# Special Correspondence

## SYNERGY OR OVERLAP: NON-ALCOHOLIC FATTY LIVER DISEASE AND CORONARY HEART DISEASE A PLEA FOR COMPUTATIONAL EPIDEMIOLOGY AND MULTI-OMICS APPROACHES

*Guglielmo M. Trovato[1] and Simon D. Taylor-Robinson[2]*

[1] University of Catania, Department of Clinical and Experimental Medicine (Catania, Italy)

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

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

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

Guglielmo Trovato (Corresponding Author)

Department of Clinical and Experimental Medicine

Catania, Italy

Email: trovato.eu@gmail.com

**Abstract**

Non-alcoholic fatty liver disease (NAFLD) is increasing in prevalence around the world. It is associated with the metabolic syndrome with cardiovascular disease the most usual cause of death. Clinical methods used to screen for NAFLD on a preliminary basis include the use of multiple algorithms, based on non-invasive serum biomarkers.

The presence of NAFLD has been linked to 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 scores. The issue of whether NAFLD is a cardiovascular risk factor itself needs to be fully determined, but it frequently coexists with insulin resistance, the metabolic syndrome and type 2 diabetes.

There is no specific medical treatment for NAFLD and the evidence is incomplete with respect to the efficacy of interventions that reduce cardiovascular risk. Management of patients with NAFLD should involve identification of risk factors with key points being lifestyle change and the treatment of underlying diabetes, dyslipidemia and hypertension. Managing big data - which must be achieved and validated in advance as much as possible – should be processed by comprehensive computational epidemiology and "-omics" approaches, taking into account advanced research challenging heterogeneity. Validation and replication are mandatory before utilizing "-omic" biomarkers in diagnostics to identify patients at risk of advanced disease, including coronary heart disease.

**Keywords:** NAFLD - Diabetes - Cardiovascular risk markers - Surrogate markers of cardiovascular disease- Fatty liver disease

## Introduction

Non-alcoholic fatty liver disease (NAFLD) and diabetes mellitus (DM) are global problems that frequently coexist and have the potential to act in more than an additive manner to increase risk of both liver and non-liver clinical outcomes. The presence of DM in the NAFLD clinical pathway increases the risk of developing more severe forms of liver disease with the evolution of hepatic fibrosis and ultimately cirrhosis. NAFLD in turn influences the development of chronic vascular complications of DM [1].

NAFLD is typified by accumulation of hepatic steatosis in the absence of immoderate alcohol intake. The condition can be sub-categorized based on microscopic appearances into simple fatty liver (hepatic steatosis) and non-alcoholic steatohepatitis (NASH), which is characterized by hepatocellular damage and the development of fibrosis as a hallmark. NASH can be associated with progression to cirrhosis with its attendant complications [2] [3]. At an etiopathogenic level, cytokines, adipocytokines and lipotoxins have been postulated to play a crucial role in both type 2 diabetes [T2DM] and NAFLD development [2]. The problem of NAFLD is growing around the world with clinically relevant fibrosis affecting up to 20% of those with both NAFLD and T2DM [4]. Because of this global pattern, it should be noted that NAFLD is becoming steadily more common as an indication for liver transplantation [5]. However, uncomplicated hepatic steatosis is usually thought to follow a benign course in adults [6], although some studies suggest that it may become a more progressive problem in children as they grow into adulthood [7].

## Epidemiology

The reported prevalence of NAFLD differs around the world from 11-46% between populations, but as a rule, it is most prevalent in groups of people with a high pre-existing rate of T2DM [8] and obesity [9]. The frequency of NAFLD increases with age [10], and most, but not all reported literature suggests that NAFLD more commonly occurs in men [11]. The usual causes of death in NAFLD are liver-related, malignancy and ischemic heart disease [12]. Alarmingly, over the past few decades, the numbers of people affected with T2DM has doubled globally [13][14]. Despite the association between NAFLD and obesity, not all obese people have NAFLD, but it is possible that the condition has the potential to

be a distinguishing feature between so called “metabolically healthy” and unhealthy obese people.

## Associations

It is generally held that fatty liver is seen to be a troubling health problem, because of its robust links with the metabolic syndrome [15], T2DM [8], insulin resistance [16], dyslipidemia [8], obesity [17] and increased mortality [18] [19]. Assessment of a person’s risk of cardiovascular disease or of the development of progressive liver disease is critical to the development of individualized treatment. However, the translation of serum biomarker panels for NAFLD to cardiovascular endpoints, direct associations between NAFLD and cardiovascular disease, and the effectiveness of interventions aimed at reducing liver fat and thus lowering cardiovascular risk are not very well established in the literature.

## Screening and diagnosis

NAFLD is a significant complication of T2DM, but diabetes guidelines around the world provide conflicting recommendations as to whether diabetic patients should be screened for NAFLD [20] [21]. The diagnosis and risk stratification of NAFLD can be technically challenging. Liver biopsy has been conventionally considered as a diagnostic gold standard, but it has significant drawbacks including sampling error of an often heterogeneous underlying condition [22] [23]. Since liver biopsy requires a high level of operator skill, there has been pressure to develop non-invasive serum biomarkers for risk stratification. Evidence-based data suggest that non-invasive serum biomarker panels such as Fibro-Test$^{TM}$ (FT) might reasonably be used as alternatives to liver biopsy for the first line assessment of fibrosis stage [21].

## Serum markers

Multi-biomarkers panels have been developed to estimate risk of hepatic steatosis or NASH. The Fatty Liver Index [24] (Equation 1), the lipid accumulation product (LAP) [25] (Equation 2) and the Liver Fat Score [26] (Equation 3) were developed to identify

increased risk of fatty liver, while the NAFLD Fibrosis Score [27] (Equation 4) was designed to enable discrimination of patients with significant fibrosis or cirrhosis from those with little or no fibrosis. The Enhanced Liver Fibrosis (ELFTM), which uses a panel of nine biomarkers for liver fibrosis, has been suggested to predict clinical outcomes of liver disease [28]. The HAIR score incorporates arterial hypertension, ALT and insulin resistance index to distinguish NASH from NAFLD [29] (Equation 5). As DM is associated with more severe forms of NAFLD, and the condition has often progressed silently to liver fibrosis, the use of such screening algorithms seems to be particularly important when coexisting DM is present. Identification of simple, inexpensive biomarkers may aid in predictable and reliable estimates of prevalence of NAFLD worldwide and may aid in the monitoring of responses to therapeutic interventions [30]. (Table 1)[24-27, 29,31-37].

**Association with the metabolic syndrome**

Insulin resistance provides a plausible link between the pathogenesis of the metabolic syndrome and NAFLD. To this end, NAFLD is now considered to be the hepatic manifestation of the metabolic syndrome, rather than a primary liver disease [38]. It is therefore important to note that most NAFLD patients (60.5%) meet the United States National Cholesterol Education Program Adult Treatment Panel-III criteria for the diagnosis of the metabolic syndrome [39]. Factors commonly associated with the metabolic syndrome and with fatty liver, include abdominal obesity [40]. A high prevalence of NAFLD is found in those with polycystic ovarian syndrome [41] and gestational diabetes [42], both conditions being strongly linked to insulin resistance.

Insulin resistance in adipose tissue increases the availability of free fatty acids to the liver, while systemic hyperinsulinemia increases hepatic lipogenesis [43]. Furthermore, peripheral insulin resistance causes hypertriglyceridemia and consequent fat accumulation within the liver [44]. An 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 [45]. There is a growing literature suggesting that hepatic steatosis has stronger associations with the metabolic complications of obesity, such as insulin resistance than does visceral fat [15]. Recent studies also highlight that it is these very metabolic alterations which are important underpinning etiological factors in

myocardial systolic and diastolic dysfunction [15]. Given that the metabolic syndrome comprises a collection of cardiovascular disease risk factors related to insulin resistance [46], the hypothesis of whether NAFLD contributes directly to the etiopathogenesis of cardiovascular disease can be postulated.

**Associations with cardiovascular risk factors and coronary artery disease.**

In an early study, 40 individuals with ultrasound-diagnosed NAFLD, the condition was associated with a significant increase in ultrasound-diagnosed carotid intima-media thickness (0.70 mm vs. 0.54 mm, $p < 0.0001$) and the prevalence of ultrasound-detected atherosclerotic plaque (20 vs. 10, $p=0.021$)[47]. In a cohort of 4,222 randomly-selected German people, the prevalence of carotid atherosclerotic plaque disease was significantly greater in individuals with ultrasound appearances of fatty liver (29.9%) [48]. NAFLD was also found to be related to a reduction in brachial artery vasodilatory response to ischemia and a moderate increase in the Framingham cardiovascular risk score [49]. It should be noted that the Framingham risk score does not take into account visceral or liver fat content. This is principally because of the fact that most of the data collected in the Framingham study predates the current NAFLD trend around the world. The presence of NAFLD has been associated with an increase in coronary artery calcification score in a study of 1,854 South Korean people, who did not have previously-identified liver or coronary artery disease, when adjusted for the degree of visceral adiposity in multivariate analysis [50]. In another cohort of 10,153 middle-aged South Korean people, who underwent liver ultrasound and cardiac computed tomography as part of a routine occupational screening program, NAFLD was associated with increased coronary artery calcium score, independent of insulin resistance, features of the metabolic syndrome, coexisting cardiovascular risk factors, and previous evidence of cardiovascular disease [51]. In another study, 60 patients who underwent liver transplantation for NASH-related cirrhosis had a 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 of any other etiology [52]. In a study of 161 Turkish people, the level of ultrasound-graded steatosis was correlated to the level of carotid intima-media thickness and negatively-associated with brachial flow-mediated dilatation [53]. However, to date there has been an overall lack of consensus as to

answering the hypothesis that NAFLD is associated with increased cardiovascular risk that is independent of other traditionally established risk factors [46-53].

Links have also been made between non-alcoholic fatty liver, steatohepatitis and serum markers associated with cardiovascular endpoints. Plasminogen activator inhibitor-1 (PAI-1), which characteristically inhibits fibrinolysis, is associated with an increased incidence of first-time myocardial infarction [54]. Furthermore, in a retrospective, cross-sectional analysis of 44 children undergoing liver biopsy, increased plasma PAI-1 was not only associated with both more significant hepatic fat content and the presence of steatohepatitis, but also with total and non-HDL cholesterol and estimated insulin resistance [55]. Of note, 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 T2DM [56].

**Associations with coronary artery disease and cardiovascular outcomes**

A meta-analysis of randomized controlled trials and cohort studies indicates that the risk of cardiovascular disease increases with the presence of the metabolic syndrome [57], but evidence for an association between NAFLD and cardiovascular endpoints is not so strong. In a study of 2,103 individuals with T2DM followed-up for an average of 6.5 years, simple fatty liver detected using ultrasound was significantly associated with non-fatal myocardial infarction, non-fatal ischemic stroke and cardiovascular death [58]. Data from the United States NHANES III cohort showed 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 [59]. A retrospective review of 286 European patients with type 1 diabetes (of whom 150 had ultrasound-detected 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 [60].

In a Japanese prospective study of 1637 people undergoing routine health screening, 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 5-year follow-up period [60]. NAFLD

was found to be an independent predictor of cardiovascular disease in this study [60]. 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 [61]. A similar association between NAFLD and increased CIMT in younger (age <45 years), but not older people, has been reported in a cross-sectional study of a cohort of people of Mexican descent in the USA [62], which suggests that NAFLD may be associated with an earlier onset of cardiovascular disease.

In 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 [63]. A meta-analysis of 40 studies assessing the natural history of NAFLD showed an overall increase in non-fatal and fatal coronary artery and cerebrovascular disease, in addition to a two-fold increase in the risk of DM. The key point is, however, if any relationship of causality may be attributed to NAFLD for the occurrence of coronary artery disease [64]. Actually, such a direct relationship is not present, and some association mediated by the co-existence of diabetes mellitus may be inferred [65-66].

Coronary microvascular dysfunction was more prevalent in patients with nonalcoholic fatty liver disease and predicted major adverse cardiac events independently of nonalcoholic fatty liver disease [67]. However, it appears that plasma lipids are an important mediator between NAFLD and CVD risk of coronary artery disease. These findings have important clinical implications, particularly for the design of anti-NAFLD drugs that also affect lipid metabolism. Managing big data - which must be achieved and validated in advance as much as possible – should be processed jointly by comprehensive computational epidemiology and “-omics” approaches, taking into account advanced research challenging heterogeneity. Validation and replication are mandatory before utilizing “-omics” biomarkers in diagnostics to identify patients at risk of advanced disease, including coronary heart disease [68-70]. Despite the verification that NAFLD and cardiovascular disease are strongly interrelated, current evidence is that NAFLD may be a useful indicator for flagging early arteriosclerosis, and not a likely causative factor. Greater sustainable contribution by precision medicine tools, by validated bioinformatics approaches, is needed for substantiating conjectures, assumptions and inferences related to the

management of big data and addressed to intervention for behavioral changes within an explicit theory of change [71].

## Conclusions

Non-alcoholic fatty liver disease is very common around the world. It is increasing in prevalence on all inhabited continents. Biomarker panels offer reasonable sensitivity and specificity for the preliminary detection of NAFLD, but there is a lack of evidence for their usefulness apart from in the initial screening stage. It should be noted that no treatment has thus far been demonstrated to directly influence the progression to steatohepatitis. Furthermore, evidence for a direct effect of NAFLD on cardiovascular risk and outcomes over and above its association with the metabolic syndrome is very scarce. Further elucidation of this potential link requires the use of imaging, rather than serum 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 is recommended of traditionally-held risk factors for arterial disease according to generally accepted international guidelines.

## Acknowledgments:

SDT-R is 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).

**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[24] |
| | Rule in FL (US) | ≥ 60 | Se 61%, Sp 86%, AR 0.85 (0.81-0.88) | Bedogni 2006[24] |
| | Steatosis > 5% (biopsy) | > 60 | PP 99%, NP 15%, AR 0.83 (0.72-0.91) | Fedchuk 2014[33] |
| | Steatosis > 33% (biopsy) | > 60 | PP 71%, NP 56%, AR 0.65 (0.59-0.71) | Fedchuk 2014 [33] |
| | Steatosis (US) | ≥ 60 | PP 72.2%, NP 81.5% | Zelber-Sagi 2013[37] |
| | HRI ≥ 1.5 | ≥ 60 | PP 57.0%, NP 86.1% | Zelber-Sagi 2013[37] |
| | HCL > 5.56 % (MRS) | >29.2 | PP 33%, NP 94%, AR 0.72 (0.59-0.85) | Kahl 2014 [34] |
| | US steatosis grade ≥ 2 | >48 | PP 74.4%, NP 88.7%, AR 0.91 (0.87-0.95) | Carvalhana 2014 [32] |
| LAP | Steatosis (US) | N/A | AR 0.79 (0.76-0.83) | Bedogni 2010 [25] |
| | Severe steatosis (US) | N/A | AR 0.79 (0.76-0.83) | Bedogni 2010[25] |
| 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 [26] |
| | Steatosis > 5% (biopsy) | > 0.16 | PP 99%, NP 16%, AR 0.80 (0.69-0.88) | Fedchuck 2014[33] |
| | Steatosis > 33% (biopsy) | > 0.16 | PP 71%, NP 67%, AR 0.72 (0.66-0.77) | Fedchuck 2014 [33] |
| | HCL > 5.56% (MRS) | -1.02 | PP 56%, NP 55%, AR 0.70 (0.53-0.87) | Kahl 2014[34] |
| N-FS | Advanced fibrosis (biopsy) | 0.676 | PP 90%, NP 82%, AR 0.82 | Angulo 2007[27] |
| | Advanced fibrosis (biopsy) | -1.455 | PP 30%, NP 92%, AR 0.81 (0.71-0.91) | McPherson 2010[35] |
| | Advanced fibrosis (biopsy) | 0.676 | PP 79%, NP 86%, AR 0.81 (0.71-0.91) | McPherson 2010[35] |
| | Severe fibrosis (biopsy) | 0.676 | PP 100%, NP 81.3%, AR 0.68 (0.57-0.78) | Ruffillo 2011[36] |
| | Fibroscan stage ≥ F2 | | Se 52.3%, Sp 88.6%, AR 0.67 (0.61-0.73) | Aykut 2014[31] |
| HAIR | NASH (biopsy) | ≥2 | Se 80%, Sp 89% | Dixon 2001[29] |

**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/m2), GGT = Gamma-Glutamyl Transferase (U/L), WC = Waist Circumference (cm) [24].

$$LAP = (WC - 65(MEN)|58(Women))*TG)$$

**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].

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

**Equation 4:** The NAFLD Fibrosis Score. Age = age in years, BMI = Body Mass Index (kg/m2), 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]

## References

[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] Dam-Larsen S, Becker U, Franzmann MB, Larsen K, Christoffersen P, Bendtsen F. Final results of a long-term, clinical follow-up in fatty liver patients. Scand J Gastroenterol. 2009;44:1236-43.

[7] Widhalm K, Ghods E. Nonalcoholic fatty liver disease: a challenge for pediatricians. Int J Obes (Lond). 2010;34:1451-67.

[8] 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.

[9] 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.

[10] 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.

[11] 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.

[12] 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.

[13] Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nature Reviews Endocrinology. 2012;8:228.

[14] Bhatt HB, Smith RJ. Fatty liver disease in diabetes mellitus. Hepatobiliary Surgery and Nutrition. 2015;4:101-8.

[15] 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.

[16] Lee JJ, Beretvas SN, Freeland-Graves JH. Abdominal adiposity distribution in diabetic/prediabetic and nondiabetic populations: a meta-analysis. J Obes. 2014;2014:697264.

[17] 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.

[18] 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.

[19] 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.

[20] 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.

[21] 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.

[22] Lackner C. Hepatocellular ballooning in nonalcoholic steatohepatitis: the pathologist's perspective. Expert Rev Gastroenterol Hepatol. 2014;5:223-31.

[23] 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.

[24] 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.

[25] 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.

[26] 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.

[27] 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.

[28] 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.

[29] 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.

[30] 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.

[31] 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.

[32] 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.

[33] 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.

[34] 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.

[35] 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.

[36] 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.

[37] 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.

[38] Gastaldelli A. Fatty liver disease: the hepatic manifestation of metabolic syndrome. Hypertens Res. 2010;33:546-7.

[39] 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.

[40] 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.

[41] 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.

[42] 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.

[43] Stefan N, Haring HU. The metabolically benign and malignant fatty liver. Diabetes. 2011;60:2011-7.

[44] 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.

[45] 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.

[46] 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.

[47] 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.

[48] 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.

[49] 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.

[50] 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.

[51] 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.

[52] 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.

[53] 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.

[54] 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.

[55] 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.

[56] 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.

[57] 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.

[58] 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.

[59] 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.

[60] 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.

[61] 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.

[62] 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.

[63] Mirbagheri SA, Rashidi A, Abdi S, Saedi D, Abouzari M. Liver: an alarm for the heart? Liver Int. 2007;27:891-4.

[64]: Fiorentino TV, Succurro E, Sciacqua A, Andreozzi F, Perticone F, Sesti G.Non-alcoholic fatty liver disease is associated with cardiovascular disease in subjects with different glucose tolerance. Diabetes Metab Res Rev. 2020 May1:e3333.

[65] VanWagner LB, Wilcox JE, Ning H, Lewis CE, Carr JJ, Rinella ME, Shah SJ, Lima JAC, Lloyd-Jones DM. Longitudinal Association of Non-Alcoholic Fatty Liver Disease With Changes in Myocardial Structure and Function: The CARDIA Study. J Am Heart Assoc. 2020; 18;9(4):e014279.

[66] Brouwers MCGJ, Simons N, Stehouwer CDA, Isaacs A. Non-alcoholic fatty liver disease and cardiovascular disease: assessing the evidence for causality.Diabetologia. 2020;63:253-260.

[67] Vita T, Murphy DJ, Osborne MT, Bajaj NS, Keraliya A, Jacob S, Diaz Martinez AJ, Nodoushani A, Bravo P, Hainer J, Bibbo CF, Steigner ML, Taqueti VR, Skali H, Blankstein R, Di Carli MF, Dorbala S. Association between Nonalcoholic Fatty Liver Disease at CT and Coronary Microvascular Dysfunction at Myocardial Perfusion PET/CT. Radiology. 2019;291:330-337.

[68] Pirola CJ, Sookoian S. Multiomics biomarkers for the prediction of nonalcoholic fatty liver disease severity. World J Gastroenterol. 2018 21;24:1601-1615.

[69] Mardinoglu A, Uhlen M, Borén J. Broad Views of Non-alcoholic Fatty Liver Disease. Cell Syst. 2018;6:7-9.

[70]Martel C, Esposti DD, Bouchet A, Brenner C, Lemoine A. Non-alcoholic steatohepatitis: new insights from OMICS studies. Curr Pharm Biotechnol. 2012;13:726-35.

[71] Trovato GM. Non-alcoholic fatty liver disease and Atherosclerosis at a crossroad: The overlap of a theory of change and bioinformatics. World J Gastrointest Pathophysiol. 2020;11:57-63.