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Clinical significance of elevated liver transaminases in HIV-infected patients

Running head: Elevated transaminases in HIV

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Abstract

Elevation of liver transaminases is common in patients infected with the Human Immunodeficiency Virus (HIV). Although this is usually an incidental finding during regular work-up, HIV-infected patients with transaminases elevations require additional visits for laboratory studies and clinical assessments, and often undergo interruptions and changes in antiretroviral therapy (ART). Alanine aminotransferase (ALT) is present primarily in the liver, thus being a surrogate marker of hepatocellular injury. Aspartate aminotransferase (AST) is present in the liver and other organs, namely cardiac and skeletal muscle, kidney and brain. Serum levels of both liver transaminases predict liver-related mortality. Moreover, serum fibrosis biomarkers based on ALT and AST predict all-cause mortality. In a busy clinical setting, a diagnostic approach to elevated liver transaminases could be complicated given the frequency and non-specificity of this finding. Indeed, HIV-infected individuals present multiple risk factors for liver damage and chronic elevation of transaminases, including co-infection with hepatitis B and C viruses, alcohol abuse, hepatotoxicity due to ART, HIV itself, and frequent metabolic comorbidities leading to nonalcoholic fatty liver disease. This review provides an update on epidemiology of elevated liver transaminases, summarizes the main etiologic contributors and discusses the prognostic significance and a pragmatic approach to this frequent finding in the clinical practice of HIV medicine. With the aging of the HIV-infected population following the successful implementation of ART in Western countries, liver-related conditions are now a major comorbidity in this setting. As such, clinicians should be aware of the frequency, clinical significance and diagnostic approach to elevated liver transaminases.

Keywords: HIV, ALT, AST, Antiretroviral therapy, Liver fibrosis, Biomarkers, Mortality.

Introduction

In medicine, elevated transaminases refer to the elevation of aspartate aminotransferase (AST) or alanine aminotransferase (ALT). Their function is to catabolize amino acids, permitting them to enter the citric acid cycle[1]. AST is found in the liver, cardiac and skeletal muscle, pancreas, brain, kidneys, lungs, erythrocytes and leukocytes. ALT is found in liver, kidney, heart, and muscle. The concentration of ALT in the liver is significantly higher than in other tissues. Therefore, ALT levels are the most specific indicators of liver damage and serum ALT is the most commonly used test to assess for liver diseases. Serum ALT and AST levels may also be affected by gender, age, alcohol consumption, strenuous exercise, nutrition, metabolic parameters, coinfections and medications[2]. The range of the upper limit of normal (ULN) for ALT or AST level spans between 30 and 50 International Units (IU)/L and it varies among laboratories and assays[3-10] (Table 1). Conventional normal range may have been set too high, because the reference people presumed to be healthy may have asymptomatic liver disease. Indeed, guidelines suggest that the optimal cutoff for ALT should be lower (33 IU/L for men and 25 IU/L for women) than the upper limit usually adopted by laboratories[11].

The prevalence of elevated liver transaminases depends on the definition and population but is likely to be between 10 and 20% of the adult general population[12-14]. In approximately 10% of the cases, no cause of chronic hypertransaminasemia is found and the prognosis of this finding is unknown[15]. Elevated liver transaminases are particularly common in HIV-infected patients, occurring in 20%-93% of patients on antiretroviral therapy (ART), even in the absence of viral hepatitis coinfection[16, 17]. Causes of elevated transaminases in HIV-infected adults include coinfection with hepatitis viruses A, B, C, D, E, alcohol abuse, and ART toxicity. A possible contribution to chronic liver injury by HIV infection itself or the associated immunodeficiency has also been hypothesized[18, 19]. Furthermore, in HIV mono-

infected patients transaminases elevations may suggest the presence of an emerging and highly prevalent liver disease, namely nonalcoholic fatty liver disease (NAFLD), with associated hepatic fibrosis and cirrhosis[20-23]. In the general population, elevated ALT is associated with liver-related mortality[12, 24]. Likewise, in HIV-infected patients elevated liver transaminases are independently associated with all-cause and liver-related mortality[25, 26]. Patients with transaminase elevations require additional visits for laboratory and clinical assessments and often undergo interruptions and changes in ART[27].

Serum ALT and AST are readily available, inexpensive and routine biochemical assay used in clinical practice. Considering their accessibility, especially in developing countries or limited-resource settings, it is important to understand the clinical significance of elevated liver transaminases in the diagnosis, follow-up, prognosis, and treatment of HIV-infected patients. The American College of Gastroenterology and the British Gastroenterology Association have recently endorsed two guidelines on management of abnormal liver tests[11, 28]. This article aims to review existing literature regarding epidemiology and etiology of elevated transaminases in the specific context of HIV infection. We also provide evidence for association of elevated ALT and AST with frequent liver conditions in HIV-infected patients, namely NAFLD and liver fibrosis/cirrhosis. Finally, a pragmatic stepwise algorithm providing clinical guidance into management of elevated transaminases in the context of HIV infection is proposed.

Epidemiology of elevated transaminases in HIV-infected patients

The prevalence of elevated transaminases in HIV-infected patients varies by country, ethnicity, population characteristics and concomitant diseases (Table 2). According to the AIDS Clinical Trials Group Criteria, liver test abnormalities can be graded as follows: grade 1 (1.25 to 2.5 times ULN); grade 2 (2.6 to 5 times ULN); grade 3 (5.1 to 10 times ULN); and

grade 4 (more than 10 times ULN)[29].

In studies of HIV mono-infection, the prevalence of elevated transaminases ranged from 6.4% to 32%[17, 30-33], with significant variation by study population and design. In most cases, the abnormalities were grade 1 or 2, although grade 3 elevations of ALT have been reported in 13.5% of HIV+ patients coinfected with HBV[34]. Longitudinal studies reported an incidence of ALT elevation ranging between 3.9 and 7.6 per 100 person-years[30, 33, 35]. In HCV or HBV coinfected patients, the prevalence of elevated liver transaminases is higher, ranging from 13% to 51.8%[16, 36-41]. Interestingly, in the study by Shah and colleagues, reporting elevated ALT in 23.2% of HIV/HCV coinfected patients, 40% of women and 43% of men had ALT values <50 IU/L and <60 IU/L, respectively, while only 6% had ALT< 30 IU/l for men or <19 IU/l for women[36]. Similarly to HIV mono-infection, elevated transaminases were grade 1-2 in most studies, with only 1-4% having elevations in the grade 3-4 range[16].

Several factors may explain the different prevalence of elevated liver transaminases across studies, including different proportion of males included, distribution of ethnicity, metabolic factors and ART. For example, several studies have found an inverse relationship between black ethnicity and chronic ALT elevation in HIV-infected patients[30, 42]. The distribution of factors encompassed into the definition of metabolic syndrome, particularly obesity and diabetes, in the study population may also account for those differences[16, 32]. Finally, geographical variations in ART uptake and regimens may influence the prevalence of elevated liver transaminases.

Causes of elevated transaminases in HIV-infected patients

Elevated transaminases are widely prevalent in HIV–infected patients, even without viral hepatitis coinfection. Just as the burden of non-AIDS morbidity and mortality has changed in

the ART-era, the types of liver disease that clinicians may encounter in the practice of HIV care have also changed[43]. Before the ART-era, most of the hepatic diseases in HIV-infected patients were of infectious etiology, including bacteria (e.g. mycobacterium), fungi (e.g. cryptococcus) and viral (e.g. cytomegalovirus [CMV]) infections. HIV-related tumors, such as Kaposi sarcoma and lymphoma, were also associated with liver involvement[44]. However, since the ART-era and resulting aging of the HIV-infected population, the spectrum of liver disease has been significantly transformed by chronic infections with HCV and HBV, ART-induced hepatotoxicity, alcoholic liver disease and NAFLD[27, 45-48] (Table 3). Another possible contributor to chronic liver injury is HIV infection itself or the associated immunodeficiency (Figure 1a)[18, 19].

HIV

A number of studies showed that poorly controlled HIV infection, defined as lower CD4 cell count and/or higher HIV viremia, is an independent risk factor for chronic elevated transaminases[30, 49]. Several underlying mechanisms may explain the direct effect of HIV in causing liver injury. There are numerous studies demonstrating direct HIV infection of hepatic cells. HIV has a directly cytopathic effect on hepatocytes, primarily triggering apoptosis via the HIV gp120 protein-receptor signalling pathway[18, 50]. Kupffer cells, differentiated tissue macrophages that reside in the liver, can be infected by HIV *in vivo*[51]. HIV may also cause immune-mediated injury by altering the functions of the hepatic stellate cells and the Kupffer cells, the two primary immune cells in the liver[18]. Moreover, HIV induces liver fibrosis by entering the hepatic stellate cells[52]. HIV directly activates hepatic stellate cells via the gp120 receptor, activating metabolic pathways resulting in oxidative stress and liver injury[18]. Finally, HIV causes pro-inflammatory responses in the liver-immune cell milieu in mouse models[53].

Alcoholic liver disease

Chronic alcohol consumption is frequently observed in HIV-infected patients and is associated with adverse health effects[54]. In a survey of 951 patients interviewed at 14 HIV primary care sites in the US, 40% and 11% of the sample reported any alcohol and hazardous alcohol use, respectively[55]. Another study of 2,864 HIV-infected adults reported that 8% of the entire cohort and 15% of current alcohol drinkers were heavy drinkers, almost twice as prevalent as in the general population[56]. Chronic alcohol use is associated with decreased ART adherence and virological suppression and may result in alcoholic hepatitis or cirrhosis, leading to liver failure[57]. In patients coinfected with HCV, the immunosuppression of chronic alcohol consumption enhances viral replication, and the combination of alcohol and chronic viral infection has a synergistic, detrimental effect. Active alcohol intake is associated with faster liver disease progression and liver-related mortality levels in HIV/HCV coinfected patients[58]. Specific patterns of elevated transaminases have been associated with alcoholic liver injury, including an AST:ALT ratio of at least 2:1, with values of AST rarely exceeding 300 IU/L (Figure 1b)[59]. A higher ratio of AST:ALT, exceeding 3:1, further increases the likelihood of alcoholic liver disease. Zone 3 of the hepatic acinus has a higher concentration of AST and damage to this zone, usually ischemic or toxic (alcohol), results in greater alteration to AST levels[1].

Viral hepatitis coinfection

A significant proportion of liver disease among HIV-infected individuals is secondary to coinfection with HCV and/or HBV. Among the 35 million HIV-infected individuals globally, it is estimated that 4-5 million (11-14%) are coinfected with HCV[60]. The prevalence rates of HIV/HCV coinfection vary depending on the route of HIV transmission, from 5-20% among those with high-risk sexual behavior (sex between men) to over 50% in regions where

injection drug use is the main driver of the HIV epidemic[60, 61]. Up to 64% of patients with HIV/HCV coinfection have elevation of transaminases[16]. The typical pattern of liver transaminases in chronic hepatitis C is normal to less than twice the ULN, rarely more than 10 times ULN, with ALT higher than AST[62]. An AST:ALT ratio more than 1 suggests presence of advanced liver fibrosis or concomitant alcoholic disease (Figure 1b). Approximately 10% of the HIV-infected population has concurrent chronic hepatitis B, with coinfection more common in areas of high prevalence for both viruses. In countries where the two viruses are highly endemic, the rate can be as high as 25%[63]. People coinfected with HIV and HBV have faster liver disease progression to cirrhosis and hepatocellular carcinoma[63]. In chronic hepatitis B, transaminases elevation may occur in up to 58% and the pattern may vary from normal AST and ALT to mild to moderate elevations (approximately twice the ULN)[64]. Interestingly, the prevalence of elevated transaminases is higher in ART naïve patients as compared to those started on ART[34].

Beside the frequent coinfections with HCV and/or HBV, other viral infections should be ruled out in case of elevated liver transaminases, such as hepatitis D in patients infected with HBV, hepatitis A, hepatitis E and CMV (Table 3).

ART-induced hepatotoxicity

Hepatotoxicity is one of the most common side effects associated with ART. The clinical manifestation can range from a mild and asymptomatic picture to liver failure (Figure 1b). Hepatotoxicity may affect up to 23% of HIV-infected patients receiving ART[65]. The incidence of ART-related severe hepatotoxicity ranges between 2 and 18%[29, 30, 65]. All ART have a certain risk of hepatotoxicity, but some cause it more commonly than others (Table 3). Hepatic failure and fatalities have also been described, for example with abacavir, didanosine, stavudine, nevirapine and tipranavir[29]. Moreover, several factors may affect the

frequency and severity of drug-related liver injuries, such as viral hepatitis coinfection, older age, female sex, high body mass index (BMI), excessive alcohol consumption, low platelet count and high HIV viral load[29]. Acute or progressive ART-induced liver damages show different patterns: immune reconstitution syndrome, hypersensitivity reactions, mitochondrial toxicity, direct cell stress, liver steatosis. Non-nucleoside reverse transcriptase inhibitors (NNRTIs), abacavir, darunavir, fosamprenavir and maraviroc are the main drugs inducing hypersensitivity reactions. Late hypersensitivity reactions have been reported, but these immune-related effects usually occur within the first days after starting the drug[29]. Positivity for human leukocyte antigen-B*5701 has been associated with abacavir-induced hypersensitivity, and systematic screening has led to a significant decrease of such complications. Nucleoside reverse transcriptase inhibitors (NRTIs), particularly zidovudine, stavudine and didanosine, can induce mitochondrial damage leading to impaired fatty acid oxidation and respiratory chain responsible for microvesicular steatosis and lactic acidosis. Importantly, this toxicity never completely recovers[66]. Immune reconstitution is suspected in HIV-infected subjects with abnormal liver transaminases after the recent start of ART and it can lead to severe hepatitis requiring the interruption of ART[29]. Liver damage in association with use of protease inhibitors (PIs) has been observed in 2%–9% of HIV-infected patients[29]. Use of ritonavir-boosted PIs is particularly associated with elevations in transaminases. Patients with baseline fibrosis and cirrhosis appear to have an increased risk of developing transaminases abnormalities when exposed to PIs, possibly through their association with metabolic complications, such as hypertriglyceridemia, insulin resistance and hepatic steatosis[42]. Some ART regimens, particularly older PIs and NRTIs still in use in low- and middle-income countries, may also contribute to elevated transaminases by inducing lipodystrophy. The term refers to metabolic complications resulting in the accumulation of visceral and central fat in the abdomen, with associated insulin resistance[67]. Elevation of

liver transaminases have been observed in patients treated with integrase inhibitors. Hurt *et al* reported an incidence of grade 3-4 elevated transaminases of 5 per 100 person-years, with HIV/HCV coinfected patients having a 2.7 increased hazard compared to mono-infected patients[68]. Cases of hepatotoxicity and sporadic liver failure have also been reported in patients receiving dolutegravir-containing regimen without pre-existing hepatic disease[69]. Overall, hepatotoxicity related to ART could be an important contributor to the risk of hepatic fibrosis and disease progression in HIV-infected patients[66].

NAFLD and hepatic steatosis

NAFLD is the most frequent cause of unexplained elevation of liver transaminases[70]. NAFLD is a spectrum of clinical and pathologic changes characterized by a fatty overload involving over 5% of the liver weight in the absence of other causes of liver disease. This can evolve to non-alcoholic steatohepatitis (NASH), a progressive disease characterized by liver fibrosis leading to cirrhosis and related complications[71]. Importantly, NAFLD is a risk factor for all-cause mortality, due to excess cardiovascular disease and cancer[72]. NAFLD is the most common liver disease in Western countries, affecting 25% of the general population due largely to the epidemic of obesity and type 2 diabetes[71]. In HIV-infected people, this prevalence is significantly higher. As a consequence, NAFLD is increasingly recognized as the most frequent liver disease in persons aging with HIV mono-infection[73, 74]. In studies conducted in diverse patient populations and employing different diagnostic methods, the prevalence of NAFLD ranges from 13% to 65%[75]. NASH, the evolutive counterpart of NAFLD, has been reported in up to 65% of HIV mono-infected patients with chronically elevated ALT and in 10% of those attending a routine screening program[22, 23, 76]. Generally, the elevation of transaminases caused by NAFLD is mild to moderate, with AST and ALT consistently <4 times ULN. Liver tests can be normal in up to 79% of patients with NAFLD[77]. When elevated, enzymes show hepatocellular pattern, often with an AST/ALT

ratio of less than 1 (Figure 1b). Therefore, liver tests are not useful to make a diagnosis of NAFLD. However, persistently chronic elevation of ALT in absence of other causes of liver disease strongly suggest NAFLD, particularly in patients with the classical metabolic phenotype[11, 78].

Similarly to the general population, NAFLD is the most frequent finding in case of unexplained elevated liver transaminases in HIV-infected patients[22]. Overall, several studies reported that the levels of ALT or AST were significantly higher in HIV mono-infected patients with NAFLD or NASH as compared to those without[20, 76, 79, 80]. Moreover, elevated transaminases were independently associated with NAFLD or NASH in HIV-infected patients[20, 76]. However, in other studies metabolic factors, particularly BMI and high triglycerides, had the strongest association with NAFLD[20, 31, 79, 81]. Taken together, these findings suggest that, in HIV mono-infected patients, persistent elevation of transaminases should prompt investigation for NAFLD (Table 4). Consistently, recent guidelines from the European AIDS Clinical Society (EACS) recommend further assessment for liver disease severity and specialist referral in case of abnormal liver enzymes, with and without NAFLD[82].

In the setting of HIV/HCV coinfection, several studies found that ALT or AST levels were significantly higher in patients with hepatic steatosis as compared to those without[16, 83, 84]. Moreover, a few studies reported that elevated transaminases were independently associated with hepatic steatosis[83, 84]. However, in other studies BMI, ART exposure >4 years and histological activity had the strongest association with hepatic steatosis[73, 81, 85-87].

Specific considerations in the setting of low- middle-income countries

Few studies have investigated the burden of liver disease and, consequently, the epidemiology of elevated liver transaminases in low- middle-income countries. This is particularly true for noncommunicable liver diseases, such as alcoholic liver disease, NAFLD

and drug-induced liver injury. Data from Uganda suggested that other factors may contribute to liver disease in this setting, such as occupational exposure to schistosomiasis and frequent use of herbal medications[88, 89]. There is also a potential for increased rates of hepatotoxicity given that nevirapine-based regimens are more frequently used and the prevalence of tuberculosis is higher, with consequent increased use of isoniazid[90]. Despite this, some studies have suggested a low rate of grade 3 transaminases elevations attributable to ART in Uganda, Cameroon and Malawi[90, 91]. In addition, excess alcohol consumption has been reported in many low- and middle-income countries, such as Uganda and Nigeria[90, 92]. Finally, although NAFLD is increasingly recognized as a major contributor to elevated liver transaminases in Western countries, data are scarce for low- middle-income countries[90]. Overall, there is a need for studies addressing noncommunicable liver diseases contributing to elevated liver transaminases in the specific setting of low- and middle-income countries.

Association of elevated transaminases with liver fibrosis and cirrhosis

Given the frequency of elevated liver transaminases in HIV-infected patients, it is pivotal to understand how this finding translates in significant liver histologic damage. Independently of the etiology, chronic liver diseases have a common histopathological pathway that is the formation and accumulation of fibrosis. This mechanism eventually leads to the development of progressive distortion of the hepatic architecture, that is the hallmark of evolution to liver cirrhosis and associated end-stage liver complications, such as hepatocellular carcinoma and liver failure[93]. Natural history studies indicate that advanced fibrosis and cirrhosis develop in about 20%-40% of patients with chronic hepatitis B or C and in a similar proportion of those with alcoholic liver disease or NASH[94]. Importantly, liver fibrosis stage is the single most important prognostic factor impacting on the natural history of chronic liver disease of any etiology[56]. Liver fibrosis predicts not only liver-related death, but also all-cause mortality in chronic liver diseases[72, 95]. In HIV-infected patients, the proportion of deaths

attributed to liver-related etiologies has increased between 8 to 10-fold in the post-ART era while AIDS-related mortality has fallen more than 90-fold[96]. Cirrhosis and its complications now represent a leading cause of death in HIV-infected patients[48]. While coinfections with HCV and HBV are largely responsible for this excess liver-related morbidity and mortality, NAFLD seems to be a significant contributor to this trend in HIV mono-infected patients[45, 97]. Since liver transaminases are readily available tests in the clinical practice of HIV care, it is relevant to understand how these tests correlate with liver fibrosis and cirrhosis (Table 5). Several studies found elevated transaminases to be independently associated with significant liver fibrosis in HIV mono-infected patients[20, 32, 73, 98, 99]. However, in other studies metabolic factors were the strongest predictors of significant fibrosis or cirrhosis[31, 79]. On the same line, several studies reported an independent association between transaminases and liver fibrosis in HIV/HCV coinfected patients[73, 98, 99]. In the study by Bani-Sadr and colleagues, none of the patients with persistently normal ALT level had cirrhosis, while 5.8% of patients with elevated ALT had cirrhosis[100]. Several longitudinal studies showed that liver transaminases strongly predicted progression to liver fibrosis and cirrhosis in this setting[101-104].

On the basis of the association between liver transaminases and liver fibrosis, a number of simple serum biomarkers have been developed as surrogates of liver histology to predict presence or absence of significant liver fibrosis (stage F2-F4), advanced liver fibrosis (stage F3-F4) and cirrhosis (stage F4)[105]. These non-invasive biomarkers include serum parameters that are generally economic and routinely measured in HIV-infected patients (Table 6). The AST-to-ALT ratio was one of the first indirect markers for staging liver fibrosis in patients with chronic liver disease. An increase in AST-to-ALT ratio reflects progressive impairment of liver functional (normal value <0.8), while a ratio >1 is indicative of cirrhosis[106]. The AST-to-platelet ratio index (APRI) is calculated through AST and platelet count, thus it has

virtually no cost[107]. APRI can be used to confirm or exclude both significant fibrosis and cirrhosis. However, there is a grey area of values in which the performance of the test is not satisfactory and 30–50% of cases cannot be classified. To date, APRI is one of the most validated noninvasive biomarkers for liver fibrosis, applied to various etiologies of liver disease[108]. Another combination of simple parameters, named fibrosis-4 (FIB-4), is based on AST, ALT, age and platelet count. FIB-4 uses cut-off values to rule in or rule out advanced liver fibrosis[109]. The NAFLD fibrosis score is a combination of age, BMI, presence of diabetes, AST, ALT, platelet count and albumin developed to diagnose advanced liver fibrosis in the specific context of NAFLD[110]. FIB-4 and NAFLD fibrosis score are included in the diagnostic algorithm proposed by the EACS in case of suspected NAFLD[82].

Several studies have validated these simple fibrosis biomarkers in HIV/HCV coinfected patients against liver biopsy, demonstrating that the diagnostic accuracy is similar to that reported in HCV mono-infection[111, 112]. Fewer studies have instead validated these tests against liver biopsy in the setting of HIV mono-infection. In a detailed study employing liver histology as reference standard, Morse and colleagues reported an area under the curve (AUC) of 0.61, 0.64 and 0.70 for APRI, FIB-4 and NAFLD fibrosis score, respectively for the diagnosis of significant liver fibrosis in 62 HIV mono-infected patients[113]. Some studies demonstrated a significant association between these simple biomarkers and liver fibrosis diagnosed with transient elastography[114].

Taken together, current evidences indicate that elevation of liver transaminases in HIV-infected patients suggest presence of liver fibrosis. A chronic elevation could be particularly predictive of a progressive liver disease[115]. Although not yet extensively validated in the HIV-infected population, simple serum biomarkers based on liver transaminases seem to have a good diagnostic accuracy in this setting. Available data suggest that these biomarkers have similar diagnostic accuracy than in HIV-uninfected patients with chronic liver diseases,

even though inferior to that of more advanced tests, such as patented serum biomarkers and transient elastography[112]. Simple fibrosis and steatosis biomarkers are readily available and convenient to implement in the busy setting of HIV clinics. As such, they could be employed for risk stratification and prioritization for specialist referral, as also recommended by the recent EACS guidelines[82].

Prognostic value of elevated liver transaminases

In the general population, elevated ALT is associated with liver-related mortality, while elevated AST has been associated with all-cause mortality[11]. Moreover, both ALT and AST levels are independent predictors of the risk of developing hepatocellular carcinoma[116].

Several studies demonstrated a similar trend in the context of HIV infection. In a study of 193 HIV/HCV coinfected and 720 HIV mono-infected persons, Scherzer and colleagues reported that higher AST was associated with 41% increased odds of all-cause mortality. This association was particularly strong for HIV/HCV coinfected patients, with an odds ratio of 2.5 (95% confidence interval [CI] 1.5-4.1)[25]. The D.A.D cohort study found that elevated ALT was significantly associated with liver-related mortality in 31,235 HIV-infected patients. Interestingly, ALT levels were U-shaped in relation to all-cause mortality: ALT level less than 18 IU/L or more than 60 IU/L was associated with higher all-cause mortality[26]. Similar results have been reported in a large study from the general population, where ALT in the normal range displayed an inverse relationship with total mortality, cardiovascular and non-cardiovascular events[117]. The biological mechanism for this last finding is unclear, but possible explanations may include low ALT as a marker for poor nutrition or its association with frailty[118].

Simple serum fibrosis biomarkers based on transaminases have been repeatedly shown to be associated with liver-related and all-cause mortality. In a study of 57 HIV/HCV coinfected

men, Price *et al* reported that APRI increases significantly 3 years prior to the development of liver-related death. Another study conducted in 673 HIV-infected patients, followed for a median of 4.6 years, found that the baseline natural logarithm of APRI was predictive of liver complications (adjusted hazard ratio [aHR] = 4.0, 95% CI: 2.5 to 6.4 per log)[119]. Similarly, Nunes and colleagues found that APRI and FIB-4 predicted 3-year liver mortality in 207 HIV/HCV coinfected patients, with an AUC of 0.88 and 0.87, respectively[120]. Interestingly, Jain *et al* reported that an increase in FIB-4 was predictive of 7-year all-cause mortality (aHR = 1.02, 95% CI: 1.00-1.03) in HIV+ patients coinfected with HCV and/or HBV[121]. In a cohort study of 20,308 HIV-infected patients on ART from Zambia, APRI $\geq$1.5 was predictive of all-cause mortality during a total of 49,058 person-years of follow-up (aHR = 1.41, 95% CI: 1.21-1.64). On the same line, patients with FIB-4 $\geq$3.25 experienced increased all-cause mortality during ART (aHR = 1.44, 95% CI, 1.25–1.65)[122].

Current evidence indicates that elevated ALT and AST levels are significantly associated with increased liver-related mortality. Moreover, elevated AST, APRI and FIB-4 are predictive of all-cause mortality. As such, these simple and readily available tests could be used for prognostication and risk stratification, particularly in busy HIV clinics, primary care and low resource settings. Besides liver disease, clinicians should be aware of the link between liver transaminases and other diseases, particularly cardiovascular disease and diabetes, and follow-up the relevant indicators regularly.

Pragmatic approach to elevated liver transaminases

Elevated transaminases should be investigated through a pragmatic stepwise approach to the differential diagnosis and management, particularly in case of a chronic pattern (Figure 2). Demographic characteristics including age, BMI, sex and ethnicity should be obtained to confirm the specific ULN of the patient[11]. The initial medical history should assess risk

factors for liver diseases, including viral hepatitis, components of the metabolic syndrome, recent changes in ART regimens, exposures to medications, toxins such as alcohol, and complementary alternative medications. Other infections, autoimmune and uncommon genetic liver diseases should be looked for if other investigations are inconclusive. When simple fibrosis biomarkers, namely APRI, FIB-4 and NAFLD fibrosis score, are indeterminate or suggestive of significant liver disease, another non-invasive staging tool for liver fibrosis should be obtained, such as transient elastography. Imaging examinations may also include computerized tomography, magnetic resonance elastography, acoustic radiation force impulse, magnetic resonance imaging-derived proton density fat fraction, according to local availability. A liver biopsy should be used as a definitive diagnostic method when the transaminases elevation remains unexplained or in case of discordance between non-invasive tests[123]. Once the etiologic diagnosis of elevated transaminases is established, the management for common diseases in HIV-infected patients depends on the underlying disease. The diagnosis of drug-induced liver injury is difficult to establish as this is usually an exclusionary diagnosis. It is essential to obtain a clear timeline of the exposure to medications and appearance of elevated transaminases. Moreover, a complete list of medications and supplements should be compiled and all non-essential agents removed. Liver function should be performed at ART initiation and patients with symptoms associated with liver damage should be regularly scheduled for ALT and AST[124]. Anti-tubercolosis medications are also a relative common cause of drug-induced liver injury and elevation of transaminases[47].

Conclusion

The proportion of deaths attributed to liver-related etiologies has undergone a 10-fold increase in the post-ART era[48, 125]. Elevated liver transaminases are now a common finding in HIV-infected patients. The main etiologic contributors to the burden of liver diseases in HIV-infected patients include viral hepatitis coinfection, NAFLD, alcohol abuse, ART-

related liver injury and infection-related factors. Chronic liver transaminase elevations are independently associated with hepatic steatosis and liver fibrosis in both HIV mono-infected and HIV/HCV coinfected patients. Several simple fibrosis biomarkers based on liver transaminases, including APRI, FIB-4 and NAFLD fibrosis score, can help guide the clinician in the management of the individual patient. Moreover, considering the clear association between elevated liver transaminases and related biomarkers with both liver-related and all-cause mortality, these simple and inexpensive tests can help in risk stratification, resources utilization, and prioritization for specialist referral to hepatology. Overall, clinicians practicing HIV care should promptly approach the finding of elevated liver transaminases for its diagnostic and prognostic implications.

Authors contributions

JC contributed to study design, data, interpretation of the data and first draft of the manuscript. MO contributed to interpretation of the data and first draft of the manuscript. GS contributed to conception, study design, data and interpretation of the data, and first draft of the manuscript. All authors approved the final version of the article.

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Table 1. Studies proposing ULN for ALT and/or AST levels.

Reference	ULN (male)	ULN (female)
Lee[9]	33 IU/L (ALT)	25 IU/L (ALT)
Neuschwander-Tetri[5]	40 IU/L (ALT)	40 IU/L (ALT)
Piton[6]	42 IU/L if BMI≤23 66 IU/L if BMI>23 (ALT)	31 IU/L if BMI≤23 44 IU/L if BMI>23 (ALT)
Prati[7]	30 IU/L (ALT)	19 IU/L (ALT)
Pratt[8]	40 IU/L (ALT and AST)	40 IU/L (ALT and AST)
Ruhl[10]	29 IU/L (ALT)	22 IU/L (ALT)
Siest[3]	56 IU/L (ALT), 31 IU/L (AST)	34 IU/L (ALT), 31 IU/L (AST)
Zhang[4]	22IU/L (ALT), 25 IU/L (AST)	22 IU/L (ALT), 24 IU/L (AST)

Legend: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; IU, International units; ULN, upper limit of normal.

Table 2. Prevalence of elevated liver transaminases in HIV-infected patients.

Reference/ Country	N	Design	Age	Male (%)	Ethnicity (%)	HCV or HBV coinfection (%)	On ART (%)	Prevalence (%)
Alghamdi[33] / Saudi Arabia	387	Retrospective, longitudinal	40.0	93	Asian (100)	0	83	ALT (32)
Audsley[34]/ Australia, US, Thailand	143	Prospective, cross-sectional	>40 in 62.9%	90.2	NA	HBV (100)	100	ALT (10.6)
Bailey[40]/ Ukraine	2,050	Retrospective,cross-sectional	27.7 (24-31)	0	White (100)	HCV (33), HBV (17)	28	ALT (24), AST (34)
Crum-Cianflone[17] / US	299	Retrospective,cross-sectional	39 (30-46)	93	White (49)	0	67	ALT (20), AST (10)
Jha[38]/ Nepal	144	Retrospective,cross-sectional	34 (18-61)	56	Asian (100)	HCV (6), HBV (0.7)	100	ALT (42.4)
Kovari[30]/ US	2,365	Prospective, longitudinal	38 (33-45)	66	White (84)	0	56	ALT (16)
Lombardi[32] / UK	156	Retrospective,cross-sectional	47.5±8.5	92	NA	0	97	ALT (18.6), AST (6.4)
Lombardi[31] / UK	125	Prospective, cross-sectional	39.5±10.3	91	NA	0	68	ALT (16.8), AST (5.6)

Nagu[39]/ Tanzania	41,891	Retrospective,cross-sectional	36±10	29	Black (100)	HCV (2), HBV (6)	0	ALT (13)
Pathania[41]/ India	247	Retrospective,cross-sectional	40.5±11.2	70	NA	HCV (0.4), HBV (0.8)	86	ALT or AST (51.8)
Puri[37]/ India	320	Prospective, cross-sectional	35.4±7.3	88	Asian (100)	HCV (9), HBV (12)	79	ALT or AST (44.6)
Shah[36]/ US	237	Retrospective,cross-sectional	46±8	74	Black (79)	HCV (100)	84	ALT (23.2)
Shur[115]/ UK	222	Retrospective, cross-sectional	47.6 ± 10.1	90.8	White (83.7)	0	100	ALT (27)
Sterling[16]/ US	1,208	Retrospective,cross-sectional	42.1±9.7	63	White (24.7)	HCV (24), HBV (7.1)	66	ALT (23.8), AST (31.5)

Legend: Continuous variables are expressed as mean±standard deviation or median (interquartile range) and categorical variables are presented as numbers (%). ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; ULN, upper limit of normal; NA, not available.

Table 3. Causes and patterns of elevated transaminases in HIV infection in the ART-era.

Cause	**Pattern**	**Clinical presentation/implicated agent or drug**
Infection		
Viral hepatitis: HCV, HBV, HDV, HAV, HEV, CMV, Epstein Barr Virus, Herpes Simplex Virus, Varicella Zoster Virus, Human Herpes Virus-6.	ALT >AST in viral infections.	- 11-14% and 10% of HIV-infected individuals are coinfected with HCV and HBV, respectively[60, 63]. - HDV should be considered in case of HBV infection. - HAV can cause fulminant hepatitis (if underlying liver disease). - Chronic HEV infection reported in HIV-infected patients[47]. - CMV is one of the most common opportunistic infection in HIV-infected patients with AIDS.
Opportunistic infections: Mycobacterium avium complex, CMV, Cryptococcus	Alkaline phosphatase is usually disproportionally increased, with mild to	Hepatic involvement in advanced AIDS.

neoformans, Microsporidium, Pneumocystis jirovecii, Histoplasma capsulatum, Schistosoma mansoni, Bartonella henselae	moderate elevations of liver transaminases[47].	
NAFLD	AST:ALT ratio usually <1	Prevalence ranges from 13% to 65%[75].
Alcoholic liver disease	AST:ALT ratio of at least 2:1[59]	Prevalence of any and hazardous alcohol use is at 40-53% and 11-15%, respectively[55, 56].
Nodular regenerative hyperplasia	Normal to mildly elevated liver transaminases. Biochemical features of portal hypertension.	Rare but severe condition leading to noncirrhotic portal hypertension, usually associated with use of didanosine[126].
Other liver diseases	Liver transaminases elevated, associated with cholestatic pattern elevation in primary biliary cholangitis and primary sclerosing cholangitis.	Primary biliary cholangitis, Primary sclerosing cholangitis, Autoimmune hepatitis, Hemochromatosis, Wilson's disease, Alpha-1 antitrypsin.
Medication toxicity		
ART	- ALT and/or AST.	- Incidence of ART-related

	- Mechanisms: hypersensitivity reactions, mitochondrial toxicity, lipid/sugar metabolism disturbances and steatosis, direct liver cell stress, immune reconstitution.	severe hepatotoxicity ranges between 1 and 14%. - Chronic viral hepatitis increases the risk. - Most implicated drugs: Abacavir, Didanosine, Stavudine, Zidovudine (NRTI); Delavirdine, Efavirenz, Nevirapine (NNRTI); Atazanavir, Darunavir, Indinavir, Lopinavir, Ritonavir, Tipranavir (PI)[29].
Antifungals	ALT and/or AST.	Frequently used in HIV-infected patients: Ketoconazole, Fluconazole, Amphotericin B[47].
Antibiotics	Hepatotoxicity can present as hepatocellular injury (elevated ALT and/or AST), cholestatis injury or mixed hepatocellular-cholestatic pattern.	Frequently used in HIV-infected patients: Ciprofloxacin, Dapsone, Trimethoprim-sulfamethoxazole[47].
Tubercolosis treatment	Elevated ALT and/or AST	Frequently used in HIV-infected patients: Isoniazid, Rifampin, Pyrazinamide,

		Ethambutol[47].
Anti-virals	Elevated ALT and/or AST	Frequently used in HIV-infected patients: Ganciclovir, Acyclovir[47].
Anabolic/Androgenic steroids	Cholestatic pattern, peliosis hepatis, liver masses.	Frequently used in HIV-infected patients: Testosterone, Nandrolone, Oxandrolone[47].
Recreational drugs	Cocaine hepatotoxicity presents with marked elevation of liver transaminases and elevated lactate dehydrogenase[127]. Liver involvement in MDMA intoxication usually presents with hepatocellular pattern and marked elevation of liver transaminases[128].	Recreational drugs, such as cocaine and MDMA (Ecstasy), are more frequently used by HIV-infected patients[129].
Neoplasm		
Lymphoma	Mild to moderate elevations of liver transaminases, sometimes associated with abdominal pain or jaundice.	Non-Hodking lymphoma and Kaposi's sarcoma involve the liver in 33% and 9% of cases, respectively[47].
Kaposi's sarcoma		
Hepatocellular carcinoma	Mild to marked elevation	Mostly occurring in the context

	of liver transaminases, alpha-fetoprotein ranges between normal to values in the thousands.	of liver cirrhosis. Incidence rate reported at 2.8 cases per 1000 person-years[130].
AIDS cholangiopathy	Markedly increased alkaline phosphatase, less elevated bilirubin, normal or slightly increased transaminases.	Most commonly caused by Cryptosporidium, followed by CMV. Other reported pathogens involved: Microsporidium, Cyclospora cayetanensis, Mycobacterium avium intracellulare, Histoplasma capsulatum[47].
Acalculous cholecystitis	Elevation of cholestatic indices and liver transaminases.	Usually caused by CMV or Cryptosporidium. Microsporidium and Isospora also reported[47].

Legend: AIDS, acquired immunodeficiency syndrome; ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; CMV, c*ytomegalovirus*; EBV, E*pstein–Barr virus;* HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; *MDMA, 3,4-methylenedioxymethamphetamine;* NAFLD, nonalcoholic fatty liver disease; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Table 4. Association between elevated transaminases and NAFLD or hepatic steatosis in HIV-infected patients.

Reference	N	Study design	Age	Male (%)	Ethnicity White/Black (%)	HCV or HBV Coinfection (%)	On ART (%)	Diagnostic method for NAFLD	Association With NAFLD or NASH
Benmassaoud[76]	202	Prospective cross-sectional	53.8± 10.5	77	48/36	0	90.6	Transient elastography with CAP	ALT: OR=2.39 (1.50-3.79) for NASH
Ryan[131]	225	Cross-sectional case-control study	43.8± 7	81	NA	HCV (48), HBV (12)	NA	Ultrasound	ALT and AST not associated with hepatic steatosis
Castera[86]	137	Retrospective cross-sectional	39.3± 5.8	65	NA	HCV (100)	91	Liver biopsy	ALT not associated with hepatic steatosis
Guaraldi[21]	225	Prospective cross-sectional	48 (range 19-74)	72.4	NA	0	100	CT scan	ALT to AST ratio: OR= 4.59 (2.09–10.08) for NAFLD
Lombardi[32]	156	Retrospective cross-sectional	47.5± 8.5	92	NA	0	97	Ultrasound	ALT not associated with hepatic steatosis
Lombardi[31]	125	Prospective cross-sectional	39.5± 10.3	91	NA	0	68	Transient elastography with CAP	ALT not associated with hepatic steatosis
Lui[79]	80	Retrospective cross-sectional	53.9± 11.2	92.5	93.5 (Chinese)	0	100	Transient elastography with CAP	ALT not associated with hepatic steatosis
Macias[81]	505	Prospective cross-sectional	46 (41-49)	69	95/1.4	HCV (50), HBV (6.7)	94	Transient elastography with CAP	ALT not associated with hepatic steatosis

Morse[22]	62	Prospective cross-sectional	50 (17-67)	94	65/8	0	97	Liver biopsy	ALT and AST associated with NASH in univariate analysis; multivariate NA
Neau[84]	148	Prospective cross-sectional	38.8 (24-70)	64	NA	HCV (100)	90.5	Liver biopsy	AST＞2.5 ULN: OR=2.9 (1.1-7.4) for hepatic steatosis
Pembroke[73]	726	Prospective cross-sectional	50 (42-56)	75	52/32	HCV (22.7), HBV (3.2)	92	Transient elastography with CAP	ALT not associated with hepatic steatosis
Price[132]	465	Prospective, cross-sectional	53 (48-58)	100	52/36	HCV (12)	92	CT scan	ALT>40 IU/L: OR= 2.54 (1.20- 5.36) for NAFLD
Rodriguez-Torres[83]	283	Prospective cross-sectional	40.2	84	NA	HCV (100)	NA	Liver biopsy	ALT: OR=1.20 (1.02-1.41) for hepatic steatosis
Sulkowski[87]	112	Retrospective cross-sectional	38	64	NA/94	HCV (100)	74	Liver biopsy	ALT and AST not associated with hepatic steatosis
Vecchi[133]	118	Prospective cross-sectional	45.1	72.9	NA	HCV (51.7)	83.9	Ultrasound	ALT and AST not associated with hepatic steatosis
Verma[134]	60	Retrospective cross-sectional	39.4± 8.2	90	40/NA	HCV (100)	72	Liver biopsy	ALT not associated with hepatic steatosis
Vuille-Lessard[20]	300	Prospective cross-sectional	50	77	42/40	0	88.7	Transient elastography with CAP	ALT: OR=3.17 (1.43-7.07) for NAFLD

Legend: Continuous variables are expressed as mean±standard deviation or median (interquartile range) and categorical variables are presented as numbers (%). Associations between transaminases and a variable are reported as odds ratio (OR) and 95% confidence interval. ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; HBV, hepatitis B virus; HCV, hepatitis C virus; NA, not available; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OR, odds ratio; ULN, upper limit of normal.

Table 5. Association between elevated transaminases and liver fibrosis in HIV-infected patients.

Reference	N	Study design	Age	Male (%)	Ethnicity White/ Black (%)	HCV or HBV Coinfection (%)	On ART (%)	Diagnostic method for fibrosis	Association with liver fibrosis
Barreiro[98]	283	Retrospective cross-sectional	42±5	71	NA	HCV (100)	94	Transient elastography	ALT: OR=1.02 (1.01-1.03) for significant liver fibrosis
Charpentier[99]	60	Prospective, cross-sectional	50 (46-53)	75	NA	HCV (100)	100	Transient elastography	AST: OR=1.06 (1.02-1.10) for advanced liver fibrosis
Leite[101]	30	Retrospective longitudinal	41.5 (29-56)	83	NA	HCV (100)	100	Liver biopsy	ALT and AST not associated with liver fibrosis
Lemoine[135]	405	Retrospective cross-sectional	53±9	89	NA	0	54.8 PI	Transient elastography	ALT and AST not associated with liver fibrosis

Lombardi[32]	156	Retrospective cross-sectional	47.5±8.5	92	NA	0	97	FIB-4 APRI	ALT: OR=1.033 (1.015-1.510) for significant liver fibrosis
Lombardi[31]	125	Prospective cross-sectional	39.5±10.3	91	NA	0	68	Transient elastography	ALT and AST not associated with liver fibrosis
Lui[79]	74	Retrospective cross-sectional	53.9±11.2	92.5	93.5 (Chinese)	0	100	Transient elastography	ALT and AST not associated with liver fibrosis
Pembroke[73]	726	Prospective cross-sectional	50 (42-56)	75	52/32	HCV (22.7), HBV (3.2)	92	Transient elastography	ALT: OR=1.13 (1.04-1.24) for significant liver fibrosis
Vuille-Lessard[20]	300	Prospective cross-sectional	50	77	42/40	0	88.7	Transient elastography	ALT: OR=3.30 (1.27-8.59) for significant liver fibrosis

Legend: Continuous variables are expressed as mean±standard deviation or median (interquartile range) and categorical variables are presented as numbers (%). Associations

between transaminases and a variable are reported as odds ratio (OR) and 95% confidence interval. Significant liver fibrosis is defined as stage F2-F4; advanced liver fibrosis is defined as stage F3-F4; cirrhosis is defined as stage F4[105]. ALT, alanine aminotransferase; APRI, AST-to-Platelets Ratio Index; ART, antiretroviral therapy; AST, aspartate aminotransferase; FIB-4, fibrosis-4; NA, not available; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OR, odds ratio.

Table 6. Simple biomarkers based on liver transaminases for the diagnosis of hepatic steatosis and liver fibrosis/cirrhosis employed in HIV-infected patients.

Reference	Biomarker	Formula	Validated population	End-point (Cut-off)	AUC	Reference method
[111, 136-144]	APRI	[(AST/ULN AST) x 100] /Platelet count	HIV mono-infected, HIV/HCV coinfected	Significant liver fibrosis (0.5 / 1.5), cirrhosis (1 / 2)	0.61-0.89	Liver biopsy
[111, 138, 140-142, 144]	FIB-4	(Age [yr]x AST [IU/L]) / ((Platelet count) x (√ALT [IU/L]))	HIV mono-infected, HIV/HCV coinfected	Advanced liver fibrosis (1.45 / 3.25)	0.64-0.80	Liver biopsy
[111]	AST/ALT ratio	AST / ALT	HIV/HCV coinfected	Cirrhosis (1)	0.60	Liver biopsy
[113]	NAFLD fibrosis score	-1.675 + 0.037 × age (year) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet count - 0.66 × albumin (g/dL)	HIV mono-infected	Advanced liver fibrosis (-1.455 / 0.676)	0.70	Liver biopsy
[145]	Hepatic steatosis index	8 x ALT/AST + BMI (+2, if female; +2, if diabetes)	HIV mono-infected, HIV/HCV coinfected	Hepatic steatosis (30 / 36)	0.88	Ultrasound

Legend: Significant liver fibrosis is defined as stage F2-F4; advanced liver fibrosis is defined as stage F3-F4; cirrhosis is defined as stage F4[105]. ALT, alanine aminotransferase; APRI, AST-to-Platelets Ratio Index; ART, antiretroviral therapy; AST, aspartate aminotransferase;

AUC, area under the curve; BMI, body mass index; FIB-4, fibrosis-4; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IFG, impaired fasting glucose; NA, not available; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; ULN, upper limit of normal.