Liver test abnormalities in patients with HIV mono-infection: assessment with simple non-invasive fibrosis markers

NAFLD in HIV mono-infected patients

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Abstract

Background: Patients with HIV mono-infection may develop chronic liver disease due to a number of factors including hepatic steatosis. We estimated prevalence and predictors of hepatic steatosis and fibrosis in a cohort of HIV mono-infected patients with persistently deranged LFTs.

Methods: Out of 2398 consecutive patients at one UK clinical centre, 156 (6.5%) had persistently abnormal transaminases in at least two measurements six months apart. We used APRI and FIB4 scores to determine the presence of significant and/or advanced fibrosis in this group and potential associations.

Results and Conclusions: Mean age was 47.5±8.5 years and 91% (142/156) were males. Diabetes (DM) was present in 11% of, hypertension in 18% and dyslipidemia in 52%. Almost all were on antiretroviral therapy (ART) (97%) and most were virologically suppressed (94%). Steatosis was detected at ultrasound in 71% of patients. Prevalence of FIB4≤1.45, 1.46-3.24 and >3.25 was 67%, 29% and 4% respectively, and of APRI≤0.5, 0.51-1.49 and >1.5 was 52%, 45% and 3% respectively. In multivariate analysis, only cumulative ART exposure was associated with FIB4>1.45 (OR 1.008, 95%CI 1.000-1.016) while APRI>0.5 was associated with higher ALT levels (OR 1.033, 95%CI 1.015-1.510). Twenty patients had a liver biopsy, of whom 13 had non-alcoholic fatty liver disease (NAFLD). Elevated transaminases are often present in HIV mono-infected patients and this may be associated with NAFLD and/or ART. Non-invasive screening for the presence of NAFLD and fibrosis to all HIV mono-infected patients as part of their routine clinical management should be further explored.

Introduction

In the era of effective antiretroviral therapy (ART), chronic liver disease is an important cause of morbidity and mortality among HIV-positive people, even in the absence of HCV and HBV co-infections [1, 2].

Mild transaminitis in HIV-positive individuals on ART is frequently reported [3], although the aetiology is often indeterminate. In a study by Crum-Cianflone, elevated transaminases were found in 80 (27%) of 299 HIV mono-infected patients on ART and steatosis detected on ultrasound in 30%, though in 51% the cause of liver dysfunction remained unexplained [4].

Fatty liver due to metabolic syndrome and/or ART exposure is an increasingly recognized cause of liver test abnormality in HIV mono-infected patients [3]. Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome (MS) [5], but could also represent a long-term toxicity of ART as nucleoside reverse-transcriptase inhibitors (NRTIs) and to a lesser extent protease inhibitors (PIs), are associated with insulin resistance and mitochondrial toxicity [6]. Moreover, specific HIV-related factors may predispose to NAFLD, such as lipodistrophy or the HIV virus per se [7, 8]. NAFLD has a prevalence of 20% in the general population of industrialized countries, which rises to more than 50% in HIV infected people [9].

Since NAFLD encompasses a wide spectrum of disease ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and eventually cirrhosis [10], the early detection of liver fibrosis prior to onset of complications associated with decompensated liver disease is crucial. Non-invasive fibrosis tests are increasingly used for the assessment of fibrosis in patients with liver disease [11], including in HIV positive people [12].

In this study, we retrospectively evaluated the prevalence and predictors of liver fibrosis in a cohort of HIV mono-infected patients with persistently elevated transaminases by using simple serum non-

invasive fibrosis panels, namely FIB4 and APRI index. Moreover, we assessed the presence of hepatic steatosis and associated features in this cohort.

Material and Methods

Study population

This is a retrospective cross-sectional audit of consecutive HIV mono-infected patients that attended the HIV dedicated outpatient service at Royal Free Hospital, London, UK, from January to December 2014. A clinical database was used to identify patients with persistently elevated transaminases in at least two measurements six months apart. Serum levels more than 39 IU/L and 41 IU/L were considered as abnormal for aspartate aminotransferase (AST) and alanine aminotransferase (ALT), respectively. Patients with evidence of HCV or HBV co-infection, as well as other documented causes of liver disease other than NAFLD were excluded. Further information for patients with persistently abnormal transaminases was retrieved from their clinical notes.

Laboratory investigations and epidemiological and clinical features were retrieved from the clinical database for all included patients. The presence of diabetes, hypertension and dyslipidemia, as well as relevant medications were documented.

HIV specific information was also obtained; this included time since diagnosis, duration of ART, most recent CD4+ count(n/mm^3) and HIV viral load (RNA copies/mm^3). HIV RNA copies <40/mmc defined virologic suppression.

Liver fibrosis and steatosis assessment

We obtained the results of abdominal ultrasound scans and transient elastography (TE) with Fibroscan® which were performed as part of the routine clinical care to further investigate the liver test abnormalities and stage fibrosis respectively. US and TE was included if within 6 months of the date of transaminase measurement.

The presence of significant (≥F2 METAVIR stage) or advanced (≥F3 METAVIR stage) liver fibrosis was evaluated by APRI and FIB4 scores respectively. APRI consists of AST and platelet count in the formula *[AST levels (IU/L)/AST ULN (IU/L)/Platelets (10^9/L)]*100* and It has dual cut-offs for ruling out (<0.5) or diagnosing (>1.5) significant fibrosis[13]. FIB4 consists of age, AST, ALT and platelet count in the formula *[(Age (years) x AST (IU/L)) / (Platelets (10^9/L)*√ALT (IU/L))]*and it also has dual cut-offs and for ruling out (<1.45) or diagnosing (>3.25) advanced fibrosis [14]. These tests usually have dual cut-offs: a high cut-off with high specificity and a low cut-off with high sensitivity. Depending on the clinical scenario and the disease prevalence, the low or high cut-off is used at the expense of increased false positives and false negatives respectively. If these cut-offs are combined, then the number of false positive and false negatives are minimized, however, a number of patients will fall in the indeterminate range of fibrosis (i.e. their score will be indeterminate ie between the low and the high cut-off) and will need either further non-invasive testing or a liver biopsy [15].

In a small subgroup of 19 patients, transient elastography (TE) was performed with FibroScan®(Echosens; Paris, France), after at least 6 hours of fasting. Results in Kilopascals (KPa), were 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. Only determinations of liver stiffness with 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 reliable. Liver stiffness values >7.4 kPa were considered suggestive of significant fibrosis.

Finally, 20 patients in the same cohort had previous liver biopsies as part of their routine clinical care. The presence and severity of steatosis, as well as histological grading and staging were assessed according to the Brunt score [16].

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 to assess predictors of steatosis and fibrosis with APRI and FIB-4 values of >0.5 and >1.45 respectively. Only variables with a P value $\leq$0.10 at univariate analysis were entered in the multivariate analysis models. A two-tailed P value <0.05 was considered to indicate a statistically significant difference between groups.

Results

Baseline characteristics

Out of 2398 consecutive patients with HIV mono-infection between January and December 2014, 156 (6.5%) had persistently elevated transaminases in at least two measurements six months apart and were included in the analysis.

The mean age of those included was 47.5 years and 91% were males. Clinical, biochemical and virological characteristics are reported in Table 1. In particular, 28 patients (17.9%) had hypertension, 17 (10.9%) had diabetes and 80 (51.9%) had hyperlipidaemia, while 47 (30.5%) were on lipid-lowering medication.

AST and ALT values <2 times upper limit of normal (ULN) were found in 93.6% and 81.4% of patients respectively, 2-3 times ULN in at least one measurement in 3.8% and 14.1% and >3 times ULN in at least one measurement in 2.6% and 4.5% of the cohort.

Almost all patients were on ART (n= 154, 97%), with a median length of treatment of 11 years (IQR 0-26 years), and the majority of them had virological suppression (n=146, 93.6%). Mean time since

diagnosis was 14 years (IQR 2-30 years). CD4 counts <200/mm^3 were present only in 3 (1.9%) patients and median viral load was 39 copies/mm^3 (IQR 39-2622).

Information about the specific treatment regimen were available only in 97 patients. The most common drugs used were NRTIs in 86 (88.7%) of treated patients, while PIs and non-nucleoside reverse transcriptase inhibitors (NNRTIs) were prescribed in 41 (42.3%) and 46 (47.4%) of patients, respectively.

Prevalence and predictors of hepatic steatosis

Ultrasound examination was performed in 66 (42.3%) patients, and fatty liver was detected in the majority of these (n=47, 71.2%) as shown in table 2. Nevertheless, no association was found between the presence of US steatosis and patients' clinical features, as shown in table 3. In particular, there was no association between the presence of steatosis and metabolic features, namely hypertension, diabetes and dyslipidemia. Importantly, fatty liver was not significantly associated with the since diagnosis, the length of ART, or the category of the antiviral drugs used. At univariate analysis, fenofibrate use was significantly associated with the absence of US steatosis (p=0.022), but this association was lost in the multivariate model.

Prevalence and predictors of significant and advanced liver fibrosis

Considering the high cut-off of >1.5 for APRI, we categorised 5 (3.2%) patients as having significant fibrosis (≥F2), whereas values <0.5 excluded this diagnosis in 51.9% of them. On the other hand, FIB4>3.25 suggested advanced fibrosis (≥F3) in 6 (3.8%) patients while values ≤1.45 (<F3) were found in 67.3% of the cohort. Forty-five (28.8%) and 70 (44.9%) patients fell into the "indeterminate" diagnostic zone of FIB4 and APRI, respectively, as shown in table 2.

For further analysis, and in keeping with the use of NITs as screening tests, we considered a threshold of 1.45 for FIB4 and of 0.5 of APRI to rule out the presence of advanced or significant fibrosis respectively in our cohort of HIV mono-infected patients.

In univariate analysis, FIB4 >1.45 (n=51, 32.7%), was significantly associated with both time since HIV diagnosis (14±7 vs. 16±6 years, p=0.019) and duration of ART (10±6 vs.13±6 years, p= 0.002). Moreover, diabetes was significantly more prevalent in patients with a FIB4 >1.45 compared to those with values <1.45 (17.6% vs. 7.6%, p=0.098). Nevertheless, in the multivariate analysis, only duration of ART maintained this association (OR 1.008, 95%CI 1.000-1.016; p=0.04) (Table 4).

On the other hand, APRI values >0.5 were found in 75 (48%) patients and only ALT levels were significantly increased in patients with evidence of significant fibrosis (78±49 vs. 56±13; OR 1.033, 95% CI 1.015-1.510, p=0.01). In contrast to FIB4, neither the time since HIV diagnosis nor the use of ART or the presence of diabetes was associated with increased APRI score. Similarly to FIB4, no association with demographic and metabolic features was noted (Table 5).

Interestingly, no relation was found between the presence of liver fibrosis assessed by both FIB4 and APRI index and US evidence of steatosis.

A small subset of patients underwent also a TE (n=19, 12.1%) and a liver biopsy (n=20, 12.8%). Considering a threshold stiffness value of 7.4 kPa, 4 (21.1%) patients were classified as having significant fibrosis, while stiffness values more than 10 Kpa were found only in 1 patient, suggesting more advanced liver disease.

Despite the small number of liver biopsies, histology showed non-alcoholic fatty liver disease in 13 (65%) patients, of who one was also diagnosed with cirrhosis. Moreover, features of drug reaction were present in 2 cases, while 4 patients had non-specific histological changes and 1 biopsy was normal.

Discussion

In this study, we demonstrated that persistently abnormal transaminases are fairly prevalent in unselected HIV mono-infected patients (156/2398, 6.5%). More importantly, 4% of these patients had evidence of advanced fibrosis based on simple non-invasive fibrosis tests. Despite the presence of persistently deranged liver tests in this group, there was inconsistent further investigation of these abnormalities; just 66 (42%) patients had undergone US examination and only 19 (12%) had a TE, despite the non-invasiveness and relatively low cost of these techniques.

A rise in liver enzymes in HIV mono-infected individuals on ART has been previously reported. A cross-sectional study by Sterling et al. reported elevated transaminases in 20% of 679 patients and a positive association with high viral load and features of metabolic syndrome [17]. Maida et al. studied a cohort of 3200 individuals with long history of HIV infection and ART exposure and found abnormal LFTs in only 17 (0.5%) patients, of whom 10 (58.8%) had histologically advanced fibrosis (F3-F4) and clinically advanced liver disease. However, in this study harmful alcohol use and medications predisposing to steatosis were excluded [18]. On the other hand, in a prospective study of 2365 HIV mono-infected individuals who were followed for a 5-year period, 385 (16%) developed a persistent ALT elevation, which was associated with increased body mass index (BMI), high HIV viral load and exposure to NRTIs, in particular zidovudine and stavudine [19].

The underlying aetiology of increased LFTs in people who are HIV positive often remains unrecognized, as liver biopsy is not routinely performed. Nevertheless, many studies have indicated that NAFLD is more prevalent in this category of patients, potentially progressing to NASH and liver fibrosis.

A histological evaluation of a cohort of 20 HIV mono-infected individuals with chronically increased LFTs demonstrated the presence of steatosis in 18 (60%) patients, of who 16 (88.9%) had NASH and 13 (72.2%) had fibrosis [20]. Morse et al. biopsied a cohort of 62 patients with similar features and

found steatosis in 45 (73%), NASH in 34 (55%) and bridging fibrosis in 11 (17%) patients respectively [21].

These data are similar to our study and indicate the necessity of screening HIV positive people for the presence of steatosis and fibrosis, especially when LFTs are deranged. In fact, in the subgroup of patients who underwent a liver US, NAFLD was present in the majority of them (47/66, 71.2%). Moreover, even if liver biopsy was performed in only in 20 patients, histology showed non-alcoholic fatty liver disease in 13 (65%), and NASH in 5 (25%) patients, of who one had cirrhosis.

The prevalence of steatosis is likely to be even higher than 70%, as ultrasound can detect steatosis only when more than 20-30% of hepatocytes are involved. Importantly, in our study there was no significant association with metabolic, anthropometric and either infection or ART related features. However, as US reports were available for less than a half of our cohort, this likely reflects under-powering of the study to detect any significant associations.

Conversely, two other studies reported a lower prevalence of NAFLD assessed by imaging techniques. Guaraldi et al. detected steatosis by CT scan in 37% of 225 HIV mono-infected patients, and found an association with cumulative NRTI exposure (OR 1.12) and obesity (OR 1.07) [22]. On the contrary, steatosis was present on ultrasound in 31% and at histology in 33% of 216 HIV mono-infected patients but there was no association with either duration of HIV infection or ART, as only obesity (OR 2.1) and hypertriglyceridemia (OR 1.2) reached statistical significance [23]. Nevertheless, in both studies less than 30% of patients had deranged LFTs, thereby this possibly explaining the lower prevalence of steatosis compared to our findings.

Using APRI index and FIB4 score, we classified 5 (3.2%) and 6 (3.8%) patients as potentially having significant and advanced fibrosis, respectively. Although TE appears to be the best non-invasive tool in HIV infected patients, showing an excellent accuracy for the detection of liver cirrhosis and a

moderate accuracy for significant fibrosis [24, 25], it was available only in a small subset of the cohort and suggested significant fibrosis in 4 patients. On the other hand, our data suggest that 81 (51.2%) and 105 (67.3%) patients could be excluded from having significant and advanced fibrosis according to APRI and FIB4, respectively. Indeed, APRI and FIB4 have a higher diagnostic accuracy in ruling out rather than ruling in the presence of significant/advanced fibrosis and are ideal screening tests as they have a negative predictive value of >90%. [14][26].

The low prevalence of significant and advanced fibrosis based on simple non-invasive tests is compatible with previously published data. In a cohort of 818 HIV positive women, the majority of patients (86.6%) had FIB-4 values ≤1.45, while increasing FIB4 values were associated with higher HIV viral load [27]. Similarly, in a cohort of 225 HIV mono-infected patients, 28 were classified as not having fibrosis on FIB-4 [22], while in another cohort of 62 patients FIB4 ≤1.45 and >3.25 were found in 37 (59.7%) and 5 (%) patients respectively [21]. Conversely, a slightly higher prevalence of 8% of APRI values >1.5 was found in 432 HIV mono-infected patients and both diabetes and detectable HIV viraemia were confirmed as risk factors for significant fibrosis [28]. However, the presence of obesity in 50% of the cohort could have contributed to the higher prevalence of significant fibrosis.

In our study, the duration of ART was the only factor independently associated with the presence of fibrosis based on FIB-4. The impact of ART on liver fibrosis is controversial as highlighted by conflicting reports on worsening with long term use of ART, especially didanosine or stavudine [9, 29], absence of significant effect [21], or even improvement following commencement of PIs [30, 31]. Furthermore, Mendeni at al. followed a cohort of 1112 HIV mono-infected patients for approximately 6 years and found that progression of fibrosis, assessed by APRI and FIB4, was prevented by viral control by early ART, provided that didanosine use was avoided [31].

Interestingly, in this study neither FIB4 nor APRI showed any association with metabolic features or increasing age. Diabetes was significantly associated with increased FIB4 values, but only in the

univariate analysis. These data reflect the characteristics of our population, as HIV patients were relatively young (mean age 47 years) and both diabetes and hypertension had a low prevalence thus limiting the power of this study to detect any associations with fibrosis.

This study has limitations. Firstly it is a cross sectional study, so it is not possible to define the dynamic process underlying the development of both steatosis and fibrosis in HIV positive patients. Therefore, these data should be prospectively assessed, in order to evaluate the true prognostic impact of hepatic steatosis and fibrosis in HIV . Secondly, the selection of patients on the basis of elevated transaminases could have led to an underestimation of fatty liver disease, given their lack of sensitivity in diagnosing NAFLD or NASH [32]. Thirdly, detection of steatosis by ultrasound could also have underestimated the prevalence of NAFLD, as the accuracy of this technique is suboptimal. In addition, an element of undisclosed alcohol abuse could have also contributed to the high steatosis prevalence in this cohort.

In conclusion, the presence of liver disease is under-investigated in HIV mono-infected patients despite deranged LFTs. Such abnormalities are often associated with the presence of NAFLD, and could potentially evolve into progressive liver fibrosis. A potential role for ART in this scenario has yet to be determined, but it is likely that NAFLD could represent a long term toxicity of antiviral therapy, as suggested by the association of the length of antiviral treatment and liver fibrosis and evidence from other studies. A wide series of non-invasive tools for the assessment of liver fibrosis are currently available, therefore a non-invasive screening for the presence of NAFLD and fibrosis should be evaluated in the routine clinical management of HIV mono-infected patients receiving antiretroviral treatment. Moreover, aggressive treatment of the metabolic syndrome components should be undertaken in HIV positive patients with steatosis, given the well-established association of NAFLD with increased cardiovascular morbidity and mortality [33].

Summary box

What is already known:

- Liver disease is an important cause of morbidity and mortality among HIV infected patients, even in the absence of HCV and HBV co-infections.
- Abnormal liver tests in HIV mono-infection is frequently reported and NAFLD is increasingly recognized as potential cause.
- Non-invasive fibrosis tests are widely used for the assessment of fibrosis in liver disease, including HIV positive people.

New findings:

- Despite the high prevalence of liver tests abnormalities in HIV patients, liver disease is frequently under-investigated.
- Duration of antiretroviral therapy seems to have an impact on the onset of hepatic steatosis and its progression to fibrosis. Therefore, NAFLD could represent a long term toxicity of this pharmacological treatment.
- Simple non-invasive tests such as APRI and FIB-4 can be used for the triaging of patients for dedicated hepatological follow-up and/or liver biopsy.

REFERENCES

1. Palella, F.J., Jr., et al., Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998; **338**: 853-860.

2. Bica, I., et al., Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. *Clin Infect Dis* 2001; **32**: 492-497.

3. Pol, S., P. Lebray, and A. Vallet-Pichard, HIV infection and hepatic enzyme abnormalities: intricacies of the pathogenic mechanisms. *Clin Infect Dis* 2004; **38**: S65-S72.

4. theCrum-Cianflone, N., et al., Prevalence and factors associated with liver test abnormalities among human immunodeficiency virus-infected persons. *Clin Gastroenterol Hepatol* 2010; **8**: 183-191.

5. Tsochatzis, E.A., et al., Insulin resistance and metabolic syndrome in chronic liver diseases: old entities with new implications. *Scand J Gastroenterol* 2009; **44**: 6-14.

6. Nunez, M., Hepatotoxicity of antiretrovirals: incidence, mechanisms and management. *J Hepatol* 2006; **44**: S132-S139.

7. McGovern, B.H., et al., Hepatic steatosis is associated with fibrosis, nucleoside analogue use, and hepatitis C virus genotype 3 infection in HIV-seropositive patients. *Clin Infect Dis* 2006; **43**: 365-372.

8. Leow, M.K., C.L. Addy, and C.S. Mantzoros, Clinical review 159: Human immunodeficiency virus/highly active antiretroviral therapy-associated metabolic syndrome: clinical presentation, pathophysiology, and therapeutic strategies. *J Clin Endocrinol Metab* 2003; **88**: 1961-1976.

9. Blanco, F., et al., Risk factors for advanced liver fibrosis in HIV-infected individuals: role of antiretroviral drugs and insulin resistance. *J Viral Hepat* 2011; **18**: 11-16.

10. Vuppalanchi, R. and N. Chalasani, Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: Selected practical issues in their evaluation and management. *Hepatology* 2009; **49**: 306-317.

11. Buzzetti, E., et al., Noninvasive Assessment of Fibrosis in Patients with Nonalcoholic Fatty Liver Disease. *Int J Endocrinol* 2015; 2015: 343828.

12. Tsochatzis, E.A. and L. Castera, Assessing liver disease in HIV-HCV coinfected patients. *Curr Opin HIV AIDS* 2014.

13. Wai, C.T., et al., A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526.

14. Sterling, R.K., et al., Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325.

15. Crossan, C., et al., Cost-effectiveness of non-invasive methods for assessment and monitoring of liver fibrosis and cirrhosis in patients with chronic liver disease: systematic review and economic evaluation. *Health Technol Assess* 2015; **19**: 401-409.

16. Brunt, E.M., et al., Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474.

17. Sterling, R.K., et al., The prevalence and risk factors for abnormal liver enzymes in HIV-positive patients without hepatitis B or C coinfections. *Dig Dis Sci* 2008; **53**: 1375-1382.

18. Maida, I., et al., Severe liver disease associated with prolonged exposure to antiretroviral drugs. *J Acquir Immune Defic Syndr* 2006; **42**: 177-182.

19. Kovari, H., et al., Incidence and risk factors for chronic elevation of alanine aminotransferase levels in HIV-infected persons without hepatitis b or c virus co-infection. *Clin Infect Dis* 2010; **50**: 502-511.

20. Ingiliz, P., et al., Liver damage underlying unexplained transaminase elevation in human immunodeficiency virus-1 mono-infected patients on antiretroviral therapy. *Hepatology* 2009; **49**: 436-442.

21. Morse, C.G., et al., Nonalcoholic Steatohepatitis and Hepatic Fibrosis in HIV-1-Monoinfected Adults With Elevated Aminotransferase Levels on Antiretroviral Therapy. *Clin Infect Dis* 2015; **60**: 1569-1578.

22. Guaraldi, G., et al., Nonalcoholic fatty liver disease in HIV-infected patients referred to a metabolic clinic: prevalence, characteristics, and predictors. *Clin Infect Dis* 2008; **47**: 250-257.

23. Crum-Cianflone, N., et al., Nonalcoholic fatty liver disease among HIV-infected persons. *J Acquir Immune Defic Syndr* 2009; **50**: 464-473.

24. de Ledinghen, V., et al., Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2006; **41**: 175-179.

25. Vergara, S., et al., The use of transient elastometry for assessing liver fibrosis in patients with HIV and hepatitis C virus coinfection. *Clin Infect Dis* 2007; **45**: 969-974.

26. Vallet-Pichard, A., et al., FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**: 32-36.

27. Blackard, J.T., et al., HIV mono-infection is associated with FIB-4 - A noninvasive index of liver fibrosis - in women. *Clin Infect Dis* 2011; **52**: 674-680.

28. DallaPiazza, M., et al., Prevalence and risk factors for significant liver fibrosis among HIV-monoinfected patients. *BMC Infect Dis* 2010; **10**: 116.

29. Al-Mohri, H., et al., Evaluating liver fibrosis progression and the impact of antiretroviral therapy in HIV and hepatitis C coinfection using a noninvasive marker. *J Acquir Immune Defic Syndr* 2007; **44**: 463-469.

30. Han, S.H., et al., Abnormal liver stiffness assessed using transient elastography (Fibroscan(R)) in HIV-infected patients without HBV/HCV coinfection receiving combined antiretroviral treatment. *PLoS One* 2013; **8**: e52720.

31. Mendeni, M., et al., Evaluation of liver fibrosis: concordance analysis between noninvasive scores (APRI and FIB-4) evolution and predictors in a cohort of HIV-infected patients without hepatitis C and B infection. *Clin Infect Dis* 2011; **52**: 1164-1173.

32. Mofrad, P., et al., Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* 2003; **37**: 1286-1292.

33. Adams, L.A., et al., The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113-121.

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

Characteristics	**N=156**
Gender, male (%)	143 (91.7)
Age, years	47.5 ± 8.5
AST, IU/L	41 (22-299)
ALT, IU/L	56 (29-372)
GGT, U/L	74 (13-960)
ALP, U/L	86 ± 34
PTL, /mm^3	217± 5
Diabetes, n (%)	17 (11)
Hypertension, n (%)	28 (18.2)
Dyslipidaemia, n (%)	81 (51.9)
Currently Receiving Statin, n (%)	47 (30.5)
Currently Receiving Fenofibrate, n (%)	7 (4.5)
Time since diagnosis, years	14 (2-30)
Current treatment, n (%)	154 (97)
PIs*, n (%)	41 (42.3)
NRTIs*, n (%)	86 (88.7)
NNRTs*, n (%)	40(41.2)
Duration of ART years (amongst those currently exposed)	11 (0-26)
HIV-RNA, copies/ mm^3>100000	0
Virologic suppression	146 (93.6)
CD4+, cells/mm3	683 (4-1900)

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

Information on drug category available only in 97 patients (62%)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltranspeptidase; ALP, alkaline phosphatase; PLT, platelets; PIs, protease inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors;

Table 2. Prevalence of liver disease in cohort of HIV mono-infected patients with deranged transaminases

Variables	N=156
Ultrasound examinations, n (%)	66 (42.3)
Ultrasound steatosis, n (%)	47 (71.2)
FIB4 score	
1.45, n (%)	105 (67.3)
1.46-3.24, n (%)	45 (28.8)
≥3.25, n (%)	6 (3.8)
APRI index	
0.5, n (%)	81 (51.9)
0.51-1.49, n (%)	70 (44.9)
≥1.5, n (%)	5 (3.2)

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

Patient characteristics	No steatosis (N= 19)	Steatosis (N=47)	Univariate analysis P value	Multivariate analysis P value	Multivariate analysis OR (95% CI)
Age, years	46.9 ± 9.5	47.9 ± 8.5	0.700		
Sex, males (%)	16(84.2)	43(91.5)	0.401		
Diabetes mellitus, n (%)	0(0)	6(12.8)	0.171		
Hypertension, n (%)	5 (26.3)	9 (19.4)	0.529		
Dyslipidaemia, n (%)	11 (57.9)	25 (53.2)	0.790		
Statin, n (%)	7 (36.8)	15 (31.9)	0.779		
Fenofibrate, n (%)	3 (15.8)	0 (0)	0.022	NS	
PLT, x103/mm3	218 ± 51	220 ± 52	0.836		
AST, IU/L	46± 14	46± 22	0.466		
ALT, IU/L	61± 15	66± 28	0.832		
ALP, U/L	92 ± 37	91± 29	0.835		
GGT, U/L	194 ± 273	97 ± 86	0.428		

Duration since diagnosis, years	15 ± 8	14 ± 7	0.451
Duration of treatment, years	12± 6	16± 6	0.729
PIs, n (%)	4(21)	13(27.7)	1.000
NRTIs, n (%)	11 (57.9)	32 (68.1)	1.000
NNRTs, n (%)	6 (31.6)	14 (29.8)	0.737
Virologic suppression, n (%)	18 (94.7)	44 (93.6)	1.000
CD4+ <200/mm3, n (%)	0	0	-
FIB4 >1.45, n (%)	7 (36.8)	17 (36.2)	1.000
APRI >0.5, n (%)	10 (52.6)	25 (53.2)	1.000

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltranspeptidase; ALP, alkaline phosphatase; PLT, platelets; PIs, protease inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; US, ultrasound.

Table 4. Variables associated with the presence of liver fibrosis assessed by FIB4 in the whole cohort of HIV mono-infected patients.

Patient characteristics	FIB4 ≤1.45 (N= 105)	FIB4 >1.46 (N=51)	Univariate analysis P value	Multivariate analysis	
				P value	OR (95% CI)
Age, years	-	-	-		
Sex, males (%)	94 (89.5)	49 (96.1)	0.224		
Diabetes mellitus, n (%)	8 (7.6)	9 (17.6)	0.098	NS	
Hypertension, n (%)	16 (15.2)	12 (23.5)	0.268		
Dyslipidaemia, n (%)	58 (55.2)	23 (45.1)	0.305		
Statins, n (%)	32 (30.5)	15 (29.4)	1.000		
Fenofibrate, n (%)	5 (4.8)	2 (4)	1.000		
PLT, x103/mm3	-	-	-		
ALT, IU/L	-	-	-		
AST, IU/L	-	-	-		
ALP, U/L	88 ± 33	80 ± 31	0.138		
GGT, U/L	99 ± 123	156 ± 191	0.104		

Time since HIV diagnosis, years	14 ± 7	16 ± 6	0.019	NS	
Duration of ART, years	10 ± 6	13 ± 6	0.002	0.041	1.008 (1.000-1.016)
PIs, n (%)	23 (21.9)	18 (35.3)	0.202		
NRTIs, n (%)	55 (52.4)	31 (60.8)	1		
NNRTs, n (%)	28 (26.7)	12 (23.5)	0.391		
Virologic suppression, n (%)	98 (9.3)	48 (94.1)	1.000		
CD4+ <200/mm3, n (%)	1 (0.9)	2 (3.9)	0.249		
US steatosis, n (%)	30 (28.6)	17(33.3)	1		

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltranspeptidase; ALP, alkaline phosphatase; PLT, platelets; PIs, protease inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; US, ultrasound.

Table 5. Variables associated with the presence of liver fibrosis assessed by APRI index in the whole cohort of HIV mono-infected patients.

Patient characteristics	APRI ≤0.5 (N= 81)	APRI>0.51 (N=75)	Univariate analysis P value	Multivariate analysis P value	Multivariate analysis OR (95% CI)
Age, years	47.1 ± 8.3	47.9 ± 8.8	0.57		
Sex, males (%)	73 (89.5)	70 (96.1)	0.568		
Diabetes mellitus, n (%)	9 (7.6)	8 (17.6)	1		
Hypertension, n (%)	14 (15.2)	14 (23.5)	0.836		
Dyslipidaemia, n (%)	45 (55.2)	36 (45.1)	0.423		
PLT, x103/mm3	-	-	-		
Statin, n (%)	23 (30.5)	24 (29.4)	0.601		
Fenofibrate, n (%)	4 (4.9)	3 (4)	1.000		
ALT, IU/L	56 ± 13	78 ± 49	0.001	0.01	1.033 (1.015-1.510)
AST, IU/L	-	-	-		
ALP, U/L	87 ± 28	85 ± 37	0.751		
GGT, U/L	85 ± 64	153 ± 201	0.223		

Duration of infection, years	15 ± 7	14 ± 7	0.425			
Duration of treatment, years	11 ± 6	11 ± 6	0.767			
PIs, n (%)	19 (23.4)	22 (29.3)	0.311			
NRTIs, n (%)	45 (55.5)	41 (54.7)	1.000			
NNRTs, n (%)	20 (24.7)	20 (26.7)	0.685			
CD4+ <200/mm3, n (%)	1 (0.9)	2 (3.9)	0.608			
Virologicalsuppression, n (%)	76 (93.8)	70 (93.3)	1.000			
US steatosis, n (%)	22 (28.6)	25 (33.3)	1.000			

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltranspeptidase; ALP, alkaline phosphatase; PLT, platelets; PIs, protease inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; US, ultrasound.