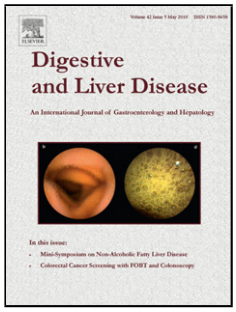

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Author: Rosa Lombardi Helen Sambatakou Ilias Mariolis Demosthenis Cokkinos George V. Papatheodoridis Emmanuel A. Tsochatzis

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Prevalence and predictors of liver steatosis and fibrosis in unselected patients with HIV mono-infection

Liver fibrosis in unselected HIV mono-infected patients

Rosa Lombardi[1], Helen Sambatakou[2], Ilias Mariolis[2], Demosthenis Cokkinos[3], George V. Papatheodoridis[4*], Emmanuel A. Tsochatzis[1*]

1. Sheila Sherlock Liver Unit and UCL Institute for Liver and Digestive Health, Royal Free Hospital and UCL, London, UK

2. 2nd Department of Internal Medicine, Hippokration General Hospital, Athens, Greece

3. Department of Radiology, Evangelismos General Hospital, Athens, Greece

4. Academic Department of Gastroenterology, Laiko General Hospital, Athens Greece

Corresponding author: Emmanuel A. Tsochatzis, MD, PhD, UCL Institute for Liver and Digestive Health, Royal Free Hospital and UCL.

Email: e.tsochatzis@ucl.ac.uk, phone: (0044)2077940500 ext 31142

*These two authors contributed equally and are joint senior authors

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Abstract:

Objectives: Significant liver disease may develop in HIV mono-infected patients, usually associated with fatty liver and/or cART exposure. We estimated the prevalence and predictors of hepatic steatosis and fibrosis as assessed by ultrasound and transient elastography (TE).

Methods: We enrolled 125 consecutive HIV mono-infected patients who underwent ultrasound and TE. Clinical, biochemical, immunological, virological features and medication history were analysed.

Results: Mean age was 39.5±10.3 years and 91% were male. Metabolic syndrome (MS) was present in 9.8%, diabetes in 5.6%, hypertension in 9.7%, dyslipidemia in 32.8%. Increased AST and ALT were found in 5.6% and 16.8% respectively. Eighty-five (68%) patients were on cART (median length of treatment of 3 years, IQR 0-17). Hepatic steatosis was detected in 61 (55%) patients and was independently associated with male sex (OR 14.6, 95%CI 1.44-**148.17**), age (OR **1.082**, 95%CI 1.01-**1.16**), HOMA (OR 2.56, 95%CI **1.101-5.96**) and GGT (OR **1.037**, 95%CI **1.007**-1.075). Significant fibrosis (stiffness >7.4 KPa) was present in 22 patients (17.6%) and was significantly associated with MS (OR 3.99, 95%CI 0.992-16.09).

Conclusions: Liver fibrosis can develop in asymptomatic HIV mono-infected patients. This is likely associated with NAFLD and usually manifests with normal transaminases. Non-invasive screening for the presence of NAFLD and fibrosis should be considered in the routine care of such patients.

Key words: Non-invasive assessment; elastography; FIB4; APRI; diagnostic accuracy; NAFLD

Introduction

Liver disease is an increasingly important issue in patients with HIV infection and is a leading cause of mortality second only to AIDS (1). This increase in liver-related morbidity and mortality is a consequence of the dramatic decrease in early AIDS-related deaths due to the introduction of effective highly active antiretroviral therapy (HAART) (2). Apart from viral co-infections with HCV and/or HBV, non-alcoholic fatty liver disease (NAFLD) in the setting of metabolic syndrome or antiretroviral therapy exposure is an emerging cause.

NAFLD is the hepatic manifestation of the metabolic syndrome (MS) and consists of a broad spectrum of diseases ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) that could potentially lead to cirrhosis and hepatocellular cancer (3). It has a prevalence of 20% in the general population of industrialized countries but can rise up to 50 % in HIV-infected people, due to the additive effects of antiretroviral treatment, lipodystrophy and the HIV virus per se (4, 5). Indeed, NAFLD could represent a long-term toxicity of HAART as both nucleoside reverse-transcriptase inhibitor (NRTIs) and protease inhibitors (PIs) are associated with the onset of insulin resistance and/or mitochondrial toxicity (6).

Non-invasive fibrosis tests (NITs) are increasingly used for the assessment of fibrosis in patients with NAFLD (7). Of these, transient elastography (TE) has advantages over serum markers in patients with HIV, as it is not influenced by concomitant antiretroviral medication, HIV replication, CD4 cell count, or non-liver inflammatory processes (8, 9).

In this study, we evaluated the prevalence and predictors of hepatic steatosis and fibrosis as assessed by ultrasound and simple NITs and TE respectively in a consecutive unselected cohort of HIV mono-infected patients.

Methods

Study population

Over a 6-month period, we included 125 consecutive patients with HIV mono-infection who were followed up at the dedicated HIV outpatient clinic at Hippokration General Hospital in Athens. Patients with HCV or HBV co-infection were excluded. The study was approved by the hospital ethical committee.

All patients had baseline laboratory investigations, and epidemiological and anthropometric features were recorded. BMI was calculated as weight (Kg)/height (m^2). Waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest. **The presence of lipoatrophy and/or lipohypertrophy was assessed during clinical examination**.

An increase in transaminases level was defined as values of AST and ALT more than 39 U/L and 41 U/L, respectively. MS was diagnosed according to the American Heart Association/National Heart, Lung and Blood Institute criteria (10). Insulin resistance (IR) was assessed by HOMA index (fasting glucose mg/dl x fasting insulin mg/dl/22.5), and values more than 3 were considered diagnostic. **All patients had a full liver screen performed, including viral hepatitis serology, autoimmune serology, ferritin and transferrin saturation, a1-antithrypsin levels and ceruloplasmin levels. Commercially available enzyme immunoassays were used for the detection of HBV markers (HBsAg, HBeAg, anti-HBe, anti-HBc), anti-HCV and anti-HDV and commercially available polymerase chain reaction assays for the detection of serum HBV DNA and HCV RNA. In patients who were anti-HBc and anti-HBsAg positive, HBV DNA was determined.**
Patients were interviewed about their smoking habits (packs/year) and drinking pattern; an alcohol consumption of more than 20g/day for women and 30g/day for men was considered as alcohol abuse.

A detailed HIV infection history was available, including duration of infection, CD4+ count(n°/μL) and HIV viral load (RNA copies/μL), current and previous antiretroviral therapy and duration of treatment.

Liver stiffness and steatosis assessment

All patients underwent ultrasound and TE by dedicated operators (DC and ET respectively) after at least six hours of fasting. The presence and severity of hepatic steatosis was evaluated by ultrasound; in particular, the grade of HS was diagnosed according to characteristic imaging features, namely bright liver pattern, liver-kidney contrast, vascular blurring and/or deep hepatic attenuation.

TE was performed with FibroScan®(Echosens; Paris, France) and the results were expressed in Kilopascals (KPa). The final value was calculated as the mean of ten valid measurements, obtained by placing the probe on the patient's skin between the ribs at the level of the right lobe of the liver in dorsal decubitus position. In order to ensure a reliable determination of liver stiffness, an interquartile range for measurements within 30% and ratio of success rate of measurements (number of total measurements/number of valid acquisition) >60% were considered adequate. Liver stiffness values >7.4 kPa were considered suggestive of significant fibrosis.

The presence of significant fibrosis (≥F2) was evaluated with the APRI index, which consists of AST and platelet count, with validated cut-off values of <0.5 and >1.5 for ruling out or diagnosing the target condition respectively (11). The presence of advanced fibrosis (≥F3) was evaluated by the FIB4 score, which consists of age, AST, ALT and platelet count and validated cut-offs values of <1.45 and >3.25 for ruling out or in advanced fibrosis respectively were used (12).

Statistical analysis

All data were analysed using the statistical package SPSS (version 22.0, IBM, New York, NY, USA). Statistical analysis was performed using t-test, ANOVA, Mann-Whitney test or Kruskal-Wallis test for comparisons of continuous variables between or among groups, corrected chi-squared method or two-tailed Fisher's exact test for comparisons of qualitative data and Spearman's co-efficient for correlations of quantitative data, when appropriate. Multivariate analysis was performed using logistic regression models. Only variables with a P value ≤0.10 at univariate analysis were entered in the multivariate analysis models. A two-tailed P value <0.05 was considered to be statistically significant.

Results

Baseline characteristics

In total, 125 HIV mono-infected patients were enrolled in the study. The mean age was 39.5 years and 91% were males. Baseline characteristics are shown in Table 1. The corresponding mean values of BMI and waist circumference were 24.6±2.8 kg/m^2 and 91.8±14.3 cm, while MS was present in 12 patients (9.8%). In particular, 12 patients (9.7%) had hypertension, 7 (5.6%) had diabetes and 6 (7.1%) were insulin resistant, with a median HOMA index of 1.44 (IQR 0.26-7.07). Dyslipidaemia was present in 41 patients (32.8%), abnormal LDL in 65 patients (52%) and hypertriglyceridemia in 49 patients (39%). **Patients on fibrates (n=2) were considered to have hypertriglyceridaemia) and patients on statins (n=5) were considered to have high LDL irrespective of lipid levels.**

Five patients reported drinking above the recommended limits, however as their intake was only moderately increased (<60 units per week) we included them in the analysis.

Abnormal transaminases were prevalent only in a minority of patients, with increased AST and ALT values in 7 and 21 cases respectively (5.6% and 16.8%).

Eighty-five (68%) patients have been on anti-HIV treatment for a median duration of 3 (IQR 0-17) years. The most common drugs used were PIs in 45.1% of treated patients, while thymidine analogues, NNRTIs and nevirapine accounted for the 29.5%, 27% and 4.8% of treatment, respectively. The median duration of HIV viral infection was 6 (IQR 0-26) years, while thirty-five (36.8%) patients had lipodystrophy. No patients presented with AIDS and **median CD4 count was 480 copies/mmc (9-1740)** , while mean HIV viral load was 333732 ± 144520 copies/mL.

Prevalence and predictors of hepatic steatosis

Fatty liver was detected by ultrasound in 61 patients (55%), of who 3.6% had severe steatosis. As shown in table 2, in univariate analysis, steatosis was associated with male sex, presence of metabolic syndrome and increasing age, waist circumference and BMI. MS had a prevalence of 18% and 2% in patients with and without steatosis (p=0.006), while BMI values were 25.59 ± 2.99 and 23.37 ± 2.19 kg/m^2, respectively (p=0.001). HOMA values were significantly increased in the subset of patients with steatosis (2.01 ± 1.37 vs. 1.26 ± 0.65, p=0.002), however the presence of IR, defined by HOMA values more than 3, was not significantly different between the two groups. No association with lipid profile was found, apart from the presence of hypertrygliceridaemia. Of all liver enzymes, only GGT was higher in patients with steatosis. Neither the duration nor the severity of HIV was associated with steatosis, as well as the use of antiviral agents. **Prevalence of steatosis on ultrasound was lower in treatment-naïve patients however this did not reach statistical significance.** In the multivariate analysis, steatosis was independently associated with male sex (OR 14.6; 95% CI 1.44-147.9; p=0.02), increasing age (OR 1.083; CI 95% 1.012-1.158; p=0.02), increasing HOMA index (OR 2.56; CI 95% 1.102-5.93; p=0.03) and GGT (OR 1.038; CI 95% 1.002-1.075; p= 0.039). **Interestingly, the association with either MS or the use of past or current antiviral treatment, was no longer significant. Results did not significantly change when categorical variables were analysed as continuous (data not shown).**

Prevalence and predictors of liver fibrosis

Considering as threshold a stiffness value of 7.4 KPa, significant fibrosis (≥F2) was present in 22 patients (17.6%), whereas 54 patients (43%) had stiffness values >5.5 KPa. On the other hand, values >10 Kpa, suggestive of advanced fibrosis (≥F3), were detected in only 5 patients (4%). Interestingly, only a minority of patients with significant fibrosis as assessed by TE had abnormal transaminases, with deranged AST and ALT in 2 (9.1%) and 4 (18.1%) patients respectively.

In the univariate analysis, no association between significant fibrosis and fatty liver, IR and the lipid profile was noted. Similarly, there was no significant association with the severity or duration of the HIV infection, the duration of treatment or different treatment regimens**, as well as the treatment status.** Conversely, increasing age and BMI were significantly associated with significant fibrosis. Moreover, MS was significantly associated with stiffness values >7.4 KPa (27.3% vs. 5.8 %, p=0.04). In the multivariate analysis, the only

factor independently associated with fibrosis was presence of the MS (OR 3.99: 95% CI 0.992-16.09; p=0.05). **Results did not significantly change when categorical variables were analysed as continuous (data not shown).** All the above is summarized in table 3.

FIB4 score values of >3.25, suggestive of advanced fibrosis (≥F3), were seen in just 1 patient (0.8%), whereas more than 95% of patients (n=119) had normal values, namely <1.45. Similarly, APRI index > 1.5, suggestive of significant fibrosis (≥F2), was seen only in 2 patients (1.6%), while normal values <0.5 were found in 117 subjects (93.6%).

Among patients with FIB4<1.45 (n=119), TE values >7.4 KPa and >10 KPa were present in 19 (16%) and 4 (3.4%) subjects, respectively. In patients with APRI values <0.5 (n=117), TE was suggestive of significant and advanced fibrosis in 20 (17%) and 4 (3.4%) patients respectively.

Among patients with Fibroscan values >7.4 KPa, the majority had FIB4 <1.45 (19/22, 86.4 %) and APRI <0.5 (20/22 patients, 90.9 %). Conversely, FIB4 was <1.45 in 100 (97%) patients with TE values <7.4 KPa and in 115 (95.8%) of patients with TE values <10 KPa. All the above is summarized in Table 4.

In the subgroup of 50 patients without ultrasonographic evidence of fatty liver, 7 had evidence of significant fibrosis (14%) based on elastography, while the FIB4 was suggestive of advanced fibrosis in only 1 subject, but no predictive factors could be found.

Discussion

In our study, we showed that NAFLD is prevalent in more than 50% of patients with HIV mono-infection and more importantly that significant liver fibrosis is prevalent in 17.6% of these otherwise asymptomatic patients. These results are of concern, particularly as they were observed in young and non-obese patients with no clear risk factors for NAFLD and fibrosis. Therefore, they emphasize the

emerging significance of liver disease and in particular the importance of previously under-recognized NAFLD in patients with HIV infection.

The current “gold standard” for the assessment of non-alcohol related liver injury is histology. Nevertheless, liver biopsy is an invasive procedure that cannot be used for screening asymptomatic individuals, therefore we used transient elastography (TE) with Fibroscan®, which has demonstrated an acceptable diagnostic accuracy in the detection of advanced fibrosis and cirrhosis in NAFLD (13).

Data on the prevalence and predictors of NAFLD in HIV mono-infected patients are limited, as most of existing studies have focused on patients with HIV/HCV co-infection. In our study, NAFLD as detected by ultrasound was prevalent in more than half of the included patients. However, one could safely assume that the true prevalence of NAFLD is even higher, as ultrasound can detect steatosis only when more than 20-30% of hepatocytes are affected. Interestingly, most patients with NAFLD had normal transaminases, confirming their lack of accuracy in diagnosing NAFLD or NASH (14). NAFLD was independently associated with male sex, increasing age and HOMA values. Surprisingly, neither MS nor obesity were significantly associated with the presence of steatosis. This probably reflects the evaluated cohort, as the majority of patients were non-obese and did not have metabolic syndrome. The association of NAFLD with insulin resistance confirms previous observations that the HIV status predisposes to steatosis, through the virus itself, the direct or metabolic effects of the anti-retroviral medication and lipodystrophy (15). All these factors may indeed contribute to an even more severe liver disease compared to uninfected subjects, as shown by Vodkin et al, who compared liver histology of 33 patients with HIV associated NAFLD and 33 patients with primary NAFLD and found higher rates of NASH and of features of liver disease, namely lobular inflammation and acidophil bodies (16). Finally, an element of undisclosed alcohol abuse could have also contributed to the higher than expected steatosis prevalence in our cohort.

In our study, there was no association **of either the duration and severity of HIV infection or of the past or present** use of HAART with steatosis. In a study by Crum-Cianflone, steatosis was found on ultrasound in 31% and at histology in 33% of 216 HIV mono-infected patients and there was no association with HIV viral load, duration of infection, or antiretroviral therapy (17). A similar prevalence of

NAFLD at ultrasound (31%) was found in an Asian cohort of 435 HIV mono-infected patients and was associated with high BMI, dyslipidemia, and high ALT/AST ratio, but not with HIV-related factors (18). Conversely, in a cohort of 225 HIV mono-infected patients, steatosis detected by CT scan was prevalent in 37% of patients and was associated with cumulative NRTI exposure (OR 1.12) (19). However, in the latter study the mean time exposure to NRTI was approximately 10 years, compared to 4.8 years reported in our study. Therefore, the lack of association between NRTIs treatment and hepatic steatosis in the current study might be related to the shorter exposure to these drugs.

Moreover, data are also emerging on steatosis in HIV mono-infected patients assessed by controlled attenuation parameter (CAP) measurements with Fibroscan. In particular, Macias et al demonstrated the presence of steatosis by CAP in 201 (40%) of 505 HIV mono-infected patients, with BMI being the main predictor of fatty liver. A similar prevalence of 49% was reported by Sulyok et al, who found a significant association between high CAP values and BMI, diabetes and hypertension. These results are in line with our data even if they are reported in a cohort of more obese patients (20, 21).

For the non-invasive evaluation of fibrosis using TE, we considered stiffness values >7.4 kPa as suggestive of significant fibrosis, thus classifying 22 patients (17.6%) in this category. Although data about the use of TE in HIV mono-infected patients are limited and no validated cut-off values are available, in principle TE appears to be the best non-invasive tool in such patients. TE has excellent accuracy for the detection of liver cirrhosis and is moderately accurate for significant fibrosis (22, 23). However, it is safe to assume that patients with stiffness values >7.4 would have some degree of fibrosis, even if this did not reach F2. In our analysis, the presence of significant fibrosis using TE was independently associated with MS but not with any parameters of the HIV infection. The impact of antiretroviral therapy on liver fibrosis is controversial as evidenced by conflicting reports on worsening with long term use of HAART, especially didanosine or stavudine (24, 25), absence of significant effect (26), or even improvement in the course of antiretroviral treatment with PIs (27, 28).

Few other studies have evaluated the presence of fibrosis in unselected mono-infected patients with HIV and none correlated this with metabolic risk factors. In a cohort of 258 patients with HIV mono-infection, stiffness of >7.2 KPa was prevalent in 11.2%, however there were no data on the presence of steatosis and no correlation with metabolic parameters (29). In a similar cohort of 93 patients, stiffness of >5.3 KPa was prevalent in 42% with no information on NAFLD and/or metabolic comorbidities (27). In a group of 234 mono-infected patients that had Fibroscan for clinical reasons, 20 (8%) were reported with stiffness values >9.3 KPa, although there is an evident selection bias (24). Finally, literature reporting data on liver biopsy is limited. In a cohort of 62 patients with increased transaminases in HIV mono-infection, 73% of patients had NAFLD, 55% had NASH and 31% had evidence of fibrosis (26). In a prospective study of 198 HIV mono-infected subjects, the prevalence of liver fibrosis assessed by Fibroscan was 10.6% (21 patients) and NAFLD detected at histology accounted for 50% of all etiologies (30). Our data are consistent with the above results and further provide the missing link of high stiffness values in such patients with NAFLD and the metabolic syndrome.

On the other hand, there was a discordance of TE with APRI and FIB4 in the diagnosis of significant and advanced fibrosis respectively, as the majority of them had normal FIB4 and APRI values (95.2% and 93.6%, respectively), while the prevalence of significant and advanced fibrosis as assessed by APRI and FIB-4 was 1.2% and 0.8% respectively. The FIB4 results are in line with previous data, as in a cohort of 1310 HIV infected women values ≥3.25 were found in only 1.3% of the mono-infected cohort of patients, rising up to 8.6% in the co-infected one (31). Similarly, in a cohort of 83 HIV mono-infected patients, only one of them was diagnosed with advanced fibrosis (19). In another retrospective observational study FIB4 values >3.25 were found in 31 (4%) of 796 HIV mono-infected patients, but an undisclosed element of alcohol consumption might have contributed this slightly higher rate (32). Conversely, a higher prevalence at 8% of APRI values >1.5 was found in 432 HIV mono-infected patients (33). However, in this study fibrosis was associated with diabetes and the cohort was made of more than 50% of overweight subjects, thus these metabolic features could possibly have contributed to the higher prevalence of fibrosis. **Moreover, in an observational prospective study on 1112 HIV mono-infected subjects, FIB4 values >3.25 and APRI values >1.45 were found in the 3.4% and 2.9% of the cohort respectively, with a moderate concordance between the scores.**

Interestingly, the progression of liver fibrosis, as assessed by the transition to higher values of both scores, was prevented by an early antiviral therapy initiation (28).

We believe that this discordance is attributed mostly to false negative results of the simple NITs rather than TE. While the majority of our cohort had normal transaminases, FIB4 and APRI have been developed and validated in patients with abnormal liver enzymes. Therefore, their diagnostic accuracy in patients with normal transaminases is unknown and possibly suboptimal. Moreover, APRI is suboptimal in patients with NAFLD and has only been sufficiently validated in patients with HCV or HBV. Finally, TE is superior to simple non-invasive serum markers for the diagnosis of significant liver fibrosis (22, 34).

This study has some limitations. Firstly, it is a cross sectional study, so it is not possible to define the consecutive steps leading to the development of both steatosis and fibrosis in HIV infected patients. Moreover, a long-term follow-up longitudinal study would be needed to truly assess the significance of steatosis and fibrosis in this cohort and the potential effects of modifications in the anti-retroviral therapy regime. TE would be of value in such a scenario as it would provide serial non-invasive measurements, with each patient serving as his own control. Secondly, the detection of steatosis by ultrasound could have led to an underestimation of the actual prevalence of NAFLD, as the specificity of this technique is suboptimal. Although histology was not available, it would be unethical to biopsy otherwise healthy patients. Furthermore, non-invasive fibrosis tests are increasingly used and are now part of diagnostic algorithms and routine clinical practice (35, 36). **Thirdly, the small number of patients considered does not allow our results to be conclusive, as they need to be further confirmed in larger cohorts.** Finally, the treatment used in this cohort does not reflect current practice and could be in part responsible for the relatively high prevalence of steatosis in this cohort. Although we cannot exclude that high stiffness values in some patients, particularly those on didanosine, were due to nodular regenerative hyperplasia, the prevalence of this condition is very low and therefore would not influence the main conclusions (37).

In conclusion, liver fibrosis is likely to develop in asymptomatic HIV infected patients independently of HCV or HBV co-infection. This is more likely associated to NAFLD and usually manifests with normal transaminases even in patients with abnormal stiffness values.

Possibly HAART or HIV infection itself might play a key role, even if a significant correlation was not supported by our data. Therefore, non-invasive screening for the presence of NAFLD and fibrosis should be offered in HIV mono-infected patients as part of their routine clinical care. This would allow the timely diagnosis of chronic liver disease and would allow for the instigation of preventative measures (38). Given the association of NAFLD with cardiovascular morbidity and mortality, aggressive treatment of the MS components should be offered in patients with HIV and steatosis (39).

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Table 1. Characteristics of the cohort of HIV mono-infected patients

Characteristics	*N= 125*
Gender, male (%)	114 (91)
Age, years	39.5 ± 10.3
Current smoker, n(%)	49 (45.4)
BMI, Kg/m^2	24.6 ± 2.9
WC, cm	91.8 ± 14.3
AST, IU/L	23 (11-188)
ALT, IU/L	26 (9-315)
GGT, U/L	27 (10-200)
ALP, U/L	76 ± 21
Hb, g/dL	15.1 ± 2.4
PTL, $/cm^3$	250 ± 61
WC (n°/10^3)	6956 ± 2365
HOMA index	1.45 (0.26-7.07)

IR, n (%)	6 (7.1)
Total Cholesterol, mmol/L	210 ± 50
LDLc, mmol/L	136 ± 45
HDL, mmol/L	42 ± 14
Tryglicerides, mmol/L	137 (25-1340)
MS, n(%)	12 (9.8)
Diabetes, n(%)	5 (6.3)
Hypertension, n(%)	12 (9.7)
Lipodystrophy, n(%)	35 (36.8)
Age at infection years	29.1 ± 8.5
Duration of infection , years	6 (0-26)
No previous HIV treatment, n (%)	**35 (28%)**
Current treatment, n(%)	85 (68)
thymidine analogues, n(%)	36 (29.5)
PIs, n(%)	55 (45.1)
NNRTIs, n(%)	33 (27)

Duration of treatment, years	3 (0-17)
HIV-RNA, copies/mL	333732 ± 144520
Lymphocytes CD4+, /mm^3	**480 (9-1740)**
US steatosis, n(%)	61 (55)
Grade I, n(%)	40 (36)
Grade II, n(%)	17 (15.3)
Grade III, n(%)	4 (3.6)
Stiffness, KPa	5.8 ± 1.9
Stiffness >7.4 KPa, n(%)	22 (17.6)
Stiffness > 10 KPa, n(%)	5 (4)
FIB4 score	
≤1.45, n(%)	119 (95.2)
1.46-3.24, n(%)	5 (4)
≥3.25, n(%)	1 (0.8)
APRI index	
≤0.5, n(%)	117 (93.6)

0.51-1.49, n(%)	6 (4.8)
≥1.5, n(%)	2 (1.6)

§data expressed as mean ± SD for quantitative variables with normal distribution; data expressed as median (IQR) for quantitative variables without normal distribution.

Abbreviations: BMI, body mass index; WC, waist circumference; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; Hb, haemoglobin; PLT, platelets; IR, insulin resistance; MS, metabolic syndrome; PIs, protease inhibitors; NNRTIs, non-nucleoside reverse transcriptases.

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Table 2. Variables associated with the presence of liver steatosis in the cohort of HIV mono-infected patients

	No steatosis	Steatosis	Univariate analysis	Multivariate analysis	
Patient characteristics	(N= 50)	(N=61)	P value	P value	OR (95% CI)
Age, years	36.7 ± 10.5	42.6 ± 9.5	0.002	**0.03**	**1.082 (1.01-1.16)**
Sex, males (%)	42 (84)	59 (96.7)	0.041	0.02	**14.64 (1.44-148.17)**
Smoking, n(%)	15 (30)	28 (45.9)	0.247		
Alcohol abuse, n (%)	1 (2)	4 (6.5)	0.378		
BMI, Kg/m^2	23.4 ± 2.2	25.6 ± 3	0.001	-	
Waist circumference, cm	84.8 ± 17.9	97.7 ± 8.7	<0.001	-	
HOMA index	1.26 ± 0.65	2.01 ± 1.37	0.002	0.03	2.56 **(1.101-5.96)**
Insulin resistance, n (%)	1 (2)	5 (8.2)	0.216		
Diabetes mellitus, n (%)	1 (2)	6 (9.8)	0.124		
PLT, $x10^3/mm^3$	253 ± 54	248 ± 65	0.589		

Serum LDL cholesterol, mg/dL	134 ± 45	139 ± 48	0.532		
Serum HDL cholesterol <40 mg/dl (male) and < 50 mg/dl (female), n(%)	26 (52)	35 (57.3)	0.702		
Serum triglycerides > 150 mg/dl, n(%)	12 (24)	35 (57.4)	<0.001	-	
Hypertension, n(%)	3 (6)	7 (11.5)	0.342		
Metabolic syndrome, n(%)	1 (2)	11 (18)	0.006	NS	
Lipodystrophy, n(%)	12 (24)	21 (34.4)	0.125		
ALT, IU/L	28 ± 13	35 ± 38	0.1		
AST, IU/L	29 ± 24	27 ± 21	0.487		
ALP, U/L	74 ± 24	76 ± 20	0.738		
GGT, U/L	28 ± 18	43 ± 35	0.001	**0.04**	**1.037 (1.007-1.075)**
Duration of infection, years	7 ± 6.5	8.2 ± 6.8	0.418		
No previous HIV treatment, n (%)	**18 (36)**	**13 (22)**	**0.066**	**NS**	
Current treatment, n (%)	30 (60)	45 (73.8)	0.155		
Protease inhibitors	19 (38)	28 (45.9)	0.437		

Thymidine analogues	15 (30)	16 (26.2)	0.831
NNRTIs	13 (26)	18 (29.5)	0.675
Duration of treatment, years	4.3 ± 4.6	5.3 ± 4.8	0.191
CD4+ <200/mm^3	4 (8)	4 (6.5)	1
HIV-RNA > 100000 copies/mL	3 (6)	5 (8.2)	0.728

Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; Hb, haemoglobin; PLT, platelets; NNRTIs, non-nucleoside reverse transcriptases.

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Table 3. Variables associated with the presence of liver fibrosis in the cohort of HIV mono-infected patients

	Stiffness < 7.4 KPa	Stiffness > 7.41 KPa	Univariate analysis	Multivariate analysis	
Patient characteristic	(N= 103)	(N=22)	P value	P value	OR (95% CI)
Age, years	38.4 ± 9.8	44.8 ± 11.2	0.008	NS	

Sex, males (%)	92(89.3)	22(100)	0.108		
Smoking, n(%)	42 (40.8)	7 (11.7)	0.8		
Alcohol abuse, n (%)	3 (2.9)	2 (9)	0.232		
BMI, Kg/m^2	24.2 ± 2.5	26 ± 3.9	0.011	-	
Waist circumference, cm	90.5 ± 14.2	100.6 ± 11.4	0.036	-	
HOMA index	1.71 ± 1.18	1.3 ± 0.62	0.33		
Insulin resistance, n(%)	6 (5.8)	0 (0)	0.419		
Diabetes mellitus, n(%)	4 (3.9)	3 (13.6)	0.094		
PLT, $x10^3/mm^3$	254 ± 61	224 ± 56	0.033	NS	
Serum LDL cholesterol, mg/dL	136 ± 44	136 ± 50	0.994		
Serum HDL cholesterol <40 mg/dl (male) and < 50 mg/dl (female), n(%)	52 (50.4)	13 (59)	0.491		
Serum triglycerides > 150 mg/dl, n(%)	39 (37.9)	10 (4.5)	0.631		
Hypertension, n(%)	6 (5.8)	6 (27.3)	0.006	-	
Metabolic syndrome, n(%)	6 (5.8)	6 (27.3)	0.004	0.05	3.99 (0.992-16.09)

Lipodystrophy, n(%)	31 (30)	4 (18.1)	0.279	
ALT, IU/L	28 ± 13	43 ± 62	0.159	
AST, IU/L	26 ± 18	31 ± 32	0.699	
ALP, U/L	76 ± 23	75 ± 14	0.901	
GGT, U/L	34 ± 27	44 ± 34	0.137	
Duration of infection, years	7.5 ± 6.5	6.3 ± 6.5	0.66	
No previous HIV treatment , n (%)	**28 (27)**	**7 (32)**	**0.42**	
Current treatment, n(%)	70 (68)	15 (68.2)	0.599	
Protease inhibitors	43 (41.7)	12 (54.5)	0.352	
Thymidine analogues	30 (29.1)	6 (27.3)	1	
NNRTIs	30 (29.1)	3 (13.6)	0.184	
Duration of treatment, years	4.5 ± 4.3	6.4 ± 6.1	0.252	
CD4+ <200/mm^3	7 (6.8)	3 (13.6)	0.379	
HIV-RNA > 100000 copies/mL	7 (6.8)	2 (9)	0.658	
US steatosis, n(%)	48 (46.6)	13 (59)	0.457	
Grade of steatosis			0.026	NS

Grade I, n(%)	35	5
Grade II, n(%)	11	6
Grade III, n(%)	2	2

Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; Hb, haemoglobin; PLT, platelets; NNRTIs, non-nucleoside reverse transcriptases.

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Table 4. Liver stiffness values stratified for APRI index and FIB4 score in the whole cohort of HIV mono-infected patients (n=125)

	Liver stiffness values (KPa)			
	≤7.4 (n=103)	>7.4 (n=22)	≤10 (n=120)	>10 (n=5)
APRI<0.5 (n=117)	97	20	113	4
APRI>1.5 (n=2)	1	1	2	0
FIB4<1.45 (n=119)	100	19	115	4
FIB4>3.25 (n=1)	1	0	1	0