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The Effect of Hepatitis C Virologic Clearance on Cardiovascular Risk Biomarkers in HIV/Hepatitis C Coinfection

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by

Kara W. Chew

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ABSTRACT OF THE THESIS

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by

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Master of Science in Clinical Research

University of California, Los Angeles, 2013

Professor Robert M. Elashoff, Chair

Background: Hepatitis C (HCV) may increase cardiovascular disease (CVD) risk in HIV-infected persons. We hypothesized that HCV virologic clearance reduces CVD risk, as manifested by reduction in non-hepatic CVD biomarkers.

Methods: Of 54 HIV/HCV coinfected subjects who received 72 weeks of pegylated interferon/ribavirin, 27 with and 27 without sustained virologic response (SVR) matched by race/ethnicity and sex, stored serum/plasma before treatment and 24 weeks after end of treatment

(EOT) were tested for non-hepatic (sICAM-1, sP-selectin, IL-6, D-dimer, and lipoprotein-associated phospholipase A2 [Lp-PLA2]) and hepatic markers of CVD (cholesterol and hsCRP). Baseline characteristics and biomarkers were compared between SVRs and non-SVRs by Wilcoxon rank sum test. Changes in each biomarker were examined within SVRs/non-SVRs and between groups by t-tests and regression models.

Results: The cohort included 54 subjects, 30 white, 24 black, and 44 male. Baseline levels of non-hepatic markers were not significantly different between groups, including sICAM-1 (overall median [Q1, Q3]=439.2 [365.6, 592.8] ng/mL), sP-selectin (146.7 [94.1, 209.9] ng/mL) and IL-6 (2.32 [1.61, 3.49] pg/mL). Of 52 subjects with baseline Lp-PLA2, 37 (71%) had Lp-PLA2>235 ng/mL. SVRs had a significant decrease in $\log_{10}$ sICAM-1, but not non-SVRs (mean [sd] = -0.09 [0.13] vs -0.01 [0.14], p=0.047 for between group comparison). Adjusting for baseline AST and ALT, SVR was significantly associated with decrease in sICAM-1 (p=0.033), but not after adjusting for change in ALT (p=0.105). At 24 weeks after EOT, 17 (63%) SVRs had Lp-PLA2>235 ng/mL compared to 25 (93%) non-SVRs (p=0.021).

Conclusions: High baseline levels of non-hepatic CVD markers suggest high CVD risk in HIV/HCV coinfection. SVR was associated with decrease in sICAM-1 and lower CVD risk by Lp-PLA2 level. HCV virologic clearance may lower CVD risk through reduction in hepatic, and subsequently systemic, inflammation.

The thesis of Kara W. Chew is approved.

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Table of Contents

List of Figures and Tables

CHAPTER 1: BACKGROUND

The burden of hepatitis C infection. Approximately 170 million individuals are chronically infected with hepatitis C virus (HCV) worldwide,[1] including up to 3.9 million in the United States (U.S.).[2] HCV is a blood-borne virus with transmission in developed countries today occurring primarily through injection drug use (IDU). In developing countries, transmission continues to occur through unsafe injection practices in healthcare settings and unscreened blood transfusions, as well as through IDU.[3] There has also been increasing recognition of sexual transmission in HIV-infected men who have sex with men (MSM).[4] In the U.S., approximately three-quarters of infections are borne by "baby boomers," those individuals born in the years 1945-1965.[2] Such individuals are aging and increasingly at risk for HCV cirrhosis, which typically presents between 20-30 years following initial infection, and its complications of end-stage liver disease, hepatocellular carcinoma and death. These same individuals are further at risk for non-HCV, age-related morbidity and mortality, such as cardiovascular disease.[5]

The impact of HCV and HIV coinfection on liver and non-liver outcomes. Approximately 30% of HIV-infected persons in the U.S. and Europe are coinfected with HCV.[6] Liver disease is accelerated with HIV/HCV coinfection, with HIV-infected persons at three times greater risk for progression to cirrhosis or decompensated liver disease as compared to persons with HCV alone.[7] Antiretroviral therapy (ART) appears to slow liver disease progression, but not fully.[8,9] Overall and liver-related mortality are increased in HIV/HCV-coinfected persons as compared to HIV and HCV monoinfection, despite effective ART.[10-12] In one large cohort comparing mortality by HCV infection status, persons with chronic HCV were at 50% increased risk of

death; only 20% of deaths were liver-related, leaving an excess of non-liver related deaths, including cardiovascular disease (CVD)-related deaths.[11]

Cardiovascular disease risk and risk assessment in HIV/hepatitis C coinfection. Large observational studies suggest an increased risk of cardiovascular disease (CVD) events in HCV-infected and HIV/HCV-coinfected persons.[13-18] Multiple potential mechanisms for increased CVD risk with HCV infection exist, including hepatic steatosis, insulin resistance, and diabetes mellitus.[19,20] Chronic immune activation, implicated in HIV-associated accelerated atherosclerosis, is further increased with HCV coinfection.[21,22] The optimal method for CVD risk assessment in HCV-infected persons is unknown. Lipid and hsCRP levels are widely used in routine clinical practice for CVD risk assessment.[23,24] However, hsCRP, total cholesterol, low-density lipoprotein (LDL), and triglycerides are reduced in the setting of HCV-associated chronic liver disease,[25,26] related not only to impaired hepatic function, but also an incompletely understood interaction between HCV and host lipid metabolism that is necessary for HCV replication.[27] This favorable risk profile stands in contrast to the increased CVD risk seen with HCV infection. Thus, routine markers may underestimate CVD risk in HCV-coinfected persons.

The potential role of non-hepatic biomarkers for CVD risk assessment in HCV-coinfected persons. Non-hepatically produced markers of endothelial dysfunction, immune activation, and inflammation may be better predictors of CVD risk in HIV/HCV-coinfected persons, as their levels would be less susceptible to perturbation due to active liver disease. There are a number of non-hepatic markers that have been evaluated in the general, non-HIV-infected population with regard to their predictive value for CVD risk. Such markers include soluble intercellular

adhesion molecule (sICAM-1), soluble P-selectin (sP-selectin), D-dimer, interleukin-6 (IL-6), and lipoprotein-associated phospholipase A2 (Lp-PLA2). ICAM-1 and P-selectin are vascular adhesion molecules expressed on the surface of activated endothelial cells and intimately involved in the early stages of atherogenesis and plaque formation in arterial walls.[28-36] Levels of sICAM-1 and sP-selectin are elevated at baseline in HCV coinfection.[37-40] In large prospective studies, circulating sICAM-1 and sP-selectin levels both independently predicted future CVD events and were consistently associated with classical risk factors.[41-45]

D-dimer and IL-6 are markers of inflammation and activated coagulation, processes that are critical to atherosclerosis and atherothrombosis. The association between D-dimer and IL-6 with risk of future CVD events in the general population is quite robust.[46-49] Both D-dimer and IL-6 are elevated in HIV infection,[50,51] and emerging evidence suggests such markers of thrombosis and chronic inflammation may improve CVD risk stratification in HIV-infected individuals.[52]

An additional novel, non-hepatically produced CVD biomarker is Lp-PLA2, a pro-inflammatory enzyme expressed by leukocytes in atherosclerotic plaques and under investigation as a therapeutic target for atherosclerosis.[53,54] Lp-PLA2 hydrolyzes oxidized phospholipids to promote endothelial dysfunction, plaque inflammation, and the formation of necrotic core in plaque. In a recent meta-analysis of 32 prospective studies in the general population, Lp-PLA2 had continuous associations with risk of CVD similar in magnitude to that with non-HDL cholesterol or systolic blood pressure in the study populations.[55] Recently published ACCF/AHA guidelines found reasonable evidence to support use of Lp-PLA2 for CVD risk stratification in intermediate risk asymptomatic adults.[24] Lp-PLA2 has never been described in

the HCV literature. It predominantly associates with LDL in circulation[56] and it is unclear if, given lower LDL levels in HCV infection, Lp-PLA2 would be a useful marker in the presence of HCV coinfection.

The potential role of HCV treatment for cardiovascular risk reduction. Berenguer et al. recently found that not only liver, but non-liver mortality in HIV-infected persons is reduced with successful HCV treatment and HCV viral eradication.[57] In this cohort, the second leading cause of non-liver, non-AIDS deaths was cardiovascular disease. Further, other studies have demonstrated that cardiovascular risk factors such as hepatic steatosis and insulin resistance improve with HCV clearance.[58-60] The potential benefit of HCV treatment on CVD risk reduction in coinfection is also suggested by one cross-sectional study that demonstrated decreased levels of sICAM-1 in subjects who responded to HCV treatment compared to those who did not, although this study did not control for potential confounders such as age, sex, and hypertension.[38] Of note, successful HCV treatment may also unmask underlying CVD risk, through rise in hsCRP and lipids. One recently published study showed that HCV clearance with PEG/RBV therapy was associated with an increase in total cholesterol and LDL, reaching levels with indication for lipid-lowering therapy in some patients.[61]

Optimization of cardiovascular risk assessment and risk reduction in HIV/HCV coinfected persons is needed. Cardiovascular disease has emerged as a major cause of morbidity and mortality in the aging HIV-infected population. HCV coinfection may contribute to accelerated CVD and better characterization of CVD risk in HIV/HCV-coinfected persons is needed. It is not widely recognized that the traditional, hepatically-produced VD risk markers used in routine

clinical practice are reduced and may be unreliable for risk assessment in HCV-coinfected persons. There is also a paucity of data characterizing non-hepatic CVD risk markers in the HIV/HCV-coinfected population. Further, identifying methods to reduce the observed increased CVD risk in HCV-infected persons is crucial. Our hypotheses were: 1) HCV is additive or synergistic in promoting CVD risk through increased immune activation; 2) Markers of immune activation that are not produced by the liver will provide more accurate and novel biomarkers for assessment of CVD risk in HIV-1 co-infected persons; and 3) Successful treatment of HCV in HIV/HCV co-infected persons will reduce CVD outcomes in correlation with improvement in these novel biomarkers and paradoxical rise in hepatically-produced biomarkers reflecting improved liver function.

CHAPTER 2: MANUSCRIPT

Abstract

Background: Hepatitis C (HCV) may increase cardiovascular disease (CVD) risk in HIV-infected persons. We hypothesized that HCV virologic clearance reduces CVD risk, as manifested by reduction in non-hepatic CVD biomarkers.

Methods: Of 54 HIV/HCV coinfected subjects who received 72 weeks of pegylated interferon/ribavirin, 27 with and 27 without sustained virologic response (SVR) matched by race/ethnicity and sex, stored serum/plasma before treatment and 24 weeks after end of treatment (EOT) were tested for non-hepatic (sICAM-1, sP-selectin, IL-6, D-dimer, and lipoprotein-associated phospholipase A2 [Lp-PLA2]) and hepatic markers of CVD (cholesterol and hsCRP). Baseline characteristics and biomarkers were compared between SVRs and non-SVRs by Wilcoxon rank sum test. Changes in each biomarker were examined within SVRs/non-SVRs and between groups by t-tests and regression models.

Results: The cohort included 54 subjects, 30 white, 24 black, and 44 male. Baseline levels of non-hepatic markers were not significantly different between groups, including sICAM-1 (overall median [Q1, Q3]=439.2 [365.6, 592.8] ng/mL), sP-selectin (146.7 [94.1, 209.9] ng/mL) and IL-6 (2.32 [1.61, 3.49] pg/mL). Of 52 subjects with baseline Lp-PLA2, 37 (71%) had Lp-PLA2>235 ng/mL. SVRs had a significant decrease in $\log_{10}$ sICAM-1, but not non-SVRs (mean [sd] = -0.09 [0.13] vs -0.01 [0.14], p=0.047 for between group comparison). Adjusting for baseline AST and ALT, SVR was significantly associated with decrease in sICAM-1 (p=0.033),

but not after adjusting for change in ALT (p=0.105). At 24 weeks after EOT, 17 (63%) SVRs had Lp-PLA2>235 ng/mL compared to 25 (93%) non-SVRs (p=0.021).

Conclusions: High baseline levels of non-hepatic CVD markers suggest high CVD risk in HIV/HCV coinfection. SVR was associated with decrease in sICAM-1 and lower CVD risk by Lp-PLA2 level. HCV virologic clearance may lower CVD risk through reduction in hepatic, and subsequently systemic, inflammation.

Introduction

Studies in the current era of effective antiretroviral therapy demonstrate increased overall, and not just liver-specific, mortality in HIV/HCV-coinfected persons as compared to HIV-monoinfected control groups, with cardiovascular disease being a leading cause of non-liver, non-AIDS deaths.[11,12] A number of large retrospective, longitudinal, observational database studies and cross-sectional studies suggest an increased risk of cardiovascular disease (CVD) events in HCV-infected and coinfected persons.[13-18] Multiple potential mechanisms for increased CVD risk with HCV infection exist, including hepatic steatosis, insulin resistance, diabetes mellitus, and chronic immune activation, which is further increased in HCV-coinfected persons.[19-22]

The optimal method for CVD risk assessment in HIV/HCV-coinfected persons is unknown. Total cholesterol, low-density lipoprotein (LDL) cholesterol, and hsCRP levels are widely used in routine clinical practice for CVD risk assessment,[23,24] but are reduced in the setting of HCV-associated chronic liver disease,[25,26] related not only to impaired hepatic function, but also an

incompletely understood interaction between HCV and host lipid metabolism that is necessary for HCV replication.[27] Such routine hepatic markers may underestimate CVD risk in coinfected persons and non-hepatically produced markers of endothelial dysfunction, immune activation, and inflammation may be better predictors of CVD risk. Potentially useful markers which have primarily been evaluated in the general, non-HIV-infected population include soluble intercellular adhesion molecule-1 (sICAM-1), soluble P-selectin (sP-selectin), lipoprotein-associated phospholipase A2 (Lp-PLA2), D-dimer, and interleukin-6 (IL-6). These markers, in large prospective studies, independently predicted future CVD events and were consistently associated with classical CVD risk factors.[41-49,55] Both D-dimer and IL-6 are elevated in HIV infection,[50,51] and emerging evidence suggests such markers of thrombosis and chronic inflammation may improve CVD risk stratification in HIV-infected individuals.[52] Soluble ICAM-1 and sP-selectin levels are elevated at baseline in HIV/HCV coinfection as compared to HIV-monoinfected and uninfected controls, but their predictive value in coinfected patients remains unknown.[37-39,62]

HCV treatment and viral eradication may provide benefits beyond reduction in liver-specific morbidity and mortality. Berenguer et al. recently found that not only liver, but also non-liver mortality in HIV-infected persons is reduced with successful HCV treatment.[57] In this cohort, the second leading cause of non-liver, non-AIDS deaths was cardiovascular disease. Further, other studies have demonstrated that cardiovascular risk factors such as hepatic steatosis and insulin resistance improve with HCV clearance.[58-60] Notably, successful HCV treatment may also unmask underlying CVD risk, through rise in hsCRP and lipids. One recently published study showed that HCV clearance with PEG/RBV therapy was associated with an increase in

total cholesterol and LDL, reaching levels with indication for lipid-lowering therapy in some patients.[61]

HCV coinfection may contribute to accelerated CVD in HIV-infected persons and the optimal approach to CVD risk assessment and risk reduction in coinfected patients remains unknown. Our aim was to characterize non-hepatic CVD biomarker levels in a well-characterized HIV/HCV-coinfected cohort and explore the potential benefit of HCV treatment on cardiovascular outcomes, hypothesizing that HCV clearance would be associated with a favorable reduction in non-hepatic CVD biomarkers.

Methods

Study Design and Subject Selection: This was a retrospective case control study analyzing stored serum and plasma samples and clinical data of HIV/HCV-coinfected subjects who participated in the AIDS Clinical Trials Group study A5178. All subjects were selected from the Step 3 arm of A5178, for which the study design and treatment outcomes have been published.[63] In brief, all subjects achieved a 12-week early virologic response and were assigned to receive a 72-week PEG/RBV treatment course. Serum and plasma samples were collected and frozen during the study, including at entry (week 0) and 24 weeks after end of treatment (week 96). Twenty-seven responders (those with sustained virologic response, or SVR) and 27 nonresponders/relapsers (non-SVR) to PEG/RBV therapy were selected randomly from those with available paired (week 0 and week 96) serum and plasma samples. The SVR and non-SVR groups were frequency-matched on race/ethnicity and sex, as race/ethnicity and sex have been shown to be related to the biomarkers as well as to treatment response.[43]

Clinical Data: Baseline and on-treatment data were abstracted from the original A5178 dataset, including: age; gender; race/ethnicity; presence or absence of hypertension; presence or absence of diabetes; medication use, including those that might affect biomarker levels, such as lipid-lowering, aspirin and other antiplatelet or nonsteroidal anti-inflammatory drugs (NSAIDs), antihypertensives, hormone use, and antiretroviral therapy (ART); body mass index (BMI); intravenous drug use history; fasting glucose and insulin; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels; comorbid disease which may affect biomarker levels, including vascular or coronary disease and opportunistic infection; HCV treatment history; baseline, end of treatment (EOT) and 24 weeks after end of treatment HCV RNA level; HCV genotype; hepatic fibrosis scores; CD4 cell count; and HIV RNA level. HOMA-IR as a measure of insulin resistance was calculated as fasting glucose(mg/dl) x fasting insulin(μU/mL) / 405. All subjects had liver biopsy available at baseline, with different scoring systems used, including Knodell, Ludwig, METAVIR, Modified HAI, and Scheuer.

Outcomes: The primary outcome was change in sICAM-1 levels from week 0 to 24 weeks after end of treatment. Secondary outcomes were changes in sP-selectin, IL-6, D-dimer, Lp-PLA2, total cholesterol, high-density lipoprotein cholesterol (HDL), LDL, and triglyceride levels between the same timepoints. The primary predictor was sustained virologic response (SVR), defined as undetectable HCV viral load at 24 weeks after end of treatment.

Biomarkers: Serum and EDTA plasma specimens were collected and stored at a central repository. Soluble ICAM-1, sP-selectin, Lp-PLA2, D-dimer, IL-6, hsCRP, and lipid panel (total

cholesterol, HDL, triglycerides, calculated LDL) were measured at Quest Diagnostics Laboratories, Clinical Trials Division. Serum sICAM-1 and plasma sP-selectin was measured by microarray fluorescent immunoassay, plasma Lp-PLA2 mass by microplate-based enzyme immunoassay, plasma D-dimer and serum IL-6 by enzyme-linked immunosorbent assay (ELISA), serum hsCRP by chemiluminescence, and serum lipid panel by automated spectrophotometry. Forty-one of the 54 available subjects had samples collected under A5178 protocol version 1 and had fasting lipids (mixed direct and calculated LDL), insulin, and glucose available for analysis. A lipid panel was repeated on all subjects, including non-fasting lipids on the 13 subjects without fasting samples.

Data Analysis: For data analysis, biomarkers below the limit of detection were assigned the lowest level of detection. For lipid levels, analyses were done separately for fasting lipids collected during A5178 and combining re-testing of fasting samples and non-fasting samples. Lp-PLA2 levels were analyzed as a continuous variable and categorized into high (>235 ng/mL), intermediate (200-235), or low CVD risk (<200 ng/mL). Liver fibrosis scores were dichotomized as <3 or ≥3, regardless of scoring method. Baseline characteristics and biomarker levels between the SVR and non-SVR groups were compared by Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables. Log_{10} transformations were explored for biomarkers with skewed distribution. For baseline analyses, biomarkers in their original scale are reported. Parametric analysis was considered primary. Within each group, change in each biomarker from baseline to 24 weeks after end of treatment was compared to zero by paired sample t-test and between groups by two-sample test. For those biomarkers for which there was a significant association between change in biomarker and SVR

status, linear regression models with change is the biomarker as the outcome were fit to further quantify the association between the change in the biomarker and SVR status, adjusting for different covariates. Model 1 adjusted for sex and race/ethnicity, model 2 included backwards selection, adjusting for baseline variables that were significantly different by SVR status at a 0.10 significance level, model 3 further adjusted for change in ALT from baseline to 24 weeks after end of treatment, and model 4 for duration of PEG/RBV treatment. Additional regression models adjusted for occurrence of opportunistic infection and concomitant medication use (grouped *a priori* as immunomodulators, antiplatelet/aspirin, antihypertensives, nonsteroidal anti-inflammatories, and lipid-lowering agents), as both might influence biomarker levels. In sensitivity analyses, if more than 20% (11) of subjects had on-treatment CVD events, opportunistic infections, or concomitant medication use that might affect biomarker levels, the above analyses were repeated excluding each group of subjects.

Results

Baseline characteristics of the cohort by SVR status are summarized in Table 1. Mean age (SD) overall was 47.7 (6.2) years. 44 of the 54 subjects were male, 30 white non-Hispanic, and 24 black. Median CD4 cell count was similar between the groups and the majority (78%) had HIV-1 RNA <50 copies/mL at baseline. Forty-eight subjects (25 in the SVR group and 23 in the non-SVR group) had baseline ART information available. ART did not differ between the groups comparing categorically by unboosted protease inhibitor (PI) (9 or 36% in SVR group vs 6 or 26% non-SVR), ritonavir-boosted PI (5 or 20% vs 8 or 35%), or non-PI (11 or 44% vs 8 or 35%) regimen (p=0.498). One subject in the non-SVR group was not on ART. Of 38 subjects with available fasting insulin and fasting glucose (22 SVR, 16 non-SVR), HOMA-IR, AST, and ALT

levels were significantly higher in non-SVR subjects at baseline (see Table 1). Few subjects had known CVD at baseline and most subjects (96%) did not have cirrhosis, though 5 (19%) of the SVR group and 13 (48%) of the non-SVR group had significant fibrosis, with a fibrosis score of at least 3. In the overall cohort, 19 (35%) were taking antihypertensives, 5 (9%) antiplatelet medications including aspirin, 24 (44%) NSAIDs, 4 (7%) HMG Co-A reductase inhibitors (statins), and 4 (7%) other lipid-lowering agents at baseline. Use of these medications remained similar during the study. Twenty-four subjects (44%) received immunomodulators on study, primarily granulocyte colony-stimulating factor.

Baseline levels of the non-hepatic and hepatic CVD biomarkers by SVR status are given in Table 2. One subject in each of the SVR and non-SVR groups did not have sufficient sample volume for Lp-PLA2 testing and one subject in the SVR group did not have sufficient sample volume for IL-6 testing. Overall levels of the non-hepatic biomarkers were high. 37 of 52 (71%) subjects had Lp-PLA2 level >235 ng/mL, which by routine interpretation would be classified as high CVD risk. 24 (44%) subjects had undetectable D-dimer (<0.22 mcg/mL) at baseline (13 SVR, 11 non-SVR) and 21 subjects had undetectable D-dimer at 24 weeks after end of treatment (13 SVR, 8 non-SVR). Total cholesterol and LDL levels were significantly higher in the SVR group (median [IQR] 188 [169, 222] vs 153 [135, 167] mg/dL and 112 [84, 124] vs 81 [68, 108] mg/dL). Total cholesterol and LDL levels correlated with degree of fibrosis, with fibrosis stage ≥ 3 associated with lower total cholesterol (median [IQR] 157 [135, 157] vs 177 [146, 207] mg/dL for fibrosis stage <3) and lower LDL (86 [51, 109] vs 103 [78, 124] mg/dL). There were no differences by SVR status in baseline levels of sICAM-1, sP-selectin, IL-6, Lp-PLA2, D-dimer, triglycerides, HDL, or hsCRP.

Levels of sICAM-1 decreased significantly from week 0 to 24 weeks after EOT in the SVR group (p=0.001), but remained unchanged in the non-SVR group (mean ratio of $\log_{10}$ sICAM-1 = -0.09 [0.13] vs -0.01 [0.14]). The change in sICAM-1 comparing SVR and non-SVR groups was statistically significant (p=0.047). Box plots depicting the change in sICAM-1 levels by SVR status are provided in Figure 1. In model 1, regression analysis controlling for sex and race/ethnicity, SVR status was significantly associated with decrease in $\log_{10}$ sICAM-1 (p=0.042). In model 2, baseline characteristics that were significant at <0.10 level and included in backwards variable selection included liver fibrosis stage (<3 vs ≥3), HCV RNA level, HCV treatment history (naïve vs experienced), and baseline AST and ALT level. Only baseline AST and ALT were retained in the model; adjusting for baseline AST and ALT, SVR status remained statistically significantly associated with decrease in $\log_{10}$ sICAM-1 (p=0.033), Figure 2A. In model 3 (Figure 2B), the regression model was further adjusted for change in ALT from baseline to 24 weeks after EOT, resulting in loss of the association between SVR status and change in $\log_{10}$ sICAM-1 (p=0.105). Additional regression analysis found no association between duration of PEG/RBV and change in sICAM-1.

No subjects had a CVD event during the course of the study. Two subjects, both in the non-SVR group, developed diabetes during the study. Given few subjects with on-study CVD or diabetes, no regression analyses were conducted to further adjust for these conditions. Eight (15%) of subjects experienced an opportunistic infection (3 SVR, 5 non-SVR), including bacterial pneumonia, sepsis, and deep infection, pulmonary histoplasmosis, and cutaneous varicella zoster. Regression models adjusting for occurrence of on-study opportunistic infections found no

association between OIs and change in log10 sICAM-1 and persistence of the association between SVR status and change in log10 sICAM-1 (p=0.025). As more than 20% of subjects received medications that may have affected biomarker levels (24 with immunomodulators, 26 with antihypertensives, and 32 with NSAIDS), sensitivity analyses were conducted excluding these subjects sequentially. Statistically significant between-group differences in $\log_{10}$ sICAM-1 persisted with exclusion of subjects with antihypertensive use. No statistically significant between-group differences were seen after excluding subjects with immunomodulator use or with NSAID use, but the sample size was substantially limited (n=29 excluding immunomodulator use, 20 excluding NSAID use). However, similar point estimates for change in $\log_{10}$ sICAM-1 were seen in the SVR and non-SVR groups as with the whole cohort.

Given concern that HIV viremia might confound biomarker levels, sensitivity analyses were conducted limiting sICAM-1 analysis to those with documented virologic suppression (<50 copies/mL) for the entire duration of the study. This limited the analysis to 14 SVR subjects and 8 non-SVR subjects. By two-sample t-test for difference in change in sICAM-1 from week 0 to 24 weeks after EOT, there was no statistically significant difference between SVR and non-SVR groups (p=0.129), but the point estimates for change in sICAM-1 were similar for the groups as was seen in analysis including the whole cohort (mean [57] change in log10 sICAM-1-0.09 [0.12] vs 0.02 [0.17]).

There were no statistically significant changes from baseline to 24 weeks after EOT in the other non-hepatic and hepatic markers. Numerically there was a decrease in Lp-PLA2 levels in the SVR group as compared to the non-SVR group (-17.5 ([-67.0, 47.0] vs 9.50 [-49.0, 67.0]

ng/mL), though this was not statistically significant (p=0.334). However, there was a statistically significant difference in CVD risk class distribution by Lp-PLA2 level at 24 weeks after EOT (Figure 3), with 25 of 27 (93%) of the non-SVR group in the high-risk category as compared to 17 of 27 (63%) of the SVR group (p=0.021).

Discussion

In our cohort, we found high baseline levels of the non-hepatic CVD biomarkers sICAM-1, sP-selectin, IL-6, and Lp-PLA2, suggesting high CVD risk in HIV/HCV coinfection. Levels were higher than those reported in the general population, exceeding levels in HIV-uninfected persons at high risk for CVD or with known baseline CVD.[42,43,47,49,64] Levels of sICAM-1, sP-selectin, and IL-6 in our analysis were consistent with those seen in other studies of coinfected persons.[40,62] Also consistent with other studies, total cholesterol and LDL levels were low or optimal in the cohort, with higher levels in the SVR as compared to non-SVR group, driven in part by degree of hepatic fibrosis. The high levels of non-hepatic CVD markers, independent of SVR status, suggest they may be less confounded by liver disease effects and more readily interpretable in HCV-coinfected patients. In the general population, the association between levels of sICAM-1, sP-selectin, and IL-6 and incident CVD events, as well as with classical CVD risk factors, has consistently been shown. Their incorporation into risk prediction paradigms for the general population has been limited, as they do not, in non-HIV/HCV-infected persons, provide incremental risk discrimination. However, in the context of perturbed traditional risk markers such as cholesterol in chronic HCV infection, there may be a distinct role for these biomarkers in HIV/HCV-coinfected or HCV-monoinfected persons.

We further found that HCV virologic clearance, as measured by SVR, was associated with a significant decrease in sICAM-1. The magnitude of this change was greater than the difference in sICAM-1 observed in studies of the general population comparing subjects who went on to have a coronary disease event and those who did not.[43,65] This association no longer existed after adjusting for change in ALT, suggesting that the effect of SVR on sICAM-1 levels may be mediated by reduction in hepatic inflammation. This is a distinct, and new, finding from other studies that have explored the effect of HCV treatment and eradication on biomarkers such as sICAM-1.[38,39] We also found SVR was associated with stable Lp-PLA2 levels over 96 weeks, whereas non-SVR was associated with a shift to higher CVD risk class by Lp-PLA2 level. This is a suggestive finding: perhaps HCV virologic clearance attenuates atherosclerotic disease progression in HIV-infected persons. This is the first report describing Lp-PLA2 levels in HCV-infected persons.

Liver deaths alone do not explain excess mortality in HIV/HCV-coinfected patients on effective antiretroviral therapy. Chronic and high levels of viral replication and increased gut permeability and microbial translocation/endotoxemia are associated with systemic immune activation in HIV, and thought to be major drivers of HIV-1 disease progression. HCV coinfection and ongoing HCV viremia may augment such immune activation, complicating the interaction between HIV and host innate immune response.[66] Impaired lipopolysaccharide tolerance in the setting of chronic HCV infection may lead to chronic intrahepatic monocyte and macrophage activation, hepatic inflammation, and further systemic immune activation and inflammation. As such, it may be that HCV acts additively, or even synergistically, to drive immune activation and

extra-hepatic complications. Treatment and clearance of HCV may reduce hepatic inflammation and, subsequently, systemic inflammation and its associated complications.

Strengths of our study include the well-characterized cohort, the uniformity of HCV treatment and collection and processing of specimens as subjects were enrolled in a clinical trial, the centralized testing of biomarkers to reduce inter-assay variability, and its study design, including matching for potential confounding by race/ethnicity and sex.

Limitations include the retrospective nature of the study, with use of frozen samples for which the stability of all the assays has not been demonstrated; lack of all data relevant to the outcomes of interest, such as smoking history, though we would not expect there to be between group differences in smoking status that would affect biomarker levels or changes; and confounding by HIV viremia, which we explored in sensitivity analysis (reassuringly finding similar point estimates, with substantially reduced sample size likely limiting our power to find a statistically significant difference between the groups). The study was also powered to detect a significant change in sICAM-1 levels, and may have been underpowered for evaluation of the other biomarkers. We also made a large number of comparisons, such that it is possible that our findings were due to chance, but the reduction in sICAM-1 with SVR has now been seen consistently in several studies in coinfected subjects. Interpretation of the favorable changes in sICAM-1 and Lp-PLA2 is limited given the small size of our cohort, lack of validation of these biomarkers for CVD risk prognostication in HIV/HCV coinfection, and inability to correlate biomarker levels with hard outcomes such as CVD events or validated surrogate measures of cardiovascular risk. Masia et al, in a cross-sectional analysis, approached correlation of sICAM-

1 and another vascular adhesion molecule, sVCAM-1, with carotid intima-media thickness and brachial artery flow-mediated dilation in HIV/HCV-coinfected subjects and HIV-monoinfected controls, finding no association, but were limited by heterogeneous HIV status.[62] The effect of HCV treatment was also not explored in their study.

Despite these limitations, our data remain suggestive and further investigation into characterizing the predictive utility of both non-hepatic and hepatic CVD biomarkers and quantifying the potential benefit of HCV treatment on non-liver outcomes such as cardiovascular disease is warranted. Today, aging HIV-infected patients on effective antiretroviral therapy are at increasing risk for non-AIDS complications including cardiovascular disease, which may be further accelerated by HCV coinfection. Successful HCV treatment may be a viable method for CVD risk reduction and an additional indication for earlier HCV treatment in HIV/HCV-coinfected persons.

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Table 1. Baseline characteristics of the study cohort

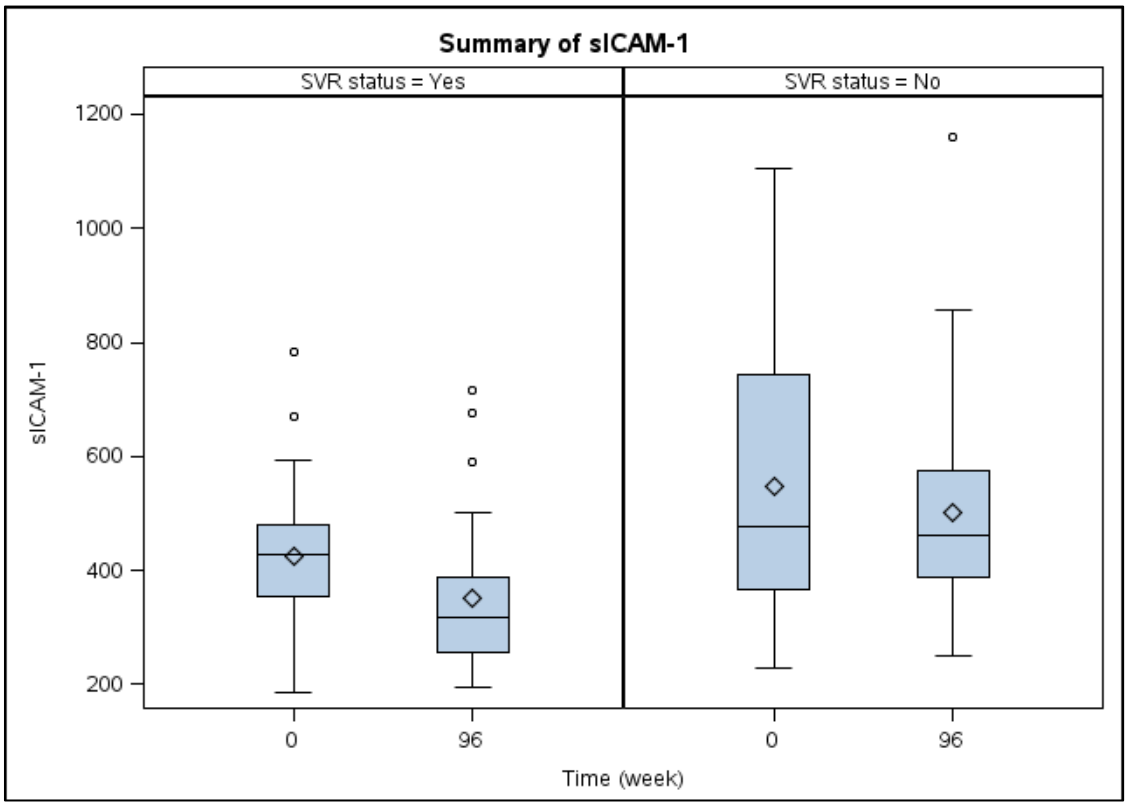
Characteristic	SVR (n=27) Median (Q1, Q3) or n (%)	Non-SVR (n=27) Median (Q1, Q3) or n (%)
Age (years)	47 (42, 52)	48 (45, 52)
Sex		
Male	22 (81%)	22 (81%)
Race/ethnicity		
White Non-Hispanic	15 (56%)	15 (56%)
Black	12 (44%)	12 (44%)
Body mass index (BMI)	26.1 (23.2, 30.0)	25.5 (24.1, 28.5)
HIV-1 RNA (copies/mL)		
Undetectable (<50)	20 (74%)	22 (81%)
CD4 cell count	571 (378, 747)	536 (357, 734)
HCV genotype		
1	18 (87%)	23 (85%)
2	7 (26%)	2 (7%)
3	2 (7%)	1 (4%)
4	0 (0%)	1 (4%)
Cirrhosis	0 (0%)	2 (7%)
Fibrosis stage ≥ 3	5 (19%)	13 (48%)
HCV RNA (log10 copies/mL)	6.41 (5.73, 6.92)	6.70 (6.31, 7.04)
History of prior HCV treatment	4 (15%)	11 (41%)
History of CVD	3 (11%)	4 (15%)
HTN	5 (19%)	9 (33%)
Diabetes	2 (7%)	5 (19%)
HOMA-IR (SVR, n=22; non-SVR, n=16)	2.97 (1.43, 4.49)	6.11 (3.73, 8.77)
AST (U/L)	41 (30, 60)	63 (42, 85)
ALT (U/L)	58 (37, 73)	68 (51, 91)

Table 2. Baseline biomarker levels by SVR status

Biomarker	SVR (n=27) Median (Q1, Q3)	Non-SVR (n=27) Median (Q1, Q3)	P-value*
sICAM-1 (ng/mL)	428.6 (355.1, 478.8)	476.6 (366.9, 744.5)	0.156
sP-selectin (ng/mL)	153.6 (110.8, 225.9)	146.4 (89.4, 170.6)	0.276
IL-6 (pg/mL) (SVR n=26)	2.43 (1.55, 3.60)	2.32 (1.61, 3.49)	0.943
Lp-PLA2 (ng/mL) (SVR and non-SVR n=26)	315.5 (231.0, 394.0)	297.5 (235.0, 371.0)	0.934
D-dimer (mcg/mL)	0.24 (0.22, 0.36)	0.31 (0.22, 0.52)	0.239
Total cholesterol (mg/dL)	188 (169, 222)	153 (135, 167)	0.002
LDL (mg/dL) (SVR, n=23, non-SVR, n=22)	112 (85, 125)	81 (68, 108)	0.015
Triglycerides (mg/dL)	144 (107, 243)	141 (97, 215)	0.665
HDL (mg/dL)	38 (31, 50)	36 (31, 46)	0.545
hsCRP	1.15 (0.70, 3.20)	1.00 (0.60, 2.60)	0.476

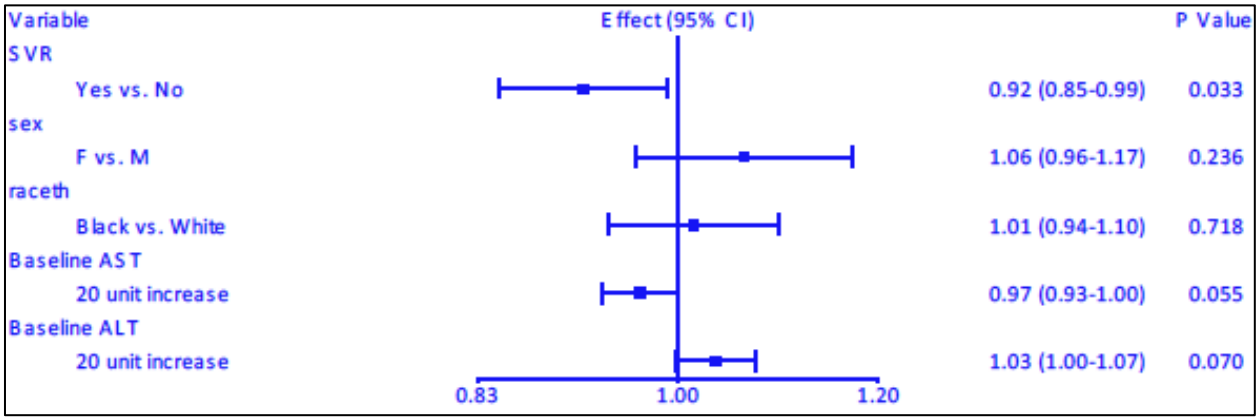
*Wilcoxon rank sum test
SVR=sustained virologic response; sICAM-1=soluble ICAM-1; sP-selectin = soluble P-selectin,; IL-6= interleukin-6; Lp-PLA2 = lipoprotein-associatd phospholipase A2; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; hsCRP = high-sensitivity CRP

Figure 1. Box plots of effect of SVR on sICAM-1 level in ng/mL



Week 0 = baseline, Week 96 = 24 weeks after end of HCV treatment

Figure 2A. Regression of change in log10 sICAM-1 after backward variable selection



Variables included in backward variable selection: baseline hepatic fibrosis stage, baseline HCV RNA level, HCV treatment history (naïve or experienced), baseline AST and baseline ALT level.

Figure 2B. Regression of change in log10 sICAM-1 further adjusting for change in alanine aminotransferase (ALT) level from baseline to 24 weeks after end of HCV treatment

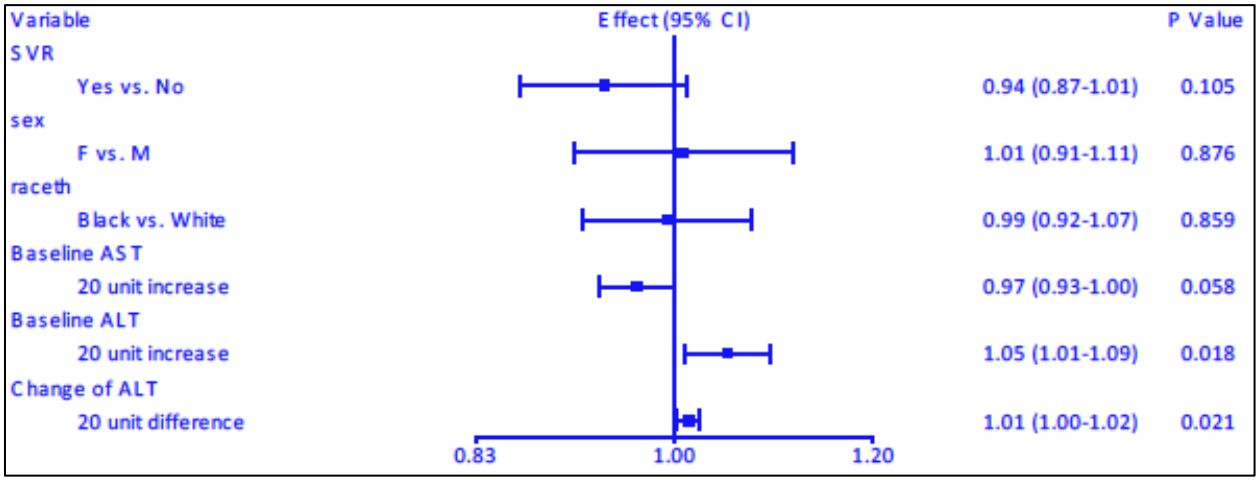
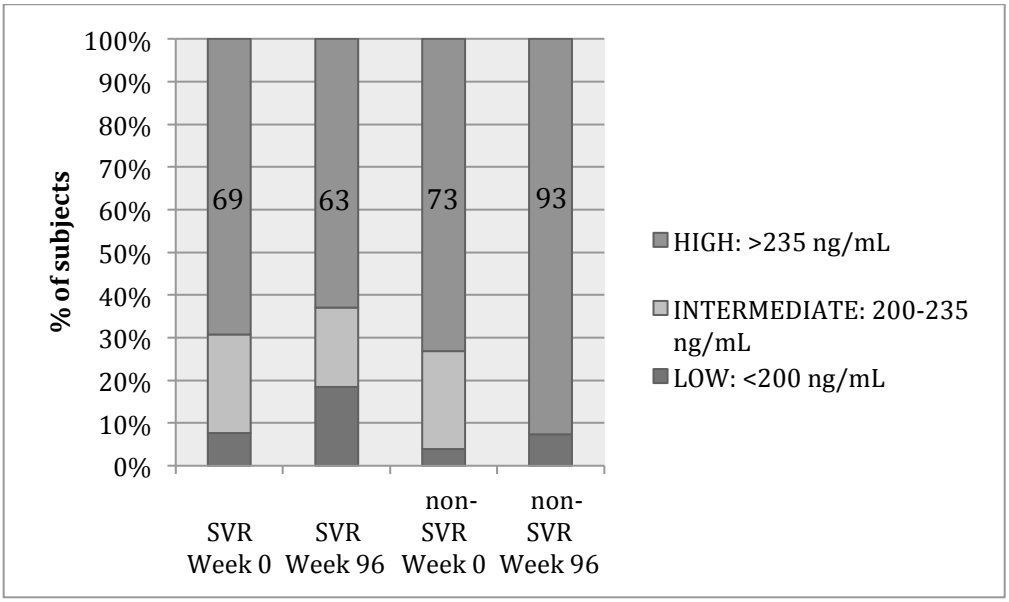


Figure 3. Effect of SVR on cardiovascular risk class by Lp-PLA2 level



Numbers overlying bars reflect percentage in each group with Lp-PLA2 level in the high CVD risk category.
p=0.021 by Fisher's exact test for difference in risk class at week 96, SVR vs non-SVR

CHAPTER 3: STATISTICAL APPENDIX

Change in selected biomarkers from baseline to week 96

Analyses comparing change in each biomarker (sICAM-1, sP-selectin, D-dimer, IL-6, Lp-PLA2, total cholesterol, LDL, triglycerides, and hsCRP) from baseline to week 96 (24 weeks after end of treatment [EOT]) within each group (SVR and non-SVR) and comparing the change between groups were conducted. Only sICAM-1 data were provided in the manuscript as a significant change in biomarker level was found only with sICAM-1, and not with the other biomarkers. For illustration, summary statistics from selected non-hepatic (sP-selectin, IL-6, Lp-PLA2) and hepatic biomarkers (total cholesterol, LDL) are provided below (Tables 3A-E). For the within-group comparisons for change in these biomarkers, paired t-test was used for all biomarkers except IL-6. For the between-group comparison for change in these biomarkers, two-sample t-test was used for all biomarkers except IL-6. Non-parametric analyses were selected for statistical inferences for IL-6 as the distribution of IL-6 remained skewed even with $\log_{10}$ transformation. There was no statistically significant within-group change in $\log_{10}$ sP-selectin from baseline to 24 weeks after EOT (p=0.426 and p=0.845 for SVR and non-SVR groups, respectively). There was no between-group difference in change in $\log_{10}$ sP-selectin (p=0.581). By Wilcoxon signed rank test, there was no statistically significant change in IL-6 from baseline to 24 weeks after EOT in the SVR group (p=0.671). Subjects without SVR had a statistically significant increase in IL-6 (p=0.044). There was no between-group difference in change in IL-6 by two-sample Wilcoxon test (p=0.282). One subject had an extremely large baseline IL-6 measurement; excluding this subject did not change the findings. There was no statistically significant within-group change in Lp-PLA2 from baseline to 24 weeks after EOT (p=0.187 and p=0.806 for SVR and non-SVR groups, respectively). There was no between-group difference in

change in Lp-PLA2 (p=0.334). There was no statistically significant within-group change in total cholesterol from baseline to 24 weeks after EOT (p=0.323 and p=0.960 for SVR and non-SVR groups, respectively). There was no between-group difference in change in total cholesterol by (p=0.464). There was no statistically significant within-group change in LDL from baseline to 24 weeks after EOT (p=0.820 and p=0.782 for SVR and non-SVR groups, respectively). There was no between-group difference in change in LDL (p=0.719)

Table 3A. Summary statistics for sP-selectin

Characteristic		**SVR (N=27)**	**Non-SVR (N=27)**	**Total (N=54)**	**P-Value***
Week 0: log10 sP-selectin	N	27	27	54	0.276
	Mean (SD)	5.19 (0.28)	5.12 (0.23)	5.16 (0.26)	
	Min, Max	4.40, 5.69	4.75, 5.66	4.40, 5.69	
	Median (Q1, Q3)	5.19 (5.04, 5.35)	5.17 (4.95, 5.23)	5.17 (4.97, 5.32)	
Week 96: log10 sP-selectin	N	27	27	54	0.904
	Mean (SD)	5.14 (0.28)	5.12 (0.23)	5.13 (0.26)	
	Min, Max	4.59, 5.74	4.59, 5.77	4.59, 5.77	
	Median (Q1, Q3)	5.10 (4.94, 5.30)	5.09 (4.99, 5.22)	5.10 (4.99, 5.23)	
Diff: log10 sP-selectin	N	27	27	54	
	Mean (SDa)	0.05 (0.34)	0.01 (0.23)	0.03 (0.29)	
	Min, Max	-0.73, 0.81	-0.56, 0.57	-0.73, 0.81	
	Median (Q1, Q3)	0.04 (-0.10, 0.26)	-0.01 (-0.11, 0.05)	0.01 (-0.10, 0.20)	

*Wilcoxon test

Table 3B. Summary statistics for IL-6

Characteristic		SVR (N=27)	Non-SVR (N=27)	Total (N=54)	P-Value*
Week 0: IL-6	N	26	27	53	0.943
	Mean (SD)	2.96 (2.82)	49.36 (243.39)	26.60 (173.70)	
	Min, Max	0.84, 15.60	0.72, 1,267.20	0.72, 1,267.20	
	Median (Q1, Q3)	2.43 (1.55, 3.60)	2.32 (1.61, 3.49)	2.32 (1.61, 3.49)	
Week 96: IL-6	N	23	25	48	0.076
	Mean (SD)	4.22 (7.43)	5.95 (8.60)	5.12 (8.03)	
	Min, Max	0.52, 37.12	1.28, 43.52	0.52, 43.52	
	Median (Q1, Q3)	1.99 (1.25, 4.37)	3.21 (1.95, 6.67)	2.75 (1.63, 4.66)	
Diff: IL-6	N	22	25	47	
	Mean (SDa)	1.24 (4.95)	-47.20 (245.12)	-24.53 (178.76)	
	Min, Max	-2.85, 21.52	-1,223.68, 10.11	-1,223.68, 21.52	
	Median (Q1, Q3)	0.02 (-0.86, 1.79)	0.74 (-0.17, 3.20)	0.54 (-0.60, 1.82)	

*Wilcoxon test

Table 3C. Summary statistics for Lp-PLA2.

Characteristic		SVR (N=27)	Non-SVR (N=27)	Total (N=54)	P-Value*
Week 0: Lp-PLA2	N	26	26	52	0.934
	Mean (SD)	311.65 (92.72)	311.00 (86.18)	311.33 (88.63)	
	Min, Max	140, 457	193, 537	140, 537	
	Median (Q1, Q3)	315.50 (231.00, 394.00)	297.50 (235.00, 371.00)	312.50 (234.50, 382.50)	
Week 96: Lp-PLA2	N	27	27	54	0.276
	Mean (SD)	293.93 (109.96)	314.15 (81.92)	304.04 (96.58)	
	Min, Max	100, 491	144, 497	100, 497	
	Median (Q1, Q3)	286 (223, 395)	312 (261, 366)	292.50 (241.00, 366.00)	
Diff: Lp-PLA2	N	26	26	52	
	Mean (SDa)	-17.42 (65.41)	4.62 (94.63)	-6.40 (81.30)	
	Min, Max	-149, 89	-225, 224	-225, 224	
	Median (Q1, Q3)	-17.50 (-67.00, 47.00)	9.50 (-49.00, 67.00)	-5 (-59, 51)	

*Wilcoxon test

Table 3D. Summary statistics for total cholesterol (re-testing of samples, including fasting and non-fasting samples).

Characteristic		SVR (N=27)	Non-SVR (N=27)	Total (N=54)	P-Value*
Week 0: Cholesterol	N	27	27	54	0.002
	Mean (SD)	188.89 (43.63)	155.11 (34.93)	172.00 (42.70)	
	Min, Max	98, 288	88, 235	88, 288	
	Median (Q1, Q3)	188 (169, 222)	153 (135, 167)	168 (139, 192)	
Week 96: Cholesterol	N	26	26	52	0.002
	Mean (SD)	196.92 (48.54)	158.00 (35.94)	177.46 (46.63)	
	Min, Max	124, 322	97, 242	97, 322	
	Median (Q1, Q3)	192 (157, 223)	155 (125, 189)	175.00 (138.00, 207.50)	
Diff: Cholesterol	N	26	26	52	
	Mean (SDa)	7.35 (37.13)	0.31 (31.32)	3.83 (34.20)	
	Min, Max	-98, 67	-62, 81	-98, 81	
	Median (Q1, Q3)	9.50 (-14.00, 35.00)	2 (-25, 17)	4.50 (-18.00, 24.00)	

*Wilcoxon test

Table 3E. Summary statistics for LDL cholesterol (re-testing of samples, including fasting and non-fasting samples).

Characteristic		SVR (N=27)	Non-SVR (N=27)	Total (N=54)	P-Value*
Week 0: LDL	N	24	25	49	0.015
	Mean (SD)	107.13 (28.22)	86.28 (28.97)	96.49 (30.20)	
	Min, Max	42, 161	40, 143	40, 161	
	Median (Q1, Q3)	111.50 (84.50, 124.50)	81 (68, 108)	95 (76, 124)	
Week 96: LDL	N	23	22	45	<.001
	Mean (SD)	108.57 (26.68)	79.82 (26.75)	94.51 (30.14)	
	Min, Max	71, 166	39, 156	39, 166	
	Median (Q1, Q3)	106 (82, 126)	74.50 (62.00, 101.00)	92 (73, 111)	
Diff: LDL	N	22	21	43	
	Mean (SDa)	1.50 (30.50)	-1.95 (31.83)	-0.19 (30.83)	
	Min, Max	-50, 49	-54, 72	-54, 72	
	Median (Q1, Q3)	-4.50 (-19.00, 34.00)	-6 (-33, 17)	-6 (-20, 27)	

*Wilcoxon test

HIV viremia on study

HIV viremia has been associated with sICAM-1, IL-6, and D-dimer levels. Viremia at any point over the course of the study (from week 0 to 24 weeks after treatment) may confound these and other biomarker levels and the effect of SVR on change in the biomarkers. We found that only 14 SVR subjects and 8 non-SVR subjects had documented undetectable HIV RNA (<50 copies/mL) for the entire duration of the study. Table 4A provides the number of subjects in each group (SVR, non-SVR) with detectable HIV RNA at different time points during the study, as well as summary statistics for degree of viremia at each time point. Table 4B provides summary statistics from sensitivity analysis for the effect of SVR on sICAM-1, limiting the cohort to only those subjects with undetectable HIV RNA for the entire duration of the study. Among these 22 subjects, those with SVR had a significant reduction in sICAM-1 from baseline to 96 weeks, whereas those without SVR did not (p=0.015 for change in $\log_{10}$ sICAM-1 within the SVR group, p=0.750 for non-SVR group), with point estimates for change in $\log_{10}$ sICAM-1 similar to those seen in the overall cohort. There was not a statistically significant difference comparing the change in $\log_{10}$ sICAM-1 between groups (p=0.129 by two-sample t-test), likely due to lack of power.

Table 4A. Summary statistics for HIV viral load in copies/mL during the course of the study for all subjects with HIV viremia (HIV RNA >50 copies/mL).

Week	SVR		Non-SVR	
	# subjects with HIV viremia	Median viral load (IQR)	# subjects with HIV viremia	Median viral load (IQR)
0	5	689 (90, 2688)	3	5811 (800, 19354)
12	3	191 (110, 8410)	3	4085 (61, 17454)
24	3	1995 (56, 8108)	4	19673 (5120, 173222)
36	6	1137 (86, 1198)	3	2093 (95, 61227)
48	4	1888 (82, 9144)	5	666 (297, 2381)
72	6	9832 (56, 33770)	5	10872 (392, 20259)
84	9	262 (100, 922)	5	12951 (213, 27641)
96	7	922 (696, 55072)	8	445 (143, 3800)

Table 4B. Summary statistics for log_{10} sICAM-1, limited to subjects with undetectable HIV RNA.

Characteristic		SVR (N=14)	Non-SVR (N=8)	Total (N=22)	P-Value*
Week 0: log10 sICAM-1	Mean (SD)	5.61 (0.16)	5.65 (0.24)	5.62 (0.19)	0.973
	Min, Max	5.27, 5.89	5.36, 6.02	5.27, 6.02	
	Median (Q1, Q3)	5.64 (5.45, 5.68)	5.58 (5.49, 5.85)	5.62 (5.45, 5.71)	
Week 96: log10 sICAM-1	Mean (SD)	5.51 (0.14)	5.67 (0.15)	5.57 (0.16)	0.027
	Min, Max	5.29, 5.77	5.40, 5.83	5.29, 5.83	
	Median (Q1, Q3)	5.51 (5.37, 5.63)	5.70 (5.59, 5.79)	5.56 (5.43, 5.70)	
Diff: log10 sICAM-1	Mean (SDa)	-0.09 (0.12)	0.02 (0.17)	-0.05 (0.15)	
	Min, Max	-0.29, 0.10	-0.25, 0.32	-0.29, 0.32	
	Median (Q1, Q3)	-0.13 (-0.17, 0.02)	0.02 (-0.08, 0.11)	-0.04 (-0.16, 0.06)	

Correlation analyses

We were interested in exploring the associations between individual biomarkers (comparing non-hepatic with hepatic markers) at baseline, at 24 weeks after EOT, and their change from baseline. Spearman correlation was used for these comparisons. Spearman correlation was selected as it is robust to the normality assumption. Pearson correlation could have been used if normality assumptions for the two biomarkers being compared were met. All analyses were conducted at a two-sided 0.05 significance level, without adjustment for multiple comparisons. Given the large number of comparisons made, inflation of overall type I error is likely, and interpretation of significant correlation between any one pair of biomarkers is limited.

We hypothesized that there would be significant positive correlation between the non-hepatic biomarkers, with negative correlations between the non-hepatic biomarkers and the hepatic biomarkers. We found only modest correlations (0.30-0.44) amongst a few biomarkers,

including positive correlation between some of the non-hepatic markers (sICAM-1 with IL-6, Lp-PLA2 with IL-6, and sP-selectin with D-dimer) and negative correlation between the non-hepatic sICAM-1, D-dimer, and IL-6 with hepatic total cholesterol and LDL. Surprisingly, there was positive correlation of the non-hepatic markers of Lp-PLA2, sP-selectin, and IL-6 with the hepatic marker hsCRP, which was the opposite of what we expected to find. Selected Spearman correlations are provided in Table 5A and 5B for illustration.

Table 5A. Correlations between sICAM-1 and hepatic markers

Variable	**Time point analyzed**	**Spearman correlation coefficient**	**P-value**
sICAM-1 vs. cholesterol	baseline	-0.31	0.021
	24 weeks after completion of treatment	-0.40	0.004
	change from baseline to 24 weeks after completion of treatment	-0.17	0.220
sICAM-1 vs. HDL	baseline	-0.21	0.137
	24 weeks after completion of treatment	-0.05	0.745
	change from baseline to 24 weeks after completion of treatment	-0.30	0.030
sICAM-1 vs. hsCRP	baseline	0.10	0.461
	24 weeks after completion of treatment	0.26	0.066
	change from baseline to 24 weeks after completion of treatment	0.21	0.137
sICAM-1 vs. LDL	baseline	-0.30	0.037
	24 weeks after completion of treatment	-0.37	0.013
	change from baseline to 24 weeks after completion of treatment	-0.12	0.455
sICAM-1 vs. triglyceride	baseline	-0.05	0.703
	24 weeks after completion of treatment	-0.16	0.254
	change from baseline to 24 weeks after completion of treatment	0.11	0.455

Table 5B. Selected correlations between non-hepatic biomarkers

Variable	Time point analyzed	Spearman correlation coefficient	P-value
sICAM-1 vs. D-dimer	Baseline	0.13	0.341
	24 weeks after completion of treatment	0.19	0.182
	change from baseline to 24 weeks after completion of treatment	0.22	0.125
sICAM-1 vs. IL-6	baseline	0.21	0.136
	24 weeks after completion of treatment	0.32	0.028
	change from baseline to 24 weeks after completion of treatment	0.34	0.020
Lp-PLA2 vs. D-dimer	baseline	0.27	0.057
	24 weeks after completion of treatment	-0.09	0.522
	change from baseline to 24 weeks after completion of treatment	0.19	0.173
Lp-PLA2 vs. IL-6	baseline	0.09	0.550
	24 weeks after completion of treatment	0.04	0.807
	change from baseline to 24 weeks after completion of treatment	-0.34	0.023
sP-selectin vs. D-dimer	baseline	-0.07	0.626
	24 weeks after completion of treatment	0.27	0.053
	change from baseline to 24 weeks after completion of treatment	0.39	0.003
sP-selectin vs. IL-6	baseline	-0.16	0.250
	24 weeks after completion of treatment	-0.07	0.653
	change from baseline to 24 weeks after completion of treatment	0.02	0.908

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