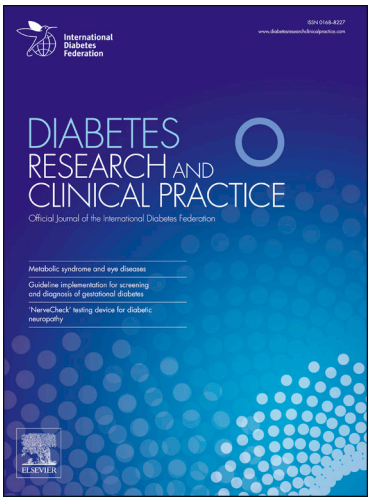

Review

Non-alcoholic fatty liver disease: relationship with cardiovascular risk markers and clinical endpoints

Adam Buckley, E. Louise Thomas, Nader Lessan, Francesca M. Trovato, Guglielmo M. Trovato, Simon D. Taylor-Robinson

PII: S0168-8227(18)30954-9
DOI: https://doi.org/10.1016/j.diabres.2018.08.011
Reference: DIAB 7483

To appear in: *Diabetes Research and Clinical Practice*

Received Date: 18 June 2018
Revised Date: 8 August 2018
Accepted Date: 14 August 2018

Please cite this article as: A. Buckley, E. Louise Thomas, N. Lessan, F.M. Trovato, G.M. Trovato, S.D. Taylor-Robinson, Non-alcoholic fatty liver disease: relationship with cardiovascular risk markers and clinical endpoints, *Diabetes Research and Clinical Practice* (2018), doi: https://doi.org/10.1016/j.diabres.2018.08.011

Non-alcoholic fatty liver disease: relationship with cardiovascular risk markers and clinical endpoints

Adam Buckley[1, 2], E. Louise Thomas[3], Nader Lessan[1, 2], Francesca M. Trovato[4], Guglielmo M. Trovato[5] and Simon D. Taylor-Robinson[6]

1 Imperial College London, Division of Diabetes, Endocrinology and Metabolism, Department of Medicine (London, United Kingdom);

2 Imperial College London Diabetes Centre, Research Department (Abu Dhabi, United Arab Emirates);

3 University of Westminster, Department of Life Sciences (London, United Kingdom);

4 University of Catania, Department of Clinical and Experimental Medicine (Catania, Italy)

5 University of Catania, Department of Clinical and Experimental Medicine (Catania, Italy)

6 Imperial College London, Division of Integrative Systems Medicine and Digestive Health, Department of Surgery and Cancer (London, United Kingdom)

Adam J Buckley - a.buckley13@imperial.ac.uk

Elizabeth Louise Thomas - L.Thomas3@westminster.ac.uk

Francesca M. Trovato - trovatofrancesca@gmail.com

Guglielmo M. Trovato - trovato.eu@gmail.com

Simon D. Taylor-Robinson - s.taylor-robinson@imperial.ac.uk

Nader Lessan MD, FRCP (Corresponding Author)

Consultant Endocrinologist

Imperial College London Diabetes Centre

PO Box 48338

Abu Dhabi, United Arab Emirates

Telephone: 009712 4040800, Ext 928

Fax: 009712 4040900

Email: nlessan@icldc.ae

Abstract

Non-alcoholic fatty liver disease (NAFLD) is a common diagnosis and is increasing in prevalence worldwide. NAFLD is usually asymptomatic at presentation; progression of the disease is unpredictable, leading to the development of a variety of techniques for screening, diagnosis and risk stratification. Clinical methods in current use include serum biomarker panels, hepatic ultrasound, magnetic resonance imaging, and liver biopsy.

A NAFLD is strongly associated with the metabolic syndrome, and the most common cause of death for people with the condition is cardiovascular disease. Whether NAFLD is an independent cardiovascular risk factor needs exploration. NAFLD has been associated with surrogate markers of cardiovascular disease such as carotid intima-media thickness, the presence of carotid plaque, brachial artery vasodilatory responsiveness and CT coronary artery calcification score.

There is no effective medical treatment for NAFLD and evidence is lacking regarding the efficacy of interventions in mitigating cardiovascular risk. Health care professionals managing patients with NAFLD should tackle the issue with early identification of risk factors and aggressive modification. Current management strategies therefore comprise lifestyle change, with close attention to known cardiovascular risk factors.

Keywords: NAFLD and Diabetes - Cardiovascular risk markers – Carotid intima-media thickness – Surrogate markers of cardiovascular disease- Fatty liver disease – NAFLD screening process

Introduction

Non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM) are common conditions and are both associated with increased insulin resistance and vascular disease. The coexistence of diabetes mellitus and NAFLD increases the risk both of developing more severe forms of NAFLD and also of the evolution of chronic vascular complications of diabetes mellitus [1]. NAFLD is characterized by the accumulation of liver fat in the absence of excessive alcohol intake. The liver has a vital role in systemic metabolism, contributing significantly to the development of insulin resistance and type 2 diabetes mellitus (T2DM) [2]. Hepatic insulin signaling is essential to maintain the carbohydrate and lipid homeostasis [3]. Cytokines, adipocytokines and lipotoxins have been postulated to play a crucial part in the pathogenesis of both T2DM and NAFLD [2]. Clinically relevant fibrosis affects up to 20% of individuals with both diagnoses [4]. Non-alcoholic steatohepatitis (NASH), which is distinguished from simple steatosis by the presence of progressive hepatocyte injury and fibrosis, is increasingly common as a primary or secondary indication for liver transplantation, as decompensated cirrhosis is the end result of the progressive condition [5]. As a consequence, the combination of both T2DM and NAFLD is of increasing clinical concern, as a potential cause of both the exacerbation of liver injury and the potential risk of cardiovascular disease.

Epidemiology

Globally, the reported prevalence of NAFLD varies from 11-46% between different populations, but in general it is highest in populations with a high pre-existing prevalence of type 2 diabetes [6] and obesity [7]. The prevalence of NAFLD increases with age [8], and most, though not all studies report that NAFLD is more prevalent in males [9]. The most common causes of death in the NAFLD are liver-related complications, malignancy and ischemic heart disease [10]. Over

the past several decades, the amount of people affected with diabetes has doubled globally, making it one of the cardinal public health challenges [11]. Over 11 years of follow-up, individuals with NAFLD are more likely to develop diabetes and the metabolic syndrome when compared with matched controls [12]. The occurrence of glycogen hepatopathy is associated with liver abnormalities in type 1 diabetes mellitus (T1DM) [12]. Despite the association between NAFLD and obesity, the two conditions do not necessarily coexist and it is there possible that the presence of NAFLD may help to distinguish between metabolically healthy and unhealthy obese individuals.

Associations

Although it is rarely implicated in the progression to chronic liver disease, simple hepatic steatosis without significant hepatic inflammation or scarring remains a health concern, owing to its strong associations with the metabolic syndrome [13], type 2 diabetes [6], insulin resistance [14], dyslipidemia [6], obesity [15] and increased mortality [16]. In a longitudinal study conducted in the Pima Indian population over 7 years of follow-up, serum alanine aminotransferase (ALT) was a significant predictor of T2DM with the association remaining significant after adjusting for percentage of body fat and direct measures of insulin sensitivity and secretion [17].

Treatment of NAFLD with lifestyle interventions may provide prognostic benefit, and hence stratification of a patient's individual risk of cardiovascular disease or progressive liver disease is of key importance for developing and implementing therapy. This process is complicated by a lack of evidence in the literature regarding the relevance of currently available NAFLD

biomarker panels to cardiovascular endpoints, direct associations between NAFLD and cardiovascular disease, and the effectiveness of interventions aimed at reducing liver fat in mitigating cardiovascular risk.

Screening and diagnosis of NAFLD

Despite the strong associations between the two conditions, current guidelines provide conflicting recommendations as to whether patients with T2DM should be screened for NAFLD [18]. A high prevalence of NAFLD and/or advanced fibrosis has been found in patients with T2DM [18], and hence the data support screening for NAFLD and/or advanced fibrosis with biomarkers, such as Fibro Test (FT) in this population. Evidence-based data suggest that biomarkers can be used as alternatives to liver biopsy for the first line assessment of fibrosis stage [19].

The diagnosis and risk stratification of NAFLD both pose significant technical challenges. Liver biopsy has traditionally been considered the gold standard for the detection of features differentiating NASH from simple steatosis [20], but it has significant drawbacks including sampling error of an often patchy condition, leading to under- or over-representation in the histopathological specimen. Analysis of paired percutaneous liver biopsies show good agreement for the degree of steatosis, but show much poorer agreement for features of steatohepatitis or fibrosis, further indicating that liver biopsy may significantly underestimate the severity of patchily-distributed disease [21]. Liver biopsy and imaging techniques may be time-consuming and expensive and require a high level operator skill; consequently there has been considerable interest in the use of biomarkers in risk stratification.

Serum markers

Single markers provide poor sensitivity and specificity for NASH, and consequently panels of multiple biomarkers have been developed to estimate an individual patient's risk of simple hepatic steatosis or NASH. The Fatty Liver Index [22] (Equation 1), the lipid accumulation product (LAP) [23] (Equation 2) and the Liver Fat Score [24] (Equation 3) aim to identify increased risk of liver steatosis, while the NAFLD Fibrosis Score [25] (Equation 4) was designed to enable discrimination of patients with advanced fibrosis or cirrhosis from those with mild or no fibrosis. The Enhanced Liver Fibrosis (ELF), using a panel of nine biomarkers for liver fibrosis, initially designed to stage fibrosis, and is claimed to predict clinical outcomes of liver disease [26]. The HAIR score incorporates arterial hypertension, ALT and insulin resistance index to distinguish NASH from NAFLD [27] (Equation 5). As diabetes is associated with more severe forms of NAFLD, and the condition has often progressed silently to liver fibrosis, this is particularly important when coexisting diabetes is present. Identification of simple, inexpensive biomarkers would aid in predictable and reliable estimates of prevalence of NAFLD worldwide in people with diabetes and would provide a diagnostic tool for the monitoring of responses to therapeutic interventions [28]. While biomarker panels are reported to provide good sensitivity and specificity, their performance in further validation studies is limited and the initially-published cut-off values do not reliably produce the best discrimination in other study populations (Table 1)[22-25, 27],[29]-[30, 31].

Imaging

Imaging techniques offer a more direct and potentially more reliable measurement of both liver fat content and fibrosis stage. Many of these techniques can differentiate between mild and severe disease, but fewer can accurately quantify levels of fat infiltration, or distinguish between degrees of mild and moderate fibrosis. While standard ultrasound provides sensitivity of 89% and specificity of 93% for the detection of hepatic steatosis [32], it provides limited quantification of liver fat content and cannot reliably detect mild fibrosis [33]. Moreover, ultrasound does not reliably detect hepatic fat content of less than 20% [34] and has limited sensitivity in individuals with body mass index (BMI) of greater than 40 kg/m² for reasons of technical visibility [35]. Ultrasound-based transient elastography, performed either separately or during ultrasound examination, can distinguish mild or absent fibrosis from severe fibrosis or cirrhosis, although the technique has several significant confounders and it is challenging to perform in people with BMI > 30 kg/m² [36]. ^{1}H-magnetic resonance spectroscopy (H-MRS) assessment of triglyceride content is widely considered to be a 'non-invasive gold standard' and has seen a huge increase in its application in recent years, although it is more common in a research contexts, rather than a clinical environment [37]. More recently the use of a combination of sequences based on T_1 and T_2* mapping to predict liver inflammation, iron accumulation and fibrosis (the “LIF” score) has been described [38].

Association with the metabolic syndrome

NAFLD is now considered by many researchers to be the hepatic manifestation of the metabolic syndrome, rather than a primary liver disease [39]**.** The majorities (60.5%) of patients with NAFLD meet the National Cholesterol Education Program Adult Treatment Panel-III criteria for the metabolic syndrome [40], and several factors commonly associated with the metabolic

syndrome, such as increased abdominal obesity, are associated with steatosis [41]. Similarly, a high prevalence of NAFLD is reported in women diagnosed with polycystic ovarian syndrome [42] and in those with gestational diabetes [43], both conditions strongly associated with insulin resistance.

Insulin resistance provides a plausible link between the pathogenesis of the metabolic syndrome and NAFLD. Adipose tissue insulin resistance increases influx of free fatty acids into the liver and systemic hyperinsulinaemia increases hepatic lipogenesis [44], whereas peripheral insulin resistance contributes to hypertriglyceridemia and intrahepatic fat accumulation [45]. Elevation of serum alanine aminotransferase (ALT) and an increased serum ALT, compared to the serum aspartate aminotransferase (AST) level – the ALT:AST ratio is associated with reduced insulin sensitivity and increased incidence of the metabolic syndrome [46]. There is increasing recognition that liver fat is more closely related to metabolic complications of obesity, such as insulin resistance and systemic inflammation than visceral fat, and that it is these metabolic alterations which underlie the myocardial systolic and diastolic dysfunction, which is notably absent in people with "metabolically healthy" obesity [13]. Since the metabolic syndrome is essentially a constellation of cardiovascular disease risk factors linked to insulin resistance [47], the question arises as to whether NALFD directly contributes to the pathogenesis of cardiovascular disease.

Associations with cardiovascular risk factors

In 40 individuals with ultrasound-diagnosed NAFLD matched for sex and age, but not diabetes status, to healthy individuals, NAFLD was associated with significant increases in carotid intima-media thickness (0.70 mm vs. 0.54 mm, $p < 0.0001$) and the prevalence of atherosclerotic

plaque (20 vs. 10, p=0.021) detected by ultrasound of the carotid artery [48]. In a group of 4,222 randomly-selected people of German descent, the prevalence of carotid atherosclerotic plaque disease was significantly higher in individuals with ultrasound diagnosis of hepatic steatosis (29.9%), when corrected for age, sex, diabetes status, hypertension, BMI, total cholesterol:HDL cholesterol ratio, and plasma fibrinogen, although no significant difference in carotid intima-media thickness was found when adjusted for the same covariates [49]. NAFLD was found to be associated with a reduction in the vasodilatory response to ischemia of the brachial artery, a marker of endothelial dysfunction, and a moderate increase in Framingham cardiovascular risk score [50]. It should perhaps be noted that the Framingham risk score does not take account of visceral or liver fat and that, to a large extent, the data collected in the Framingham study predates the NAFLD epidemic. The presence of NAFLD was associated with an increase in coronary artery calcification score in 1,854 South Korean individuals without previously identified liver or coronary artery disease, when adjusted for the degree of visceral adiposity in multivariate analysis [51]. In another cohort of 10,153 middle-aged South Korean adults, who underwent liver ultrasound and cardiac computed tomography as part of occupational screening, NAFLD was associated with increased coronary artery calcium score independent of HOMA-IR, features of the metabolic syndrome, coexisting cardiovascular risk factors, and previous evidence of cardiovascular disease [52]. Sixty patients who underwent liver transplantation for NASH-related or cryptogenic cirrhosis had significantly higher prevalence of obesity, hypertension, diabetes mellitus, the metabolic syndrome and coronary artery disease than an age- and sex-matched control group of 60 patients with cirrhosis due to any other etiology [53]. Among 161 individuals referred for liver evaluation at a hospital in Ankara, the degree of ultrasound-graded steatosis was positively associated with carotid intima-media thickness and negatively-associated

with brachial flow-mediated dilatation, although multicollinearity introduced into the linear regression analysis by the inclusion of metabolic syndrome as diagnosed by NCEP ATP-III criteria along with the individual NCEP ATP-III components as covariates is likely to have significantly biased the outcomes of these analyses [54]. However, there is a lack of overall consensus as to whether or not NAFLD is associated with increased cardiovascular risk independently of other established risk factors [50]-[53].

More recently, associations have been demonstrated between non-alcoholic fatty liver, steatohepatitis and serum markers associated with cardiovascular endpoints. Plasminogen activator inhibitor-1 (PAI-1), which inhibits fibrinolysis, is associated with an increased incidence of first myocardial infarction [55]. In a retrospective, cross-sectional analysis of 44 children undergoing liver biopsy, increased plasma PAI-1 was associated with both higher grades of steatosis and the presence of steatohepatitis, and with total and non-HDL cholesterol and estimated insulin resistance, after adjustment for age, sex and ethnicity [56]. Higher plasma levels of adiponectin, an adipokine hormone, which is negatively associated with obesity and insulin resistance, independently predict a reduced risk of coronary artery disease in men with type 2 diabetes [57].

Associations with cardiovascular outcomes

A meta-analysis of randomized controlled trials and cohort studies indicates that the risk of incident cardiovascular disease increases in the presence of the metabolic syndrome [58], but evidence for an association between NAFLD and cardiovascular endpoints is more limited. In 2,103 individuals with type 2 diabetes followed up for an average of 6.5 years, hepatic steatosis

diagnosed using ultrasound was significantly associated with a composite endpoint of non-fatal myocardial infarction, coronary revascularization, non-fatal ischemic stroke and cardiovascular death in multivariate regression, when adjusted for age, sex, smoking status, duration of diabetes, HbA1c, LDL cholesterol and the use of hypoglycemic, antihypertensive, lipid-lowering and antiplatelet drugs [59]. Data from the NHANES III cohort demonstrated increased overall mortality in individuals with NAFLD after a median of 8.7 years follow-up, with the three most common causes of death being cardiovascular disease, malignancy and chronic liver disease [60]. A retrospective review of 286 European patients with type 1 diabetes, of whom 150 met ultrasound criteria for any grade of NAFLD, found an increased hazard ratio for a composite endpoint of cerebrovascular, coronary artery or peripheral vascular disease of 6.73 (95% CI 1.2 - 38.1, p = 0.031) after a mean of 5.3 years' follow-up, adjusted for age, sex, body mass index, smoking, diabetes duration, HbA1c, dyslipidemia, hypertension, chronic kidney disease, prior ischemic heart disease and serum gamma-glutamyltransferase [47]. In a cohort of 317 adults undergoing elective coronary artery angiography, ultrasound-detected fatty liver disease was a significant predictor of >30% stenosis of at least one coronary artery in multivariate regression when corrected for sex, waist-hip ratio, body mass index, diabetes mellitus, hypertension, serum triglycerides and LDL [61].

In a prospective study of 1637 Japanese individuals attending a health screening service, 12 of 231 individuals (5.1%) with ultrasound-diagnosed NAFLD reported a new cardiovascular event (5 coronary artery disease, 6 ischemic stroke, 1 hemorrhagic stroke), compared with 10 of 990 (1.0%) individuals without NAFLD, during a five year follow-up period; NAFLD remained an independent predictor of cardiovascular endpoints after correction for known risk factors [62].

In a longitudinal cohort study, cardiovascular events occurred in 17 of 91 patients (19%) with ultrasound-diagnosed NAFLD and 18 of 182 age-matched controls; NAFLD was associated with greater carotid intima-media thickness (CIMT) at enrolment, but a smaller increase in CIMT during follow-up [63]. A similar association between NALFD and increased CIMT in younger (age <45 years), but not older people, has been reported in a cross-sectional study of a cohort of Mexican people in the USA [64], suggesting that NAFLD may be associated with earlier onset of cardiovascular disease.

Management of NAFLD

To date there are no licensed treatments for NAFLD and lifestyle modification is currently considered to be the only effective intervention. However, several drugs licensed for other indications are now being tested in NAFLD [28]. The severity of type 2 diabetes and liver disease influence the therapy. Management of type 2 diabetes in patients with liver diseases is, for the most part, the same as without liver disease. First-line therapy with metformin is appropriate in most patients, but not recommended in patients with advanced hepatic disease, because of a perceived increased risk of lactic acidosis. Recent trials have shown some benefit in patients with fatty liver and type 2 diabetes [18]. Decreased alcohol intake should be recommended [28]. A meta-analysis of trials on diet and exercise showed that performing exercise 3 times a week for 4 weeks was associated with a reduction in intrahepatic triglyceride content or serum ALT in the absence of overall weight change. Moreover, the combination of

both diet and exercise produced a 10% reduction in weight over 6 months, resulting in improvement of histological findings and reduction of steatosis on repeat liver biopsy [65]. The UK National Health Service advises weight loss with a BMI target of 18.5-24.9 [66]. Both resistance training and aerobic training improved MRI-assessed hepatic fat content and insulin resistance in sedentary people with type 2 diabetes [67]. A meta-analysis of nine studies of dietary supplementation of omega-3 polyunsaturated fatty acid indicates a potential for reduction of liver fat, as assessed by ultrasound or magnetic resonance spectroscopy, but no benefit in terms of normalization of liver enzymes [68].

The evidence that treatment of NAFLD can reduce cardiovascular risk is modest. A 16-week moderate-intensity supervised exercise program was associated with significant reductions in liver fat and serum transaminases, along with increased cardiorespiratory fitness and flow-mediated brachial artery dilatation, in ten obese Caucasian individuals with MRI-measured liver fat between 5.5% and 80%, but these changes were not sustained 12 months, following the cessation of the intervention [69].

Conclusions

Non-alcoholic fatty liver disease is both highly prevalent and increasing in prevalence worldwide. Biomarker panels offer reasonable sensitivity and specificity for the diagnosis of NAFLD, but there is a lack of evidence for their utility outside of the initial screening process. No treatment has been demonstrated to directly influence progression to steatohepatitis. Evidence for a direct effect of NAFLD on cardiovascular risk and outcomes over and above its association with the metabolic syndrome is very limited. Further elucidation of this potential link requires the use of imaging, rather than biomarker panels, for the assessment of liver fat and

fibrosis, and hard cardiovascular outcomes instead of estimated cardiovascular risk as study endpoints. At present, careful modification of acknowledged risk factors for arterial disease according to generally accepted guidelines is recommended.

Declarations

Ethics approval and consent to participate:

The study followed the ethical guidelines approved by the ICLDC Research Ethics Committee.

Consent for publication:

All authors have approved the manuscript and agree with submission.

Availability of data and material:

Not applicable

Competing interests:

The authors declare that they have no competing interests.

Funding:

Imperial College London Diabetes Centre (ICLDC), a Mubadala Healthcare company.

Authors contributions:

A.J.B. took the lead in writing the manuscript and is the guarantor of this work. N.L. reviewed and contributed to the manuscript. S.T.R. reviewed and contributed to the manuscript. L.T reviewed and contributed to the manuscript. F. M. T. and G. M. T reviewed and contributed to the manuscript.

Acknowledgments:

AB is funded by a Mubadala Healthcare. AB and SDT-R are grateful to the United Kingdom National Institute for Health Research (NIHR) Biomedical Facility at Imperial College London for infrastructure support. SDTR holds grants from the British Medical Research Council and the Wellcome Trust (London, United Kingdom).

The authors would like to thank Dr Tanveer Ashraf and Dr Saradalekshmi K R for their efforts in completion of the manuscript and submission.

Performance of clinical scores

Score	Endpoint	Cut	Performance	Source
FLI	Rule out FL (US)	≤ 30	Se 87%, Sp 64%, AR 0.85 (0.81-0.88)	Bedogni 2006[22]
	Rule in FL (US)	≥ 60	Se 61%, Sp 86%, AR 0.85 (0.81-0.88)	Bedogni 2006[22]
	Steatosis > 5% (biopsy)	> 60	PP 99%, NP 15%, AR 0.83 (0.72-0.91)	Fedchuk 2014[70]
	Steatosis > 33% (biopsy)	> 60	PP 71%, NP 56%, AR 0.65 (0.59-0.71)	Fedchuk 2014 [70]
	Steatosis (US)	≥ 60	PP 72.2%, NP 81.5%	Zelber-Sagi 2013[31]
	HRI ≥ 1.5	≥ 60	PP 57.0%, NP 86.1%	Zelber-Sagi 2013[31]
	HCL > 5.56 % (MRS)	>29.2	PP 33%, NP 94%, AR 0.72 (0.59-0.85)	Kahl 2014 [71]
	USS steatosis grade ≥ 2	>48	PP 74.4%, NP 88.7%, AR 0.91 (0.87-0.95)	Carvalhana 2014 [72]
LAP	Steatosis (US)	N/A	AR 0.79 (0.76-0.83)	Bedogni 2010 [23]
	Severe steatosis (US)	N/A	AR 0.79 (0.76-0.83)	Bedogni 2010[23]
LFS	Liver fat ≥ 55.6 mg TG/g liver tissue by MRS	-0.640	Se 86%, Sp 71%, AR 0.86 (0.83-0.89)	Kotronen 2009 [24]
	Steatosis > 5% (biopsy)	> 0.16	PP 99%, NP 16%, AR 0.80 (0.69-0.88)	Fedchuck 2014[70]
	Steatosis > 33% (biopsy)	> 0.16	PP 71%, NP 67%, AR 0.72 (0.66-0.77)	Fedchuck 2014 [70]
	HCL > 5.56% (MRS)	-1.02	PP 56%, NP 55%, AR 0.70 (0.53-0.87)	Kahl 2014[71]
N-FS	Advanced fibrosis (biopsy)	0.676	PP 90%, NP 82%, AR 0.82	Angulo 2007[25]
	Advanced fibrosis (biopsy)	-1.455	PP 30%, NP 92%, AR 0.81 (0.71-0.91)	McPherson 2010[73]
	Advanced fibrosis (biopsy)	0.676	PP 79%, NP 86%, AR 0.81 (0.71-0.91)	McPherson 2010[73]
	Severe fibrosis (biopsy)	0.676	PP 100%, NP 81.3%, AR 0.68 (0.57-0.78)	Ruffillo 2011[30]
	Fibroscan stage ≥ F2		Se 52.3%, Sp 88.6%, AR 0.67 (0.61-0.73)	Aykut 2014[29]
HAIR	NASH (biopsy)	≥2	Se 80%, Sp 89%	Dixon 2001[27]

Table 1 Reported performance of clinical scores for diagnosis of liver steatosis, non-alcoholic steatohepatitis or fibrosis. FLI = Fatty Liver Index, LAP = Lipid Accumulation Product, LFS = Liver Fat Score, NAFLD = Non-Alcoholic Fatty Liver Disease, NASH = Non-Alcoholic Steatohepatitis, N-FS = NAFLD Fibrosis Score, HAIR = Hypertension, ALT, Insulin Resistance , FL = Fatty Liver, US = Ultrasound, HRI = Hepatorenal Ultrasound Index (ratio of US brightness of liver to renal cortex in same sonographic plane), HCL = Hepatocellular Lipid, Se = Sensitivity, Sp = Specificity, AR = Area Under the Receiver Operator Curve, PP = Positive Predictive Value, NP = Negative Predictive Value, TG = triglyceride, MRS = ^{1}H-Magnetic Resonance Spectroscopy.

Clinical risk scoring;

$$FLI = \left(\frac{e^{0.953*\log(TG)+0.139*BMI+0.718*\log(GGT)+0.053*WC-15.745}}{1+e^{0.953*\log(TG)+0.139*BMI+0.718*\log(GGT)+0.053*WC-15.745}} \right) * 100$$

Equation 1: Fatty Liver Index. TG = Triglycerides (mg/dL), BMI = Body Mass Index (kg/m^{2}), GGT = Gamma-Glutamyl Transferase (U/L), WC = Waist Circumference (cm) [24].

$$LAP = \left(WC - 65(Men) \middle| 58(Women)\right) * TG$$

Equation 2: The Lipid Accumulation Product. WC = Waist Circumference (cm), TG = Triglycerides (mmol/L) [25].

$$LFS = -2.89 + 1.18 * MetS + 0.9 * T2DM + 0.15 * Ins + 0.04 * AST - 0.94 * AST/ALT$$

Equation 3: The Liver Fat Score. MetS = presence of the Metabolic Syndrome (yes = 1, no = 0), T2DM = presence of Type 2 Diabetes Mellitus (yes = 1, no = 0), Ins = serum Insulin (mU/L), AST = Aspartate aminotransferase (U/L), ALT = Alanine aminotransferase (U/L) [26].

$$NFS = -1.675 + 0.037 * Age + 0.094 * BMI + 1.13 * HG + 0.99 * \frac{AST}{ALT} - 0.013 * Plt - 0.66 * Alb$$

Equation 4: The NAFLD Fibrosis Score. Age = age in years, BMI = Body Mass Index (kg/m^2), HG = Impaired Fasting Glucose, Impaired Glucose Tolerance or Diabetes Mellitus (yes = 1, no = 0), AST = Aspartate aminotransferase (U/L), ALT = Alanine aminotransferase (U/L), Plt = platelets (1000/µcL), Alb = serum Albumin (g/dL). [27]

$$HAIR = Hypertension + (IRI > 5) + (ALT > 40)$$

Equation 5: The HAIR score. Hypertension = previously diagnosed hypertension, IRI = Insulin Resistance Index (log (Fasting Glucose (mg/dL) + log (Fasting Insulin (mIU/L)), ALT = Alanine aminotransferase (U/L). A HAIR of ≥2 is reported to provide sensitivity of 80% and specificity of 89% for the presence of non-alcoholic steatohepatitis. [29]

1. Bibliography

[1] Targher G, Lonardo A, Byrne CD. Nonalcoholic fatty liver disease and chronic vascular complications of diabetes mellitus. Nature Reviews Endocrinology. 2018;14:99.
[2] Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. Nature Reviews Gastroenterology and Hepatology. 2017;14:32.
[3] Honma M, Sawada S, Ueno Y, Murakami K, Yamada T, Gao J, et al. Selective insulin resistance with differential expressions of IRS-1 and IRS-2 in human NAFLD livers. International Journal of Obesity. 2018:1.
[4] Koehler EM, Plompen EP, Schouten JN, Hansen BE, Darwish Murad S, Taimr P, et al. Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: the Rotterdam study. Hepatology. 2016;63:138-47.
[5] Charlton MR, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. Gastroenterology. 2011;141:1249-53.
[6] Leite NC, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. Liver Int. 2009;29:113-9.

[7] Chen CH, Huang MH, Yang JC, Nien CK, Yang CC, Yeh YH, et al. Prevalence and risk factors of nonalcoholic fatty liver disease in an adult population of taiwan: metabolic significance of nonalcoholic fatty liver disease in nonobese adults. J Clin Gastroenterol. 2006;40:745-52.
[8] Kagansky N, Levy S, Keter D, Rimon E, Taiba Z, Fridman Z, et al. Non-alcoholic fatty liver disease--a common and benign finding in octogenarian patients. Liver Int. 2004;24:588-94.
[9] Lee JY, Kim KM, Lee SG, Yu E, Lim YS, Lee HC, et al. Prevalence and risk factors of non-alcoholic fatty liver disease in potential living liver donors in Korea: a review of 589 consecutive liver biopsies in a single center. J Hepatol. 2007;47:239-44.
[10] Adams LA, Harmsen S, Sauver JLS, Charatcharoenwitthaya P, Enders FB, Therneau T, et al. Nonalcoholic fatty liver disease increases risk of death among patients with diabetes: a community-based cohort study. The American journal of gastroenterology. 2010;105:1567.
[11] Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nature Reviews Endocrinology. 2012;8:228.
[12] Bhatt HB, Smith RJ. Fatty liver disease in diabetes mellitus. Hepatobiliary Surgery and Nutrition. 2015;4:101-8.
[13] Dobson R, Burgess MI, Sprung VS, Irwin A, Hamer M, Jones J, et al. Metabolically healthy and unhealthy obesity: differential effects on myocardial function according to metabolic syndrome, rather than obesity. Int J Obes (Lond). 2016;40:153-61.
[14] Lee JJ, Beretvas SN, Freeland-Graves JH. Abdominal adiposity distribution in diabetic/prediabetic and nondiabetic populations: a meta-analysis. J Obes. 2014;2014:697264.
[15] Bellentani S, Saccoccio G, Masutti F, Croce LS, Brandi G, Sasso F, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. Ann Intern Med. 2000;132:112-7.
[16] Soderberg C, Stal P, Askling J, Glaumann H, Lindberg G, Marmur J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology. 2010;51:595-602.
[17] Hanley AJ, Williams K, Festa A, Wagenknecht LE, D'Agostino RB, Kempf J, et al. Elevations in markers of liver injury and risk of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes. 2004;53:2623-32.
[18] Nair S, Diehl A, Wiseman M, Farr G, Perrillo R. Metformin in the treatment of non-alcoholic steatohepatitis: a pilot open label trial. Alimentary pharmacology & therapeutics. 2004;20:23-8.
[19] Poynard T, Morra R, Ingiliz P, Imbert-Bismut F, Thabut D, Messous D, et al. Assessment of liver fibrosis: noninvasive means. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association. 2008;14:163.
[20] Lackner C. Hepatocellular ballooning in nonalcoholic steatohepatitis: the pathologist's perspective. Expert Rev Gastroenterol Hepatol. 2014;5:223-31.
[21] Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128:1898-906.
[22] 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 Gastroenterol. 2006;6:33.
[23] Bedogni G, Kahn HS, Bellentani S, Tiribelli C. A simple index of lipid overaccumulation is a good marker of liver steatosis. BMC Gastroenterol. 2010;10:98.
[24] Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology. 2009;137:865-72.
[25] Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45:846-54.
[26] Parkes J, Roderick P, Harris S, Day C, Mutimer D, Collier J, et al. Enhanced liver fibrosis test can predict clinical outcomes in patients with chronic liver disease. Gut. 2010;59:1245-51.

[27] Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology. 2001;121:91-100.
[28] Byrne CD. Dorothy Hodgkin Lecture 2012 Non-alcoholic fatty liver disease, insulin resistance and ectopic fat: a new problem in diabetes management. Diabetic Medicine. 2012;29:1098-107.
[29] Aykut UE, Akyuz U, Yesil A, Eren F, Gerin F, Ergelen R, et al. A comparison of FibroMeter NAFLD Score, NAFLD fibrosis score, and transient elastography as noninvasive diagnostic tools for hepatic fibrosis in patients with biopsy-proven non-alcoholic fatty liver disease. Scand J Gastroenterol. 2014;49:1343-8.
[30] Ruffillo G, Fassio E, Alvarez E, Landeira G, Longo C, Dominguez N, et al. Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in nonalcoholic fatty liver disease. J Hepatol. 2011;54:160-3.
[31] 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 J Gastroenterol. 2013;19:57-64.
[32] Joseph AE, Saverymuttu SH, al-Sam S, Cook MG, Maxwell JD. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. Clin Radiol. 1991;43:26-31.
[33] Mehta SR, Thomas EL, Patel N, Crofton ME, McCarthy J, Eliahoo J, et al. Proton magnetic resonance spectroscopy and ultrasound for hepatic fat quantification. Hepatol Res. 2010;40:399-406.
[34] Fishbein M, Castro F, Cheruku S, Jain S, Webb B, Gleason T, et al. Hepatic MRI for fat quantitation: its relationship to fat morphology, diagnosis, and ultrasound. J Clin Gastroenterol. 2005;39:619-25.
[35] European Association for the Study of the L, European Association for the Study of D, European Association for the Study of O. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016;64:1388-402.
[36] Cosgrove D, Piscaglia F, Bamber J, Bojunga J, Correas J-M, Gilja O, et al. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 2: Clinical applications. Ultraschall in der Medizin-European Journal of Ultrasound. 2013;34:238-53.
[37] Machann J, Thamer C, Schnoedt B, Stefan N, Haring HU, Claussen CD, et al. Hepatic lipid accumulation in healthy subjects: a comparative study using spectral fat-selective MRI and volume-localized 1H-MR spectroscopy. Magn Reson Med. 2006;55:913-7.
[38] Banerjee R, Pavlides M, Tunnicliffe EM, Piechnik SK, Sarania N, Philips R, et al. Multiparametric magnetic resonance for the non-invasive diagnosis of liver disease. J Hepatol. 2014;60:69-77.
[39] Gastaldelli A. Fatty liver disease: the hepatic manifestation of metabolic syndrome. Hypertens Res. 2010;33:546-7.
[40] Yilmaz Y, Senates E, Ayyildiz T, Colak Y, Tuncer I, Ovunc AO, et al. Characterization of nonalcoholic fatty liver disease unrelated to the metabolic syndrome. Eur J Clin Invest. 2012;42:411-8.
[41] Fracanzani AL, Valenti L, Bugianesi E, Vanni E, Grieco A, Miele L, et al. Risk of nonalcoholic steatohepatitis and fibrosis in patients with nonalcoholic fatty liver disease and low visceral adiposity. J Hepatol. 2011;54:1244-9.
[42] Gambarin-Gelwan M, Kinkhabwala SV, Schiano TD, Bodian C, Yeh HC, Futterweit W. Prevalence of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. Clin Gastroenterol Hepatol. 2007;5:496-501.
[43] Forbes S, Taylor-Robinson SD, Patel N, Allan P, Walker BR, Johnston DG. Increased prevalence of non-alcoholic fatty liver disease in European women with a history of gestational diabetes. Diabetologia. 2011;54:641-7.
[44] Stefan N, Haring HU. The metabolically benign and malignant fatty liver. Diabetes. 2011;60:2011-7.
[45] Flannery C, Dufour S, Rabol R, Shulman GI, Petersen KF. Skeletal muscle insulin resistance promotes increased hepatic de novo lipogenesis, hyperlipidemia, and hepatic steatosis in the elderly. Diabetes. 2012;61:2711-7.

[46] Hanley AJ, Williams K, Festa A, Wagenknecht LE, D'Agostino RB, Jr., Haffner SM. Liver markers and development of the metabolic syndrome: the insulin resistance atherosclerosis study. Diabetes. 2005;54:3140-7.
[47] Mantovani A, Mingolla L, Rigolon R, Pichiri I, Cavalieri V, Zoppini G, et al. Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular disease in adult patients with type 1 diabetes. Int J Cardiol. 2016;225:387-91.
[48] Brea A, Mosquera D, Martin E, Arizti A, Cordero JL, Ros E. Nonalcoholic fatty liver disease is associated with carotid atherosclerosis: a case-control study. Arterioscler Thromb Vasc Biol. 2005;25:1045-50.
[49] Volzke H, Robinson DM, Kleine V, Deutscher R, Hoffmann W, Ludemann J, et al. Hepatic steatosis is associated with an increased risk of carotid atherosclerosis. World J Gastroenterol. 2005;11:1848-53.
[50] Villanova N, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, et al. Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. Hepatology. 2005;42:473-80.
[51] Kim D, Choi SY, Park EH, Lee W, Kang JH, Kim W, et al. Nonalcoholic fatty liver disease is associated with coronary artery calcification. Hepatology. 2012;56:605-13.
[52] Sung KC, Wild SH, Kwag HJ, Byrne CD. Fatty liver, insulin resistance, and features of metabolic syndrome: relationships with coronary artery calcium in 10,153 people. Diabetes Care. 2012;35:2359-64.
[53] Kadayifci A, Tan V, Ursell PC, Merriman RB, Bass NM. Clinical and pathologic risk factors for atherosclerosis in cirrhosis: a comparison between NASH-related cirrhosis and cirrhosis due to other aetiologies. J Hepatol. 2008;49:595-9.
[54] Kucukazman M, Ata N, Yavuz B, Dal K, Sen O, Deveci OS, et al. Evaluation of early atherosclerosis markers in patients with nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol. 2013;25:147-51.
[55] Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation. 1998;98:2241-7.
[56] Jin R, Krasinskas A, Le NA, Konomi JV, Holzberg J, Romero R, et al. Association between plasminogen activator inhibitor-1 and severity of liver injury and cardiovascular risk in children with non-alcoholic fatty liver disease. Pediatr Obes. 2016;13:23-9.
[57] Schulze MB, Shai I, Rimm EB, Li T, Rifai N, Hu FB. Adiponectin and future coronary heart disease events among men with type 2 diabetes. Diabetes. 2005;54:534-9.
[58] Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, et al. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol. 2007;49:403-14.
[59] Targher G, Bertolini L, Rodella S, Tessari R, Zenari L, Lippi G, et al. Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. Diabetes Care. 2007;30:2119-21.
[60] Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. J Hepatol. 2008;49:608-12.
[61] Mirbagheri SA, Rashidi A, Abdi S, Saedi D, Abouzari M. Liver: an alarm for the heart? Liver Int. 2007;27:891-4.
[62] Hamaguchi M, Kojima T, Takeda N, Nagata C, Takeda J, Sarui H, et al. Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. World J Gastroenterol. 2007;13:1579-84.
[63] Fracanzani AL, Tiraboschi S, Pisano G, Consonni D, Baragetti A, Bertelli C, et al. Progression of carotid vascular damage and cardiovascular events in non-alcoholic fatty liver disease patients compared to the general population during 10 years of follow-up. Atherosclerosis. 2016;246:208-13.

[64] Gill C, Vatcheva KP, Pan JJ, Smulevitz B, McPherson DD, Fallon M, et al. Frequency of Nonalcoholic Fatty Liver Disease and Subclinical Atherosclerosis Among Young Mexican Americans. Am J Cardiol. 2017;119:1717-22.
[65] Johnson NA, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW, et al. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. Hepatology. 2009;50:1105-12.
[66] Choices N-. Non-alcoholic fatty liver disease (NAFLD) - NHS Choices[Internet]. Department of Health; 2016.
[67] Bacchi E, Negri C, Targher G, Faccioli N, Lanza M, Zoppini G, et al. Both resistance training and aerobic training reduce hepatic fat content in type 2 diabetic subjects with nonalcoholic fatty liver disease (the RAED2 Randomized Trial). Hepatology. 2013;58:1287-95.
[68] Parker HM, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J. Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. J Hepatol. 2012;56:944-51.
[69] Pugh CJ, Sprung VS, Jones H, Richardson P, Shojaee-Moradie F, Umpleby AM, et al. Exercise-induced improvements in liver fat and endothelial function are not sustained 12 months following cessation of exercise supervision in nonalcoholic fatty liver disease. Int J Obes (Lond). 2016;40:1927-30.
[70] Fedchuk L, Nascimbeni F, Pais R, Charlotte F, Housset C, Ratziu V, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. Aliment Pharmacol Ther. 2014;40:1209-22.
[71] Kahl S, Strassburger K, Nowotny B, Livingstone R, Kluppelholz B, Kessel K, et al. Comparison of liver fat indices for the diagnosis of hepatic steatosis and insulin resistance. PLoS One. 2014;9:e94059.
[72] Carvalhana S, Leitao J, Alves AC, Bourbon M, Cortez-Pinto H. How good is controlled attenuation parameter and fatty liver index for assessing liver steatosis in general population: correlation with ultrasound. Liver Int. 2014;34:e111-7.
[73] McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut. 2010;59:1265-9.