Non-alcoholic fatty liver disease predicts development of metabolic comorbidities in HIV-infected patients

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Key-Points:

- People with HIV are at high risk for non-alcoholic fatty liver disease (NAFLD)
- NAFLD is associated with incident metabolic complications
- Diabetes and dyslipidemia develop at more than double rate in people with HIV and NAFLD compared to those without NAFLD

Footnote page

Conflict of interest

NK reports grants from the Canadian Institutes of Health Research (CIHR) and the CIHR Canadian HIV Trials Network, a grant for an investigator-initiated study from Gilead Sciences and personal fees from ViiV Healthcare, Gilead Sciences and Merck, outside the submitted work. BL has acted as consultant for ViiV, Gilead and Merck and received research funding from Merck and Gilead. JF has received consulting fees from Theratechnologies Inc., and has received payment for lectures from ViiV Canada, Gilead Canada, and Abbott Canada. GG has acted as speaker for Merck, Gilead, ViiV, served as an advisory board member for Merck and ViiV and has received research funding from Merck, Gilead and ViiV. BL has acted as consultant for ViiV, Gilead and Merck and received research funding from Merck and Gilead. MBK has acted as consultant for ViiV, Gilead, Janssen and Merck and received research funding from Merck and ViiV. PW has acted as consultant for BMS, Gilead, Merck, Novartis. MD has served as an advisory board member for Merck, Janssen, Gilead. PG has acted as consultant for Merck and Gilead. GS has acted as speaker for Merck, Gilead, Abbvie, Novonordisk, Novartis served as an advisory board member for Merck, Novartis, Gilead and Intercept and has received unrestricted research funding from Merck and Theratechnologies Inc. TK, MM and RSP have nothing to disclose.

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Authors contributions

TK and GS contributed to conception, study design, data and interpretation of the data, statistical analysis and first draft of the manuscript. MM contributed to statistical analysis. RSP, NK, JF, GG, BL, MBK, PW, MD and PG contributed to data and interpretation of data. All authors approved the final version of the article. Part of this work has been presented at the Liver Meeting of the American Association for the Study of Liver Diseases (Boston, US; November 2019).

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Abstract

Background

Cardiovascular and liver disease are main contributors to mortality in people with HIV (PWH). In HIV-uninfected patients, non-alcoholic fatty liver disease (NAFLD) is associated with incident metabolic complications. We investigated the effect of NAFLD on development of metabolic comorbidities in PWH.

Methods

We included PWH undergoing a screening program for NAFLD using transient elastography. NAFLD was defined as controlled attenuation parameter ≥248 dB/m and exclusion of other liver diseases. Incident diabetes, hypertension, dyslipidemia and chronic kidney disease were investigated using survival analysis and Cox proportional hazards.

Results

485 HIV mono-infected patients were included. During a median follow-up of 40.1 months (interquartile range 26.5-50.7), patients with NAFLD had higher incidence of diabetes (4.74, 95% confidence interval [CI] 3.09-7.27 vs. 0.87, 95% CI 0.42-1.83 per 100 person-years [PY]) and dyslipidemia (8.16, 95% CI 5.42-12.27 vs. 3.99, 95% CI 2.67-5.95 per 100 PY) compared to those without NAFLD. On multivariable analysis, NAFLD was an independent predictor of diabetes (adjusted hazard ratio [aHR] 5.13, 95% CI 2.14-12.31) and dyslipidemia (aHR 2.35, 95% CI 1.34-4.14) development.

Conclusions

HIV mono-infected patients with NAFLD are at higher risk of incident diabetes and dyslipidemia. Early referral strategies and timely management of metabolic risk may improve outcomes.

Keywords: diabetes, dyslipidemia, transient elastography, controlled attenuation parameter, liver fibrosis.

Introduction

Non-alcoholic fatty liver disease (NAFLD), defined as fat accumulation in the liver in the absence of excessive alcohol consumption, is an epidemic entity affecting approximately 25% of the world's population[1]. In the general population, NAFLD is not only associated with liver-related morbidity and mortality related to cirrhosis and hepatocellular carcinoma, but has also been linked with increased mortality from extra-hepatic diseases, particularly cardiovascular disease[2]. Moreover, NAFLD is strongly associated with the metabolic syndrome, and has been linked with increased risk of developing diabetes, hypertension, dyslipidemia, and chronic kidney disease (CKD)[3-5].

NAFLD is expected to increasingly comprise the burden of liver disease in people with HIV (PWH) due to the widespread availability and effectiveness of direct-acting antivirals for the treatment of chronic hepatitis C. The prevalence of NAFLD in HIV mono-infection is currently estimated to be at least 35%[6]. The prolonged exposure to HIV infection and antiretroviral therapy (ART), as well as increased intestinal dysbiosis and bacterial translocation, are thought to contribute to the development of NAFLD in PWH in addition to risk factors shared with the general population, including obesity, insulin resistance, and dyslipidemia[7]. Importantly, the classic metabolic risk factors for NAFLD are more frequent in PWH. In HIV positive men, a four times higher prevalence of type 2 diabetes mellitus (T2DM) has been described vs. HIV negative men[8]. Dyslipidemia is also common due to both HIV chronic infection and lifelong use of ART[9]. Hypertension and a higher cardiovascular risk have also been consistently reported[10, 11]. HIV-infected patients are also at higher risk for CKD[12].

Given that HIV infection and NAFLD independently increase the risk of metabolic comorbidities, the aim of this study was to investigate the effect of NAFLD on development of T2DM, hypertension, dyslipidemia and CKD in a cohort of PWH by means of transient elastography with associated controlled attenuation parameter (CAP)[13]. This diagnostic tool for NAFLD and associated liver fibrosis has been validated against liver biopsy in both HIV uninfected and HIV-infected patients[14-16].

Patients and methods

Study design and population

We conducted a retrospective analysis of the LIVEr in HIV (LIVEHIV), which is an established prospective cohort of PWH followed at the McGill University Health Centre (MUHC). From September 2013 to August 2018, 798 PWH were enrolled at the university-based clinic of the Chronic Viral Illness Service (CVIS). Patients are regularly followed by their treating physician for their HIV care every 6 months. Moreover, they are consecutively screened for liver disease, including hepatitis C virus (HCV) and hepatitis B virus (HBV) serology, Alcohol Use Disorders Identification Test (AUDIT-C) questionnaire, and undergo annual measurement of transient elastography with CAP using the FibroScan (EchoSens, Paris, France). For the present study, we included all consecutive patients who met the following criteria: 1) age ≥18 years; 2) HIV infection, as documented by positive enzyme-linked immunosorbent assay (ELISA) with Western blot confirmation; 3) valid measurement of transient elastography with available CAP; 4) availability of relevant biochemical and physiologic parameters. Exclusions criteria were: 1) positivity for HCV antibody or hepatitis B surface antigen; 2) hazardous alcohol intake (AUDIT-C ≥7); 3) lack of longitudinal follow-up (at least 6 months); 4) contraindications (pacemaker or pregnancy), failure or unreliable transient elastography examination; 5) decompensated cirrhosis, or hepatocellular carcinoma at enrolment.

Ethics

The study was approved by the Research Ethics Board of the Research Institute of the MUHC (code 14-182-BMD) in accordance with the declaration of Helsinki. Participants in the study provided written informed consent prior to enrolment.

Clinical and biological parameters

Relevant data were collected during routine follow-up clinic appointments and within 3 months from the transient elastography examination. Demographic and clinical information included age, sex, ethnicity, body mass index (BMI), risk factors for HIV infection, time since HIV diagnosis, exposure to ART. ART drugs were classified as: protease inhibitors (PIs), non-nucleoside reverse-transcriptase inhibitors (NNRTIs), nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and integrase inhibitors. Laboratory data included aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), platelet count, total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglycerides, CD4 cell count and nadir, serum creatinine, bilirubin, albumin. In addition, metabolic comorbidities (history of diabetes, hypertension, dyslipidemia) were documented.

TE examination with controlled attenuation parameter

Measurement of transient elastography with CAP was performed in patients fasting for at least 3 hours by two experienced operators (>500 examinations before the study). The M probe was used routinely, with XL probe used if the M probe failed, or the patient had a BMI greater than 30 kg/m^2. Given recent published data on lack of influence of probe type on liver stiffness measurement, the same cut-off value was used for M and XL probe to define suspected significant liver fibrosis[15]. The following criteria were applied to define the result of transient elastography examination as reliable: at least 10 validated measures and an

interquartile range (IQR) less than 30% of the median[17]. Suspected significant liver fibrosis (stages F2-F4) and cirrhosis (stage F4) were defined as liver stiffness measurement ≥7.1 kPa and ≥13 kPa, respectively[14, 16, 18]. As previously reported in the setting of HIV infection, any grade (involving >10% of hepatocytes) and severe (involving >66% of hepatocytes) steatosis were defined as CAP ≥248 dB/m and >292 dB/m, respectively[19, 20].

Exposure and outcome measures

The main exposure of this study was the diagnosis of NAFLD at baseline (time zero). Any grade hepatic steatosis (CAP ≥248 dB/m) was used to diagnose NAFLD[19, 20]. As secondary exposure, we also explored suspected significant liver fibrosis[14, 20]. The main study outcomes were the development of metabolic comorbidities, as defined below. Hypertension and dyslipidemia were diagnosed as per Canadian Cardiovascular Society guidelines[21, 22]. T2DM was defined as an HbA1C of 6.5% or greater, or as previously diagnosed by an endocrinologist/treating physician[23]. CKD was defined as estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73m^2 (eGFR category G3), as calculated using serum creatinine by the CKD-Epi formula[24] or albuminuria category A2 or above obtained on two occasions ≥ 3 months apart as per the KDIGO guidelines[25]. Albuminuria was measured using spot urine microalbumin to creatinine ratio and/or by 24h protein urine collection for protein. As secondary outcome, we reported the incidence of all-cause mortality, which was collected by means of dedicated outcome measures form.

Statistical analysis

Baseline (time zero) corresponded to the first visit after September 1st, 2013 when transient elastography examination was performed. We compared characteristics of participants at baseline by exposure status using Student's *t* test for continuous variables and Pearson's χ^2 or Fisher's exact test for categorical variables. Patients in the incidence cohort were observed until August 2018 or were censored either when they died or at their last clinic visit. T2DM, hypertension, dyslipidemia and CKD were recorded as binary outcomes. Participants with the respective outcomes of interest at baseline were excluded from the subgroup calculation of incidence rate, time-to-failure plots, and

hazard ratios (HRs). Incidence rates of T2DM, hypertension, dyslipidemia and CKD were estimated by dividing the number of participants developing the outcome by the number of person-years (PY) of follow-up. Poisson count models were used to calculate confidence intervals (CIs) for incidence rates. Kaplan-Meier plots and log-rank tests were used to illustrate time to metabolic outcomes by presence or absence of NAFLD and by presence or absence of suspected significant liver fibrosis. Multivariable time-dependent Cox regression models were constructed to assess predictors of T2DM, hypertension, dyslipidemia and CKD. Results were reported as adjusted HR (aHR) with 95% CI. Robust variance estimation was used in all Cox regression analyses to account for the correlation of data contributed by the same participant at multiple visits. Multivariable models included covariates that were determined *a priori* to be clinically important, namely age, sex, ethnicity. Given the known effect of PIs on lipid metabolism, models were also adjusted for current use of PIs, except for the CKD model, which was adjusted for current use of NRTIs given the known effect of tenofovir on kidney function, and for the T2DM model given the smaller number of outcomes[12, 26, 27]. Moreover, all models were adjusted for either pre-existing hypertension (for the outcomes of T2DM and dyslipidemia) or T2DM (for the outcomes of hypertension and CKD). A complete case analysis was used for the multivariable models and the percentage of missing data was less than 10%, unless specified. A two tailed alpha=0.05 was used as a threshold to determine statistical significance. Statistical analyses were performed using SAS (SAS Institute Inc) and STATA 13.1 (STATA Corp. LP, College Station, Texas, USA).

Results

After applying exclusion criteria (Fig. 1), 485 patients were included in this study. At baseline, the prevalence of NAFLD was 38.1%. Severe hepatic steatosis affected 81 (16.7%) patients. Suspected significant liver fibrosis and cirrhosis affected 72 (14.8%) and 12 (2.5%) patients, respectively. Overall, 57 (11.9%) and 102 (21%) patients were exposed to didanosine and stavudine, respectively. Table 1 reports the main demographic, clinical, biochemical and immuno-virological characteristics of the study population by NAFLD status at baseline. Patients with NAFLD were older, more likely to be Caucasian and had higher BMI. Moreover, they had longer time since HIV diagnosis. Finally, they had higher prevalence of hypertension, lower HDL cholesterol, higher triglycerides and glucose, higher ALT and AST, higher albumin and higher liver stiffness measurement. At baseline, patients with NAFLD had higher prevalence of hypertension and dyslipidemia (Table 1), while those with suspected significant liver fibrosis had higher prevalence of all metabolic comorbidities (Fig. 2).

Incidence and predictors of metabolic comorbidities

Patients were followed for a median of 40.1 months (IQR 26.5-50.7). There were 68, 86, 190, and 84 patients with T2DM, hypertension, dyslipidemia, and CKD, respectively, who were excluded from the longitudinal analysis for having the outcome at baseline. Overall, incidence rates of T2DM, hypertension, dyslipidemia and CKD were 2.2 (95% CI 1.6-3.3), 4.2 (95% 3.2-5.5), 5.3 (95% CI 4.0-7.1) and 2.7 (95% CI 1.9-3.8) per 100 PY, respectively. Table 2 reports incidence rates of metabolic comorbidities by NAFLD and suspected significant liver fibrosis status. Patients with NAFLD had higher incidence rate of T2DM and dyslipidemia as compared to those without NAFLD (Fig. 3a and 3c). There was also a tendency for a higher incidence rate of hypertension (Fig. 3b), while there was no difference for CKD (Fig. 3d). Patients with suspected significant liver fibrosis had a higher incidence

rate of T2DM, while no difference was found for hypertension, dyslipidemia and CKD (Fig. 4). Supplemental Table S1 depicts the characteristics of patients who developed metabolic comorbidities during the follow-up period. Compared to the whole cohort, patients who developed T2DM were more likely to be females and of black ethnicity, and had a higher BMI. They were also less likely to be MSM and to be on NRTIs. Patients who developed dyslipidemia were more likely to be on NRTIs. Those who developed CKD were more likely to be diabetic and to have higher liver stiffness measurement. During the follow-up period, there were a total of 8 deaths, of which three were related to non-liver cancer, two to infections and two were not known. The corresponding incidence rate was 0.4 (95% CI, 0.2-0.9) per 100 PY.

After adjustments, NAFLD and pre-existing hypertension were independent predictors of T2DM development (Table 3). Incident hypertension was independently predicted by older age. NAFLD and current use of PIs as ART regimen were independent predictors of development of dyslipidemia. Incident CKD was independently predicted by older age and pre-existing T2DM. We also conducted a multivariable analysis including suspected significant liver fibrosis as exposure. After adjusting for age (per 10 years; aHR 1.14, 95% CI 0.78-1.67), male sex (aHR 0.84, 95% CI 0.35-1.97), and black ethnicity (aHR 2.15, 95% CI 0.93-4.97), independent predictors of T2DM development were suspected significant liver fibrosis (aHR 2.71, 95% CI 1.11-6.61; p=0.029) and pre-existing hypertension (aHR 4.22, 95% CI 1.81-9.80; p=0.001). There was no effect of suspected significant liver fibrosis on development of other metabolic comorbidities (results not shown). We also conducted a sensitivity analysis for the subgroup of patients with available BMI. The relative univariable Cox regression analysis showed HR of 1.20 (95% CI, 0.99-1.45; p=0.05) for incident T2DM, 1.18 (95% CI, 0.99-1.41; p=0.06) for incident hypertension, 1.01 (95% CI, 0.87-1.17; p=0.91) for incident dyslipidemia and 1.24 (95% CI 0.99-1.55; p=0.06) for incident CKD.

Discussion

This study, based on a cohort of HIV-infected patients consecutively screened for liver disease, shows that NAFLD predicts the development of important metabolic comorbidities, including T2DM and dyslipidemia. Furthermore, NAFLD severity, represented by suspected significant liver fibrosis, predicts development of T2DM. Our findings mirror what has already been reported in the general population and shape fatty liver as a central barometer of metabolic health also in PWH[28]. To the best of our knowledge, this is the first cohort study linking NAFLD with the development of important metabolic comorbidities.

NAFLD is increasingly recognized as the most frequent liver disease in people aging with HIV[13, 29-32]. A recent meta-analysis situates the prevalence of NAFLD in HIV mono-infected at 35%, higher than the global prevalence reported for the general population at 25%[1, 6]. In the same study, the pooled prevalence of significant liver fibrosis was 22%. One of the reason for this excess, may be that PWH present with particularly high frequency of metabolic conditions. A higher incidence of T2DM in the HIV-infected population has been well described, though the role of the liver in this process has not been investigated. A longitudinal study with a median follow-up of 4 years reported a cumulative incidence of T2DM of 10% in PWH, compared to 3% in uninfected controls[8]. A recent meta-analysis reported a pooled incidence rate for T2DM of 13.7 per 1000 PY of follow-up (95% CI 13-20)[33]. Frequent hypertension and a higher cardiovascular risk have also been consistently reported[10, 11]. Several virologic and ART-related factors have been implicated in the pathophysiology of T2DM and hypertension in HIV infection, including chronic inflammation, immune reconstitution and lipodystrophy[34]. Dyslipidemia is also common due to both HIV chronic infection and lifelong use of ART, particularly PIs[9]. CKD is a frequent complication of HIV infection, occurring in 3.5-48.5% of the patients, due to HIV infection itself and as a consequence of ART, such as tenofovir disoproxil fumarate[12, 27].

Our study provides novel longitudinal data on the increased risk of T2DM, hypertension and dyslipidemia in PWH with NAFLD. This is consistent with findings in the general population with NAFLD. A large meta-analysis including 19 observational studies and 296,439 individuals followed for at least 1 year reported a twofold increased risk of incident T2DM in patients with NAFLD[4]. A community study including 3,869 NAFLD subjects and 15,209 controls followed for a median of 7 years found that a subject with NAFLD and without T2DM, hypertension or dyslipidemia was over two times (relative risk 2.62, 95% CI 5 2.31-2.96) more likely to develop one or more of these comorbidities than an age- and sex-matched control[35]. In our study, we found that PWH with NAFLD were more likely to develop T2DM and dyslipidemia, with aHR of 5.13 (95% CI 2.14-12.31) and 2.35 (95% CI 1.34-4.14), respectively. Moreover, suspected significant liver fibrosis was an independent predictor of T2DM development, with an aHR of 2.71 (95% CI 1.11-6.61). A recent cross-sectional study of factors associated with liver fibrosis and steatosis in patients with HIV mono-infection demonstrated an association of T2DM with liver fibrosis (odds ratio 3.78, 95% 1.48-9.68)[36]. Our finding suggests a possible link between the progression of NAFLD-associated liver disease and insulin resistance in the context of HIV infection. Current use of PIs was an independent predictor of developing dyslipidemia. This class of ART is associated with a less favorable lipid profile, in particular elevated triglycerides and total cholesterol, especially when boosted with ritonavir[37]. We also observed a trend for NAFLD to predict development of hypertension. Conversely, we did not observe any link between NAFLD and incident CKD. In the general population, a cohort study of 41,430 adults in Korea reported an aHR for NAFLD diagnosed by ultrasound of 1.22 (95% CI 1.04-1.43) for the development of CKD. We speculate that the

pathogenesis of CKD in PWH may be more complex, and HIV-related factors may overcome the effect of NAFLD in this setting[3].

Liver and cardiovascular disease are the primary non-AIDS related causes of morbidity and mortality in PWH[38]. While HCV coinfection has been driven much of the liver-related mortality in the past, the implementation of effective direct antiviral-agents has inverted this trend. The increasing burden of NAFLD in the HIV-infected population, combined with our findings of its impact on metabolic comorbidities, indicate that PWH with NAFLD may be at particularly high risk of cardiovascular morbidity and mortality. Indeed, cardiovascular events are the leading cause of mortality in patients with NAFLD, and PWH have higher cardiovascular risk than the general population[39-41]. This is thought to be as a result of the effects of systemic inflammation and endothelial dysfunction, as well as the disproportionate presence of the traditional risk factors of insulin resistance and dyslipidemia. Additionally, the effect of NAFLD on long-term outcomes in the HIV-infected population is still not completely understood. Importantly, in HIV-negative patients NAFLD the severity of liver fibrosis impacts not only on liver-related, but also on all-cause mortality[39]. As such, guidelines recommend cardiovascular risk stratification for all patients with NAFLD, and particularly those with liver fibrosis[42, 43]. Management of NAFLD in HIV infection may include treating modifiable metabolic risk factors, diet and exercise, nutritional supplements as well as considering optimizing HIV-related factors and ART[44-46].

The main strength of our study is the longitudinal design, able to capture dynamics over time and providing novel data regarding the effects of NAFLD on development of metabolic comorbidities in PWH. Moreover, we included only consecutive patients as part of an ongoing screening program for liver disease at a single center. We wish to acknowledge several limitations of our study. First, the study was retrospective. Second, we did not have more robust diagnostic tools to diagnose hepatic steatosis, such as liver biopsy or magnetic resonance[14, 32]. However, it would be costly and time-consuming to use these approaches in a large prospective cohort. Moreover, transient elastography has not been widely validated against liver biopsy in the context of HIV infection. Although CAP $\geq$248 dB/m has a good sensitivity for detection of any grade steatosis, higher cut-off values have been proposed and its accuracy for grading steatosis is lower[14]. Third, we could not correlate our findings with actual cardiovascular outcomes. Fourth, given the limited number of outcomes, we were unable to study the influence of specific ART drugs and to include all metabolic comorbidities in the multivariable models. Fifth, BMI was missing for 22% of cases, so we could not account for its effect on multivariable models, although we found a tendency to predict incident T2DM, hypertension and CKD in univariable model. BMI might be an easy predictor to classify patients at risk of metabolic complications in clinical practice. Sixth, the observational design of this study does not allow for conclusions regarding causality. Specifically, our findings do not necessarily implicate NAFLD as a cause of diabetes. Finally, shared risk factors could contribute to both NAFLD, T2DM and dyslipidemia, including medication effects, genetics, type of diet and other lifestyle factors.

In conclusion, our study suggests that NAFLD is an independent predictor for the development of important metabolic comorbidities in PWH. This finding configures NAFLD as a barometer for metabolic health also in the setting of HIV infection. Metabolic comorbidities might perpetuate the NAFLD spectrum and contribute to increased cardiovascular risk in PWH. In the context of HIV infection, patients with NAFLD should be closely monitored for the development of metabolic outcomes and modifiable risks be managed in a timely fashion. Our findings might be considered a further argument to advocate for screening for NAFLD in PWH, as it is the case for patients with diabetes[47]. Indeed, the European AIDS Clinical Society guidelines recommended screening for NAFLD in PWH with metabolic syndrome, and expansion of these criteria to PWH with any metabolic comorbidity has been proposed[48, 49]. Implications of our findings on long-term cardiovascular outcomes should be investigated in future studies.

Table 1. Characteristics of HIV mono-infected patients at baseline by NAFLD status (n=485).

	Whole cohort (n=485)	**NAFLD** (n=185)	**No NAFLD** (n=300)	**p-value**
Age (years)	49.5 (10.9)	51.0 (9.7)	48.7 (11.6)	0.011
Males (%)	367 (75.7)	146 (78.9)	221 (73.7)	0.190
Ethnicity (%) °				
Caucasian	226 (47.9)	101 (55.5)	125 (43.1)	0.012
Black	165 (35.0)	47 (25.8)	118 (40.7)	
Hispanic	50 (10.6)	17 (9.3)	33 (11.4)	
Asian	16 (3.4)	9 (5.0)	7 (2.4)	
Other	15 (3.1)	8 (4.4)	7 (2.4)	
BMI (Kg/m^2) °	26.5 (5.1)	28.1 (4.5)	25.6 (5.2)	<0.001
AUDIT-C score	1.3 (1.7)	1.3 (1.7)	1.4 (1.7)	0.444
Active tobacco smoker (%)	75 (15.5)	29 (15.7)	46 (15.3)	0.740
Hypertension (%)	86 (17.7)	43 (23.2)	43 (14.3)	0.003
T2DM (%)	68 (14.0)	32 (17.3)	36 (12.0)	0.080
MSM (%)	165 (34.8)	66 (37.7)	99 (33.1)	0.232
Active IDU (%)	17 (3.6)	7 (4.0)	10 (3.3)	0.724
Time since HIV diagnosis (years)	13.3 (8.4)	14.7 (8.4)	12.5 (8.3)	0.003
CD4 cell count (cells/µL)	672.2 (278.5)	668.8 (271.6)	674.3 (282.9)	0.905
Undetectable HIV viral load (≤ 50 copies) (%)	421 (86.8)	157 (84.9)	259 (86.3)	0.653
Current ART regimen (%)				
NRTI	411 (84.7)	148 (80.0)	263 (87.7)	0.018
NNRTI	170 (35.0)	65 (35.1)	105 (35.0)	0.960
PI	173 (35.7)	74 (40.0)	99 (33.0)	0.134
Integrase inhibitors	178 (36.7)	65 (35.1)	113 (37.7)	0.587
INR	1.08 (0.37)	1.09 (0.46)	1.08 (0.30)	0.821
Platelets (10^9/L)	209.6 (59.7)	215.5 (58.7)	206.1 (60.2)	0.100
Total cholesterol (mmol/L)	4.74 (1.08)	4.81 (1.05)	4.70 (1.10)	0.342
LDL cholesterol (mmol/L)	2.78 (0.89)	2.77 (0.93)	2.79 (0.87)	0.786
HDL cholesterol (mmol/L)	1.23 (0.42)	1.12 (0.34)	1.30 (0.46)	<0.001
Triglycerides (mmol/L)	1.84 (1.74)	2.37 (2.28)	1.50 (1.17)	<0.001
Glucose (mmol/L)	5.5 (1.2)	5.7 (1.4)	5.3 (1.0)	0.039
Creatinine (µmol/L)	85.4 (22.4)	84.8 (20.2)	85.9 (23.6)	0.643
ALT (IU/L)	31.1 (25.0)	37.7 (34.4)	27.2 (16.1)	<0.001
AST (IU/L)	26.6 (14.5)	29.3 (20.2)	25.1 (9.4)	<0.001
Total bilirubin (µmol/L)	13.5 (11.7)	14.7 (13.3)	12.7 (10.6)	0.160
Albumin (g/L)	42.2 (3.5)	43.0 (3.3)	41.7 (3.6)	<0.001
Liver stiffness measurement (kPa)	5.60 (3.72)	6.75 (5.45)	4.89 (1.67)	<0.001
CAP (dB/m)	237.4 (57.9)	293.7 (38.0)	202.1 (36.2)	<0.001

Legend: Continuous variables are expressed as mean (standard deviation) and categorical variables are expressed as frequencies (%). The p-values refer to Student t-test or χ^2 test between NAFLD and no NAFLD. ° Data on BMI and ethnicity was available for 376 patients and 472 patients, respectively. Abbreviations; ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; AUDIT-C, Alcohol Use Disorders Identification Test; BMI, body mass index; CAP, controlled attenuation parameter; HIV, human immunodeficiency virus; HDL, high-density lipoprotein cholesterol; IDU, injection drug use; IU, international units; INR, international normalized ratio; LDL, low-density lipoprotein cholesterol; MSM, men who have sex with men; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PI, Protease Inhibitors; T2DM, type 2 diabetes mellitus.

Table 2. Incidence rates of metabolic complications during follow-up by NAFLD status after exclusion of patients with the outcome of interest at baseline.

	NAFLD (n=185)		**No NAFLD** (n=300)	
	Cases	**Incidence rate** (95% CI)	**Cases**	**Incidence rate** (95% CI)
T2DM	21	4.74 (3.09-7.27)**	7	0.87 (0.42-1.83)**
Hypertension	22	5.25 (3.45-7.98)	28	3.59 (2.48-5.21)
Dyslipidemia	23	8.16 (5.42-12.27)*	24	3.99 (2.67-5.95)*
CKD	13	2.82 (1.64-4.86)	20	2.67 (1.72-4.14)
	Fibrosis (n=72)		**No fibrosis** (n=413)	
	Cases	**Incidence rate** (95% CI)	**Cases**	**Incidence rate** (95% CI)
T2DM	8	5.01 (2.50-10.01)*	20	1.85 (1.19-2.86)*
Hypertension	9	5.97 (3.11-11.48)	41	3.92 (2.88-5.32)
Dyslipidemia	5	5.00 (2.08-12.01)	42	5.37 (3.97-7.27)
CKD	7	4.20 (2.00-8.81)	26	2.50 (1.70-3.67)

Legend: Incidence rates are presented as 100 person-years with 95% CI. *p<0.05; **p<0.001. The p-values refer to log-rank test between patients with and without NAFLD. Abbreviations: CI, confidence interval; CKD, chronic kidney disease; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus.

Table 3. Multivariable analysis of predictors of development of diabetes, hypertension and dyslipidemia.

Variable	**aHR** (95% CI)	**p-value**
T2DM (n=417)		
Age (per 10 years)	1.15 (0.76-1.72)	0.505
Male sex (yes vs. no)	0.71 (0.30-1.71)	0.446
Black ethnicity (yes vs. no)	1.90 (0.83-4.31)	0.127
Hypertension (yes vs. no)	3.69 (1.56-8.74)	0.003
NAFLD (yes vs. no)	5.13 (2.14-12.31)	<0.001
Hypertension (n=399)		
Age (per 10 years)	1.62 (1.25-2.10)	<0.001
Male sex (yes vs. no)	0.93 (0.44-1.95)	0.850
Black ethnicity (yes vs. no)	1.62 (0.88-2.98)	0.123
Current PIs use (yes vs. no)	0.78 (0.45-1.35)	0.377
T2DM (yes vs. no)	1.53 (0.71-3.32)	0.281
NAFLD (yes vs. no)	1.64 (0.93-2.88)	0.088
Dyslipidemia (n=295)		
Age (per 10 years)	1.05 (0.79-1.40)	0.723
Male sex (yes vs. no)	1.19 (0.57-2.48)	0.643
Black ethnicity (yes vs. no)	0.59 (0.32-1.10)	0.094
Current PIs use (yes vs. no)	2.08 (1.17-3.70)	0.012
Hypertension (yes vs. no)	1.02 (0.46-2.26)	0.963
NAFLD (yes vs. no)	2.35 (1.34-4.14)	0.003

CKD (n=401)		
Age (per 10 years)	1.99 (1.46-2.70)	<0.001
Male sex (yes vs. no)	1.18 (0.51-2.80)	0.702
Black ethnicity (yes vs. no)	1.36 (0.64-2.87)	0.427
Current NRTIs use (yes vs. no)	0.54 (0.25-1.13)	0.101
T2DM (yes vs. no)	2.21 (1.04-4.69)	0.039
NAFLD (yes vs. no)	1.16 (0.56-2.38)	0.688

Legend: Hazard ratios (HR) and 95% confidence interval (CI) are presented for each variable analyzied in multivariable Cox regression analysis. *p<0.05; **p<0.001. Abbreviations: aHR, adjusted hazard ratio; CKD, chronic kidney disease; NAFLD, non-alcoholic fatty liver disease; NRTI, nucleoside reverse transcriptase inhibitors; PI, Protease Inhibitors; T2DM, type 2 diabetes mellitus.

Legend

Figure 1. Flow chart displaying the selection of study participants. Liver stiffness measures by Fibroscan were considered reliable if the ratio of the interquartile range (IQR) over the median of the 10 measures was no more than 30%. Abbreviations: AUDIT-C, Alcohol Use Disorders Identification Test; CAP, controlled attenuation parameter; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LIVEHIV, LIVEr in HIV.

Figure 2. Prevalence of diabetes, dyslipidemia, hypertension and CKD by: a) NAFLD status at baseline; b) fibrosis status at baseline.

Figure 3. Survival curves of incidence of: (a) diabetes by NAFLD status; (b) hypertension by NAFLD status; (c) dyslipidemia by NAFLD status; (d) CKD by NAFLD status. The p-values refer to log-rank test.

Figure 4. Survival curves of incidence of: (a) diabetes by fibrosis status; (b) hypertension by fibrosis status; (c) dyslipidemia by fibrosis status; (d) CKD by fibrosis status. The p-values refer to log-rank test.

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Figure 1

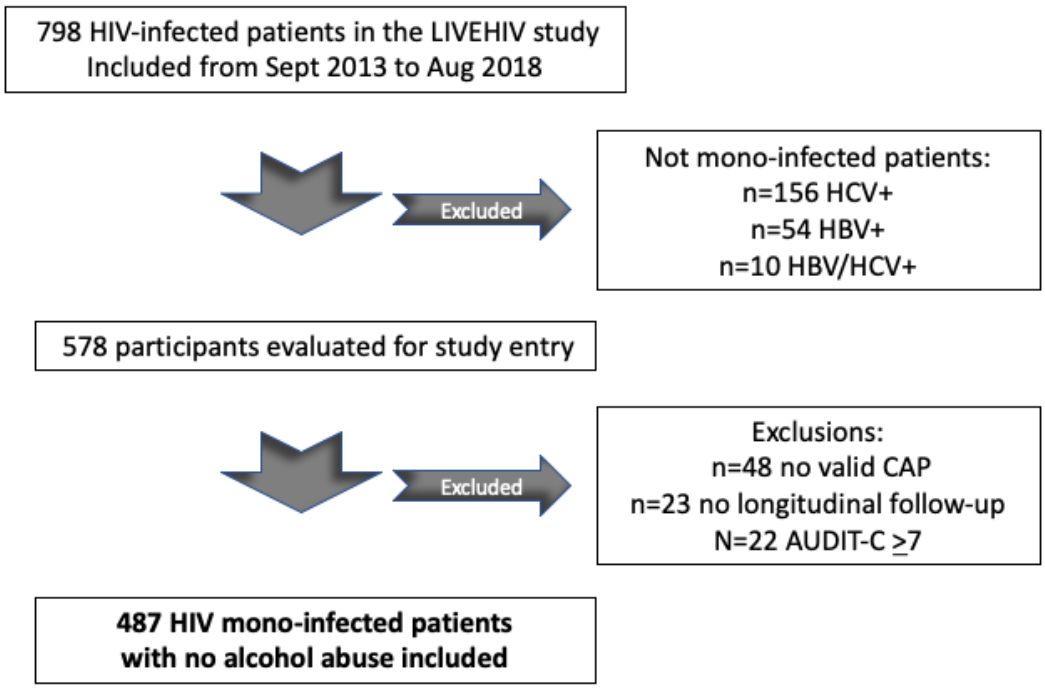


Figure 2a

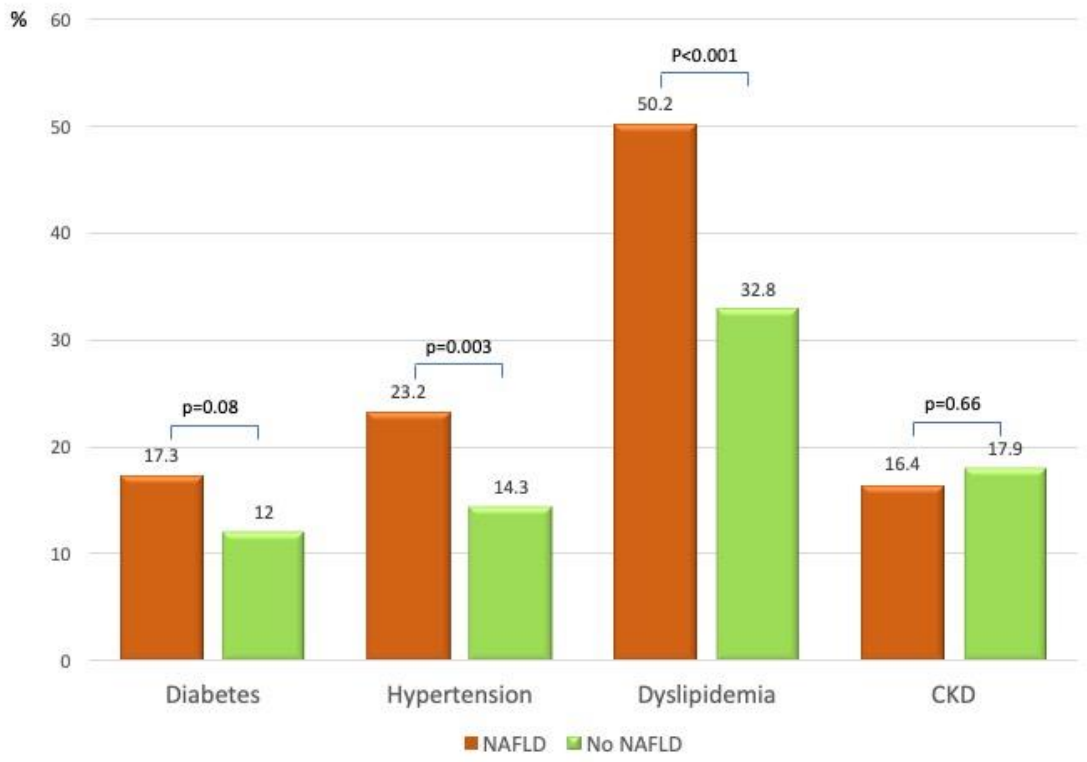
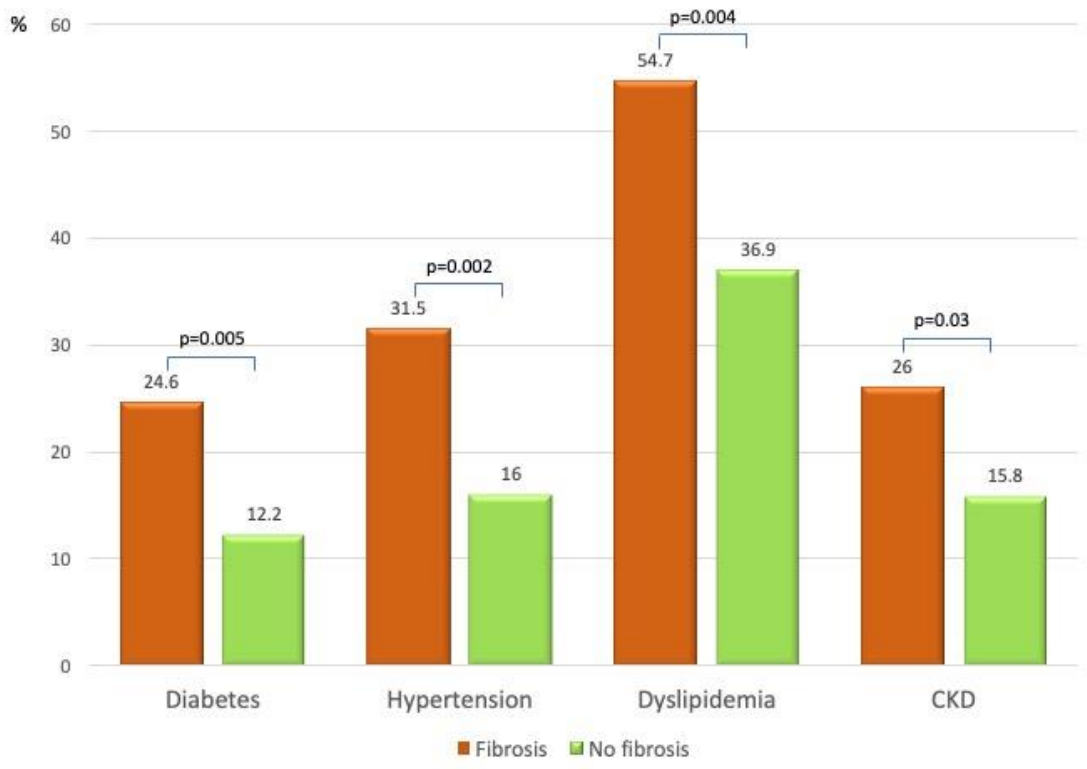


Figure 2b



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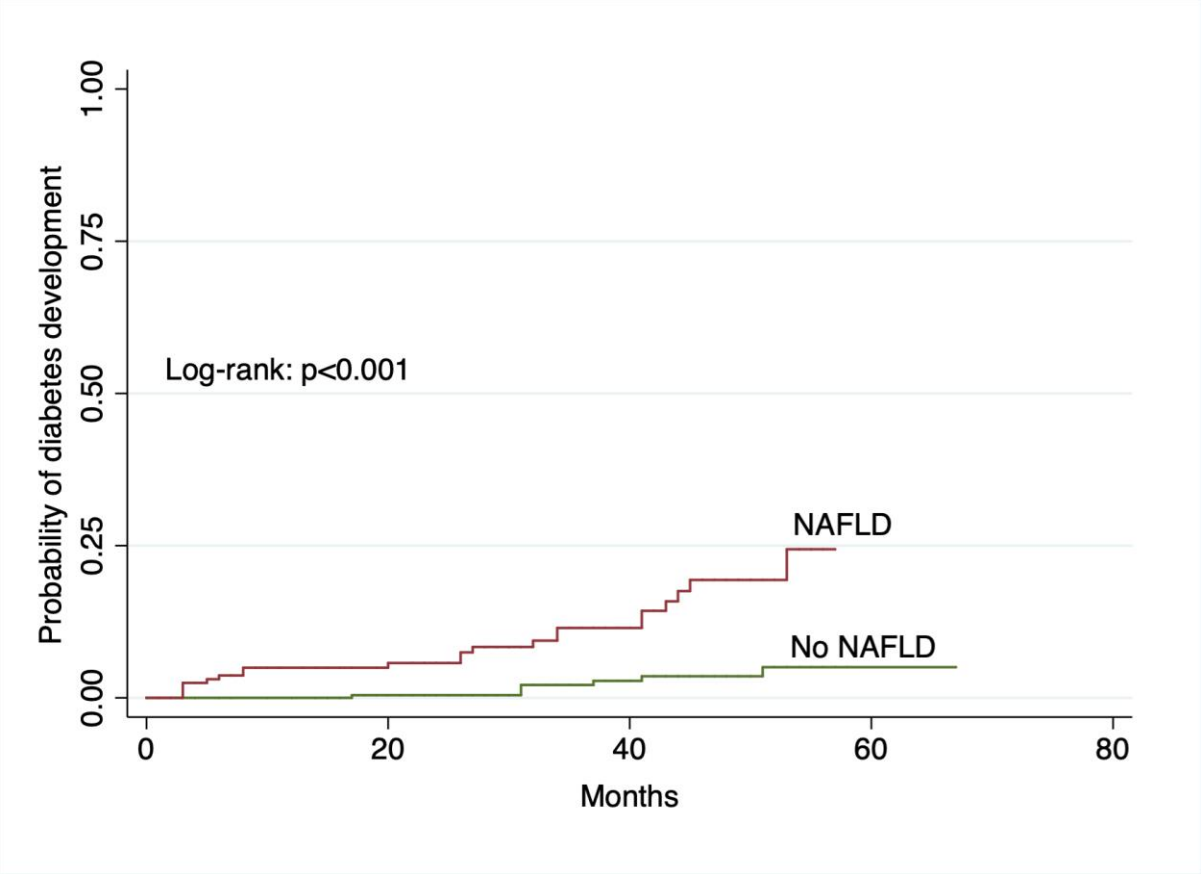

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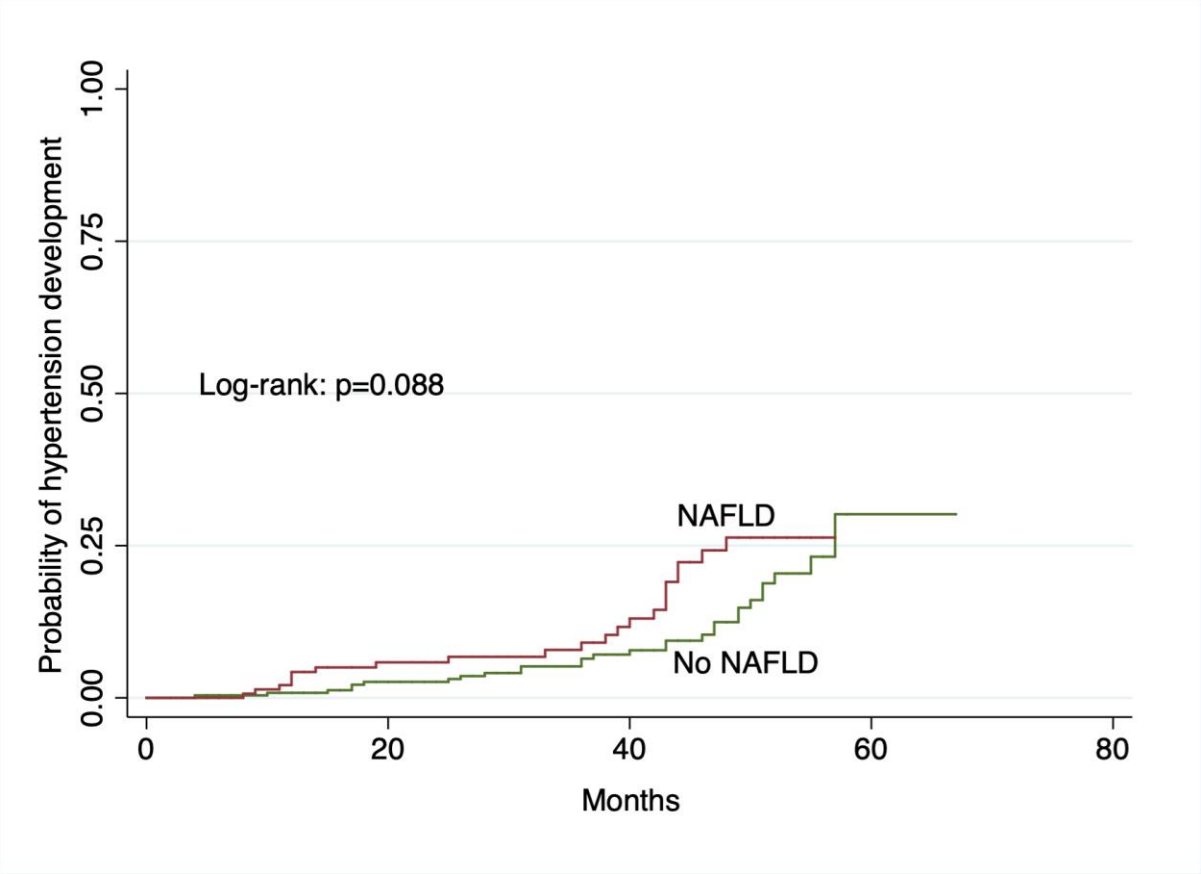


Figure 3c

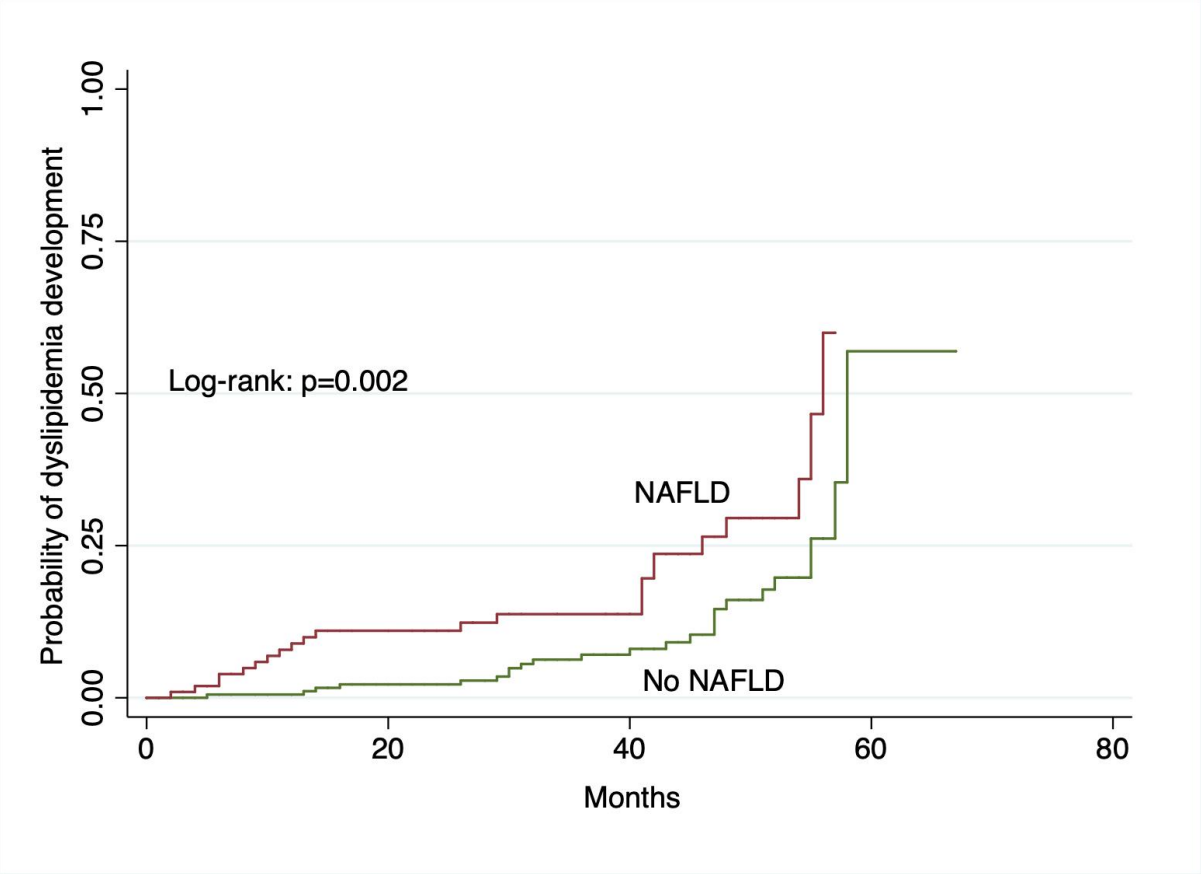
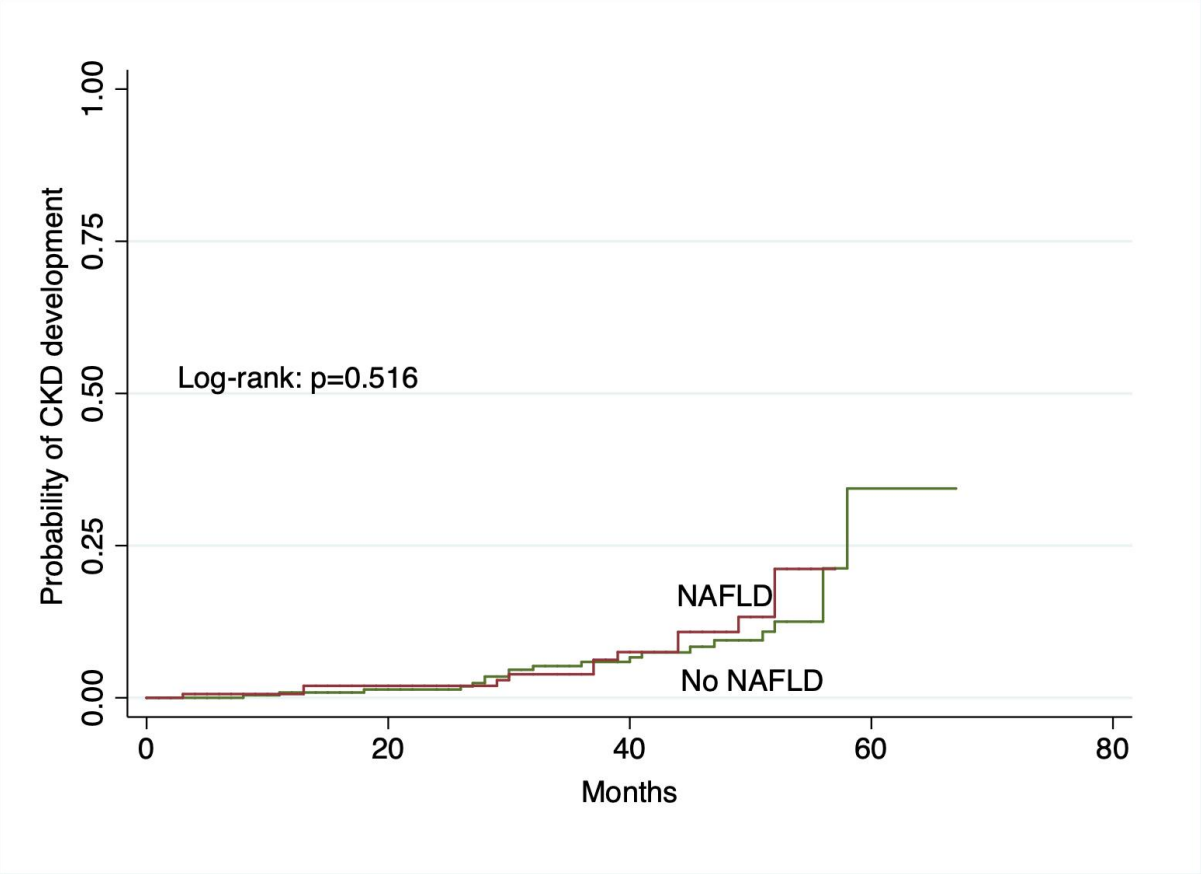


Figure 3d



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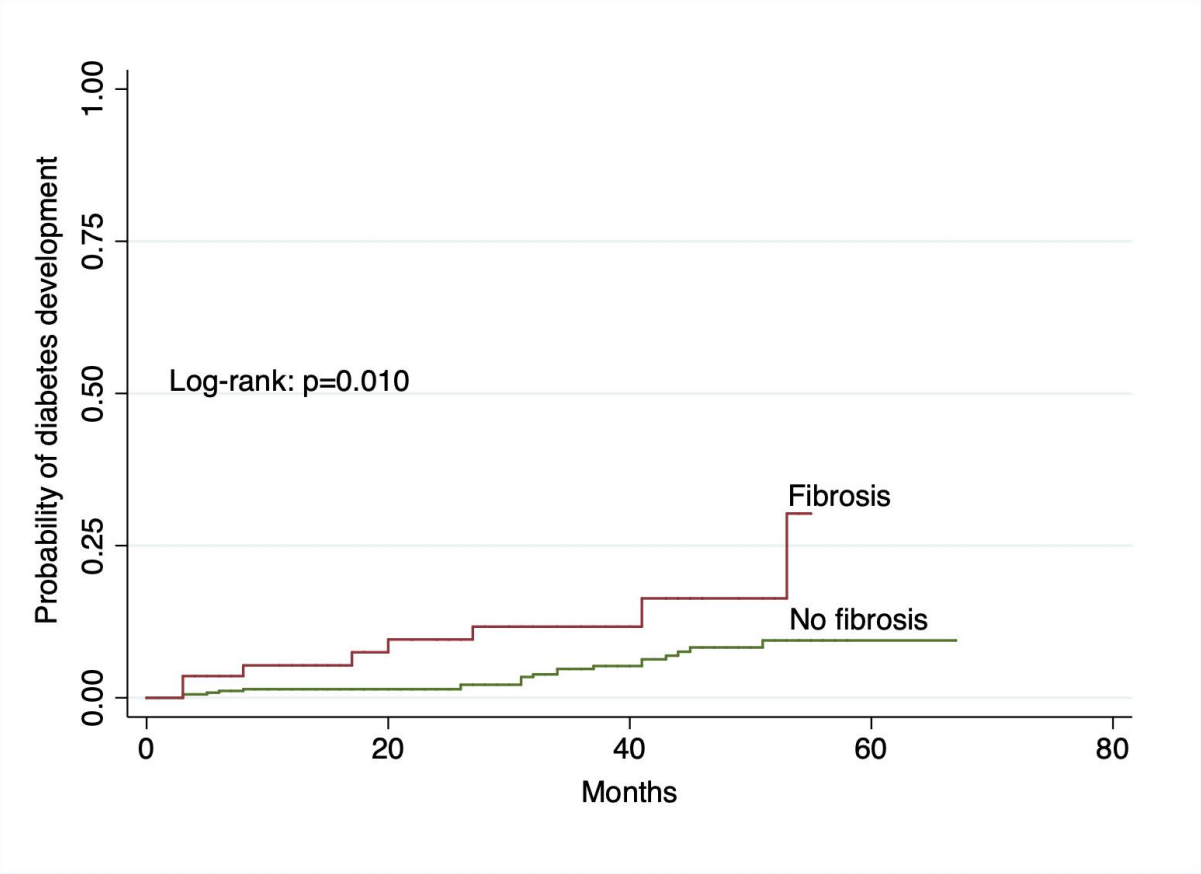
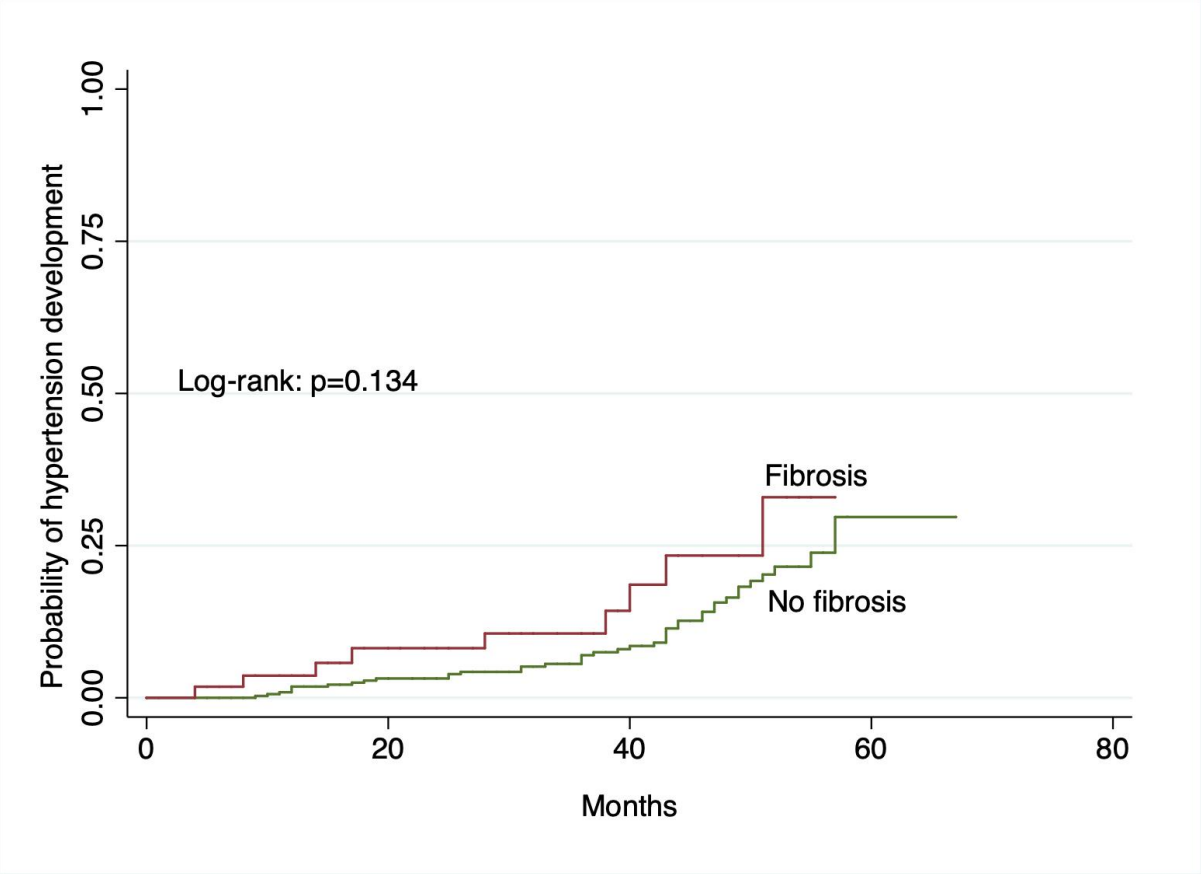


Figure 4b



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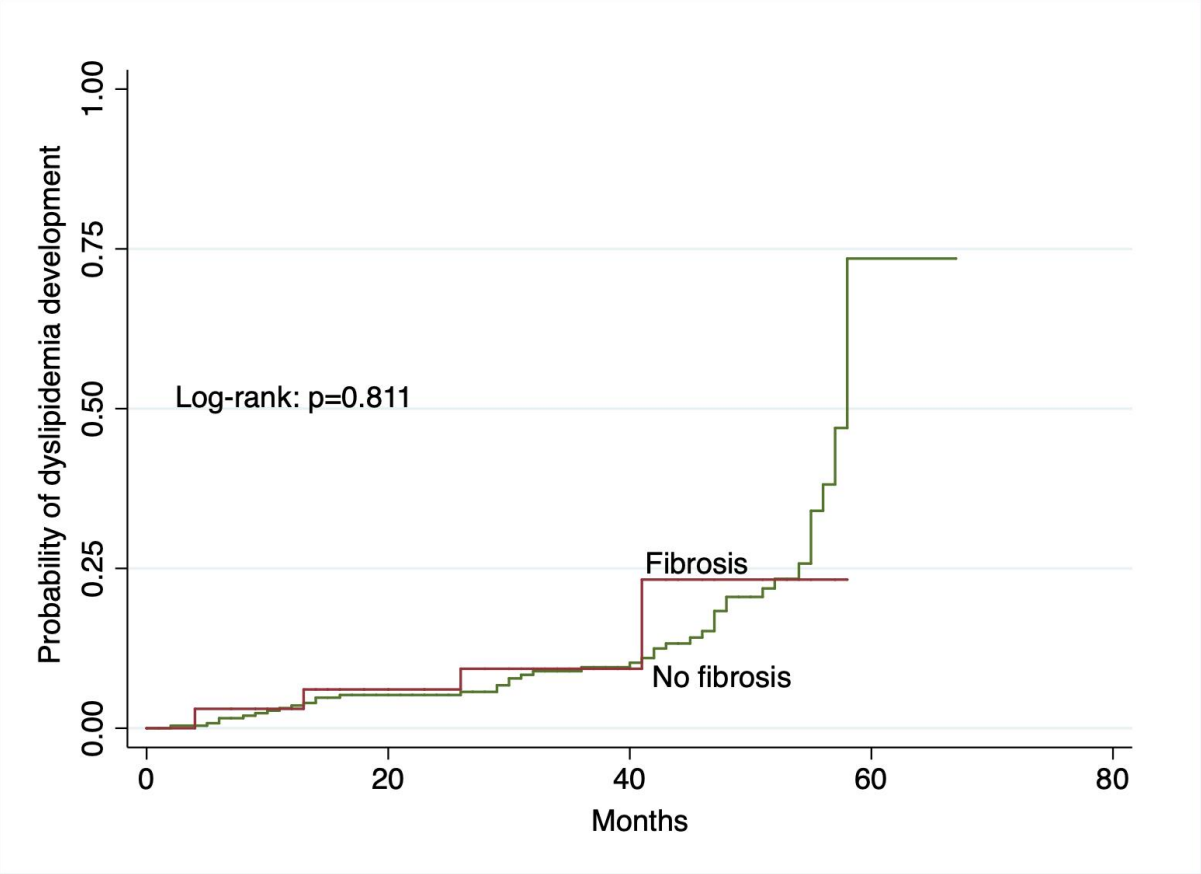

Figure 4d