Aetiology and severity of liver disease in HIV positive patients with suspected NAFLD: lessons from a cohort with available liver biopsies

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Running Head: Aetiology of liver disease in HIV patients

ABSTRACT

Background: Spectrum of liver injury among HIV positive people is wide; in particular prevalence of non-alcoholic fatty liver disease (NAFLD) seems to be higher compared to HIV-negative people.

Methods: We retrospectively evaluated all liver biopsies performed at Royal Free Hospital from 2000 to 2017 in HIV mono-infected patients with abnormal transaminases, in order to assess the underlying cause of liver disease and to characterize the extent of fibrosis. We furthermore evaluated the diagnostic accuracy of FIB4 and Fibroscan™ as non-invasive tools for fibrosis assessment.

Results: 97 patients were included. Most common histological findings were NAFLD (28%), non-specific changes (26%) and normal histology (13%). 20% patients had significant fibrosis, 11% had advanced fibrosis. FIB4, at a cut-off of 1.3, had a specificity of 82% and NPV of 95% for exclusion of advanced fibrosis. Fibroscan was available in 28% patients and 33% had a liver stiffness ≥7.5 kPa. Fibroscan showed a specificity of 77% and NPV of 94% for exclusion of significant fibrosis. Among patients with NAFLD (n=27), 18% had advanced fibrosis while the majority (56%) did not have any fibrosis. The NPV of FIB4 and Fibroscan for advanced and significant fibrosis in these patients was 93% and 100% respectively.

Conclusions: Among HIV positive patients with elevated transaminases, a surprisingly high number of patients had non-significant changes or even normal histological findings. The prevalence of NAFLD was lower than reported in other series. Use of non-invasive tools with a high NPV for significant fibrosis can help reduce the number of required biopsies.

Key words: acquired immunodeficiency syndrome, liver histology, liver fibrosis, FIB4, Fibroscan

Introduction

HIV infection is a major global health issue with an estimated 36.7 million people living with HIV (PLWH) worldwide by the end of 2016[1]. The widespread availability of combination antiretroviral therapy (cART) in particular in resource rich settings countries has led to near normalisation of life expectancy in HIV-positive people with access to testing and early ART. The "Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D)" study demonstrated the reduction in HIV related mortality among PLWH but at the same time highlighted the increase in the proportion of patients dying from other causes and importantly, chronic liver disease (CLD) accounted for 10% of deaths during the years 2009-2011[2].

HIV-positive people with HBV/HCV co-infection, are at higher risk of developing hepatic complications particularly at the commencement of ART therapy[3,4]. Nevertheless, irrespective of the presence of co-infection, liver disease and abnormal liver blood tests (LFTs) are more prevalent in people who are HIV-positive compared to the general population[5]. Despite the growing attention to this issue, clinicians still struggle to characterize aetiology of abnormal LFTs in HIV mono-infected people[6,7], as the extent of liver abnormalities that can affect PLWH is vast[8]. Non-alcoholic fatty liver disease (NAFLD) is the most frequent underlying cause of abnormal LFTs among HIV mono-infected people[9-11] with a prevalence that seems to be higher than in the general population[12]. Moreover, there are efforts to validate tools of non-invasive fibrosis assessment among HIV positive people, particularly in those with NAFLD[13] .

We therefore performed a retrospective review of all liver biopsies performed in our centre from 2000 to 2017 in HIV mono-infected patients with abnormal LFTs; the aims were firstly to assess the underlying cause of abnormal LFTs and to characterize the extent of liver fibrosis and, secondly, to test the performance of non-invasive tests in assessing fibrosis severity.

Patients and methods

This is a retrospective study in HIV mono-infected patients with available liver histology and any grade of abnormal LFTs for ≥3 months at the time of biopsy. Abnormal LFTs were defined as an ALT value >35 U/L and/or AST >31 U/L according to the normal range values of the laboratory. We considered all the liver biopsies performed at Royal Free Hospital, an acute Trust in North London with tertiary HIV and liver centres, from January 2000 to June 2017 in patients with diagnosed HIV who were aged ≥18 years old. Liver biopsies were identified using the Trust pathology departmental clinical database with "HIV" and "liver" as searching terms. Clinical and patient data were obtained from the HIV clinical database. Exclusion criteria were: HBsAg positivity; previous or current HCV infection (anti-HCV positivity); concurrent infection (opportunistic or non-opportunistic) or treatment for suspected infection at time of biopsy; liver malignancy; liver biopsies performed on transplanted or explanted livers or on focal liver lesions; samples deemed suboptimal by the pathologist; biopsies performed in patients with hepatic decompensation or lack of sufficient clinical data.

For each patient we collected demographic, anthropometric and clinical data (presence of diabetes, dyslipidaemia, hypertension, cardiovascular disease, lipodystrophy, duration since HIV diagnosis, exposure to cART, time between HIV diagnosis and cART initiation, duration of cART, exposure to nucleoside/tide reverse transcriptase inhibitors (NRTI), non-nucleoside/tide reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), integrase inhibitors (II), d-drugs (stavudine, didanosine, zalcitabine) at the time of biopsy. Diabetes, hypertension and dyslipidaemia were identified from listed diagnoses or when relevant medication was prescribed.

Use of tobacco, illicit drugs and the degree of alcohol consumption were retrieved from the clinical records; alcohol abuse was defined as a daily consumption >30 g/day for men >20 g/d for women.

Blood test results at the time of biopsy (or within ±6 months if not available) were collected (full blood count, AST, ALT, ALP, GGT, bilirubin, albumin, INR, creatinine, urea, total cholesterol, HDL, LDL, triglycerides, HbA$_1$C, fasting glycaemia, TSH, fT4, CD4 cell count, CD8 cell count, CD4/CD8 ratio, HIV viral load).

Histological findings were classified in 11 categories: normal findings, non-specific changes, non-alcoholic fatty liver disease (NAFLD), drug-induced liver injury (DILI), alcohol related liver disease, mixed conditions (alcohol-related damage associated to other causes of liver damage), autoimmune liver diseases, metabolic liver diseases (haemochromatosis, alpha-1 antitrypsin deficiency), nodular regenerative hyperplasia (NRH), biliary diseases, cryptogenic cirrhosis.

Fibrosis stage was assessed using a 0-4 point scale based on the report from the pathologist (0= absent, 1=mild, 2=significant, 3=advanced, 4=cirrhosis) and disease-specific scoring systems used by the pathologist were converted into this scale. Biopsies with non-specific changes were assessed for grade of inflammation on a 0-3 point scale (0=absent, 1=mild, 2=moderate, 3=severe). Biopsies in keeping with NAFLD were sub-classified in simple steatosis (NAFL, steatosis in ≥5% of hepatocytes) and non-alcoholic steatohepatitis (NASH, concurrent presence of steatosis, lobular inflammation and ballooning degeneration).

To assess the accuracy of non-invasive measures of liver fibrosis, FIB4[14] was calculated for each patient (age (y)*AST (U/L) /platelet count (10^9/L)*√ALT (U/L))[15]. A cut-off of <1.3 was used for exclusion of advanced fibrosis.

Liver stiffness assessed by FibroScan[TM] (Echosens, France) was also considered for patients who had FibroScan[TM] performed within 1 year of the biopsy. FibroScan[TM] has been widely demonstrated a reliable tool to detect liver fibrosis but no established cut-offs exist for differentiating fibrosis stages. We considered a cut off of 7.5 kPa for detection/exclusion of significant fibrosis[16].

Statistical Analysis

Distribution of continuous variables were assessed and presented as mean ±SD (parametric data) or median (IQR) (non-parametric data). Tests of normality were used to assess the distribution of variables. Categorical variables were presented as frequencies and percentages (n,%). Univariate analysis was performed to find determinants of significant and advanced fibrosis. Comparison between categorical (fibrosis severity) and continuous variables was performed using T-student test for normal variables and Mann-Whitney test for not normal ones. Comparison between categorical variables was performed with Chi-square test. A two-tailed P-value ≤0.05 was considered statistically significant. Only variables with P-value ≤0.05 were entered in the multivariate analysis. Multivariable logistic regression was used to identify independent predictors of significant and advanced fibrosis. All data were analysed using the statistical package SPSS (version 22, IBM, New York, USA).

RESULTS

Selection of biopsies and baseline characteristics

We collected a total of 177 biopsies. From this initial pool, we excluded biopsies performed in patients with opportunistic and non-opportunistic infections (n=26), missing clinical data (n=17), co-infection with HCV/HBV (n=16), explanted/transplanted livers (n=7), multiple biopsies performed in the same patient, in which cases the most recent biopsy was used (n=7), hepatic decompensation (n=4), malignancy (n=2) and inadequate sample quality (n=1). Finally, 97 biopsies fulfilled our selection criteria. All included biopsies were performed to investigate abnormal LFTs.

Table 1 summarizes the baseline characteristics of the population. Mean age was 47±10 years, 81% were male, 66% of Caucasian ethnicity. Mean BMI was 27±6 Kg/m^2. Documentation on alcohol consumption was available for 85 (88%) patients, 20% of which reported hazardous alcohol use. 47% of patients had dyslipidaemia, 11% diabetes and 4% had a history of cardiovascular disease (3 acute

myocardial infarction and 1 chronic myocardial ischaemia). Most of the patients had undetectable HIV viral load (74%) and were on ART at the time of biopsy (89%). Three patients stopped cART before the biopsy (2 patients 5 months before and 1 patient 3 years before) whereas 8 patients had never been on cART at the time of the biopsy; thus 92% patients were cART experienced (past or current exposure to cART) with a median duration of cART of 100 months. Median duration since HIV diagnosis was 126 months. The majority of the patients were NRTI (92%), NNRT (72%), PI (68%) and d-drugs (55%) experienced.

Histological findings

The most common histological findings were NAFLD (n=27, 28%) and non-specific changes (n=25, 26%). Twelve biopsies (13%) showed a completely normal picture. Less common findings were consistent with DILI (n=8, 8%), alcoholic liver disease (n=5, 5%), nodular regenerative hyperplasia (n=5, 5%), metabolic liver disease (n=4, three with haemochromatosis and one with alpha-1 antitrypsin deficiency), mixed conditions associated with some degree of alcohol damage (n=4, of which three in keeping with alcohol and NASH based on clinical history and metabolic risk factors and one showing alcohol damage plus signs of porphyria cutanea tarda), autoimmune disease (n=3, 3% of which 2 in keeping with treated autoimmune hepatitis and 1 consistent with autoimmune cholangitis), biliary disease (n=2, one secondary biliary cirrhosis due to gallstones and one HIV cholangiopathy) and cryptogenic cirrhosis (n=2, of which one probably associated with heart failure).

Significant fibrosis, defined as ≥F2 stage, was present in 19 (20%) patients whereas advanced fibrosis, defined as ≥F3 stage, was present in 11 (11%) patients. The majority of patients had no fibrosis (n=61, 63%). Among biopsies that showed non-specific changes, more than half had mild inflammation (n=19, 76%) and absence of fibrosis (n=18, 72%). Table 2 summarizes the histological findings.

Factors associated with the presence of significant fibrosis in the univariate analysis were history of coronary artery disease, lower HDL cholesterol levels, detectable HIV viral load and diagnosis of NAFLD; of these, only HDL cholesterol level remained significantly associated in the multivariate analysis. No significant association was found with any other variable.

NAFLD subgroup

Compared to the non-NAFLD population, the NAFLD cohort was composed only of men (100%) with a higher BMI (29 Kg/m^2) and a higher prevalence of dyslipidaemia (81%). Baseline characteristics of patients with NAFLD are shown in table 1. Among the 27 biopsies in keeping with NAFLD, 15 (55%) had simple steatosis, 8 (30%) met the diagnostic criteria for NASH and 4 (15%) had NASH-cirrhosis. Significant and advanced fibrosis were present in 9 (33%) and 5 (18%) biopsies respectively. More than a half of the biopsies (n=15, 56%) showed no fibrosis. Table 3 summarizes the histological findings in the NAFLD subgroup.

The presence of NAFLD of any severity was associated with the presence of significant fibrosis only in the univariate analysis.

Accuracy of FIB4 in excluding advanced fibrosis

The prevalence of FIB4 scores <1.3, 1.3-2.67 and >2.67 was 38%, 46% and 16% respectively. Table 4 illustrates the matching between histology and FIB4 values in our cohort: FIB4 had 82% specificity and 95% a negative predictive value (NPV) for ruling out advanced fibrosis. In the NAFLD subgroup, the prevalence of FIB4 scores <1.3, 1.3-2.67 and >2.67 was 52%, 41% and 7%. Specificity and NPV for the exclusion of advanced fibrosis were similar to the general cohort (80% and 93% respectively).

Accuracy of liver stiffness assessed by FibroScan^{TM} in detecting significant fibrosis

FibroScan^{TM} values within 1 year of the biopsy were available for 27 (28%) patients and 9 (33%) of them had a liver stiffness >7.5 kPa. FibroScan^{TM} had a sensitivity of 80% (4/5), specificity of 77% (17/22) and NPV of 94% for significant fibrosis. In the NAFLD subgroup, 9 patients had valid FibroScan^{TM} values of which 6 (67%) were >7.5 kPa. Sensitivity, specificity and NPV were 100%, 60% and 100% respectively.

Discussion

In this study we evaluated the underlying cause of abnormal LFTs in HIV mono-infected patients referred for liver biopsy and assessed the severity of liver injury. Our study highlighted that NAFLD and non-specific changes were the most common findings in this population. We also found that a significant proportion of HIV people with raised transaminases had a completely normal histological picture, and the majority undergoing a liver biopsy had mild or no fibrosis. Based on this, we examined our cohort for the potential utility of a simple serological test (FIB4) in excluding severe liver disease and we demonstrated its excellent performance in ruling out advanced fibrosis (NPV 95%). Additionally, in a small subgroup of patients with available FibroScan™ results, we showed that FibroScan™ had an equally excellent performance in excluding significant fibrosis (NPV 94%).

Although this study was not designed to establish the overall prevalence of NAFLD in HIV mono-infected patients, it did confirm the significant prevalence of NAFLD among people with HIV (28% in those biopsied in our study). Interestingly, a recent meta-analysis shows that the global prevalence of NAFLD among the general population detected by different imaging techniques is 25.2% [17]: taken together, these data suggest that the prevalence of NAFLD among HIV patients might not significantly differ compared to the general population.

In line with our findings, several other studies have demonstrated that the burden of NAFLD in HIV mono-infected patients may be similar to the HIV negative population[12]. Of note, studies that included HIV patients with elevated LFTs and available liver histology, documented a high prevalence of NAFLD, in some cases almost three times higher than observed in our study [9-11,18]. Ingiliz et al. showed that in a cohort of 30 mono-infected patients with abnormal LFTs, after exclusion of other concomitant causes of CLD, NAFLD accounted for 60% of the cases and among these, steatosis was associated with inflammatory injury in 90% (NASH, 53% of the total cohort) [9]. A more substantial

number of liver biopsies were analysed by Morse et al. who investigated 62 HIV mono-infected people because of unexplained chronic elevated LFTs; NAFLD was diagnosed in 73% of cases and NASH in 55% [11].

Other studies, which used imaging techniques to detect steatosis among people with HIV, showed generally a lower prevalence of NAFLD compared to studies based on histology [19-22], with prevalence ranging from 29% using H-MRS [21] to 37% using CT [20] and 48% using the Fibroscan CAP [23].

The discrepancy between these data and ours is partially due to different diagnostic methods, patient inclusion criteria and, for older studies, different histological criteria used to define NASH. In our study we did not exclude a priori patients with alcohol abuse (20% of our cohort) or patients with known history of CLD, although only a small percentage (20%) had a diagnosis or high suspicion of CLD before undergoing liver biopsy. Exact definition of NASH has to be carefully evaluated when considering older studies, as several changes has been made over the years in the diagnosis of NASH, before the current histological criteria [24].

Taking all this into account, we believe that the prevalence of NAFLD in HIV cohorts identified from published studies, especially those with histological endpoints, may be an overestimation due to selection bias.

The second most common histological finding in our study was consistent with non-specific changes. This includes biopsies with only minimal steatosis, inflammation or fibrosis and non-diagnostic features, which did not allow the pathologist to formulate a specific diagnosis. Moreover, more than a half of our biopsies (72%) showed no evidence of fibrosis. These findings are not uncommon: in other studies on HIV mono-infected patients with available liver histology, non-specific abnormalities or biopsies without significant steatosis/inflammation were found in 13%-35% of cases [9-11]. Moreover, in our study a substantial number of biopsies reassuringly showed a completely normal picture (13%). Minimal lesions or normal histological pictures are also a common finding in studies

involving HIV-negative populations. For example de Ledinghen et al reported liver biopsy results in 272 HIV negative patients with unexplained chronically elevated ALT, which showed normal/almost normal liver in 20% of cases [25].

These results raise two main issues. First, as already noticed by Morse [11] there is a considerable overlap in the clinical, biochemical and imaging background among patients with non-specific/non diagnostic abnormalities and frequent pathological conditions such as NAFLD and NASH suggesting the need to develop further reliable criteria to discriminate between these two liver conditions. Secondly, it is important to re-evaluate the criteria clinicians rely on to perform a liver biopsy and to incorporate a more widespread use of non-invasive tests for diagnosis and fibrosis assessment before performing a liver biopsy, in order to prevent unnecessary risks.

Therefore, we evaluated the extent and the severity of liver fibrosis among HIV mono-infected people, as fibrosis is the strongest predictor of liver related mortality and is independently associated with long-term overall mortality in NAFLD [26,27]. More than half of included patients had no evidence of fibrosis (63%), whereas significant and advanced fibrosis was detected in 19% and 11% respectively, in line with the prevalence reported in similar studies [12]. In our study, the only factor independently associated with significant fibrosis was a lower level of HDL, while we could not identify predictive factors for advanced fibrosis. This is likely due to a type II error due to the relatively small number of patients and the heterogeneity in the diagnoses. Data from literature showed that abnormal lipid profile, especially low HDL and high triglycerides levels, have a higher prevalence in metabolic syndrome (MS) in HIV-positive compared to HIV-negative people with MS. In addition, not only is MS more prevalent in HIV-positive people, but also the composition of it differs compared to the general population [28,29]. On the other hand, there is some evidence that HIV-positive people with NAFLD are more likely to have features of severe liver injury than HIV negative people with NAFLD [18,30] despite a lower BMI and more intense physical activity [31]. This suggests that factors other than

those traditionally linking NAFLD and MS components can drive liver injury in these patients, such as the HIV infection per se or the prior use of ART.

Neither diabetes nor BMI were found to predict liver fibrosis in our cohort, likely due to the small sample size or the relatively low BMI and prevalence of diabetes in our population. Our findings contrast with the data from other studies [23,32-34], a meta-analysis [12] and from data on HIV-negative patients where insulin resistance is associated with severe fibrosis [35,36]. No factor associated with HIV infection or cART correlated with the presence of significant or advanced fibrosis in our population. Although we had a small sample size to be able to detect such differences, the majority of studies in HIV positive people has so far failed to convincingly associate HIV infection and its related therapy to NAFLD and fibrosis. Some exceptions are the studies of Guaraldi, who correlated NRTI exposure to the presence of NAFLD detected by CT[20] and studies by Blanco, Matthews and Pembroke, who found an association respectively between DDI/D4T exposure, HIV viremia, longer duration of HIV and detectable HIV viral load with higher fibrosis stages assessed with FibroScan[TM] [33,37,38]. These discrepancies could be due to patient exposure to earlier generations of ART with greater liver toxicity.

Considering the importance of detecting fibrosis in patients with suspected liver injury, we tested two different non-invasive tools, FIB4 and FibroScan[TM], looking at their potential utility in avoiding unnecessary biopsies.

FIB4 was initially validated in a cohort of HIV-HCV co-infected patients[14] showing a good performance in ruling out advanced fibrosis. In our cohort, a cut-off of 1.3 had a sensitivity of 41% and a specificity of 82% respectively for advanced fibrosis and NPV was 95% in the general cohort and 93% in the NAFLD subgroup. Using FIB4 to triage our patients we could have spared almost 40% (37/97) biopsies at the expense of a minor percentage of patients (5%, 2/37) who would have been incorrectly

classified. This obviously translates in a double benefit as it saves costs and reduces the risk related to invasive procedure for the patient.

The drawback of such kind of test is the relatively high percentage (46% in our cohort) of results included in the grey zone, which need to be further investigated. FibroScan™, as already demonstrated in HIV mono-infecteded patients[39], had a sensitivity of 80%, specificity of 77% and NPV of 94% in ruling out significant fibrosis. Importantly, non-invasive fibrosis tests perform better in excluding rather than detecting fibrosis[40].

Overall, these results support the validity of using non-invasive tests to triage HIV patients with suspected liver injury according to their risk of significant or advanced fibrosis[41] in order to select the appropriate patients for a liver biopsy. As there were a significant number of HIV mono-infected patients with normal histology or non-specific minimal changes, the use of non-invasive fibrosis tests would ensure a more careful selection before proceeding to a biopsy.

Limitations of the study are firstly its retrospective nature and secondly the lack of central reading for liver biopsies as we relied only on the final report made by the local pathologist. The small sample size limited the statistical power to detect associations with NAFLD and liver fibrosis. Since the referral for liver biopsy was based on an individual clinical decision, our population was also characterized by heterogeneity.

In conclusion, we showed that the most common causes of abnormal LFTs among HIV mono-infected patients referred for liver biopsy are NAFLD and non-specific changes and that these two conditions could not be distinguished based on LFTs elevation only. Indeed, raised LFTs should not trigger automatically a decision for a liver biopsy as the probability of finding normal/near normal histology is high. Non-invasive tools of fibrosis assessment, such as FIB4 and FibroScan™ can help to rule out

significant fibrosis and detect patients who do not require further investigation or specialist hepatology input.

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Table captions

Table 1. Characteristics of the study population

Table 2. Distribution of samples according to histological diagnosis and fibrosis stage (n=97)

Table 3. Histological findings of patients with NAFLD (n= 27)

Table 4. Matching between FIB4 values and fibrosis stage in all patients (n = 97) and in the NAFLD subgroup (n=27)