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Associations of Hepatosteatosis with Cardiovascular Disease in HIV Positive and HIV Negative Patients: The Liverpool HIV-Heart Project

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Introduction

Hepatosteatosis (HS) is the most common cause of liver disease in patients living with HIV (PLWHIV), affecting between 13 to 65% (1–3) individuals. HS describes hepatic ectopic fat accumulation and is present when it affects >5% of the liver by weight. HS encompasses a spectrum of clinically entities including non-alcoholic fatty liver disease (NAFLD). The prevalence of HS is under reported.

Histologically, progressive hepatic fat accumulation is associated with lipotoxicity and chronic inflammation, progressing in many cases to cirrhotic liver disease, and a threefold increase in mortality (4). The relationship of obesity, insulin resistance, type II diabetes and hepatosteatosis (HS) is well defined in non-HIV populations (5). The estimated prevalence of NAFLD in the United States is predicted to reach 33% of the adult population by 2030 (6).

PLWHIV have unique risk factors for the development of HS compared to non-HIV populations. They have been shown to develop lean NAFLD, defined as NAFLD in BMI< 25Kg/m^2, at increased rates compared to non-HIV populations (7). The complex interplay of viral related factors, antiretroviral (ARV) medications and chronic inflammation may cause PLWHIV to be more susceptible to the development of HS. Liver disease represents a huge source of morbidity and mortality in PLWHIV with up to 13% of deaths in the Data Collection on Adverse events of Anti-HIV Drugs (D:A:D) cohort attributable to liver disease (8). In both HIV-positive and HIV-negative populations dyslipidaemia, insulin resistance and overt type II diabetes are strongly associated with the presence of HS.

HS has been shown to be associated with CVD in HIV-negative populations (9–11) although this is not universal (12–14). Given the increasing burden of HS in HIV-positive populations

we sought to examine if HS was independently associated with CVD in HIV-positive compared to HIV-negative populations.

Methods

We conducted a real-world retrospective analysis to compare the associations of HS and CVD in both HIV positive and HIV negative patients. Data was collected from the Royal Liverpool University Hospital HIV clinical database and CT Coronary Angiography (CTCA) clinical database. Both the HIV and CTCA databases exist for both clinical use and quality improvement purposes and are approved by the host institution's audit committee. All demographic and clinical variables present on the databases were cross checked by the research team using the Trusts electronic patient record (clinical notes). The Royal Liverpool University Hospital audit committee approved this study. The HIV database includes all patients under follow up with service. It contains clinical co-morbidities, current and previous medications, anthropometric measurements, blood chemistry and CVD risk.

The HIV database was cross checked for patients that had a received a CT thorax within the last 10 years. The images were inspected by an independent imaging cardiologist for the presence of coronary calcification which was recorded in a binary fashion (yes or no). HS was assessed using non-contrast CT scans or venous phase CT scans in those with liver parenchyma visible on the images. Both imaging techniques are established methods for quantifying liver fat content. Two regions of interest circles covering 100mm^2 were drawn on the right lobe of the liver and one on the left lobe. The mean attenuation (Hounsfield unit [HU]) was recorded for liver parenchyma. Regions with non-uniform attenuation and hepatic vessels were avoided. A further 100mm^2 region of interest was drawn on the spleen

and the HU recorded. HS was confirmed if the liver to spleen ratio (L/S ratio) was less than 1 and/or the mean hepatic measurement was <40HU in non-contrast scans (15–18). Liver attenuation of 20HU less than spleen attenuation was used for the venous phase imaging (19). Hepatosteatosis was also confirmed in patients that had a prior imaging (ultrasound) or biopsy confirmed diagnosis of fatty liver. The HIV-positive group were taken exclusively from this database.

The CTCA database contains demographic and clinical variables for all patients referred for cardiac CT (either CAC scoring or CTCA) between January 2014 and November 2016. The majority of patients were referred for the investigation of atypical angina and were considered low to medium risk in line with guidelines from the National Institute for Clinical Excellence that were established at the time (20). The presence of HS had previously been calculated by two independent radiologists and added to the clinical database. This was done using gated, non-contrast studies from the CAC protocol. The methodology and criteria used to establish HS was the same as outlined with the HIV-positive cohort. Patients were labelled as having coronary calcification if the calcium score was >0.

Variables were collected by reviewing patients case notes, including consultation letters, for prior and current diagnoses. Patients with prior diagnoses of cardiovascular disease (including clinical diagnosis, imaging diagnosis of coronary plaque or coronary event or intervention) were excluded. Patients were also excluded if no quantification of coronary calcifications or quantification of HS was not possible.

Statistical Analysis

Summary statistics were calculated to compare the difference in clinical and demographic covariates between HIV-positive and HIV-negative groups. The prevalence of categorical

variables was presented in absolute prevalence and percentages. All data were inspected using graphical representation for normality (histograms and Q-Q plots) and Shapiro-Wilk test. The proportions between categorical variables were compared using Chi Squared test. Continuous variables were presented as means and standard deviations where normally distributed. The means were compared using an independent t test. P-values were considered statistically significant if <0.05. The data used was complete. Any case with missing values was excluded from the analysis.

A multiple logistic regression (LR) model was developed to ascertain the association between coronary calcifications and CVD risk co-variates in HIV-positive and HIV-negative patient groups, using odd ratios (OR) as a measure of association. Additionally, we performed a sensitivity analysis using random forest (RF), to assess variables of importance in both HIV-positive and HIV-negative patients. Variables of importance were determined by Gini index. The Gini index is an established measure of accumulated nodal impurity for each variable in random forest algorithms. A high mean decrease in Gini indicates a high variable importance (21).

For the development of the models we performed bootstrapping, with 1000 repetitions (including replacements), each with the same size as the original datasets. This was done to calculate the standard errors and confidence intervals. For the sensitivity analysis we split the datasets into 70% training and 30% test in both group of patients. The area under the receiver operator curve (AUC) for the test sets were calculated, and 95% confidence intervals (95% CI) were estimated from the standard error.

The statistical analysis and development of the machine learning models were performed using RStudio, version 1.3.1056.

Results

The HIV database contains data on 1294 patients who are under follow up. After removing those with prior documented CVD and those where assessment of coronary calcification or HS was not possible there were 209 cases available for analysis. There were 1744 patients within the CTCA database. Again, after removing those with prior CVD, there were 1097 cases available for assessment. This left an overall total of 1306 cases (mean age 52.32; 46.6% male) to be included in the analysis.

The clinical and demographic co-variates for the whole cohort and stratified by HIV status are displayed in table 1. The groups differed in terms of the proportion of clinical and demographic covariates. Mean age was lower in the HIV-positive group (49.9 versus 52.3, p=0.002) and the proportion of males was higher (76.6% versus 40.9%, p<0.001). The proportion of patients with hypertension, type II diabetes and dyslipidaemia were significantly higher in the HIV-negative group (p<0.005 in all). The proportion of current smokers was higher in the HIV-positive group (28.7% versus 18.8%, p=0.002).

The overall prevalence of HS was 44.6% and was significantly higher in the HIV-negative group (46% versus 36.8%, p=0.018). The rate of coronary calcification was similar between the 2 groups (37.4% in HIV-negative versus 32.5% in HIV-positive, p=0.128). Within the HIV-positive group 91.9% had HS quantified by CT.

Multivariate analysis

In the multivariate model, including all clinically relevant demographic and clinical risk factors, HS was significantly associated with CVD in HIV-positive patients (OR: 3.13, 95% CI: 1.51-6.63 p=0.005). The only other significant co-variates associated with CVD were

increasing age (OR: 1.15, 95% CI: 1.10-1.20, p<0.005) and male sex (OR: 3.77, 95% CI:1.37-11.69 p=0.014 (table 2). The associations of clinical covariates with CVD differed in the HIV-negative group. Current smoking (OR:1.96, 95% CI: 1.37-2.81p<0.005), dyslipidaemia (OR:1.66, 95% CI: 1.24-2.22 p <0.005) along with increasing age and male sex were significantly associated with CVD. HS was not significantly associated with CVD in this group (OR:1.08, 95%CI: 0.81-1.44 p=0.60) (table 3). The AUC for the logistic regression was 0.841 (95% CI: 0.785– 0.897) for the HIV-positive model and 0.796 (95% CI: 0.770 – 0.822) for the HIV-negative model. Upon removing HS from the model the AUC dropped to 0.819 (95% CI: 0.758-0.881) for the HIV-positive model and 0.796 (95% CI: 0.770-0.822) (figure 1). There was no statistically significant difference between either AUC (HIV positive: 0.619, HIV-negative: 0.993, Wilcoxon test).

Sensitivity analysis

For the HIV-positive model age, HS and male sex were top three variables of importance using, according to the mean decrease in Gini index. In the HIV-negative group the top three variables of importance were age, male sex and hypertension (figure 2). The mean AUC for the HIV-positive and HIV negative models were 0.877 (95% CI: 0.755-0.959) and 0.828 (95% CI: 0.780-0.873), respectively. The logistic regression models and the random forest models were significantly different for both cohorts (p<0.001, Wilcoxon test) (supplementary material).

Discussion

In this retrospective analysis we sought to assess the differences in the association of HS with CVD between HIV-positive and HIV-negative patients. The principal finding from this study is HS is independently associated with CVD in HIV-positive patients whilst there was no significant association in HIV-negative patients. To our knowledge, our study is the first to directly compare the effect of HS on CVD in both HIV-positive and HIV-negative patients. The finding that HS was independently associated with CVD in HIV-positive patients but not HIV-negative patients was confirmed in our sensitivity analysis.

Despite a higher proportion of HS in the HIV-negative group (p=0.018) and similar rates of coronary calcification (p=0.128) we found HS to be independently associated with CVD in the HIV-positive group (table 2) but not in HIV-negative group (table 3) after adjusting for CVD risk factors. We also observed a significant association of the traditional risk factors of age and male sex with subclinical CVD.). The association of HS and CVD in HIV-positive patients has been assessed in previous analyses. However, there have been no study comparing HS and CVD in HIV-positive and HIV-negative groups. Kaplan et al (22) compared 232 HIV-positive patients with and without NAFLD and found that NAFLD, as assessed by ICD-9 codes, was independently associated with CVD (OR: 3.08, 95%CI: 1.37-6.94). Our HIV-positive patients were similar in age and proportions of male sex. However, the definition of CVD differed from this study as they used a broad composite which included coronary artery disease, heart failure, peripheral vascular disease, stroke, transient ischaemic attack, myocardial infarction and revascularisation. In our analysis we assessed the association with subclinical CVD found on CT rather than hard clinical outcomes.

In a separate analysis, using a mainly male HIV-positive cohort, Crum-Cianflone et al found that HS diagnosed on non-contrast CT was significantly associated with coronary calcification (OR: 3.8, p<0.01) (23). In a further analysis, Guaraldi et al assessed HIV-positive patients and found no significant association with NAFLD and coronary calcium (24).

Studies assessing the impact of HS as an independent CVD risk factor in HIV-negative groups demonstrate conflicting results. In their meta-analysis Kapuria et al concluded that NAFLD was associated with increased prevalence of subclinical atherosclerosis (based largely on CAC score) (13). Vanwagner et al analysed the association of NAFLD from CT scans, defined as liver attenuation <40HU, and found no significant association with coronary calcium after adjustment for obesity (12). In this current study we found that HS was not significantly associated with CVD in our multivariate analysis (OR 1.08, 95%CI: 0.81-1.44).

Current smoking, hypertension, type II diabetes and dyslipidaemia are established risk factors for CVD in both HIV-positive and HIV-negative groups. The relatively low incidence of these risk factors within this study makes it difficult to interpret the magnitude of effect. In the case of dyslipidaemia only 7.2% (n=15) of the HIV-positive had this risk factor documented. This is reflected in the wide confidence interval produced by the multivariate analysis (95% CI: 0.85-10.58). Although these results did not show an association between traditional risk factors and CVD the study was underpowered to demonstrate any association. The purpose of this retrospective analysis was to compare the associations of HS with CVD in HIV-positive and HIV-negative groups. It was not designed to determine the impact of traditional risk factors.

In the sensitivity analysis we utilised a random forest algorithm to investigate the variables of importance in HIV-positive and HIV-negative groups (figure 2). The benefit of using this technique in a sensitivity analysis is that it is not constrained by the same assumptions that logistic regression and offers an alternative methodology to examine the results. In both groups age was the most important variable. In the HIV-positive group HS was the second most important variable followed by dyslipidaemia. In the HIV-negative group HS was the fifth most important variable. This further illustrates the difference in the impact of HS as an independent risk factor between HIV-positive and HIV-negative groups.

We utilised random forest as an alternative statistical technique as it does not require the same assumptions from the data as logistic regression. The random forest models demonstrated good discriminatory ability and was statistically significantly superior to logistic regression performed on the same testing data (supplementary data). The performance of each model is summarised in the box plots in supplementary data. Although the random forest models demonstrated superior discriminatory ability compared to logistic regression models, they demonstrated similar results. By utilising this contemporary alternative analysis, it increases the robustness of the finding that HS is significantly associated with CVD in HIV-positive groups and not associated in the HIV-negative group.

The association of HS as an independent risk factor may be attenuated by adjustment for other metabolic disease related conditions such as diabetes, obesity, dyslipidaemia and hypertension. Our finding, showing that HS is an independent predictor of CVD in HIV-positive but not HIV-negative, suggest potential differences in drivers of CVD between these two cohorts. HS that manifests in HIV-positive populations may represent a more severe adverse metabolic phenotype compared to non-HIV populations.

HS drives cardiovascular risk through increased atherogenic lipid profiles, inflammation and insulin resistance. HIV-positive patients have been shown to have increased rates of lean adiposity compared to HIV-negative groups (7). Ectopic fat deposition has also been shown to be associated with prior CVD events (25). An individual's susceptibility to this process may also be derived from genetic factors (26). The findings from this study further demonstrate the unique pathophysiological process underpinning the increased CVD risk seen in PLWHIV.

Limitations and strengths

There were several limitations to our study. First, the way in which patients had coronary calcifications assessed in the HIV-positive group was based on the presence of a non-dedicated CT scan. These scans had taken place historically for different indications. Whilst it is recognised that assessment of coronary calcification using non-dedicated CT thorax (27) the assessment between groups weas not homogenous. Second, by opportunistically selecting patients who had received CT scans of the thorax for alternative indications we may have introduced selection bias into the study. We attempted to reduce any differences between groups by undertaking the sensitivity analysis. Third, the HIV-negative group were a pre-defined group of patients with low to intermediate risk chest pain. This may affect the generalisability of this result. We did not adjust for HIV-related co-variates including ARV medications. However, this study was designed to assess the differences between two different cohort rather than determinants of CVD risk specific to HIV-positive patients. In addition, prior studies did not demonstrate any significant HIV-related associations with HS and CVD (22).

Finally, we were not able to adjust for alcohol consumption or other secondary causes of hepatosteatosis (such as hepatitis C) in either group due to the retrospective design of the study. Although these influences are of significant interest it is outside the scope of this retrospective study to assess their impact on the presence of hepatosteatosis. Crum Cianflone et al performed a sensitivity analysis in which participants with significant alcohol consumption were excluded. This did not significantly alter their result (23). Further prospective work is required to assess their impact on the presence of HS and CVD.

Despite these limitations there were multiple strengths to our analysis. Both cohorts used in this comparison were extremely well characterised and data used within this analysis was 100% complete. This study is also unique in the fact that it is the first to compare the impact of HS on CVD between HIV-positive and HIV-negative cohorts. We were able to confirm the validity of the result by utilising a random forest algorithm in our sensitivity analysis. The broad agreement between logistic regression models and random forest models confirms the robustness of the result. This study was designed to be hypothesis generating rather than show definitive causality. Future studies quantifying CVD risk, plaque burden and homogenous assessment of HS and coronary calcium scores are required to further delineate this emerging field. These findings may have important clinical implications for the way in which CVD risk quantified in HIV-positive patients.

In conclusion, in these well characterised cohorts we have demonstrated a significant difference in the impact of HS as an independent CVD predictor between HIV-positive and HIV-negative groups. This may represent a unique metabolic process that drives the excess CVD risk seen in PLWHIV.

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Table 1: Summary statistics of the cohort and stratified by HIV serostatus.

	Overall	HIV negative	HIV positive	P Value
n	1306	1097	209	
Age (mean (SD))	52.32 (±12.00)	52.77 (±12.26)	49.92 (±10.22)	<0.005
Male Sex (%)	609 (46.6)	449 (40.9)	160 (76.6)	<0.005
Current smoker (%)	266 (20.4)	206 (18.8)	60 (28.7)	<0.005
HTN (%)	375 (28.7)	342 (31.2)	33 (15.8)	<0.005
DMII (%)	124 (9.5)	113 (10.3)	11 (5.3)	0.032
Dyslipidaemia (%)	389 (29.8)	374 (34.1)	15 (7.2)	<0.005
Statin (%)	343 (26.3)	304 (27.7)	39 (18.7)	0.008
Coronary calcium (%)	489 (37.4)	421 (38.4)	68 (32.5)	0.128
HS (%)	582 (44.6)	505 (46.0)	77 (36.8)	0.018
Obesity (%)	135 (10.3)	72 (6.6)	66 (31.2)	<0.005

SD, standard deviation; HTN, hypertension; DMII, type II diabetes; HS, hepatosteatosis

Table 2: Multivariate analysis in HIV-positive patients for the association of coronary calcification

	Odds Ratio (95% CI)	P Value
Age	1.15 (1.10-1.20)	<0.005*
Male Sex	3.77 (1.37-11.69)	0.014*
Current smoker	2.14 (0.93-5.06)	0.077
HTN	0.58(0.19-1.67)	0.317
DMII	0.75 (0.14-3.45)	0.718
Dyslipidaemia	2.89 (0.84-10.73)	0.097
HS	3.13 (1.51-6.63)	0.005*
Obesity	1.58 (0.70-3.56)	0.269

HTN, hypertension; DMII, type II diabetes; HS, hepatosteatosis

* Denotes significant association

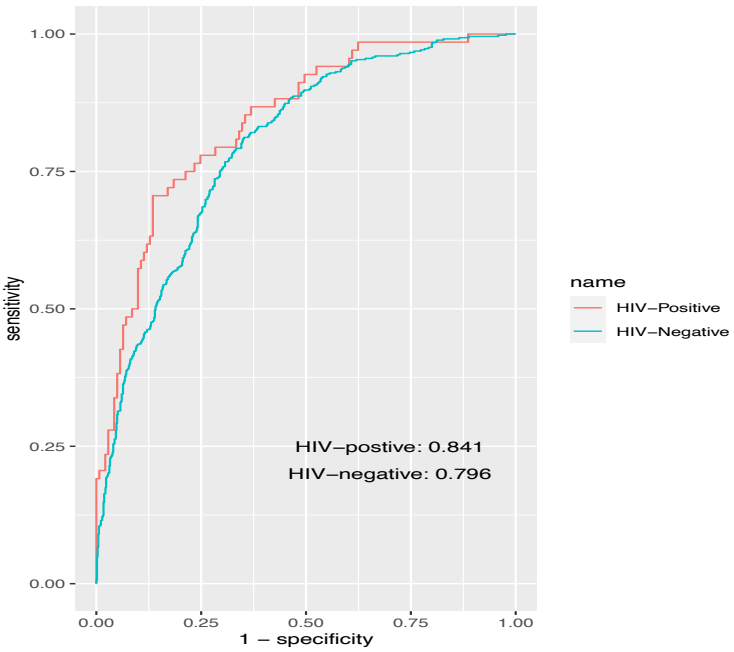
Table 3: Multivariate Analysis in HIV Negative Patients for the association of coronary calcification

	Odds Ratio (95% CI)	P value
Age	1.11 (1.09-1.13)	<0.005*
Male Sex	2.97 (2.19-4.05)	<0.005*
Current Smoker	1.96 (1.37-2.81)	<0.005*
HTN	1.39 (1.02-1.90)	0.04
DMII	1.14 (0.72-1.82)	0.58
Dyslipidaemia	1.66 (1.24-2.22)	<0.005
HS	1.08 (0.81-1.44)	0.60
Obesity	0.95 (0.54-1.65)	0.87

HTN, hypertension; DMII, type II diabetes; HS, hepatosteatosis

* Denotes significant association

Figure 1: Comparison of HIV-positive and HIV-negative models including and excluding HS



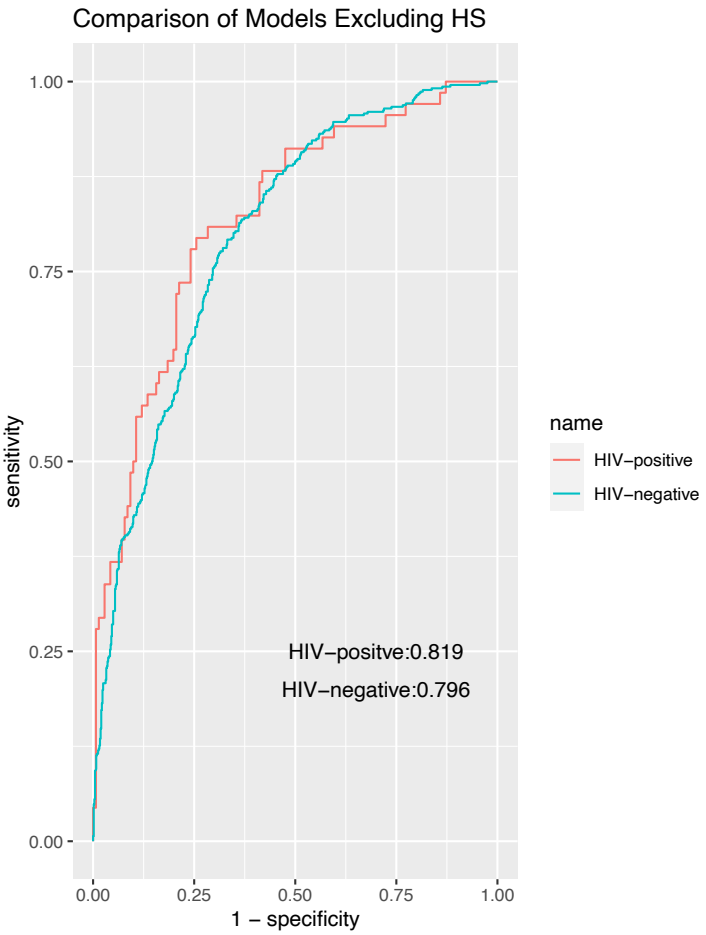


Figure Legend: Comparison of receiver operator characteristic curves for the logistic regression models. The first plot demonstrates the predictive ability between HIV-positive and HIV negative groups. The second plot demonstrates the predictive ability of the models not including HS.

Figure 2: Random forest analysis of the variables of importance in HIV-positive and HIV-negative patients

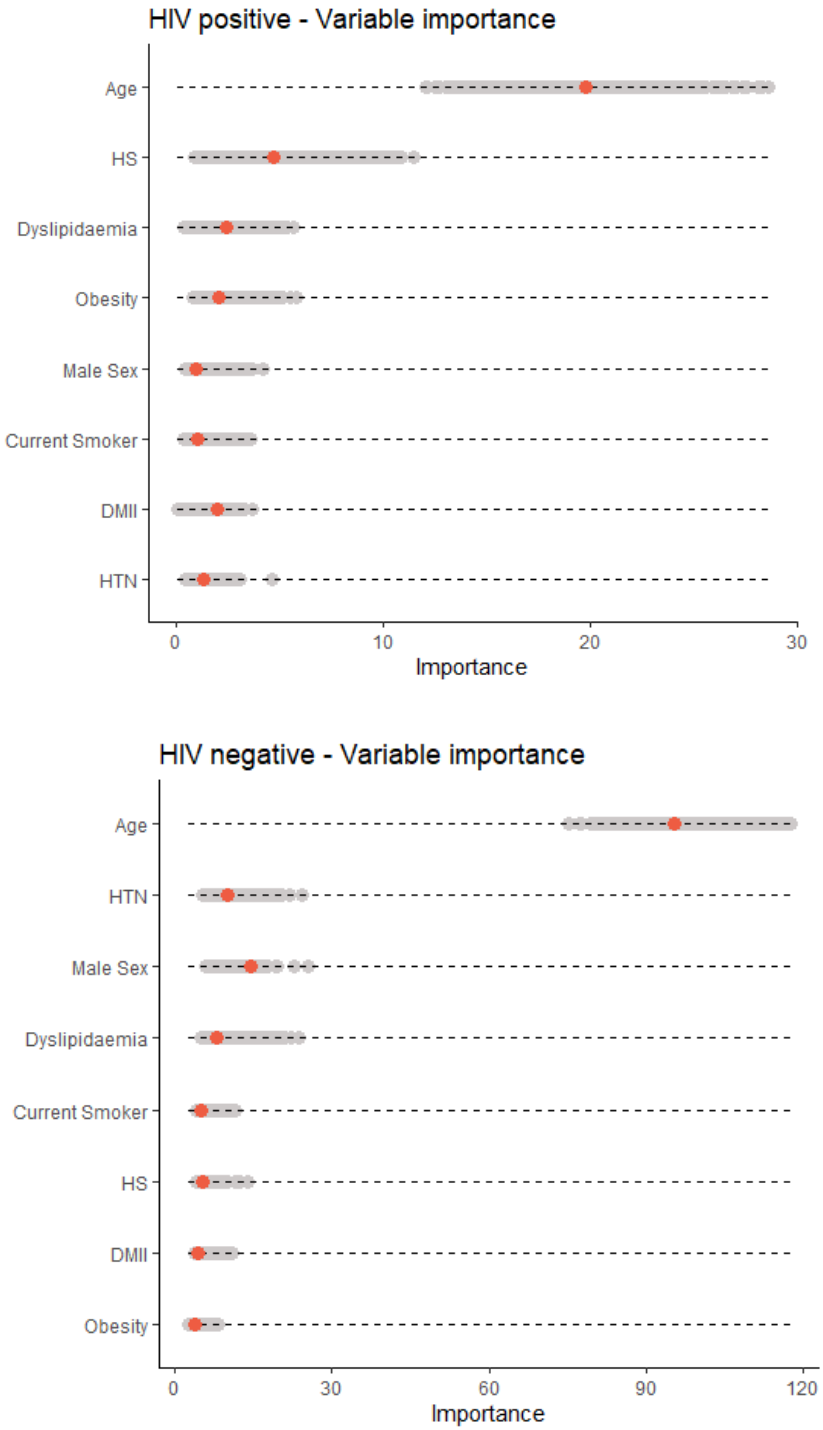
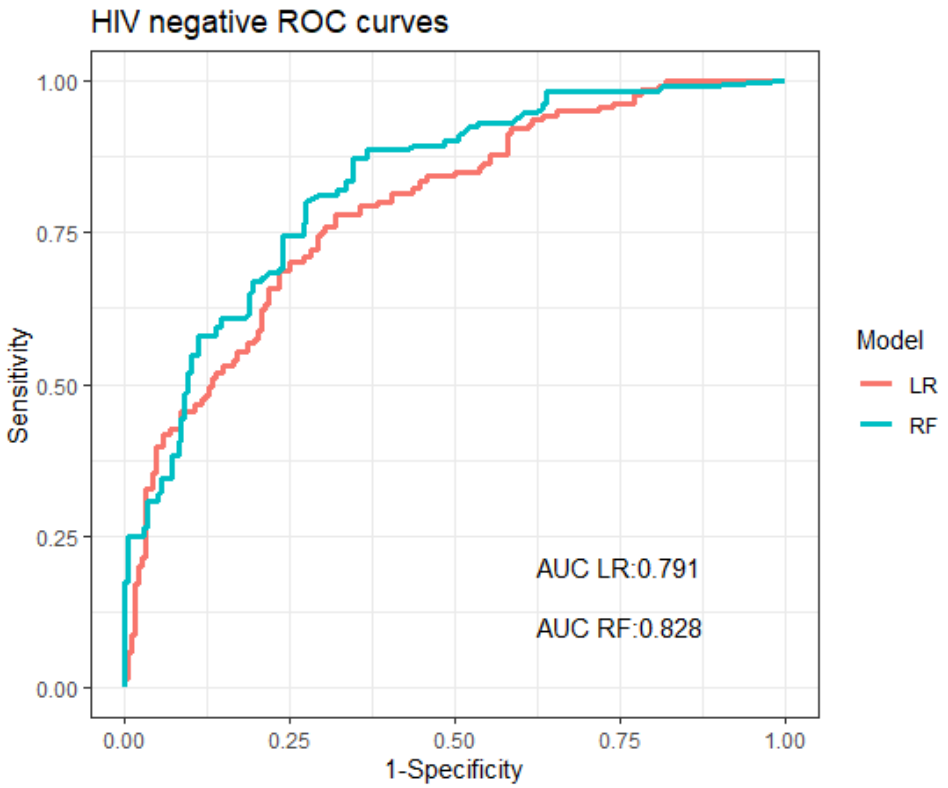


Figure legend:

Variables of importance given by mean decrease in Gini index. The shaded area (grey points) is the variable importance given by all models. The variables of importance of the model with the best AUC is highlighted in red. Variable abbreviations are the same as table 1.

Supplementary Figure 1: Receiver operator characteristic curves demonstrating the predictive ability of regression and random forest models in HIV-positive and HIV-negative groups.



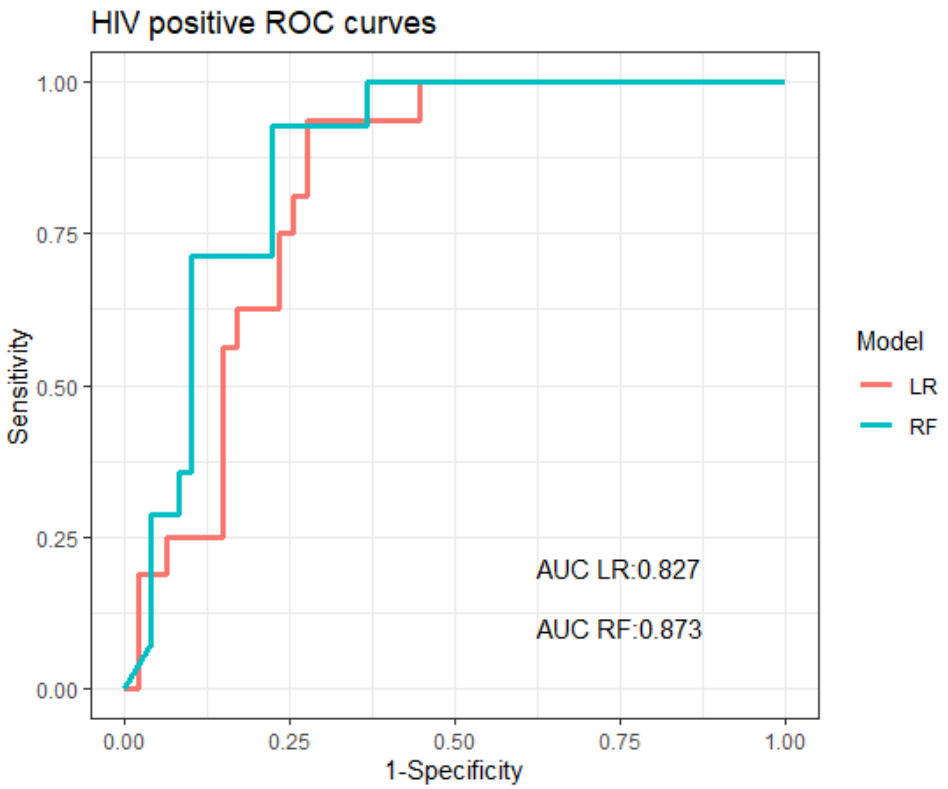


Figure Legend: Comparison of receiver operator characteristic curves and corresponding area under the curves for LR models and RF models. In the HIV negative plot, the mean AUC for LR was 0.791 (95% CI: 0.741-0.842) and for RF a mean AUC of 0.828 (95% CI: 0.781-0.872). The difference between these models was also statistically significant ($p<0.001$, Wilcoxon test). The HIV positive plot shows a mean AUC for LR of 0.827 (95% CI: 0.705-0.933) and for RF a mean AUC of 0.873 (95% CI: 0.768-0.959. The difference between the models was statistically significant ($p<0.001$, Wilcoxon test).

Abbreviations; AUC: area under the curve; LR: logistic regression; RF: random forest

Supplementary Figure 2: Box plots to show the performance of models in HIV-positive and HIV-negative groups

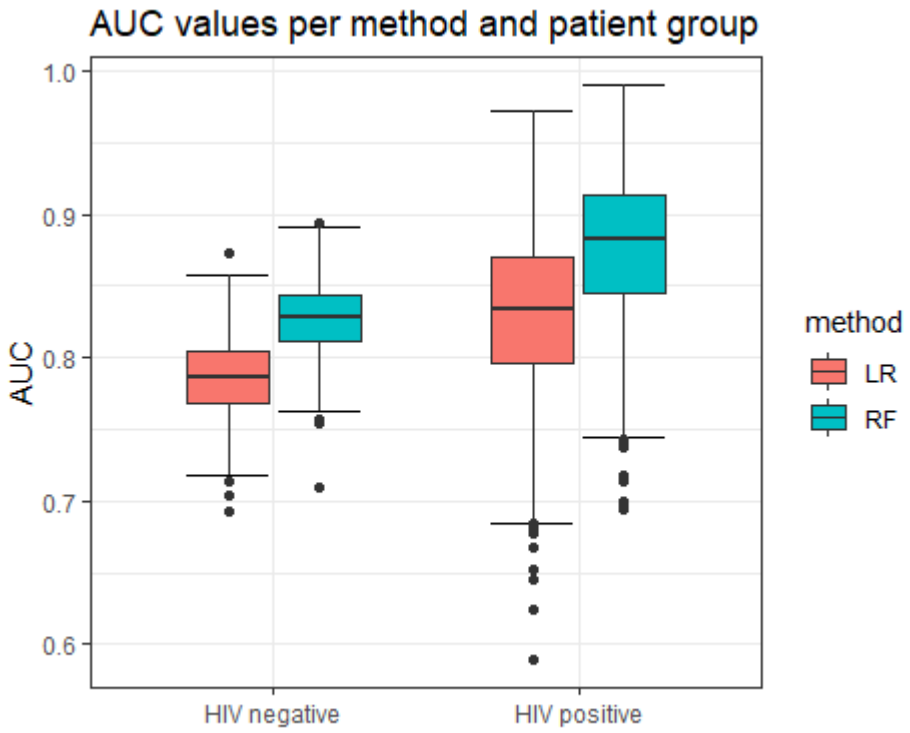


Figure Legend: Box plots to summarise the performance of RF and LR models in HIV-positive and HIV-negative groups.