AIDS

A gathering storm: HIV infection and non-alcoholic fatty liver disease (NAFLD) in low and middle-income countries (LMIC)

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Abstract:	Liver disease remains one of the major causes of morbidity and mortality amongst people living with HIV and a significant proportion of liver disease in these individuals can be attributed to non-alcoholic fatty liver disease (NAFLD). NAFLD in HIV infection is a growing problem in view of increasing life expectancy associated with the use of effective anti-retroviral therapy (ART), wider uptake of ART and increasing rates of obesity in many Asian as well as Western countries. The problem may be more pronounced in developing countries where there are limited resources available for mass screening and diagnosis of NAFLD. There is a small but growing body of literature examining NAFLD in the setting of HIV, with data from low and middle-income countries (LMIC) particularly lacking. In this paper, we discuss the epidemiology, risk factors, diagnostic approaches and therapeutic options available for NAFLD in the setting of HIV, with a special emphasis on the situation in LMICs.
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Abstract

Liver disease remains one of the major causes of morbidity and mortality amongst people living with HIV and a significant proportion of liver disease in these individuals can be attributed to non-alcoholic fatty liver disease (NAFLD). NAFLD in HIV infection is a growing problem in view of increasing life expectancy associated with the use of effective anti-retroviral therapy (ART), wider uptake of ART and increasing rates of obesity in many Asian as well as Western countries. The problem may be more pronounced in developing countries where there are limited resources available for mass screening and diagnosis of NAFLD. There is a small but growing body of literature examining NAFLD in the setting of HIV, with data from low and middle-income countries (LMIC) particularly lacking. In this paper, we discuss the epidemiology, risk factors, diagnostic approaches and therapeutic options available for NAFLD in the setting of HIV, with a special emphasis on the situation in LMICs.

A gathering storm: HIV infection and non-alcoholic fatty liver disease (NAFLD) in low and middle-income countries (LMIC)

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Introduction

Liver disease remains one of the major causes of morbidity and mortality amongst people living with HIV.[1, 2]. A significant proportion of the liver disease in these individuals can be attributed to non-alcoholic fatty liver disease (NAFLD), which has been defined as hepatic steatosis without significant alcohol intake (less than 30 g/day for men and 20 g/day for women) or co-infection with such as hepatitis B (HBV) and hepatitis C (HCV) [3-5].

The disease spectrum of NAFLD can range from mild steatosis to an inflammatory state known as non-alcoholic steato-hepatitis (NASH) which may progress to cirrhosis, and hepatocellular carcinoma (HCC) (Figure 1) [6]. NAFLD in HIV infection is a growing problem in view of increasing life expectancy associated with the use of effective anti-retroviral therapy (ART), wider uptake of ART and increasing rates of obesity in many Asian as well as Western countries. Increasing rates of obesity have also been observed in people living with HIV[7] . The problem may be more pronounced in developing countries where there are limited resources available for mass screening and diagnosis of NAFLD [8]. There is a small but growing body of literature examining NAFLD in the setting of HIV, with data from low and middle-income countries (LMIC) particularly lacking.

In this review, we discuss the epidemiology, risk factors, diagnostic approaches and therapeutic options available for NAFLD in the setting of HIV, with a special emphasis on the situation in LMICs.

Pathophysiology - Risk Factors and Natural History

The risk factors that predispose individuals with HIV to NAFLD include traditional risk factors that are also observed in the general population and others which are more specific to HIV infection. Traditional risk factors include most components of metabolic syndrome (metS) such as obesity, Type 2 diabetes mellitus (T2DM) associated with underlying insulin resistance due to reduced physical activity and over nutrition [9-11], dyslipidemia and hypertension. When compared to HIV-negative individuals, the risk of having metS is almost double in those with HIV and a prevalence of 15–60% has been reported in some studies.

This increased incidence has been observed in both ART-naïve and HIV-infected individuals on ART [12, 13].

Certain drugs, other than ART, are also responsible for the development of steatohepatitis. Commonly used medications such as amiodarone, methotrexate, tamoxifen, sodium valproate and glucocorticoids are associated with developing fatty liver; the exact pathophysiologic mechanisms, which are multi-factorial, are not fully elucidated [14]. It is postulated that not only do these drugs predispose to metS and thereby increase the chances of developing fatty liver, but may also escalate hepatocyte lipogenesis, reduce the secretion of fatty acids and interrupt mitochondrial beta oxidation [14]. The concurrent use of these medications with ART may result in added risk of developing NAFLD.

HIV-associated risk factors for NAFLD are related to ART and lipodystrophy. Antiretrovirals which precipitate an adverse lipid profile include the nucleotide reverse transcriptase inhibitors (NRTI) these may indirectly increase the risk of NAFLD via lipid profile alterations. Didanosine (ddI) and stavudine (d4T) are the most commonly implicated NRTI associated with NAFLD, however their use has been phased out in nearly all treatment guidelines and national programs [15]. Switching to NRTI-sparing regimens has been associated with an increase in subcutaneous fat and there is some evidence that those on efavirenz (EFV)-based regimens had higher loss of limb fat compared to those on PI-containing regimens [16]. ART-associated lipodystrophy has primarily occurred with the ARVs ddI, d4T, zidovudine (ZDV) and to a lesser extent EFV [16]; is associated with insulin resistance and dyslipidemia and is also a major factor driving NAFLD in HIV-infected individuals. Trunk to lower limb fat ratio, as measured using dual-energy x-ray absorptiometry (DEXA), can be used as a surrogate measure of lipodystrophy. In a South Asian HIV cohort receiving ART, a trunk to lower limb fat ratio cut-off > than 2.28 identified lipodystrophy and had the highest odds ratio (OR) for predicting metS - an additional cardiovascular risk [17].

The pathogenesis of hepatic steatosis, NAFLD and NASH

Insulin resistance mediates the increased flux of free fatty acids to hepatocytes from adipose tissue. This induces oxidative stress at the level of the endoplasmic reticulum (ER),

promoting steatosis and inflammation. This, coupled with cellular dysfunction, is now referred to as a 'multiple parallel hit model' to explain the development of NASH, which precedes NAFLD [18]. This cellular dysfunction is impacted by the adipocytokines generated in adipose tissue and gut microflora [19]. The relationship between obesity and adipocytokines is complex, with increased adipose tissue secretion of TNF-α and interleukin-6 but decreased secretion of adiponectin in HIV-negative populations [20]. Recent data has shown that increased adiponectin level is associated with a lower risk of fatty liver regardless of HIV status [20].

The relationship between NAFLD, insulin resistance and visceral fat is complex and not fully understood; however pro-inflammatory cytokines such as interleukin (IL) 6, C-reactive protein (CRP) and pro-fibrogenics such as fibrinogen may play an important role in this complex interplay [21].

In the setting of HIV infection, increased levels of adiponectin have been associated at the univariate level with lower levels of liver fibrosis, but not when adjusted for MetS [22]. NASH is defined by steatosis, inflammation and hepatocyte damage; and increased bacterial translocation associated with altered gut permeability has been proposed as a trigger for liver inflammation [23]. As HIV infection leads to altered gut permeability and increased bacterial translocation, together with mitochondrial toxicity secondary to NRTI's, this may further add to inflammation in the setting of HIV.

Natural history of NAFLD and NASH in HIV infection

The early stages of NAFLD usually begin with hepatic steatosis which is largely benign, however this may be a forerunner in some individuals to the development of NASH. NASH, when coupled with fibrosis, may in turn lead to more serious consequences like cirrhosis and, in some, even HCC which can occur in the absence of cirrhosis[9]. Approximately 25-30% of HIV-negative individuals with NAFLD develop NASH [6]. In HIV-positive individuals, however, the severity both at initial presentation and that of progression is higher[24].

Though there is limited data available on histopathological correlation, in a cohort of HIV-positive individuals (91% on ART) with biopsy-proven NAFLD, the prevalence of NASH was 65% and the majority had fibrosis [15, 24]

Co-infection with HCV genotype 3 is also associated with insulin resistance and a higher prevalence of hepatic steatosis, and it is unclear how effectively this regresses after achieving a sustained virological response [25]. However, with effective treatment now available for HCV, the risk of ongoing inflammation and contribution to development of NAFLD from HCV may reduce over time, although this is dependent on access to directly acting antivirals for HCV which is variable in LMIC [26, 27].

HIV-associated NAFLD seems to be more severe in clinical and biochemical severity. Compared to HIV-uninfected individuals, in the setting of HIV there is significantly higher liver enzyme and serum triglyceride levels and higher rates of steatohepatitis with more features of liver injury, such as the presence of acidophil bodies and lobular inflammation [24]. Risk factors associated with progression of fibrosis included advancing age, a higher HIV viral load, increased liver enzymes and the use of ddI/d4T [24]. As ddI/d4T are now rarely used, the role of contemporary NRTIs merits further investigation and there is currently insufficient published data for metanalysis of exposure by drug class [28]. Meta-analyses have shown that in the general NAFLD population, those with fibrosis have an increased risk for all-cause mortality and that risk increases with stage of fibrosis [29]. HIV can infect hepatic stellate cells, hepatocytes and Kupffer cells [30, 31], and given that significant fibrosis has been observed in treated HIV mono-infection [30, 32, 33], prospective fibrosis assessment is very important in HIV-NAFLD. In HIV mono-infection duration of ddI and AZT have been associated with cirrhosis, undetectable HIV (<40copies/ml) with the absence of significant of fibrosis and duration of boosted-PIs had a negative correlation with abnormal liver-stiffness values [32, 33].

Management – Diagnostic modalities and available treatment

The diagnostic criteria and therapeutic approach to NAFLD in HIV-positive individuals is the same as for HIV-negative individuals. A range of biochemical and imaging tools have been used both to diagnose NAFLD and to determine the severity, especially in those HIV-positive individuals with metS, lipodystrophy and/or abnormal liver function tests (LFTs). Of the currently available biomarkers/panels, there is no single ideal biomarker that can be used to diagnose NAFLD, however there are certain non-invasive models utilising routine clinical parameters that have been proposed to assist diagnosis of steatosis. These are summarised in Table 1 (serum biomarkers) and Table 2 (diagnostic/imaging modalities).

Current non-invasive diagnostic and imaging techniques have limited success in identifying those individuals with NAFLD who are likely to progress to NASH. The development and validation of reliable, cost-effective and widely available non-invasive biomarkers and imaging techniques for use in clinical care are essential to screen and closely follow individuals with NAFLD to diagnose NASH earlier in the course of the disease and to enable the initiation of life style measures to reduce the risk factors for progression [34]. A combined approach, using the various advantages of different biomarkers/modalities to individual requirements, may provide an efficient way forward.

Cohort studies examining NAFLD in the setting of HIV

To date there have been more than 30 studies examining NAFLD in the setting of HIV. We have reviewed the fourteen studies which all excluded co-infection with HCV and/or HBV as well as excessive alcohol intake (Table 3). All but one were cross-sectional [35] and cohort size ranged from 14 - 796, with nine studies having cohorts of more than 60 participants. None of these studies were performed in LMICs indicating the significant gap of our understanding of NAFLD in this setting.

Modalities used for the diagnosis of NAFLD/NASH/steatosis varied, including ultrasound, liver biopsy, computed tomography (CT), magnetic resonance spectroscopy (MRS), hepatic steatosis index (HSI) and controlled attenuation parameter (CAP). Reported prevalence of NAFLD and NASH in HIV ranged from 26-65% and 31-72% respectively. Only two studies

reported statistically significant associations between HIV-related factors and NAFLD or NASH after adjustment, which included duration of HIV infection [24] and longer exposure to NRTIs [36]. All other significant associations were non-HIV related, including traditional risk factors that have been identified in the general population: waist circumference [36, 37], age [38], lower HDL [37], higher triglycerides [37, 39], ALT/AST ratio and/or ALT [36, 40, 41], HOMA-IR [12, 38, 42, 43], higher fasting glucose and insulin [12], male gender [36, 38], black ethnicity [35], lower albumin [35], BMI [40, 41], visceral adipose tissue (VAT) [42], dyslipidemia [40], higher APRI and FIB-4 scores [24] and γ glutamyl transferase (GGT) [38, 43].

Two studies identified associations with metabolic genetic factors. The over-expression of sterol regulatory element binding protein 1 (SREBP-1), involved in the regulation of lipid metabolism, was associated with steatosis [44] and the presence of single-nucleotide polymorphisms in the gene coding for patatin-like phospholipase domain-containing 3 protein (PNPLA3) was associated with NASH [45]. However, these polymorphisms are general risk factors and not HIV-specific.

While all fourteen study cohorts had a lower proportion of women participants (0-29%) compared to men, male gender was reported as a risk factor in two cohorts (24% and 8% female [36, 38]). One cohort (35% female) presented major analyses by gender [42]. They reported that while HIV-infected women (73% on ART) had a higher liver fat fraction (LFF) than HIV-negative women, LFF was similar in HIV-positive (97% on ART) and HIV-negative men. LFF was defined as ratio of total lipids to total lipids and unsuppressed water, as measured by MRS and expressed as a percentage. There was less VAT in women than men, and HIV-infected women had 25% less LFF than HIV-infected men.

Only one published study to date has examined hepatic steatosis longitudinally, however while the cohort was large (n=796) the study was retrospective and diagnosed steatosis using the hepatic steatosis index (HSI) [35]. Median follow-up was 4.9 years and they found an incidence of steatosis of 6.9/100 person-years (95%CI 5.9-7.9). They reported associations between development of hepatic steatosis with black ethnicity and lower albumin, in adjusted models.

A recent systematic review and meta-analysis that examined 10 of these studies and reported a prevalence of NAFLD and NASH in the setting of HIV of 35% (95% CI 29-42) and 42% (95% CI 22-64), respectively [28]. While the key risk for factors for NAFLD were related to metS (BMI, waist circumference, T2D, hypertension and triglycerides), they did report an association between NAFLD and higher CD4 count (mean difference of 54.83 (95% CI 11.55-98.11, *p*=0.0)), based on data from four studies [36, 37, 39, 40].

While NAFLD is more prevalent in HIV than in the general population, studies in HIV mono-infection cohorts to date have not consistently found statistically significant associations with HIV-related factors. This may likely be due to the limited numbers of studies that have investigated NAFLD in HIV and excluded viral hepatitis/excess alcohol, and the diversity in the cohorts. There is an obvious need for prospective longitudinal studies sufficiently powered to robust outcomes.

Treatment for NAFLD and NASH

There are no treatments for NAFLD or NASH that are specific to people living with HIV. Presently, lifestyle modification and dietary changes are the main interventions for NAFLD, in conjunction with the specific treatment of associated metabolic disorders. A multi-disciplinary team approach is beneficial to manage such cases [46].

Life style changes including weight loss achieved by both dietary changes and enhanced physical exercise remains the first line of treatment [47]. Weight loss of about 3 to 5% of body weight is recommended to improve steatosis and about 10% to improve histological features of NASH, including portal inflammation and fibrosis [47]. Pharmacologic therapy is currently offered only to those with NASH, and in particular those with NASH and significant fibrosis. Current European guidelines suggests the use of glitazones or Vitamin E, or in combination [48]. There are no drugs to date that have completed phase III trials and none approved for NASH by regulatory authorities [48]. The underlying risk factors should be managed as per the standard of care for individual disorders [49].

There are several new agents in development in the drug pipeline. Of interest in HIV-NAFLD is cenicriviroc (CVC), a chemokine receptor type 5 (CCR5) and chemokine receptor type 2

(CCR2) antagonist that has anti-HIV activity both in vitro and in vivo [50, 51]. In addition, CVC has been shown to reduce circulating markers of monocyte inflammation in HIV-infected individuals on ART, including soluble CD14 [51]. In a large randomised phase 2b study of CVC in HIV-negative individuals with NASH (CENTAUR) [52], CVC resulted in a reduction in multiple markers of inflammation, including IL1b, high-sensitivity CCRP, IL6 and fibrinogen. In addition, a reduction in soluble (s)CD14 and increase in the ligands for CCR2 (CCL2) and CCR5 (CCL3 and 4) increased. Most importantly, this was associated with a significant decline in hepatic fibrosis [53]. CVC is now in Phase 3 clinical trials and is not yet approved.

Specific challenges in LMICs

Most LMIC ART programs routinely offer TDF-based therapy as first line treatment due toxicity associated with ddI, d4T and AZT. However, the availability of newer NRTIs, such as TAF and abacavir-based regimens are still out of reach for many in LMICs due to cost. In addition, most government programs do not currently have any defined guidelines to detect ongoing liver inflammation. Most liver dysfunction is diagnosed either incidentally by assessment of liver function tests (often in the stage of steatohepatitis) or on presentation with decompensated liver disease. Cost effective screening algorithms for LMICs based on sound scientific data are needed to screen for NAFLD related disorders.

In India specifically, as South Asians have higher total body fat for a given BMI than Caucasians, including at normal range BMI [54, 55], healthy BMI may not be indicative of total body fat in this population. Rates of metabolic syndrome and insulin resistance are high in Asian populations, and in particular in South Asia [56]. Data in HIV-infection from some Asian countries have shown an incidence of NALFD among HIV-infected individuals of Asian origin similar to that observed in Western countries, and an association with conventional NAFLD related risk factors[40]. There are some studies with published data from HIV-infected cohorts, including published Japan [40], China [39] and Thailand [57]. Data from the Indian subcontinent is sparse and mainly confined to autopsy in the general population, where in one study 9.2% of the cohort was HIV positive [58]. Data from African countries is not uniform, with both a lower prevalence than in Caucasians reported in HIV-infected

individuals on ART (range 6-42 months) in Nigeria [59] while a similar prevalence to that in the West was reported in an HIV-infected individuals on ART (minimum 6 months) in Cameroon [60]. There is a great need for prospective cohort studies generating data in LMICs, which would then lead to specific policies and guidelines, tailored to these countries [61, 62].

Management and monitoring of NAFLD in HIV-infected individuals, particularly in LMIC where there is increased visceral fat in the healthy weight range, increasing levels of obesity and pre-diabetes combined with large numbers of people living with HIV, could be a very significant health burden that requires management and prevention strategies. Screening individuals newly diagnosed with HIV using a cost-effective and widely available modality such as liver ultrasound would be beneficial, although effective treatment options for NAFLD beyond modification of life-style factors remain elusive. An initial focus on monitoring those diagnosed with NAFLD in the setting of HIV in LMICs would be useful, particularly tracking fibrosis progression with T/E or CAP to identify those at risk of NASH and/or HCC.

Gaps in existing literature/Knowledge

While there is a small but growing body of literature on HIV-associated NAFLD, there are still large gaps regarding the optimal guidelines for NAFLD screening and management in the setting of HIV, particularly in LMIC. Guidelines in those who are HIV-infected may need to differ from the HIV-negative population as NAFLD prevalence in the setting of HIV appears higher, progression to NASH more frequent and a role for HIV-related factors has not been fully elucidated. Importantly, there is no data to date prospectively examining NAFLD progression in HIV-positive individuals and very little published from LMIC and in HIV-infected women. Further research is also required to develop non-invasive markers for diagnosis and monitoring of NAFLD and its progression to NASH to identify those at highest risk for advanced liver disease. An increased role of genetic testing for predicting progression and new therapeutic interventions could potentially play a role in the future.

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References

1. Smith C, Sabin CA, Lundgren JD, Thiebaut R, Weber R, Law M, et al. **Factors associated with specific causes of death amongst HIV-positive individuals in the D:A:D Study**. *AIDS (London, England)* 2010; 24(10):1537-1548.
2. Weber R, Ruppik M, Rickenbach M, Spoerri A, Furrer H, Battegay M, et al. **Decreasing mortality and changing patterns of causes of death in the Swiss HIV Cohort Study**. *HIV Medicine* 2012.
3. Lemoine M, Serfaty L, Capeau J. **From nonalcoholic fatty liver to nonalcoholic steatohepatitis and cirrhosis in HIV-infected patients: diagnosis and management**. *Current opinion in infectious diseases* 2012; 25(1):10-16.
4. Sanyal AJ, Brunt EM, Kleiner DE, Kowdley KV, Chalasani N, Lavine JE, et al. **Endpoints and clinical trial design for nonalcoholic steatohepatitis**. *Hepatology (Baltimore, Md)* 2011; 54(1):344-353.
5. The Antiretroviral Therapy Cohort C. **Causes of Death in HIV-1–Infected Patients Treated with Antiretroviral Therapy, 1996–2006: Collaborative Analysis of 13 HIV Cohort Studies**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2010; 50(10):1387-1396.
6. McCullough AJ. **Pathophysiology of nonalcoholic steatohepatitis**. *Journal of clinical gastroenterology* 2006; 40 Suppl 1:S17-29.
7. Crum-Cianflone N, Roediger MP, Eberly L, Headd M, Marconi V, Ganesan A, et al. **Increasing Rates of Obesity among HIV-Infected Persons during the HIV Epidemic**. *PLOS ONE* 2010; 5(4):e10106.
8. Ahmed MH, Noor SK, Bushara SO, Husain NE, Elmadhoun WM, Ginawi IA, et al. **Non-Alcoholic Fatty Liver Disease in Africa and Middle East: An Attempt to Predict the Present and Future Implications on the Healthcare System**. *Gastroenterology research* 2017; 10(5):271-279.
9. Angulo P. **GI epidemiology: nonalcoholic fatty liver disease**. *Alimentary pharmacology & therapeutics* 2007; 25(8):883-889.
10. Bonfanti P, Giannattasio C, Ricci E, Facchetti R, Rosella E, Franzetti M, et al. **HIV and metabolic syndrome: a comparison with the general population**. *Journal of acquired immune deficiency syndromes (1999)* 2007; 45(4):426-431.
11. Grunfeld C. **Insulin resistance in HIV infection: drugs, host responses, or restoration to health?** *Topics in HIV medicine : a publication of the International AIDS Society, USA* 2008; 16(2):89-93.
12. Ingiliz P, Valantin M-A, Duvivier C, Medja F, Dominguez S, Charlotte F, et al. **Liver damage underlying unexplained transaminase elevation in human immunodeficiency virus-1 mono-infected patients on antiretroviral therapy**. *Hepatology (Baltimore, Md)* 2009; 49(2):436-442.
13. Price JC, Seaberg EC, Latanich R, Budoff MJ, Kingsley LA, Palella FJ, Jr., et al. **Risk factors for fatty liver in the Multicenter AIDS Cohort Study**. *The American journal of gastroenterology* 2014; 109(5):695-704.
14. Rabinowich L, Shibolet O. **Drug Induced Steatohepatitis: An Uncommon Culprit of a Common Disease**. *BioMed research international* 2015; 2015:168905.
15. Blanco F, Barreiro P, Ryan P, Vispo E, Martín-Carbonero L, Tuma P, et al. **Risk factors for advanced liver fibrosis in HIV-infected individuals: role of antiretroviral drugs and insulin resistance**. *Journal of viral hepatitis* 2011; 18(1):11-16.
16. de Waal R, Cohen K, Maartens G. **Systematic Review of Antiretroviral-Associated Lipodystrophy: Lipoatrophy, but Not Central Fat Gain, Is an Antiretroviral Adverse Drug Reaction**. *PLOS ONE* 2013; 8(5):e63623.
17. Asha HS, Seshadri MS, Paul TV, Abraham OC, Rupali P, Thomas N. **Human immunodeficiency virus-associated lipodystrophy: an objective definition based on dual-energy x-ray absorptiometry-derived regional fat ratios in a South Asian population**. *Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists* 2012; 18(2):158-169.
18. Tilg H, Moschen AR. **Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis**. *Hepatology (Baltimore, Md)* 2010; 52(5):1836-1846.

19. Stojsavljevic S, Gomercic Palcic M, Virovic Jukic L, Smircic Duvnjak L, Duvnjak M. **Adipokines and proinflammatory cytokines, the key mediators in the pathogenesis of nonalcoholic fatty liver disease**. *World journal of gastroenterology* 2014; 20(48):18070-18091.
20. Price JC, Wang R, Seaberg EC, Budoff MJ, Kingsley LA, Palella FJ, et al. **The Association of Inflammatory Markers With Nonalcoholic Fatty Liver Disease Differs by Human Immunodeficiency Virus Serostatus**. *Open forum infectious diseases* 2017; 4(3):ofx153.
21. Byrne CD, Targher G. **NAFLD: a multisystem disease**. *J Hepatol* 2015; 62(1 Suppl):S47-64.
22. Lemoine M, Lacombe K, Bastard JP, Sebire M, Fonquernie L, Valin N, et al. **Metabolic syndrome and obesity are the cornerstones of liver fibrosis in HIV-monoinfected patients**. *AIDS (London, England)* 2017; 31(14):1955-1964.
23. Schuster S, Cabrera D, Arrese M, Feldstein AE. **Triggering and resolution of inflammation in NASH**. *Nature reviews Gastroenterology & hepatology* 2018; 15(6):349-364.
24. Vodkin I, Valasek MA, Bettencourt R, Cachay E, Loomba R. **Clinical, biochemical and histological differences between HIV-associated NAFLD and primary NAFLD: a case-control study**. *Alimentary pharmacology & therapeutics* 2015; 41(4):368-378.
25. McGovern Barbara H, Ditelberg Jeremy S, Taylor Lynn E, Gandhi Rajesh T, Christopoulos Katerina A, Chapman S, et al. **Hepatic Steatosis Is Associated with Fibrosis, Nucleoside Analogue Use, and Hepatitis C Virus Genotype 3 Infection in HIV-Seropositive Patients**. *Clinical Infectious Diseases* 2006; 43(3):365-372.
26. Ford N, Wiktor S, Kaplan K, Andrieux-Meyer I, Hill A, Radhakrishnan P, et al. **Ten priorities for expanding access to HCV treatment for people who inject drugs in low- and middle-income countries**. *International Journal of Drug Policy* 2015; 26(11):1088-1093.
27. Walsh N, Durier N, Khwairakpam G, Sohn AH, Lo Y-R. **The hepatitis C treatment revolution: how to avoid Asia missing out**. *Journal of Virus Eradication* 2015; 1(4):272-275.
28. Maurice JB, Patel A, Scott AJ, Patel K, Thursz M, Lemoine M. **Prevalence and risk factors of nonalcoholic fatty liver disease in HIV-monoinfection**. *AIDS (London, England)* 2017; 31(11):1621-1632.
29. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, et al. **Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis**. *Hepatology (Baltimore, Md)* 2017; 65(5):1557-1565.
30. Mohr R, Schierwagen R, Schwarze-Zander C, Boesecke C, Wasmuth JC, Trebicka J, et al. **Liver Fibrosis in HIV Patients Receiving a Modern cART: Which Factors Play a Role?** *Medicine* 2015; 94(50):e2127.
31. Singh KP, Crane M, Audsley J, Avihingsanon A, Sasadeusz J, Lewin SR. **HIV-hepatitis B virus coinfection: epidemiology, pathogenesis, and treatment**. *AIDS (London, England)* 2017; 31(15):2035-2052.
32. Anadol E, Lust K, Boesecke C, Schwarze-Zander C, Mohr R, Wasmuth JC, et al. **Exposure to previous cART is associated with significant liver fibrosis and cirrhosis in human immunodeficiency virus-infected patients**. *PLoS One* 2018; 13(1):e0191118.
33. Han SH, Kim SU, Kim CO, Jeong SJ, Park JY, Choi JY, et al. **Abnormal Liver Stiffness Assessed Using Transient Elastography (Fibroscan®) in HIV-Infected Patients without HBV/HCV Coinfection Receiving Combined Antiretroviral Treatment**. *PLOS ONE* 2013; 8(1):e52720.
34. Loomba R, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, et al. **Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis**. *Hepatology (Baltimore, Md)* 2012; 56(3):943-951.
35. Sebastiani G, Rollet-Kurhajec KC, Pexos C, Gilmore N, Klein MB. **Incidence and predictors of hepatic steatosis and fibrosis by serum biomarkers in a large cohort of human immunodeficiency virus mono-infected patients**. *Open forum infectious diseases* 2015; 2(1):ofv015.
36. Guaraldi G, Squillace N, Stentarelli C, Orlando G, D'Amico R, Ligabue G, et al. **Nonalcoholic fatty liver disease in HIV-infected patients referred to a metabolic clinic: prevalence, characteristics, and**

predictors. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2008; 47(2):250-257.
37. Crum-Cianflone N, Dilay A, Collins G, Asher D, Campin R, Medina S, et al. **Nonalcoholic fatty liver disease among HIV-infected persons**. *Journal of acquired immune deficiency syndromes (1999)* 2009; 50(5):464-473.
38. Lombardi R, Sambatakou H, Mariolis I, Cokkinos D, Papatheodoridis GV, Tsochatzis EA. **Prevalence and predictors of liver steatosis and fibrosis in unselected patients with HIV mono-infection**. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver* 2016; 48(12):1471-1477.
39. Lui G, Wong VW, Wong GL, Chu WC, Wong CK, Yung IM, et al. **Liver fibrosis and fatty liver in Asian HIV-infected patients**. *Alimentary pharmacology & therapeutics* 2016; 44(4):411-421.
40. Nishijima T, Gatanaga H, Shimbo T, Komatsu H, Nozaki Y, Nagata N, et al. **Traditional but not HIV-related factors are associated with nonalcoholic fatty liver disease in Asian patients with HIV-1 infection**. *PLoS One* 2014; 9(1):e87596.
41. Vuille-Lessard E, Lebouche B, Lennox L, Routy JP, Costiniuk CT, Pexos C, et al. **Nonalcoholic fatty liver disease diagnosed by transient elastography with controlled attenuation parameter in unselected HIV monoinfected patients**. *AIDS (London, England)* 2016; 30(17):2635-2643.
42. Kardashian A, Ma Y, Scherzer R, Price JC, Sarkar M, Korn N, et al. **Sex differences in the association of HIV infection with hepatic steatosis**. *AIDS (London, England)* 2017; 31(3):365-373.
43. Sterling RK, Smith PG, Brunt EM. **Hepatic steatosis in human immunodeficiency virus: a prospective study in patients without viral hepatitis, diabetes, or alcohol abuse**. *Journal of clinical gastroenterology* 2013; 47(2):182-187.
44. Lemoine M, Barbu V, Girard PM, Kim M, Bastard JP, Wendum D, et al. **Altered hepatic expression of SREBP-1 and PPARgamma is associated with liver injury in insulin-resistant lipodystrophic HIV-infected patients**. *AIDS (London, England)* 2006; 20(3):387-395.
45. Morse CG, McLaughlin M, Matthews L, Proschan M, Thomas F, Gharib AM, et al. **Nonalcoholic Steatohepatitis and Hepatic Fibrosis in HIV-1-Monoinfected Adults With Elevated Aminotransferase Levels on Antiretroviral Therapy**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2015; 60(10):1569-1578.
46. Loria P, Adinolfi LE, Bellentani S, Bugianesi E, Grieco A, Fargion S, et al. **Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian Association for the Study of the Liver (AISF) Expert Committee**. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver* 2010; 42(4):272-282.
47. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. **The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases**. *Hepatology (Baltimore, Md)* 2018; 67(1):328-357.
48. **EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease**. *Journal of Hepatology* 2016; 64(6):1388-1402.
49. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. **The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association**. *Hepatology (Baltimore, Md)* 2012; 55(6):2005-2023.
50. Klibanov OM, Williams SH, Iler CA. **Cenicriviroc, an orally active CCR5 antagonist for the potential treatment of HIV infection**. *Current opinion in investigational drugs (London, England : 2000)* 2010; 11(8):940-950.
51. Thompson M, Saag M, DeJesus E, Gathe J, Lalezari J, Landay AL, et al. **A 48-week randomized phase 2b study evaluating cenicriviroc versus efavirenz in treatment-naive HIV-infected adults with C-C chemokine receptor type 5-tropic virus**. *AIDS (London, England)* 2016; 30(6):869-878.

52. Friedman S, Sanyal A, Goodman Z, Lefebvre E, Gottwald M, Fischer L, et al. **Efficacy and safety study of cenicriviroc for the treatment of non-alcoholic steatohepatitis in adult subjects with liver fibrosis: CENTAUR Phase 2b study design**. *Contemporary Clinical Trials* 2016; 47:356-365.
53. Sanyal AJ, Ratziu V, Harrison S, Abdelmalek MF, Aithal GP, Caballeria J, et al. **Cenicriviroc Versus Placebo for the Treatment of Nonalcoholic Steatohepatitis with Liver Fibrosis: Results from the Year 1 Primary Analysis of the Phase 2b CENTAUR Study (AASLD abtsract)**. *Hepatology (Baltimore, Md)* 2016; 64(6).
54. Kesavachandran C, Bihari V, Mathur N. **The normal range of body mass index with high body fat percentage among male residents of Lucknow city in north India**. *Indian Journal of Medical Research* 2012; 135(1):72-77.
55. Shah A, Kanaya AM. **Diabetes and associated complications in the South Asian population**. *Current cardiology reports* 2014; 16(5):476.
56. Ramachandran A, Wan Ma RC, Snehalatha C. **Diabetes in Asia**. *The Lancet* 2010; 375(9712):408-418.
57. Peonim V, Sujirachato K, Srisont S, Udnoon J. **Pathology of HIV seropositive: forensic autopsy study in a tertiary care hospital, Bangkok, Thailand**. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet* 2012; 95(8):1059-1065.
58. Amarapurkar A, Ghansar T. **Fatty liver: experience from western India**. *Annals of hepatology* 2007; 6(1):37-40.
59. Lesi OA, Soyebi KS, Eboh CN. **Fatty liver and hyperlipidemia in a cohort of HIV-positive Africans on highly active antiretroviral therapy**. *Journal of the National Medical Association* 2009; 101(2):151-155.
60. Ongolo-Zogo P, Nkodo Mbia N, Mvogo Minkala TL, Biwole Sida M, Kouanfack C, Nko Amvene S. **[Lipodystrophy and echographic hepatic steatosis in HIV-positive patients under highly active antiretroviral therapy (HAART) in Yaounde (Cameroon)]**. *Bulletin de la Societe de pathologie exotique (1990)* 2012; 105(5):353-360.
61. Kelly P, Saloojee H, Chen JY, Chung RT. **Noncommunicable diseases in HIV infection in low- and middle-income countries: gastrointestinal, hepatic, and nutritional aspects**. *Journal of acquired immune deficiency syndromes (1999)* 2014; 67 Suppl 1:S79-86.
62. Sonderup MW, Wainwright HC. **Human Immunodeficiency Virus Infection, Antiretroviral Therapy, and Liver Pathology**. *Gastroenterology clinics of North America* 2017; 46(2):327-343.
63. Poynard T, Ratziu V, Naveau S, Thabut D, Charlotte F, Messous D, et al. **The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis**. *Comparative hepatology* 2005; 4:10.
64. Munteanu M, Tiniakos D, Anstee Q, Charlotte F, Marchesini G, Bugianesi E, et al. **Diagnostic performance of FibroTest, SteatoTest and ActiTest in patients with NAFLD using the SAF score as histological reference**. *Alimentary pharmacology & therapeutics* 2016; 44(8):877-889.
65. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. **The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population**. *BMC gastroenterology* 2006; 6:33.
66. Zelber-Sagi S, Webb M, Assy N, Blendis L, Yeshua H, Leshno M, et al. **Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification**. *World journal of gastroenterology* 2013; 19(1):57-64.
67. Cuthbertson DJ, Weickert MO, Lythgoe D, Sprung VS, Dobson R, Shoajee-Moradie F, et al. **External validation of the fatty liver index and lipid accumulation product indices, using 1H-magnetic resonance spectroscopy, to identify hepatic steatosis in healthy controls and obese, insulin-resistant individuals**. *European journal of endocrinology* 2014; 171(5):561-569.
68. Lee JH, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, et al. **Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease**. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver* 2010; 42(7):503-508.

69. Kahn HS. **The "lipid accumulation product" performs better than the body mass index for recognizing cardiovascular risk: a population-based comparison**. *BMC Cardiovasc Disord* 2005; 5.
70. Bedogni G, Kahn HS, Bellentani S, Tiribelli C. **A simple index of lipid overaccumulation is a good marker of liver steatosis**. *BMC gastroenterology* 2010; 10(1):98.
71. Siddiqui MS, Patidar KR, Boyett S, Smith PG, Sanyal AJ, Sterling RK. **Validation of noninvasive methods for detecting hepatic steatosis in patients with human immunodeficiency virus infection**. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2015; 13(2):402-405.
72. Otgonsuren M, Estep MJ, Hossain N, Younossi E, Frost S, Henry L, et al. **Single non-invasive model to diagnose non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH)**. *Journal of gastroenterology and hepatology* 2014; 29(12):2006-2013.
73. Long MT, Pedley A, Colantonio LD, Massaro JM, Hoffmann U, Muntner P, et al. **Development and Validation of the Framingham Steatosis Index to Identify Persons With Hepatic Steatosis**. *Clinical Gastroenterology and Hepatology* 2016; 14(8):1172-1180.e1172.
74. Shen YN, Yu MX, Gao Q, Li YY, Huang JJ, Sun CM, et al. **External validation of non-invasive prediction models for identifying ultrasonography-diagnosed fatty liver disease in a Chinese population**. *Medicine* 2017; 96(30):e7610.
75. Jimba S, Nakagami T, Takahashi M, Wakamatsu T, Hirota Y, Iwamoto Y, et al. **Prevalence of non-alcoholic fatty liver disease and its association with impaired glucose metabolism in Japanese adults**. *Diabetic medicine : a journal of the British Diabetic Association* 2005; 22(9):1141-1145.
76. Lee DH. **Imaging evaluation of non-alcoholic fatty liver disease: focused on quantification**. *Clinical and molecular hepatology* 2017; 23(4):290-301.
77. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. **The utility of radiological imaging in nonalcoholic fatty liver disease**. *Gastroenterology* 2002; 123(3):745-750.
78. Wu J, You J, Yerian L, Shiba A, Schauer PR, Sessler DI. **Prevalence of liver steatosis and fibrosis and the diagnostic accuracy of ultrasound in bariatric surgery patients**. *Obesity surgery* 2012; 22(2):240-247.
79. Borges VF, Diniz AL, Cotrim HP, Rocha HL, Andrade NB. **Sonographic hepatorenal ratio: a noninvasive method to diagnose nonalcoholic steatosis**. *Journal of clinical ultrasound : JCU* 2013; 41(1):18-25.
80. de Ledinghen V, Hiriart JB, Vergniol J, Merrouche W, Bedossa P, Paradis V. **Controlled Attenuation Parameter (CAP) with the XL Probe of the Fibroscan((R)): A Comparative Study with the M Probe and Liver Biopsy**. *Digestive diseases and sciences* 2017; 62(9):2569-2577.
81. Shi KQ, Tang JZ, Zhu XL, Ying L, Li DW, Gao J, et al. **Controlled attenuation parameter for the detection of steatosis severity in chronic liver disease: a meta-analysis of diagnostic accuracy**. *Journal of gastroenterology and hepatology* 2014; 29(6):1149-1158.
82. Fierbinteanu-Braticevici C, Dina I, Petrisor A, Tribus L, Negreanu L, Carstoiu C. **Noninvasive investigations for non alcoholic fatty liver disease and liver fibrosis**. *World journal of gastroenterology* 2010; 16(38):4784-4791.
83. Johnston RJ, Stamm ER, Lewin JM, Hendrick RE, Archer PG. **Diagnosis of fatty infiltration of the liver on contrast enhanced CT: limitations of liver-minus-spleen attenuation difference measurements**. *Abdominal imaging* 1998; 23(4):409-415.
84. Kinner S, Reeder SB, Yokoo T. **Quantitative Imaging Biomarkers of NAFLD**. *Digestive diseases and sciences* 2016; 61(5):1337-1347.
85. Bohte AE, van Werven JR, Bipat S, Stoker J. **The diagnostic accuracy of US, CT, MRI and 1H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis**. *European radiology* 2011; 21(1):87-97.
86. Livingstone RS, Grunnet LG, Thomas N, Eapen A, Antonisamy B, Mohan VR, et al. **Are hepatic and soleus lipid content, assessed by magnetic resonance spectroscopy, associated with low birth weight or insulin resistance in a rural Indian population of healthy young men?** *Diabetic medicine : a journal of the British Diabetic Association* 2016; 33(3):365-370.

87. Middleton MS, Heba ER, Hooker CA, Bashir MR, Fowler KJ, Sandrasegaran K, et al. **Agreement Between Magnetic Resonance Imaging Proton Density Fat Fraction Measurements and Pathologist-Assigned Steatosis Grades of Liver Biopsies From Adults With Nonalcoholic Steatohepatitis**. *Gastroenterology* 2017; 153(3):753-761.
88. Moreno-Torres A, Domingo P, Pujol J, Blanco-Vaca F, Arroyo JA, Sambeat MA. **Liver triglyceride content in HIV-1-infected patients on combination antiretroviral therapy studied with 1H-MR spectroscopy**. *Antiviral therapy* 2007; 12(2):195-203.
89. Nasr P, Forsgren MF, Ignatova S, Dahlstrom N, Cedersund G, Leinhard OD, et al. **Using a 3% Proton Density Fat Fraction as a Cut-Off Value Increases Sensitivity of Detection of Hepatic Steatosis, Based on Results From Histopathology Analysis**. *Gastroenterology* 2017; 153(1):53-55.e57.
90. Park CC, Nguyen P, Hernandez C, Bettencourt R, Ramirez K, Fortney L, et al. **Magnetic Resonance Elastography vs Transient Elastography in Detection of Fibrosis and Noninvasive Measurement of Steatosis in Patients With Biopsy-Proven Nonalcoholic Fatty Liver Disease**. *Gastroenterology* 2017; 152(3):598-607.e592.
91. Qayyum A. **MR spectroscopy of the liver: principles and clinical applications**. *Radiographics : a review publication of the Radiological Society of North America, Inc* 2009; 29(6):1653-1664.
92. Thomas EL, Fitzpatrick JA, Malik SJ, Taylor-Robinson SD, Bell JD. **Whole body fat: content and distribution**. *Progress in nuclear magnetic resonance spectroscopy* 2013; 73:56-80.
93. Loomba R, Sirlin CB, Ang B, Bettencourt R, Jain R, Salotti J, et al. **Ezetimibe for the treatment of nonalcoholic steatohepatitis: assessment by novel magnetic resonance imaging and magnetic resonance elastography in a randomized trial (MOZART trial)**. *Hepatology (Baltimore, Md)* 2015; 61(4):1239-1250.
94. Loomba R, Wolfson T, Ang B, Hooker J, Behling C, Peterson M, et al. **Magnetic resonance elastography predicts advanced fibrosis in patients with nonalcoholic fatty liver disease: a prospective study**. *Hepatology (Baltimore, Md)* 2014; 60(6):1920-1928.
95. Cassinotto C, Boursier J, de Ledinghen V, Lebigot J, Lapuyade B, Cales P, et al. **Liver stiffness in nonalcoholic fatty liver disease: A comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy**. *Hepatology (Baltimore, Md)* 2016; 63(6):1817-1827.
96. Cui J, Heba E, Hernandez C, Haufe W, Hooker J, Andre MP, et al. **MRE is superior to ARFI for the diagnosis of fibrosis in patients with biopsy-proven NAFLD: A prospective study**. *Hepatology (Baltimore, Md)* 2016; 63(2):453-461.
97. Liu H, Fu J, Hong R, Liu L, Li F. **Acoustic Radiation Force Impulse Elastography for the Non-Invasive Evaluation of Hepatic Fibrosis in Non-Alcoholic Fatty Liver Disease Patients: A Systematic Review & Meta-Analysis**. *PLOS ONE* 2015; 10(7):e0127782.
98. Palmeri ML, Wang MH, Dahl JJ, Frinkley KD, Nightingale KR. **Quantifying Hepatic Shear Modulus In Vivo Using Acoustic Radiation Force**. *Ultrasound in medicine & biology* 2008; 34(4):546-558.
99. Palmeri ML, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B, et al. **Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease**. *Journal of Hepatology* 2011; 55(3):666-672.
100. Yoneda M, Suzuki K, Kato S, Fujita K, Nozaki Y, Hosono K, et al. **Nonalcoholic Fatty Liver Disease: US-based Acoustic Radiation Force Impulse Elastography**. *Radiology* 2010; 256(2):640-647.
101. Mohammed SS, Aghdassi E, Salit IE, Avand G, Sherman M, Guindi M, et al. **HIV-positive patients with nonalcoholic fatty liver disease have a lower body mass index and are more physically active than HIV-negative patients**. *Journal of acquired immune deficiency syndromes (1999)* 2007; 45(4):432-438.
102. Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. **The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis**. *Hepatology (Baltimore, Md)* 2010; 51(6):1972-1978.

103. Kawamura Y, Arase Y, Ikeda K, Seko Y, Imai N, Hosaka T, et al. **Large-Scale Long-Term Follow-Up Study of Japanese Patients With Non-Alcoholic Fatty Liver Disease for the Onset of Hepatocellular Carcinoma**. *The American journal of gastroenterology* 2011; 107:253.
104. McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. **Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: Implications for prognosis and clinical management**. *Journal of Hepatology* 2015; 62(5):1148-1155.

Table 1: Serum biomarker panels used in NAFLD/NASH

Bio-marker	Algorithm parameters	Performance	Advantages
SteatoTest [63] (Logistic regression model)	Age, sex, BMI, ALT, α-2 macro globulin, apolipoprotein A1, haptoglobulin, total bilirubin, GGT, cholesterol, serum triglyceride and blood glucose	- Diagnosis of steatosis*, AUROC 0.80 (n=884, training& validation cohort) [63] - Non-binary AUROC of 0.82 against biopsy scored steatosis* (n=600) [64]	- easily available parameters
Fatty Liver Index (FLI- validated algorithm-based index) [65]	BMI, WC, serum triglyceride levels and GGT	- Diagnosis of steatosis*, AUROC 0.97 (95% CI: 0.95-0.98), compared against SteatoTest [66]	- simple algorithm - fewer parameters - validation against MRS [67]
Hepatic Steatosis Index (HSI) [68]	AST:ALT ratio, BMI and DM	- HSI <30.0 excluded NAFLD with 93.1% (95% CI 92.1-94.1) sensitivity - HSI >36 detected NAFLD with 93.1% (95% CI 92.0-94.0) specificity	- simple algorithm - minimal blood parameters - developed in a large (n=10,724) Korean cohort [68] - published data in the setting of HIV [35]
Lipid accumulation product (LAP) [69], modified to include gender [70]	WC and fasting triglycerides and gender	- AUROC 0.78 (IQR 0.72, 0.83) against MRI - in HIV+ (89% also HCV+ve): LAP >42 had 74% sensitivity and 89% specificity [71]	- simple algorithm - modified to include gender [70] - validation against MRS [67] - validated in a cohort of HIV positive individuals [71]
Index of NASH (ION) [72]	Triglycerides, ALT, HOMA-IR for women; waist:hip ratio for men only	- cut-off ≥22 had 60% sensitivity and 82% specificity for NAFLD [72] - ≥50 identified NASH from simple steatosis with 92% sensitivity and 60% specificity [72]	- gender-specific - biopsy-proven validation cohort - developed using NHANES III survey - can identify NASH from simple steatosis
Framingham Steatosis Index (FSI) [73]	Age, sex, BMI, triglycerides, hypertension, DM, and ALT:AST ratio	- cut-off 23 had 71% specificity and 79% sensitivity for hepatic steatosis in the development cohort - AUROC 0.85 (95% CI: 0.84-0.86) against U/S in a large Chinese cohort [74]	- developed using Framingham Third Generation Cohort [73] - published data in a Chinese cohort

* - steatosis >5%

ALT - Alanine aminotransferase; AST - Aspartate aminotransferase; AUROC – area under the receiver operator curve; BMI - body mass index; CI – confidence interval; DM- diabetes mellitus; GGT - Gamma Glutamyl transferase; HOMA - homeostasis model for assessment for insulin resistance; MRS - H1 magnetic resonance spectroscopy; NHANES III - National Health and Nutrition Examination Survey III; U/S – ultrasound; WC - waist circumference

Table 2: Diagnostic and imaging modalities used in NAFLD/NASH

Diagnostic/Imaging modalities	Sensitivity	Cost	Complications	Patient acceptability	Availability	Comments
Liver biopsy (LB)	✓✓✓✓	✓✓✓	✓✓✓	✓	✓✓✓	Gold standard, small area sampled
Ultrasonography (U/S) [75-78]	✓✓	✓	✓	✓✓✓✓	✓✓✓✓	Most widely used NAFLD screening technique
HepatoRenal Ratio (HRR) [79]	✓✓	✓	✓	✓✓✓✓	✓✓	U/S based technique
Transient elastography (TE)	✓✓	✓	✓	✓✓✓✓	✓✓✓	Used for staging fibrosis in NAFLD, not NAFLD diagnosis
Controlled attenuation parameter (CAP) [80, 81]	✓✓	✓	✓	✓✓✓✓	✓✓	T/E based technique, also provides fibrosis assessment
Computed Tomography (CT) [82-84]	✓✓	✓✓	✓✓	✓✓	✓	Radiation exposure
Magnetic resonance spectroscopy (^{1}H-MRS) & magnetic resonance imaging (MRI) [85-92]	✓✓✓	✓✓✓✓	✓	✓✓	✓	Mostly used in research, published NAFLD data in HIV+ve and in Indian populations
Two-dimensional MR elastography (2D-MRE) [90, 93, 94]	✓✓	✓✓✓	✓	✓✓✓	✓	Used for staging fibrosis in NAFLD, not NAFLD diagnosis
Acoustic radiation force impulse (ARFI) elastography [95-100]	✓✓	✓✓✓	✓	✓✓✓	✓	Used for staging fibrosis in NAFLD, not NAFLD diagnosis

KEY:	Sensitivity/Acceptability/Availability	Cost	Complications
✓	= somewhat	= moderate	= rarely/never
✓✓	= moderately	= expensive	= unlikely, also radiation exposure
✓✓✓	= very good	= very expensive	= possible
✓✓✓✓	= excellent	= extremely expensive	

Table 3: Cohort studies examining NAFLD in the setting of HIV mono-infection that excluded HBV and HCV and excess alcohol use

Study	Location, n	Female (%)	Age (med)	Med CD4	HIV RNA	Duration HIV+ve	ART (%)	Major assessment method	Prevalence of NAFLD/steatosis / NASH	Associations with NAFLD/steatosis/ NASH[1]
Crum-Cianfione [37]	US, n=216	5.6	40	535	50% <50 copies/ml	10 yrs	66	U/S	31% NAFLD	• NAFLD: waist circumference, TG, HDL • ART not significant
Guaraldi [36]	Italy, n=255	24.3	48	586[2] 509[3]	2.15 $\log_{10}$ copies/ml[2] 2.23 $\log_{10}$ copies/ml[3]	147.5 months	100	CT	37% NAFLD	• NAFLD: ALT:AST ratio, WC, male gender & longer NRTI exposure
Ingiliz [12]	France, n=30	3	46	365	200 copies /ml	13 yrs	100	Liver biopsy	60% steatosis (89% of whom with NASH)	• NASH (univariate): fasting glucose, fasting insulin, HOMA • ART not significant
Kardashian [42]	US, n=229	35	F: 50 M: 53	F: 569 M: 610	F: 60% U/D M: 86% U/D	N/S	F: 73 M: 97	MRS	F: 17% steatosis M: 41% steatosis	• LFF: VAT & HOMA-IR • HIV-related factors not significant
Lemoine [44]	France, n=33	14.3[3]	43.5[3]	377	50 copies/ml	10.6 yrs	100	Liver biopsy	57% NASH	• Steatosis: expression of SREBP-1
Lombardi [38]	Greece, n=125	8	39.5	N/S	333,732 copies/ml	6 yrs	68	U/S	55% steatosis	• Steatosis: male gender, age, HOMA index, GGT • ART not significant
Lui [39]	Hong Kong, n=240	7	54[3]	503[3]	95% U/D	8yrs[3]	100	MRS	29% FL	• FL: TG • HIV-related factors not significant
Mohammed [101]	Canada, n=51	0	46.2[3]	N/S	N/S	N/S	96	Liver biopsy	Steatosis 35% NASH 65%	-
Morse [45]	US, n=62	6	50	548	<40 copies/ml	17.7 yrs	100	Liver biopsy	NAFLD 73% NASH 55%	• NASH: Insulin resistance, obesity, PNPLA3 gene SNPs • HIV-related factors not significant
Nishijima [40]	Japan, n=435	7	40	349	52% <50 copies/ml	N/S	65	U/S	NAFLD 31%	• NAFLD: BMI, dyslipidemia, ALT:AST ratio • HIV-related factors not significant
Sebastiani [35] [4]	Canada, n=796	16	43.5	353.5	40% <50 copies/ml	6.3 yrs	76	HIS	Steatosis 24% Incidence 6.9 per 100 PY (95%CI 5.9-7.9)	• Steatosis: black ethnicity, albumin
Sterling [43]	USA, n=14	29	45	614	100% <50 copies/ml	N/S	100	Liver biopsy	Steatosis 65% NASH 26%	• Steatosis: GGT • NASH: HOMA-IR

Study	Location, n	Female (%)	Age (med)	Med CD4	HIV RNA	Duration HIV+ve	ART (%)	Major assessment method	Prevalence of NAFLD/steatosis / NASH	Associations with NAFLD/steatosis/ NASH[1]
Vodkin [24]	USA, n=66	21.2	44.8[3]	612.5	78.8% <400 copies/ml	118.4m	91	Liver biopsy	64% NASH HIV+ve	• NAFLD: APRI, FIB-4 • NASH: duration of HIV
Vuille-Lessard [41]	Canada, n=300	23.3	51.5[1] 49[2]	N/S	92.4%[2] 87.83[3] <40copies /ml	13 yrs[2] 11 yrs[3]	88[2] 90[3]	CAP	48% NAFLD	• NAFLD: BMI, ALT

ALT - alanine aminotransferase; AST - aspartate aminotransferase; APRI - AST to Platelet Ratio Index; ART – antiretroviral therapy; CAP – controlled attenuation parameter; CI – confidence interval; CT – computed tomography; F – female; FIB-4 – fibrosis 4 score; F/L – fatty liver; GGT - γ-glutamyl transpeptidase; HDL - high-density lipoprotein; HOMA-IR – homeostasis model for assessment of insulin resistance; HSI - hepatic steatosis index; LFF – liver fat fraction; M - male; MRS – magnetic resonance spectroscopy; MVA – multivariable analysis; NASH non-alcoholic steatohepatitis); NAS – NAFLD activity score; N/S- not stated; PY – person years; SREBP-1 - sterol regulatory element binding protein 1; SNP – single-nucleotide polymorphisms; T/E – transient elastography; TG – triglyceride; U/D – undetectable; U/S – ultrasound; VAT – visceral adipose tissue; WC – waist circumference.

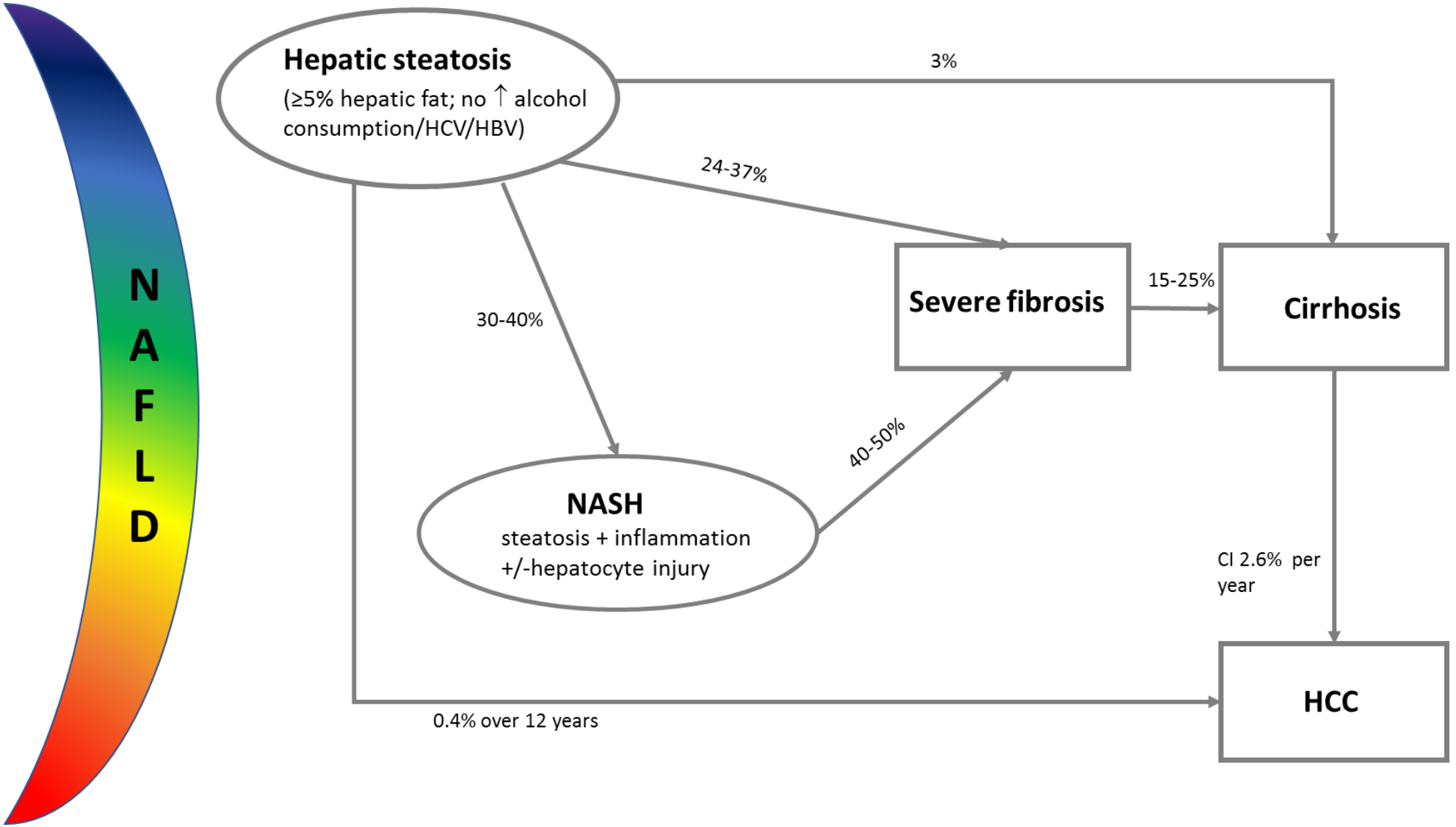
1: multivariate analyses unless otherwise stated

2: subgroup with NAFLD

3: subgroup without NAFLD

4: the only retrospective longitudinal study. All other studies are cross sectional.

Figure 1: The disease spectrum of NAFLD in the general population



CI: cumulative incidence; HCC: hepatocellular carcinoma; HBV: hepatitis B virus; HCV: hepatitis C virus; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steato-hepatitis

Based on [6, 21, 102-104]