D-dimer and sCD14. For each biomarker, we assessed the association between the biomarker and 1) HIV status, 2) HIV stratified by RNA, 3) HIV stratified by CD4 count, and 4) HIV stratified by CD4 count or RNA,

and antiretroviral therapy (ART). Uninfected Veterans were the reference group for all HIV comparisons. All regression models were adjusted for 1) age, race/ethnicity and 2) age, race/ethnicity, prevalent CVD, hypertension, diabetes, smoking, BMI, cholesterol lowering medication use, HDL, LDL, triglycerides, cocaine use in the past year, alcohol use, HCV, and renal disease. We analyzed two-way interactions between HIV and each covariate and included significant interactions ($p<0.05$) in the final regression models.

We also performed secondary analyses excluding comorbid conditions associated with our outcomes to better understand the independent effect of HIV infection on prevalence of elevated IL-6, D-dimer, and sCD14. Specifically, we excluded Veterans with confounding comorbid behaviors (current hazardous drinking or smoking) or diseases (CVD, uncontrolled hypertension, diabetes, BMI$\geq$30 kg/m^2, BMI<18 kg/m^2, HCV, renal disease).

To address missing covariate data, we used multiple imputation to generate 10 complete datasets to increase the robustness of estimated associations. Multiple imputation was done using the ICE command [80] (Stata v11.0 StataCorp).

2.4 RESULTS

We collected 2,345 IL-6 measurements, 2,352 D-dimer measurements, and 2,357 sCD14 measurements. Compared to uninfected Veterans, HIV infected Veterans were slightly younger (51.8 vs. 53.5 years; $p<0.01$), more likely to be male (97.3% vs. 90.4%), and had less prevalent CVD (6.3% vs. 12.8%), uncontrolled hypertension (24.3% vs. 28.7%), diabetes (19.5% vs. 31.8%), BMI $\geq$30 kg/m^2 (14.2% vs. 42.9%), cholesterol lowering medication use (26.9% vs. 40.1%), current hazardous drinking (24.7% vs. 27.2%), and renal disease (8.1% vs. 10.2%) ($p<0.05$ for all; Table 3).

HIV infected Veterans had more prevalent current smoking (50.6% vs. 47.3%), low HDL (44.8% vs. 33.6%), high/very high LDL (10.4% vs. 5.2%), high/very high triglycerides (22.7% vs. 12.0%), cocaine use in the past year (20.3% vs. 17.6%) and HCV (45.1% vs. 30.8%) ($p<0.05$ for all; Table 3).

Differences in median biomarker levels between infected and uninfected Veterans were most apparent when HIV was stratified by HIV-1 RNA or CD4 count (Figure 4). Median IL-6 was greater in HIV infected compared to uninfected Veterans (2.08 vs. 1.79 pg/mL; $p<0.01$), while the reverse was true for median D-dimer (0.26 vs. 0.30 μg/mL; $p<0.01$). There was no difference between the two groups for median sCD14 (1.72 vs. 1.73 μg/mL; $p=0.95$) (Table 3).

Median biomarker levels varied significantly by covariate and co-morbid disease status (Table 4) with many moderate but significant correlations (Table 5) between these measures.

Table 3. Baseline characteristics of study population

	HIV infected (N=1525)	HIV uninfected (N=843)	p-value
Demographics (%)			
Age, years (mean ± SD)	51.8 ± 8.2	53.5 ± 9.3	<0.01
Male	97.3	90.4	<0.01
Race			0.69
White	19.0	20.9	
African American	69.0	67.3	
Hispanic	8.3	7.8	
Other	3.8	4.0	
Cardiovascular disease risk factors (%)			
Prevalent cardiovascular disease	6.3	12.8	<0.01
Blood pressure, mmHg			
Mean systolic (SD)	128.9 (14.4)	132.1 (14.6)	<0.01
Mean diastolic (SD)	78.7 (9.1)	78.8 (9.3)	0.82
Hypertension			<0.01
No hypertension	17.8	9.9	
Pre-hypertension	25.2	18.9	
Controlled hypertension	32.5	42.6	
Uncontrolled hypertension	24.3	28.7	
Diabetes	19.5	31.8	<0.01
Smoking [a]			<0.01
Current	50.6	47.3	
Past	28.3	29.1	
Never	8.9	22.1	
BMI, kg/m^2 [a]			
Mean (SD)	25.5 (4.7)	29.5 (6.0)	<0.01
<18	2.0	0.6	
18-24.9	45.3	22.1	<0.01
25-29.9	36.5	33.1	
≥30	14.2	42.9	
Cholesterol lowering agent use	26.9	40.1	<0.01
HDL cholesterol, mg/dL [a]			
Median (mean ± SD)	41.0 (44.0±16.7)	44.5 (46.4±14.7)	<0.01
High (≥60)	11.2	10.6	
Medium (40-59)	40.7	51.4	<0.01
Low (<40)	44.8	33.6	
LDL cholesterol, mg/dL [a]			
Median (mean ± SD)	103.5 (142.3±163.4)	100.0 (102.5±59.4)	0.03
Optimal (<100)	33.3	31.6	
Near optimal (100-129)	18.8	18.3	<0.01
Borderline high (130-159)	8.8	8.8	
High/Very high (≥160)	10.4	5.2	
Triglycerides, mg/dL [a]			
Median (mean ± SD)	140.0 (180.4±151.5)	111.0 (146.3±137.0)	<0.01
Normal (<150)	42.3	44.5	
Borderline high (150-199)	13.2	9.1	<0.01
High/Very high (≥200)	22.7	12.0	
Other risk factors (%)			
Cocaine in past year (%)	20.3	17.6	0.03
Hazardous drinking(%) [a]			0.04

Table 3 continued

	HIV infected (N=1525)	HIV uninfected (N=843)	p-value
Current infrequent/moderate drinking	24.4	19.2	
Abuse/dependence; no current hazardous drinking	8.3	9.3	
Current hazardous drinking	24.7	27.2	
Past drinking	9.9	9.0	
Hepatitis C (%)	45.1	30.8	<0.01
eGFR, mL/min/1.73m^2			
Median (mean ± SD)	96.5 (98.8±33.0)	94.6 (97.8±43.2)	0.12
<60 (%)	8.1	10.2	0.03
Laboratory analysis			
CD4+ T lymphocyte count, cells/mm^3 [a]			
Median (mean ± SD)	392 (432.0 ± 278.1)	--	
>= 500 (%)	33.4	--	
200-499.9 (%)	43.8	--	
<200 (%)	20.4	--	
HIV-1 RNA, copies/mL [a]			
Median (mean ± SD)	75 (20633 ± 73540)	--	
<500 (%)	65.3	--	
>500 (%)	33.0	--	
Class of antiretroviral therapy (%)			
Protease inhibitors	45.7	--	
Non-nucleoside reverse transcriptase inhibitors	34.7	--	
Nucleoside analog reverse transcriptase inhibitors	76.7	--	
Biomarkers of inflammation, altered coagulation, monocyte activation			
IL-6,pg/ml median (mean ± SD) [a]	2.08 (3.36 ± 7.47)	1.79 (3.70 ± 14.82)	<0.01
>75th percentile IL-6 (%)	25.8	22.9	<0.01
D-dimer, μg/ml median (mean ± SD) [a]	0.26 (0.50 ± 1.08)	0.30 (0.54 ± 0.93)	<0.01
>75th percentile D-dimer (%)	23.7	26.0	<0.01
sCD14, μg/ml median (mean ± SD) [a]	1.72 (1.82 ± 0.55)	1.73 (1.81± 0.49)	0.95
>75th percentile sCD14 (%)	25.8	23.3	<0.01

Abbreviations: HIV, human immunodeficiency virus; CVD, cardiovascular disease; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HCV, hepatitis C virus; eGFR, estimated glomerular filtration rate; HIV-1, human immunodeficiency virus type-1. *For Smoking (N=1340 HIV+, 830 HIV-); BMI (N=1493 HIV+, 832 HIV-); HDL cholesterol (N=1173 HIV+, 590 HIV-); LDL cholesterol (N=1086 HIV+, 538 HIV-); Triglycerides (N=1191 HIV+, 553 HIV-); cocaine (N=1468 HIV+, 795 HIV-); Hazardous drinker (N=1017 HIV+; 536 HIV-); EGFR (N=1525 HIV+, 841 HIV-); CD4+ lymphocyte count (N=1488); HIV-1 RNA (N=1499); IL-6 (N=1521 HIV+, 824 HIV-); D-dimer (N=1523 HIV+, N=829 HIV-); sCD14 (N=1525 HIV+; N=832 HIV-).

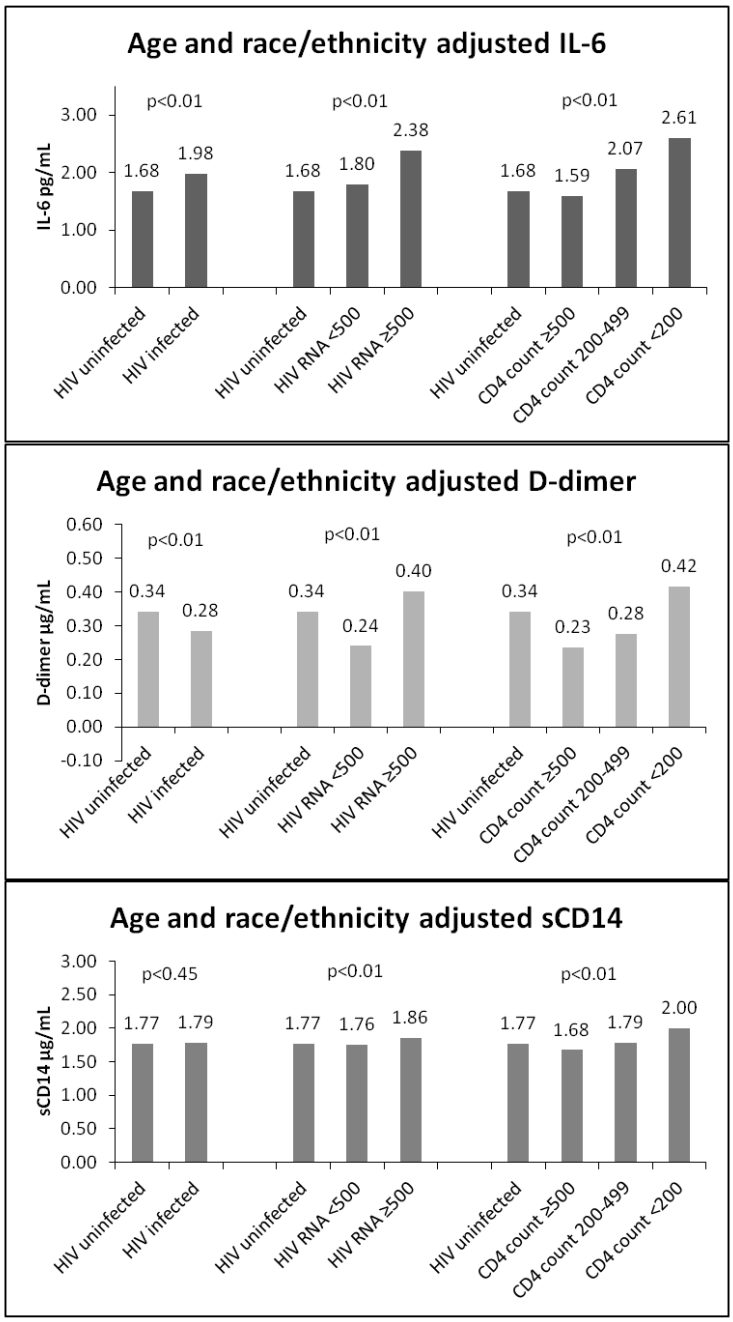

Figure 4: Distribution of age- and race/ethnicity-adjusted interleukin 6 (IL-6), D-dimer, and soluble CD14 (sCD14) levels by human immunodeficiency virus (HIV) status, HIV-1 RNA level, and CD4 lymphocyte count

Table 4. Distributions of interleukin-6, D-dimer and soluble CD14 by HIV status and categorical covariates

	Median (mean±SD)		
	IL-6 (pg/mL)	**D-dimer (µg/ml)**	**sCD14 (µg/ml)**
Demographics			
Male	1.74 (2.59±2.51)	0.32 (0.46±0.77)	1.74 (1.77±0.45)
Female	2 .00 (3.53±10.91)	0.28 (0.51±1.04)	1.72 (1.82±0.54)
Race			
White	2.02 (3.04±4.18)	0.26 (0.42±0.55)	1.80* (1.90±0.50)
African American	1.97 (3.48±11.14)	0.29 (0.55±1.19)	1.69* (1.79±0.54)
Hispanic	2.17 (4.06±15.17)	0.26 (0.44±0.53)	1.75* (1.86±0.58)
Other	2.04 (4.47±12.71)	0.29 (0.48±0.55)	1.74* (1.83±0.44)
Cardiovascular disease risk factors			
No prevalent cardiovascular disease	1.95* (3.44±11.01)	0.26* (0.5±1.05)	1.72* (1.81±0.53)
Prevalent cardiovascular disease	2.58* (3.9±5.35)	0.41* (0.68±0.78)	1.83* (1.93±0.56)
Hypertension			
No hypertension	1.95* (3.32±7.19)	0.25* (0.5±1.28)	1.68* (1.78±0.55)
Pre-hypertension	1.68* (2.47±3.63)	0.24* (0.42±1.01)	1.68* (1.75±0.48)
Controlled hypertension	2.11* (3.78±10.12)	0.29* (0.5±0.63)	1.76* (1.88±0.54)
Uncontrolled hypertension	2.1* (4.02±15.88)	0.32* (0.62±1.29)	1.73* (1.82±0.54)
No diabetes	1.91* (3.18±6.25)	0.26* (0.49±1.02)	1.69* (1.79±0.52)
Diabetes	2.22* (4.42±18.71)	0.34* (0.58±1.07)	1.81* (1.92±0.57)
Smoking [a]			
Current	2.1* (3.77±9.18)	0.29 (0.52±1.02)	1.77* (1.86±0.54)
Past	2.00* (3.63±15.55)	0.29 (0.55±1.25)	1.71* (1.82±0.54)
Never	1.66* (2.57±3.58)	0.28 (0.43±0.48)	1.64* (1.72±0.47)
BMI, kg/m^2 [a]			
<18	2.36* (3.2±2.84)	0.37* (0.58±0.7)	1.84* (1.90±0.52)
18-24.9	2.02* (3.75±10.00)	0.29* (0.56±1.31)	1.80* (1.92±0.57)
25-29.9	1.79* (3.23±13.66)	0.26* (0.46±0.95)	1.67* (1.75±0.49)
≥30	2.22* (3.49±6.5)	0.32* (0.51±0.58)	1.68* (1.76±0.50)
No cholesterol lowering agent use	2.02 (3.3±6.2)	0.26* (0.51±1.12)	1.71 (1.81±0.53)
Cholesterol lowering agent use	1.91 (4.03±18.57)	0.31* (0.51±0.68)	1.75 (1.84±0.55)
HDL cholesterol, mg/dL [a]			
High (≥60)	1.74* (2.58±2.74)	0.26* (0.44±0.55)	1.68* (1.79±0.57)
Medium (40-59)	1.83* (3.74±16.32)	0.26* (0.49±1.21)	1.68* (1.78±0.53)
Low (<40)	2.21* (3.45±6.19)	0.31* (0.58±1.24)	1.79* (1.88±0.56)
LDL cholesterol, mg/dL [a]			
Optimal (<100)	2.14* (3.82±14.43)	0.29 (0.54±0.98)	1.75* (1.88±0.56)
Near optimal (100-129)	1.82* (3.22±10.38)	0.26 (0.58±1.77)	1.72* (1.8±0.52)
Borderline high (130-159)	1.96* (2.96±8.42)	0.28 (0.46±0.55)	1.66* (1.72±0.48)
High/Very high (≥160)	1.96* (3.44±5.97)	0.29 (0.47±0.57)	1.72* (1.8±0.56)
Triglycerides, mg/dL [a]			
Normal (<150)	1.91* (3.16±4.96)	0.28* (0.57±1.42)	1.68* (1.78±0.53)
Borderline high (150-199)	2.1* (3.11±4.63)	0.29* (0.51±0.62)	1.71* (1.82±0.51)
High/Very high (≥200)	2.16* (3.73±11.85)	0.26* (0.4±0.57)	1.82* (1.93±0.60)
Other risk factors (%)			
No cocaine in past year	1.96 (3.56±11.96)	0.28 (0.48±0.79)	1.72 (1.81±0.53)
Cocaine in past year	2.09 (3.24±4.46)	0.29 (0.61±1.44)	1.74 (1.85±0.53)
Hazardous drinking(%) a			
Current infrequent/moderate drinking	1.70* (2.49+/-2.92)	0.25* (0.45+/-0.78)	1.67* (1.76+/-0.49)
Abuse/dependence; no current hazardous drinking	2.08* (2.98+/-3.45)	0.25* (0.45+/-0.52)	1.73* (1.85+/-0.53)
Current hazardous drinking	2.09* (3.37+/-7.15)	0.29* (0.52+/-1.27)	1.73* (1.82+/-0.50)

Table 4 continued

	Median (mean±SD)		
	IL-6 (pg/mL)	**D-dimer (µg/ml)**	**sCD14 (µg/ml)**
Past drinking	1.94* (5.21+/-26.02)	0.31* (0.58+/-1.40)	1.75* (1.87+/-0.56)
No hepatitis C	1.81* (3.47±12.97)	0.28 (0.50±0.99)	1.67* (1.76±0.51)
Hepatitis C	2.17* (3.5±5.59)	0.29 (0.53±1.09)	1.81* (1.91±0.55)
eGFR≥60	1.95* (3.19±7.29)	0.26* (0.49±1.05)	1.70* (1.78±0.50)
eGFR<60	2.63* (6.51±27.11)	0.46* (0.77±0.83)	2.08* (2.23±0.71)

Abbreviations: HIV, human immunodeficiency virus; CVD, cardiovascular disease; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HCV, hepatitis C virus; eGFR, estimated glomerular filtration rate; HIV-1, human immunodeficiency virus type-1.
*p<0.05

[a]For Smoking (N=1340 HIV+, 830 HIV-); BMI (N=1493 HIV+, 832 HIV-); HDL cholesterol (N=1173 HIV+, 590 HIV-); LDL cholesterol (N=1086 HIV+, 538 HIV-); Triglycerides (N=1191 HIV+, 553 HIV-); cocaine (N=1468 HIV+, 795 HIV-); Hazardous drinker (N=1017 HIV+; 536 HIV-); EGFR (N=1525 HIV+, 841 HIV-); CD4+ lymphocyte count (N=1488); HIV-1 RNA (N=1499); IL-6 (N=1521 HIV+, 824 HIV-); D-dimer (N=1523 HIV+, N=829 HIV-); sCD14 (N=1525 HIV+; N=832 HIV-).

Table 5: Correlations between biomarkers of inflammation, altered coagulation, immune activation HIV-related biomarkers and biomarkers of co-comorbid disease

	IL6	D-dimer	sCD14
IL6	1.00		
D-dimer	0.37*	1.00	
sCD14	0.38*	0.22*	1.00
Age	0.15*	0.16*	0.12*
HIV-1 RNA	0.17*	0.25*	0.11*
CD4+ T cell count	-0.28*	-0.21*	-0.22*
Systolic blood pressure	0.04	0.09*	0.01
Diastolic blood pressure	0.02	0.02	0.01
Body mass index	0.01	0.04	-0.14*
High density lipoprotein cholesterol	-0.15*	-0.12*	-0.11*
Low density lipoprotein cholesterol	-0.11*	-0.06	-0.12*
Triglycerides	0.09*	-0.05	0.11*
Estimated glomerular filtration rate	-0.04	-0.06	-0.15*

*$p<0.05$ for pairwise comparison with Bonferroni correction for multiple comparisons

In age and race/ethnicity adjusted logistic regression models, HIV status was not associated with a higher prevalence of elevated IL-6 (OR:1.18; 95%CI=0.96-1.44), D-dimer (0.91; 0.74-1.11), or sCD14 (1.16; 0.95-1.42). After full covariate adjustment, the odds ratios (95% CI) for the association with IL-6, D-dimer, and sCD14 were 0.81 (0.55-1.19), 0.95 (0.75-1.20) and 0.79 (0.62-1.02) respectively (Table 6).

However, compared to uninfected Veterans, HIV infected Veterans with HIV-1 RNA ≥500 copies/ml or CD4 count <200 cells/mm^3 had a significantly higher prevalence of elevated IL-6 (OR: 1.54; 95%CI=1.14-2.09, OR:2.25; 95%CI:1.60-3.16 respectively) and D-dimer (OR: 1.97; 95%CI:1.44-2.71, OR:1.68; 95%CI: 1.22-2.32 respectively) after adjusting for confounders (Table 7). HIV infected Veterans with a CD4 cell count <200 cells/mm^3 had significantly higher prevalence of elevated sCD14 compared to uninfected Veterans (OR: 2.60; 95%CI:1.64-4.14; Table 7).

In the adjusted HIV status model, the covariates age ≥60 years, current smoking, low HDL, current hazardous or past drinking were also associated with higher prevalence of elevated IL-6 ($p<0.05$ for all; Table 6). Age ≥70 years, black race/ethnicity, prevalent CVD, uncontrolled hypertension, low HDL, and renal disease were significantly associated with D-dimer ($p<0.05$ for all; Table 6). High/very high triglycerides, HCV, and renal disease were significantly associated with sCD14 ($p<0.05$ for all; Table 6). These covariate associations were of comparable (or greater) magnitude to associations for the HIV variables. The magnitudes of the covariate associations were similar in the models stratified by HIV-1 RNA or CD4 cell count.

HIV status had significant interactions with black race and renal disease ($p<0.05$ for both; Table 6). HIV-1 RNA had significant interactions with renal disease, diabetes and HCV ($p<0.05$

for all; Table 7). CD4 count had significant interactions with renal disease, current hazardous drinking and HCV ($p<0.05$ for all; Table 7).

When we stratified HIV-1 RNA and CD4 counts by current ART status, only modest differences in the prevalence of elevated IL-6 and D-dimer between those on ART and not on ART were observed (Table 8).

To assess the impact of HIV on elevated biomarker independent of confounding comorbidities, we excluded Veterans with prevalent comorbid behaviors or diseases (Table 9) that were associated with elevated biomarkers. Among these subsets of "healthier" Veterans, the magnitude of the association between HIV infection and prevalence of elevated biomarkers persisted and in some cases, was increased.

Table 6: The association between HIV infection and elevated (>75th percentile) biomarkers of inflammation (IL-6), altered coagulation (D-dimer) and monocyte activation (sCD14).

	IL-6		D-dimer		sCD14	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
HIV uninfected	**1.00**	--	**1.00**	--	**1.00**	--
HIV infected	0.81	(0.55-1.19)	0.95	(0.75-1.20)	0.79	(0.62-1.02)
Age <40, years	**1.00**	--	**1.00**	--	**1.00**	--
40-49	0.93	(0.57-1.52)	0.86	(0.53-1.40)	1.10	(0.67-1.82)
50-59	1.36	(0.84-2.22)	1.28	(0.79-2.07)	1.24	(0.75-2.04)
60-69	1.80	(1.05-3.08)	1.51	(0.88-2.57)	1.26	(0.72-2.20)
≥70	2.15	(1.07-4.31)	2.23	(1.15-4.32)	1.30	(0.64-2.65)
White race	**1.00**	--	**1.00**	--	**1.00**	--
African American[a]	0.59	(0.40-0.88)	1.63	(1.22-2.18)	0.85	(0.65-1.11)
Hispanic	1.22	(0.81-1.82)	1.29	(0.83-2.01)	0.98	(0.65-1.46)
Other	0.73	(0.42-1.28)	1.21	(0.68-2.14)	0.76	(0.44-1.31)
Prevalent CVD	1.39	(0.98-1.98)	1.89	(1.35-2.65)	1.17	(0.82-1.66)
Normal BP, mmHg	**1.00**	--	**1.00**	--	**1.00**	--
Pre-hypertension	0.68	(0.48-0.96)	0.86	(0.60-1.22)	0.88	(0.63-1.23)
Controlled hypertension	1.01	(0.73-1.40)	1.14	(0.82-1.58)	1.19	(0.86-1.64)
Uncontrolled hypertension	1.21	(0.87-1.68)	1.44	(1.02-2.02)	0.91	(0.64-1.28)
Diabetes	1.19	(0.93-1.54)	0.99	(0.77-1.28)	1.26	(0.98-1.62)
Never smoker	**1.00**	--	**1.00**	--	**1.00**	--
Current	1.67	(1.17-2.37)	1.28	(0.92-1.79)	1.23	(0.88-1.73)
Past	1.06	(0.74-1.53)	1.19	(0.83-1.72)	1.15	(0.81-1.63)
BMI 18-25, kg/m^2	**1.00**	--	**1.00**	--	**1.00**	--
<18	1.23	(0.58-2.60)	1.72	(0.83-3.58)	0.78	(0.35-1.73)
25-29	0.85	(0.67-1.09)	0.72	(0.57-0.93)	0.55	(0.43-0.70)
>30	1.33	(1.00-1.78)	0.98	(0.74-1.31)	0.43	(0.32-0.59)
Cholesterol lowering medication	0.64	(0.50-0.83)	0.89	(0.69-1.14)	1.10	(0.86-1.41)
High HDL (>60), mg/dL	**1.00**	--	**1.00**	--	**1.00**	--
Medium (40-59)	1.39	(0.98-1.98)	1.01	(0.72-1.41)	1.14	(0.78-1.65)
Low (<40)	1.69	(1.16-2.48)	1.79	(1.25-2.56)	1.28	(0.88-1.87)
Optimal LDL(<100), mg/dL	**1.00**	--	**1.00**	--	**1.00**	--
Near optimal (100-129)	0.88	(0.66-1.18)	0.77	(0.58-1.02)	0.91	(0.69-1.18)
Borderline high (130-159)	0.84	(0.57-1.26)	1.23	(0.84-1.80)	0.86	(0.58-1.27)
High or very high (≥160)	0.82	(0.57-1.19)	0.94	(0.65-1.36)	0.81	(0.59-1.11)
Normal triglyceride(<150), mg/dL	**1.00**	--	**1.00**	--	**1.00**	--
Borderline high (150-199)	1.05	(0.76-1.44)	0.79	(0.56-1.11)	1.36	(1.00-1.84)
High or very high (≥200)	0.90	(0.68-1.20)	0.74	(0.55-0.99)	1.60	(1.19-2.14)
Cocaine use in last year	1.25	(0.94-1.65)	1.25	(0.94-1.65)	0.93	(0.70-1.23)

Table 6 continued

	IL-6		D-dimer		sCD14	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Infrequent/Moderate drinking	**1.00**	**--**	**1.00**	**--**	**1.00**	**--**
Abuse/dependence; no current hazardous drinking	1.24	(0.81-1.91)	0.94	(0.61-1.43)	1.20	(0.79-1.83)
Current hazardous drinking	1.67	(1.22-2.29)	1.21	(0.88-1.67)	1.30	(0.95-1.77)
Past drinking	1.94	(1.45-2.58)	1.25	(0.95-1.65)	1.19	(0.90-1.57)
Hepatitis C [a]	1.05	(0.84-1.32)	0.89	(0.71-1.13)	1.51	(1.20-1.89)
eGFR<60 mL/min/1.73 m^2 [a]	1.06	(0.61-1.86)	2.54	(1.84-3.48)	1.86	(1.11-3.11)

IL-6 (N=2345); D-dimer (N=2352); sCD14 (N=2357)

HIV, human immunodeficiency virus; CVD, cardiovascular disease; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HCV, hepatitis C virus; eGFR, estimated glomerular filtration rate.

*Significant interactions with HIV infection: IL-6 (African American race and eGFR<60 mL/min/1.73 m^2); D-dimer (none); sCD14 (eGFR<60 mL/min/1.73 m^2)

Table 7: The association between HIV infection stratified by a) HIV-1 RNA b) CD4 cell count and elevated (>75th percentile) biomarkers of inflammation (IL-6), altered coagulation (D-dimer), and monocyte activation (sCD14).

a)	**IL-6**		**D-dimer**		**sCD14**	
i) Age and race/ethnicity adjusted model	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95%CI**
HIV uninfected	**1.00**	--	**1.00**	--	**1.00**	--
HIV infected; RNA <500	0.97	(0.77-1.21)	0.64	(0.51-0.81)	1.09	(0.88-1.35)
HIV infected; RNA ≥500	1.69	(1.31-2.18)	1.61	(1.26-2.06)	1.40	(1.08-1.81)
	IL-6		**D-dimer**		**sCD14**	
ii) Fully adjusted model	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95%CI**
HIV uninfected	**1.00**	--	**1.00**	--	**1.00**	--
HIV infected; RNA <500	1.04	(0.79-1.36)	0.71	(0.53-0.97)	0.89	(0.63-1.26)
HIV infected; RNA ≥500	1.54	(1.14-2.09)	1.97	(1.44-2.71)	1.48	(1.00-2.20)

b)	**IL-6**		**D-dimer**		**sCD14**	
i) Age and race/ethnicity adjusted model	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95%CI**
HIV uninfected	**1.00**	--	**1.00**	--	**1.00**	--
HIV infected; CD4+ count ≥500	0.57	(0.42-0.76)	0.56	(0.42-0.74)	0.76	(0.58-1.01)
HIV infected; CD4+ count 200-499	1.31	(1.04-1.66)	0.89	(0.70-1.14)	1.06	(0.83-1.35)
HIV infected; CD4+ count <200	2.30	(1.73-3.06)	1.76	(1.33-2.34)	2.51	(1.89-3.32)
	IL-6		**D-dimer**		**sCD14**	
ii) Fully adjusted model	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95%CI**
HIV uninfected	**1.00**	--	**1.00**	--	**1.00**	--
HIV infected; CD4+ count ≥500	0.58	(0.41-0.82)	0.61	(0.45-0.84)	1.04	(0.67-1.60)
HIV infected; CD4+ count 200-499	1.41	(1.06-1.88)	0.97	(0.74-1.27)	1.25	(0.83-1.87)
HIV infected; CD4+ count <200	2.25	(1.60-3.16)	1.68	(1.22-2.32)	2.60	(1.64-4.14)

Models are adjusted for i) age and race/ethnicity ii) all covariates.
Reference for all comparisons is HIV uninfected.
HIV-1 RNA in copies/mL; CD4 count in cells/mm^3
a) IL-6 (N=2319); D-dimer (N=2326); sCD14 (N=2331)
Significant interactions in fully adjusted model: IL-6 (HIV-1 RNA < or ≥500 and eGFR<60 mL/min/1.73 m^2; $p<0.05$), D-dimer (HIV-1 RNA≥500 and diabetes; $p<0.05$), sCD14 (HIV-1 RNA < or ≥500 and HCV, HIV-1 RNA<500 and eGFR<60 mL/min/1.73 m^2; $p\leq0.05$ for all)
Abbreviations: OR, odds ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus.
b) IL-6 (N=2308); D-dimer (N=2315); sCD14 (N=2320)

Significant interactions in fully adjusted model: IL-6 (CD4≥200 and eGFR<60 mL/min/1.73 m^2; p≤0.05), sCD14 (CD4≥200 and current hazardous drinking, any CD4 count and hepatitis C; p<0.05 for all)

Table 8: The association between HIV infection and elevated (>75%) biomarkers of inflammation (IL-6), altered coagulation (D-dimer), and monocyte activation (sCD14).

a)	**IL-6**		**D-dimer**		**sCD14**	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
HIV uninfected	1.00	--	1.00	--	1.00	--
HIV-1 RNA <500; ART-	0.82	(0.47-1.42)	0.58	(0.33-1.03)	0.57	(0.33-0.99)
HIV-1 RNA <500; ART+	1.19	(0.92-1.55)	0.71	(0.54-0.92)	0.87	(0.67-1.12)
HIV-1 RNA ≥500; ART-	1.40	(0.97-2.02)	1.54	(1.08-2.19)	0.62	(0.42-0.93)
HIV-1 RNA ≥500; ART+	2.04	(1.45-2.88)	1.62	(1.15-2.27)	1.54	(1.10-2.15)
b)	**IL-6**		**D-dimer**		**sCD14**	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
HIV uninfected	1.00	--	1.00	--	1.00	--
CD4≥200; ART-	0.95	(0.66-1.38)	1.03	(0.73-1.47)	0.37	(0.24-0.57)
CD4≥200; ART+	1.16	(0.89-1.50)	0.73	(0.56-0.95)	0.82	(0.63-1.06)
CD4<200; ART-	2.73	(1.60-4.65)	1.85	(1.08-3.18)	1.96	(1.14-3.36)
CD4<200; ART+	2.31	(1.62-3.27)	1.60	(1.12-2.26)	1.82	(1.29-2.56)

Models stratified by a) HIV-1 RNA (copies/mL) b) CD4 lymphocyte count (cells/mm^3) and use of antiretroviral therapy (ART) and adjusted for all covariates.
a) IL-6 (N=2319); D-dimer (N=2326); sCD14 (N=2331).
b) IL-6 (N = 2308); D-dimer (N = 2315); sCD14 (N = 2320).
OR, odds ratio; CI, confidence interval; HIV-1, human immunodeficiency virus type-1; ART, antiretroviral therapy.

Table 9: Effect of excluding Veterans with selected comorbid behaviors and diseases on the association between HIV infection and prevalence of elevated biomarkers of inflammation (IL-6), altered coagulation (D-dimer), and monocyte activation (sCD14).

Biomarker	Comorbidities Omitted	Adjusted odds ratios (95% CI)						
		Reference	Model 1	Model 2		Model 3		
		HIV uninfected	HIV infected	HIV RNA <500	HIV RNA ≥500	CD4 count ≥500	CD4 count 200-499	CD4 count <200
IL-6	None	1.00 --	0.81 (0.55-1.19)	1.04 (0.79-1.36)	1.54 (1.14-2.09)	0.58 (0.41-0.82)	1.41 (1.06-1.88)	2.25 (1.60-3.16)
	Behaviors	1.00 --	1.39 (0.91-2.12)	1.24 (0.79-1.94)	1.84 (1.03-3.27)	0.73 (0.41-1.28)	1.61 (0.98-2.64)	2.82 (1.53-5.19)
	Diseases	1.00 --	1.36 (0.77-2.39)	0.92 (0.49-1.73)	2.37 (1.23-4.57)	0.64 (0.29-1.41)	1.26 (0.65-2.44)	3.95 (1.91-8.2)
D-dimer	None	1.00 --	0.95 (0.75-1.20)	0.71 (0.53-0.97)	1.97 (1.44-2.71)	0.61 (0.45-0.84)	0.97 (0.74-1.27)	1.68 (1.22-2.32)
	Behaviors	1.00 --	0.71 (0.48-1.05)	0.56 (0.36-0.85)	1.27 (0.75-2.17)	0.49 (0.29-0.82)	0.75 (0.47-1.22)	1.21 (0.66-2.25)
	Diseases	1.00 --	1.33 (0.76-2.32)	0.67 (0.35-1.26)	3.04 (1.61-5.74)	0.60 (0.28-1.30)	1.14 (0.60-2.18)	3.67 (1.82-7.38)
sCD14	None	1.00 --	0.79 (0.62-1.02)	0.89 (0.63-1.26)	1.48 (1.00-2.20)	1.04 (0.67-1.60)	1.25 (0.83-1.87)	2.60 (1.64-4.14)
	Behaviors	1.00 --	1.39 (0.91-2.14)	1.23 (0.79-1.92)	2.08 (1.17-3.72)	1.17 (0.7-1.94)	1.28 (0.78-2.12)	2.43 (1.32-4.49)
	Diseases	1.00 --	0.92 (0.56-1.52)	0.72 (0.42-1.24)	1.51 (0.83-2.75)	0.76 (0.41-1.42)	0.67 (0.36-1.22)	2.36 (1.21-4.58)

Models stratified by a) HIV status b) HIV-1 RNA and c) CD4 count and adjusted for all covariates
Reference for all models is HIV uninfected
Independent variables: Model 1 (HIV status); Model 2 (HIV status stratified by HIV-1 RNA (copies/mL)) Model; 3 (HIV status stratified by CD4 lymphocyte count (cells/mm^3))
Covariates: All models were adjusted for the following covariates excluding the comorbidities omitted: Age, race/ethnicity, prevalent CVD, hypertension, diabetes, smoking, BMI, cholesterol lowering medication use, HDL, LDL, triglycerides, cocaine use in the past year, hazardous drinking, hepatitis C, and renal disease

Comorbid behaviors omitted: current smoking, current hazardous alcohol drinking. Regression sample size ranges from 891 to 908 due to covariate variation between imputed datasets and missingness of outcomes and independent variables.
Comorbid diseases omitted: CVD, uncontrolled hypertension, diabetes, BMI≥30 kg/m^2, BMI<18 kg/m^2, HCV, renal disease. Regression sample size ranges from 574 to 587 due to covariate variation between imputed datasets and missingness of outcomes and independent variables.
OR, odds ratio; CI, confidence interval; HIV-1, human immunodeficiency virus type-1; CVD, cardiovascular disease; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HCV, hepatitis C virus; eGFR, estimated glomerular filtration rate.

2.5 DISCUSSION

Compared to uninfected Veterans, infected Veterans with HIV-1 RNA ≥500 copies/mL had a higher prevalence of elevated IL-6 and D-dimer. HIV infected Veterans with CD4 counts <200 cells/mm^3 had a higher prevalence of elevated IL-6, D-dimer, and sCD14 compared to uninfected Veterans. Importantly, the increased prevalence of these elevated biomarkers among HIV infected Veterans occurred despite the fact that the uninfected Veterans had similar or higher prevalence of comorbid conditions that were also strongly and significantly associated with prevalence of elevated biomarkers.

Our data confirm that comorbid conditions like CVD, and renal disease are important contributors to the prevalence of elevated biomarkers. However, excluding Veterans with confounding comorbid behaviors or diseases did not diminish the associations between HIV and increased prevalence of elevated biomarkers. The magnitude of some associations actually increased among Veterans with high viremia or immune depletion. Together, these findings point to the importance of addressing both HIV- and non HIV-related morbidity in clinical practice to potentially minimize elevations in these biomarkers that are associated with mortality and cardiovascular disease.

Our findings are consistent with prior studies demonstrating a significant association between HIV infection and elevated IL-6 and D-dimer. Neuhaus et al [67] showed IL-6 and D-dimer levels were respectively 152% and 94% higher among HIV infected individuals in the Strategies for Management of Antiretroviral Therapy

(SMART) study compared to levels in uninfected individuals of similar age in the Multi Ethnic Study of Atherosclerosis (MESA) ($p<0.001$ for both). Our study of Veterans found age and race/ethnicity adjusted IL-6 and D-dimer levels were 18% higher and lower respectively in infected Veterans. The smaller difference in these biomarkers in the VACS may be explained by the fact that our uninfected Veterans came from the same healthcare clinics as our HIV infected Veterans, and thus had a more comparable burden of comorbid disease. For example, smoking and dyslipidemia were less prevalent among uninfected Veterans, but they had a higher burden of CVD obesity, and diabetes. In contrast Neuhaus et al [67] reported that uninfected participants in MESA had a lower prevalence of CVD, diabetes, and smoking as compared to HIV infected participants in the SMART study. Selecting population based controls or participants from established cohort studies could result in a relatively "healthier" uninfected referent group. Because comorbidities like CVD risk factors are associated with elevated biomarkers of inflammation [81-83], altered coagulation [84-86], and/or monocyte activation [87,88], the effects of HIV on these biomarkers in HIV infected Veterans may have been balanced partially by the effects of these comorbidities among uninfected Veterans in the VACS.

Our results are also consistent with findings by Kuller et al [61] demonstrating that higher levels of IL-6 and D-dimer occurred in participants randomized to the SMART study drug conservation arm (intermittent ART), compared to those in the viral suppression arm (ongoing ART). In our study, unsuppressed HIV-1 RNA and low CD4 counts were each associated with a significantly higher prevalence of elevated IL-6 and D-dimer. It should be noted that our reference group (uninfected individuals) was

different from the reference group in SMART (virally suppressed HIV infected individuals).

Prior studies in HIV infected and uninfected people reported higher biomarkers of monocyte activation among those infected with HIV [69,89,90]. Our results suggest that this phenomenon is specific to those with low CD4 counts as there was no difference in the prevalence of elevated sCD14 by HIV status unless the CD4 count was <200 cells/mm^3. Although sCD14 is a biomarker of monocyte activation rather than a specific biomarker for microbial translocation, this finding among immune depleted Veterans is consistent with prior work demonstrating that microbial translocation is associated with chronic HIV infection and substantial CD4 cell depletion in the gastrointestinal tract [91-93]. Differences between our study and prior reports may partially be explained by their smaller sample sizes, restricted generalizability, and the greater comorbid disease burden among their infected versus uninfected participants. Our lack of a significant difference in sCD14 between infected Veterans with a CD4 count ≥200 cells/mm^3 and uninfected Veterans may be partially explained by the relatively balanced number of stimulators for immune activation between the two groups; infected Veterans had a higher prevalence of HCV while uninfected Veterans had a higher prevalence of obesity and diabetes, with current hazardous drinking being fairly similar between the two groups (Table 1).

Strengths of our study include a large cohort providing excellent power to detect clinically important differences between HIV infected and uninfected participants. The cohort also contains important subgroups – particularly Blacks, and people with HCV and/or substance use disorders. Our uninfected Veterans have a comparable burden of comorbid conditions to infected Veterans. We know this because we have detailed data

on important comorbidities such as prevalent CVD, diabetes, blood pressure values, lipid levels, smoking data and pharmacy refill data on antiretroviral therapy.

There are limitations that warrant discussion. First, the cross-sectional study design does not allow us to determine cause and effect. Second, the time window between ART ascertainment and blood specimen collection may have resulted in some misclassification by ART status. Third, it is likely that each biomarker is neither unique to, nor does it completely describe the complex biologic process of inflammation, altered coagulation or monocyte activation. The inclusion of lipopolysaccharide and 16S rDNA in combination with sCD14 may provide more accurate insights into the microbial translocation process. Fourth, this study also lacked biomarkers of immune activation (e.g. TNF alpha, IL-1 beta, IL-10). Such biomarkers would be valuable for providing insights into the immune response to the inflammatory processes studied. Finally, as our cohort is overwhelmingly male, our results may not be generalizable to women.

2.6 CONCLUSION

In conclusion, HIV infected Veterans with unsuppressed HIV viremia or a low CD4 count had a significantly higher prevalence of elevated IL-6 and D-dimer as compared to uninfected Veterans. This was true despite the fact that uninfected Veterans had a similar or higher burden of comorbid diseases that were associated with elevations in these biomarkers. Increased monocyte activation as measured by sCD14 only differed by HIV status when infected Veterans had CD4 counts <200 cell/mm^3. These data suggest that both ongoing HIV replication and immune depletion and comorbid

conditions like CVD and renal disease, contribute to elevated biomarkers associated with inflammation, altered coagulation, and monocyte activation. Focusing on viremia reduction, CD4 cell restoration and treatment of non-HIV related comorbidity may be an important strategy to reduce mortality and CVD risk.

3.0 PAPER 2: HIV, HEPATITIS C AND INFLAMMATORY BIOMARKERS IN INDIVIDUALS WITH ALCOHOL PROBLEMS

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3.1 ABSTRACT

Background: Assessing whether hepatitis C (HCV) co-infection with human immunodeficiency virus (HIV) is associated with increased inflammation is complex. The liver, integral to inflammatory biomarker synthesis, is compromised by HCV and alcohol abuse. Using single liver-synthesized biomarkers (e.g. C-reactive protein) to represent inflammation may not be appropriate in HIV/HCV co-infection. We hypothesized that 1) detectable HIV/HCV RNA was independently associated with increased inflammation; 2) a composite inflammation measure describes inflammation differently from single inflammatory biomarkers.

Methods: We compared inflammation by HIV/HCV group in a cohort of 361 HIV infected participants from the HIV-Longitudinal Interrelationships of Viruses and Ethanol study. Inflammatory biomarkers >75th percentile were considered elevated. Associations between HIV/HCV group and elevated biomarkers were analyzed as a composite measure (inflammatory burden) or individually. We defined inflammatory burden as number of concurrently elevated biomarkers. Biomarkers included interleukin-6 (IL-6), C-reactive protein (CRP), cystatin C, serum amyloid-A (SAA), tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10). Covariates: alcohol, liver fibrosis, comorbidities, CD4 count, antiretroviral therapy, substance use.

Results: Detectable HIV and HCV RNA (OR=2.49; 95%CI=1.05–5.89) and detectable HCV RNA alone (2.95; 1.08–8.01) were independently associated with increased odds of having a greater inflammatory burden compared to undetectable viremia. Elevated IL-10 (7.79; 1.90–31.97) and TNF-α (7.70; 1.42–41.83) were independently associated with

detectable HIV and HCV RNA. Elevated IL-10 was also associated with detectable HCV RNA alone (5.51; 1.17, 25.84).

Conclusion: Detectable HIV and HCV replication versus undetectable replication was associated with inflammatory burden and certain inflammatory biomarkers independently of alcohol consumption, liver fibrosis and other comorbidities.

3.2 INTRODUCTION

Several reports suggest that human immunodeficiency virus (HIV) infection and hepatitis C (HCV) co-infection with HIV are associated with increased cardiovascular disease (CVD) risk [94-97]. Prior studies also link chronic inflammation, monocyte activation and/or altered coagulation with acute myocardial infarction and death in HIV infected people [61,62,65,98]. Whether HIV and HCV mediate their effects on CVD risk through these mechanisms is not known. Assessing whether HIV mono- and HIV/HCV co-infection are associated with increased inflammation is therefore important, though not straightforward. Liver damage caused by alcohol consumption and HCV may alter serum levels of inflammatory biomarkers that are synthesized in the liver (e.g., C reactive protein) and possibly confound the association between viremia and biomarkers of systemic inflammation. Moreover, using a single biomarker, particularly one synthesized in the liver, to represent systemic levels of inflammation may not adequately represent this complex process. Whether a composite measure involving multiple elevated inflammatory biomarkers, including those synthesized in the liver, provides a more complete representation of the state of inflammation in the setting of HIV/HCV infection is not clear.

The objective of this study, therefore, was to examine the association between HIV and HCV viremia and biomarkers of inflammation while accounting for alcohol consumption and liver fibrosis.

3.3 METHODS

3.3.1 Study sample

Baseline data were collected from 361 HIV infected participants in the HIV-Longitudinal Interrelationships of Viruses and Ethanol (HIV-LIVE) study. HIV-LIVE is a prospective cohort of HIV infected people with current or past alcohol problems. As previously reported [99], 400 HIV-LIVE participants were enrolled from four different sources from August 2001 to July 2003: (1) an existing cohort of HIV-infected participants with alcohol problems; (2) Boston Medical Center (BMC)'s Diagnostic Evaluation Unit; (3) Beth Israel Deaconess Medical Center (BIDMC) primary care and specialty clinics; and (4) local health care sites or shelters in the Boston area. Participants were included if they had a positive HIV antibody test (ELISA, confirmed by Western blot), had two or more affirmative responses to the CAGE (Cut down, Annoyed, Guilty, and Eye opener) alcohol screening questionnaire [100] or by physician-investigator diagnosis of alcoholism, spoke English or Spanish, and had at least one contact person likely to know the participant's whereabouts. Individuals were excluded if the 30-item Folstein Mini-Mental State Examination score [101] was less than 21 or a trained interviewer deemed the patient incapable of comprehending the informed consent or answering interview questions.

3.3.2 Ethics statement

The Institutional Review Boards of Boston Medical Center, Beth Israel Deaconess Medical Center, and the University of Pittsburgh approved this study.

3.3.3 Dependent variable

We defined an elevated biomarker as a serum biomarker level >75th percentile based on prior studies [61,102]. We examined the following seven biomarkers: interleukin-6 (IL-6), C-reactive protein (CRP), cystatin C, serum amyloid A (SAA), tumor necrosis factor-alpha (TNF-α), monocyte chemotactic protein-1 (MCP-1), and interferon gamma (IFN-γ). The primary outcome, inflammatory burden score, was defined as the presence of zero, one, two, or three or more elevated biomarkers. For example, an inflammatory burden score of 0 corresponds to having none of the seven biomarkers elevated. We defined a score of 3 as having at least three of the seven biomarkers elevated since few people had between four and seven biomarkers elevated. Our secondary outcomes were elevated individual inflammatory biomarkers and included interleukin-10 (IL-10). Importantly, IL-10 was not included in the inflammatory burden score because of its anti-inflammatory properties. For both outcomes, these biomarkers were chosen due to their associations with cardiovascular morbidity and mortality [60,103-105], HIV [61,67,106], or their synthesis in the liver [107,108].

IL-6 was measured by ELISA (Quantiglo Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN) with an assay range of 0.48–1500 pg/mL. The intra-assay and inter-assay coefficients of variation (CVs) ranged from 3.0–5.8% and 6.3–9.6%,

respectively. CRP, cystatin C and SAA were measured using a particle enhanced immunonepholometric assay (BNII nephelometer; Dade Behring Inc., Deerfield, IL). The CRP assay range was 0.16–1100 ug/mL. Intra-assay CVs ranged from 2.3–4.4% and inter-assay CVs ranged from 2.1–5.7%. The cystatin C assay range was 0.195–7.330 mg/L. Intra- and inter-assay CVs were <5%. The SAA assay range and minimum detectable level were determined by the lower limit of the reference curve and were dependent on the concentration of the SAA standard used in the assay (N SAA Standard SY). Intra-assay CVs ranged from 4.3–6.2% and inter-assay CVs ranged from 2.8–4.7%. TNF-α, MCP-1, IL-10 and INF-γ were measured using the Human Serum Adipokine Panel B LINCOplex Kit (Linco Research, Inc. St. Charles, MO). The TNF-α and MCP-1 assay ranges were 3.2–50,000 pg/mL. Intra- and inter-assay CVs ranged from 1.4-7.9% and < 21%, respectively. The IL-10 assay range was 3.2–10,000 pg/mL. Intra- and inter-assay CVs ranged from 4.8–9.0% and 3.1–18.4%, respectively. For INF-γ, the assay range was 3.2–10,000 pg/mL. Intra- and inter-assay CVs ranged from 4.8–9.0% and 3.1–18.4%, respectively. All assays were performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT).

3.3.4 Independent variable

Detectable viremia was the independent variable categorized into one of four groups: HIV and HCV RNA undetectable (undetectable), HIV RNA but not HCV RNA detectable (HIV mono-detectable), HIV RNA undetectable but HCV RNA detectable (HCV mono-detectable), and HIV and HCV RNA detectable (HIV/HCV detectable). The

undetectable group was the referent group for all analyses. HCV RNA was determined from serum collected at the time of enrollment or from participants' medical records. All participants were HIV antibody positive. HIV RNA testing was performed using a branched-chain DNA assay or polymerase chain reaction (PCR) [109]. The lower threshold of detection was 50-75 copies/mL. All subjects were tested for HCV infection by measuring HCV antibodies and antibody-positive subjects were tested for HCV RNA if this was unavailable from medical records. HCV RNA was measured either by branched chain DNA or PCR-based assays. The lower level of detection of the assays was 615 IU/mL. HCV antibody-negative subjects were assumed to be HCV RNA negative [110].

3.3.5 Covariates

Demographic covariates were age; gender; race (white vs. non-white). Liver fibrosis was assessed as a fibrosis index-4 (FIB-4) score ≥1.45 [111]. We detailed alcohol use data using the 30-day TimeLine Follow Back instrument [112]. Current at-risk alcohol consumption was defined per National Institute on Alcohol Abuse and Alcoholism criteria: drinking >14 standard drinks for men (>seven for women) per week or >four drinks on one occasion for men (>three drinks for women) in the last 30 days [113]. Other covariates were CD4+ T-cell count <200 cells/mm^3 and self-reported antiretroviral therapy (ART) use at time of assessment, and obesity (body mass index (BMI) ≥30 kg/m^2). Self-reported comorbid disease was defined as a "yes" response to any of the following questions: "Has a doctor ever told you that you had: CVD (peripheral vascular disease, hardening of the arteries in your neck or legs, atherosclerosis; a stroke, cerebrovascular accident, blood clot or bleeding in the brain, or transient ischemic attack;

or a heart attack or myocardial infarction); diabetes or high blood sugar or sugar; hypertension or high blood pressure; high cholesterol; renal disease (poor kidney function or blood tests showing high creatinine); or anemia (low red blood cell count, hemoglobin)?"

For other substance use variables, we defined current smoking as a "yes" response to the question, "Do you currently smoke cigarettes every day or on some days?"; cocaine use as self-reported use of "cocaine, crack or free base"; and injection drug use as a "yes" response to the question, "In your lifetime, have you ever injected drugs?"

3.3.6 Analysis

Baseline characteristics and biomarker distributions were described and compared by HIV/HCV group using ANOVA, Kruskal Wallis tests, or chi-square tests as appropriate. Biomarker distributions were also described and compared by covariates.

The primary analysis (labeled A) used a proportional odds model to estimate the odds of more elevated (>75th percentile) biomarkers. Two models were fit: (1) an unadjusted model with HIV/HCV status only; (2) an adjusted model with HIV/HCV status, age, gender, FIB-4 score, at-risk drinking, CD4 count, ART use, and self-reported comorbidity. The proportional odds model estimates the proportional odds (P_{odds}) of having more than *N* concurrently elevated biomarkers versus *N* or fewer. For example, compared to those in the undetectable group, the odds of having more than two versus two or fewer elevated biomarkers is P_{odds} greater for those in the HIV/HCV detectable

group. The assumption of proportional odds implies that the coefficients that describe the relationship between an inflammatory burden score of 0 compared to a score of 1 or more are the same as those for an inflammatory burden score of 1 compared to 2 or more. This assumption was assessed by the Score Test. Our secondary analyses used logistic regression to model the odds of having an elevated individual biomarker (labeled B-I) adjusted for the covariates in the models above. Spearman correlation was used to assess potential collinearity in the regression models. No pair of variables within a regression model was highly correlated ($r>0.40$). Analyses were conducted using two-sided tests and a significance level of 0.05 and performed using SAS 9.3 (Cary, NC).

Table 10: Sources, targets and effects of biomarkers investigated in this study.

Biomarker	Secreted by	Targets and effects
IL-6	Macrophages, endothelial cells	Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell linkage)
CRP	Hepatocytes	Activates complement, deposits an opsonin on surface of microorganisms to facilitate phagocytosis
Cystatin C	All nucleated cells	Inhibits cysteine proteinases from breaking down proteins. Cleared by glomerular filtration so used as a marker of renal disease. Correlated with inflammatory biomarkers (IL-6, CRP, TNF-α in MESA).
SAA	Hepatocytes, adipocytes	During acute phase reaction, liver is major site of SAA synthesis, but in non-acute conditions, adipocytes secrete SAA. SAA is expressed in response to pro-inflammatory cytokines and associates with HDL particles to alter reverse cholesterol transport.
TNF-alpha	Monocytes, macrophages, other (activated T cells, NK cells, neutrophils, fibroblasts)	Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation
MCP-1	Hepatic stellate cells[61]	Cytokine that recruits monocytes, memory T cells, and dendritic cells to the sites of inflammation produced by either tissue injury or infection.
IL-10	Activated subsets of $CD4^+$ and $CD8^+$ T cells (T_H2 cells)	Stimulates or enhances B-cell proliferation (as well as thymocytes, mast cells); IgA synthesis and secretion by B-cells; antagonizes generation of T_H1 cells.
IFN-gamma	T_H1 cells, $CD8^+$ cells, NK cells	Activates macrophages; increases expression of MHC class I and class II molecules; increases antigen presentation; weak antiviral and anti-proliferative activities

3.4 RESULTS

Mean age (range: 40 – 46 years) was different ($p<0.01$) across the four HIV/HCV groups and study participants were two-thirds non-white and three quarters male. Evidence of liver fibrosis, diabetes, and CVD was highest among those with HCV whereas at-risk alcohol consumption and immunodeficiency were highest among those with detectable HIV RNA (Table 11). Median biomarker levels were generally highest among those with both HIV and HCV detectable (Table 12) – likewise for those with highest levels of inflammatory burden (Table 13). Significant ($p<0.05$) correlations were observed between HIV RNA and IL-10, TNF-α, cystatin C (Table 14).

Participants in the undetectable group were least likely to have concurrently elevated biomarkers (inflammatory burden score = 2 or 3), while those in the HIV/HCV detectable group were most likely (Figure 5). For individual biomarkers, the prevalence of elevated IL-10, TNF-α, cystatin C, and IL-6 was significantly different across the four HIV/HCV groups ($p<0.05$, Figure 5). The highest proportions of elevated IL-10, TNF-α, and cystatin C occurred in the HIV/HCV detectable group (Figure 5). The HCV mono-detectable group had the highest proportion of elevated IL-6 (Figure 5). The prevalence of elevated CRP, SAA, IFN-γ and MCP-1 was similar across the four groups ($p>0.05$, Figure 5).

Table 11: Characteristics of 361 HIV-LIVE participants with HIV infection and alcohol problems stratified by detectable HIV and HCV viremia.

Subject characteristics	HIV/HCV RNA both undetectable (N=59)	HIV RNA only detectable (N=122)	HCV RNA only detectable (N=53)	HIV/HCV RNA both detectable (N=127)	p-value
Age in years, mean (SD)	43 (7)	40 (8)	46 (7)	43 (7)	<0.01
Female	16 (27.1)	26 (21.3)	11 (20.8)	35 (27.6)	0.59
Race, Non-White	42 (71.2)	84 (68.9)	34 (64.2)	84 (66.1)	0.84
FIB-4 ≥ 1.45	7 (16.3)	28 (28.2)	22 (59.4)	52 (54.7)	<0.01
FIB-4, Median (Min, Max)	0.9 (0.5, 3.1)	1.1 (0.2, 7.5)	1.6 (0.7, 14.9)	1.6 (0.4, 17.1)	0.30
Mean (Standard Deviation)	1.1 (0.5)	1.3 (1.0)	2.8 (2.9)	2.3 (2.2)	
N	43	99	37	95	
Current at-risk alcohol consumption	15 (25.4)	46 (38.0)	9 (17.0)	43 (33.9)	0.03
Current CD4+ T-lymphocyte count <200 cells/mm^3	4 (6.8)	27 (22.9)	6 (11.8)	34 (26.8)	<0.01
Current antiretroviral medication	52 (88.1)	56 (45.9)	50 (94.3)	65 (51.2)	<0.01
BMI ≥30kg/ m^2	13 (22.8)	23 (19.8)	8 (15.1)	25 (21.2)	0.76
Prevalent cardiovascular disease	3 (5.1)	2 (1.6)	6 (11.3)	15 (11.9)	<0.01
Ever had diabetes	2 (3.4)	6 (4.9)	9 (17.0)	9 (7.1)	0.02
Ever had hypertension	16 (27.1)	28 (23.0)	14 (26.4)	33 (26.0)	0.91
Ever had high cholesterol	29 (49.2)	32 (26.2)	11 (20.8)	25 (19.7)	<0.01
Ever had kidney disease	2 (3.4)	5 (4.1)	5 (9.4)	9 (7.1)	0.40
Ever anemic	17 (28.8)	28 (23.0)	12 (22.6)	31 (24.4)	0.84
Current smoker	39 (66.1)	93 (76.2)	40 (75.5)	102 (80.3)	0.22
Ever cocaine use	23 (39.0)	53 (43.4)	18 (34.0)	65 (51.2)	0.14
Ever injection drug use	10 (17.0)	26 (21.5)	46 (86.8)	115 (90.6)	<0.01
Ever treated hepatitis C	0 (0)	3 (2.5)	8 (15.1)	6 (4.7)	<0.01

All values are n (% of HIV/HCV group) unless otherwise specified

HIV-human immunodeficiency virus; HCV-hepatitis C virus; FIB-4-liver fibrosis index-4; BMI-body mass index

Table 12: Distribution of biomarkers by HIV/HCV viremia status

	HIV & HCV Detectable	HIV Only Detectable	HCV Only Detectable	HIV & HCV Undetectable
IL-10 pg/ml	6.50 (6.76)	5.00 (4.88)	6.30 (4.85)	2.85 (2.27)
TNFa pg/ml	7.96 (6.35)	7.64 (4.52)	6.59 (3.6)	4.88 (3.23)
Cystatin C mg/l	0.87 (0.24)	0.76 (0.21)	0.77 (0.18)	0.67 (0.19)
IL-6 pg/ml	3.24 (3.48)	2.35 (2.12)	3.86 (4.89)	1.89 (2.36)
CRP mg/l	1.08 (2.63)	1.73 (2.86)	1.89 (3.82)	2.06 (2.47)
SAA mg/l	3.00 (5.1)	3.35 (4.65)	3.00 (4.2)	3.20 (4.8)
IFNg pg/ml	0.12 (0)	0.12 (0)	0.12 (0)	0.12 (0)
MCP-1 pg/ml	563.13 (393.62)	623.08 (474.23)	520.78 (425.81)	544.39 (347.77)

Table 13: Distribution of biomarkers by inflammatory burden score

	No Elevated Biomarkers	1 Elevated Biomarker	2 Elevated Biomarkers	3+ Elevated Biomarkers
IL-10 pg/ml	3.13 (2.67)	4.49 (4.32)	5.46 (4.61)	8.39 (8.68)
TNFa pg/ml	4.89 (2.21)	6.23 (4.01)	7.86 (5.37)	9.84 (6.33)
Cystatin C mg/l	0.65 (0.17)	0.77 (0.18)	0.82 (0.21)	0.93 (0.4)
IL-6 pg/ml	1.45 (1.54)	2.05 (1.96)	3.25 (2.8)	5.53 (6.91)
CRP mg/l	0.82 (1.73)	1.13 (1.55)	1.67 (3.33)	3.49 (5.94)
SAA mg/l	2.15 (2.2)	2.60 (3.4)	3.65 (4.4)	6.35 (8.9)
IFNg pg/ml	0.12 (0.0)	0.12 (0.0)	0.12 (0.0)	0.12 (0.2)
MCP-1 pg/ml	460.69 (270.33)	543.83 (392.52)	644.18 (544.09)	746.73 (483.7)

Table 14: Correlations between biomarkers and HIV/HCV viremia

Spearman Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations		
	HIV-1 RNA	**Hepatitis C RNA**
IL-10 pg/mL	0.31 <0.01 313	0.07 0.36 192
TNFa pg/mL	0.31 <0.01 313	0.04 0.59 192
Cystatin C_mg/L	0.28 <0.01 313	0.09 0.23 192
IL-6_pg/ml	0.09 0.11 281	0.04 0.62 170
CRP mg/L	0.02 0.68 313	-0.11 0.15 192
SAA_mg/L	0.05 0.42 298	0.11 0.16 183
IFNg pg/mL	-0.03 0.55 313	-0.04 0.57 192
MCP-1 pg/mL	0.09 0.11 313	0.16 0.03 192

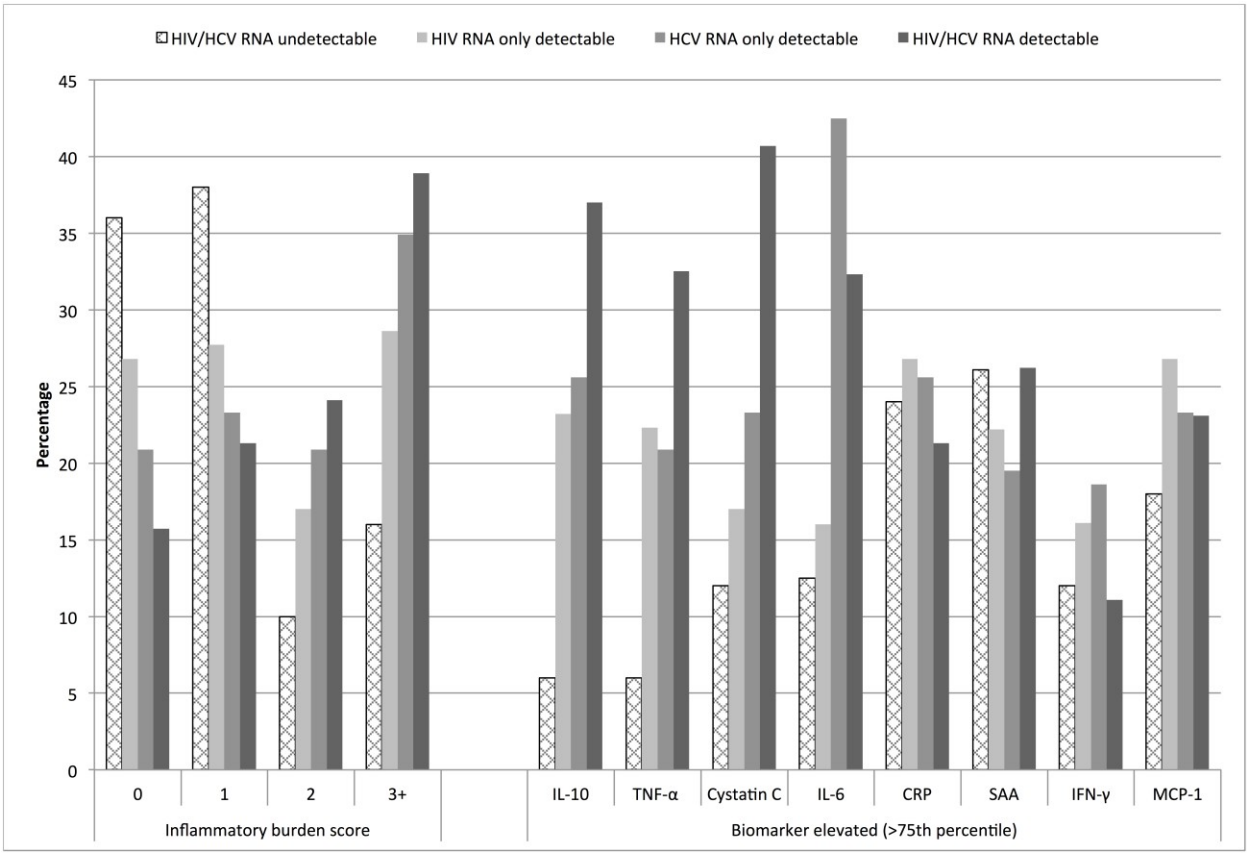

Figure 5: Inflammatory burden scores (number of elevated biomarkers) and individually elevated biomarkers by HIV/HCV group.

Elevated individual biomarkers were defined as a serum biomarker level >75th percentile. Inflammatory burden score was defined as the presence of zero, one, two, or three or more elevated biomarkers. For individual biomarkers, the prevalence of elevated IL-10, TNF-α, IL-6 and cystatin C was significantly different across the four HIV/HCV groups ($p<0.05$).

Compared to participants with undetectable HIV and HCV RNA, those in the HIV mono-detectable group (proportional odds ratio (P_{OR})=1.89 (95% confidence interval (CI) 1.03-3.46), HCV mono-detectable group (P_{OR}=2.70, 95% CI=1.29-5.68), and HIV/HCV detectable group (P_{OR}=3.48, 95% CI=1.87-6.46) had a significantly higher inflammatory burden (Table 15). This association persisted among participants in the HCV mono detectable and HIV/HCV detectable groups after adjusting for potential confounders (Table 15). FIB4 score ≥1.45 was also significantly associated with an increased burden of inflammatory biomarkers ($p<0.01$, Table 15).

Compared to the undetectable group, the HIV/HCV RNA detectable group had higher prevalence of elevated IL-10, TNF-α, IL-6, and cystatin C. After adjustment for potential confounders, this association remained significant for IL-10 (OR=7.79, 95% CI=1.90-31.97) and TNF-α (OR=7.70, 95% CI=1.42-41.83, Table 16). Elevations in CRP, SAA, IFN-γ, and MCP-1 were not significantly different in any HIV/HCV group compared to the undetectable group in either the unadjusted or adjusted models (Table 16).

FIB-4 ≥1.45 was significantly associated with elevated cystatin C (OR=3.43, 95% CI= 1.45-8.10), IL-6 (OR=3.22, 95% CI= 1.44-7.20) [114] and MCP-1 (OR=2.39, 95% CI=1.10- 5.20). Age above the median (42 years) was associated with lower odds of elevated IL-10, self-reported high cholesterol and diabetes with higher odds of elevated TNF-α, and current ART use with lower odds of elevated TNF-α. Self-reported CVD and smoking were associated with higher odds of elevated cystatin C, and self-reported renal disease with higher odds of elevated SAA and cystatin C. BMI ≥30 kg/m^2

was associated with higher odds of elevated CRP, and female gender with lower odds of elevated MCP-1 ($p<0.05$ for all; Table A2).

Table 15: Association of HIV/HCV group with concurrently elevated (>75th percentile) inflammatory biomarkers.

Model A (N=218)	**Unadjusted**		**Adjusted[a]**	
	P_{OR} (95% CI)	**p-value**	**P_{OR} (95% CI)**	**p-value**
Undetectable	**1**	**--**	**1**	**--**
HIV mono-detectable	1.89 (1.03, 3.46)	0.04	1.52 (0.68, 3.41)	0.31
HCV mono-detectable	2.70 (1.29, 5.68)	<0.01	2.95 (1.08, 8.01)	0.03
HIV/HCV detectable	3.48 (1.87, 6.46)	<0.01	2.49 (1.05, 5.89)	0.04
FIB-4 $\geq$ 1.45	--	--	2.56 (1.39, 4.35)	<0.01
High cholesterol	--	--	1.74 (0.97, 3.14)	0.06
Age greater than median (42 yrs)	--	--	0.61 (0.36, 1.04)	0.07
BMI $\geq$ 30 kg/m^2	--	--	1.81 (0.93, 3.54)	0.08
Ever smoker	--	--	1.53 (0.85, 2.78)	0.16
CD4 > 200 cells/mm^3	--	--	0.63 (0.31, 1.26)	0.19
Renal disease	--	--	2.15 (0.67, 6.86)	0.20
Diabetes	--	--	1.90 (0.58, 6.21)	0.29
Current antiretroviral therapy use	--	--	0.74 (0.42, 1.31)	0.30
Prevalent cardiovascular disease	--	--	1.82 (0.56, 5.97)	0.32
Hypertension	--	--	0.78 (0.42, 1.45)	0.44
At-risk alcohol consumption	--	--	0.94 (0.55, 1.62)	0.83
Female	--	--	1.03 (0.55, 1.94)	0.93

[a]Model adjusted for age, gender, FIB-4 score, at-risk drinking, CD4 count, ART use, and self-reported comorbidity.

The ordinal logistic regression model estimates the proportional odds of having more than N concurrently elevated biomarkers versus N or fewer where N=0, 1, 2, or 3 or more.

POR-proportional odds ratio; HIV-human immunodeficiency virus; HCV-hepatitis C virus; FIB-4- liver fibrosis index-4; BMI-body mass index; CD4-CD4+ T-lymphocyte count

Table 16: Association of HIV/HCV group with individually elevated (>75th percentile) biomarkers.

Biomarker elevated	HIV/HCV group	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Model B (N=218)	**Undetectable**	1	1
Interleukin-10	HIV mono-detectable	4.74* (1.36, 16.48)	2.95 (0.74, 11.85)
	HCV mono-detectable	5.39* (1.39, 20.84)	5.51* (1.17, 25.84)
	HIV/HCV detectable	9.22* (2.69, 31.55)	7.79* (1.90, 31.97)
Model C (N=218)	**Undetectable**	1	1
Tumor necrosis factor-α	HIV mono-detectable	4.50* (1.29, 15.70)	4.44 (0.86, 22.82)
	HCV mono-detectable	4.15* (1.04, 16.47)	4.45 (0.68, 29.02)
	HIV/HCV detectable	8.50* (2.48, 29.16)	7.70* (1.42, 41.83)
Model D (N=196)	**Undetectable**	1	1
Interleukin-6	HIV mono-detectable	1.33 (0.49, 3.66)	0.57 (0.15, 2.16)
	HCV mono-detectable	5.17* (1.79, 14.94)	2.99 (0.75, 11.98)
	HIV/HCV detectable	3.33* (1.28, 8.70)	1.47 (0.40, 5.35)
Model E (N=218)	**Undetectable**	1	1
Cystatin-C	HIV mono-detectable	1.50 (0.56, 4.01)	0.49 (0.12, 2.02)
	HCV mono-detectable	2.22 (0.73, 6.73)	0.40 (0.07, 2.34)
	HIV/HCV detectable	5.04* (1.98, 12.85)	1.60 (0.39, 6.57)
Model F (N=218)	**Undetectable**	1	1
C-reactive protein	HIV mono-detectable	1.16 (0.54, 2.51)	0.99 (0.35, 2.84)
	HCV mono-detectable	1.09 (0.42, 2.80)	1.41 (0.41, 4.90)
	HIV/HCV detectable	0.86 (0.39, 1.90)	0.69 (0.22, 2.14)
Model G (N=210)	**Undetectable**	1	1
Serum amyloid A	HIV mono-detectable	0.81 (0.36, 1.80)	0.58 (0.19, 1.79)
	HCV mono-detectable	0.69 (0.25, 1.89)	0.38 (0.09, 1.66)
	HIV/HCV detectable	1.01 (0.46, 2.22)	0.72 (0.22, 2.31)
Model H (N=218)	**Undetectable**	1	1
Interferon-γ	HIV mono-detectable	1.40 (0.52, 3.78)	1.65 (0.46, 5.87)
	HCV mono-detectable	1.68 (0.53, 5.28)	2.63 (0.59, 11.76)
	HIV/HCV detectable	0.92 (0.32, 2.60)	1.03 (0.25, 4.30)
Model I (N=218)	**Undetectable**	1	1
Monocyte chemoattractant protein-1	HIV mono-detectable	1.67 (0.72, 3.84)	2.96 (0.80, 10.95)
	HCV mono-detectable	1.38 (0.50, 3.79)	3.71 (0.83, 16.53)
	HIV/HCV detectable	1.37 (0.59, 3.21)	3.48 (0.88, 13.69)

[a]Models adjusted for age, gender, FIB-4 score, at-risk drinking, CD4 count, ART use, and self-reported comorbidity. See Table A2 for odds ratios for covariates.

3.5 DISCUSSION

This study suggests that HIV and HCV viremia contribute to elevations in inflammatory burden score, IL-10 and TNF-α, independently of and in addition to the contribution from comorbid conditions. Our results also suggest that a composite measure, comprising multiple inflammatory biomarkers, may suggest an inflammatory state is present even when individual biomarkers do not. The fact that there was no significant association between any HIV/HCV group and CRP or SAA, two biomarkers synthesized in the liver, suggests a need for caution when using these biomarkers to assess inflammation in this population with high potential for liver morbidity.

This study differs from prior work [115-123] in that it attempts to classify people as having more or less inflammation using a concomitantly elevated panel of inflammatory biomarkers rather than emphasizing one individual biomarker of inflammation. Additionally, our results may be more definitive these prior studies because of our detailed data on alcohol consumption and the inclusion of a validated measure of liver fibrosis.

Findings from our study are consistent with those from prior studies investigating the association between HIV/HCV status and TNF-α [115-118], CRP [119,120], IL-10 [118,121], IFN-γ [118,122,124], IL-6 [116] and cystatin C [123]. However, some differences between our work and these prior studies may reflect different biomarker outcome categorization (quartiles versus detection, secretion, ormeans), HIV/HCV categorization (viremia versus antibody detection, *in vitro* stimulation with viral proteins) sources of biomarkers (serum versus intrahepatic),

viral proteins) sources of biomarkers (serum versus intrahepatic), referent groups (participants with undetectable HIV and HCV viremia versus HIV or HCV mono-infected participants) and adjustment covariates. The strong associations we reported between detectable viremia and the anti-inflammatory cytokine, IL-10, are consistent with prior research linking persistent viral infection with increased IL-10 production [125]. The lack of data comparing MCP-1, or SAA levels by HIV/HCV status suggests that our findings for these particular cytokines may be novel.

The present study and other work has shown that factors including viral replication, immunocompetence, comorbidity [61,67,68,102], ART [61,67] and ART hepatotoxicity [126], contribute to alterations in some inflammatory biomarkers but not others. If the current study had only investigated CRP, a clinically used inflammatory biomarker [127], we may have concluded that detectable HIV and HCV viremia were not associated with increased inflammation. To more completely describe inflammation, we propose that research into the inflammatory basis of morbidity and mortality should also use composite measures of inflammatory biomarkers. These biomarkers often reflect overlapping biological processes involved in the immune response. Concurrently using multiple biomarkers potentially reduces the variability (intra and inter-person) inherent in measuring and analyzing individual biomarkers. Using single biomarkers to quantify systemic inflammation may be most appropriate in populations with minimal inflammatory comorbid disease. However, a composite measure of inflammation may be more appropriate in HIV-infected populations, where multi-morbidity contributes strongly to chronic systemic inflammation. Future research should compare the contributions of composite versus individual inflammatory biomarker measures to CVD morbidity and mortality prediction.

Strengths of our study include a large, diverse panel of inflammatory biomarkers. Our study sample had well characterized measures of current or past alcohol problems, an important comorbid condition in HIV populations. We had a large proportion of non-white individuals (>50%) enabling generalizability to important minority populations. Limitations that warrant discussion include the lack of HIV uninfected controls, the cross sectional nature of this study, self-reported measures of health conditions, and lack of direct cell surface immune activation data for comparison to serum cytokine data. Sample size limitations may explain the strong but non-significant associations of covariates like high cholesterol, renal disease and smoking with inflammatory burden. This limitation may also explain the stronger association of the HCV mono-detectable group with inflammatory burden versus the HIV/HCV detectable group. As with all observational studies, we cannot exclude the potential for residual confounding.

3.6 CONCLUSION

In a cohort of HIV infected people with current or past alcohol problems, detectable HIV and HCV RNA compared to undetectable viremia was associated with greater systemic inflammation as measured by an inflammatory burden score and elevations in certain inflammatory biomarkers. This association was independent of at-risk drinking, liver fibrosis and other comorbidities.

4.0 PAPER 3: PREHYPERTENSION, HYPERTENSION AND THE RISK OF ACUTE MYOCARDIAL INFARCTION IN HIV INFECTED AND UNINFECTED VETERANS

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4.1 ABSTRACT

Introduction: HIV infection is an independent predictor of acute myocardial infarction (AMI) with a magnitude of association similar to that of diabetes mellitus. In the general population, current guidelines recommend more aggressive treatment of prehypertension among those with diabetes or renal disease to prevent future cardiovascular disease (CVD). Similar rationale may apply to HIV infection. The objective of this study was to examine whether the association between elevated blood pressure and risk for AMI differed by HIV status.

Methods: We analyzed data on 81,030 people from the observational Veterans Aging Cohort Study Virtual Cohort (VC), who were free of CVD at baseline. HIV infected (HIV+) and uninfected Veterans were matched 1:2 on age, gender, race/ethnicity, and clinical site. We collected data on systolic and diastolic blood pressure, antihypertensive medications, diabetes, dyslipidemia, smoking, hepatitis C, body mass index, renal disease, and substance abuse at baseline and on the incidence of clinically confirmed AMI from 10/2003-9/2008. Blood pressure was the average of the three outpatient routine clinical blood pressure measurements performed closest to the baseline date (first clinical visit after 4/2003). Blood pressure categories used in the analyses were based on JNC- VI and VII criteria. Analyses were performed using Cox proportional hazards regression.

Results: Over a median of 5.9 years, 860 incident AMIs occurred. AMI rates were higher among HIV+ compared to uninfected Veterans within almost all blood pressure categories. Low/high prehypertensive and untreated/treated hypertensive individuals had increased AMI risk compared to uninfected untreated normotensive individuals [Hazard Ratio [HR]: 1.60; 95% confidence interval (CI): 1.07-2.39; HR:1.80 (1.21-2.68); HR: 2.57 (1.76-3.76); HR: 2.75 (1.89-4.00) respectively]

Conclusions: Given the increased risk of AMI, diabetes, and renal disease associated with HIV infection and if our results are confirmed in other studies, future research should explore whether lower blood pressure targets among HIV+ people translates into lower AMI risk in a randomized controlled trial.

4.2 INTRODUCTION

For certain high cardiovascular disease (CVD) risk populations like people with diabetes or chronic kidney disease, more aggressive blood pressure control is recommended to prevent future CVD events[128]. Compared to uninfected people, HIV infected (HIV+) people have an increased risk of acute myocardial infarction (AMI)[129-131] as well as diabetes[132] and chronic renal disease[133]. However there are no specific guidelines for blood pressure management among HIV+ people. Whether HIV+ people have increased AMI risk even at prehypertension levels compared to uninfected people is not known. The objective of this study was to examine the relationship among HIV status, blood pressure, and the risk of AMI and to determine if HIV infected people with prehypertension had an increased risk of AMI compared with uninfected people.

4.3 METHODS

4.3.1 Subject selection

The Veterans Aging Cohort Study Virtual Cohort (VACS VC)[134] is a prospective longitudinal cohort of HIV+ Veterans each matched on age, race/ethnicity, and clinical site to two uninfected Veterans in care. Subjects have been continuously enrolled in each year since 1998 using a validated existing algorithm from United States Department of Veterans Affairs

(VA) national electronic medical record system [134]. The institutional review boards at the University of Pittsburgh, Yale University, and the West Haven VAMC approved this study.

All VACS VC participants who were alive and enrolled in VACS VC on or after 2003 were eligible for this study. Baseline was a participant's first clinical encounter on or after 4/1/2003. All participants were followed from their baseline date to an AMI event, death, or the date of last follow-up (1/1/2010).

These AMI data were merged with AMI data from Medicare and the Ischemic Heart Disease Quality Enhancement Research Initiative (IHD-QUERI), an initiative designed to improve the quality of care and health outcomes of Veterans with IHD [71,135]. We excluded any participant who had prevalent CVD based on ICD-9 codes for AMI, unstable angina, cardiovascular revascularization, stroke or transient ischemic attack, peripheral vascular disease or heart failure up to six months after their baseline line date (N=17,229) [72,136]. After this exclusion, 81,030 Veterans (33% HIV+) with available blood pressure data were included for this study.

4.3.2 Independent variable

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and antihypertensive medication prescription were used to create the independent variables. SBP and DBP were averaged across the three routine outpatient clinical blood pressure measurements performed closest to the baseline date. Data on antihypertensive medication were obtained from the pharmacy management benefits package. Blood pressure status was based on the Sixth and Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC VI and VII) [128,137]: Normal (SBP 90-120 and DBP 60-

80 mmHg and no antihypertensive medication prescription), low-hypertension (SBP 120-129 or DBP 80-84 mmHg and no antihypertensives), high-prehypertension (SBP 130-139 or DBP 85-89 mmHg and no antihypertensives), hypertension (SBP≥ 140 or DBP ≥100 mmHg and no antihypertensives), treated hypertension. Participants were classified as being in the higher category if SBP and DBP fell in different categories.

While JNC VII represents the most current blood pressure management guidelines, we applied the prehypertension criteria from JNC VI for a more detailed description of people with elevated blood pressure but without frank hypertension. JNC VII incorporated the normal and high-normal blood pressure categories from JNC VI into the prehypertension category. We renamed these as low and high prehypertension to avoid confusion with JNC VII terminology.
HIV infection was present if a participant had ≥1 inpatient and/or ≥2 outpatient ICD-9 codes for HIV infection and was included in the VA Immunology Case Registry.[134]

4.3.3 Dependent variable

The definition of AMI incidence has previously been described [129]. Briefly AMI incidence was determined using VA, Medicare and death certificate data. AMI events in the VA were collected by trained abstractors from the VA External Peer Review program [71,138]. Clinical confirmation required documentation of AMI in the discharge summary followed by a review of the physician notes and medical chart. Medical information abstracted included evidence of elevated serum markers of myocardial damage and EKG findings. Non-VA AMI events were captured using ICD-9 code, 410, which had strong agreement with adjudicated AMI outcomes in the Cardiovascular Health Study (CHS)[72]. Based on CHS criteria we defined definite or possible fatal AMI as a death within four weeks of a clinically confirmed AMI or a death certificate

documenting AMI as the underlying cause (ICD-10 code I21.0-I21.9) respectively. Deaths were identified using the VA vital status file, the Social Security Administration death master file, the Beneficiary Identification and Records Locator Subsystem, and the Veterans Health Administration medical Statistical Analysis Systems inpatient datasets. Cause of death was obtained from the National Death Index on 94.2% and 95.5% of HIV+ and uninfected decedents, respectively.

4.3.4 Covariates

Sociodemographic data included age, sex, and race/ethnicity. Framingham CVD risk factors including diabetes[139], hyperlipidemia (i.e., low density (LDL), high density (HDL) lipoprotein cholesterol and triglycerides) were measured using outpatient and clinical laboratory data collected closest to the baseline date. Smoking was measured from the VA Health Factors data repository[75] using a standardized form within the VA. For other CVD risk factors: HMG-CoA reductase inhibitor use was based on pharmacy data, body mass index (BMI) was measured from Health Factors data, renal disease and anemia were measured using outpatient and clinical laboratory data collected closest to the baseline date. Hepatitis C (HCV) infection was defined as a positive HCV antibody test or ≥1 inpatient and/or ≥2 outpatient ICD-9 codes for this diagnosis[78]. History of cocaine and alcohol abuse or dependence was defined using ICD-9 codes[140].

Specifically, diabetes was diagnosed using glucose measurements, use of insulin or oral hypoglycemic agents, and/or ≥1 inpatient and/or 2 outpatient ICD-9 codes[74]. Hyperlipidemia variables were categorized based on National Cholesterol Education Program Adult Treatment Panel III criteria [76]. Cholesterol measurements were obtained from the VA Decision Support

System (DSS). Renal disease was defined as an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73m^2 per National Kidney Foundation Kidney Disease Outcomes Quality Initiative thresholds for chronic kidney disease[79]. Smoking was categorized into current, past, and never smoking.

For HIV specific covariates, we collected data on HIV-1 RNA, CD4+ T-lymphocyte counts (CD4+ cell counts), and current use of highly active antiretroviral therapy (HAART). We used CD4+ cell counts and HIV-1 RNA measurements obtained as part of clinical care from within 180 days of our baseline date. We included all ART medications that were on VA formulary during the study period. We have previously shown in a nested sample that 98% of HIV+ Veterans on ART obtain their medications from the VA[134].

4.3.5 Statistical Analyses

Variables were compared by HIV and blood pressure category using Kruskall-Wallis, Wilcoxon rank sum tests, or chi-square tests as appropriate. We calculated unadjusted and age and race/ethnicity adjusted AMI rates per 10,000 person years (py) for each blood pressure category overall and by HIV status using Poisson regression models. Using Cox proportional hazard models, we estimated hazard ratios (HR) and 95% confidence intervals (CI) to assess whether HIV combined with blood pressure category was an independent risk factor for incident AMI after adjusting for age, sex and race/ethnicity. We additionally adjusted these analyses for traditional CVD risk factors (diabetes, lipids, smoking), comorbid diseases (HMG CoA reductase inhibitor use, HCV infection, renal disease, anemia, obesity) and substance use or abuse (cocaine, alcohol). We accounted for antiretroviral therapy, HIV viremia and immune status in models restricted to HIV infected participants.

In secondary analyses, we investigated the impact of widening pulse pressure (systolic minus diastolic blood pressure) and HIV on AMI risk.

Missing covariate data were included in the analyses using multiple imputation techniques that generated five data sets with complete covariate values to increase the robustness of the estimated hazard ratios. Analyses were performed using (Stata v11.0 StataCorp).

4.4 RESULTS

Of 81,030 Veterans with available blood pressure data, 16% were categorized as normal, 44% as prehypertensive (half of whom had high-prehypertension) and 39% as hypertensive. Mean age ranged from 47 to 53 years. Compared to Veterans with normal blood pressure, those with prehypertension or hypertension had greater prevalence of diabetes, and triglycerides, HMG CoA inhibitor use, BMI $\geq$30 kg/m^2 but less prevalent cocaine use. Hypertensive Veterans were older and had more prevalent renal dysfunction than normotensive Veterans (Table 17). HIV+ Veterans had a higher prevalence of low HDL, high triglycerides, low hemoglobin and hepatitis C, but less diabetes and BMI $\geq$30 kg/m^2 than uninfected Veterans (Table 17). HAART use was similar across blood pressure categories. Median CD4+ T-cell counts were higher and HIV-1 RNA was lower among those with elevated blood pressure compared to those with normal blood pressure (Table 17).

Table 17: Baseline characteristics of study population

	Normotension† (90-119)/(60-79) mmHg		Prehypertension (120-139)/(80-89) mmHg		Hypertension ≥140/90 no BP meds		Hypertension (on BP meds)	
	HIV uninfected	**HIV infected**	**HIV uninfected**	**HIV infected**	**HIV uninfected**	**HIV infected**	**HIV uninfected**	**HIV infected**
N	7634	5722	23477	12065	11021	4613	11425	4257
Demographics								
Age in years, mean (SD)	47.0 (8.5)	47.0 (8.7)	47.0 (9.0)	47.0 (9.3)	49.0 (9.0)	49.0 (9.6)	53.0 (8.9)	53.0 (8.9)
Female	5	4	3	3	2	2	2	2
Race								
African American	47	48	44	45	48	47	57	58
White	38	36	41	41	38	39	32	31
Hispanic	10	10	8	7	7	6	7	6
Other	6	7	7	7	7	8	4	5
Framingham risk factors								
Systolic blood pressure in mmHg, mean (SD)	114.0 (5.3)	113.3 (5.8)	129.3 (5.9)	128.5 (6.0)	145.7 (10.0)	145.0 (9.4)	140.0 (15.6)	140.0 (16.9)
Diastolic blood pressure in mmHg, mean (SD)	70.3 (4.9)	70.0 (4.9)	78.3 (6.2)	78.0 (6.2)	87.7 (8.2)	87.7 (8.1)	83.3 (10.0)	83.3 (10.8)
Diabetes	11	8	16	11	20	16	39	30
LDL cholesterol								
Optimal (<100 mg/dL)	32	49	30	44	29	44	37	50
Near optimal (100-129 mg/dL)	35	30	33	30	33	31	33	28
Borderline high (130-159mg/dL)	22	14	24	17	24	16	20	14
High/Very high (≥160 mg/dL)	11	7	13	9	14	9	10	7
HDL cholesterol								
High (≥60 mg/dL)	18	11	14	11	16	12	13	11
Medium (40-59	49	37	48	38	47	38	45	36

Table 17 continued

	Normotension† (90-119)/(60-79) mmHg		Prehypertension (120-139)/(80-89) mmHg		Hypertension ≥140/90 no BP meds		Hypertension (on BP meds)	
	HIV uninfected	**HIV infected**	**HIV uninfected**	**HIV infected**	**HIV uninfected**	**HIV infected**	**HIV uninfected**	**HIV infected**
mg/dL)								
Low (<40 mg/dL)	34	53	38	51	37	50	42	52
Triglycerides ≥150 mg/dL	29	41	38	47	41	53	42	50
HMG-CoA reductase inhibitor use	6	4	8	6	8	7	18	11
Smoking								
Current	59	66	54	59	56	58	48	56
Past	12	11	15	13	16	13	20	16
Never	28	23	30	27	28	29	33	28
Other risk factors								
BMI ≥30 kg/m^2	21	8	37	15	45	21	53	24
Renal disease								
EGFR≥60 mL/min/1.73 m^2	98	96	97	96	96	94	89	82
EGFR 30-59 mL/min/1.73 m^2	2	4	3	4	4	5	9	12
EGFR <30 mL/min/1.73 m^2	0	0	0	1	0	1	2	6
Anemia								
Hemoglobin ≥14 mg/dL	71	49	76	60	75	61	64	45
Hemoglobin 12-13.9 mg/dL	24	35	21	30	21	30	29	35
Hemoglobin 10-11.9 mg/dL	4	12	2	7	3	7	6	14
Hemoglobin <10 mg/dL	1	4	1	2	1	2	2	6
HIV-related risk factors								
HCV infection	17	36	15	32	16	34	17	44
Cocaine	10	13	7	11	6	9	6	13

Table 17 continued

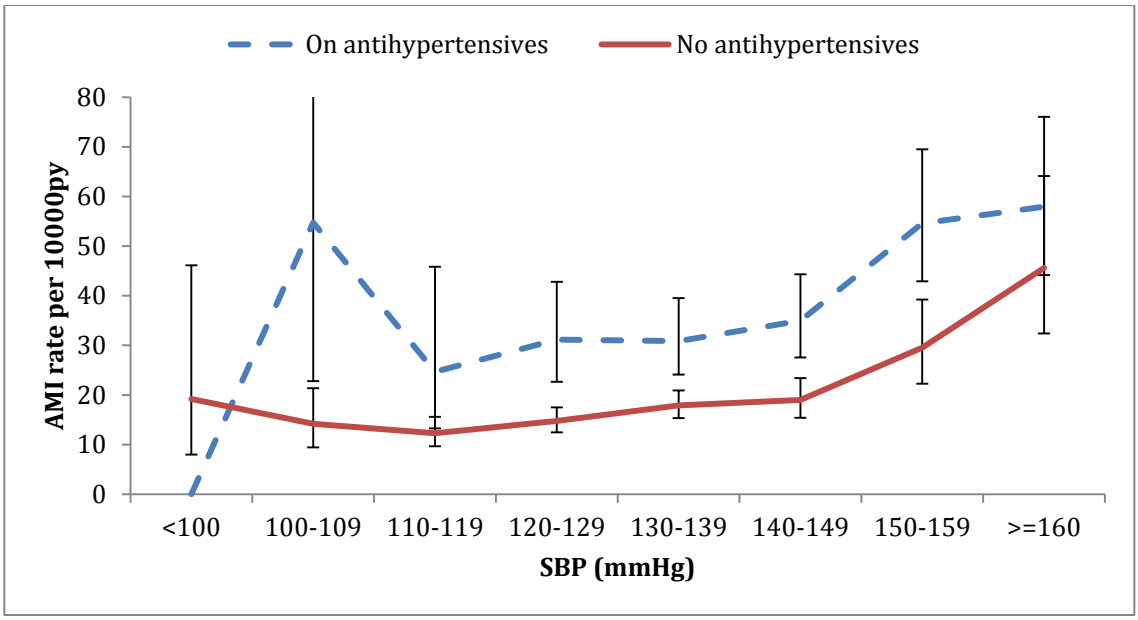
	Normotension† (90-119)/(60-79) mmHg		Prehypertension (120-139)/(80-89) mmHg		Hypertension ≥140/90 no BP meds		Hypertension (on BP meds)	
	HIV uninfected	**HIV infected**	**HIV uninfected**	**HIV infected**	**HIV uninfected**	**HIV infected**	**HIV uninfected**	**HIV infected**
abuse/dependence								
Alcohol abuse/dependence	15	15	13	13	13	12	14	18
On HAART at baseline		43		45		44		45
HIV specific biomarkers								
CD4+ T lymphocyte count (cells/mm^3)								
≥ 500		27		34		34		32
200-499		39		41		40		41
<200		34		26		26		27
Median, mean ± SD		314 (359±295)		369 (415±303)		374 (413±298)		365 (414±304)
HIV RNA (copies/mL)								
≥ 500		60		55		54		52
Median, (mean ± SD *10^4)		0.3 (7.9±22.0)		0.1 (5.6±18.4)		0.09 (46.2±12.1)		0.07 (49.4±13.3)

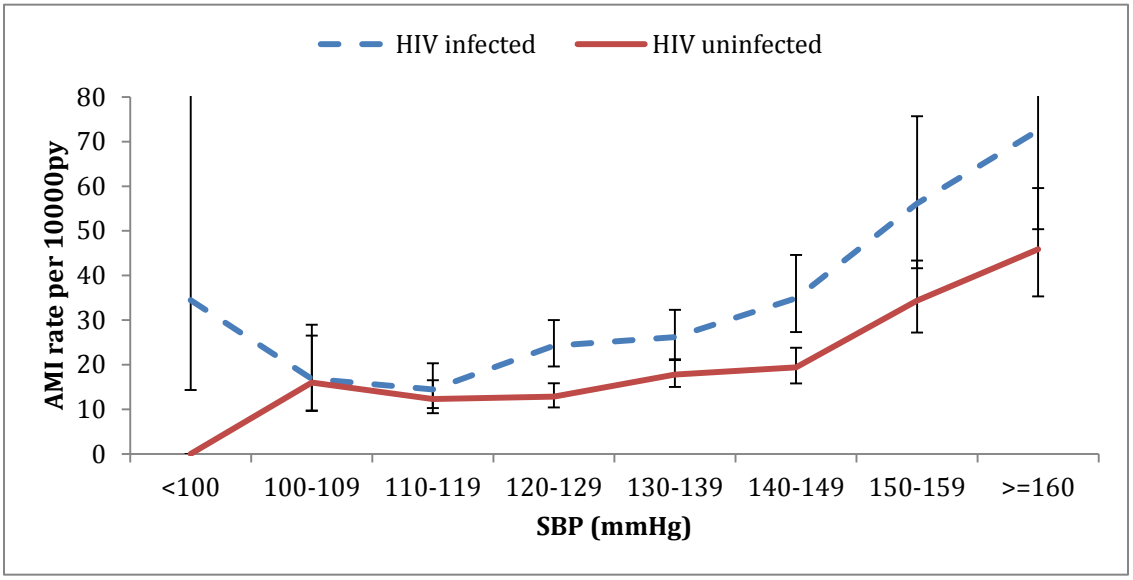
Over a median of 5.9 years, 860 incident AMIs occurred. AMI rates and risk increased with increasing blood pressure (Figure 6, Table 18) among HIV infected and uninfected Veterans (Table 19). These associations persisted among HIV infected Veterans even after adjustment for HIV-1 RNA, CD4+ cell count and/or HAART use (Table A3).

There was evidence of additive interaction between blood pressure and HIV status on AMI since AMI rates were higher than would have been expected from HIV or elevated blood pressure alone (Figure 7). However, no significant multiplicative interactions ($p>0.05$) were observed even when blood pressure was considered as a continuous systolic or diastolic measure.

Compared to uninfected Veterans with normal blood pressure, HIV+ Veterans with low or high prehypertension or hypertension had significantly increased AMI risk [Hazard Ratio [HR]: 1.60; 95% confidence interval (CI): 1.07-2.39; HR:1.80 (1.21-2.68); HR: 2.57 (1.76-3.76); HR: 2.75 (1.89-4.00) respectively] after adjustment for confounders (Table 20). Results were similar when systolic and diastolic blood pressure were analyzed separately (Table A4).

A 10 mmHg increase in pulse pressure was associated with a small but significantly increased risk of AMI: 1.12 (1.06-1.19); $p<0.001$) after adjusting for confounders. HIV infection did not modify the association between pulse pressure and AMI risk [HR (95% CI) for interaction: 0.92 (0.83-1.03), data otherwise not shown].


On antihypertensives
No antihypertensives
AMI rate per 10000py
80
70
60
50
40
30
20
10
0
<100
100-109
110-119
120-129
130-139
140-149
150-159
>=160
SBP (mmHg)


HIV infected
HIV uninfected
AMI rate per 10000py
80
70
60
50
40
30
20
10
0
<100
100-109
110-119
120-129
130-139
140-149
150-159
>=160
SBP (mmHg)

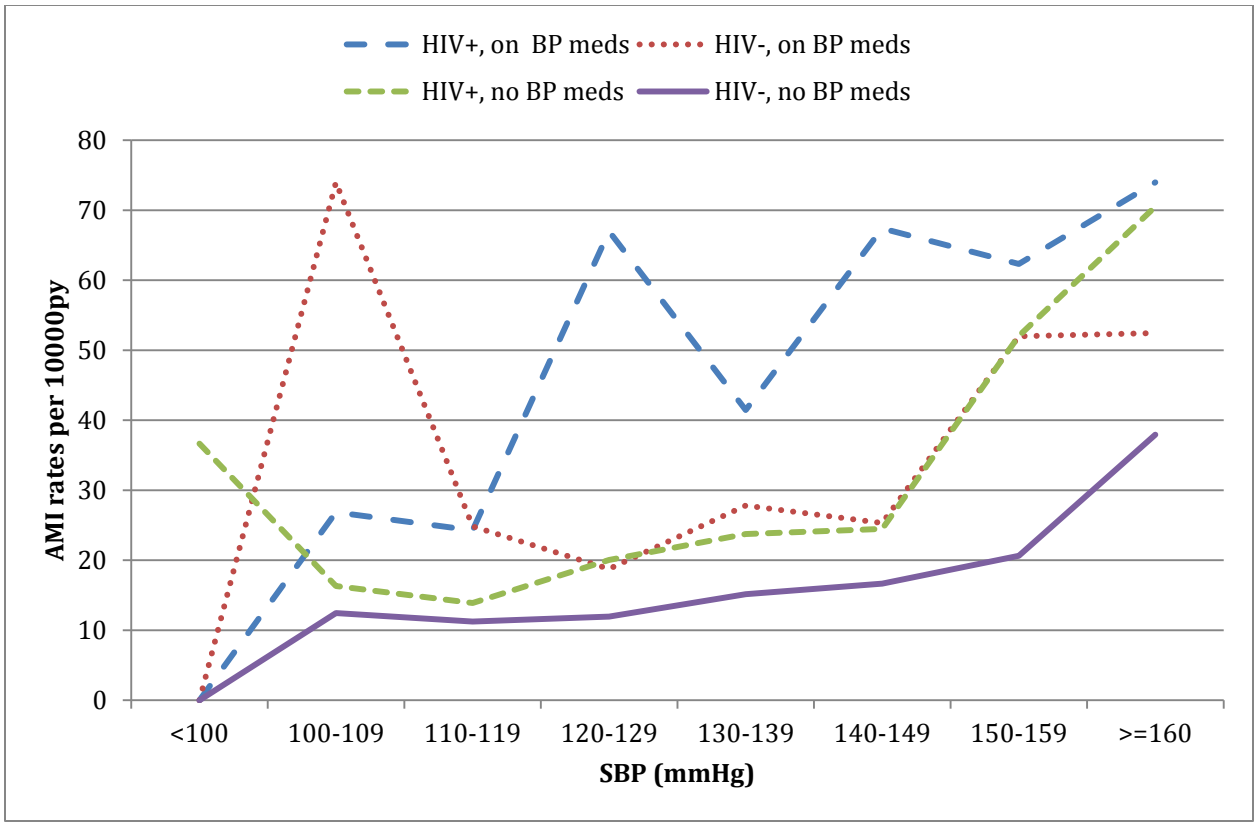


Figure 6: Unadjusted rate of incident AMI by systolic and diastolic blood pressure increments stratified by HIV status or antihypertensive therapy

Table 18: Rate & risk of incident AMI (95% CI) by blood pressure status

Blood Pressure Category (mmHg)	Definition		N	# of AMIs	Age/race adjusted AMI rate/10,000py	Hazard Ratio (95% CI)	
	JNC VII	JNC VI				Adjusted for age, sex, race/ethnicity	Adjusted for all covariates*
<90/60†	Hypotension	Hypotension	846	7	18.6 (8.8-39.7)	1.34 (0.62-2.90)	1.31 (0.61-2.84)
90-119/60-79†	Normal	Optimal	13356	82	12.8 (9.9-16.4)	1	1
120-129/80-84	Pre hypertension	Low prehypertension	17813	123	13.8 (11.1-17.3)	1.09 (0.83-1.44)	1.11 (0.84-1.46)
130-139/85-89		High-prehypertension	17699	156	17.5 (14.3-21.5)	1.33 (1.01-1.73)	1.35 (1.03-1.77)
≥140/90	Hypertension (Stages 1 -2)	Hypertension (Stages 1-3)	15634	190	24.5 (20.3-29.8)	1.72 (1.32-2.23)	1.69 (1.29-2.20)
Use of antihypertensive medication	Hypertension (Stages 1 -2)	Hypertension (Stages 1-3)	15682	302	39.1 (33.1-46.1)	2.34 (1.82-3.00)	2.03 (1.57-2.64)

All covariates includes age, sex, and race/ethnicity, LDL, HDL, triglycerides, diabetes, HMG CoA reductase inhibitor use, HCV infection, renal disease, anemia, BMI, cocaine, and alcohol use

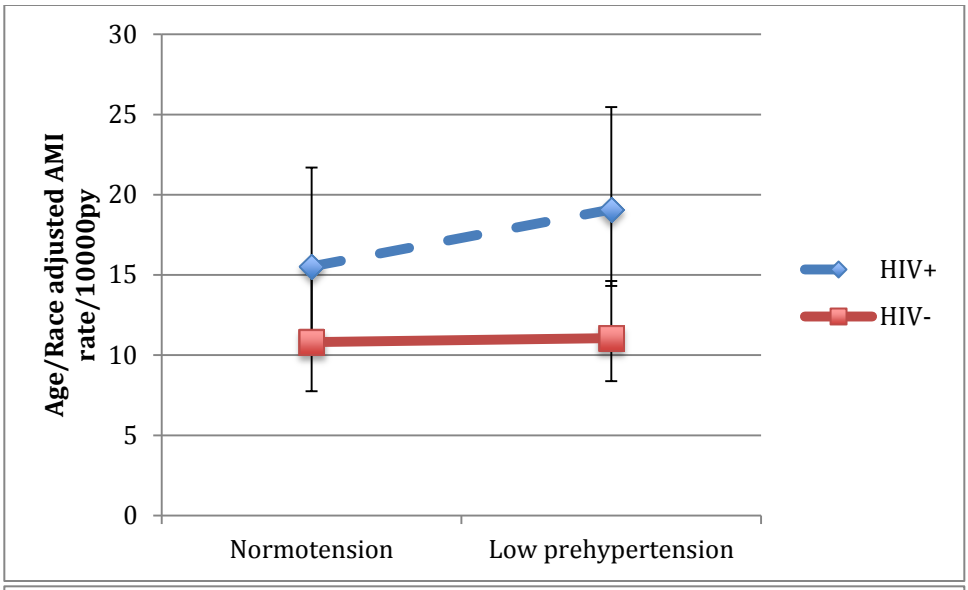
† We included people with SBP<90 mmHg or DBP<60 mmHg as a separate category because in our cohort, such low blood pressure indicates hypotension instead of normotension.

Table 19: Rate & risk of incident AMI (95% CI) by blood pressure status among HIV infected and uninfected Veterans (separate reference groups)

Blood Pressure Category (mmHg)	Definition	Hazard Ratio (95% CI) Adjusted for all covariates*	
		HIV-	HIV+
<90/60†	Hypotension	1.67 (0.6-4.67)	1.05 (0.32-3.39)
90-119/60-79†	Normal	1	1
120-129/80-84	Low prehypertension	0.99 (0.67-1.47)	1.23 (0.82-1.83)
130-139/85-89	High-prehypertension	1.29 (0.89-1.87)	1.38 (0.93-2.04)
≥140/90 (no antihypertensive medication)	Hypertension (Stages 1-2)	1.44 (1.00-2.08)	2.01 (1.37-2.94)
Use of antihypertensive medication	Hypertension (Stages 1-2)	1.83 (1.28-2.62)	2.24 (1.54-3.28)

All covariates includes age, sex, and race/ethnicity, LDL, HDL, triglycerides, diabetes, HMG CoA reductase inhibitor use, HCV infection, renal disease, anemia, BMI, cocaine, and alcohol use

† We included people with SBP<90 mmHg or DBP<60 mmHg as a separate category because in our cohort, such low blood pressure indicates hypotension instead of normotension.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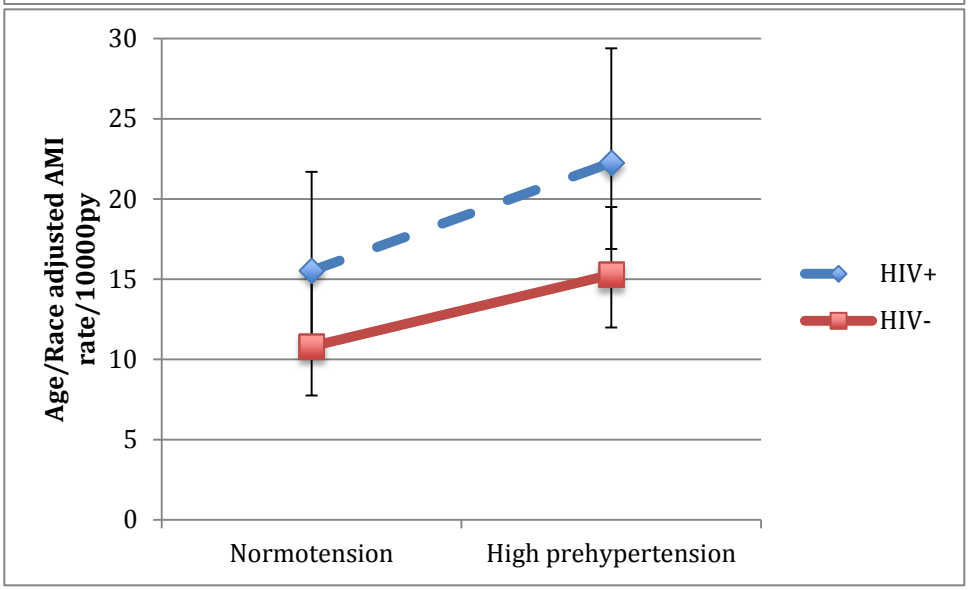
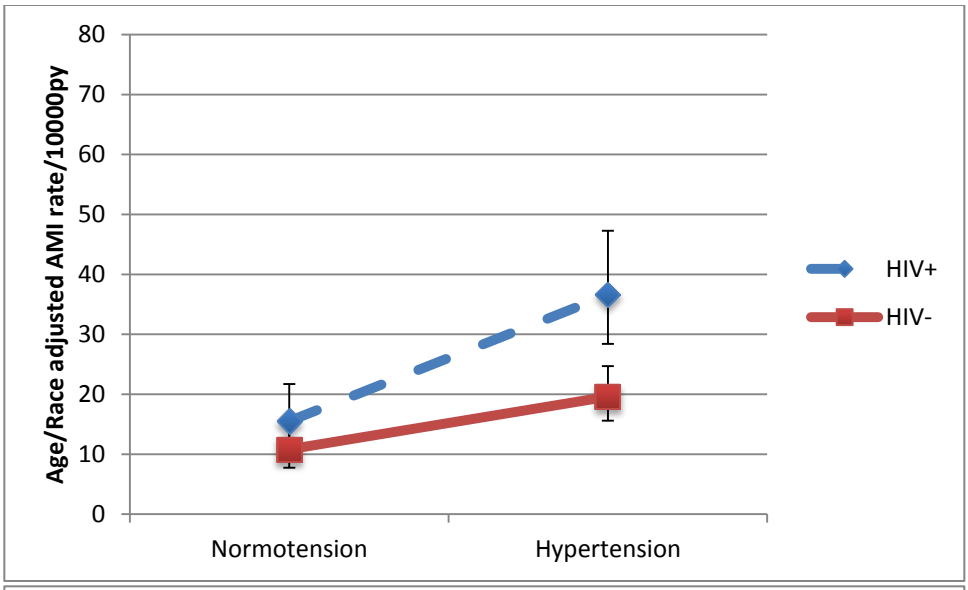
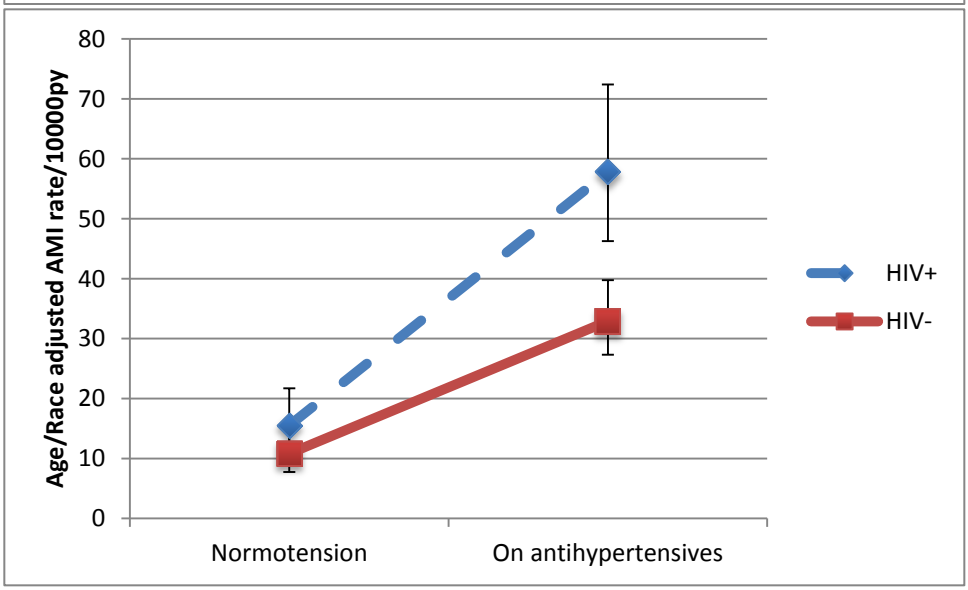
25
20
15
10
5
0
Age/Race adjusted AMI rate/10000py
Normotension
Low prehypertension
HIV+
HIV-


30
25
20
15
10
5
0
Age/Race adjusted AMI rate/10000py
Normotension
High prehypertension
HIV+
HIV-


Age/Race adjusted AMI rate/10000py
80
70
60
50
40
30
20
10
0
Normotension
Hypertension
HIV+
HIV-


Age/Race adjusted AMI rate/10000py
80
70
60
50
40
30
20
10
0
Normotension
On antihypertensives
HIV+
HIV-

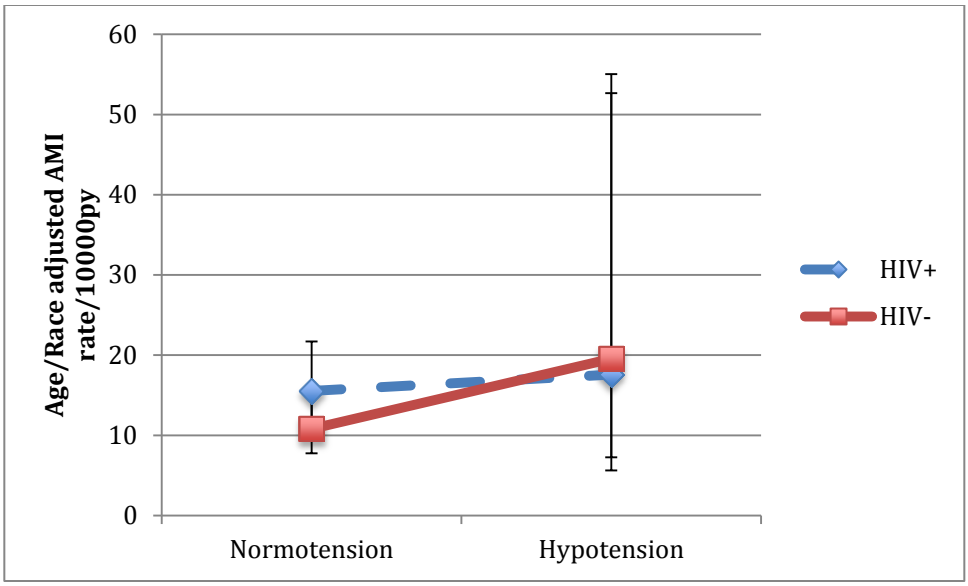


Figure 7: AMI rates per 10,000py by blood pressure category and HIV status illustrating additive interaction between blood pressure and HIV status on AMI rates.

Bars represent 95% confidence intervals for rates.

Table 20: Rate & risk of incident AMI (95% CI) by blood pressure and HIV status (common reference group)

Blood Pressure Category (mmHg)	Definition	HIV status	N	# of AMIs	Age/race adjusted AMI rate/10,000py	Hazard Ratio (95% CI) Adjusted for all covariates*
<90/60†	Hypotension	HIV-	441	4	19.54 (7.25-52.66)	1.68 (0.60-4.69)
		HIV+	405	3	17.58 (5.61-55.04)	1.34 (0.41-4.34)
90-119/60-79†	Normal	HIV-	7634	41	10.81 (7.75-15.1)	1
		HIV+	5722	41	15.53 (11.13-21.7)	1.30 (0.84-2.01)
120-129/80-84	Low prehypertension	HIV-	11503	64	11.06 (8.37-14.62)	1.00 (0.67-1.47)
		HIV+	6310	59	19.08 (14.31-25.47)	1.60 (1.07-2.39)
130-139/85-89	High prehypertension	HIV-	11944	92	15.28 (11.99-19.5)	1.30 (0.90-1.88)
		HIV+	5755	64	22.26 (16.88-29.4)	1.80 (1.21-2.68)
≥140/90, no anti-hypertensive medication	Hypertension (Stages 1-2)	HIV-	11021	108	19.61 (15.6-24.69)	1.47 (1.02-2.12)
		HIV+	4613	82	36.61 (28.4-47.29)	2.57 (1.76-3.76)
Use of anti-hypertensive medication	Hypertension (Stages 1-2)	HIV-	11425	192	32.92 (27.31-39.77)	1.92 (1.35-2.73)
		HIV+	4257	110	57.83 (46.27-72.41)	2.75 (1.89-4.00)

All covariates includes age, sex, and race/ethnicity, LDL, HDL, triglycerides, diabetes, HMG CoA reductase inhibitor use, HCV infection, renal disease, anemia, BMI, cocaine, and alcohol use

† We included people with SBP<90 mmHg or DBP<60 mmHg as a separate category because in our cohort, such low blood pressure indicates hypotension instead of normotension.

4.5 DISCUSSION

In this cohort of Veterans, high prehypertension and hypertension were associated with an increased risk of AMI. Compared to normotensive uninfected Veterans, low and high prehypertension and hypertension were associated with increased risk of AMI. Both the absolute rates and relative risks of AMI were higher for HIV+ Veterans compared to uninfected Veterans within these elevated blood pressure categories.

This is the first study to report an association between prehypertension and AMI risk among HIV+ Veterans. In the general population, prehypertension has been independently associated with cardiovascular events [141,142]. We extend these findings to HIV+ Veterans using an adjudicated, outcome, AMI.

Whether HIV infection and/or its treatment interact with blood pressure to increase the risk of AMI is not clear. Prior studies examining the association between HIV, ART, and blood pressure are inconsistent [143-145]. Our data suggest the presence of additive interaction between HIV status and blood pressure on AMI risk. Such an interaction would be consistent with prior mechanistic studies reporting an association between HIV and renal disease[146], endothelial dysfunction[147], reduced arterial elasticity[148], and progression of atherosclerosis [149-151]. The indirect nature of this biological interaction may have contributed to the lack of a significant multiplicative interaction.

Prior research has reported an association between hypertension and AMI risk among HIV+ people [152,153]. This study extends those results by demonstrating that the relative rates and risk of AMI are progressively higher with elevations in blood pressure among HIV+ compared to

uninfected Veterans. This study may not have had enough power to detect significant differences in AMI risk in regression models additionally stratified by HIV status (Table 19), though the trend in hazard ratios was similar to that seen for regression models using a common HIV uninfected normotensive reference group.

Higher rates and risks of AMI observed among treated versus untreated hypertensive Veterans are challenging to interpret in the absence of data on duration of hypertension and/or treatment.

Our findings have important clinical implications for HIV+ people. Prior work in the VACS and other studies demonstrate that HIV infection, ART use, and traditional CVD risk factors, including hypertension, are associated with an increased risk of AMI [129]. Although HIV+ people have an increased risk of AMI (including those who achieve HIV-1 RNA levels <500 copies/ml over time), as well as an increased risk of diabetes and renal disease compared to uninfected people, there are no HIV specific guidelines for AMI risk reduction beyond those for the general population[154]. In contrast, people with diabetes and/or chronic kidney disease, have lower recommended blood pressure targets (i.e., blood pressure <130/80 compared to <140/90 mmHg) in order to reduce the risk of future CVD events. Whether such recommendations would improve outcomes for HIV infected people is not known. However our data showing increased AMI risk among prehypertensive HIV+ Veterans suggests that such targets may be beneficial in this population.

There are limitations that warrant discussion. First, as this is an observational study, our findings alone are insufficient to change current guidelines for blood pressure management for HIV infected people. Second, as our population is overwhelmingly male, our results may not be generalizable to women. Third, there is always the possibility of residual confounding present in

this analysis. For example, alcohol is associated with blood pressure and coronary heart disease outcomes. In this study, alcohol abuse and dependence was assessed with ICD-9 codes, which can result in some misclassification. However, the fact that alcohol use is common in both HIV+ and uninfected Veterans suggests that misclassification would not be differential. Fourth, blood pressure, HIV-1 RNA, and CD4+ cell count were assessed only at baseline. Participants could have moved between blood pressure, HIV-1 RNA, and/or CD4+ cell count categories between baseline and censoring.

4.6 CONCLUSION

In summary, prehypertensive and hypertensive blood pressure were associated with an increased risk of AMI in a cohort of HIV infected and uninfected Veterans. HIV may modify the association between hypertension and AMI risk. Given the increased risk of AMI, diabetes, and renal disease associated with HIV infection and if our results are confirmed in other studies, future research should explore whether lower blood pressure targets among HIV+ people translates into lower AMI risk in a randomized controlled trial.

5.0 THEMATIC SUMMARY

In summary, this dissertation contributes insights into the pathogenesis of CHD in the setting of HIV infection.

Prior studies have shown strong associations between inflammation and CHD risk and mortality. We have demonstrated that ongoing HIV replication and immune depletion and comorbid conditions like prevalent CVD and renal disease, contribute to elevated biomarkers associated with inflammation, altered coagulation, and monocyte activation. We have also described a different and potentially more complete method to describe an individual's inflammatory state using a composite score with multiple inflammatory biomarkers. Our data suggest an interaction between blood pressure and HIV status on AMI risk such that people with elevated blood pressure and HIV infection have higher rates of AMI than would be expected from either alone.

Taken together, these studies emphasizes the importance of studying HIV as a disease with pleiotropic effects – on multiple biomarkers and organ systems – that can interact with each other, modifying their independent contributions to CHD risk. An important question for future study is whether co-morbid diseases, traditionally associated with CVD, contribute to CVD risk to a similar degree and in a similar manner in HIV infected versus uninfected people, given differences in etiology of these co-morbid diseases.

APPENDIX

SUPPLEMENTARY TABLES

Table A1: PubMed search concepts and search terms for literature review describing effects of HIV infection on lipid and lipoprotein metabolism

Search concept	PubMed search terms
Kinetic studies	(((((((kinetic*) OR rate of reaction) OR rates of reaction)) OR ((rate*) AND biochemical) OR tracer) OR compartment models) OR clearance rate) OR synthesis rate
HIV	((((((hiv) OR hiv infection) OR human immunodeficiency virus) OR hiv seropositivity) OR hiv 1) OR hiv 2) OR acquired immune deficiency syndrome
HIV uninfected	((((((seronegativity) OR seronegative) OR uninfected)) OR seroconversion) OR noninfected) OR hiv control group OR hiv negative
Antiretroviral therapy naïve	(hiv therapy naive) OR ((((((art naive) OR haart naive) OR untreated hiv) OR pre art) OR pre haart) OR antiretroviral

Table A1 continued

Search concept	**PubMed search terms**
	therapy naive)
Lipids/lipoproteins	(((((((((lipid) OR lipids) OR lipid metabolism) OR lipid biosynthesis) OR lipid clearance) OR lipid immunology)) OR ((((((lipoprotein) OR lipoproteins) OR lipoprotein metabolism) OR lipoprotein biosynthesis) OR lipoprotein clearance) OR lipid immunology))) OR (((((((chylomicron) OR vldl) OR low density lipoprotein) OR ldl) OR high density lipoprotein) OR triglyceride*) OR fatty acid) OR cholesterol)
Title pertaining to lipids or lipoproteins	((((((((((((((((((((lipid[Title]) OR lipids[Title]) OR lipoprotein*[Title]) OR apolipoprotein*[Title]) OR hdl[Title]) OR ldl[Title]) OR vldl[Title]) OR idl[Title]) OR dyslipidemia*[Title]) OR dylipidaemia) OR lipodystrophy[Title]) OR lipoatrophy[Title]) OR lipohypertrophy[Title]) OR cholesterol*[Title]) OR hypercholesterolemia*[Title]) OR triglycerides*[Title]) OR hypertriglyceridemia*[Title]) OR hyperlipidemia*[Title]) OR metabolic[Title]) OR lipolysis[Title]) OR adipocyte*[Title]) OR fatty acid*[Title]) OR lipogenesis[Title]) OR hypocholesterolemia[Title] OR acute*phase[Title]
Hypertriglyceridemia	Hypertriglyceridemia
Elevated free fatty acids	(elevated OR high) AND (blood free fatty acids OR serum free fatty acids OR plasma free fatty acids)

Table A1 continued

Search concept	**PubMed search terms**
High density lipoprotein cholesterol reduction	high density lipoprotein cholesterol reduction OR high density lipoprotein cholesterol decrease
Low density lipoprotein cholesterol reduction	low density lipoprotein cholesterol reduction OR low density lipoprotein cholesterol decrease
Very low density lipoprotein cholesterol reduction	very low density lipoprotein cholesterol reduction OR very low density lipoprotein cholesterol decrease
Triglycerides	triglycerides
High density lipoprotein triglycerides	high density lipoprotein triglycerides
Low density lipoprotein triglycerides	low density lipoprotein triglycerides
Very low density lipoprotein triglycerides	Very low density lipoprotein triglycerides
Cell membrane structure	((((cell membrane structure) OR membrane fluidity) OR membrane microdomains)) OR lipid rafts

Table A2: Association of HIV/HCV group and covariates with individually elevated (>75th percentile) biomarkers.

	Odds Ratio (95% Confidence Interval)							
	IL-10	**TNF-α**	**Cystatin C**	**IL-6**	**CRP**	**SAA**	**IFN-γ**	**MCP-1**
Undetectable	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
HIV mono-detectable	2.95 (0.74, 11.85)	4.44 (0.86, 22.82)	0.49 (0.12, 2.02)	0.57 (0.15, 2.16)	0.99 (0.35, 2.84)	0.58 (0.19, 1.79)	1.65 (0.46, 5.87)	2.96 (0.80, 10.95)
HCV mono-detectable	5.51* (1.17, 25.84)	4.45 (0.68, 29.02)	0.40 (0.07, 2.34)	2.99 (0.75, 11.98)	1.41 (0.41, 4.90)	0.38 (0.09, 1.66)	2.63 (0.59, 11.76)	3.71 (0.83, 16.53)
HIV/HCV detectable	7.79* (1.90, 31.97)	7.70* (1.42, 41.83)	1.60 (0.39, 6.57)	1.47 (0.40, 5.35)	0.69 (0.22, 2.14)	0.72 (0.22, 2.31)	1.03 (0.25, 4.30)	3.48 (0.88, 13.69)
FIB-4 $\geq$ 1.45	1.26 (0.61, 2.62)	2.07 (0.93, 4.61)	3.43* (1.45, 8.10)	3.22* (1.44, 7.20)	1.12 (0.54, 2.32)	1.13 (0.51, 2.48)	0.63 (0.25, 1.61)	2.39* (1.10, 5.20)
High cholesterol	1.27 (0.58, 2.77)	4.22* (1.85, 9.62)	1.71 (0.71, 4.11)	0.90 (0.37, 2.17)	1.20 (0.58, 2.51)	1.13 (0.50, 2.54)	1.58 (0.66, 3.80)	1.81 (0.79, 4.18)
Age greater than median (42 years)	0.42* (0.20, 0.87)	0.47 (0.21, 1.02)	1.05 (0.46, 2.41)	0.74 (0.33, 1.68)	0.57 (0.28, 1.14)	1.82 (0.87, 3.77)	0.49 (0.20, 1.17)	0.97 (0.46, 2.05)
BMI $\geq$ 30 kg/m^2	0.57 (0.22, 1.48)	0.82 (0.32, 2.10)	1.11 (0.40, 3.08)	1.37 (0.52, 3.60)	2.33 (1.03, 5.24)	2.05 (0.85, 4.95)	0.47 (0.15, 1.49)	1.67 (0.64, 4.38)
Ever smoker	1.02 (0.45, 2.29)	1.12 (0.47, 2.69)	3.71* (1.17, 11.72)	1.33 (0.52, 3.41)	1.92 (0.83, 4.45)	1.57 (0.66, 3.76)	0.50 (0.20, 1.23)	0.73 (0.32, 1.65)
CD4 > 200 cells/mm^3	1.39 (0.58, 3.34)	1.72 (0.64, 4.63)	0.43 (0.16, 1.17)	0.87 (0.32, 2.38)	0.65 (0.27, 1.57)	0.50 (0.20, 1.26)	1.57 (0.47, 5.26)	0.53 (0.22, 1.27)
Renal disease	1.24 (0.32, 4.83)	2.18 (0.54, 8.79)	9.47* (2.06, 43.48)	2.09 (0.45, 9.69)	1.15 (0.30, 4.35)	5.17* (1.23, 21.69)	2.79 (0.58, 13.38)	0.14 (0.02, 1.23)
Diabetes	0.91 (0.20, 4.04)	4.88* (1.18, 20.21)	4.91 (0.98, 24.65)	3.33 (0.69, 16.11)	3.68 (0.97, 14.02)	1.26 (0.27, 5.93)	--** (--)	1.08 (0.25, 4.60)
Current antiretroviral therapy use	0.62 (0.29, 1.30)	0.38* (0.16, 0.86)	0.44 (0.18, 1.07)	0.81 (0.34, 1.92)	1.04 (0.50, 2.20)	1.07 (0.49, 2.33)	1.43 (0.56, 3.65)	1.30 (0.59, 2.90)

Table A2 continued

	Odds Ratio (95% Confidence Interval)							
	IL-10	**TNF-α**	**Cystatin C**	**IL-6**	**CRP**	**SAA**	**IFN-γ**	**MCP-1**
Prevalent cardiovascular disease	0.34 (0.06, 1.84)	3.64 (0.88, 14.98)	4.56* (1.02, 20.43)	0.32 (0.03, 3.08)	1.83 (0.44, 7.64)	1.61 (0.38, 6.89)	2.73 (0.54, 13.66)	0.35 (0.06, 2.13)
Hypertension	0.97 (0.42, 2.26)	0.79 (0.32, 1.92)	1.09 (0.43, 2.80)	0.59 (0.22, 1.58)	0.66 (0.29, 1.51)	0.51 (0.20, 1.28)	1.10 (0.41, 2.96)	0.84 (0.33, 2.08)
At-risk alcohol consumption	0.75 (0.36, 1.55)	0.80 (0.37, 1.74)	0.99 (0.43, 2.29)	1.79 (0.78, 4.08)	1.06 (0.52, 2.14)	1.13 (0.53, 2.38)	1.50 (0.61, 3.65)	0.63 (0.29, 1.38)
Female	0.79 (0.34, 1.82)	1.26 (0.53, 3.03)	1.47 (0.59, 3.66)	1.21 (0.50, 2.92)	1.01 (0.45, 2.26)	1.62 (0.69, 3.80)	1.36 (0.49, 3.80)	0.17* (0.05, 0.60)

*p-value <0.05;
**There were no elevated IFN-γ outcomes among those with diabetes.

Table A3: AMI risk and blood pressure category adjusted for HIV-1 RNA (< or ≥500 copies/mL) or CD4+ T-lymphocyte count (cells/mm3) and HAART use among HIV infected Veterans

Blood Pressure Category (mmHg)	Definition	HIV status	Hazard Ratio (95% CI)	
			All covariates +HAART	All covariates, HAART, CD4 cell count, HIV-1 RNA
<90/60†	Hypotension	HIV+	1.05 (0.32-3.38)	1.04 (0.32-3.37)
90-119/60-79†	Normal	HIV+	1	1
120-129/80-84	Low prehypertension	HIV+	1.23 (0.82-1.83)	1.24 (0.83-1.85)
130-139/85-89	High-prehypertension	HIV+	1.38 (0.93-2.04)	1.39 (0.94-2.05)
≥140/90	Hypertension (Stages 1-2)	HIV+	2.01 (1.37-2.93)	2.02 (1.38-2.96)
Use of antihypertensive medication		HIV+	2.24 (1.54-3.28)	2.28 (1.56-3.33)

All models adjust for age, sex, and race/ethnicity, LDL, HDL, triglycerides, diabetes, HMG CoA reductase inhibitor use, HCV infection, renal disease, anemia, BMI, cocaine, and alcohol use.

† We included people with SBP<90 mmHg or DBP<60 mmHg as a separate category because in our cohort, such low blood pressure indicates hypotension instead of normotension.

Table A4: Rate & risk of incident AMI (95% CI) by a) systolic blood pressure (SBP) or b) diastolic blood pressure (DBP) and HIV status

a) Systolic Blood Pressure Category (mmHg)	Definition	HIV status	N	# of AMIs	Age/race adjusted AMI rate/10,000py	Hazard Ratio (95% CI) Adjusted for all covariates*
<90†	Hypotension	HIV-	19	0	--	--
		HIV+	41	2	179.93 (44.62-725.64)	9.79 (2.32-41.34)
90-119†	Normal	HIV-	8735	48	11.13 (8.15-15.22)	1
		HIV+	6521	45	15.02 (10.9-20.72)	1.22 (0.81-1.84)
120-129	Low prehypertension	HIV-	11945	71	11.85 (9.08-15.49)	1.04 (0.72-1.5)
		HIV+	6466	64	20.24 (15.33-26.78)	1.65 (1.13-2.4)
130-139	High-prehypertension	HIV-	11975	91	15.07 (11.81-19.25)	1.24 (0.87-1.76)
		HIV+	5712	68	23.85 (18.21-31.28)	1.87 (1.29-2.71)
≥140	Hypertension (Stages 1-2)	HIV-	9869	99	19.98 (15.78-25.36)	1.43 (1.01-2.03)
		HIV+	4065	70	35.11 (26.8-46.09)	2.34 (1.61-3.39)
Use of antihypertensive medication		HIV-	11525	193	32.87 (27.28-39.69)	1.86 (1.34-2.59)
		HIV+	4256	110	57.53 (46.03-72.03)	2.68 (1.88-3.81)

Table A4 continued

b) Diastolic Blood Pressure Category (mmHg)	Definition	HIV status	N	# of AMIs	Age/race adjusted AMI rate/10,000py	Hazard Ratio (95% CI) Adjusted for all covariates*
<60†	Hypotension	HIV-	554	6	22.87 (10.12-51.68)	1.75 (0.77-3.96)
		HIV+	463	3	15.06 (4.8-47.23)	0.98 (0.31-3.1)
60-79†	Normal	HIV-	23063	145	12.49 (10.13-15.44)	1
		HIV+	13753	125	18.78 (15.09-23.42)	1.43 (1.11-1.82)
80-84	Low prehypertension	HIV-	8869	70	15.52 (11.88-20.32)	1.23 (0.92-1.64)
		HIV+	4211	52	25.07 (18.54-33.95)	1.89 (1.37-2.61)
85-89	High-prehypertension	HIV-	5586	49	17.63 (12.96-24.03)	1.42 (1.03-1.96)
		HIV+	2511	35	28.29 (19.84-40.38)	2.17 (1.5-3.15)
≥90	Hypertension (Stages 1-2)	HIV-	4471	39	18.15 (12.96-25.44)	1.42 (1-2.03)
		HIV+	1867	34	39.91 (27.83-57.3)	2.91 (2-4.24)
Use of antihypertensive medication		HIV-	11525	193	32.89 (27.29-39.73)	1.78 (1.42-2.23)
		HIV+	4285	110	57.56 (46.04-72.08)	2.57 (1.97-3.35)

All covariates includes age, sex, and race/ethnicity, LDL, HDL, triglycerides, diabetes, HMG CoA reductase inhibitor use, HCV infection, renal disease, anemia, BMI, cocaine, and alcohol use

† We included people with SBP<90 mmHg or DBP<60 mmHg as a separate category because in our cohort, such low blood pressure indicates hypotension instead of normotension.

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