U.PORTO

FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2022/2023

Sara Rodrigues Fernandes

The impact of integrase inhibitors on the development of hepatic steatosis and fibrosis in HIV-monoinfected patients

MARÇO, 2023

Sara Rodrigues Fernandes

The impact of integrase inhibitors on the development of hepatic steatosis and fibrosis in HIV-monoinfected patients

Mestrado Integrado em Medicina

Área: Doenças Infeciosas

Tipologia: Artigo de Investigação

Trabalho efetuado sob a Orientação de:

Doutora Paula Isabel Marques Simões Freitas

sob a Coorientação de:

Dra. Maria do Rosário de Valadares Souto Pinto Serrão Brito da Cunha

Trabalho organizado de acordo com as normas da revista:
BMC Infectious Diseases

MARÇO, 2023

UC Dissertação/Projeto (6º Ano) - DECLARAÇÃO DE INTEGRIDADE

Eu, Sara Rodrigues Fernandes, abaixo assinado, nº mecanográfico 201707139, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 24/03/2023

Assinatura conforme cartão de identificação:

Sara Rodrigues Fernandes

UC Dissertação/Projeto (6º Ano) – DECLARAÇÃO DE REPRODUÇÃO

NOME

Sara Rodrigues Fernandes

NÚMERO DE ESTUDANTE	E-MAIL
201707139	sararfernandes.vnc@gmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

Doenças Infeciosas

TÍTULO DISSERTAÇÃO

The impact of integrase inhibitors on the development of hepatic steatosis and fibrosis in HIV-monoinfected patients

ORIENTADOR

Doutora Paula Isabel Marques Simões Freitas

COORIENTADOR (se aplicável)

Dra. Maria do Rosário de Valadares Souto Pinto Serrão Brito da Cunha

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	☒

Faculdade de Medicina da Universidade do Porto, 24/03/2023

Assinatura conforme cartão de identificação:

Sara Rodrigues Fernandes

Um grande agradecimento à Doutora Paula Freitas e à Dra. Rosário Serrão, por me terem dado a oportunidade de realizar este projeto e, assim, conjugar duas áreas que tanto me fascinam. Muito obrigada pela disponibilidade e orientação.

À Dra. Ana Rita Leite, pela paciência e pela ajuda incansável e imprescindível.

Aos meus pais, pelo apoio incondicional e por me darem sempre o empurrãozinho que falta.

À minha família, por acreditar sempre em mim, quer esteja perto ou esteja longe.

Aos melhores colegas de turma e amigos, pelas histórias e conselhos, pela ajuda e motivação e por sofrermos, mas, acima de tudo, sermos felizes em conjunto. Obrigada por partilharem estes 6 anos comigo.

Aos meus amigos cerveirenses, por todos os momentos, pelas pausas, pelas verdinhas e por nunca duvidarem de mim.

Ao Francisco, pela calma, a paciência e as palavras nas horas mais difíceis.

Para os meus pais

The impact of integrase inhibitors on the development of hepatic steatosis and fibrosis in HIV-monoinfected patients

Sara Rodrigues Fernandes

Faculdade de Medicina, Universidade do Porto, Porto, Portugal

Ana Rita Leite

Serviço de Endocrinologia, Diabetes e Metabolismo do Centro Hospitalar Universitário São João, Faculdade de Medicina, Universidade do Porto, Portugal.

Rita Lino

Departamento de Doenças Infeciosas do Centro Hospitalar Universitário São João, Porto, Portugal.

André Rodrigues Guimarães

Departamento de Doenças Infeciosas do Centro Hospitalar Universitário São João, Porto, Portugal.

Carmela Pineiro

Departamento de Doenças Infeciosas do Centro Hospitalar Universitário São João, Porto, Portugal.

Rosário Serrão

Departamento de Doenças Infeciosas do Centro Hospitalar Universitário São João, Porto, Portugal.

Paula Freitas

Serviço de Endocrinologia, Diabetes e Metabolismo do Centro Hospitalar Universitário São João, Faculdade de Medicina, Investigação e Inovação em Saúde (i3s), Universidade do Porto, Portugal.

Abstract

Background: Non-alcoholic Fatty Liver Disease (NAFLD) has a high prevalence among patients with HIV infection. Since Integrase Strand Transfer Inhibitors (INSTIs) are used worldwide and have been associated with weight gain, we must determine their effect in the development of NAFLD and Non-alcoholic Steatohepatitis (NASH) in these patients. The aim of this study was to explore the impact of INSTIs in the development of liver steatosis and fibrosis in the patient with HIV infection, using Hepatic Steatosis Index (HSI), Fibrosis-4 Index (FIB-4), BARD score and NAFLD Fibrosis Score (NFS).

Methods: We performed a monocentric, retrospective cohort study in HIV-monoinfected cART-naïve patients that initiated INSTI based regimens between December 2019 and January 2022. Data was collected at baseline, 6 and 12 months after initiation. Demographic, clinical and laboratory characteristics, hepatic steatosis, and fibrosis scores were compared between baseline and last visit at 12 months. Linear regression models were performed to analyse the associations between analytical data at baseline and hepatic scores variation during the 12 months of treatment. Models were performed unadjusted and adjusted for age and sex.

Results: 99 patients were included in our study. Eighty-two percent were male and median age was 36 years. We observed a significant increase in body mass index (BMI), HDL, platelet count, albumin, and creatinine and a significant decrease in AST levels. HSI showed no statistically significant differences during follow-up (p=0.114). We observed a significant decrease in FIB-4 (p=0.007) and NFS (p=0.002). BARD score showed a significant increase (p=0.006). The linear regression model demonstrated a significant negative association between baseline HIV RNA and FIB-4 change (β= -0.08, 95% CI [-0.16 to -0.00], p=0.045), suggesting that higher HIV RNA loads at baseline were associated with a greater decrease in FIB-4.

Conclusion: INSTIs seem to have no impact on hepatic steatosis, even though they

were associated with a significant increase in BMI. This might be explained by the direct effect of a dolutegravir-containing regimen and/or by the “return-to-health effect” observed with cART initiation. Furthermore, INSTIs were associated with a reduction in risk of liver fibrosis in HIV-monoinfected patients, possibly due to their effect on viral suppression.

Keywords: HIV, integrase strand transfer inhibitors, non-alcoholic fatty liver disease, steatosis, liver fibrosis.

Background

Improvements in Human Immunodeficiency Virus (HIV) infection treatment has shifted the priorities in the clinical care of patients with this infection. Due to the increased access to combined Antiretroviral Therapy (cART), mortality amongst HIV-positive people has declined and life expectancy has been approaching that of the general population. Even though it remains the leading cause of death in this group of patients, Acquired Immunodeficiency Syndrome (AIDS)-related mortality has decreased, hence increasing the importance of non-AIDS related morbidities, such as non-AIDS cancers, liver disease, cardiovascular diseases, and stroke. (1, 2)

Non-alcoholic Fatty Liver Disease (NAFLD) is characterized by evidence of hepatic steatosis, without secondary causes for hepatic fat accumulation, and is related to metabolic comorbidities. NAFLD is divided into two categories, Non-alcoholic fatty liver (NAFL) and Non-alcoholic Steatohepatitis (NASH). NAFL is defined as the presence of steatosis in ≥5% of hepatocytes without hepatocyte ballooning. NASH is defined as the presence of steatosis in ≥5% of hepatocytes and inflammation with hepatocyte injury, associated or not to fibrosis. (3)

Although the true prevalence of NAFLD in the HIV infected patient is still unknown, Maurice et al. showed a prevalence of NAFLD and NASH, in these patients, of 35% and 42%, respectively. (4) According to Vodkin et al., there is a higher proportion of NASH and features of more severe liver injury in patients with HIV-associated NAFLD, when compared with patients with primary NAFLD, despite having similar metabolic characteristics. (5)

Multiple risk factors have been associated with the development of NAFLD in the HIV infected patient. These include factors that also have an association with NAFLD in the general population, such as sex, obesity, hypertriglyceridemia, and insulin resistance. However, factors associated with HIV itself, such as lipodystrophy and cART, contribute

to the disease as well. (6)

Previous studies have suggested the contribution of cART in the development of hepatic steatosis, due to its metabolic side effects. (7) In particular, various HIV protease inhibitors (PIs) have been associated with higher levels of insulin resistance. Most PIs, some Non-Nucleoside Reverse-Transcriptase Inhibitors (NNRTIs) such as efavirenz and some Nucleoside/nucleotide Reverse-Transcriptase Inhibitors (NRTIs) such as abacavir have been related to dyslipidemia. Stavudine and didanosine have been shown to induce mitochondrial toxicity, which also contributes to the development of NASH. (8)

Bischoff et al., demonstrated that the use of Integrase Strand Transfer Inhibitors (INSTIs) and/or Tenofovir-alafenamid (TAF) contributes to the occurrence of hepatic steatosis and progression to NASH, in the context of increased body weight. (9)

Liver biopsy is the gold standard for identifying both NASH and NAFLD. However, it has various limitations, as it is an invasive procedure with high costs, low acceptability, and sampling variability. Therefore, multiple non-invasive strategies have been studied and developed, as alternatives to this technique, including blood biomarkers and imaging techniques. (10) Scores based on blood biomarkers available to diagnose or grade steatosis include the Hepatic Steatosis Index (HSI), and to stage fibrosis include NAFLD Fibrosis Score (NFS) and BARD, which are more specific of NAFLD, and Aspartate Transaminase (AST)/Alanine Transaminase (ALT) Ratio and Fibrosis-4 Index (FIB-4), which have been developed in the context of hepatitis C. (11)

According to EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease, NFS, FIB-4, Enhanced Liver Fibrosis (ELF) or FibroTest calculation should be performed in every NAFLD patient to exclude significant fibrosis. If fibrosis is not excluded, then transient elastography should be performed. Only if this exam confirms significant fibrosis, should liver biopsy be done in order to establish the final diagnosis. (12)

Currently, INSTIs are recommended worldwide as first line treatment in HIV infection.

(13) With the growing number of patients under this treatment and the high prevalence of liver disease in the HIV infected patient, it becomes essential to determine the effect of these drugs in the development of NAFLD and liver fibrosis.

Therefore, we performed a retrospective cohort study with the aim of evaluating the impact of INSTIs in the risk of developing liver steatosis and fibrosis, using HSI, FIB-4, BARD and NFS indexes, in the patient with HIV infection.

Methods

Subjects

We performed an observational monocentric, retrospective cohort study in HIV-infected patients followed at the Infectious Diseases Outpatient Clinic of Centro Hospitalar Universitário de São João. This study included all treatment-naïve adults (age ≥ 18 years) that initiated an INSTI based regimen between December 2019 and January 2022 and maintained it during at least 12 months. Patients with reported Hepatitis C Virus (HCV) and/or Hepatitis B Virus (HBV) infection, pregnant at the beginning or during follow-up and with excessive alcohol use were excluded. This study was approved by the Ethics Committee for Health of Centro Hospitalar Universitário de São João and the requirement for a signed informed consent was waived.

Clinical assessment

For each patient the following information was collected: demographic data (age, sex), clinical comorbidities, duration of HIV infection, HIV infection risk factors, duration of cART, cART regimen and characterization of the infection. We used the “Centers for Disease Control and Prevention” (CDC) criteria for classifying the degree of the infection. (14) Weight and height were measured in routine consultation at baseline, before starting cART, and during follow-up. These data were collected through clinical records stored at

the hospital's electronic platform.

Laboratory analysis

Serum samples were tested at baseline, before starting cART (T0), and six months (T6) and twelve months (T12) after initiating cART. $CD4^+$ T cell count, type 1 HIV Ribonucleic Acid (RNA), platelet count, albumin, AST, ALT, total bilirubin, total cholesterol, High-density Lipoprotein (HDL) cholesterol, Low-density Lipoprotein (LDL) cholesterol, Triglycerides (TG), fasting glucose, creatinine, uric acid, and C Reactive Protein (CRP) levels were retrieved from clinical records through the hospital's electronic platform.

Hepatic steatosis and fibrosis evaluation

The HSI values were calculated automatically using the formula: 8 x (ALT/AST ratio) + Body Mass Index (BMI) (+2, if female; +2, if diabetes mellitus). The categories considered were NAFLD ruled out with HSI<30.0 and NAFLD detected with HSI>36.0. (15)

The FIB-4 values were calculated automatically using the formula: age (years) × AST [U/l] / (platelets [10^9/l] ×√ (ALT [U/l])). FIB-4 < 1.45 was considered as no or moderate fibrosis (F0-F1-F2-F3), and FIB-4 > 3.25 was considered as extensive fibrosis or cirrhosis (F4-F5-F6) (in the ISHAK classification of fibrosis). (16)

The BARD score was calculated as BMI ≥28 kg/m2 (1 point) + AST/ALT ratio ≥0.8 (2 points) + presence of diabetes (1 point). The categories considered were low risk of advanced fibrosis (0-1 score) or high risk of advanced fibrosis (2-4 score). (17)

The NFS values were calculated automatically using the formula: -1.675 + (0.037 x age [years]) + (0.094 x BMI [kg/m2]) + (1.13 x IFG/diabetes [yes = 1, no = 0]) + (0.99 x AST/ALT ratio) – (0.013 x platelet count [×10^9/L]) – (0.66 x albumin [g/dl]). We divided

the individuals in categories based on NFS score as low risk of advanced fibrosis with NFS<-1,455, intermediate risk with NFS between -1,455 and 0,672 and high risk with NFS>0,672. (11)

Statistical analysis

Demographic, clinical and laboratory characteristics and hepatic steatosis and fibrosis scores were compared between baseline and last visit at 12 months. Categorical variables were presented as absolute and relative frequencies. Continuous variables were expressed as means (standard deviation), if normally distributed, or as median (25^{th} to 75^{th} percentile), if non-normally distributed. Variables with skewed distribution were transformed to their natural logarithm.

Persons with missing baseline or follow-up data for the variables needed to calculate each score were excluded from the analysis of the respective score.

Differences in continuous variables between baseline and the last visit were assessed using paired t-test or Wilcoxon test, according to the distribution of the variables. McNemar test was used for categorical data.

Linear regression models were performed to analyse the associations between analytical data at baseline and the hepatic scores variation during the 12 months of treatment. Regression models were performed unadjusted and adjusted for age (18) and sex (19).

The statistical analysis was performed using SPSS version 27.0 (IBM Corporation, Armonk, NY). Two-sided *p* values <0.05 were considered significant.

The manuscript was prepared in adherence to the STROBE guidelines for cohort studies. (20)

Results

Characteristics of the Study Population

Overall, as demonstrated in Figure 1, 99 patients were included in our analysis, both at baseline and through follow-up, until last visit at 12 months. Eighty-two percent were male, and the median age was 36 years (28 to 50). (Table 1) The most frequent routes of transmission were men who have sex with men (60.4%) and heterosexual contact (29.7%). Thirty-nine percent of patients had a nadir CD4 cell count <200/ μL and 17.2% were diagnosed as having HIV stage C.

We were able to calculate BMI in both baseline and last visit only in 59 patients, due to weight and height data availability. At baseline, overweight, defined by a BMI of at least 25 and less than 30 kg/m^2, was observed in 19 (27.9%) patients, and obesity, defined by a BMI of at least 30 kg/m^2, was observed in 4 (5.9%).

We observed a significant increase in BMI, HDL, platelet count, albumin, and creatinine during follow-up. Furthermore, we observed a significant decrease in AST levels.

Hepatic fibrosis and steatosis scores

The median HSI values were 31.30 (26.78 to 34.82) at baseline and 31.48 (28.21 to 36.37) at the last visit, showing no statistically significant differences (p=0.114). The median difference in HSI score between baseline and last visit was 0.56 (-1.33 to 2.30). HSI scores <30, ruling out the presence of NAFLD, were observed in 31 (46.27%) and 26 (38.24%) of patients at baseline and last visit, respectively. HSI values >36, indicating presence of NAFLD, were observed in 13 (19.40%) and 17 (25.00%) of patients at baseline and last visit, respectively.

The median FIB-4 values were 1.02 (0.64 to 1.40) at baseline and 0.79 (0.60 to 1.20) at the last visit, showing a significant decrease (p=0.007). The median difference in FIB-4 values between baseline and last visit was -0.058 (-0.357 to 0.097). FIB-4 values <1.45,

indicating none or moderate fibrosis, were observed in 71 (76.34%) and 78 (82.98%) of patients at baseline and last visit, respectively. FIB-4 values >3.25, indicating extended fibrosis or cirrhosis, were observed in 4 (4.30%) and 3 (3.19%) of patients at baseline and last visit, respectively.

The mean of BARD scores was 1.82 (0.85) at baseline and 2.09 (0.73) at the last visit, showing a significant increase of this score during follow-up (p=0.006). The mean difference in BARD values between baseline and last visit was 0.37 (0.93). Eleven (16.4%) and 5 (7.4%) patients had BARD scores of either 0 or 1, representing a low risk for advanced fibrosis, at baseline and last visit, respectively. BARD scores between 2 and 4, representing a high risk of advanced fibrosis, were observed in 56 (83.6%) and 63 (92.7%) patients at baseline and last visit, respectively. However, only 13 (22%) patients had a different BARD score value between baseline and last visit. 46 (78%) patients showed no alteration in BARD score.

The median NFS values were -1.95 (-3.35 to -0.75) at baseline and -2.15 (-3.29 to -1.16) at the last visit, displaying a significant decrease in this score (p=0.002). The median difference in NFS values was -0.42 (-0.93 to 0.18) between baseline and last visit. NFS scores <-1.455, indicating low risk of advanced fibrosis, were observed in 38 (63.3%) and 45 (66.2%) of patients at baseline and last visit, respectively. NFS values between -1.455 and 0.672, representing intermediate risk, were found in 18 (30.0%) and 20 (29.4%) patients at baseline and last visit, respectively. NFS values >0.672, indicating high risk of advanced fibrosis, were observed in 4 (6.67%) and 2 (2.94%) patients at baseline and last visit, respectively.

In Figure 2, we show a decrease in FIB-4 and NFS throughout time, at baseline, 6 and 12 months, and an increase in BARD. HSI did not vary over time.

Analytical predictors of changes in hepatic fibrosis scores

In the unadjusted linear regression model (Table 2), there was a significant negative association between baseline HIV RNA and FIB-4 change, suggesting that higher HIV RNA loads at baseline are associated with a decrease in FIB-4 (β=-0.08 [-0.16 to 0.00]; p=0.045). After adjusting for age and sex, this association was no longer significant, although a trend for a negative association was found (β=-0.08 [-0.16 to 0.00]; p=0.062).

A significant positive association was observed between total bilirubin at baseline and BARD score change (β=1.09 [0.18 to 2.00]; p=0.019 in the adjusted model), suggesting that higher baseline bilirubin is associated with an increase in BARD.

The unadjusted linear regression model showed no association between HDL and NFS change, but, when adjusted for age and sex, there was a significant positive association with NFS change (β=0.03 [0.00 to 0.05]; p=0.036), indicating that higher baseline HDL cholesterol is associated with an increase in NFS.

No associations were found between any of the fibrosis scores and CD4 cell count, fasting glucose, total and LDL cholesterol, TG and CRP.

Discussion

In our single-center retrospective assessment of previously naïve HIV monoinfected patients on an INSTI based regimen, we observed a significant decrease in the values of FIB-4 and NFS scores, indicating a reduction in the risk of developing fibrosis in these patients. Also, we found a significant negative association between HIV RNA load at baseline and FIB-4 variation between baseline and 12 months, suggesting higher HIV RNA at baseline was significantly associated with a greater decrease in FIB-4.

Although, we did not see any significant changes in the HSI, that would indicate a change in steatosis, our findings supported that NAFLD is highly prevalent in HIV-infected patients, as demonstrated in previous studies. (4)

Macias et al. compared HIV-infected patients with NALFD who switched from efavirenz to raltegravir (RAL) with patients maintaining efavirenz-based therapy. After 48 weeks, they found that the patients who switched to RAL showed a reduction in the degree of hepatic steatosis, as measured by Controlled Attenuation Parameter (CAP) as well as a greater proportion of patients without significant steatosis. (21) This study agrees with our findings in suggesting that INSTIs do not contribute to the progression of hepatic steatosis. However, we did not find a similar reduction in hepatic steatosis. The mentioned study measures hepatic steatosis using CAP, a much more sensitive method of evaluating this parameter when compared to the HSI score used in our study, which might explain the differences in results.

On the other hand, Bischoff et al. showed that patients receiving INSTIs had a greater development and progression of steatosis and evolution towards NASH, in relation to increased body weight gain, which is contrary to our findings. (9) Similarly, a prospective cohort study showed that INSTIs were related to greater odds of moderate-to-severe hepatic steatosis. However, they did not find this relation to be true for every INSTI. This association was present for exposure to elvitegravir and RAL, but not to dolutegravir

(DTG), even though the patients receiving DTG had the highest weight gain. (22)

In our study, the INSTI 99% of patients was receiving was DTG. This way, the previously mentioned study comes to support our findings, and propose a hypothesis as to why they are not congruent with previous studies, such as the one performed by Bischoff et al., in which INSTIs used are not specified. Although INSTIs appear to contribute to the progression of hepatic steatosis in HIV monoinfected patients, this might not be true for DTG, despite its effect on weight gain. Riebensahm et al. suggested the same explanation for their findings of lack of relation between INSTIs and hepatic steatosis. (7) Therefore, to support this claim, more studies comparing the various INSTIs and their individual effects on hepatic steatosis are needed.

The patients in the present study showed a significant increase in BMI, which could be explained by multiple factors. On the one hand, several studies demonstrated a greater weight gain in patients receiving INSTI based regimens, especially DTG and RAL. (23, 24) On the other hand, studies have shown that the initiation of cART in treatment-naïve HIV-infected patients is associated with a short period of weight gain. Considering this is true particularly in patients with lower baseline CD4+ T-cell count and higher HIV RNA viral load, this is consistent with a “return to health effect”. (25, 26)

Contrary to the significant decrease in values of FIB-4 and NFS scores, we observed a significant increase in BARD score. These first two scores are continuous variables and BARD score is an ordinal variable, obtained from an addition of points. Although BARD score showed a significant increase, 80% of patients had the same BARD score at baseline and at the last visit, meaning differences were only visible in 13 patients out of 59 in total. Since the calculation of this score includes only BMI, AST/ALT ratio and the presence of diabetes, the fact that BMI showed a significant increase might have had a great impact in BARD score, possibly explaining its elevation. Such an impact would not be so visible in the other scores, since FIB-4 does not include BMI in its calculation and NFS is a much more complex index with various other liver function parameters.

Additionally, McPherson et al. compared multiple simple non-invasive fibrosis scoring systems, including the three scores we used in our study, and found FIB-4 score to have the best diagnostic accuracy for advanced fibrosis, with an Area Under Receiver Operator Characteristic Curve (AUROC) of 0.86. The AUROC for NFS was 0.81 and 0.77 for BARD. (27) Imajo et al. compared elastography and various risk scores to histology and found NFS and FIB-4 to be better than other indexes, including BARD, in predicting advanced fibrosis in patients with NAFLD. (28) Accordingly, both the guidelines by the European Association for the Study of the Liver and by the American Association for the Study of the Liver Diseases advocated the use of FIB-4 and NFS to rule out advanced liver fibrosis. (3, 12)

The decrease we observed in the risk of developing liver fibrosis, as demonstrated by the reduction in NFS and FIB-4 values, can probably be explained by the effects of cART in the suppression of HIV infection.

HIV infection alone contributes to the development of liver fibrosis, through multiple processes, such as mitochondrial injury, oxidative stress, fatty acid accumulation, gut microbial translocation and immune-activation and proapoptotic effects on hepatocytes. (29, 30) With viral suppression from cART, these mechanisms are reduced, thus decreasing hepatic fibrosis markers and scores in the patients receiving treatment.

Our linear regression model supported this hypothesis by showing that higher HIV RNA at baseline was significantly associated with a greater decrease in FIB-4. This indicates that patients with a higher activity of HIV at baseline, and consequently more liver damage induced by the above-mentioned mechanisms, had a greater reduction in risk of fibrosis with the initiation of treatment. Therefore, these findings support the early initiation of cART.

Multiple previous studies come to support our conclusions. Blackard et al. found an association between plasma HIV RNA levels and increased FIB-4 in HIV mono-infected women with no cART or alcohol use, as well as a negative association between CD4 cell

count and FIB-4. (31)

This was also true in HIV-coinfected patients, as shown by Bräu et al., who demonstrated that HIV suppression with cART led to a slower progression rate of HCV-induced fibrosis (32), and by Yang et al. who associated cART initiation with a significant reduction in fibrosis scores in HIV/HBV coinfected patients. (33)

Additionally, the findings of our linear regression model suggested that higher baseline bilirubin is associated with an increase in BARD, which is in line with previous studies that associate advanced liver fibrosis with increased bilirrubin. (34) Furthermore, this model, when adjusted for age and sex, suggested that higher baseline HDL cholesterol is associated with an increase in NFS, which is contrary to what has been shown in prior studies that associate HDL to regeneration and suppression of liver fibrosis. (35)

Our study had several limitations. It was a retrospective assessment of a small predominantly male cohort from one center in the north of Portugal, with no control group, therefore the results may not be generalizable to other populations. Our short follow-up time of 12 months allows us only to evaluate the short-term impact of the INSTIs and may underestimate their effect on liver steatosis and fibrosis on the long run. We used serum biomarkers to evaluate the presence of steatosis and fibrosis that have lower sensitivity and specificity than the gold standard test, liver biopsy. Other limitations were present in the availability of patient's data, possibly due to the COVID-19 period and the use of telephonic or virtual consultations. Weight and height information were not available for every patient at the three evaluation times, which led to BMI calculation only being possible in 59 patients. Additionally, only self-reported, not quantitatively specified, alcohol consumption was available, which might have led us to underestimate the presence of alcohol consumption in a small percentage of patients. Furthermore, data on waist and hip circumferences were not available. Consequently, we evaluated weight gain only considering BMI, which does not give information regarding the distribution of fat and presence of visceral fat, important factors in NAFLD.

Conclusion

In this monocenter cohort of HIV-monoinfected patients, INSTIs had no impact on hepatic steatosis, mainly driven by the use of a DTG-containing regimen. Additionally, INSTIs were associated with a significant increase in BMI, that might be explained by the direct effect of DTG and/or by the “return-to-health effect” observed with cART initiation. Furthermore, INSTIs were associated with a reduction in the risk of liver fibrosis in HIV-monoinfected patients, probably due to their effect on viral suppression.

Therefore, our study highlights the need for early initiation of cART, namely INSTI, as well as a close monitorization of patients with NAFLD, a disease with high prevalence among HIV-infected patients, in order to prevent the progression towards NASH and liver fibrosis.

List of abbreviations

AIDS – Acquired Immunodeficiency Syndrome

ALT – Alanine Transaminase

AST – Aspartate Transaminase

AUROC – Area Under Receiver Operator Characteristic Curve

BMI – Body Mass Index

CAP – controlled attenuation parameter

cART – combined Antiretroviral Therapy

CDC – Centers for Disease Control and Prevention

CRP – C Reactive Protein

DTG – Dolutegravir

ELF – Enhanced Liver Fibrosis

FIB-4 – Fibrosis-4 Index

HBV – Hepatitis B Virus

HCV – Hepatitis C Virus

HDL – High-density Lipoprotein

HIV – Human Immunodeficiency Virus

HSI – Hepatic Steatosis Index

INSTIs – Integrase Strand Transfer Inhibitors

LDL – Low-density Lipoprotein

NAFL – Non-alcoholic fatty liver

NAFLD – Non-alcoholic Fatty Liver Disease

NASH – Non-alcoholic Steatohepatitis

NFS – NAFLD Fibrosis Score

NNRTIs – Non-Nucleoside Reverse-Transcriptase Inhibitors

NRTIs – Nucleoside/nucleotide Reverse-Transcriptase Inhibitors

PIs – HIV protease inhibitors

RAL – Raltegravir

RNA – Ribonucleic Acid

T0 – Before starting cART

T12 – Twelve months of follow-up

T6 – Six months of follow-up

TAF – Tenofovir-alafenamid

TG – Triglycerides

References

1. Smith CJ, Ryom L, Weber R, Morlat P, Pradier C, Reiss P, et al. Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. The Lancet. 2014;384(9939):241-8.

2. Croxford S, Kitching A, Desai S, Kall M, Edelstein M, Skingsley A, et al. Mortality and causes of death in people diagnosed with HIV in the era of highly active antiretroviral therapy compared with the general population: an analysis of a national observational cohort. The Lancet Public Health. 2017;2(1):e35-e46.

3. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328-57.

4. Maurice JB, Patel A, Scott AJ, Patel K, Thursz M, Lemoine M. Prevalence and risk factors of nonalcoholic fatty liver disease in HIV-monoinfection. Aids. 2017;31(11):1621-32.

5. Vodkin I, Valasek MA, Bettencourt R, Cachay E, Loomba R. Clinical, biochemical and histological differences between HIV-associated NAFLD and primary NAFLD: a case-control study. Alimentary Pharmacology & Therapeutics. 2015;41(4):368-78.

6. Morse CG, McLaughlin M, Matthews L, Proschan M, Thomas F, Gharib AM, et al. Nonalcoholic Steatohepatitis and Hepatic Fibrosis in HIV-1-Monoinfected Adults With Elevated Aminotransferase Levels on Antiretroviral Therapy. Clin Infect Dis. 2015;60(10):1569-78.

7. Riebensahm C, Berzigotti A, Surial B, Günthard HF, Tarr PE, Furrer H, et al. Factors Associated With Liver Steatosis in People With Human Immunodeficiency Virus on Contemporary Antiretroviral Therapy. Open Forum Infectious Diseases. 2022;9(11).

8. Rockstroh JK. Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic Steatohepatitis (NASH) in HIV. Current HIV/AIDS Reports. 2017;14(2):47-53.

9. Bischoff J, Gu W, Schwarze-Zander C, Boesecke C, Wasmuth J-C, Van Bremen K, et al. Stratifying the risk of NAFLD in patients with HIV under combination antiretroviral therapy (cART). EClinicalMedicine. 2021;40:101116.

10. Makri E, Goulas A, Polyzos SA. Epidemiology, Pathogenesis, Diagnosis and Emerging Treatment of Nonalcoholic Fatty Liver Disease. Arch Med Res. 2021;52(1):25-37.

11. Castera L, Friedrich-Rust M, Loomba R. Noninvasive Assessment of Liver Disease in Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2019;156(5):1264-81.e4.

12. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016;64(6):1388-402.

13. Organization WH. Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach: World Health Organization; 2021.

14. Castro KG, Ward JW, Slutsker L, Buehler JW, Jaffe HW, Berkelman RL, et al. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. Clinical Infectious Diseases. 1993;17(4):802-10.

15. Lee J-H, Kim D, Kim HJ, Lee C-H, Yang JI, Kim W, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. Digestive and Liver Disease. 2010;42(7):503-8.

16. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. Hepatology. 2007;46(1):32-6.

17. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut. 2008;57(10):1441-7.

18. McPherson S, Hardy T, Dufour J-F, Petta S, Romero-Gomez M, Allison M, et al.

Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. The American journal of gastroenterology. 2017;112(5):740.

19. Yang JD, Abdelmalek MF, Pang H, Guy CD, Smith AD, Diehl AM, et al. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. Hepatology. 2014;59(4):1406-14.

20. Cuschieri S. The STROBE guidelines. Saudi journal of anaesthesia. 2019;13(Suppl 1):S31.

21. Macías J, Mancebo M, Merino D, Téllez F, Montes-Ramírez ML, Pulido F, et al. Changes in Liver Steatosis After Switching From Efavirenz to Raltegravir Among Human Immunodeficiency Virus–Infected Patients With Nonalcoholic Fatty Liver Disease. Clinical Infectious Diseases. 2017;65(6):1012-9.

22. Kirkegaard-Klitbo DM, Thomsen MT, Gelpi M, Bendtsen F, Nielsen SD, Benfield T. Hepatic steatosis associated with exposure to elvitegravir and raltegravir. Clinical Infectious Diseases. 2021;73(3):e811-e4.

23. Bourgi K, Jenkins CA, Rebeiro PF, Shepherd BE, Palella F, Moore RD, et al. Weight gain among treatment-naïve persons with HIV starting integrase inhibitors compared to non-nucleoside reverse transcriptase inhibitors or protease inhibitors in a large observational cohort in the United States and Canada. Journal of the International AIDS Society. 2020;23(4):e25484.

24. Kileel EM, Lo J, Malvestutto C, Fitch KV, Zanni MV, Fichtenbaum CJ, et al., editors. Assessment of obesity and cardiometabolic status by integrase inhibitor use in REPRIEVE: a propensity-weighted analysis of a multinational primary cardiovascular prevention cohort of people with human immunodeficiency virus. Open forum infectious diseases; 2021: Oxford University Press US.

25. Yuh B, Tate J, Butt AA, Crothers K, Freiberg M, Leaf D, et al. Weight change after antiretroviral therapy and mortality. Clinical Infectious Diseases. 2015;60(12):1852-9.

26. Grant PM, Kitch D, McComsey GA, Collier AC, Bartali B, Koletar SL, et al. Long-term body composition changes in antiretroviral-treated HIV-infected individuals. AIDS

(London, England). 2016;30(18):2805.

27. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut. 2010;59(9):1265-9.

28. Imajo K, Kessoku T, Honda Y, Tomeno W, Ogawa Y, Mawatari H, et al. Magnetic resonance imaging more accurately classifies steatosis and fibrosis in patients with nonalcoholic fatty liver disease than transient elastography. Gastroenterology. 2016;150(3):626-37. e7.

29. Kaspar MB, Sterling RK. Mechanisms of liver disease in patients infected with HIV. BMJ open gastroenterology. 2017;4(1):e000166.

30. Kovari H, Weber R. Influence of antiretroviral therapy on liver disease. Current Opinion in HIV and AIDS. 2011;6(4):272-7.

31. Blackard JT, Welge JA, Taylor LE, Mayer KH, Klein RS, Celentano DD, et al. HIV mono-infection is associated with FIB-4 - A noninvasive index of liver fibrosis - in women. Clin Infect Dis. 2011;52(5):674-80.

32. Bräu N, Salvatore M, Ríos-Bedoya CF, Fernández-Carbia A, Paronetto F, Rodríguez-Orengo JF, et al. Slower fibrosis progression in HIV/HCV-coinfected patients with successful HIV suppression using antiretroviral therapy. Journal of hepatology. 2006;44(1):47-55.

33. Yang R, Gui X, Ke H, Xiong Y, Gao S. Combination antiretroviral therapy is associated with reduction in liver fibrosis scores in patients with HIV and HBV co-infection. AIDS Research and Therapy. 2021;18:1-9.

34. Jarčuška P, Janičko M, Veselíny E, Jarčuška P, Skladaný Ľ. Circulating markers of liver fibrosis progression. Clinica Chimica Acta. 2010;411(15-16):1009-17.

35. Ding B-S, Liu CH, Sun Y, Chen Y, Swendeman SL, Jung B, et al. HDL activation of endothelial sphingosine-1-phosphate receptor-1 (S1P1) promotes regeneration and suppresses fibrosis in the liver. JCI insight. 2016;1(21).

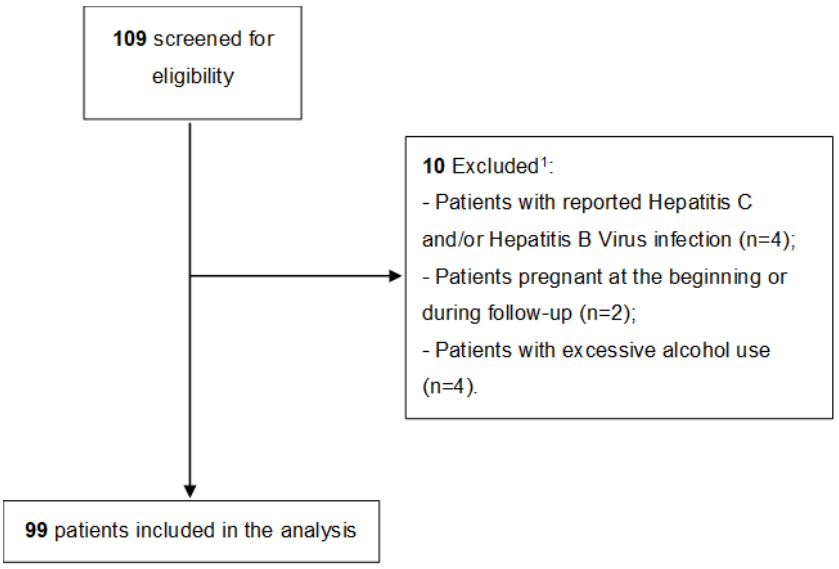

Figure 1 Flow diagram of the patients' selection.

[1]Patients could meet more than 1 exclusion criteria.

Table 1 Comparison of baseline and last visit characteristics of the study population.

Parameter	Baseline (n=99)	Last Visit (n=99)	P
Male	81 (81.8)		
Age, years	36.00 (28.00;50.00)	37.00 (29.00;51.00)	
Smoker	39 (39.4)		
BMI, kg/m^2 (n=59)	23.74 (3.74)	24.61 (3.99)	**<0.001**
25 to <30	19 (27.94)	21 (30.88)	**0.009**
≥30	4 (5.88)	9 (13.24)	
HIV-related parameters			
HIV RNA, 10^4 copies/mL	9.21 (3.18;25.10)	0.00 (0.00;0.03)	**<0.001**
HIV RNA (<50)	0	80 (79.20)	
CD4 cell count, cells/µL	259.00 (102.00;450.00)	539.50 (296.00;782.75)	**<0.001**
HIV risk factor			
Injecting drug user	1 (0.99)		
Homosexual contact	61 (60.39)		
Heterosexual contact	30 (29.70)		
CDC stage			
A	61 (61.62)		
B	21 (21.21)		
C	17 (17.17)		
cART Regimen			
TDF/FTC + DTG	36 (36.36)		
ABC/3TC/DTG	36 (36.36)		
3TC/DTG	22 (22.22)		
FTC/TAF/BIC	4 (4.04)		
TAF/FTC + DTG	1 (1.01)		

Analytical parameters			
Fasting Plasma Glucose, mg/dL	88.00 (80.00;94.00)	89.00 (83.00;100.00)	0.590
Triglycerides, mg/dl	97.50 (72.50;130.75)	90.00 (72.00;137.00)	0.773
Total cholesterol, mg/dl	156.79 (43.74)	170.67 (45.72)	0.112
HDL, mg/dl	40.23 (12.87)	48.59 (13.19)	**<0.001**
LDL, mg/dl	100.87 (31.84)	107.09 (32.67)	0.421
AST, U/L	26.00 (21.00;33.25)	24.00 (20.00;29.00)	**0.019**
ALT, U/L	22.00 (14.00;34.25)	19.00 (15.00;28.00)	0.115
Total bilirubin, mg/dL	0.60 (0.22)	0.59 (0.48;0.77)	0.076
Platelets, 10^3/µL	208.61 (78.46)	234.55 (61.02)	**<0.001**
Albumin, g/L	39.51 (7.29)	43.20 (40.80;45.10)	**<0.001**
Creatinine, mg/dL	0.78 (0.65;0.89)	0.96 (0.26)	**<0.001**
Uric acid, mg/dL	5.60 (4.70;6.35)	5.70 (4.80;6.40)	0.920
CRP, mg/L	4.15 (1.73;19.50)	2.60 (1.40;30.60)	0.515
Hepatic Fibrosis and Steatosis Scores			
HSI score (n=59)	31.30 (26.78;34.82)	31.48 (28.21;36.37)	0.114
<30	31 (46.27)	26 (38.24)	0.388
30 to 36	23 (34.33)	25 (36.76)	1.000
>36	13 (19.40)	17 (25.00)	0.453
FIB-4 score (n=92)	1.02 (0.64;1.40)	0.79 (0.60; 1.20)	**0.007**
<1.45	71 (76.34)	78 (82.98)	0.146
1.45 to 3.25	18 (19.35)	13 (13.83)	0.302
>3.25	4 (4.30)	3 (3.19)	1.000
BARD score (n=59)	1.82 (0.85)	2.09 (0.73)	**0.006**
0	9 (13.43)	4 (5.88)	0.070
1	2 (2.99)	1 (1.47)	1.000
2	50 (74.63)	50 (73.53)	1.000
3	4 (5.97)	11 (16.18)	**0.016**
4	2 (2.99)	2 (2.94)	1.000
NFS score (n=51)	-1.95 (-3.25; -0.75)	-2.15 (-3.29; -1.16)	**0.002**
<-1.455	38 (63.33)	45 (66.22)	0.146
-1.455 to 0.672	18 (30.00)	20 (29.41)	0.227
>0.672	4 (6.67)	2 (2.94)	1.000

Data are shown as mean (standard deviation), median (interquartile range) or n (%). P values were obtained using paired samples t-test, Wilcoxon test or McNemar test where appropriate. Statistical significance was set for a value of $p < 0.05$. In bold: $p < 0.05$.

Abbreviations: 3TC, lamivudine; ABC, Abacavir, ALT, alanine aminotransferase; AST, aspartate aminotransferase; BIC, bictegravir; BMI, body mass index; CRP, C-reactive protein; DTG, dolutegravir; FIB-4, fibrosis-4; FTC, emtricitabine; HDL, high-density lipoprotein; HSI, hepatic steatosis score; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; NFS, NAFLD fibrosis score; RNA, ribonucleic acid; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

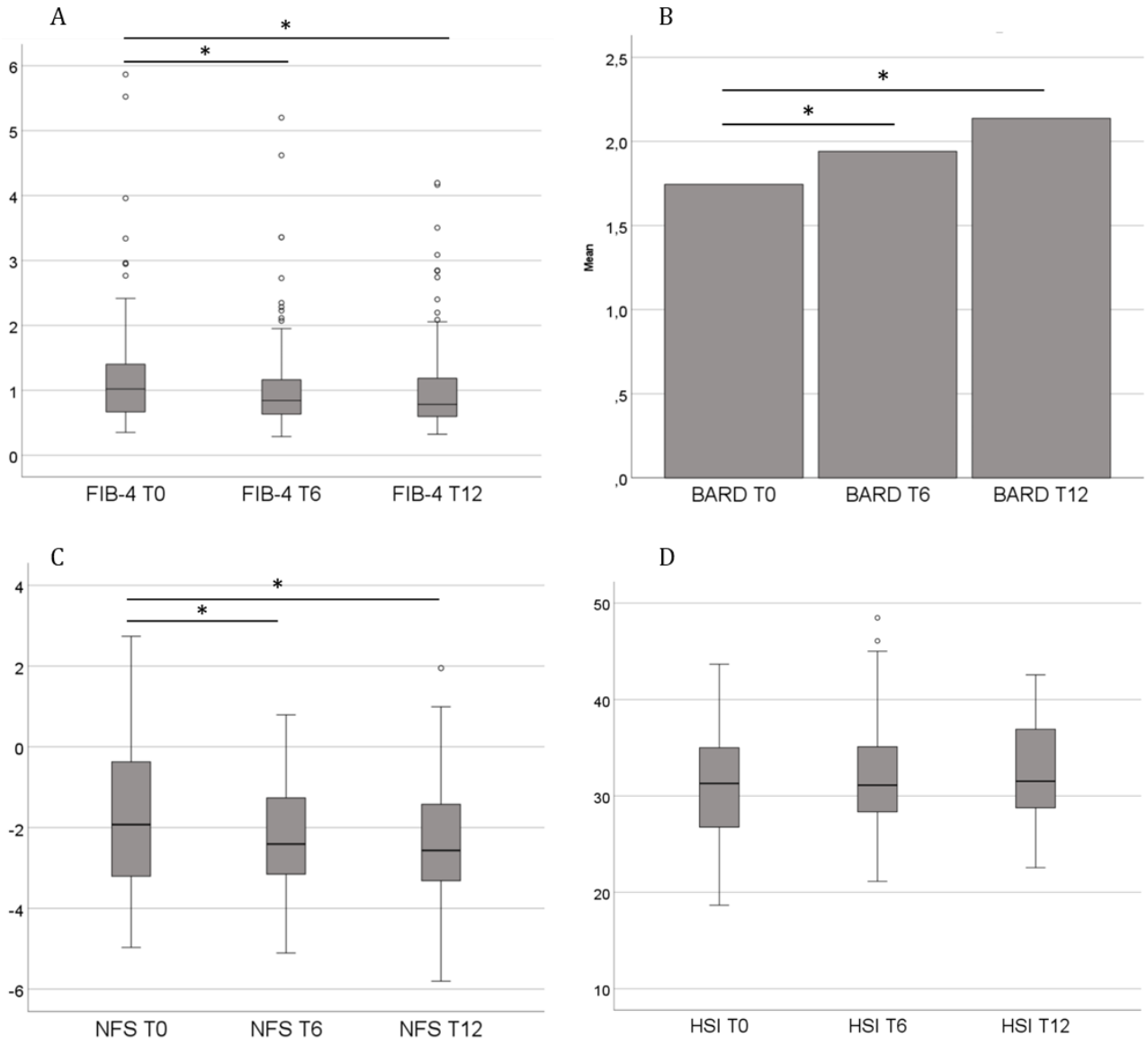

Figure 2 Boxplots and bar charts of liver steatosis and fibrosis scores at baseline, and 6 and 12 months after initiation of treatment with Integrase Strand Transfer Inhibitors.

A, Box-plot of FIB-4 values at baseline (T0), 6 months (T6) and 12 months (T12) of follow-up; B, Bar chart of mean BARD values at T0, T6 and T12; C, Box-plot of NFS values at T0, T6 and T12; D, Box-plot of HSI values at T0, T6 and T12. * $p<0.05$

Abbreviations: FIB-4, Fibrosis-4; HSI, Hepatic Steatosis Index; NFS, NAFLD Fibrosis Score.

Table 2. Associations between analytical variables at baseline and changes in hepatic fibrosis scores.

	FIB-4		BARD		NFS	
	β (95% CI)	**P value**	**β (95% CI)**	**P value**	**β (95% CI)**	**P value**
HIV RNA						
Unadjusted model	**-0.08 (-0.16 to -0.00)**	**0.045**	0.06 (-0.07 to 0.20)	0.346	-0.04 (-0.23 to 0.16)	0.691
Adjusted model	-0.08 (-0.16 to 0.00)	0.063	0.06 (-0.07 to 0.20)	0.354	-0.04 (-0.23 to 0.15)	0.672
CD4 cell count						
Unadjusted model	0.12 (-0.04 to 0.28)	0.141	-0.02 (-0.27 to 0.22)	0.854	0.13 (-0.22 to 0.48)	0.453
Adjusted model	0.10 (-0.08 to 0.28)	0.261	-0.03 (-0.28 to 0.22)	0.801	0.21 (-0.14 to 0.57)	0.238
Total bilirubin						
Unadjusted model	0.03 (-0.66 to 0.71)	0.941	**1.08 (0.22 to 1.94)**	**0.015**	-0.05 (-1.31 to 1.20)	0.931
Adjusted model	0.00 (-0.69 to 0.70)	0.993	**1.09 (0.18 to 2.00)**	**0.019**	-0.11 (-1.37 to 1.16)	0.867
Fasting glucose						
Unadjusted model	0.16 (-0.94 to 1.27)	0.772	-0.11 (-1.87 to 1.65)	0.902	-0.92 (-2.91 to 1.06)	0.350
Adjusted model	0.49 (-0.76 to 1.73)	0.439	-0.02 (-2.04 to 2.00)	0.985	-1.87 (-4.04 to 0.31)	0.090
Total cholesterol						
Unadjusted model	0.00 (-0.00 to 0.00)	0.679	0.00 (-0.00 to 0.01)	0.611	0.00 (-0.00 to 0.01)	0.292
Adjusted model	0.00 (-0.00 to 0.00)	0.698	0.00 (-0.01 to 0.01)	0.621	0.00 (-0.00 to 0.01)	0.356
HDL cholesterol						
Unadjusted model	0.00 (-0.01 to 0.01)	0.549	-0.00 (-0.03 to 0.02)	0.710	0.02 (-0.00 to 0.05)	0.059
Adjusted model	0.00 (-0.01 to 0.02)	0.491	-0.00 (-0.03 to 0.02)	0.702	**0.03 (0.00 to 0.05)**	**0.036**
LDL cholesterol						
Unadjusted model	0.00 (-0.00 to 0.01)	0.458	0.00 (-0.01 to 0.01)	0.534	0.00 (-0.01 to 0.01)	0.632
Adjusted model	0.00 (-0.00 to 0.01)	0.479	0.00 (-0.01 to 0.01)	0.540	0.00 (-0.01 to 0.01)	0.660
Triglycerides						
Unadjusted model	-0.19 (-0.50 to 0.12)	0.238	0.16 (-0.48 to 0.79)	0.616	-0.58 (-1.31 to 0.16)	0.120
Adjusted model	-0.20 (-0.52 to 0.12)	0.210	0.15 (-0.51 to 0.82)	0.645	-0.60 (-1.34 to 0.15)	0.112
C-reactive protein						
Unadjusted model	-0.07 (-0.23 to 0.09)	0.371	0.14 (-0.06 to 0.33)	0.158	-0.05 (-0.33 to 0.23)	0.720
Adjusted model	-0.08 (-0.24 to 0.08)	0.303	0.13 (-0.08 to 0.33)	0.216	-0.02 (-0.31 to 0.28)	0.913

1 Linear regression models of the association between variables at baseline (HIV RNA, CD4 cell
2 count, total bilirubin, fasting glucose, total, HDL and LDL cholesterols, triglycerides, and c-reactive

protein) and hepatic fibrosis scores (FIB-4, BARD and NFS). HIV RNA, CD4 cell count, fasting glucose, triglycerides, and c-reactive protein were log-transformed. Statistical significance was set for a value of $p < 0.05$.

Abbreviations: FIB-4, fibrosis-4; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; NFS, NAFLD fibrosis score; RNA, ribonucleic acid.

Apêndices

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1 "retrospective cohort study"
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 1 "We performed a monocentric, retrospective cohort study in HIV-monoinfected cART-naïve patients that initiated INSTI based regimens between December 2019 and January 2022. Data was collected at baseline, 6 and 12 months after initiation. Demographic, clinical and laboratory characteristics, hepatic steatosis, and fibrosis scores were compared between baseline and last visit at 12 months. Linear regression models were performed to analyse the associations between analytical data at baseline and hepatic scores variation during the 12 months of treatment. Models were performed unadjusted and adjusted for age and sex. Results: 99 patients were included in our study. Eighty-two percent were male and median age was 36 years. We observed a significant increase in body mass index (BMI), HDL, platelet count, albumin, and creatinine and a significant decrease in AST levels. HSI showed no statistically significant differences during follow-up (p=0.114). We observed a significant decrease in FIB-4 (p=0.007) and NFS (p=0.002). BARD score showed a significant increase (p=0.006). The linear regression model demonstrated a significant negative association between baseline HIV RNA and FIB-4 change (β= -0.08, 95% CI [-0.16 to -0.00], p=0.045), suggesting that higher HIV RNA loads at baseline were associated with a greater decrease in FIB-4."
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	Pages 3, 4 and 5 "Improvements in Human Immunodeficiency Virus (HIV) infection treatment has shifted the priorities in the clinical care of patients with this infection. Due to the increased access to combined Antiretroviral Therapy (cART), mortality amongst HIV-positive people has declined and life expectancy has been approaching that of the general population. Even though it remains the leading cause of death in this group of patients, Acquired Immunodeficiency Syndrome (AIDS)-related mortality has decreased, hence increasing the importance of non-AIDS related morbidities, such as non-AIDS cancers, liver disease, cardiovascular diseases, and stroke. (1, 2) Non-alcoholic Fatty Liver Disease (NAFLD) is characterized by evidence of hepatic steatosis, without secondary causes for hepatic fat accumulation, and is related to metabolic comorbidities. NAFLD is divided into two categories, Non-alcoholic fatty liver (NAFL) and Non-alcoholic Steatohepatitis (NASH). NAFL is defined as the presence of steatosis in ≥5% of hepatocytes without hepatocyte ballooning. NASH is defined as the presence of steatosis in ≥5% of hepatocytes and inflammation with hepatocyte injury, associated or not to fibrosis. (3) Although the true prevalence of NAFLD in the HIV infected patient is still unknown, Maurice et al. showed a prevalence of NAFLD and NASH, in these patients, of 35% and 42%, respectively. (4) According to Vodkin et al., there is a higher proportion of NASH and features of more severe liver injury in patients with HIV-associated NAFLD, when compared with patients with primary NAFLD, despite having similar metabolic characteristics. (5)

			Multiple risk factors have been associated with the development of NAFLD in the HIV infected patient. These include factors that also have an association with NAFLD in the general population, such as sex, obesity, hypertriglyceridemia, and insulin resistance. However, factors associated with HIV itself, such as lipodystrophy and cART, contribute to the disease as well. (6) Previous studies have suggested the contribution of cART in the development of hepatic steatosis, due to its metabolic side effects. (7) In particular, various HIV protease inhibitors (PIs) have been associated with higher levels of insulin resistance. Most PIs, some Non-Nucleoside Reverse-Transcriptase Inhibitors (NNRTIs) such as efavirenz and some nucleoside/nucleotide reverse-transcriptase inhibitors (NRTIs) such as abacavir have been related to dyslipidemia. Stavudine and didanosine have been shown to induce mitochondrial toxicity, which also contributes to the development of NASH. (8) Bischoff et al., demonstrated that the use of Integrase Strand Transfer Inhibitors (INSTIs) and/or Tenofovir-alafenamid (TAF) contributes to the occurrence of hepatic steatosis and progression to NASH, in the context of increased body weight. (9) Liver biopsy is the gold standard for identifying both NASH and NAFLD. However, it has various limitations, as it is an invasive procedure with high costs, low acceptability, and sampling variability. Therefore, multiple non-invasive strategies have been studied and developed, as alternatives to this technique, including blood biomarkers and imaging techniques. (10) Scores based on blood biomarkers available to diagnose or grade steatosis include the Hepatic Steatosis Index (HSI), and to stage fibrosis include NAFLD Fibrosis Score (NFS) and BARD, which are more specific of NAFLD, and Aspartate Transaminase (AST)/Alanine Transaminase (ALT) Ratio and Fibrosis-4 Index (FIB-4), which have been developed in the context of hepatitis C. (11) According to EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease, NFS, FIB-4, Enhanced Liver Fibrosis (ELF) or FibroTest calculation should be performed in every NAFLD patient to exclude significant fibrosis. If fibrosis is not excluded, then transient elastography should be performed. Only if this exam confirms significant fibrosis, should liver biopsy be done in order to establish the final diagnosis. (12) Currently, INSTIs are recommended worldwide as first line treatment in HIV infection. (13) With the growing number of patients under this treatment and the high prevalence of liver disease in the HIV infected patient, it becomes essential to determine the effect of these drugs in the development of NAFLD and liver fibrosis. Therefore, we performed a retrospective cohort study with the aim of evaluating the impact of INSTIs in the risk of developing liver steatosis and fibrosis, using HSI, FIB-4, BARD and NFS indexes, in the patient with HIV infection.”
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 5 “Therefore, we performed a retrospective cohort study with the aim of evaluating the impact of INSTIs in the hepatic fibrosis markers and in the risk of developing liver steatosis and fibrosis(…)”
Methods			
Study design	4	Present key elements of study design early in the paper	Page 5 “We performed an observational monocentric, retrospective cohort study”
Setting	5	Describe the setting, locations, and	Page 5

		relevant dates, including periods of recruitment, exposure, follow-up, and data collection	"HIV-infected patients followed at the Infectious Diseases Outpatient Clinic of Centro Hospitalar Universitário de São João.(…) between December 2019 and January 2022 and maintained it during at least 12 months" Page 5 "Weight and height were measured in routine consultation at baseline, before starting cART, and during follow-up." Page 6 "Serum samples were tested at baseline, before starting cART (T0), and six months (T6) and twelve months (T12) after initiating cART"
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 6 "HIV-infected patients followed at the Infectious Diseases Outpatient Clinic of Centro Hospitalar Universitário de São João. This study included all treatment-naïve adults (age ≥ 18 years) that initiated an INSTI based regimen between December 2019 and January 2022 and maintained it during at least 12 months. Patients with reported Hepatitis C Virus (HCV) and/or Hepatitis B Virus (HBV) infection, pregnant at the beginning or during follow up and with excessive alcohol use were excluded."
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Non applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 5 "demographic data (age, sex), clinical comorbidities, duration of HIV infection, HIV infection risk factors, duration of cART, cART regimen and characterization of the infection" "Weight and height" Page 6 "CD4+ T cell count, type 1 HIV Ribonucleic Acid (RNA), platelet count, albumin, AST, ALT, total bilirubin, total cholesterol, High-density Lipoprotein (HDL) cholesterol, Low-density Lipoprotein (LDL) cholesterol, triglycerides (TG), fasting glucose, creatinine, uric acid and C Reactive Protein (CRP)" Pages 6 and 7 "HSI values(…) NAFLD ruled out with HSI<30.0 and NAFLD detected with HSI>36.0(…)FIB-4 values (…) FIB-4 < 1.45 was considered as no or moderate fibrosis (F0-F1-F2-F3), and FIB-4 > 3.25 was considered as extensive fibrosis or cirrhosis (F4-F5-F6) (in the ISHAK classification of fibrosis (…) BARD score (…) low risk of advanced fibrosis (0-1 score) or high risk of advanced fibrosis (2-4 score). (…) NFS values (…) low risk of advanced fibrosis with NFS<-1,455, intermediate risk with NFS between -1,455 and 0,672 and high risk with NFS>0,672"
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 5 "These data were collected through clinical records stored at the hospital's electronic platform." Page 6 "The HSI values were calculated automatically using the formula: 8 x (ALT/AST ratio) + Body Mass Index (BMI) (+2, if female; +2, if diabetes mellitus)." "The FIB-4 values were calculated automatically using the formula: age (years) × AST [U/l] / (platelets [10^9/l] ×√ (ALT [U/l]))." "The BARD score was calculated as BMI ≥28 kg/m2 (1 point) + AST/ALT ratio ≥0.8 (2 points) + presence of diabetes (1 point)." "The NFS values were calculated automatically using the formula: -1.675 + (0.037 x age [years]) + (0.094 x BMI [kg/m2]) + (1.13 x

			IFG/diabetes [yes = 1, no = 0]) + (0.99 x AST/ALT ratio) – (0.013 x platelet count [×109/L]) – (0.66 x albumin [g/dl]).”
Bias	9	Describe any efforts to address potential sources of bias	Page 5 “Patients with reported Hepatitis C Virus (HCV) and/or Hepatit is B Virus (HBV) infection, pregnant at the beginning or during follow- up and with excessive alcohol use were excluded.” Page 7 “Regression models were performed unadjusted and adjusted for age and sex.”
Study size	10	Explain how the study size was arrived at	Page 5 “HIV-infected patients followed at the Infectious Diseases Outpatient Clinic of Centro Hospitalar Universitário de São João. This study included all treatment-naïve adults (age ≥ 18 years) that initiated an INSTI based regimen between December 2019 and January 2022 and maintained it during at least 12 months. Patients with reported Hepatitis C Virus (HCV) and/or Hepatitis B Virus (HBV) infection, pregnant at the beginning or during follow up and with excessive alcohol use were excluded.”
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 7 “Continuous variables were expressed as means (standard deviation), if normally distributed, or as median (25th to 75th percentile), if non-normally distributed. Variables with skewed distribution were transformed to their natural logarithm.”
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 7 “Differences in continuous variables between baseline and the last visit were assessed using paired t-test or Wilcoxon test, according to the distribution of the variables. Chi-squared tests were used for categorical data. Linear regression models were performed to analyse the associations between analytical data at baseline and the hepatic scores variation during the 12 months of treatment. Regression models were performed unadjusted and adjusted for age and sex.”
		(*b*) Describe any methods used to examine subgroups and interactions	Page 7 “Linear regression models were performed to analyse the associations between analytical data at baseline and the hepatic scores variation during the 12 months of treatment. Regression models were performed unadjusted and adjusted for age and sex.”
		(*c*) Explain how missing data were addressed	Page 7 “Persons with missing baseline or follow-up data for the variables needed to calculate each score were excluded from the analysis of the respective score.”
		(*d*) If applicable, explain how loss to follow-up was addressed	Non applicable
		(*e*) Describe any sensitivity analyses	No sensitivity analyses were performed
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible,	Page 8 “Overall, as demonstrated in Figure 1, 99 patients were included in our analysis, both at baseline and through follow-up, until last visit at 12 months.” Page 21 Figure 1

		examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	Page 21 Figure 1
		(c) Consider use of a flow diagram	Page 21 Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 21 Table 1 Page 8 "Overall, as demonstrated in Figure 1, 99 patients were included in our analysis, both at baseline and through follow-up, until last visit at 12 months. Eighty-two percent were male, and the median age was 36 years (28 to 50). (Table 1) The most frequent routes of transmission were men who have sex with men (60.4%) and heterosexual contact (29.7%). Thirty-nine percent of patients had a nadir CD4 cell count <200/ μL and 17.2% were diagnosed as having HIV stage C. We were able to calculate BMI in both baseline and last visit only in 59 patients, due to weight and height data availability. At baseline, overweight, defined by a BMI of at least 25 and less than 30 kg/m2, was observed in 19 (27.9%) patients, and obesity, defined by a BMI of at least 30 kg/m2, was observed in 4 (5.9%). We observed a significant increase in BMI, high-density lipoprotein (HDL), platelet count, albumin, and creatinine during follow-up. Furthermore, we observed a significant decrease in AST levels."
		(b) Indicate number of participants with missing data for each variable of interest	Pages 21 and 22 - Table 1 "BMI, kg/m2 (n=59)" "FIB-4 score (n=92)" "BARD score (n=59)" "NFS score (n=51)" "HSI score (n=59)"
		(c) Summarise follow-up time (eg, average and total amount)	Page 8 "99 patients were included in our analysis, both at baseline and through follow-up, until last visit at 12 months."
Outcome data	15*	Report numbers of outcome events or summary measures over time	Page 21 and 22 Table 1 Pages 8 and 9 "The median HSI values were 31.30 (26.78 to 34.82) at baseline and 31.48 (28.21 to 36.37) at the last visit, showing no statistically significant differences (p=0.114). The median difference in HSI score between baseline and last visit was 0.56 (-1.33 to 2.30). HSI scores <30, ruling out the presence of NAFLD, were observed in 31 (46.27%) and 26 (38.24%) of patients at baseline and last visit, respectively. HSI values >36, indicating presence of NAFLD, were observed in 13 (19.40%) and 17 (25.00%) of patients at baseline and last visit, respectively. The median FIB-4 values were 1.02 (0.64 to 1.40) at baseline and 0.79 (0.60 to 1.20) at the last visit, showing a significant decrease (p=0.007). The median difference in FIB-4 values between baseline and last visit was -0.058 (-0.357 to 0.097). FIB-4 values <1.45, indicating none or moderate fibrosis, were observed in 71 (76.34%) and 78 (82.98%) of patients at baseline and last visit, respectively.

			FIB-4 values >3.25, indicating extended fibrosis or cirrhosis, were observed in 4 (4.30%) and 3 (3.19%) of patients at baseline and last visit, respectively. The mean of BARD scores was 1.82 (0.85) at baseline and 2.09 (0.73) at the last visit, showing a significant increase of this score during follow-up (p=0.006). The mean difference in BARD values between baseline and last visit was 0.37 (0.93). Eleven (16.4%) and 5 (7.4%) patients had BARD scores of either 0 or 1, representing a low risk for advanced fibrosis, at baseline and last visit, respectively. BARD scores between 2 and 4, representing a high risk of advanced fibrosis, were observed in 56 (83.6%) and 63 (92.7%) patients at baseline and last visit, respectively. However, only 13 (22%) patients had a different BARD score value between baseline and last visit. 46 (78%) patients showed no alteration in BARD score. The median NFS values were -1.95 (-3.35 to -0.75) at baseline and -2.15 (-3.29 to -1.16) at the last visit, displaying a significant decrease in this score (p=0.002). The median difference in NFS values was -0.42 (-0.93 to 0.18) between baseline and last visit. NFS scores <-1.455, indicating low risk of advanced fibrosis, were observed in 38 (63.3%) and 45 (66.2%) of patients at baseline and last visit, respectively. NFS values between -1.455 and 0.672, representing intermediate risk, were found in 18 (30.0%) and 20 (29.4%) patients at baseline and last visit, respectively. NFS values >0.672, indicating high risk of advanced fibrosis, were observed in 4 (6.67%) and 2 (2.94%) patients at baseline and last visit, respectively. In Figure 2, we show a decrease in FIB-4 and NFS throughout time, at baseline, 6 and 12 months, and an increase in BARD. HSI did not vary over time.”

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 23 - Table 2 Page 7 "Regression models were performed unadjusted and adjusted for age (18) and sex (19)." Page 10 "In the unadjusted linear regression model (Table 2), there was a significant negative association between baseline HIV RNA and FIB-4 change, suggesting that higher HIV RNA loads at baseline are associated with a decrease in FIB-4 (β=-0.08 [-0.16 to 0.00]; p=0.045). After adjusting for age and sex, this association was no longer significant, although a trend for a negative association was found (β=-0.08 [-0.16 to 0.00]; p=0.062). A significant positive association was observed between total bilirubin at baseline and BARD score change (β=1.09 [0.18 to 2.00]; p=0.019 in the adjusted model), suggesting that higher baseline bilirubin is associated with an increase in BARD. The unadjusted linear regression model showed no association between HDL and NFS change, but, when adjusted for age and sex, there was a significant positive association with NFS change (β=0.03 [0.00 to 0.05]; p=0.036), indicating that higher baseline HDL cholesterol is associated with an increase in NFS. No associations were found between any of the fibrosis scores and CD4 cell count, fasting glucose, total and LDL cholesterol, TG and CRP."
		(*b*) Report category boundaries when continuous variables were categorized	Page 8 "At baseline, overweight, defined by a BMI of at least 25 and less than 30 kg/m2, (…) and obesity, defined by a BMI of at least 30 kg/m2" "HSI scores <30, ruling out the presence of NAFLD (…) HSI values >36, indicating presence of NAFLD" Page 9 "FIB-4 values <1.45, indicating none or moderate fibrosis (…) FIB-4 values >3.25, indicating extended fibrosis or cirrhosis" "BARD scores of either 0 or 1, representing a low risk for advanced fibrosis (…) BARD scores between 2 and 4, representing a high risk of advanced fibrosis" "NFS scores <-1.455, indicating low risk of advanced fibrosis (…) NFS values between -1.455 and 0.672, representing intermediate risk (…) NFS values >0.672, indicating high risk of advanced fibrosis"
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Non applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Non applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 11 "In our single-center retrospective assessment of previously naïve HIV monoinfected patients on an INSTI based regimen, we observed a significant decrease in the values of FIB-4 and NFS scores, indicating a reduction in the risk of developing fibrosis in these patients. Also, we found a significant negative association

			between HIV RNA load at baseline and FIB-4 variation between baseline and 12 months, suggesting higher HIV RNA at baseline was significantly associated with a greater decrease in FIB-4."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 14 "Our study had several limitations. It was a retrospective assessment of a small predominantly male cohort from one center in the north of Portugal, with no control group, therefore the results may not be generalizable to other populations. Our short follow-up time of 12 months allows us only to evaluate the short-term impact of the INSTIs and may underestimate their effect on liver steatosis and fibrosis on the long run. We used serum biomarkers to evaluate the presence of steatosis and fibrosis that have lower sensitivity and specificity than the gold standard test, liver biopsy. Other limitations were present in the availability of patient's data, possibly due to the COVID-19 period and the use telephonic or virtual consultations. Weight and height information were not available for every patient at the three evaluation times, which led to BMI calculation only being possible in 59 patients. Additionally, only self-reported, not quantitatively specified, alcohol consumption was available, which might have led us to underestimate the presence of alcohol consumption in a small percentage of patients. Furthermore, data on waist and hip circumferences were not available. Consequently, we evaluated weight gain only considering BMI, which does not give information regarding the distribution of fat and presence of visceral fat, important factors in NAFLD."
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 11, 12 and 13 "Although, we did not see any significant changes in the HSI, that would indicate a change in steatosis, our findings supported that NAFLD is highly prevalent in HIV-infected patients, as demonstrated in previous studies. (4) Macias et al. compared HIV-infected patients with NALFD who switched from efavirenz to raltegravir (RAL) with patients maintaining efavirenz-based therapy. After 48 weeks, they found that the patients who switched to RAL showed a reduction in the degree of hepatic steatosis, as measured by Controlled Attenuation Parameter (CAP) as well as a greater proportion of patients without significant steatosis. (21) This study agrees with our findings in suggesting that INSTIs do not contribute to the progression of hepatic steatosis. However, we did not find a similar reduction in hepatic steatosis. The mentioned study measures hepatic steatosis using CAP, a much more sensitive method of evaluating this parameter when compared to the HSI score used in our study, which might explain the differences in results. On the other hand, Bischoff et al. showed that patients receiving INSTIs had a greater development and progression of steatosis and evolution towards NASH, in relation to increased body weight gain, which is contrary to our findings. (9) Similarly, a prospective cohort study showed that INSTIs were related to greater odds of moderate-to-severe hepatic steatosis. However, they did not find this relation to be true for every INSTI. This association was present for exposure to elvitegravir and RAL, but not to dolutegravir (DTG), even though the patients receiving DTG had the highest weight gain. (22) In our study, the INSTI 99% of patients was receiving was DTG. This way, the previously mentioned study comes to support our findings, and propose a hypothesis as to why they are not congruent with previous studies, such as the one performed by Bischoff et al., in which INSTIs used are not specified. Although INSTIs appear to contribute to the progression of hepatic steatosis in HIV monoinfected patients, this might not be true for DTG, despite its effect on weight gain. Riebensahm et al. suggested the same explanation for their findings of lack of relation between INSTIs and hepatic steatosis. (7) Therefore, to support this claim, more studies comparing the various INSTIs and their individual effects on hepatic steatosis are needed.

			The patients in the present study showed a significant increase in BMI, which could be explained by multiple factors. On the one hand, several studies demonstrated a greater weight gain in patients receiving INSTI based regimens, especially DTG and RAL. (23, 24) On the other hand, studies have shown that the initiation of cART in treatment-naïve HIV-infected patients is associated with a short period of weight gain. Considering this is true particularly in patients with lower baseline CD4+ T-cell count and higher HIV RNA viral load, this is consistent with a "return to health effect". (25, 26) Contrary to the significant decrease in values of FIB-4 and NFS scores, we observed a significant increase in BARD score. These first two scores are continuous variables and BARD score is an ordinal variable, obtained from an addition of points. Although BARD score showed a significant increase, 80% of patients had the same BARD score at baseline and at the last visit, meaning differences were only visible in 13 patients out of 59 in total. Since the calculation of this score includes only BMI, AST/ALT ratio and the presence of diabetes, the fact that BMI showed a significant increase might have had a great impact in BARD score, possibly explaining its elevation. Such an impact would not be so visible in the other scores, since FIB-4 does not include BMI in its calculation and NFS is a much more complex index with various other liver function parameters. Additionally, McPherson et al. compared multiple simple non-invasive fibrosis scoring systems, including the three scores we used in our study, and found FIB-4 score to have the best diagnostic accuracy for advanced fibrosis, with an Area Under Receiver Operator Characteristic Curve (AUROC) of 0.86. The AUROC for NFS was 0.81 and 0.77 for BARD. (27) Imajo et al. compared elastography and various risk scores to histology and found NFS and FIB-4 to be better than other indexes, including BARD, in predicting advanced fibrosis in patients with NAFLD. (28) Accordingly, both the guidelines by the European Association for the Study of the Liver and by the American Association for the Study of the Liver Diseases advocated the use of FIB-4 and NFS to rule out advanced liver fibrosis. (3, 12) The decrease we observed in the risk of developing liver fibrosis, as demonstrated by the reduction in NFS and FIB-4 values, can probably be explained by the effects of cART in the suppression of HIV infection. HIV infection alone contributes to the development of liver fibrosis, through multiple processes, such as mitochondrial injury, oxidative stress, fatty acid accumulation, gut microbial translocation and immune-activation and proapoptotic effects on hepatocytes. (29, 30) With viral suppression from cART, these mechanisms are reduced, thus decreasing hepatic fibrosis markers and scores in the patients receiving treatment. Our linear regression model supported this hypothesis by showing that higher HIV RNA at baseline was significantly associated with a greater decrease in FIB-4. This indicates that patients with a higher activity of HIV at baseline, and consequently more liver damage induced by the above-mentioned mechanisms, had a greater reduction in risk of fibrosis with the initiation of treatment. Therefore, these findings support the early initiation of cART. Multiple previous studies come to support our conclusions. Blackard et al. found an association between plasma HIV RNA levels and increased FIB-4 in HIV mono-infected women with no cART or alcohol use, as well as a negative association between CD4 cell count and FIB-4. (31) This was also true in HIV-coinfected patients, as shown by Bräu et al., who demonstrated that HIV suppression with cART led to a slower progression rate of HCV-induced fibrosis (32), and by Yang et al. who associated cART initiation with a significant reduction in fibrosis scores in HIV/HBV coinfected patients. (33)

			Additionally, the findings of our linear regression model suggested that higher baseline bilirubin is associated with an increase in BARD, which is in line with previous studies that associate advanced liver fibrosis with increased bilirrubin. (34) Furthermore, this model, when adjusted for age and sex, suggested that higher baseline HDL cholesterol is associated with an increase in NFS, which is contrary to what has been shown in prior studies that associate HDL to regeneration and suppression of liver fibrosis. (35)"
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 14 "It was a retrospective assessment of a small predominantly male cohort from one center in the north of Portugal, with no control group, therefore the results may not be generalizable to other populations."
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Non applicable

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Regras de formatação da revista *BMC Infectious Diseases*

General formatting guidelines

- Preparing main manuscript text
- Preparing illustrations and figures
- Preparing tables
- Preparing additional files

Preparing figures

When preparing figures, please follow the formatting instructions below.

- Figures should be numbered in the order they are first mentioned in the text, and uploaded in this order. Multi-panel figures (those with parts a, b, c, d etc.) should be submitted as a single composite file that contains all parts of the figure.
- Figures should be uploaded in the correct orientation.
- Figure titles (max 15 words) and legends (max 300 words) should be provided in the main manuscript, not in the graphic file.
- Figure keys should be incorporated into the graphic, not into the legend of the figure.
- Each figure should be closely cropped to minimize the amount of white space surrounding the illustration. Cropping figures improves accuracy when placing the figure in combination with other elements when the accepted manuscript is prepared for publication on our site. For more information on individual figure file formats, see our detailed instructions.
- Individual figure files should not exceed 10 MB. If a suitable format is chosen, this file size is adequate for extremely high quality figures.
- **Please note that it is the responsibility of the author(s) to obtain permission from the copyright holder to reproduce figures (or tables) that have previously been published elsewhere.** In order for all figures to be open access, authors must have permission from the rights holder if they wish to include images that have been published elsewhere in non open access journals. Permission should be indicated in the figure legend, and the original source included in the reference list.

Figure file types

We accept the following file formats for figures:

- EPS (suitable for diagrams and/or images)
- PDF (suitable for diagrams and/or images)
- Microsoft Word (suitable for diagrams and/or images, figures must be a single page)
- PowerPoint (suitable for diagrams and/or images, figures must be a single page)
- TIFF (suitable for images)
- JPEG (suitable for photographic images, less suitable for graphical images)
- PNG (suitable for images)
- BMP (suitable for images)
- CDX (ChemDraw - suitable for molecular structures)

For information and suggestions of suitable file formats for specific figure types, please see our author academy.

Figure size and resolution

Figures are resized during publication of the final full text and PDF versions to conform to the BioMed Central standard dimensions, which are detailed below.

Figures on the web:

- width of 600 pixels (standard), 1200 pixels (high resolution).

Figures in the final PDF version:

- width of 85 mm for half page width figure
- width of 170 mm for full page width figure
- maximum height of 225 mm for figure and legend
- image resolution of approximately 300 dpi (dots per inch) at the final size

Figures should be designed such that all information, including text, is legible at these dimensions. All lines should be wider than 0.25 pt when constrained to standard figure widths. All fonts must be embedded.

Preparing main manuscript text

Quick points:

- Use double line spacing
- Include line and page numbering
- Use SI units: Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF
- Do not use page breaks in your manuscript

File formats

The following word processor file formats are acceptable for the main manuscript document:

- Microsoft word (DOC, DOCX)
- Rich text format (RTF)
- TeX/LaTeX (use BioMed Central's TeX template)

Please note: editable files are required for processing in production. If your manuscript contains any non-editable files (such as PDFs) you will be required to re-submit an editable file when you submit your revised manuscript, or after editorial acceptance in case no revision is necessary.

Preparing tables

When preparing tables, please follow the formatting instructions below.

- Tables should be numbered and cited in the text in sequence using Arabic numerals (i.e. Table 1, Table 2 etc.).
- Tables less than one A4 or Letter page in length can be placed in the appropriate location within the manuscript.
- Tables larger than one A4 or Letter page in length can be placed at the end of the document text file. Please cite and indicate where the table should appear at the relevant location in the text file so that the table can be added in the correct place during production.
- Larger datasets, or tables too wide for A4 or Letter landscape page can be uploaded as additional files. Please see [below] for more information.
- Tabular data provided as additional files can be uploaded as an Excel spreadsheet (.xls) or comma separated values (.csv). Please use the standard file extensions.
- Table titles (max 15 words) should be included above the table, and legends (max 300 words) should be included underneath the table.
- Tables should not be embedded as figures or spreadsheet files, but should be formatted using 'Table object' function in your word processing program.

- Color and shading may not be used. Parts of the table can be highlighted using superscript, numbering, lettering, symbols or bold text, the meaning of which should be explained in a table legend.
- Commas should not be used to indicate numerical values.

If you have any questions or are experiencing a problem with tables, please contact the customer service team at info@biomedcentral.com.

Preparing additional files

As the length and quantity of data is not restricted for many article types, authors can provide datasets, tables, movies, or other information as additional files.

All Additional files will be published along with the accepted article. Do not include files such as patient consent forms, certificates of language editing, or revised versions of the main manuscript document with tracked changes. Such files, if requested, should be sent by email to the journal's editorial email address, quoting the manuscript reference number. Please do not send completed patient consent forms unless requested.

Results that would otherwise be indicated as "data not shown" should be included as additional files. Since many web links and URLs rapidly become broken, BioMed Central requires that supporting data are included as additional files, or deposited in a recognized repository. Please do not link to data on a personal/departmental website. Do not include any individual participant details. The maximum file size for additional files is 20 MB each, and files will be virus-scanned on submission. Each additional file should be cited in sequence within the main body of text.

If additional material is provided, please list the following information in a separate section of the manuscript text:

- File name (e.g. Additional file 1)
- File format including the correct file extension for example .pdf, .xls, .txt, .pptx (including name and a URL of an appropriate viewer if format is unusual)
- Title of data
- Description of data

Additional files should be named "Additional file 1" and so on and should be referenced explicitly by file name within the body of the article, e.g. 'An additional movie file shows this in more detail [see Additional file 1]'.

For further guidance on how to use Additional files or recommendations on how to present particular types of data or information, please see How to use additional files.

Preparing your manuscript

The information below details the section headings that you should include in your manuscript and what information should be within each section.

Please note that your manuscript must include a 'Declarations' section including all of the subheadings (please see below for more information).

Title page

The title page should:

- present a title that includes, if appropriate, the study design e.g.:
    - "A versus B in the treatment of C: a randomized controlled trial", "X is a risk factor for Y: a case control study", "What is the impact of factor X on subject Y: A systematic review"
    - or for non-clinical or non-research studies a description of what the article reports
- list the full names and institutional addresses for all authors
    - if a collaboration group should be listed as an author, please list the Group name as an author. If you would like the names of the individual members of the Group to be searchable through their individual PubMed records, please include this information in the "Acknowledgements" section in accordance with the instructions below
    - Large Language Models (LLMs), such as ChatGPT, do not currently satisfy our authorship criteria. Notably an attribution of authorship carries with it accountability for the work, which cannot be effectively applied to LLMs. Use of an LLM should be properly documented in the Methods section (and if a Methods section is not available, in a suitable alternative part) of the manuscript.
- indicate the corresponding author

Abstract

The Abstract should not exceed 350 words. Please minimize the use of abbreviations and do not cite references in the abstract. Reports of randomized controlled trials should follow the CONSORT extension for abstracts. The abstract must include the following separate sections:

- **Background:** the context and purpose of the study
- **Methods:** how the study was performed and statistical tests used
- **Results:** the main findings
- **Conclusions:** brief summary and potential implications

- **Trial registration:** If your article reports the results of a health care intervention on human participants, it must be registered in an appropriate registry and the registration number and date of registration should be stated in this section. If it was not registered prospectively (before enrollment of the first participant), you should include the words 'retrospectively registered'. See our editorial policies for more information on trial registration

Keywords

Three to ten keywords representing the main content of the article.

Background

The Background section should explain the background to the study, its aims, a summary of the existing literature and why this study was necessary or its contribution to the field.

Methods

The methods section should include:

- the aim, design and setting of the study
- the characteristics of participants or description of materials
- a clear description of all processes, interventions and comparisons. Generic drug names should generally be used. When proprietary brands are used in research, include the brand names in parentheses
- the type of statistical analysis used, including a power calculation if appropriate

Results

This should include the findings of the study including, if appropriate, results of statistical analysis which must be included either in the text or as tables and figures.

Discussion

This section should discuss the implications of the findings in context of existing research and highlight limitations of the study.

Conclusions

This should state clearly the main conclusions and provide an explanation of the importance and relevance of the study reported.

List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations should be provided.

References

Examples of the Vancouver reference style are shown below.

See our editorial policies for author guidance on good citation practice

Web links and URLs: All web links and URLs, including links to the authors' own websites, should be given a reference number and included in the reference list rather than within the text of the manuscript. They should be provided in full, including both the title of the site and the URL, as well as the date the site was accessed, in the following format: The Mouse Tumor Biology Database. http://tumor.informatics.jax.org/mtbwi/index.do. Accessed 20 May 2013. If an author or group of authors can clearly be associated with a web link, such as for weblogs, then they should be included in the reference.

Example reference style:

Article within a journal

Smith JJ. The world of science. Am J Sci. 1999;36:234-5.

Article within a journal (no page numbers)

Rohrmann S, Overvad K, Bueno-de-Mesquita HB, Jakobsen MU, Egeberg R, Tjønneland A, et al. Meat consumption and mortality - results from the European Prospective Investigation into Cancer and Nutrition. BMC Medicine. 2013;11:63.

Article within a journal by DOI

Slifka MK, Whitton JL. Clinical implications of dysregulated cytokine production. Dig J Mol Med. 2000; doi:10.1007/s801090000086.

Article within a journal supplement

Frumin AM, Nussbaum J, Esposito M. Functional asplenia: demonstration of splenic activity by bone marrow scan. Blood 1979;59 Suppl 1:26-32.

Book chapter, or an article within a book

Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. In: Bourne GH, Danielli JF, Jeon KW, editors. International review of cytology. London: Academic; 1980. p. 251-306.

OnlineFirst chapter in a series (without a volume designation but with a DOI)

Saito Y, Hyuga H. Rate equation approaches to amplification of enantiomeric excess and chiral symmetry breaking. Top Curr Chem. 2007. doi:10.1007/128_2006_108.

Complete book, authored

Blenkinsopp A, Paxton P. Symptoms in the pharmacy: a guide to the management of common illness. 3rd ed. Oxford: Blackwell Science; 1998.

Online document

Doe J. Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry. 1999. http://www.rsc.org/dose/title of subordinate document. Accessed 15 Jan 1999.

Online database

Healthwise Knowledgebase. US Pharmacopeia, Rockville. 1998. http://www.healthwise.org. Accessed 21 Sept 1998.

Supplementary material/private homepage

Doe J. Title of supplementary material. 2000. http://www.privatehomepage.com. Accessed 22 Feb 2000.

University site

Doe, J: Title of preprint. http://www.uni-heidelberg.de/mydata.html (1999). Accessed 25 Dec 1999.

FTP site

Doe, J: Trivial HTTP, RFC2169. ftp://ftp.isi.edu/in-notes/rfc2169.txt (1999). Accessed 12 Nov 1999.

Organization site

ISSN International Centre: The ISSN register. http://www.issn.org (2006). Accessed 20 Feb 2007.

Dataset with persistent identifier

Zheng L-Y, Guo X-S, He B, Sun L-J, Peng Y, Dong S-S, et al. Genome data from sweet and grain sorghum (Sorghum bicolor). GigaScience Database. 2011. http://dx.doi.org/10.5524/100012.

Figures, tables and additional files

See General formatting guidelines for information on how to format figures, tables and additional files.

Submit manuscript

SÃO JOÃO

Declaração

Para os devidos efeitos declaro que o estudo 'Impacto dos Inibidores da Integrase nos marcadores de fibrose hepática no doente com infeção pelo VIH', apresentado a esta Comissão de Ética pelo Dra. Sara Rodrigues Fernandes, no âmbito do MIM da FMUP, foi avaliado e aprovado em 7 de fevereiro de 2023, autorizado pelo RAI, e enviado para parecer do EPD em 1 de março de 2023.

Porto e Centro Hospitalar Universitário de São João, 21 de março de 2023

Secretário da CE do CHUSJ/FMUP

Pedro Brito