# Myocardial Infarction, Stroke and Mortality in cART treated HIV patients on statins

A thesis

submitted by

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In partial fulfillment of the requirements

for the degree of

## Master of Science

in

Clinical and Translational Research

TUFTS UNIVERSITY

Sackler School of Graduate Biomedical Sciences

May 2014

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## Abstract:

Despite modern combination antiretroviral therapy (cART), people living with HIV (PLWH) have persistently increased systemic inflammation compared to the general population and are also at a higher risk for metabolic disturbances. The effects of inflammation and metabolic disturbances, known risk factors for atherosclerosis and organ dysfunction, may be ameliorated by statin therapy. We carried out a secondary analysis of prospective cohort data to determine the association between statins and myocardial infarction (MI), stroke and all-cause mortality in PLWH. We included 438 out of 881 HIV infected patients from the Nutrition For Healthy Living (NFHL) cohort. We included cART treated individuals, who were followed between September, 2000 and the present. We used Cox proportional hazards analysis to evaluate the association between statins and the incidence of MI, stroke and all-cause mortality as a composite outcome, where statin exposure was examined both dichotomously and by duration of exposure. For confounding control, we used propensity scoring for statin use in our sensitivity analyses. The average age of our study sample (n=438) was 44 years and 32% were women. 67 (15%) of the 438 analyzed subjects used statins during follow up. We ran 2 separate models with only one of our statin variables at the time (history or duration). There was no association between statin therapy and our composite endpoint in either model (1.26 (0.57-2.79) in statin history model and 0.93 (0.65-1.32) per year in statin duration model). We found significant associations between the composite outcome and CD4 count (HR = 0.88 (0.83-0.94) per 50 CD4 cells/mL), age (HR = 1.07 (1.03-1.1)) and smoking status (HR = 1.78 (1.04-3.19)) in both models. In our analysis, statins did

not have an effect on the incidence of MI, stroke and all-cause mortality. Larger samples are needed to further test the effects of statins on this composite outcome, and individual endpoints in PLWH. Traditional risk factors appear to be important predictors of these outcomes in this population.

## Acknowledgements

I would like to thank my advisers, Drs. Christine Wanke, David Kent, Norma Terrin and Christina Holcroft, who guided me throughout the process of developing this manuscript. I am thankful also to Drs. Cheleste Thorpe and Jose A. Caro, who were a part of my mentorship committee and provided their support in my clinical and theoretical education in various ways. Lastly, I am very grateful to all of my teachers in the Clinical and Translational Research program, who were knowledgeable, motivating and patient, which was instrumental in my successful my completion of the school requirements.

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## List of abbreviations

HIV – human immune-deficiency virus

PLWH – people living with HIV

AIDS – acquired immune-deficiency syndrome

PI – protease inhibitors

cART – combination anti-retrovirals

CRP – C-reactive protein

NNRTI – non-nucleotide reverse transcriptase inhibitors

MI – myocardial infarction

NFHL – Nutrition For Healthy Living

cIMT – carotid intima media thickness

CAC – coronary artery calcium

HDL – high-density lipoprotein

LDL – low-density lipoprotein

RNA – ribonucleic acid

MICE – Multivariate Imputation by Chained Equations

GEE – generalized estimating equation

HCV – hepatitis C virus

HBV – hepatitis B virus

DM – diabetes mellitus

HTN – hypertension

IV – intra-venous

SD – standard deviation

HR – hazard ratio

IPW – inverse probability weightin

## Introduction

HIV in the era of cART has become a chronic disease and PLWH now more frequently die from heart disease, stroke, non-AIDS defining cancers or organ failures as opposed to AIDS[1]. This evolution has been a process in flux since the introduction of AZT as the first therapeutic agent in 1987. HIV, however, did not become the chronic disease we know today until after the introduction of protease inhibitors (PI) in mid-1990s and their use in combination with nucleoside reverse transcriptase inhibitors. Reduction in morbidity and mortality brought by cART was evident by 2000[2,3]. Further evidence of modern cART benefits regarding HIV disease outcomes in the broadest sense has been reviewed in the literature since then[4,5,6].

Despite fully suppressed viral load achieved by modern cART, PLWH have persistently increased systemic inflammation compared to the general population[7]. Chronic inflammation is an independent risk factor for atherosclerosis[8] and neoplasias[9] and can lead to dysfunction in multiple organs[10]. Multiple studies have documented increased levels of inflammatory biomarkers (e.g., C-reactive protein (CRP), interleukin-6, sCD14) in HIV patients as well as their concurrent rise with HIV related disease progression[11,12,13,14]. 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins), primarily known as serum cholesterol lowering agents, have been shown to suppress inflammation[15] by, at present, incompletely understood mechanisms. Clinicians choosing to use statins in HIV patients, however, face challenges beyond complications associated with statin therapy in the general population (e.g., diabetes, myopathy)[16],

including potential toxicity associated with drug interactions between statins and certain cART agents (particularly PI and NNRTI)[17].

Though an extensive literature supports the benefits of statins on survival and lowering of inflammatory biomarkers in HIV-free subjects[18,19] , less is known about the benefits of statins in HIV infected individuals. This topic has recently started to attract the attention of HIV-focused investigator groups. One study showed a mortality benefit far beyond what has been observed in non-HIV infected patients[20], while other studies have failed to show benefits on mortality[21], or mortality and cardiovascular outcomes[22]. The latter study[22], however, showed a significant association between statins and a decreased incidence of non-AIDS defining malignancies. All of the above mentioned studies are observational and no results of randomized trials have been reported so far.

Using data from a prospective cohort of PLWH, we examined the association of statins with the risk of developing MI, stroke & all-cause mortality.

## Methods

### Cohort description

Nutrition For Healthy Living (NFHL) cohort was initiated in 1995 to examine the nutritional status and metabolism in a representative cohort of HIV infected adults from Massachusetts. Since 1995, 881 HIV infected adults have been enrolled on a rolling basis. The NFHL patients were followed for HIV (and its outcomes), other medical conditions, dietary intake, medications, body composition, quality of life, liver function,

serum glucose and insulin levels initially via 6-monthly visits, and later on annually. The exclusion criteria for NFHL included diabetes, uncontrolled hypertension, and myocardial infarction or stroke within the past 6 months, but participants who developed these conditions after enrollment continued in the study and were consented for the CARE sub-study, which focused on cardiovascular health. The CARE subset was begun in 2000 and enrolled any consenting NFHL participants (total n=327). The initiation of this sub-cohort reflected a new era for the monitoring of HIV infected patients in general. From September of 2000 on, the participants continued their regular 6-monthly study visits but the NFHL investigators began collecting data on serum lipid profiles, Framingham risk score, and CRP as well as surrogate markers of cardiovascular disease (carotid intima media thickness (cIMT) & coronary artery calcium (CAC).

Study objectives

We evaluated the association of statins with incidence of myocardial infarction (MI), stroke and all-cause mortality treated as a composite in an HIV infected cohort.

Inclusion criteria & start of follow up

In our analysis we included only those participants in the NFHL study, who at any point (prior to or at baseline) initiated cART. Reflecting the initiation of CARE, the baseline in our study was September 1$^{st}$, 2000 or the date of initiation of cART (whichever occurred later). CARE style monitoring (including lipid levels and other cardiovascular parameters) was applied to the NFHL participants beyond CARE subset, and our analyzed group is therefore larger than CARE subgroup. September 1$^{st}$, 2000 was

chosen to address a few important factors: Our analyzed population was selected to represent “modern” HIV patients by both, the cART agents used for treatment and by the way they are monitored (not only for HIV but also for cardiovascular and metabolic health). Participants, who reported MI or stroke prior to initiation of cART were excluded (9 subjects). 1 additional subject was excluded based on statin use for a period immediately prior to the baseline but not after, which would have confounded baseline parameters and caused possible misclassification of this subject. We eventually identified 438 participants from NFHL cohort for our analysis (Figure 1).

Outcome definition, censoring criteria & follow up time

Our main outcome of interest was the composite endpoint of MI, stroke and all-cause mortality.

Participants’ eligible study time continued until the time of the 1st event within the composite outcome, or until the time of censoring. Participants were censored at the last known study visit, except that death was followed up for one year past the last visit (censoring at the last visit would exclude all deaths as a possible outcome). The period of 1 year was chosen to match the predefined spacing of scheduled visits and as such did not cause significant differential follow up time. cART interruption was not considered as a basis for censoring. Follow up flow diagram is depicted in Figure 2.

Figure 1: Study flow diagram

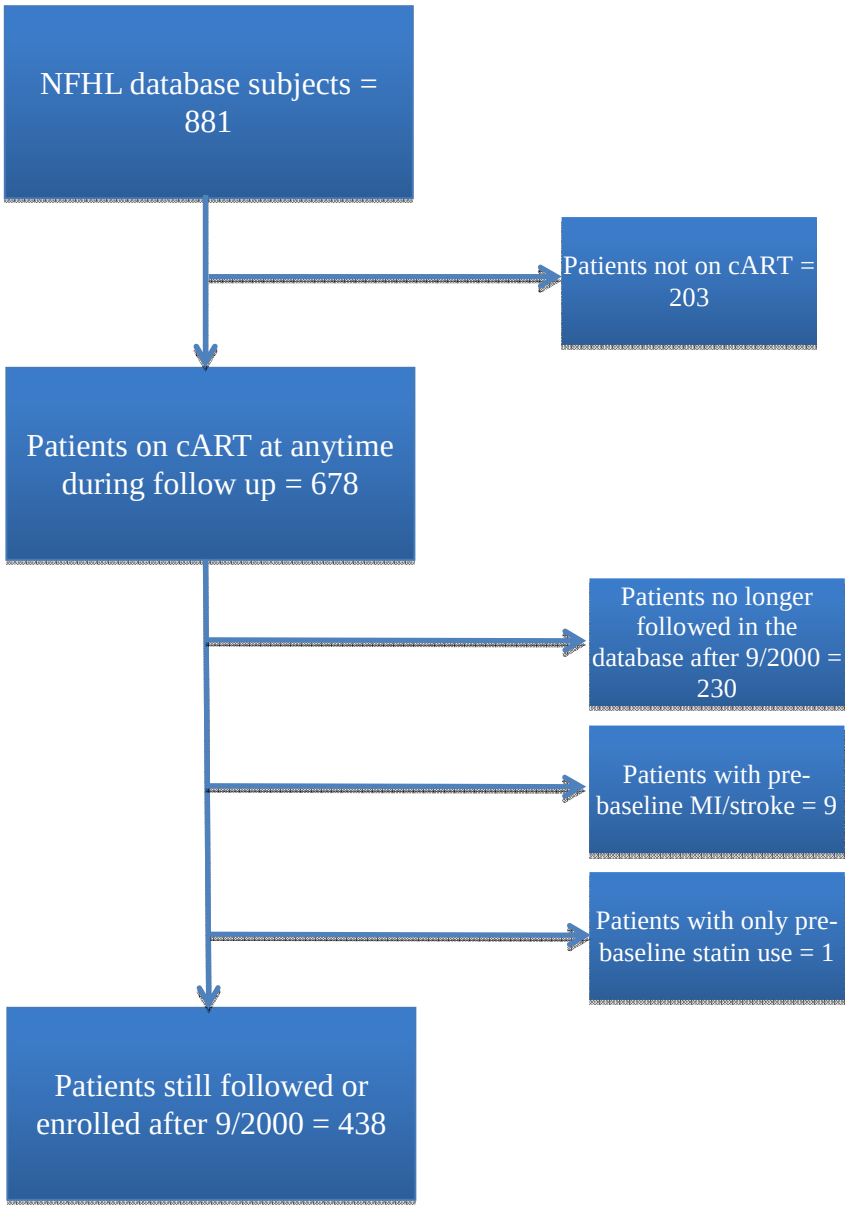
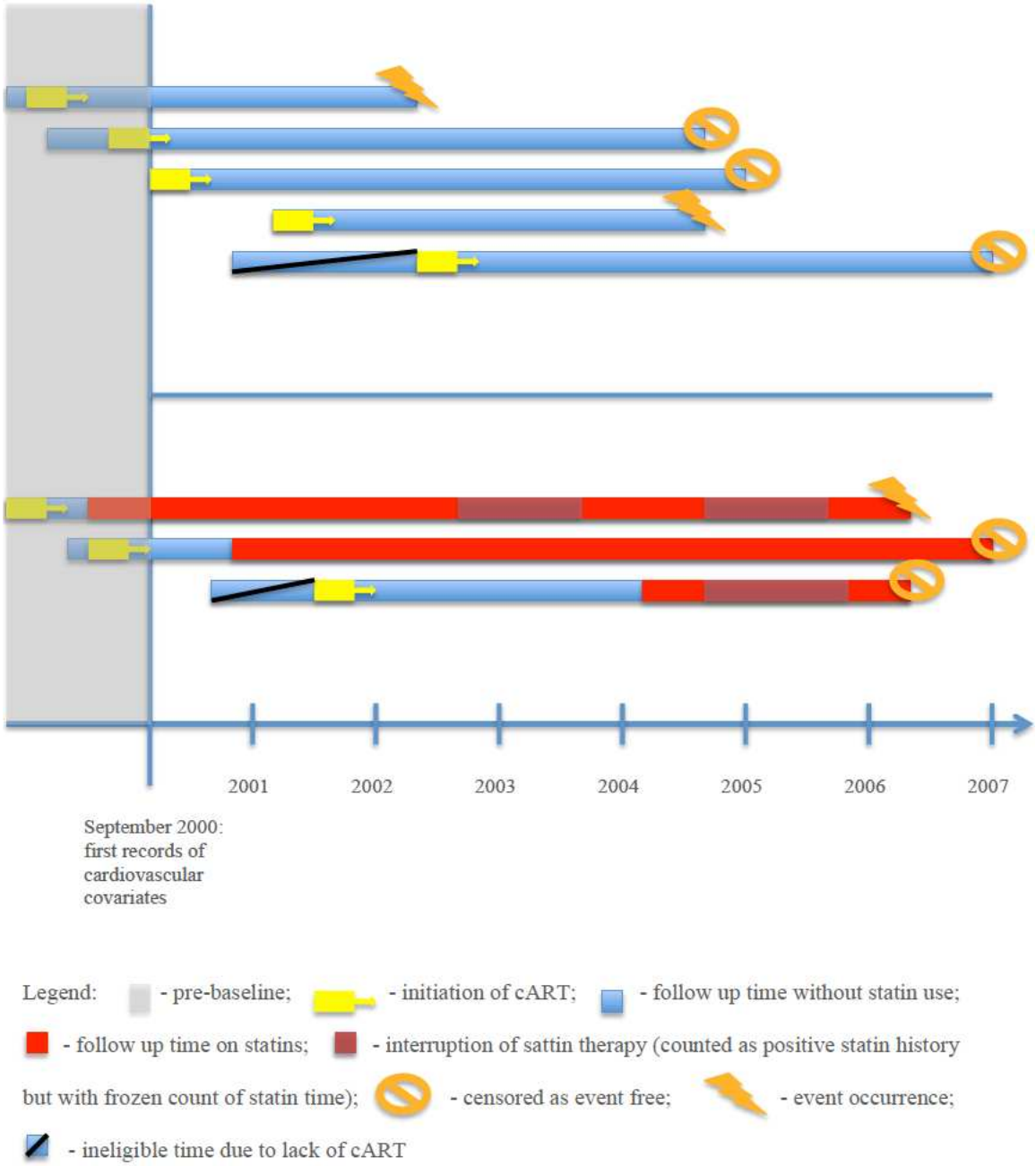


Figure 2: Study follow up flow diagram



Clinical Data

Clinical information was collected at baseline and every 12 months (initially every 6 months). Laboratory data were obtained during the same visit, or as close as possible. Demographic data were assessed via interviewer-administered questionnaires.

Laboratory Methods

Plasma levels of total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were measured via standard enzymatic methods; low-density lipoprotein (LDL) cholesterol was measured directly via Roche Diagnostics kit (Roche, Inc, Indianapolis, IN). High-sensitivity CRP was measured with a high sensitivity latex-enhanced turbidimetric assay from Wako Diagnostics with coefficients of variation <5%. CD4+ cell counts were determined by flow cytometry, HIV RNA was quantified by Roche Amplicor (Roche, Inc, Indianapolis, IN) (lower limit of detection = 400 copies/mL and lower values were set to 200 copies/mL).

Calculation of detectable difference in hazard

We calculated a statistically detectable HR needed under our sample size constrictions: Our study had 67 experimental subjects, 371 control subjects, an accrual interval of approximately 6 years, and additional follow-up of 5 years after the accrual interval. We estimated the median survival time for people not on statins to be 24 years. For 1 year of statin use, we would have been able to detect a hazard ratio of 0.48 with probability (power) of 0.8 and the type I error probability of 0.05[23].

Statistical analysis

We chose Cox proportional hazards method with time varying covariates to evaluate the association of statins with the composite outcome. Besides statin history and duration, other important variables used as time varying predictors were LDL cholesterol and CD4 count. Missing values (detailed in Table 1) were imputed using Multivariate Imputation by Chained Equations (MICE)[24] R-software package, version 2.18. We considered findings from the prior literature and chose baseline predictors based on face validity.

Table 1: Subject characteristics

| Baseline Variables | Subjects = 438 |
|---|---|
| **Age** | mean=44.3 (sd=7.7) |
| **Race:** | |
| **Black** | 139 (32%) |
| **Hispanic** | 42 (10%) |
| **White** | 234 (53%) |
| **Not reported** | 23 (5%) |
| **Female** | 141 (32%) |
| **Weeks of pre-baseline cART use** | mean=130.7 (sd=94.8) |
| **Metabolic syndrome** | 100 (23%) |
| **Framingham Risk Score %** | mean=6.5 (sd=5.5) |
| **HTN** | 151 (35%) |
| **DM** | 30 (7%) |
| **Tobacco smoking** | 207 (47%) |
| **LDL (mg/dL)** | mean=113 (sd=40.4) |
| **missing LDL data** | 57 (13%) |
| **HDL (mg/dL)** | mean=45 (sd=19) |
| **missing HDL data** | 37 (8%) |
| **TG (mg/dL)** | mean=206 (sd=218) |
| **missing TG data** | 37 (8%) |
| **CRP (mg/L)** | mean=3.8 (sd=10.6) |
| **missing CRP data** | 347 (79%) |
| **Statin use** | 25 (6%) |
| **Fibrate use** | 5 (1%) |
| **Niacin use** | 3 (<1%) |
| **Anti-HTN use** | 23 (5%) |
| **current IVDU** | 9 (2%) |
| **missing IVDU data** | 1 (<1%) |
| **HBV co-infection** | 156 (36%) |
| **missing HBV data** | 64 (15%) |
| **HCV co-infection** | 125 (29%) |
| **missing HCV data** | 64 (15%) |
| **CD4 count** | mean=426 (sd=271) |
| **missing CD4 count data** | 14 (3%) |
| **History of opportunistic infections** | 70 (16%) |
| **missing OI data** | 4 (<1%) |
| **log HIV viral load** | mean=2.895 (sd=0.918) |
| **missing HIV viral load data** | 50 (15%) |
| **current cART use** | 394 (90%) |
| **PI use** | 254 (58%) |
| **Abacavir use** | 74 (17%) |

The restriction of baseline to September 2000 or later (explained above) imposed a potential for survivor bias through the inclusion of patients started on cART before 2000. We mitigated this effect by using time from cART initiation to baseline as a continuous variable in the regression model. This variable served as a proxy for the survivor effect as well as for disease duration and stage. Statin use was categorized in two ways: as a dichotomous variable, representing current or prior statin use, which changed from 0 to 1 at the first visit with reported use and remained that way through the end of follow up; we also specified statin use as cumulative, but not necessarily continuous, time on treatment, initiated at the 1$^{st}$ visit during which the patient reported use. If the treatment was interrupted, the last value was carried forward until statin use was restarted or through the end of follow up. We chose this approach because the main effect of statins is thought to be the prevention of atherosclerotic plaque formation and this effect may stop but is unlikely to be completely reversed after treatment cessation. We chose to model the 2 statin specifications separately. One model used statin history while the other used statin duration as the main predictor of the composite endpoint. The other 2 time varying predictors, LDL and CD4 count, were specified as their respective continuous values at each follow up visit. All other predictors carried their baseline value forward. All covariates were analyzed as linear or binomial depending upon their respective way of reporting in clinical settings. No linear variables were subdivided by cut-points. The choice of predictors for the multivariate models was aided by univariate analyses shown in Table 2. For multivariate adjustment, we used all statistically significant predictors from the univariate models. We then forced the statin use variables into the model (one at the time, as outlined above), as our main predictor. We also forced LDL levels into the

model as a potential important confounder of the main predictor. The main model was applied to the pooled imputed dataset and no automated selection techniques were used to further reduce the number of predictors.

Table 2: Univariate models of the composite outcome for individual predictors considered for multivariate analysis

| Variable | HR | CI 95% (2-sided) | p-value |
|---|---|---|---|
| **Female** | 1.08 | 0.64-1.82 | 0.77 |
| **Race:** | | | 0.03 |
| **Black (reference)** | | | |
| **Hispanic** | 1.59 | 0.78-3.22 | 0.2 |
| **White** | 0.69 | 0.40-1.17 | 0.17 |
| **Not reported** | 0.21 | 0.03-1.54 | 0.12 |
| **HBV co-infection** | 0.47 | 0.25-0.86 | 0.01 |
| **HCV co-infection** | 2.26 | 1.31-3.90 | 0.004 |
| **Age** | 1.04 | 1.01-1.07 | 0.01 |
| **Smoking status** | 1.84 | 1.13-3.00 | 0.015 |
| **LDL per 10 mg/dL*** | 0.96 | 0.89-1.03 | 0.25 |
| **HDL per 10 mg/dL** | 0.93 | 0.77-1.12 | 0.42 |
| **Cumulative statin duration (years)*** | 0.87 | 0.61-1.23 | 0.44 |
| **Current or prior statin use (dichotomous)*** | 0.96 | 0.45-2.01 | 0.91 |
| **Framingham Risk Score per 1%** | 1.03 | 0.99-1.07 | 0.07 |
| **Metabolic syndrome** | 1.03 | 0.58-1.80 | 0.93 |
| **Protease inhibitor use** | 1.16 | 0.70-1.92 | 0.55 |
| **CD4 count per 50 cells/µL*** | 0.88 | 0.82-0.95 | <0.001 |
| **Duration from cART initiation in weeks** | 1 | 0.99-1.00 | 0.72 |

* - time varying predictor

To account for the "healthy user effect" associated with statins, we performed a propensity score adjusted sensitivity analysis of the composite outcome. We calculated two separate sets of propensity scores. For the dichotomously coded history of statin use, the propensity scores for each person/week in follow up was calculated via logistic generalized estimating equation (GEE) model, to account for repeated measures within subjects. . The unit of analysis was person/time in weeks. Model specifications and output from both models are outlined in Figure 6 and Table 5. The propensity score was then included in the sensitivity analysis as an additional time varying covariate. The predictors selected to predict statin use included: gender, race, age at baseline, HCV and HBV co-infection, presence of metabolic syndrome, Framingham risk score percentage, time varying LDL level, baseline HDL level, CD4 count, time from start of cART, smoking status and baseline protease inhibitor use. A time varying cART use was also added to the propensity score model. With a linear GEE model, using the same set of predictors; we also calculated a statin duration predicting score, which was done to balance our other main predictor of interest (statin duration in years) in another sensitivity analysis. The propensity score comparisons between statin users and non-users from the logistic GEE model are graphically shown in Figures 7 & 8. The overlap between propensity scores for users and non-users of statins calculated with the linear GEE model is depicted in Figure 9.

All of the programming and calculations were performed using R version 3.0.2 "Frisbee sailing" (freely available statistical software[25]).

Figure 3: Propensity score model specifications in R (family=binomial for propensity, and Gaussian for prediction)

Call:

gee(formula = history of statin use ~ gender + race + HBV +

HCV + LDL + CD4 count + cART use + weeks from cART start +

baseline age + smoking status + baseline HDL + baseline Framingham Risk +

metabolic syndrome at baseline + baseline protease inhibitor use; clustered by

participant ID; data = 3rd complete dataset from 5 iterations of multiple

imputation via MICE package)

Number of observations: 130077

Maximum cluster size : 642

Table 3: Linear and logistic GEE modeling of propensity/prediction for statin use

| Variables | β Estimate - linear | β Estimate - logistic |
|---|---|---|
| **Intercept** | -0.969 | -6.54 |
| **Female gender** | -0.036 | -0.51 |
| **Race:** | | |
| **Black (reference)** | | |
| **Hispanic** | 0.102 | 0.009 |
| **White** | 0.25 | 0.688 |
| **No race reported** | -0.097 | 0.233 |
| **HBV** | -0.06 | -0.355 |
| **HCV** | -0.297 | -1.055 |
| **LDL (per 10 mg/dL)** | -0.021 | -0.023 |
| **CD4 count (per 50 cells/μL)** | 0.0001 | 0.0005 |
| **current cART use** | 0.136 | 0.883 |
| **weeks from cART start** | 0.001 | 0.002 |
| **Age at baseline** | 0.019 | 0.061 |
| **Smoking status at baseline** | -0.207 | -0.892 |
| **baseline HDL** | 0.002 | 0.008 |
| **baseline Framingham Risk** | 0.022 | 0.029 |
| **metabolic syndrome at baseline** | 0.057 | 0.355 |
| **protease inhibitor use at baseline** | -0.067 | 0.294 |

Figure 4: Comparison of propensity scores for statin use between statin users and non-users

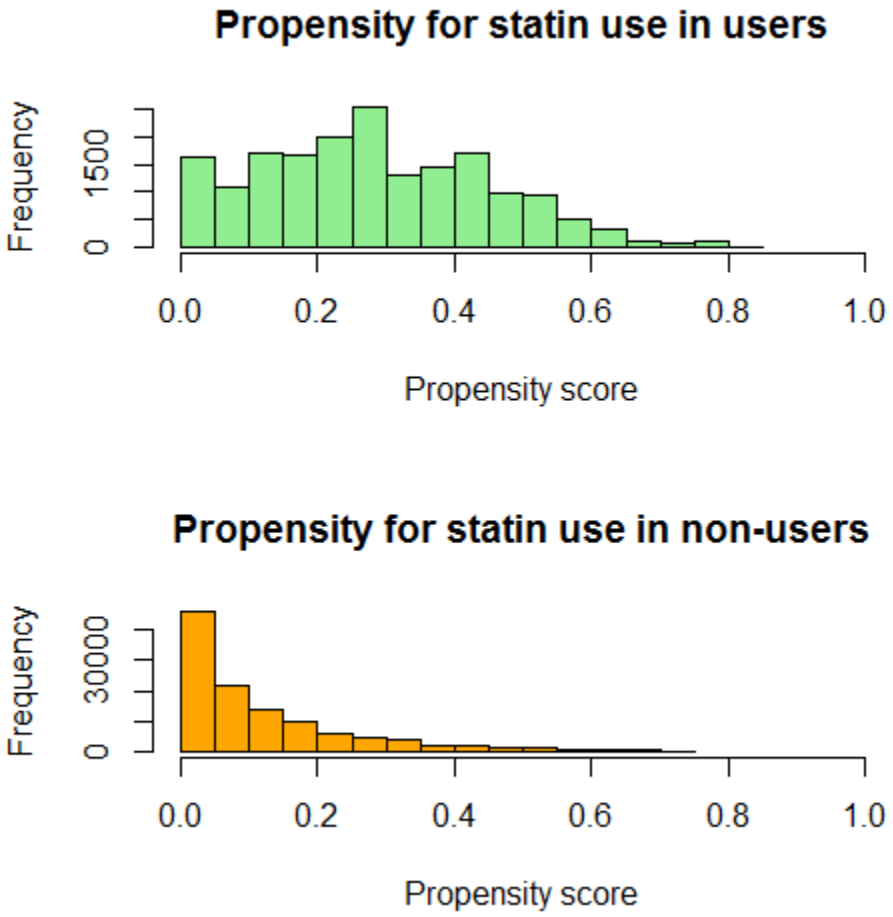


Figure 5: Propensity for statin use comparison via boxplot

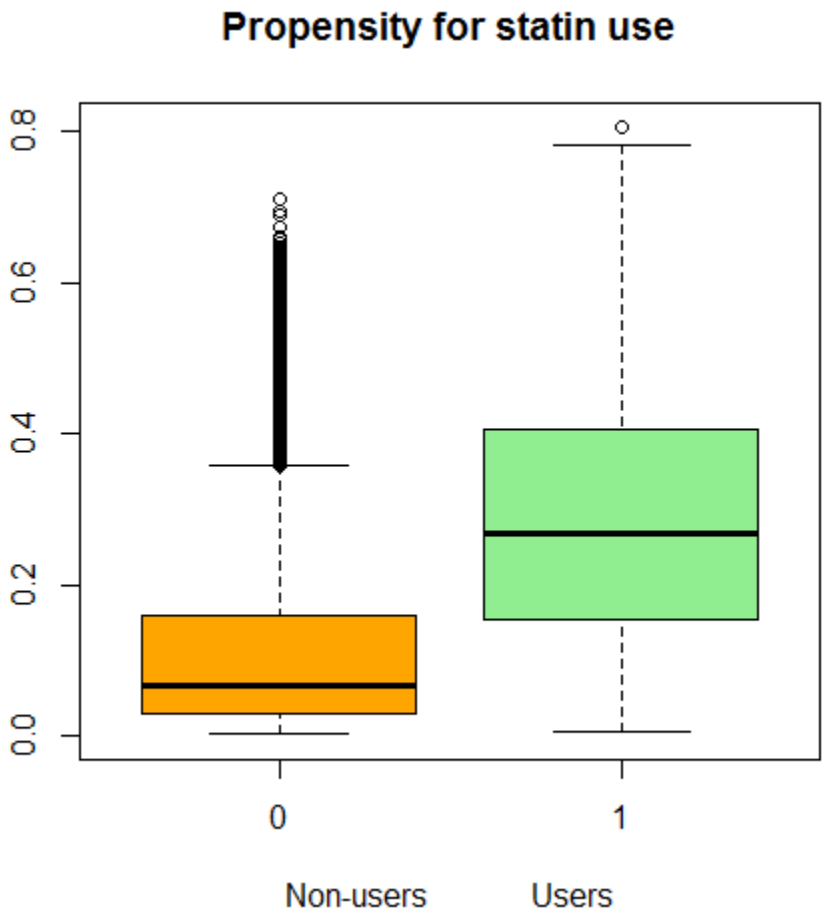
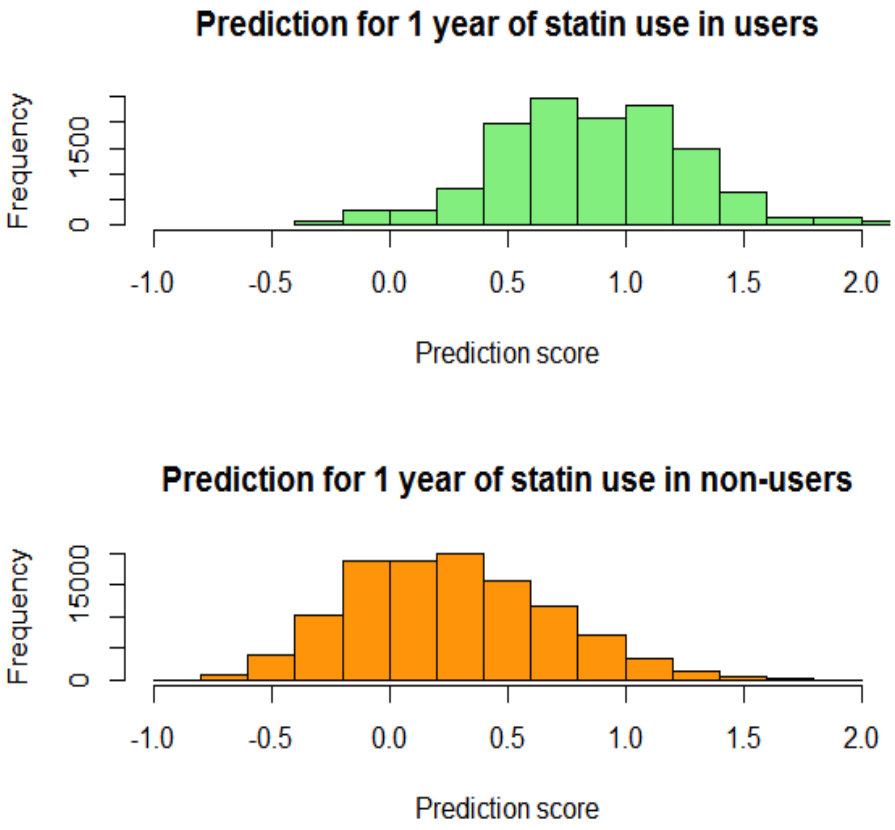


Figure 6: Prediction score for 1 year of statin use



# Results

## Descriptive subject characteristics

The average age of our study sample (n=438) was 44 years, 32% were women and the cohort included 32% black subjects, 10% Hispanic subjects and 5% subjects did not report their race. The average duration of cART prior to baseline in the whole analyzed cohort was 2.5 years and all subjects initiated cART at or prior to their respective baseline. 67 (15%) of the 438 analyzed subjects used statins during follow up. The distribution of all other baseline predictors, such as: smoking status, HDL, triglycerides, other lipid lowering medications, Framingham risk score percentage, HBV/HCV co-infection, DM, HTN, metabolic syndrome, IV substance use, and the use of protease inhibitors or abacavir is listed in Table 1. The mean follow up time in subjects, who never used statins was 275 weeks (SD=190 weeks). The mean follow up time in statin users was 411 weeks (SD=193 weeks), with the mean accumulated time on statins being 165 weeks (SD=145 weeks). Statin users, therefore, were followed for an average of 246 weeks without being on statin therapy. 141 weeks (SD=190 weeks) of these 246 weeks were analyzed as statin non-user time (prior to initiation of statins), while the remaining 105 weeks were analyzed as statin user time since these occurred during statin therapy interruptions, during which prior statin users were already classified as such (by both statin variables). 6 statin users reported statin therapy at their last known follow up visit and none of them suffered any of the events qualifying for our composite endpoint. These individuals' average follow up was 423 weeks (SD=241 weeks) and only one of these individuals had a short follow up time of 26 weeks.

There were 66 outcomes in this dataset. 20 outcomes were due to MI and/or stroke (approximately evenly distributed) and 46 were deaths. An independent panel of 4 NFHL physician investigators adjudicated the mortality cause based on all of the available information regarding the deaths. Where written documentation (e.g., death certificate and available medical records) was not conclusive with regards to whether the cause of death was due to HIV, the panel contacted clinicians for further detail. Based on this process, it was determined that 12 deaths were HIV related, 19 were unrelated to HIV and for 5 cases the HIV relatedness could not be conclusively determined. For the remaining 10 cases, the NFHL investigators had no available information as these occurred after December 31st 2006 and formal access to the data was not available at the time of this publication.

Time on statins

The follow up visit count, where our 67 statin users reported statin use is graphically depicted in Figure 4. The maximum count of visits during which a subject reported statin use was 11. This occurred in 2 individuals, 3 patients reported statin use during 10 follow up visits, and additional 12 people reported statin use more than 5 times. 50 statin users reported 5 or fewer follow up periods of statin use. 27 of these subjects (~40% of all statin users) reported statin use on only 1 or 2 occasions. Graphical translation of the number of visits with reported statin use into weeks on statins is depicted in Figure 5.

Figure 7: Number of follow up visits per person, during which subjects reported statin use

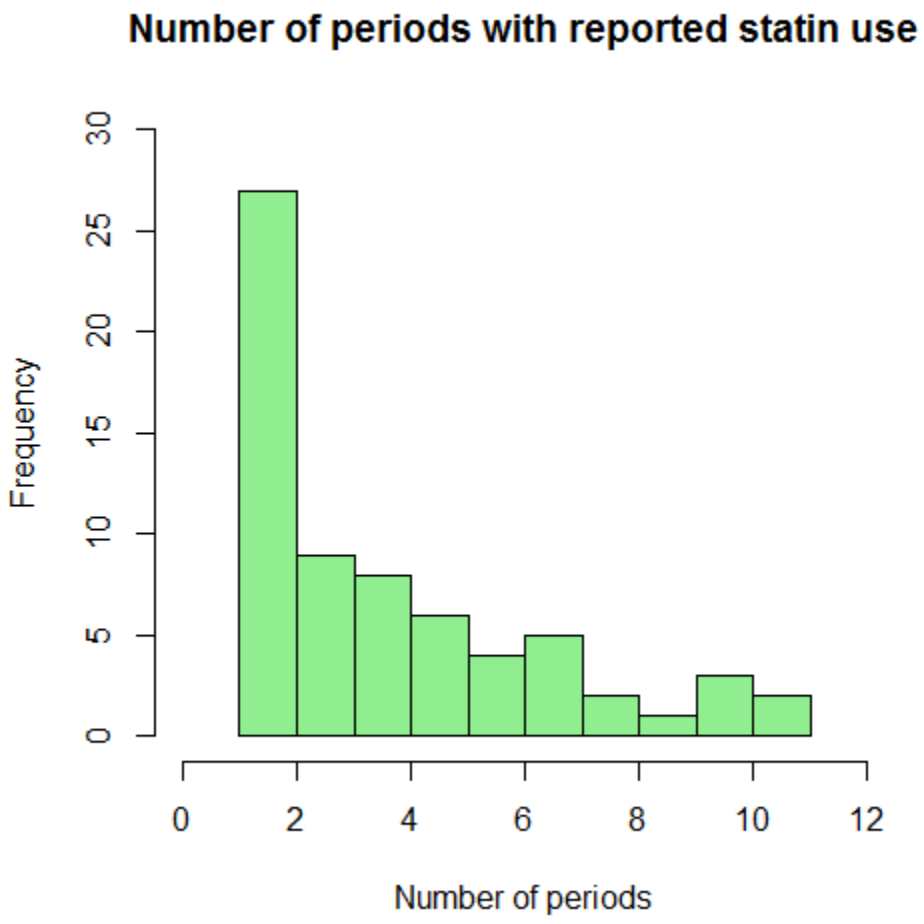
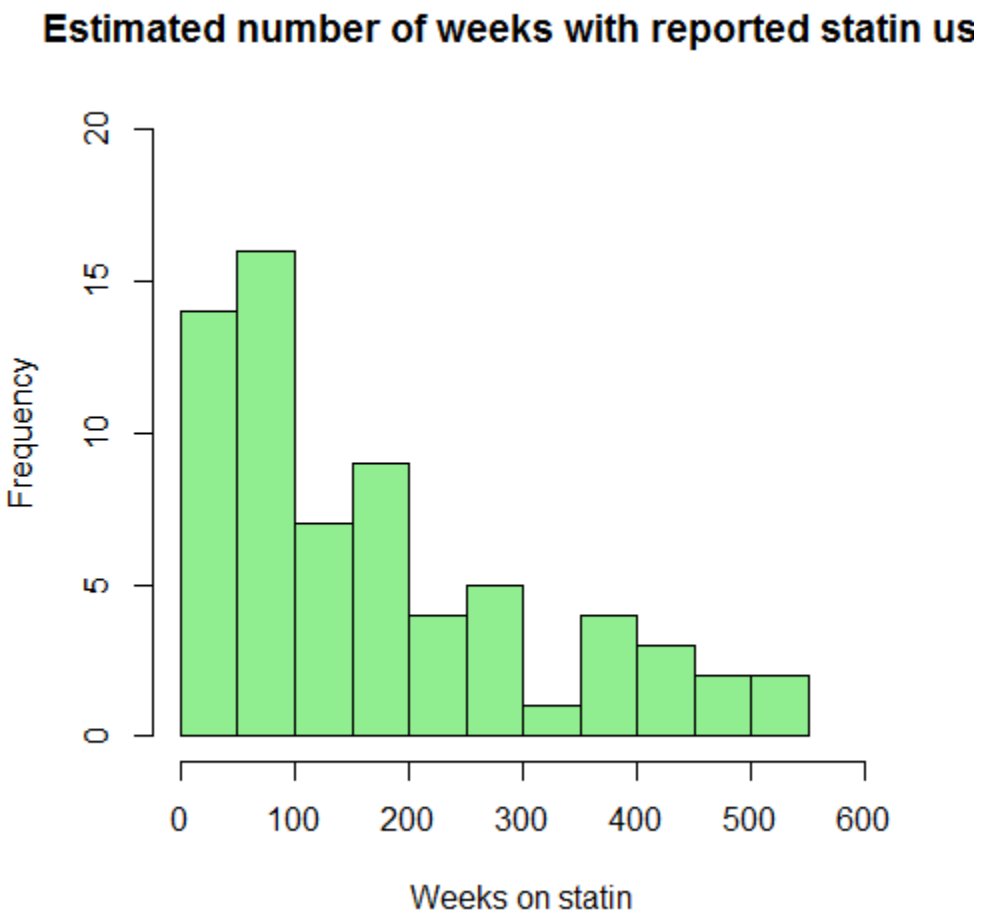


Figure 8: Number of weeks per person, during which subjects reported statin use



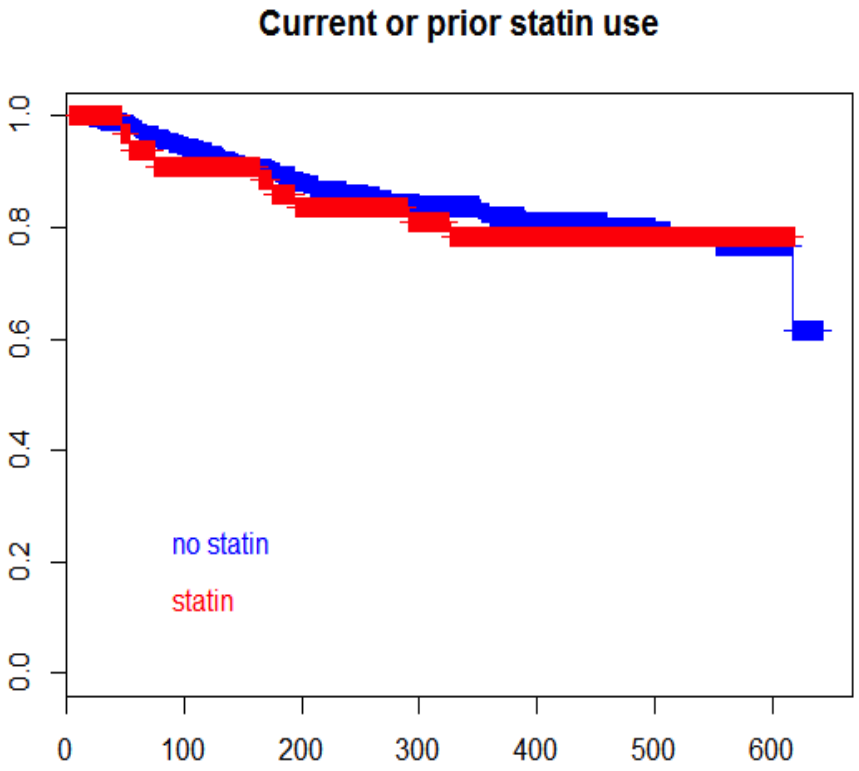
Univariate and multivariate modeling

The outcomes from our univariate models are shown in Table 2. The time from cART initiation to baseline showed no effect on our composite outcome and thus was not used in the final multivariate model. Predictors chosen for the multivariate model on the basis of their statistical significance in univariate modeling were race, age, smoking status, time varying CD4 count, HBV & HCV co-infections. Both statin variables (dichotomous statin history and cumulative duration statin therapy) were forced into the model as our main predictors of interest. Time varying LDL was also forced into the model to adjust for the lipid lowering aspect of statins. The outcomes are summarized in Table 3. Despite our original plan, we were unable to use CRP due to significant missingness of data (79% at baseline and 75% overall). The outcome of our multivariate model shows our 2 models with both statin therapy predictors to be statistically insignificant. The HR for the statin therapy predictors were 1.26 (0.57-2.79) in the statin history model and 0.93 (0.65-1.32) per year in the statin duration model. We analyzed the same models without adjustment for LDL and there was no change in the statin effect magnitude or its statistical significance: HR=0.93 (0.65-1.32) per 1 year of statin use and HR=1.26 (0.57-2.78) for positive history of statin use (data not shown). A Kaplan-Meier curve of statin history effect is depicted in Figure 3. We found significant associations between the composite outcome and CD4 count (HR = 0.88 (0.83-0.94) per 50 CD4 cells/mL), age (HR = 1.07 (1.03-1.1)) and smoking status (HR = 1.78 (1.04-3.19)) in both models. Another notable but statistically insignificant outcome was the HR of 0.66 (0.34-1.13) for HBV co-infection in both models.

Table 4: Full multivariate model

| Variables | HR-statin history model | CI 95% (2-sided) | HR-statin duration model | CI 95% (2-sided) |
|---|---|---|---|---|
| **Race:** | | | | |
| **Black (reference)** | | | | |
| **Hispanic** | 1.68 | 0.8-3.51 | 1.68 | 0.8-3.5 |
| **White** | 0.95 | 0.53-1.61 | 0.95 | 0.54-1.66 |
| **No race reported** | 0.2 | 0.03-1.48 | 0.2 | 0.03-1.47 |
| **HBV** | 0.66 | 0.35-1.29 | 0.66 | 0.34-1.29 |
| **HCV** | 1.52 | 0.87-2.85 | 1.52 | 0.84-2.74 |
| **LDL (per 10 mg/dL)** | 0.99 | 0.92-1.07 | 0.99 | 0.92-1.07 |
| **CD4 count (per 50 cells/μL)** | 0.88 | 0.83-0.94 | 0.88 | 0.83-0.94 |
| **Age at baseline** | 1.07 | 1.03-1.1 | 1.07 | 1.03-1.1 |
| **Smoking at baseline** | 1.78 | 1.08-3.19 | 1.78 | 1.04-3.04 |
| **Statin duration (years)** | n/a | n/a | 0.93 | 0.65-1.32 |
| **Statin history (dichotomous)** | 1.26 | 0.57-2.79 | n/a | n/a |

Figure 9: Kaplan-Meier curve of the composite outcome by history of statin use

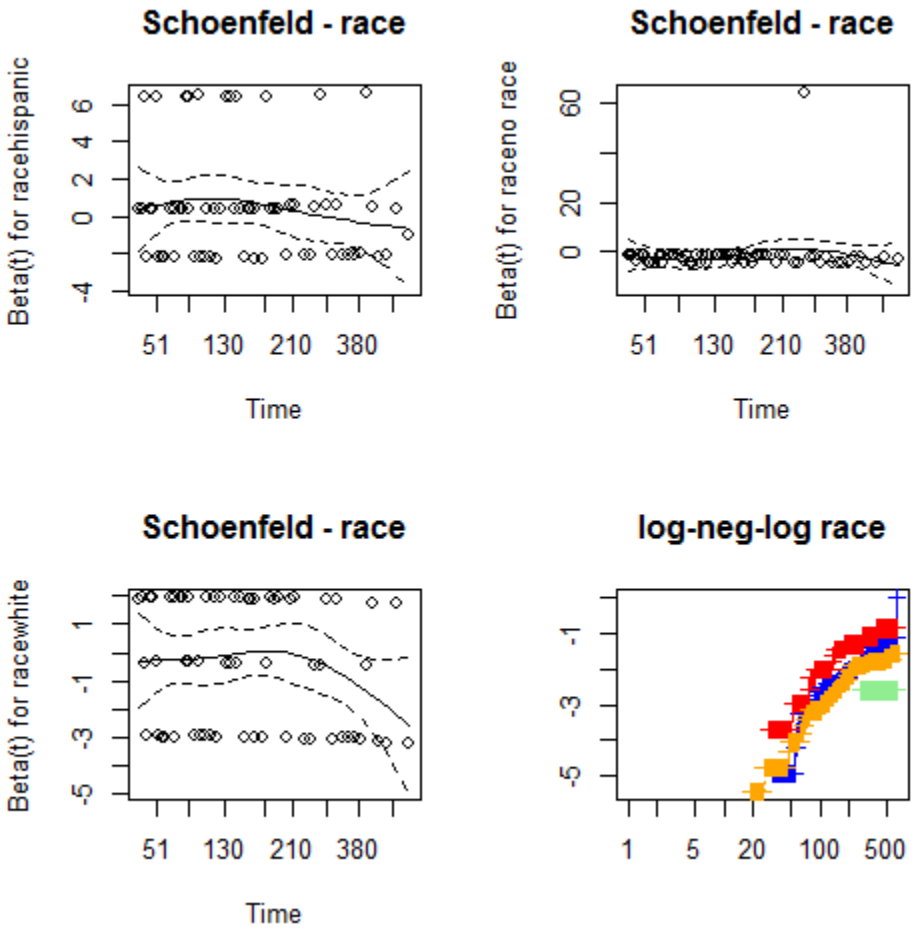



Legend: X – axis: proportion of event free subjects; Y – axis: number of weeks

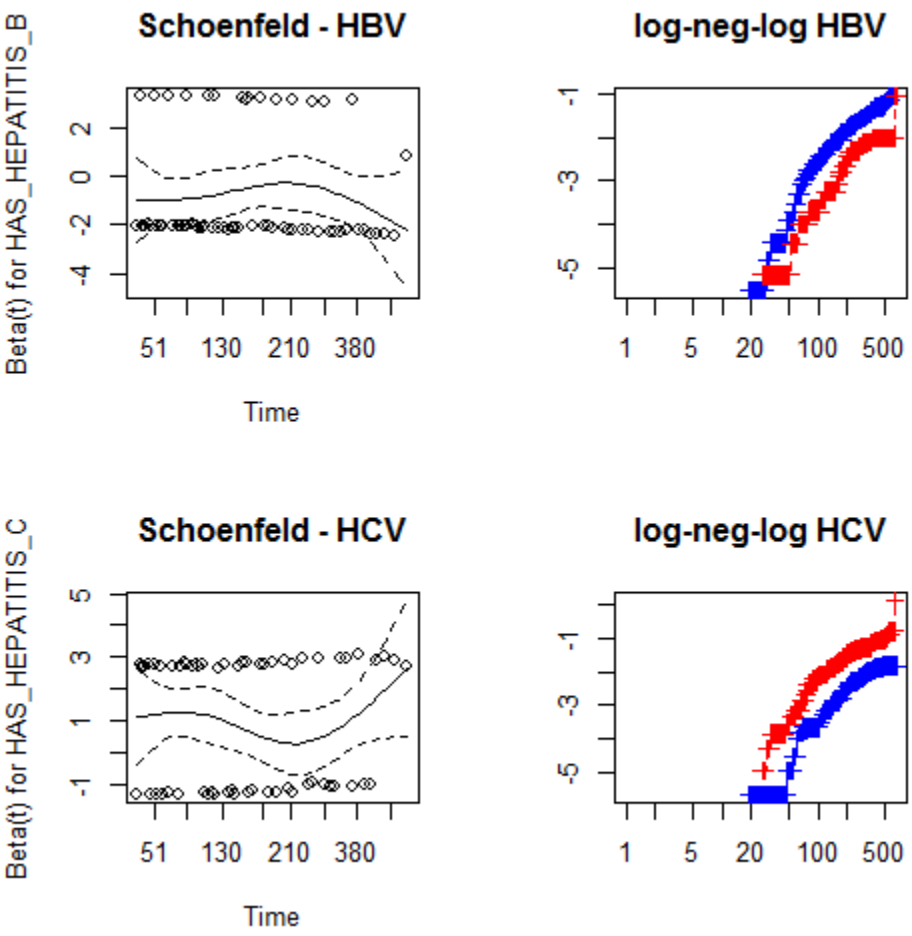
Diagnostic testing of models

In order to evaluate model assumptions regarding the proportionality of hazards, we performed Schoenfeld residuals testing as well as complementary log-log regression (Clog-log) where appropriate. With these model diagnostics, we detected minor proportionality violations in several parameters but in all of these cases, a straight line could be drawn between the confidence interval bounds, which reassured us that no significant violation appeared to be present. Schoenfeld residuals and Clog-log (where appropriate) are depicted in Figure 10. The first four graphs show Shoenfeld residuals and Clog-log for race. In addition to the disproportionality in the hazard for white race toward the end of follow up, the lines representing the white and black race (blue and orange lines respectively) cross at the beginning. After that, they appear to stay close but also proportional. HCV, more so than HBV, also shows some disproportionality towards the end of follow up but the Clog-log graphs are reassuring in both cases. Similar findings apply to the interpretation of CD4 count, LDL and age variables. Visually, there appears to be more disproportionality over time in the hazard associated with smoking but a straight line between the confidence interval bounds is still possible and the Clog-log graph is again reassuringly showing proportionality. The Schoenfeld residuals for our statin variables show reasonable proportionality but given the Clog-log for statin history and the HR<1 calculated via univariate Cox proportional hazards model, we can only state that the two lines follow each other too closely for us to determine whether they are parallel with each other or at risk for crossing.

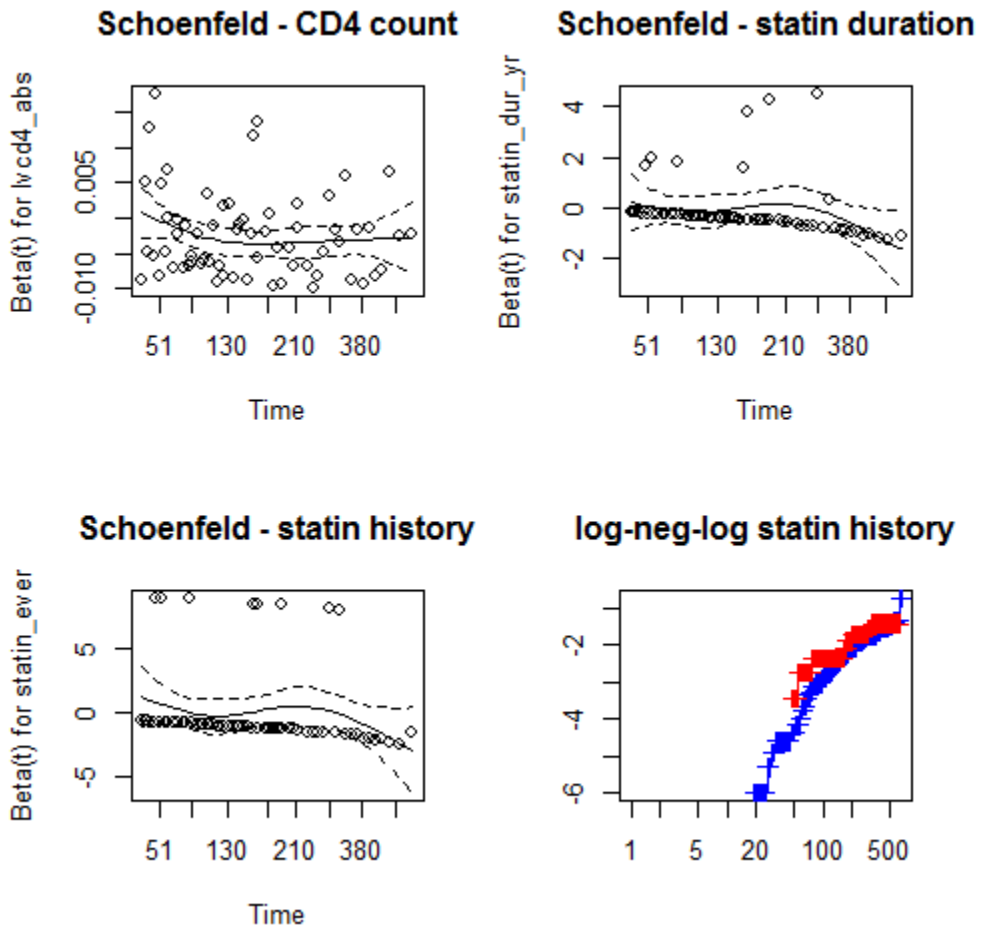
Figure 10: Schoenfeld residuals from univariate models using predictors eventually picked for the main multivariate analysis



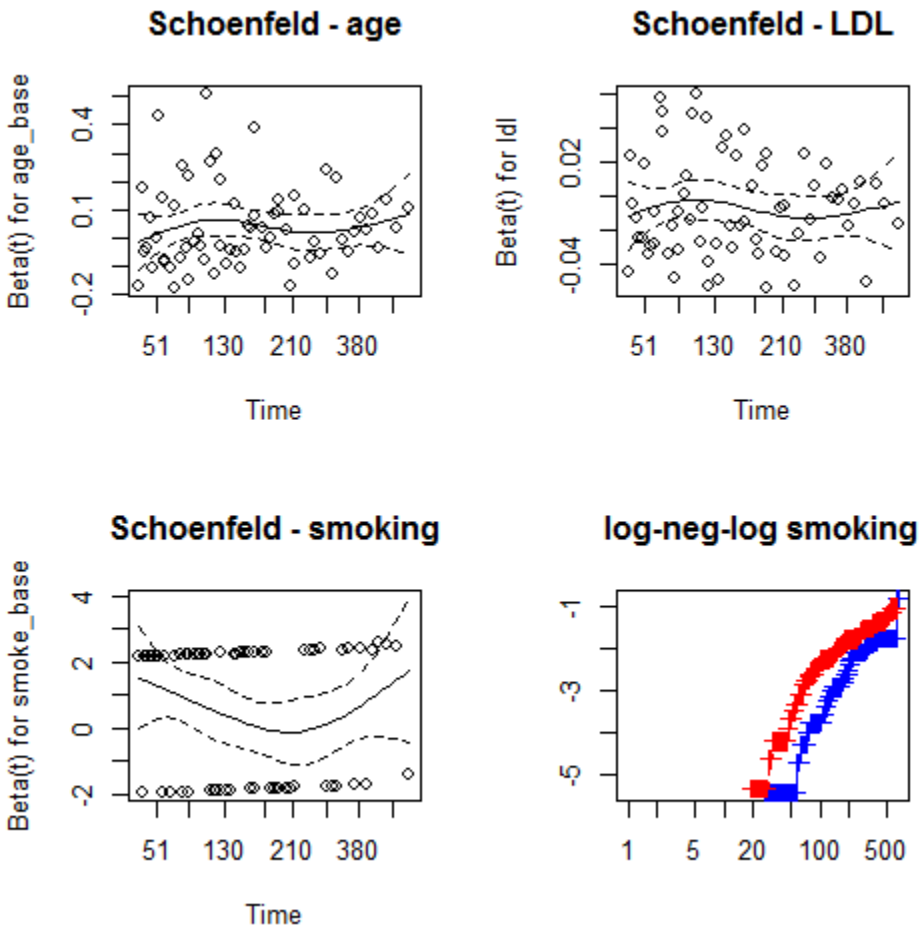

Legend: Schoenfeld residuals (from top left): 1. Hispanic race, 2. No race reported, 3. White race, Clog-log graph (bottom right): Race (red-Hispanic, blue-White, orange-Black, green-No race reported)




Legend: Schoenfeld residuals and Clog-log graphs (from top): 1. Hepatitis B co-infection at baseline (red-positive, blue-negative), 2. Hepatitis C co-infection at baseline (red-positive, blue-negative)




Legend: Schoenfeld residuals (from top left): 1. Absolute CD4 count, 2. Statin duration, 3. Statin history; Clog-log graph (bottom right): Statin history




Legend: Schoenfeld residuals (from top left): 1. Age at baseline, 2. LDL, 3. Smoking at baseline: Clog-log graph (bottom right): Smoking at baseline

Sensitivity analyses with propensity/prediction scores

With the propensity score for statin use and the statin duration predicting score we performed four sensitivity analyses. In the simple analysis, we used only the propensity score for the dichotomous determinant of statin use along with statin history as predictors. Similarly, we used statin duration predicting score along with statin duration as the only predictors in this variant of our simple sensitivity analysis. In the more complex analyses, we included all the predictors from the main model along with the propensity score (or the statin duration predicting score) along with the appropriate statin use variable. Our propensity scored sensitivity analyses consistently showed results similar to the main multivariate model (summarized in Table 4).

Table 5: Sensitivity analyses with propensity/prediction for statin use adjustment

| Variables | HR – model with propensity score for statin use | CI 95% (2-sided) | HR – model with predictive score for statin duration | CI 95% (2-sided) |
|---|---|---|---|---|
| **Propensity score** | n/a | n/a | n/a | n/a |
| **Current or prior statin use (dichotomous)** | 1.29 | 0.57-2.9 | n/a | n/a |
| **Cumulative statin duration (years)** | n/a | n/a | 0.91 | 0.63-1.3 |

| Variables | HR – model with propensity score for statin use | CI 95% (2-sided) | HR – model with predictive score for statin duration | CI 95% (2-sided) |
|---|---|---|---|---|
| **Propensity score/Prediction score** | n/a | n/a | n/a | n/a |
| **Race:** | | | | |
| **Black (reference)** | | | | |
| **Hispanic** | 1.67 | 0.6-3.5 | 1.59 | 0.76-3.33 |
| **White** | 1.02 | 0.55-1.52 | 0.81 | 0.41-1.6 |
| **No race reported** | 0.2 | 0.03-1.52 | 0.21 | 0.03-1.59 |
| **HBV** | 0.65 | 0.35-1.17 | 0.68 | 0.37-1.25 |
| **HCV** | 1.63 | 0.86-3.09 | 2.15 | 1.02-4.55 |
| **LDL (per 10 mg/dL)** | 0.98 | 0.91-1.04 | 0.98 | 0.91-1.05 |
| **CD4 count (per 50 cells/µL)** | 0.89 | 0.84-0.94 | 0.88 | 0.83-0.93 |
| **Age at baseline** | 1.07 | 1.02-1.12 | 1.04 | 0.98-1.1 |
| **Smoking status at baseline** | 1.71 | 0.96-3.08 | 2 | 1.09-3.64 |
| **Current or prior statin use (dichotomous)** | 1.36 | 0.6-3.07 | n/a | n/a |
| **Cumulative statin duration (years)** | n/a | n/a | 0.94 | 0.66-1.33 |

Propensity/prediction scored sensitivity analyses with both statin variables in the model

As suggested by Hernan et al.[28], we combined our 2 statin variables in a single model adjusted separately for either propensity or prediction scores for statin use. The effect size of the 2 statin variables was magnified but the statistical significance was further reduced. The direction of the effect remained discrepant. The outcome tables for both the simple and the complex propensity/prediction scored models are provided in Table 6.

Table 6: Propensity/Prediction for statin use adjusted multivariate models with both statin variables

| Variables | HR – model with propensity score for statin use | CI 95% (2-sided) | HR – model with prediction score for statin duration | CI 95% (2-sided) |
|---|---|---|---|---|
| **Propensity/Prediction score for statin use** | n/a | n/a | n/a | n/a |
| **Race:** | | | | |
| **Black (reference)** | | | | |
| **Hispanic** | 1.68 | 0.8-3.5 | 1.61 | 0.77-3.4 |
| **White** | 1.02 | 0.55-1.9 | 0.83 | 0.4-1.6 |
| **No race reported** | 0.2 | 0.03-1.5 | 0.22 | 0.03-1.6 |
| **HBV** | 0.64 | 0.4-1.7 | 0.68 | 0.37-1.2 |
| **HCV** | 1.6 | 0.9-3.1 | 2.12 | 0.99-4.5 |
| **LDL (per 10 mg/dL)** | 0.97 | 0.9-1.04 | 0.98 | 0.91-1.05 |
| **CD4 count (per 50 cells/µL)** | 0.89 | 0.84-0.94 | 0.88 | 0.83-0.94 |
| **Age at baseline** | 1.06 | 1.02-1.12 | 1.04 | 0.98-1.1 |
| **Smoking status at baseline** | 1.73 | 0.97-3.1 | 2.02 | 1.1-3.7 |
| **Current or prior statin use (dichotomous)** | 2.0 | 0.7-5.7 | 1.92 | 0.67-5.49 |
| **Cumulative statin duration (years)** | 0.77 | 0.47-1.33 | 0.77 | 0.45-1.3 |

| Variables | HR – model with propensity score for statin use | CI 95% (2-sided) | HR – model with prediction score for statin duration | CI 95% (2-sided) |
|---|---|---|---|---|
| **Propensity/Prediction score for statin use** | n/a | n/a | n/a | n/a |
| **Current or prior statin use (dichotomous)** | 1.55 | 0.6-5.2 | 1.65 | 0.56-4.8 |
| **Cumulative statin duration (years)** | 0.75 | 0.46-1.4 | 0.77 | 0.44-1.4 |

Modeling of pure cardiovascular outcomes

Since mortality represented the majority of our outcomes (n=46), and HIV/AIDS related complications could thus have significantly influenced outcome of our main analysis, we restricted our composite outcome to MI and stroke only (n=20), to evaluate whether age, smoking and CD4 count remain significant as predictors even in this setting. In this analysis, age and smoking no longer showed an association with the outcome but CD4 count remained significant in both univariate and multivariate analysis (summarized in Table 7). We also evaluated the performance of both statin predictors by modeling the MI & stroke combined endpoint with both of our previously described simple propensity scored models (compare to Table 3). The HR approximately 2.4 and 0.7 for history of statins and 1 year of statin use respectively in both types of our propensity scored models (Table 8). The results were not statistically significant.

Table 7: Univariate and multivariate analysis of age, smoking and CD4 count as predictors of MI and stroke only as a composite outcome

| Variables | HR - univariate | p-value | HR - multivariate | p-value |
|---|---|---|---|---|
| **Age at baseline** | 1.04 | 0.22 | 1.05 | 0.13 |
| **Smoking status at baseline** | 1.3 | 0.56 | 1.4 | 0.46 |
| **CD4 count (per 50 cells/μL)** | 0.87 | 0.009 | 0.87 | 0.008 |

Table 8: Propensity/Prediction for statin use adjusted multivariate analysis of each statin variable independently as predictors for MI and stroke only as a composite outcome

| Variables | HR – model with propensity score for statin use | CI 95% (2-sided) | HR – model with prediction score for statin duration | CI 95% (2-sided) |
|---|---|---|---|---|
| **Propensity score** | n/a | n/a | n/a | n/a |
| **Current or prior statin use (dichotomous)** | 1.53 | 0.4-5.93 | n/a | n/a |
| **Cumulative statin duration (years)** | n/a | n/a | 0.69 | 0.21-2.2 |

## Discussion

Our study failed to show a significant benefit of statin therapy on the incidence of MI, stroke and all-cause mortality in PLWH. Indeed, the only 3 variables significantly predictive of this outcome were age and smoking (traditional risk factors), as well as CD4 count, which was also associated with poor outcomes when our composite endpoint was restricted to MI and stroke only (data shown in the appendix). It is highly unlikely that these factors represent the totality of important prognostic factors. Larger studies will be needed to find additional important factors, and to further clarify the possible role of statins in averting this outcome. Ideally, given the clinical importance of non-AIDS outcomes for PLWH receiving modern cART, the role of statins would be clarified with an experimental (randomized) study.

### Strengths

Our outcome is consistent with some of the prior studies[21,22]. In our study design and statistical approach, we focused on correcting the issues that would have created significant noise or bias in this complex analysis (multiple imputation of missing data; propensity scored sensitivity analyses to account for the "healthy user effect" of statins; statistical adjustment for less than ideal baseline, imposed by the evolution of clinical practice).

We also significantly benefited from working with a prospectively collected dataset from a cohort longitudinally monitored for numerous parameters. The wide range of monitored parameters focused not only on HIV and its complications but also on cardiovascular health, nutritional status and other more general health related and social factors was essential for a complex analysis such as this one.

Limitations

Although the cohort data was prospectively collected, our study was observational. We would have included other hard clinical outcomes in our composite (e.g., neoplasias) but were unable to reliably do so. The statistically insignificant, yet discrepant HR for our two statin variables raise the question whether such result is due to the lack of effect or simply due to the lack of statistical power in this relatively small sample, in which many subjects used statins relatively briefly. This question could be addressed by evaluating a larger sample size and/or assessing a longer follow up time period. Alternatively, softer clinical outcomes leading up to MI, stroke and mortality (such as increasing cIMT or CAC) could be considered as a target for analysis.

Given the observational nature of this analysis, we also had a different follow up time between statin users and non-users as described in the results section. Generally, the statin users were followed longer but their average follow up time of interest (after the initiation of statins) was significantly shorter than the average time of follow up in subjects, who never used statins. There is an obvious potential for bias in such differential

follow up, however, in this particular case, the direction of such bias is not entirely clear. Given the overall longer follow up in the statin user group, and the fact that statin use was naturally the later part of this follow up time, we could lean toward believing in the protective effect of statins. Conversely, since the average follow up time after the initiation of statin therapy was shorter than the average total follow up time of non-users, we could also claim that statins were actually harmful by shortening the time to event in the subjects, who developed one of the eligible outcomes. Moreover, the statin interruptions, which were conservatively classified under statin user time, would to a certain degree bias the effect toward null. It is important to remember that statins, just like most other medications, are prescribed to people later in life when the morbidity burden is higher. In an observational study, such as ours, this may mean older people with more comorbidities, for whom the addition of statins may have come too late.

Additionally, allowing the count of time under statins in our analysis to start only at the baseline, disregarded certain amount of statin time in 25 individuals. This problem possibly introduced 2 types of bias: Firstly, it would attribute more power to the effect of statins, given that the amount of time under statins was underestimated. Secondly, by losing certain amount of precision, it would likely also reduce the statistical power.

In HIV patients, the use of statins is further complicated by potential drug-drug interactions with cART (mainly with PI). This may also mean that the potential benefits

are counterbalanced by additional toxicity or interactions that may lower serum levels of cART or statins.

There were several measurement imprecisions in the calculation of the time under statins. Not knowing the exact time of statin initiation resulted in choosing the time of earliest report to be time zero. This approach was conservative as it was almost certain to start the count later than it actually occurred, which could be viewed as a problem, since such time underestimation may bias the results. On the other hand, the benefit of medications like statins is unlikely to start on day one. Thus some underestimation of time on statins may actually be desirable. Adjustment for time dependent LDL level, while overall desirable, may have some residual bias, which could be addressed differently by certain techniques of causal inference, such as inverse probability weighting (IPW)[26]. These were not used in the main analysis but were, to a degree, accounted for in the propensity scored sensitivity analyses.

Insight into the interplay of inflammation, statins and important clinical outcomes has not been fully explored to date and better understanding would be essential for establishment of inflammation as an indication for statin therapy. Inflammatory biomarkers, if reliably predictive of outcomes, could also be used as an easy tool for statin treatment initiation, monitoring and dose adjustment. Some of the associations needed to clarify these complex questions have been tested in prior studies: the effect of rosuvastatin on monocytes' activation[27], assessment of inflammatory and coagulation

markers on cardiovascular and other non-AIDS outcomes[11,28,29]. We were unable to look at the association between the statin effect and the outcome via comparing unadjusted versus CRP or other inflammatory marker adjusted analyses. This was due to the volume of missing data that was simply too great and prohibitive of imputation. This disallowed direct estimation of the association between statins, inflammation and hard clinical outcomes. It is important to mention, though, that prior studies have been inconsistent about the utility of various inflammatory biomarkers as predictors of poor clinical outcomes. One of the more important reasons for this inconsistency is the low specificity of currently available biomarkers for distinguishing the cause of inflammation as well as differentiating between chronic and acute inflammation. Having a biomarker of inflammation, with properties similar to glycosylated hemoglobin A1C in diabetes monitoring, would allow for a more reliable use in clinical practice and research.

The discrepancy in hazard ratios of our two differently coded statin variables was first and foremost statistically insignificant. It nevertheless draws some attention and may be attributable to imbalance, which appeared after the introduction of HCV co-infection and/or smoking history into our multivariate models. Our statin history variable reversed its HR to >1 in HCV negative subjects and in smokers. Statin duration remained constant with HR<1 in all instances. We hypothesize, that unbalanced counts of statin users as well as unbalanced length of treatment within these groups, which may have been introduced by adjustment for HCV & smoking may be responsible for the discrepant results. For comparison we had the model adjusted also for statin treatment duration, and this variable remained consistent across all additional comparisons

performed with the above mentioned variables. An alternative hypothesis explaining this effect could be that the duration of treatment may have biased the result with a "survivor" effect. This explanation, however, seems to lack face validity in this instance for several reasons. The follow up time of statin treatment is not significantly large to begin with and that would limit the possibility of a significant survivor bias. Secondly, smokers, who typically have more cardiovascular disease, and thus have been extensively studied in the general population, have never been shown to be harmed by statins. Lastly, the "healthy user" type of survivorship would also be unlikely to contribute to this effect as we addressed it, to a certain degree, with our propensity scored analysis. Also, in the case of HCV, it would have been the subjects with less healthy lifestyles overall to benefit from statins.

Informing future research with sample size calculation based our outcome

In order to inform future research, we would have to provide an estimate of a sample size needed to show statistical significance for the result (HR for 1 year of statin use) from our analysis if true under our model specifications. Below, we performed such calculation of sample size for a trial that would otherwise have similar subject to control ratio, similar estimated survival time and similar follow-up time: Our study would have 5.5 controls per experimental subject, an accrual interval of 6 years, and additional follow-up of 5 years after the accrual interval. Based on our current analysis, the median survival time of people not on statins would be 24 years. If the true hazard ratio for one year of statin use is 0.93, we would need to study 8315 experimental subjects (with 1 year as the mean time of statin use or a proportional number with higher/lower mean time of

statin use) and 45733 control subjects to be able to reject the null hypothesis that the experimental and control survival curves are equal with probability (power) of 0.8 and the Type I error probability of 0.05[23].

Discussion summary

In summary, our small observational study failed to show significant association between statins and MI, stroke and all-cause mortality treated as a composite outcome. A larger prospective trial, monitoring patients for metabolic and inflammatory parameters, multiple hard clinical outcomes as well as for medication side-effects and drug interactions would be better suited to determine the benefits and harms in this population. It is also possible, that significant benefits or harms of statins are for various reasons (e.g., drug interactions, poly-pharmacy related compliance problems) simply harder to prove in PLWH. For this reason, cost-effectiveness analyses should also be a part future evaluation. Clinicians treating PLWH should also consider the cost-effectiveness aspect, as it relates not only to the cost burden shared by the healthcare system but also to co-pays directly affecting the patients. Our study did confirm the important role of traditional and some HIV-associated risk factors in these outcomes in HIV infected individuals.

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