**Name of journal: World Journal of Hepatology**

**ESPS Manuscript NO: 13640**

**Columns: REVIEW**

**Composite prognostic models across the non-alcoholic fatty liver disease spectrum: Clinical application in developing countries**

Lückhoff HK *et al*. Non-invasive evaluation of NAFLD severity

Hilmar K Lückhoff, Frederik C Kruger, Maritha J Kotze

**Hilmar K Lückhoff**, Division of Anatomical Pathology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, Bellville 7530, Cape Town, Western Cape, South Africa

**Maritha J Kotze**, Division of Anatomical Pathology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, Bellville 7530, Cape Town, Western Cape, South Africa

**Frederik C Kruger**, Gastroenterology Unit, Durbanville Medi-Clinic, Durbanville 7551, Cape Town, Western Cape, South Africa

**Author contributions:** Lückhoff HK, Kruger FC and Kotze MJ contributed equally to this paper.

**Conflict-of-interest:** Prof Kotze MJ is a director and shareholder of Gknowmix (Pty) Ltd. that has developed a database tool for research translation under the auspices of the Innovation Centre of the South African Medical Research Council. The other authors declared no conflict of interest and no writing assistance was obtained in the preparation of this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to: Hilmar K Lückhoff, MBChB, HonsBSc**, Division of Anatomical Pathology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, Bellville 7530, Cape Town, Western Cape, South Africa. [hilmarklausl@gmail.com](mailto:hilmarklausl@gmail.com)

**Telephone:** +27-21-9389324

**Fax:** +27-21-9389324

**Received:** August 28, 2014

**Peer-review started:** August 28, 2014

**First decision:** November 14, 2014

**Revised:** December 18, 2014

**Accepted:** March 30, 2015

**Article in press:**

**Published online:**

**Abstract**

Heterogeneity in clinical presentation, histological severity, prognosis and therapeutic outcomes characteristic of non-alcoholic fatty liver disease (NAFLD) necessitates the development of scientifically sound classification schemes to assist clinicians in stratifying patients into meaningful prognostic subgroups. The need for replacement of invasive liver biopsies as the standard method whereby NAFLD is diagnosed, graded and staged with biomarkers of histological severity injury led to the development of composite prognostic models as potentially viable surrogate alternatives. In the present article, we review existing scoring systems used to (1) confirm the presence of undiagnosed hepatosteatosis; 2) distinguish between simple steatosis and NASH; and (3) predict advanced hepatic fibrosis, with particular emphasis on the role of NAFLD as an independent cardio-metabolic risk factor. In addition, the incorporation of functional genomic markers and/or application of emerging imaging technologies are discussed as a means to improve the diagnostic accuracy and predictive performance of promising composite models found to be most appropriate for widespread clinical adoption.

**Key words:** Non-alcoholic fatty liver disease; Liver biopsy; Non-invasive biomarkers; Histological severity; Genomics; Steatohepatitis

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Non-alcoholic fatty liver disease (NAFLD) remains largely underdiagnosed and undertreated in general practice. In view of the limitations inherent to liver biopsy and peripheral surrogate biomarkers used in the diagnosis and assessment of histological severity in NAFLD, a number of composite prognostic models have entered the clinical domain as potentially viable alternatives. Lifestyle-based intervention remains the cornerstone of treatment in patients with NAFLD. The widespread clinical adoption of composite diagnostic and predictive models could however prove useful in informing clinical and therapeutic decision making with the goal of adding value to patient care across the NAFLD spectrum.

Lückhoff HK*,* Kruger FC, Kotze MJ. Composite prognostic models across the non-alcoholic fatty liver disease spectrum: Clinical application in developing countries. *World J Hepatol* 2015; In press

**INTRODUCTION**

In coming decades, the developing world is expected to bear an increasingly disproportionate share of the overall health and financial burden attributable to chronic non-communicable diseases (NCDs) once considered merely as reflections of affluence[1,2]. Economic prosperity is inexorably linked to a globalization of modernity which fuels an ongoing reversal of the social obesity gradient in non-industrialized nations[3,4]. This epidemiological trend is mirrored in the emergence of non-alcoholic fatty liver disease (NAFLD) as a major health concern in non-occidental countries, affecting individuals across boundaries for age, sex and ancestral background[5]. Despite growing evidence as to its magnitude and preventability, this “hepatic pandemic” remains largely underdiagnosed and undertreated in routine medical practice[6].

NAFLD encompasses a broad spectrum of hepatic abnormalities and is typified by marked inter-individual heterogeneity in clinical presentation, histological severity, prognosis and therapeutic outcomes. While the majority of uncomplicated steatosis is non-progressive, ~20-30% of patients will develop steotohepatitis (NASH), a more aggressive necro-inflammatory phenotype associated with increased risk for advanced fibrosis predisposing towards cirrhosis, portal hypertension, decompensated liver failure and hepatocellular carcinoma[7,8]. The pathological evaluation and classification of biopsied liver tissue remains definitive standard investigation whereby a suspected diagnosis of NAFLD is confirmed and histological severity quantified to assist prognostication and the selection of appropriate therapeutic intervention[9]. This approach is to an extent advantageous as it allows for the concurrent assessment of multiple histological parameters and may help identify unexpected hepatic pathology or comorbidities. Liver biopsy is however limited insofar as it is an invasive, expensive and time-consuming procedure which poses significant physical risk, including a 0.1% risk of mortality[9]. Moreover, it is subject to sampling error and intra- as well as inter-observer variability in interpretation, and does not adequately reflect dynamic changes in disease severity over time. Given its restricted availability in resource-limited settings, compounded by the high overall prevalence of NAFLD, liver biopsy may not always be logistically feasible in the developing world[10].

An appreciation for the abovementioned shortcomings creates an incentive to develop and validate robust and cost-effective risk classification tools as potentially viable alternatives. Growing insight into the molecular and genetic mechanisms underlying the development and pathogenic progression of NAFLD has led to the identification of novel peripheral biomarkers (Table 1) allowing for the non-invasive assessment of underlying hepatic injury. The recognition that individual risk markers have insufficient discriminatory power and limited clinical utility in stratifying patients into meaningful prognostic subgroups has led to the development and validation of composite diagnostic and predictive models as potentially viable alternatives[17-20].

A growing number of complex and often patented biomarker panels and risk classification schemes have recently entered the clinical domain. Their use is however not always applicable in resource-limited settings. In the present article, we provide an overview of non-invasive composite models used to (1) confirm the presence of undiagnosed hepatosteatosis; (2) differentiate between simple fatty liver and NASH; and (3) predict advanced hepatic fibrosis, with particular emphasis on the relationship between histological severity and cardio-metabolic risk. The advantages and shortcomings of these models are discussed in relation to the potential added value of emerging genomic applications as a means of improving their performance, weighed against the reality of its implementation. In conclusion, suggestions are provided as to how ongoing research may confirm their clinical utility as robust and cost-effective population-based screening tools used to facilitate prognostication, assist in the selection of appropriate treatment and intervention strategies, predict adverse clinical outcomes, and ultimately allow for the more goal-directed use of liver biopsy in developing nations.

**COMPOSITE DIAGNOSTIC MODELS FOR HEPATIC STEATOSIS**

Intra-hepatocyte accumulation of neutral triglycerides in excess of 5% of liver mass is the defining pathological feature of NAFLD. Once considered a “first hit” mechanism underlying its etiology, this is now rather thought to protect against oxidative stress driven by increased intra-hepatic free fatty acid (FFA) flux arising in the context of visceral adiposity and insulin resistance (IR) as components of the metabolic syndrome[21]. Alanine (ALT) and aspartate aminotransferase (AST) levels are commonly elevated in patients with this condition, and increased ALT reliably predicts the development of adverse cardiac events and cardiovascular mortality[22,23]. Although used to determine eligibility for further diagnostic work-up and imaging, liver enzymes may however remain normal in up to 80% of NAFLD cases[24]. In addition, while elevated gamma glutamyl-transferase (GGT) has greater specificity for hepatosteatosis and is a sensitive marker for early IR, it is appreciated that no single biochemical test is considered ideal for confirming a suspected diagnosis of NAFLD.

Imaging modalities such as ultrasonography (US) further have limited diagnostic utility in moderate steatosis, while the application of more complex technologies in resource-limited environments is still largely restricted by their expense. Whether routine screening for hepatic steatosis in high-risk asymptomatic patients is practical or cost-effective remains subject to debate, as the vast majority are likely to present with uncomplicated and non-progressive disease. Its identification is however of significant clinical relevance, since even uncomplicated fatty liver, once regarded as relatively benign and showing a favorable prognosis, is associated with increased risk for overall and liver-related mortality[25,26].

Emerging evidence suggests that hepatic steatosis independently predicts the development of new-onset ischemic heart disease as well as adverse cardiac events irrespective of traditional cardio-metabolic traits[27,28]. The importance of hepatosteatosis as predictor of adverse clinical outcomes and mortality in patients with established cardiovascular disease (CVD) however remains uncertain[29]. Results from prior investigation into the value of existing clinical stratification tools in estimating cardio-metabolic risk and predicting new-onset CVD in patients with the metabolic syndrome and/or NAFLD are conflicting[30]. Multiple studies have further reported positive correlations between the severity of hepatic steatosis and proportional derangements in several early markers of subclinical atherosclerotic burden and cardiovascular risk[31-33] . Composite scoring systems validated against measures of steatosis severity may therefore prove useful as non-invasive tools in the diagnosis of NAFLD.

Several diagnostic models for hepatic steatosis have been developed to date. Anthropometric correlates for central obesity, fasting triglycerides and GGT levels are utilized in the fatty liver index (FLI) commonly used in epidemiological studies, which along with its sex-specific derivative, the lipid accumulation product (LAP), accurately predicts the presence of hepatic steatosis, and could help clinicians identify patients at increased cardio-metabolic risk eligible for further diagnostic evaluation and for whom suitable lifestyle-based interventions may be indicated[34-37]. Subsequently developed models such as the NAFLD liver fat score[38] substitute GGT for the AST/ALT ratio (AAR) in addition to incorporating clinical or biochemical markers of IR. A particular advantage of recent panels is the ability to not only identify hepatic steatosis using a high cut-off value, but also reliably exclude it using a low cut-off value[39,40]. The widespread clinical use of these models is currently restricted by their limited utility in quantifying steatosis severity in obese patients in addition to not accurately predicting new-onset ischemic heart disease or cardiovascular mortality[41-43].

**COMPOSITE DIAGNOSTIC MODELS FOR NASH**

Liver-specific therapeutic interventions such as thiazelidinedione pharmacotherapy could pose specific benefit in non-cirrhotic NASH[44]. There is still however insufficient evidence to justify the routine clinical use of any specific targeted treatments at this time. Numerous studies have shown that a ~10% reduction in body weight improves both metabolic abnormalities and histological changes in patients with NAFLD/NASH and as such, a multidisciplinary lifestyle-based therapeutic approach incorporating a tailored low-calorie dietary regimen and moderate physical exercise remains the cornerstone of treatment for this condition[45-47]. To ensure the successful implementation of population-based lifestyle intervention programs aimed at preventing the onset or progression of NAFLD in resource-limited healthcare settings, it is imperative to foster collaboration between clinicians and the public sector in accordance with standardized guidelines and assisted by the necessary ethico-legal and governmental frameworks[48].

It can be argued that the abovementioned findings call into question the validity of diagnostic confirmation for NASH. Delineating between uncomplicated steatosis and this more aggressive phenotype however has important prognostic significance, since compared to patients with simple fatty liver, those with steatohepatitis are at greater risk for cardiovascular as well as liver-related mortality[49]. The association between NASH and increased cardio-metabolic risk is further evidenced by a significant correlations reported between the extent of necro-inflammatory injury and the degree of endothelial as well as diastolic dysfunction[50,51]. A number of simple composite diagnostic models for NASH have been developed to date, largely incorporating readily available clinical data in addition to several routinely performed biochemical tests (Table 2).

***Socio-demographic characteristics***

Epidemiological studies have reported marked gender-specific and population-related disparity in the prevalence and severity of NAFLD, with ethnicity and sex considered co-dependent risk modifiers further influenced by age as well as environmental exposures acting on a genetic background[57,58]. Such evidence emphasizes the importance of a population-based approach to chronic disease risk screening integrating socio-demographic variables in developing composite risk models for application as screening tools. The incidence of NASH is higher in females particularly of older age, while risk for progression to this inflammatory phenotype is inversely related to African ancestry[59]. These observations are reflected in the inclusion of non-black ethnicity and female sex in the NASH clinical scoring system for morbid obesity[54] and NASH predictive index[56], respectively.

***Cardio-metabolic risk traits***

The near-universal incorporation of cardiovascular risk traits defined by the metabolic syndrome as a component of diagnostic models for NASH accords with the well-evidenced association between these entities[60]. Direct biochemical confirmation or clinical approximation of IR is of particular relevance in this regard, as insulin-mediated intra-hepatic FFA flux potentiates cardiomyocyte ischemia contributing towards diastolic dysfunction, adaptive remodelling and subsequent cardiac injury. IR is therefore considered the primary mechanism underlying the emergence of a distinct “dysfunctional cardiovascular phenotype” of NAFLD, particularly associated with increased cardio-metabolic risk[61].

***Liver enzyme levels***

Separately, an elevation in either AST or ALT is not considered a reliable indicator of necro-inflammatory hepatic injury, and as such, normal levels do not confidently exclude a diagnosis of NASH[62]. Increased GGT however independently predicts not only progression to NASH[63] but also the new-onset CVD in addition to cardiac mortality[64].

***Obstructive sleep apnoea***

The association between obstructive sleep apnoea (OSA) and central obesity as well as the metabolic syndrome validates its incorporation into several diagnostic models for NASH. Severe chronic hypoxemia is positively correlated with steatosis grade and indirectly promotes hepatic necro-inflammation[65] although its association with other determinants of histological injury remains unclear[66,67]. Intermittent hypoxia induces intra-hepatic FFA flux which promotes progression to NASH via 1) activation of nuclear factor kappa beta (NF-KB) and increased production of pro-inflammatory adipocytokines, as well as 2) up-regulation of reactive oxygen species (ROS) synthesis mediated by the NADPH oxidase complex[68].

***Oxidative and inflammatory biomarkers***

Chronic inflammation and oxidative stress are considered important “second-hit” pathogenic mechanisms underlying progression to NASH. The relationship between the metabolic syndrome and high-sensitivity C-reactive protein (hs-CRP) is well-recognized[69] and a preponderance of evidence now supports its utility as a reliable predictive marker for new-onset CVD and subclinical atherosclerosis[70-73] . There is however general concern regarding the putative value of adipocytokines, acute phase reactants (APR) and oxidative biomarkers in the diagnosis of NASH due to their general lack of specificity[74]. While plasma caspase-generated cytokeratin-18 (CK-18) reflecting hepatocyte apoptosis is considered a highly accurate and potentially useful non-invasive diagnostic marker for NASH[75], the low sensitivity of CK-18 limits its potential viability as screening tool in the clinical setting[76]. It also remains unclear to what extent the incorporation of CK-18 into composite diagnostic models is deterministic of their accuracy.

***Iron parameters***

Emerging evidence suggests that ferritin is not only independently associated with diagnostic features of the metabolic syndrome, but reliably predicts its presentation as composite entity as well as the onset of full-blown type II diabetes mellitus (DM II) and adverse cardiac events[77-79]. It has been proposed that the well-evidenced pathogenic relationship between hyperferritinemia and the metabolic syndrome is mediated by undiagnosed hepatosteatosis[80] which in turn exacerbates the association between this condition and increased risk for DM II and atherosclerotic disease[81]. Up to 30% of NAFLD patients present with baseline hyperferritinemia[82] considered a reliable predictive marker for NASH, validating its incorporation into the NAFIC score shown to outperform both the HAIR score and Gholam’s model[83]. The utility of ferritin in the non-invasive prediction of advanced hepatic fibrosis and increased histological severity however remains contested[84-87].

In the absence of a corresponding elevation in transferrin saturation (TS), inflammation-mediated hyperferritinemia accompanied by decreased serum iron constituting a clinico-biochemical profile consistent with the “anaemia of chronic disease”[88,89] and compatible with a diagnosis of hepatosteatosis and/or the dysmetabolic iron overload syndrome (DIOS)[82]. Mendler *et al*[90] first described the presence of unexplained mild-to-moderate sinusoidal hepatic siderosis invariably associated with decreased insulin sensitivity and termed this condition insulin resistance-associated hepatic iron overload (IR-HIO). In less than two decades, DIOS has emerged as an important differential diagnosis for type I genetic hereditary hemochromatosis (HH) in patients at increased cardio-metabolic risk presenting with deranged iron profiles and a persistent elevation in liver transaminases[91]. Due to their striking similarities, it has been proposed that a superficial distinction between the “iron phenotypes of obesity” which characterize the metabolic syndrome, NAFLD and DIOS in fact belies their common pathological basis[82].

Standardized selection criteria for hemochromatosis (HFE) genotyping used to confirm a suspected diagnosis of genetic HH are already in place based on well-established diagnostic algorithms. However, there remains a pressing need to develop and validate cost-effective non-invasive pre-screen diagnostic algorithms to assist clinicians in differentiating between type I genetic HH and DIOS as common causes of hepatic siderosis with the goal of informing clinical and therapeutic decision making. Riva *et al*[91] re-evaluated the diagnostic criteria for DIOS and showed that the presence of two or more metabolic syndrome features and a normal TS percentage in patients with confirmed hepatosteatosis corresponded with mild to moderate hepatic siderosis showing a predominantly sinusoidal distribution typical of “classic” DIOS. By comparison, a significant peripheral elevation in TS dissociated from cardio-metabolic risk traits corresponded to more severe hepatocellular iron accumulation consistent with the histological presentation of genetic HH. In this context, the routine implementation of similar a validated diagnostic tool could assist clinicians in stratifying obese patients into meaningful subgroups based on the need for extended follow-up evaluation aimed at confirming a suspected diagnosis of hepatosteatosis and/or DIOS, in addition to identifying a subgroup set to derive optimal benefit from the timely implementation of suitable lifestyle-based intervention strategies and targeted therapeutic modalities aimed at decreasing cumulative cardio-metabolic risk for early on in the disease process.

**COMPOSITE PREDICTIVE MODELS FOR ADVANCED HEPATIC FIBROSIS**

Establishing the extent and severity of fibrotic injury in patients with NAFLD is of significant clinical relevance to the reliable prediction of overall and liver-related mortality[92]. In addition to its prognostic importance, confirmation thereof may identify patients eligible for enrolment in screening programs aimed at monitoring risk for progression to cirrhosis and its associated complications[93]. Fibrosis severity is moreover correlated with greater carotid intima media thickness (CIMT) measurements, decreased coronary blood flow (CBF) reserves as well as microvascular dysfunction, suggesting that patients with advanced fibrotic injury should be considered at high-risk for CVD, warranting more aggressive and sustained intervention via lifestyle-based risk reduction methods[94,95]. Several scoring panels used for the prediction of advanced hepatic fibrosis are outlined in Table 3.

***Socio-demographic characteristics***

Risk for pathogenic progression and greater histological severity increases dramatically with age[107]. Older age is therefore considered a predictor variable in multiple composite models for advanced fibrosis.

***Cardio-metabolic risk traits and liver enzyme levels***

The metabolic syndrome as composite entity, in addition to its individual components, are accurate predictors of histological severity in NASH[108]. The AAR is typically < 1 in patients with uncomplicated NAFLD; however, ALT levels decrease with resolution of necro-inflammation and as fibrotic injury progresses, resulting in decreased clearance of AST from the sinusoidal space, this gradually reverses[62]. An AAR of >1 is considered a reliable indicator of cirrhosis, while a cut-off value of 0.8 can be used to predict advanced hepatic fibrosis[109].

The abovementioned variables constitute the basis for most of the composite predictive models for advanced hepatic fibrosis developed to date. Despite the high specificity (100%) of the BAAT score, its low sensitivity largely restricts a more widespread use in clinical practice. The NAFLD fibrosis score (NFS) has proven useful in accurately predicting as well as excluding advanced hepatic fibrosis using high and low cut-off values, respectively. Although the NFS has been extensively investigated and validated in different population groups, it has limited utility in intermediate stages of non-severe fibrosis[110-112]. The BARD score compares favourably to the NFS, and is both easier to calculate and does not produce intermediary results of indeterminate significance. However, its utility is hampered by the significant proportion of patients who, despite mild disease severity, are allocated high total scores due to obesity[113,114]. Although the NFS and BARD score accurately predict the onset of cirrhotic complications and liver-related mortality, their clinical application towards these goals is undermined by the inclusion of DM II as independent predictor of adverse clinical outcome[15,116].

***Markers of impaired hepatic functioning***

Progression from severe hepatic fibrosis to cirrhosis ultimately leads to decompensated liver failure and portal hypertension, biochemically reflected as thrombocytopenia, increased prothrombin time, hypoalbuminemia and hyperbilirubinemia. Platelet count is an ideal biomarker for the prediction of advanced fibrosis in many chronic liver diseases[117] and features in multiple composite models currently available to the clinician. The clinical relevance of the AST to platelet ratio index (APRI) as a simple screening tool to predict advanced fibrosis has been demonstrated in resource-limited settings comparing favourably to the NFS with superior accuracy to the AAR. While the APRI could serve to decrease the need for liver biopsy in this context, its utility in the evaluation of intermediary stages of fibrosis severity remains unclear[118,119].

Addition of age to the APRI yields the FIB-4 index, providing a model which, while still easily and affordably calculable, consistently outperforms other non-invasive tools in comparative studies for the identification as well as exclusion of advanced hepatic fibrosis[120,121]. The international normalized ratio (INR) is not only a significant independent predictor for advanced hepatic fibrosis in NAFLD, but improves the positive predictive value of the BARD score when incorporated as an additional variable, without compromising its negative predictive value[122]. Age, platelet count and/or INR are also included in several other predictive models which have not yet been validated in adult patients with NAFLD. These scoring panels incorporate a number of components known to independently predict fibrosis severity in NAFLD, including bilirubin[123], GGT[124] and albumin[106].

***Markers of hepatocellular turnover***

Markers which reflect increased deposition or decreased degradation of extracellular matrix (ECM) components are obvious candidates for the evaluation of fibrosis severity in chronic liver disease. The accuracy of direct fibrotic biomarkers may exceed that for simple biomarker panels incorporating indirect markers of hepatic dysfunction[125]. ECM components are included in a number of complex patented biomarker panels, including the Original European Liver Fibrosis (OELF) score, simplified ELF and NASH Diagnostics panel. One of the most widely investigated direct biomarkers is the high molecular weight polysaccharide hyaluronic acid (HA), with increased levels, resulting from accelerated collagen synthesis and decreased hepatic sinusoidal clearance, shown to accurately predict advanced fibrosis in NAFLD[126,127]. However, a significant drawback to the use of ECM markers such as HA is their lack of liver-specificity, as levels are affected by diverse factors such as renal failure, extra-hepatic fibrogenesis and dietary habits[128].

**LIMITATIONS OF COMPOSITE PROGNOSTIC MODELS**

Individual biomarkers used for the peripheral evaluation of hepatic injury in NAFLD invariably fall short of the hypothetical ideal. Composite diagnostic and predictive models show greater discriminatory power compared to single-variable analysis and there is considerable interest in their potential value as non-invasive risk assessment tools. A number of important limitations however currently impede their routine use in clinical practice, as outlined below.

***Diagnostic and predictive models are defined by variability***

Different models which assess the same outcome may differ markedly in their composition as well as methods employed for risk calculation, and many were initially developed for use in other chronic liver diseases such as viral hepatitis. Existing models were constructed against histological end-points defined by variable classification schemes and evaluated using liver biopsy as an imperfect diagnostic standard. Selection bias poses a major general concern, as initial studies often utilize heterogeneous and highly selected patient cohorts as well as different reference populations. Despite promising findings supporting their clinical value, confirming the reproducibility and robustness of existing composite prognostic models will depend on their external validation in large-scale prospective studies, considered a prerequisite for extrapolation to the general population.

***Lack of consensus regarding clinically meaningful thresholds for histological severity***

Predictive model for the non-invasive assessment of the extent and severity of fibrotic injury should ideally reflect a dimensional pathogenic spectrum raging from ECM deposition though initial scarring, bridging as well as advanced fibrosis, and ultimately different stages of compensated and decompensated cirrhosis. While existing predictive models have proven useful in excluding advanced fibrosis, many require further validation in cases of intermediate severity. Their positive predictive value is also modest at best, and likely inferior to that of more complex scoring panels[129], which are again limited as the direct biomarkers they employ lack standardization as well as liver-specificity. A number of suggestions have been proposed in an attempt to address these shortcomings, including the concurrent use of multiple prognostic models towards the same goal[130]. The development of progressively more complex risk assessment schemes is intuitively a plausible solution, and while improving the performance for cirrhosis, this approach does not greatly increase their predictive accuracy for NASH or non-severe fibrosis[131].

***Limited value in predicting complications***

Composite non-invasive models have limited utility in the prediction of cirrhotic complications such as variceal bleeding[132]. There is still a disproportionate focus on liver-related as opposed to cardiovascular events, which is particularly relevant as CVD is the primary cause of mortality in NAFLD patients. Establishing the utility of composite models in predicting cardio-metabolic complications, adverse clinical outcomes and mortality risk is therefore an important research focus for future prospective studies. Moreover, an important research objective in this regard would be to focus on determining to what extent the addition of a genomics component to prognostic models allows for the accurate prediction of long-term clinical outcomes.

**IMPROVING CLINICAL RISK PREDICTION ACROSS THE NAFLD SPECTRUM**

There is ongoing research interest in determining to what extent the addition of biochemical and functional genomic markers and/or the application of emerging imaging technologies can assist in overcoming the abovementioned limitations restricting the more widespread clinical implementation of existing composite prognostic models applicable across the NAFLD spectrum by improving their diagnostic accuracy and predictive performance.

***Incorporation of personalized genomic testing to existing composite prognostic models***

Epidemiological evidence concerning the extent towards which susceptibility for NAFLD involves a substantial heritability component remains conflicted[133,134]. This has led to ongoing research interest in elucidating the genetic mechanisms which could underlie marked population-based variation in disease prevalence and inter-patient heterogeneity in histological severity characteristic of this complex disease trait[135,136].

Two seminal population-based genome-wide association studies (GWAS) conducted in 2008[137,138] identified a common non-synonymous single-nucleotide polymorphism (SNP) in the patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene (rs738409) which encodes the multifunctional lipolytic enzyme adiponutrin (ADPN)[139] as a major determinant of inter-individual variation in hepatic fat content and plasma liver enzyme levels. The rs738409 variant has subsequently been reproducibly associated with increased susceptibility towards the onset and progression of NAFLD across boundaries for age, sex and ethnic background[139-144]. Findings from at least two recent meta-analyses have also confirmed the clinical relevance of PNPLA3 rs738409 as a potent genetic risk factor for NASH, severe hepatic fibrosis and hepatocellular carcinoma[145,146].

Existing evidence concerning the exact molecular mechanisms which underlie the association between PNPLA3 rs738409 and increased susceptibility towards the onset and progression of hepatosteatosis however remains both inconclusive and conflicting[147]. ADPN possesses both triacylglycerol (TAG) lipase and acylglycerol O-acyltransferase activity with the known relationship between PNPLA3 rs738409 and decreased TAG hydrolysis[148,149] supporting a loss-of-function mechanism as underlying the promotion macrovesicular hepatosteatosis due to increased hepatic lipid accumulation and/or export associated with impaired lipidation of very low-density lipoprotein (VLDL) particles[150,151]. PNPLA3 knockout in vivo does not however lead to increased intra-hepatic TAG accumulation[152] with increased lysophosphatidic acid acyltransferase (LPAAT) activity and TAG synthesis noted for G-allele carriers rather supporting a gain-of-function mechanism contributes as contributing towards the development of hepatosteatosis[153]. Li *et al*[154] however suggested that increased susceptibility towards hepatosteatosis associated with PNPLA3 rs738409 could be explained by a combination of increased TAG synthesis as well as decreased hydrolysis in the context of excess intra-hepatic FFA accumulation.

While PNPLA3 rs738409 confers susceptibility towards hepatosteatosis independent of extra-hepatic metabolic phenotypes, visceral adiposity mediates the severity of hepatic lipid infiltration in risk-allele carriers as well as the association of this genetic variant with related cardio-metabolic risk traits[155,156]. The rs738409 variant similarly confers risk for increased histological severity dissociated from its effects on central obesity and IR[157,158] although its pathogenic role in impaired lipid homeostasis as evidenced by a peripheral decrease in serum triglyceride, total and HDL cholesterol[159,160] is unmasked in the presence of increased visceral adiposity[161] and impaired glucose tolerance[162] in addition to being modulated by lifestyle and dietary habits[161,163]. It has further been proposed that lipotoxicity and inflammatory stress resulting from impaired intra-hepatic lipid metabolism and free cholesterol deposition associated with PNPLA3 rs738409 activates dormant hepatic stellate cells (HSCs) leading to increased fibrogenesis. Severe hepatic fibrosis may however occur in the absence of pathologically evident NASH, supporting the hypothesis that a direct stimulating effect on HSCs also contributes towards hepatic fibrogenesis independent of hepatic necro-inflammation[164].

Collectively, the abovementioned findings provide a useful framework for ongoing research aimed at establishing to what extent the incorporation of PNPLA3 genotyping as a component of existing composite prognostic models used in NAFLD/NASH could improve their performance. The addition of PNPLA3 genotyping to the NAFLD liver fat score fails to significantly increase its diagnostic accuracy[38] suggesting limited utility as a diagnostic marker for hepatosteatosis. However, the combined assessment of PNPLA3 rs378409 genotype along with fasting insulin and ALT levels has been shown to reliably predict the presence of undiagnosed NASH[165]. The observation that genotype distributions and allele frequencies for PNPLA3 rs738409 closely mirror population-based prevalence rates for hepatosteatosis further supports the notion that a degree of the known variation in disease susceptibility observed between different population groups evident for NAFLD has a partly genetic basis[134,166], emphasizing the need for further research aimed at clarifying the role of ethnicity as a putative modulator of genetic risk conferred by PNPLA3 rs738409.

GWAS have identified a number of other genetic variants as potential risk modifiers for progressive necro-inflammatory injury and advanced hepatic fibrosis in NAFLD. For example, the PiS/PiZ variant of the α-1 antitrypsin (AAT) gene implicated in endoplasmic reticulum (ER) stress has been shown to predict hyperferritinemia and parenchymal iron overload independent of TS percentage despite its dissociation from risk for increased histological severity[167]. In a similar study, Valenti *et al*[168] investigated the utility of four polymorphic variants in the HFE, AAT, ferroportin-1 and beta-globin genes functioning in known iron metabolism pathways as putative predictive markers for parenchymal iron overload and increased histological severity. In this study[168], the authors demonstrated that that hepatocellular iron overload could be explained by risk-variant carriage in 63% of cases, with the beta-thalassemia trait showing the highest predictive accuracy for moderate-to-severe hepatic fibrosis in patients with NAFLD and/or DIOS.

The role of causative mutations in the HFE gene implicated in type I genetic HH as non-deterministic modifiers of susceptibility towards the onset and pathogenic progression of NAFLD remains incompletely elucidated. In an Italian study, Valenti and colleagues[88] showed that heterozygosity for the deleterious C282Y mutation confers susceptibility towards hepatosteatosis even in the absence of overt cardio-metabolic risk. The C282Y mutation has been associated with NASH[169] as well as hepatocellular iron accumulation which could promote the progression of hepatic fibrogenesis and conferring risk for increased histological severity secondary to iron-mediated oxidative stress and lipotoxicity[170-172]. Findings from conflicting studies[173,174] as well as a systematic review and meta-analysis[175] however fail to support the notion that HFE genotype is a significant genetic determinant of risk for the onset or progression of NAFLD and/or DIOS. In patients with a known diagnosis of NAFLD, confirmation that HFE genotype allows for the non-invasive prediction of parenchymal as opposed to sinusoidal iron overload could also provide evidence supporting the relevance of personalized genomic testing in the non-invasive differentiation between hepatosteatosis/DIOS and type I genetic HH. This issue was addressed in a study conducted by Valenti *et al*[176] who showed that, despite the association between C282Y mutation carriage and parenchymal siderosis typical of type I genetic HH, only ~33% of NAFLD patients exhibited this pattern of iron distribution, with HFE genotype explaining less than 50% of phenotypic variance for this trait. These findings suggest that HFE genotyping as a stand-alone genomic test most likely has limited utility as a reliable predictive marker for hepatocellular iron overload.

There is growing appreciation that genetic variants implicated in the aberrant regulation of the ferroportin-hepcidin axis play a major role in the etiopathogenesis of iron-related disorders[177]. In accordance with this notion, a polymorphic variant (rs855791) in the matriptase-2 (TMPRSS6) gene has been associated with dysfunctional down-regulation of hepcidin expression implicated in the pathogenesis of a severe atypical form of iron-refractory iron-deficiency anaemia (IRIDA). It remains unclear however to what extent the association between TMPRSS6 rs855791 and IRIDA is either mediated by or independent of hepcidin status, with existing evidence indicating that the pleiotropic effects this genetic variant exerts on serum iron profiles are likely context-dependent[178-180]. The putative role of the rs855791 as a genetic determinant of risk for the onset and progression of NAFLD has recently started to garner increasing research attention. Emerging evidence suggests that TMPRSS6 rs855791 is associated with hypoferritinemia and decreased hepatic iron stores independent of serum ferritin and HFE genotype, and may exert a protective effect against the development of hepatic siderosis and DM II in patients with NAFLD[181,182]. Ongoing investigative effort is required not only to further elucidate the clinical significance of TMPRSS6 rs855791 as a potential genetic risk modifier for NASH and/or advanced hepatic siderosis but also replicate its apparent dissociation from susceptibility towards DIOS considered in the context of known environmental and epistatic modulators of phenotypic expression in other population groups.

***Incorporation of emerging imaging methodologies as a component of existing composite prognostic models***

Findings from multiple studies and meta-analyses further support the utility of transient elastography (TE) in diagnosing and excluding advanced hepatic fibrosis and cirrhosis with excellent accuracy, providing a reliable non-invasive tool used to assess and monitor the progression of fibrogenic activity in patients with NAFLD/NASH. To what extent extra-hepatic metabolic risk phenotypes as well as underlying pathology contribute towards variation in liver stiffness measurement (LSM) and influences the efficacy of TE however remains incompletely understood[183-187]. Emerging evidence further suggests that the concurrent assessment of LSM and PNPLA3 genotype alongside existing composite prognostic models used to predict advanced hepatic fibrosis in NAFLD could improve their performance with the goal of decreasing the need for invasive liver biopsy[188,189]. Future studies should ideally aim to validate these preliminary findings in large-scale population-based on prospective studies.

**CONCLUSION**

The routine clinical adoption of composite prognostic models as viable non-invasive risk stratification tools offers distinct advantages over the use of individual peripheral biomarkers which have limited utility compared to liver biopsy as the current standard for the diagnosis, grading and staging of NAFLD. In future, improved non-invasive diagnostic and predictive models used to assess histological severity could allow for the timely implementation of tailored therapeutic intervention aimed at preventing disease onset or as well as decreasing risk for cardiovascular- and liver-related mortality in patients with NAFLD[190]. A number of important limitations however continue to impede their more widespread clinical application as part of routine patient management, including the need for external validation in large-scale prospective studies to confirm their reproducibility and robustness as well as applicability in the general population. In response, ongoing investigative effort it required in order to assess new combinations of readily available biomarkers as novel prognostic models which could serve as cost-effective screening tools in a specific target population. Future prospective studies should further aim to establish the value of existing as well as novel clinical models in the prediction of ischemic vascular events as well as cardiovascular mortality risk.

Given the decreasing cost and growing availability of personalized genomic testing in the clinical domain, their routine use in developing nations will soon become a reality. This provides motivation for future research aimed at clarifying to what extent the incorporation of functional genomic markers and/or the application of emerging imaging technologies including TE could assist in overcoming the abovementioned limitations evident for existing composite prognostic models by improving their diagnostic accuracy and predictive performance. In particular, considering the emerging role of DIOS as an important secondary cause of hepatic siderosis distinct from type I genetic HH known to pose independent risk for new-onset CVD, subclinical atherosclerosis and decompensated liver failure, there is a pressing need to develop and validate non-invasive pre-clinical diagnostic algorithms to differentiate between DIOS and type I genetic HH in patients with the metabolic syndrome and/or NAFLD. The clinical implementation of a validated diagnostic model for DIOS could potentially allow for a more comprehensive approach to cardiovascular risk screening as well, allowing clinicians to identify an obese patient subgroup set to derive optimal benefit from the timely implementation of more aggressive and sustained lifestyle-based intervention strategies as well as tailored therapeutic adjuncts aimed at decreasing cumulative cardio-metabolic risk for early on in the disease process.

In addition to allowing for a more goal-directed use of liver biopsy in resource-limited settings, clinically validated composite models may in future be used to determine eligibility for genetic testing as part of the emerging arsenal of available tools used to guide patient management and improve the standard of patient care in NAFLD. A multidisciplinary approach to genomics-based NCD risk assessment could provide a useful as a standardized pre-screening step aimed at identifying genetically uncharacterized NAFLD patients set to derive additional benefit from referral for whole exome (WES) or genome sequencing (WGS)[191]. In this context, next-generation sequencing could be used to validate common susceptibility variants implicated in the etiopathogenesis of NAFLD supporting the development and validation of a genomics-based risk panel to provide greater insight into the potential for personalized genomic testing to add value to clinical risk stratification models[192,193]. Extension of personalized genomics-based NCD screening beyond the limited scope of single-gene testing assisted by next-generation sequencing therefore has the potential to facilitate the detection of both known and novel mutations allowing for the prevention of cumulative cardio-metabolic risk across the NAFLD spectrum.

**ACKNOWLEGEMENTS**

This work is based on the research supported in part by the National Research Foundation (NRF) of South Africa (UID 83962). The Grantholder acknowledges that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF supported research are that of the authors, and that the NRF accepts no liability whatsoever in this regard.

**REFERENCES**

1 **Murray CJ**, Lopez AD. Measuring the global burden of disease. *N Engl J Med* 2013; **369**: 448-457 [PMID: 23902484 DOI: 10.1056/NEJMra1201534]

2 **Boutayeb A**, Boutayeb S. The burden of non communicable diseases in developing countries. *Int J Equity Health* 2005; **4**: 2 [PMID: 15651987 DOI: 10.1007/978-0-387-78665-0\_31]

3 **Prentice AM**. The emerging epidemic of obesity in developing countries. *Int J Epidemiol* 2006; **35**: 93-99 [PMID: 16326822 DOI: 10.1093/ije/dyi272]

4 **Egger G**. Obesity, chronic disease, and economic growth: a case for "big picture" prevention. *Adv Prev Med* 2011; **2011**: 149158 [PMID: 21991431 DOI: 10.4061/2011/149158]

5 **Chang CY**. Understanding the relationship between PNPLA3, NAFLD and insulin resistance: do ethnic differences bring more questions or more answers? *Liver Int* 2011; **31**: 1246-1249 [PMID: 22093451 DOI: 10.1111/j.1478-3231.2011.02612.x.]

6 **Bergqvist CJ**, Skoien R, Horsfall L, Clouston AD, Jonsson JR, Powell EE. Awareness and opinions of non-alcoholic fatty liver disease by hospital specialists. *Intern Med J* 2013; **43**: 247-253 [PMID: 22646061 DOI: 10.1111/j.1445-5994.2012.02848.x]

7 **Serfaty L**, Lemoine M. Definition and natural history of metabolic steatosis: clinical aspects of NAFLD, NASH and cirrhosis. *Diabetes Metab* 2008; **34**: 634-637 [PMID: 19195623 DOI: 10.1016/S1262-3636(08)74597-X]

8 **Basaranoglu M**, Basaranoglu G, Sentürk H. From fatty liver to fibrosis: a tale of "second hit". *World J Gastroenterol* 2013; **19**: 1158-1165 [PMID: 23483818 DOI: 10.3748/wjg.v19.i8.1158]

9 **Brunt EM**, Tiniakos DG. Histopathology of nonalcoholic fatty liver disease. *World J Gastroenterol* 2010; **16**: 5286-5296 [PMID: 21072891 DOI: 10.3748/wjg.v16.i42.5286]

10 **Nalbantoglu IL**, Brunt EM. Role of liver biopsy in nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 9026-9037 [PMID: 25083076 DOI: 10.3748/wjg.v20.i27.9026]

11 **El Bassat H**, Ziada DH, Hasby EA, Nagy H, Abo Ryia MH. Apoptotic and anti-apoptotic seromarkers for assessment of disease severity of non-alcoholic steatohepatitis. *Arab J Gastroenterol* 2014; **15**: 6-11 [PMID: 24630506 DOI: 10.1016/j.ajg.2014.01.009]

12 **Kawanaka M**, Mahmood S, Niiyama G, Izumi A, Kamei A, Ikeda H, Suehiro M, Togawa K, Sasagawa T, Okita M, Nakamura H, Yodoi J, Yamada G. Control of oxidative stress and reduction in biochemical markers by Vitamin E treatment in patients with nonalcoholic steatohepatitis: a pilot study. *Hepatol Res* 2004; **29**: 39-41 [PMID: 15135345]

13 **Machado MV**, Ravasco P, Jesus L, Marques-Vidal P, Oliveira CR, Proença T, Baldeiras I, Camilo ME, Cortez-Pinto H. Blood oxidative stress markers in non-alcoholic steatohepatitis and how it correlates with diet. *Scand J Gastroenterol* 2008; **43**: 95-102 [PMID: 18938777]

14 **Jarrar MH**, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, Fang Y, Elariny H, Goodman Z, Chandhoke V, Younossi ZM. Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008; **27**: 412-421 [PMID: 18081738 DOI: 10.1111/j.1365-2036.2007.03586.x]

15 **Braunersreuther V**, Viviani GL, Mach F, Montecucco F. Role of cytokines and chemokines in non-alcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 727-735 [PMID: 22371632 DOI: 10.3748/wjg.v18.i8.727]

16 **Baranova A**, Lal P, Birerdinc A, Younossi ZM. Non-invasive markers for hepatic fibrosis. *BMC Gastroenterol* 2011; **11**: 91 [PMID: 21849046 DOI: 10.1186/1471-230X-11-91]

17 **Alkhouri N**, McCullough AJ. Noninvasive Diagnosis of NASH and Liver Fibrosis Within the Spectrum of NAFLD. *Gastroenterol Hepatol* (N Y) 2012; **8**: 661-668 [PMID: 24683373 DOI: 10.1016/s0016-5085(10)62097-5]

18 **Festi D**, Schiumerini R, Marzi L, Di Biase AR, Mandolesi D, Montrone L, Scaioli E, Bonato G, Marchesini-Reggiani G, Colecchia A. Review article: the diagnosis of non-alcoholic fatty liver disease -- availability and accuracy of non-invasive methods. *Aliment Pharmacol Ther* 2013; **37**: 392-400 [PMID: 23278163 DOI: 10.1111/apt.12186]

19 **Castera L**, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 666-675 [PMID: 24061203 DOI: 10.1038/nrgastro.2013.175]

20 **Machado MV**, Cortez-Pinto H. Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical appraisal. *J Hepatol* 2013; **58**: 1007-1019 [PMID: 23183525 DOI: 10.1016/j.jhep.2012.11.021]

21 **Dowman JK**, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2011; **33**: 525-540 [PMID: 21198708 DOI: 10.1111/j.1365-2036.2010.04556.x]

22 **Schindhelm RK**, Diamant M, Dekker JM, Tushuizen ME, Teerlink T, Heine RJ. Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease. *Diabetes Metab Res Rev* 2006; **22**: 437-443 [PMID: 16832839 DOI: 10.1002/dmrr.666]

23 **Yun KE**, Shin CY, Yoon YS, Park HS. Elevated alanine aminotransferase levels predict mortality from cardiovascular disease and diabetes in Koreans. *Atherosclerosis* 2009; **205**: 533-537 [PMID: 19159884 DOI: 10.1016/j.atherosclerosis.2008.12.012]

24 **Esteghamati A**, Jamali A, Khalilzadeh O, Noshad S, Khalili M, Zandieh A, Morteza A, Nakhjavani M. Metabolic syndrome is linked to a mild elevation in liver aminotransferases in diabetic patients with undetectable non-alcoholic fatty liver disease by ultrasound. *Diabetol Metab Syndr* 2010; **2**: 65 [PMID: 21047423 DOI: 10.1186/1758-5996-2-65]

25 **Adams LA**, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113-121 [PMID: 16012941 DOI: 10.1053/j.gastro.2005.04.014]

26 **Ong JP**, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *J Hepatol* 2008; **49**: 608-612 [PMID: 18682312 DOI: 10.1016/j.jhep.2008.06.018]

27 **Misra VL**, Khashab M, Chalasani N. Nonalcoholic fatty liver disease and cardiovascular risk. *Curr Gastroenterol Rep* 2009; **11**: 50-55 [PMID: 19166659 DOI: 10.1007/s11894-009-0008-4]

28 **Ahmed MH**, Barakat S, Almobarak AO. Nonalcoholic fatty liver disease and cardiovascular disease: has the time come for cardiologists to be hepatologists? *J Obes* 2012; **2012**: 483135 [PMID: 23320150 DOI: 10.1155/2012/483135]

29 **Wong VW**, Wong GL, Yip GW, Lo AO, Limquiaco J, Chu WC, Chim AM, Yu CM, Yu J, Chan FK, Sung JJ, Chan HL. Coronary artery disease and cardiovascular outcomes in patients with non-alcoholic fatty liver disease. *Gut* 2011; **60**: 1721-1727 [PMID: 21602530 DOI: 10.1136/gut.2011.242016]

30 **Dekker JM**, Girman C, Rhodes T, Nijpels G, Stehouwer CD, Bouter LM, Heine RJ. Metabolic syndrome and 10-year cardiovascular disease risk in the Hoorn Study. *Circulation* 2005; **112**: 666-673 [PMID: 16061755 DOI: 10.1161/circulationaha.104.516948]

31 **Fracanzani AL**, Burdick L, Raselli S, Pedotti P, Grigore L, Santorelli G, Valenti L, Maraschi A, Catapano A, Fargion S. Carotid artery intima-media thickness in nonalcoholic fatty liver disease. *Am J Med* 2008; **121**: 72-78 [PMID: 18187076 DOI: 10.1016/j.amjmed.2007.08.041]

32 **Iacobellis G**, Barbarini G, Letizia C, Barbaro G. Epicardial fat thickness and nonalcoholic fatty liver disease in obese subjects. *Obesity (Silver Spring)* 2014; **22**: 332-336 [PMID: 24115757 DOI: 10.1002/oby.20624]

33 **Tarantino G**, Costantini S, Finelli C, Capone F, Guerriero E, La Sala N, Gioia S, Castello G. Carotid intima-media thickness is predicted by combined eotaxin levels and severity of hepatic steatosis at ultrasonography in obese patients with Nonalcoholic Fatty Liver Disease. *PLoS One* 2014; **9**: e105610 [PMID: 25268946 DOI: 10.1371/journal.pone.0105610]

34 **Bedogni G**, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; **6**: 33 [PMID: 17081293 DOI: 10.1186/1471-230X-6-33]

35 **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 [PMID: 20738844 DOI: 10.1186/1471-230X-10-98]

36 **Jung CH**, Lee WJ, Hwang JY, Yu JH, Shin MS, Lee MJ, Jang JE, Leem J, Park JY, Kim HK. Assessment of the fatty liver index as an indicator of hepatic steatosis for predicting incident diabetes independently of insulin resistance in a Korean population. *Diabet Med* 2013; **30**: 428-435 [PMID: 23278318 DOI: 10.1111/dme.12104]

37 **Zelber-Sagi S**, Webb M, Assy N, Blendis L, Yeshua H, Leshno M, Ratziu V, Halpern Z, Oren R, Santo E. Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification. *World J Gastroenterol* 2013; **19**: 57-64 [PMID: 23326163 DOI: 10.3748/wjg.v19.i1.57]

38 **Kotronen A**, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, Lundbom N, Rissanen A, Ridderstråle M, Groop L, Orho-Melander M, Yki-Järvinen H. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. *Gastroenterology* 2009; **137**: 865-872 [PMID: 19524579 DOI: 10.1053/j.gastro.2009.06.005]

39 **Lee JH**, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, Kim YJ, Yoon JH, Cho SH, Sung MW, Lee HS. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis* 2010; **42**: 503-508 [PMID: 19766548 DOI: 10.1016/j.dld.2009.08.002]

40 **Fuyan S**, Jing L, Wenjun C, Zhijun T, Weijing M, Suzhen W, Yongyong X. Fatty liver disease index: a simple screening tool to facilitate diagnosis of nonalcoholic fatty liver disease in the Chinese population. *Dig Dis Sci* 2013; **58**: 3326-3334 [PMID: 23900558 DOI: 10.1007/s10620-013-2774-y]

41 **Calori G**, Lattuada G, Ragogna F, Garancini MP, Crosignani P, Villa M, Bosi E, Ruotolo G, Piemonti L, Perseghin G. Fatty liver index and mortality: the Cremona study in the 15th year of follow-up. *Hepatology* 2011; **54**: 145-152 [PMID: 21488080 DOI: 10.1002/hep.24356]

42 **Borman MA,** Ladak F, Crotty P, Pollett A, Kirsch R, Pomier-Layrargues G, Beaton M, Duarte-Rojo A, Elkashab M, Myers RP. The Fatty Liver Index has limited utility for the detection and quantification of hepatic steatosis in obese patients. *Hepatol Int* 2013; **7** Suppl 2: 592-599 [DOI: 10.1111/jgh.12557]

43 **Jiang ZY**, Xu CY, Chang XX, Li WW, Sun LY, Yang XB, Yu LF. Fatty liver index correlates with non-alcoholic fatty liver disease, but not with newly diagnosed coronary artery atherosclerotic disease in Chinese patients. *BMC Gastroenterol* 2013; **13**: 110 [PMID: 23834773 DOI: 10.1186/1471-230X-13-110]

44 **Dyson J**, Day C. Treatment of non-alcoholic fatty liver disease. *Dig Dis* 2014; **32**: 597-604 [PMID: 25034293 DOI: 10.1159/000360511]

45 **Harrison SA**, Day CP. Benefits of lifestyle modification in NAFLD. *Gut* 2007; **56**: 1760-1769 [PMID: 17911352 DOI: 10.1136/gut.2006.112094]

46 **Rafiq N**, Younossi ZM. Effects of weight loss on nonalcoholic fatty liver disease. *Semin Liver Dis* 2008; **28**: 427-433 [PMID: 18956298 DOI: 10.1055/s-0028-1091986]

47 **Scaglioni F**, Marino M, Ciccia S, Procaccini A, Busacchi M, Loria P, Lonardo A, Malavolti M, Battistini NC, Pellegrini M, Carubbi F, Bellentani S. Short-term multidisciplinary non-pharmacological intervention is effective in reducing liver fat content assessed non-invasively in patients with nonalcoholic fatty liver disease (NAFLD). *Clin Res Hepatol Gastroenterol* 2013; **37**: 353-358 [PMID: 23273500 DOI: 10.1016/j.clinre.2012.10.009]

48 **Centis E**, Marzocchi R, Suppini A, Dalle Grave R, Villanova N, Hickman IJ, Marchesini G. The role of lifestyle change in the prevention and treatment of NAFLD. *Curr Pharm Des* 2013; **19**: 5270-5279 [PMID: 23394095 DOI: 10.2174/1381612811319290008]

49 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]

50 **Villanova N**, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, Zoli M, Marchesini G. Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 473-480 [PMID: 15981216 DOI: 10.1002/hep.20781]

51 **Goland S**, Shimoni S, Zornitzki T, Knobler H, Azoulai O, Lutaty G, Melzer E, Orr A, Caspi A, Malnick S. Cardiac abnormalities as a new manifestation of nonalcoholic fatty liver disease: echocardiographic and tissue Doppler imaging assessment. *J Clin Gastroenterol* 2006; **40**: 949-955 [PMID: 17063117 DOI: 10.1097/01.mcg.0000225668.53673.e6]

52 **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 [PMID: 11438497 DOI: 10.1053/gast.2001.25540]

53 **Gholam PM**, Flancbaum L, Machan JT, Charney DA, Kotler DP. Nonalcoholic fatty liver disease in severely obese subjects. *Am J Gastroenterol* 2007; **102**: 399-408 [PMID: 17311652 DOI: 10.1111/j.1572-0241.2006.01041.x]

54 **Campos GM**, Bambha K, Vittinghoff E, Rabl C, Posselt AM, Ciovica R, Tiwari U, Ferrel L, Pabst M, Bass NM, Merriman RB. A clinical scoring system for predicting nonalcoholic steatohepatitis in morbidly obese patients. *Hepatology* 2008; **47**: 1916-1923 [PMID: 18433022 DOI: 10.1002/hep.22241]

55 **Ulitsky A**, Ananthakrishnan AN, Komorowski R, Wallace J, Surapaneni SN, Franco J, Saeian K, Gawrieh S. A noninvasive clinical scoring model predicts risk of nonalcoholic steatohepatitis in morbidly obese patients. *Obes Surg* 2010; **20**: 685-691 [PMID: 20336392 DOI: 10.1007/s11695-010-0118-y]

56 **Zein CO,** Edmison JM, Schluchter M, Feldstein AE, Zein NN, McCullough A. A NASH predictive index (NPI) for use in patients with nonalcoholic fatty liver disease. *Hepatology* 2007; **46**: 747A

57 **Schwimmer JB**, McGreal N, Deutsch R, Finegold MJ, Lavine JE. Influence of gender, race, and ethnicity on suspected fatty liver in obese adolescents. *Pediatrics* 2005; **115**: e561-e565 [PMID: 15867021 DOI: 10.1542/peds.2004-1832]

58 **Pan JJ**, Fallon MB. Gender and racial differences in nonalcoholic fatty liver disease. *World J Hepatol* 2014; **6**: 274-283 [PMID: 24868321 DOI: 10.4254/wjh.v6.i5.274]

59 **Hashimoto E**, Tokushige K. Prevalence, gender, ethnic variations, and prognosis of NASH. *J Gastroenterol* 2011; **46** Suppl 1: 63-69 [PMID: 20844903 DOI: 10.1007/s00535-010-0311-8]

60 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923 [PMID: 12668987 DOI: 10.1053/jhep.2003.50161]

61 **Bhatia LS**, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? *Eur Heart J* 2012; **33**: 1190-1200 [PMID: 22408036 DOI: 10.1093/eurheartj/ehr453]

62 **Verma S**, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). *Liver Int* 2013; **33**: 1398-1405 [PMID: 23763360 DOI: 10.1111/liv.12226]

63 **Alam S**, Noor-E-Alam SM, Chowdhury ZR, Alam M, Kabir J. Nonalcoholic steatohepatitis in nonalcoholic fatty liver disease patients of Bangladesh. *World J Hepatol* 2013; **5**: 281-287 [PMID: 23717739 DOI: 10.4254/wjh.v5.i5.281]

64 **Lee DS**, Evans JC, Robins SJ, Wilson PW, Albano I, Fox CS, Wang TJ, Benjamin EJ, D'Agostino RB, Vasan RS. Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease, and mortality risk: the Framingham Heart Study. *Arterioscler Thromb Vasc Biol* 2007; **27**: 127-133 [PMID: 17095717 DOI: 10.1161/01.ATV.0000251993.20372.40]

65 **Tanné F**, Gagnadoux F, Chazouillères O, Fleury B, Wendum D, Lasnier E, Lebeau B, Poupon R, Serfaty L. Chronic liver injury during obstructive sleep apnea. *Hepatology* 2005; **41**: 1290-1296 [PMID: 15915459 DOI: 10.1002/hep.20725]

66 **Weingarten TN**, Mantilla CB, Swain JM, Kendrick ML, Oberhansley JM, Burcham RJ, Ribeiro TC, Watt KD, Schroeder DR, Narr BJ, Sprung J. Nonalcoholic steatohepatitis in bariatric patients with a diagnosis of obstructive sleep apnea. *Obes Facts* 2012; **5**: 587-596 [PMID: 22986647 DOI: 10.1159/000342677]

67 **Tahiri M**, Drighil A, Jalal Y, Ghellab D, Hliwa W, Fouad H, Badre W, Bellabah A, Habbal R, Alaoui R. Chronic permanent hypoxemia predisposes to mild elevation of liver stiffness. *World J Gastroenterol* 2014; **20**: 10564-10569 [PMID: 25132776 DOI: 10.3748/wjg.v20.i30.10564]

68 **Mirrakhimov AE**, Polotsky VY. Obstructive sleep apnea and non-alcoholic Fatty liver disease: is the liver another target? *Front Neurol* 2012; **3**: 149 [PMID: 23087670 DOI: 10.3389/fneur.2012.00149]

69 **Hirsso PK**, Timonen MJ, Jokelainen JJ, Hiltunen LA, Laakso MA, Hedberg PS, Ruokonen AO, Koskela P, Härkönen PK, Keinänen-Kiukaanniemi SM, Rajala UM. Association between high-sensitive measurement of C-reactive protein and metabolic syndrome as defined by International Diabetes Federation, National Cholesterol Education Program, and World Health Organization criteria in a population-based cohort of 55-year-old Finnish individuals. *Diabetes Care* 2006; **29**: 2177-2178 [PMID: 16936176 DOI: 10.2337/dc06-0951]

70 **Pfützner A**, Forst T. High-sensitivity C-reactive protein as cardiovascular risk marker in patients with diabetes mellitus. *Diabetes Technol Ther* 2006; **8**: 28-36 [PMID: 16472048 DOI: 10.1089/dia.2006.8.28]

71 **Ridker PM**. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. *J Am Coll Cardiol* 2007; **49**: 2129-2138 [PMID: 17531663 DOI: 10.1016/j.jacc.2007.02.052]

72 **Targher G**. High-sensitivity C-reactive protein, obesity, and subclinical atherosclerosis: implications of JUPITER from the MESA study. *Arterioscler Thromb Vasc Biol* 2011; **31**: 1251-1252 [PMID: 21593456 DOI: 10.1161/ATVBAHA.111.228320]

73 **van der Velde M**, Bello AK, Brantsma AH, El Nahas M, Bakker SJ, de Jong PE, Gansevoort RT. Do albuminuria and hs-CRP add to the International Diabetes Federation definition of the metabolic syndrome in predicting outcome? *Nephrol Dial Transplant* 2012; **27**: 2275-2283 [PMID: 22231032 DOI: 10.1093/ndt/gfr634]

74 **Pearce SG**, Thosani NC, Pan JJ. Noninvasive biomarkers for the diagnosis of steatohepatitis and advanced fibrosis in NAFLD. *Biomark Res* 2013; **1**: 7 [PMID: 24252302 DOI: 10.1186/2050-7771-1-7]

75 **Feldstein AE**, Alkhouri N, De Vito R, Alisi A, Lopez R, Nobili V. Serum cytokeratin-18 fragment levels are useful biomarkers for nonalcoholic steatohepatitis in children. *Am J Gastroenterol* 2013; **108**: 1526-1531 [PMID: 23752877 DOI: 10.1038/ajg.2013.168]

76 **Cusi K**, Chang Z, Harrison S, Lomonaco R, Bril F, Orsak B, Ortiz-Lopez C, Hecht J, Feldstein AE, Webb A, Louden C, Goros M, Tio F. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol* 2014; **60**: 167-174 [PMID: 23973932 DOI: 10.1016/j.jhep.2013.07.042]

77 **Sharifi F**, Nasab NM, Zadeh HJ. Elevated serum ferritin concentrations in prediabetic subjects. *Diab Vasc Dis Res* 2008; **5**: 15-18 [PMID: 18398807 DOI: 10.3132/dvdr.2008.003]

78 **Park SK**, Ryoo JH, Kim MG, Shin JY. Association of serum ferritin and the development of metabolic syndrome in middle-aged Korean men: a 5-year follow-up study. *Diabetes Care* 2012; **35**: 2521-2526 [PMID: 22933431 DOI: 10.2337/dc12-0543]

79 **Abril-Ulloa V**, Flores-Mateo G, Solà-Alberich R, Manuel-y-Keenoy B, Arija V. Ferritin levels and risk of metabolic syndrome: meta-analysis of observational studies. *BMC Public Health* 2014; **14**: 483 [PMID: 24884526 DOI: 10.1186/1471-2458-14-483]

80 **Zelber-Sagi S**, Nitzan-Kaluski D, Halpern Z, Oren R. NAFLD and hyperinsulinemia are major determinants of serum ferritin levels. *J Hepatol* 2007; **46**: 700-707 [PMID: 17150278 DOI: 10.1016/j.jhep.2006.09.018]

81 **Juárez-Rojas JG**, Medina-Urrutia AX, Jorge-Galarza E, González-Salazar C, Kimura-Hayama E, Cardoso-Saldaña G, Posadas-Sánchez R, Martínez-Alvarado R, Posadas-Romero C. Fatty liver increases the association of metabolic syndrome with diabetes and atherosclerosis. *Diabetes Care* 2013; **36**: 1726-1728 [PMID: 23250798 DOI: 10.2337/dc12-1276]

82 **Dongiovanni P**, Fracanzani AL, Fargion S, Valenti L. Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. *J Hepatol* 2011; **55**: 920-932 [PMID: 21718726 DOI: 10.1016/j.jhep.2011.05.008]

83 **Sumida Y**, Yoneda M, Hyogo H, Yamaguchi K, Ono M, Fujii H, Eguchi Y, Suzuki Y, Imai S, Kanemasa K, Fujita K, Chayama K, Yasui K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Okanoue T. A simple clinical scoring system using ferritin, fasting insulin, and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. *J Gastroenterol* 2011; **46**: 257-268 [PMID: 20842510 DOI: 10.1007/s00535-010-0305-6]

84 **Manousou P**, Kalambokis G, Grillo F, Watkins J, Xirouchakis E, Pleguezuelo M, Leandro G, Arvaniti V, Germani G, Patch D, Calvaruso V, Mikhailidis DP, Dhillon AP, Burroughs AK. Serum ferritin is a discriminant marker for both fibrosis and inflammation in histologically proven non-alcoholic fatty liver disease patients. *Liver Int* 2011; **31**: 730-739 [PMID: 21457446 DOI: 10.1111/j.1478-3231.2011.02488.x]

85 **Kowdley KV**, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, Sanyal AJ, Nelson JE. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2012; **55**: 77-85 [PMID: 21953442 DOI: 10.1002/hep.24706]

86 **Chandok N**, Minuk G, Wengiel M, Uhanova J. Serum ferritin levels do not predict the stage of underlying non-alcoholic fatty liver disease. *J Gastrointestin Liver Dis* 2012; **21**: 53-58 [PMID: 22457860]

87 **Yoneda M**, Thomas E, Sumida Y, Imajo K, Eguchi Y, Hyogo H, Fujii H, Ono M, Kawaguchi T, Schiff ER. Clinical usage of serum ferritin to assess liver fibrosis in patients with non-alcoholic fatty liver disease: Proceed with caution. *Hepatol Res* 2014; **44**: E499-E502 [PMID: 24628717 DOI: 10.1111/hepr.12327]

88 **Valenti L**, Dongiovanni P, Fracanzani AL, Santorelli G, Fatta E, Bertelli C, Taioli E, Fiorelli G, Fargion S. Increased susceptibility to nonalcoholic fatty liver disease in heterozygotes for the mutation responsible for hereditary hemochromatosis. *Dig Liver Dis* 2003; **35**: 172-178 [PMID: 12779071 DOI: 10.1016/s1590-8658(03)00025-2]

89 **Ausk KJ**, Ioannou GN. Is obesity associated with anemia of chronic disease? A population-based study. *Obesity* (Silver Spring) 2008; **16**: 2356-2361 [PMID: 18719644 DOI: 10.1038/oby.2008.353]

90 **Mendler MH**, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, Le Gall JY, Brissot P, David V, Deugnier Y. Insulin resistance-associated hepatic iron overload. *Gastroenterology* 1999; **117**: 1155-1163 [PMID: 10535879 DOI: 10.1016/s0016-5085(99)70401-4]

91 **Riva A**, Trombini P, Mariani R, Salvioni A, Coletti S, Bonfadini S, Paolini V, Pozzi M, Facchetti R, Bovo G, Piperno A. Revaluation of clinical and histological criteria for diagnosis of dysmetabolic iron overload syndrome. *World J Gastroenterol* 2008; **14**: 4745-4752 [PMID: 18720534 DOI: 10.3748/wjg.14.4745]

92 **Angulo P**. Diagnosing steatohepatitis and predicting liver-related mortality in patients with NAFLD: two distinct concepts. *Hepatology* 2011; **53**: 1792-1794 [PMID: 21557278 DOI: 10.1002/hep.24403]

93 **Poynard T**, Munteanu M, Deckmyn O, Ngo Y, Drane F, Castille JM, Housset C, Ratziu V, Imbert-Bismut F. Validation of liver fibrosis biomarker (FibroTest) for assessing liver fibrosis progression: proof of concept and first application in a large population. *J Hepatol* 2012; **57**: 541-548 [PMID: 22612998 DOI: 10.1016/j.jhep.2012.04.025]

94 **Targher G**, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? *Diabetologia* 2008; **51**: 1947-1953 [PMID: 18762907 DOI: 10.1007/s00125-008-1135-4]

95 **Yilmaz Y**, Kurt R, Yonal O, Polat N, Celikel CA, Gurdal A, Oflaz H, Ozdogan O, Imeryuz N, Kalayci C, Avsar E. Coronary flow reserve is impaired in patients with nonalcoholic fatty liver disease: association with liver fibrosis. *Atherosclerosis* 2010; **211**: 182-186 [PMID: 20181335 DOI: 10.1016/j.atherosclerosis.2010.01.049]

96 **Kruger FC**, Daniels CR, Kidd M, Swart G, Brundyn K, van Rensburg C, Kotze M. APRI: a simple bedside marker for advanced fibrosis that can avoid liver biopsy in patients with NAFLD/NASH. *S Afr Med J* 2011; **101**: 477-480 [PMID: 21920102]

97 **Ratziu V**, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T. Liver fibrosis in overweight patients. *Gastroenterology* 2000; **118**: 1117-1123 [PMID: 10833486 DOI: 10.1016/s0016-5085(00)70364-7]

98 **Harrison SA**, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**: 1441-1447 [PMID: 18390575 DOI: 10.1136/gut.2007.146019]

99 **Udell JA**, Wang CS, Tinmouth J, FitzGerald JM, Ayas NT, Simel DL, Schulzer M, Mak E, Yoshida EM. Does this patient with liver disease have cirrhosis? *JAMA* 2012; **307**: 832-842 [PMID: 22357834 DOI: 10.1001/jama.2012.186]

100 **Shah AG**, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1104-1112 [PMID: 19523535 DOI: 10.1016/j.cgh.2009.05.033]

101 **Sud A**, Hui JM, Farrell GC, Bandara P, Kench JG, Fung C, Lin R, Samarasinghe D, Liddle C, McCaughan GW, George J. Improved prediction of fibrosis in chronic hepatitis C using measures of insulin resistance in a probability index. *Hepatology* 2004; **39**: 1239-1247 [PMID: 15122752 DOI: 10.1002/hep.20207]

102 **Forns X**, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM, Rodés J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986-992 [PMID: 12297848 DOI: 10.1016/s0270-9139(02)00107-6]

103 **Hui AY**, Chan HL, Wong VW, Liew CT, Chim AM, Chan FK, Sung JJ. Identification of chronic hepatitis B patients without significant liver fibrosis by a simple noninvasive predictive model. *Am J Gastroenterol* 2005; **100**: 616-623 [PMID: 15743360 DOI: 10.1111/j.1572-0241.2005.41289.x]

104 **Cross TJ**, Rizzi P, Berry PA, Bruce M, Portmann B, Harrison PM. King's Score: an accurate marker of cirrhosis in chronic hepatitis C. *Eur J Gastroenterol Hepatol* 2009; **21**: 730-738 [PMID: 19430302 DOI: 10.1097/MEG.0b013e32830dfcb]

105 **Lok AS**, Ghany MG, Goodman ZD, Wright EC, Everson GT, Sterling RK, Everhart JE, Lindsay KL, Bonkovsky HL, Di Bisceglie AM, Lee WM, Morgan TR, Dienstag JL, Morishima C. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. *Hepatology* 2005; **42**: 282-292 [PMID: 15986415 DOI: 10.1002/hep.20772]

106 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]

107 **Gan L**, Chitturi S, Farrell GC. Mechanisms and implications of age-related changes in the liver: nonalcoholic Fatty liver disease in the elderly. *Curr Gerontol Geriatr Res* 2011; **2011**: 831536 [PMID: 21918648 DOI: 10.1155/2011/831536]

108 **Angulo P**, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; **30**: 1356-1362 [PMID: 10573511 DOI: 10.1002/hep.510300604]

109 **Sorbi D**, Boynton J, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. *Am J Gastroenterol* 1999; **94**: 1018-1022 [PMID: 10201476 DOI: 10.1016/s0002-9270(99)00061-1]

110 **Qureshi K**, Clements RH, Abrams GA. The utility of the "NAFLD fibrosis score" in morbidly obese subjects with NAFLD. *Obes Surg* 2008; **18**: 264-270 [PMID: 18214632 DOI: 10.1007/s11695-007-9295-8]

111 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]

112 **Treeprasertsuk S**, Björnsson E, Enders F, Suwanwalaikorn S, Lindor KD. NAFLD fibrosis score: a prognostic predictor for mortality and liver complications among NAFLD patients. *World J Gastroenterol* 2013; **19**: 1219-1229 [PMID: 23482703 DOI: 10.3748/wjg.v19.i8.1219]

113 **Ruffillo G**, Fassio E, Alvarez E, Landeira G, Longo C, Domínguez N, Gualano G. Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in nonalcoholic fatty liver disease. *J Hepatol* 2011; **54**: 160-163 [PMID: 20934232 DOI: 10.1016/j.jhep.2010.06.028]

114 **Cichoż-Lach H**, Celiński K, Prozorow-Król B, Swatek J, Słomka M, Lach T. The BARD score and the NAFLD fibrosis score in the assessment of advanced liver fibrosis in nonalcoholic fatty liver disease. *Med Sci Monit* 2012; **18**: CR735-CR740 [PMID: 23197236 DOI: 10.12659/msm.883601]

115 **Angulo P**, Bugianesi E, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Barrera F, Haflidadottir S, Day CP, George J. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2013; **145**: 782-789.e4 [PMID: 23860502 DOI: 10.1053/j.gastro.2013.06.057]

116 **Kim D**, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. *Hepatology* 2013; **57**: 1357-1365 [PMID: 23175136 DOI: 10.1002/hep.26156]

117 **Yoneda M**, Fujii H, Sumida Y, Hyogo H, Itoh Y, Ono M, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, Imajo K, Chayama K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Yoshikawa T, Okanoue T. Platelet count for predicting fibrosis in nonalcoholic fatty liver disease. *J Gastroenterol* 2011; **46**: 1300-1306 [PMID: 21750883 DOI: 10.1007/s00535-011-0436-4]

118 **Yilmaz Y**, Yonal O, Kurt R, Bayrak M, Aktas B, Ozdogan O. Noninvasive assessment of liver fibrosis with the aspartate transaminase to platelet ratio index (APRI): Usefulness in patients with chronic liver disease: APRI in chronic liver disease. *Hepat Mon* 2011; **11**: 103-106 [PMID: 22087126]

119 **Agilli M**, Aydin FN, Cayci T, Kurt YG. Is aspartate aminotransferase-to-platelet ratio index a biomarker in the evaluation of advanced fibrosis in non-alcoholic fatty liver disease? *Gastroenterol Rep* (Oxf) 2014; **2**: 323-324 [PMID: 25371481 DOI: 10.1093/gastro/gou063]

120 **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**: 1265-1269 [PMID: 20801772 DOI: 10.1136/gut.2010.216077]

121 **McPherson S**, Anstee QM, Henderson E, Day CP, Burt AD. Are simple noninvasive scoring systems for fibrosis reliable in patients with NAFLD and normal ALT levels? *Eur J Gastroenterol Hepatol* 2013; **25**: 652-658 [PMID: 23325287 DOI: 10.1097/MEG.0b013e32835d72cf]

122 **Lee TH**, Han SH, Yang JD, Kim D, Ahmed M. Prediction of Advanced Fibrosis in Nonalcoholic Fatty Liver Disease: An Enhanced Model of BARD Score. *Gut Liver* 2013; **7**: 323-328 [PMID: 23710314 DOI: 10.5009/gnl.2013.7.3.323]

123 **Demir M**, Lang S, Schlattjan M, Drebber U, Wedemeyer I, Nierhoff D, Kaul I, Sowa J, Canbay A, Töx U, Steffen HM. NIKEI: a new inexpensive and non-invasive scoring system to exclude advanced fibrosis in patients with NAFLD. *PLoS One* 2013; **8**: e58360 [PMID: 23555578 DOI: 10.1371/journal.pone.0058360]

124 **Franzini M**, Fornaciari I, Fierabracci V, Elawadi HA, Bolognesi V, Maltinti S, Ricchiuti A, De Bortoli N, Marchi S, Pompella A, Passino C, Emdin M, Paolicchi A. Accuracy of b-GGT fraction for the diagnosis of non-alcoholic fatty liver disease. *Liver Int* 2012; **32**: 629-634 [PMID: 22098947 DOI: 10.1111/j.1478-3231.2011.02673.x]

125 **Tanwar S,** Trembling PM, Ellis E, Parkes L, Herold C, Schuppan D, Rosenberg WM. Direct non-invasive serum markers of liver fibrosis predict fibrosis evolution in chronic hepatitis C but are increased by interferon-based therapy. *Gut* 2012; **61**: A143-A144 [DOI: 10.1136/gutjnl-2012-302514b.172]

126 **Sakugawa H**, Nakayoshi T, Kobashigawa K, Yamashiro T, Maeshiro T, Miyagi S, Shiroma J, Toyama A, Nakayoshi T, Kinjo F, Saito A. Clinical usefulness of biochemical markers of liver fibrosis in patients with nonalcoholic fatty liver disease. *World J Gastroenterol* 2005; **11**: 255-259 [PMID: 15633226 DOI: 10.3748/wjg.v11.i2.255]

127 **Kaneda H**, Hashimoto E, Yatsuji S, Tokushige K, Shiratori K. Hyaluronic acid levels can predict severe fibrosis and platelet counts can predict cirrhosis in patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2006; **21**: 1459-1465 [PMID: 16911693 DOI: 10.1111/j.1440-1746.2006.04447.x]

128 **Adams LA**. Biomarkers of liver fibrosis. *J Gastroenterol Hepatol* 2011; **26**: 802-809 [PMID: 21198831 DOI: 10.1111/j.1440-1746.2010.06612.x]

129 **Adams LA**, George J, Bugianesi E, Rossi E, De Boer WB, van der Poorten D, Ching HL, Bulsara M, Jeffrey GP. Complex non-invasive fibrosis models are more accurate than simple models in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2011; **26**: 1536-1543 [PMID: 21950746 DOI: 10.1111/j.1440-1746.2011.06774.x]

130 **Wong L,** Roberts M, McCorry R. Identifying NAFLD in patients attending a lipid clinic – do non-invasive scoring systems for fibrosis have a role? *Gut* 2014; **63**: 89-90 [DOI: 10.1136/gutjnl-2014-307263.189]

131 **Neuschwander-Tetri BA**, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, Zein CO, Brunt EM, Kleiner DE, McCullough AJ, Sanyal AJ, Diehl AM, Lavine JE, Chalasani N, Kowdley KV. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 913-924 [PMID: 20648476 DOI: 10.1002/hep.23784]

132 **Naveau S**, Gaudé G, Asnacios A, Agostini H, Abella A, Barri-Ova N, Dauvois B, Prévot S, Ngo Y, Munteanu M, Balian A, Njiké-Nakseu M, Perlemuter G, Poynard T. Diagnostic and prognostic values of noninvasive biomarkers of fibrosis in patients with alcoholic liver disease. *Hepatology* 2009; **49**: 97-105 [PMID: 19053048 DOI: 10.1002/hep.22576]

133 **Schwimmer JB**, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, Shiehmorteza M, Yokoo T, Chavez A, Middleton MS, Sirlin CB. Heritability of nonalcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 1585-1592 [PMID: 19208353 DOI: 10.1053/j.gastro.2009.01.050]

134 **Tarnoki AD**, Tarnoki DL, Bata P, Littvay L, Osztovits J, Jermendy G, Karlinger K, Lannert A, Preda I, Kiss RG, Molnar AA, Garami Z, Baffy G, Berczi V. Heritability of non-alcoholic fatty liver disease and association with abnormal vascular parameters: a twin study. *Liver Int* 2012; **32**: 1287-1293 [PMID: 22651705 DOI: 10.1111/j.1478-3231.2012.02823.x]

135 **Anstee QM**, Daly AK, Day CP. Genetic modifiers of non-alcoholic fatty liver disease progression. *Biochim Biophys Acta* 2011; **1812**: 1557-1566 [PMID: 21840395 DOI: 10.1016/j.bbadis.2011.07.017]

136 **Dongiovanni P**, Anstee QM, Valenti L. Genetic predisposition in NAFLD and NASH: impact on severity of liver disease and response to treatment. *Curr Pharm Des* 2013; **19**: 5219-5238 [PMID: 23394097 DOI: 10.2174/13816128113199990381]

137 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]

138 **Yuan X**, Waterworth D, Perry JR, Lim N, Song K, Chambers JC, Zhang W, Vollenweider P, Stirnadel H, Johnson T, Bergmann S, Beckmann ND, Li Y, Ferrucci L, Melzer D, Hernandez D, Singleton A, Scott J, Elliott P, Waeber G, Cardon L, Frayling TM, Kooner JS, Mooser V. Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. *Am J Hum Genet* 2008; **83**: 520-528 [PMID: 18940312 DOI: 10.1016/j.ajhg.2008.09.012]

139 **He S**, McPhaul C, Li JZ, Garuti R, Kinch L, Grishin NV, Cohen JC, Hobbs HH. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J Biol Chem* 2010; **285**: 6706-6715 [PMID: 20034933 DOI: 10.1074/jbc.M109.064501]

140 **Hotta K**, Yoneda M, Hyogo H, Ochi H, Mizusawa S, Ueno T, Chayama K, Nakajima A, Nakao K, Sekine A. Association of the rs738409 polymorphism in PNPLA3 with liver damage and the development of nonalcoholic fatty liver disease. *BMC Med Genet* 2010; **11**: 172 [PMID: 21176169 DOI: 10.1186/1471-2350-11-172]

141 **Valenti L**, Alisi A, Galmozzi E, Bartuli A, Del Menico B, Alterio A, Dongiovanni P, Fargion S, Nobili V. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 1274-1280 [PMID: 20648474 DOI: 10.1002/hep.23823]

142 **Wagenknecht LE**, Palmer ND, Bowden DW, Rotter JI, Norris JM, Ziegler J, Chen YD, Haffner S, Scherzinger A, Langefeld CD. Association of PNPLA3 with non-alcoholic fatty liver disease in a minority cohort: the Insulin Resistance Atherosclerosis Family Study. *Liver Int* 2011; **31**: 412-416 [PMID: 21281435 DOI: 10.1111/j.1478-3231.2010.02444.x]

143 **Peng XE**, Wu YL, Lin SW, Lu QQ, Hu ZJ, Lin X. Genetic variants in PNPLA3 and risk of non-alcoholic fatty liver disease in a Han Chinese population. *PLoS One* 2012; **7**: e50256 [PMID: 23226254 DOI: 10.1371/journal.pone.0050256]

144 **Dongiovanni P**, Donati B, Fares R, Lombardi R, Mancina RM, Romeo S, Valenti L. PNPLA3 I148M polymorphism and progressive liver disease. *World J Gastroenterol* 2013; **19**: 6969-6978 [PMID: 24222941 DOI: 10.3748/wjg.v19.i41.6969]

145 **Sookoian S**, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 1883-1894 [PMID: 21381068 DOI: 10.1002/hep.24283]

146 **Singal AG**, Manjunath H, Yopp AC, Beg MS, Marrero JA, Gopal P, Waljee AK. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 325-334 [PMID: 24445574 DOI: 10.1038/ajg.2013.476]

147 **Sookoian S**, Pirola CJ. PNPLA3, the triacylglycerol synthesis/hydrolysis/storage dilemma, and nonalcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 6018-6026 [PMID: 23155331 DOI: 10.3748/wjg.v18.i42.6018]

148 **Huang Y**, He S, Li JZ, Seo YK, Osborne TF, Cohen JC, Hobbs HH. A feed-forward loop amplifies nutritional regulation of PNPLA3. *Proc Natl Acad Sci U S A* 2010; **107**: 7892-7897 [PMID: 20385813 DOI: 10.1073/pnas.1003585107]

149 **Pingitore P**, Pirazzi C, Mancina RM, Motta BM, Indiveri C, Pujia A, Montalcini T, Hedfalk K, Romeo S. Recombinant PNPLA3 protein shows triglyceride hydrolase activity and its I148M mutation results in loss of function. *Biochim Biophys Acta* 2014; **1841**: 574-580 [PMID: 24369119 DOI: 10.1016/j.bbalip.2013.12.006]

150 **Perttilä J**, Huaman-Samanez C, Caron S, Tanhuanpää K, Staels B, Yki-Järvinen H, Olkkonen VM. PNPLA3 is regulated by glucose in human hepatocytes, and its I148M mutant slows down triglyceride hydrolysis. *Am J Physiol Endocrinol Metab* 2012; **302**: E1063-E1069 [PMID: 22338072 DOI: 10.1152/ajpendo.00125.2011]

151 **Pirazzi C**, Adiels M, Burza MA, Mancina RM, Levin M, Ståhlman M, Taskinen MR, Orho-Melander M, Perman J, Pujia A, Andersson L, Maglio C, Montalcini T, Wiklund O, Borén J, Romeo S. Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M (rs738409) affects hepatic VLDL secretion in humans and in vitro. *J Hepatol* 2012; **57**: 1276-1282 [PMID: 22878467 DOI: 10.1016/j.jhep.2012.07.030]

152 **Basantani MK**, Sitnick MT, Cai L, Brenner DS, Gardner NP, Li JZ, Schoiswohl G, Yang K, Kumari M, Gross RW, Zechner R, Kershaw EE. Pnpla3/Adiponutrin deficiency in mice does not contribute to fatty liver disease or metabolic syndrome. *J Lipid Res* 2011; **52**: 318-329 [PMID: 21068004 DOI: 10.1194/jlr.M01120]

153 **Kumari M**, Schoiswohl G, Chitraju C, Paar M, Cornaciu I, Rangrez AY, Wongsiriroj N, Nagy HM, Ivanova PT, Scott SA, Knittelfelder O, Rechberger GN, Birner-Gruenberger R, Eder S, Brown HA, Haemmerle G, Oberer M, Lass A, Kershaw EE, Zimmermann R, Zechner R. Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase. *Cell Metab* 2012; **15**: 691-702 [PMID: 22560221 DOI: 10.1016/j.cmet.2012.04.008]

154 **Li JZ**, Huang Y, Karaman R, Ivanova PT, Brown HA, Roddy T, Castro-Perez J, Cohen JC, Hobbs HH. Chronic overexpression of PNPLA3I148M in mouse liver causes hepatic steatosis. *J Clin Invest* 2012; **122**: 4130-4144 [PMID: 23023705 DOI: 10.1172/JCI65179]

155 **Graff M**, North KE, Franceschini N, Reiner AP, Feitosa M, Carr JJ, Gordon-Larsen P, Wojczynski MK, Borecki IB. PNPLA3 gene-by-visceral adipose tissue volume interaction and the pathogenesis of fatty liver disease: the NHLBI family heart study. *Int J Obes* (Lond) 2013; **37**: 432-438 [PMID: 22546774 DOI: 10.1038/ijo.2012.65]

156 **Guichelaar MM**, Gawrieh S, Olivier M, Viker K, Krishnan A, Sanderson S, Malinchoc M, Watt KD, Swain JM, Sarr M, Charlton MR. Interactions of allelic variance of PNPLA3 with nongenetic factors in predicting nonalcoholic steatohepatitis and nonhepatic complications of severe obesity. *Obesity* (Silver Spring) 2013; **21**: 1935-1941 [PMID: 23418085 DOI: 10.1002/oby.20327]

157 **Kantartzis K**, Peter A, Machicao F, Machann J, Wagner S, Königsrainer I, Königsrainer A, Schick F, Fritsche A, Häring HU, Stefan N. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. *Diabetes* 2009; **58**: 2616-2623 [PMID: 19651814 DOI: 10.2337/db09-0279]

158 **Speliotes EK**, Butler JL, Palmer CD, Voight BF, Hirschhorn JN. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology* 2010; **52**: 904-912 [PMID: 20648472 DOI: 10.1002/hep.23768]

159 **Goran MI**, Walker R, Le KA, Mahurkar S, Vikman S, Davis JN, Spruijt-Metz D, Weigensberg MJ, Allayee H. Effects of PNPLA3 on liver fat and metabolic profile in Hispanic children and adolescents. *Diabetes* 2010; **59**: 3127-3130 [PMID: 20852027 DOI: 10.2337/db10-0554]

160 **Zain SM**, Mohamed R, Mahadeva S, Cheah PL, Rampal S, Basu RC, Mohamed Z. A multi-ethnic study of a PNPLA3 gene variant and its association with disease severity in non-alcoholic fatty liver disease. *Hum Genet* 2012; **131**: 1145-1152 [PMID: 22258181 DOI: 10.1007/s00439-012-1141-y]

161 **Palmer CN**, Maglio C, Pirazzi C, Burza MA, Adiels M, Burch L, Donnelly LA, Colhoun H, Doney AS, Dillon JF, Pearson ER, McCarthy M, Hattersley AT, Frayling T, Morris AD, Peltonen M, Svensson PA, Jacobson P, Borén J, Sjöström L, Carlsson LM, Romeo S. Paradoxical lower serum triglyceride levels and higher type 2 diabetes mellitus susceptibility in obese individuals with the PNPLA3 148M variant. *PLoS One* 2012; **7**: e39362 [PMID: 22724004 DOI: 10.1371/journal.pone.0039362]

162 **Krarup NT**, Grarup N, Banasik K, Friedrichsen M, Færch K, Sandholt CH, Jørgensen T, Poulsen P, Witte DR, Vaag A, Sørensen T, Pedersen O, Hansen T. The PNPLA3 rs738409 G-allele associates with reduced fasting serum triglyceride and serum cholesterol in Danes with impaired glucose regulation. *PLoS One* 2012; **7**: e40376 [PMID: 22792295 DOI: 10.1371/journal.pone.0040376]

163 **Stojkovic IA**, Ericson U, Rukh G, Riddestråle M, Romeo S, Orho-Melander M. The PNPLA3 Ile148Met interacts with overweight and dietary intakes on fasting triglyceride levels. *Genes Nutr* 2014; **9**: 388 [PMID: 24563329 DOI: 10.1007/s12263-014-0388-4]

164 **Tomita K**, Teratani T, Suzuki T, Shimizu M, Sato H, Narimatsu K, Okada Y, Kurihara C, Irie R, Yokoyama H, Shimamura K, Usui S, Ebinuma H, Saito H, Watanabe C, Komoto S, Kawaguchi A, Nagao S, Sugiyama K, Hokari R, Kanai T, Miura S, Hibi T. Free cholesterol accumulation in hepatic stellate cells: mechanism of liver fibrosis aggravation in nonalcoholic steatohepatitis in mice. *Hepatology* 2014; **59**: 154-169 [PMID: 23832448 DOI: 10.1002/hep.26604]

165 **Hyysalo J**, Männistö VT, Zhou Y, Arola J, Kärjä V, Leivonen M, Juuti A, Jaser N, Lallukka S, Käkelä P, Venesmaa S, Simonen M, Saltevo J, Moilanen L, Korpi-Hyövalti E, Keinänen-Kiukaanniemi S, Oksa H, Orho-Melander M, Valenti L, Fargion S, Pihlajamäki J, Peltonen M, Yki-Järvinen H. A population-based study on the prevalence of NASH using scores validated against liver histology. *J Hepatol* 2014; **60**: 839-846 [PMID: 24333862 DOI: 10.1016/j.jhep.2013.12.009]

166 **Marzuillo P**, Miraglia del Giudice E, Santoro N. Pediatric fatty liver disease: role of ethnicity and genetics. *World J Gastroenterol* 2014; **20**: 7347-7355 [PMID: 24966605 DOI: 10.3748/wjg.v20.i23.7347./j.jhep.2013.12.009]

167 **Valenti L**, Dongiovanni P, Piperno A, Fracanzani AL, Maggioni M, Rametta R, Loria P, Casiraghi MA, Suigo E, Ceriani R, Remondini E, Trombini P, Fargion S. Alpha 1-antitrypsin mutations in NAFLD: high prevalence and association with altered iron metabolism but not with liver damage. *Hepatology* 2006; **44**: 857-864 [PMID: 17006922 DOI: 10.1002/hep.21329]

168 **Valenti L**, Canavesi E, Galmozzi E, Dongiovanni P, Rametta R, Maggioni P, Maggioni M, Fracanzani AL, Fargion S. Beta-globin mutations are associated with parenchymal siderosis and fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol* 2010; **53**: 927-933 [PMID: 20739079 DOI: 10.1016/j.jhep.2010.05.023]

169 **Bonkovsky HL**, Jawaid Q, Tortorelli K, LeClair P, Cobb J, Lambrecht RW, Banner BF. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. *J Hepatol* 1999; **31**: 421-429 [PMID: 10488699 DOI: 10.1016/s0168-8278(99)80032-4]

170 **Nelson JE**, Bhattacharya R, Lindor KD, Chalasani N, Raaka S, Heathcote EJ, Miskovsky E, Shaffer E, Rulyak SJ, Kowdley KV. HFE C282Y mutations are associated with advanced hepatic fibrosis in Caucasians with nonalcoholic steatohepatitis. *Hepatology* 2007; **46**: 723-729 [PMID: 17680648 DOI: 10.1002/hep.21742]

171 **Valenti L**, Dongiovanni P, Fracanzani AL, Fargion S. HFE mutations in nonalcoholic fatty liver disease. *Hepatology* 2008; **47**: 1794-175; author reply 1794-175; [PMID: 18438784 DOI: 10.1002/hep.22059]

172 **Nelson JE**, Klintworth H, Kowdley KV. Iron metabolism in Nonalcoholic Fatty Liver Disease. *Curr Gastroenterol Rep* 2012; **14**: 8-16 [PMID: 22124850 DOI: 10.1007/s11894-011-0234-4]

173 **Chitturi S**, Weltman M, Farrell GC, McDonald D, Kench J, Liddle C, Samarasinghe D, Lin R, Abeygunasekera S, George J. HFE mutations, hepatic iron, and fibrosis: ethnic-specific association of NASH with C282Y but not with fibrotic severity. *Hepatology* 2002; **36**: 142-149 [PMID: 12085358 DOI: 10.1053/jhep.2002.33892]

174 **Bugianesi E**, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, Massarenti P, Piga A, Marchesini G, Rizzetto M. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004; **39**: 179-187 [PMID: 14752836 DOI: 10.1002/hep.20023]

175 **Hernaez R**, Yeung E, Clark JM, Kowdley KV, Brancati FL, Kao WH. Hemochromatosis gene and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 2011; **55**: 1079-1085 [PMID: 21354231 DOI: 10.1016/j.jhep.2011.02.013]

176 **Valenti L**, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, Canavesi E, Lattuada E, Roviaro G, Marchesini G, Fargion S. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2010; **138**: 905-912 [PMID: 19931264 DOI: 10.1053/j.gastro.2009.11.013]

177 **Ganz T**, Nemeth E. The hepcidin-ferroportin system as a therapeutic target in anemias and iron overload disorders. *Hematology Am Soc Hematol Educ Program* 2011; **2011**: 538-542 [PMID: 22160086 DOI: 10.1182/asheducation-2011.1.538]

178 **Nai A**, Pagani A, Silvestri L, Campostrini N, Corbella M, Girelli D, Traglia M, Toniolo D, Camaschella C. TMPRSS6 rs855791 modulates hepcidin transcription in vitro and serum hepcidin levels in normal individuals. *Blood* 2011; **118**: 4459-4462 [PMID: 21873547 DOI: 10.1182/blood-2011-06-364034]

179 **Traglia M**, Girelli D, Biino G, Campostrini N, Corbella M, Sala C, Masciullo C, Viganò F, Buetti I, Pistis G, Cocca M, Camaschella C, Toniolo D. Association of HFE and TMPRSS6 genetic variants with iron and erythrocyte parameters is only in part dependent on serum hepcidin concentrations. *J Med Genet* 2011; **48**: 629-634 [PMID: 21785125 DOI: 10.1136/jmedgenet-2011-100061]

180 **Galesloot TE**, Geurts-Moespot AJ, den Heijer M, Sweep FC, Fleming RE, Kiemeney LA, Vermeulen SH, Swinkels DW. Associations of common variants in HFE and TMPRSS6 with iron parameters are independent of serum hepcidin in a general population: a replication study. *J Med Genet* 2013; **50**: 593-598 [PMID: 23794717 DOI: 10.1136/jmedgenet-2013-101673]

181 **Gan W**, Guan Y, Wu Q, An P, Zhu J, Lu L, Jing L, Yu Y, Ruan S, Xie D, Makrides M, Gibson RA, Anderson GJ, Li H, Lin X, Wang F. Association of TMPRSS6 polymorphisms with ferritin, hemoglobin, and type 2 diabetes risk in a Chinese Han population. *Am J Clin Nutr* 2012; **95**: 626-632 [PMID: 22301935 DOI: 10.3945/ajcn.111.025684]

182 **Valenti L**, Rametta R, Dongiovanni P, Motta BM, Canavesi E, Pelusi S, Pulixi EA, Fracanzani AL, Fargion S. The A736V TMPRSS6 polymorphism influences hepatic iron overload in nonalcoholic fatty liver disease. *PLoS One* 2012; **7**: e48804 [PMID: 23144979 DOI: 10.1371/journal.pone.0048804]

183 **Yoneda M**, Yoneda M, Fujita K, Inamori M, Tamano M, Hiriishi H, Nakajima A. Transient elastography in patients with non-alcoholic fatty liver disease (NAFLD). *Gut* 2007; **56**: 1330-1331 [PMID: 17470477 DOI: 10.1136/gut.2007.126417]

184 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077 DOI: 10.1053/j.gastro.2008.01.034]

185 **Mahadeva S**, Mahfudz AS, Vijayanathan A, Goh KL, Kulenthran A, Cheah PL. Performance of transient elastography (TE) and factors associated with discordance in non-alcoholic fatty liver disease. *J Dig Dis* 2013; **14**: 604-610 [PMID: 23859493 DOI: 10.1111/1751-2980.12088]

186 **Suzuki K**, Yoneda M, Imajo K, Kirikoshi H, Nakajima A, Maeda S, Saito S. Transient elastography for monitoring the fibrosis of non-alcoholic fatty liver disease for 4 years. *Hepatol Res* 2013; **43**: 979-983 [PMID: 23294411 DOI: 10.1111/hepr.12039]

187 **Yoshioka K**, Hashimoto S, Kawabe N. Measurement of liver stiffness as a non-invasive method for diagnosis of non-alcoholic fatty liver disease. *Hepatol Res* 2015; **45**: 142-151 [PMID: 25040931 DOI: 10.1111/hepr.12388]

188 **Burlone ME,** Rossini A, Momo E, Colletta C, Leutner M, Minisini R, Pirisi M. A composite score including BMI liver stiffness and rs738409 PNPLA3 genotype might spare liver biopsies to most NAFLD patients maintaining 95% diagnostic accuracy. [updated 2012 Nov 12]. Available from: URL: http://www.aspe.vb.it/it/downloads/Studio%20NASH.pdf

189 **Petta S,** Vanni E, Bugianesi E, Di Marco V, Cammà C, Cabibi D, Mezzabotta L, Craxì A. The combination of liver stiffness measurement and NAFLD fibrosis score improves the noninvasive diagnostic accuracy for severe liver fibrosis in patients with nonalcoholic fatty liver disease. *Liver Int* 2014 May 2; Epub ahead of print [PMID: 24798049 DOI: 10.1111/liv.12584]

190 **Sumida Y**, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2014; **20**: 475-485 [PMID: 24574716 DOI: 10.3748/wjg.v20.i2.475]

191 **Kotze MJ,** Lückhoff HK, Peeters AV, Myburgh EJ, Schoemann M, van der Merwe L, Grant K, Fisher L, van der Merwe N, Pretorius J, van Velden D, Pienaar R, van Rensburgh SJ, Yandiswa Y, September A, Moremi K, Tiffin N, Bouwens CB, Bezuidenhout J, Apffelstaedt J, Hough F, Erasmus R, Schneider J. Genomic medicine and risk prediction across the disease spectrum. *Crit Rev Clin Lab Sci* 2014; In press

192 **Lim JW**, Dillon J, Miller M. Proteomic and genomic studies of non-alcoholic fatty liver disease--clues in the pathogenesis. *World J Gastroenterol* 2014; **20**: 8325-8340 [PMID: 25024592 DOI: 10.3748/wjg.v20.i26.8325]

193 **Gerhard GS**, Chu X, Wood GC, Gerhard GM, Benotti P, Petrick AT, Gabrielsen J, Strodel WE, Still CD, Argyropoulos G. Next-generation sequence analysis of genes associated with obesity and nonalcoholic fatty liver disease-related cirrhosis in extreme obesity. *Hum Hered* 2013; **75**: 144-151 [PMID: 24081230 DOI: 10.1159/000351719]

**P-Reviewer:** Grassi A, Morales-Gonzalez JA **S-Editor:** Tian YL

**L-Editor: E-Editor:**

**Table 1 Peripheral biomarkers used for the assessment of necro-inflammation and fibrotic injury in non-alcoholic fatty liver disease**

| **Pathogenic mechanism** | **Biomarker** | | **NASH** | **Advanced**  **fibrosis** | **Ref.** |
| --- | --- | --- | --- | --- | --- |
| Hepatocyte apoptosis | Apoptotic markers | CK-18 ↑  sFas ↑ | X  X |  | El Bassat *et al*[11] |
| Oxidative stress | Lipid peroxidation products  Indicators of altered redox potential | ox-LDL ↑  TBARS ↑  MDA ↑  HNE ↑  GSH ↑  SOD ↓  TRX ↑ | X  X  X  X  X  X  X |  | Kawanaka *et al*[12], Machado *et al*[13] |
| Chronic inflammation | Acute phase reactants  Adipocytokines | CRP  Ferritin  PTX3  leptin  adiponectin  TNF-α  IL-1β  IL-6  TGF-α/β1 | X  X  X  X  X  X  X | X  X | Jarrar *et al*[14], Braunersreuther *et al*[15] |
| Increased hepatocellular turnover | Extracellular matrix components | HA  laminin  type IV collagen 7S  PIIINP  MMP-2/9  TIMP-1 |  | X  X  X  X  X  X  X | Baranova *et al*[16] |

CK-18: Cytokeratin-18; sFAS: Soluble Fas; Ox-LDL: Oxidized low-density lipoprotein cholesterol; TBARS: Thiobarbituric acid reactive substances; MDA: Malondialdehyde; HNE: Hydroxynonenal; GSH: Reduced glutathione; SOD: Superoxide dismutase; TRX: Thioredoxin; CRP: C-reactive protein; PTX3: Pentraxin 3.

**Table 2 Composite diagnostic models for non-alcoholic steatohepatitis**

| **Biomarker panel** | **Components** | **Formula** | **AUC** | **Ref.** |
| --- | --- | --- | --- | --- |
| HAIR score | Hypertension  Elevated ALT (> 40IU/L)  Insulin Resistance (index> 5) | Weighted sum (0-4) | 0.90 | Dixon *et al*[52] |
| Gholam’s model | DM II  AST level | Algorithm | 0.90 | Gholam *et al*[53] |
| NASH clinical scoring system for morbid obesity | Hypertension  DM II  Elevated AST (> 27 IU/L)  Elevated ALT (> 27 IU/L)  OSA  non-black race | Weighted sum (0-7) | Not reported | Campos *et al*[54] |
| Ulitsky’s score | DM II  Elevated ALT(> 40 IU/L)  Elevated triglycerides (> 150 mg/dL)  OSA | Weighted sum (0-5) | Not reported | Ulitsky *et al*[55] |
| NASH predictive index | Female sex  Body Mass Index  HOMA-IR  AST levels  ALT levels | Algorithm | 0.780 | Zein *et al*[56] |

AUC: Area under curve; ALT: Alanine aminotransferase; DM II: Diabetes mellitus type II; AST: Aspartate aminotransferase; OSA: Obstructive sleep apnoea; HOMA-IR: Homeostatic Measurement Assessment of Insulin Resistance.

**Table 3 Composite predictive models for advanced hepatic fibrosis in chronic liver disease**

| **Biomarker panel** | **Components** | **Formula** | **Validated in adult NAFLD** | **Ref.** |
| --- | --- | --- | --- | --- |
| **APRI index** | AST, platelet count | Ratio | ✓ | Kruger *et al*[96] |
| **BAAT score** | Age, BMI, ALT, triglycerides | Weighted sum | ✓ | Ratziu *et al*[97] |
| **BARD score** | BMI, AST, ALT, DM II | Weighted sum | ✓ | Harrison *et al*[98] |
| **Cirrhosis discrimination score** | AST, ALT, platelet count, INR | Weighted sum | 🗶 | Bonacini *et al*[99] |
| **FIB-4 index** | Age, AST, ALT, platelet count | Algorithm | ✓ | Shah *et al*[100] |
| **Fibrosis probability index** | Age, AST, previous alcohol use, HOMA-IR, cholesterol | Algorithm | 🗶 | Sud *et al*[101] |
| **Forns index** | Age, platelet count, GGT, cholesterol | Algorithm | ✓ | Forns *et al*[102] |
| **Gholam’s score** | ALT, HbA1C | Weighted score | ✓ | Gholam *et al*[53] |
| **Hui model** | BMI, bilirubin, albumin, platelet count | Algorithm | 🗶 | Hui *et al*[103] |
| **King score** | Age, AST, platelet count, INR | Algorithm | 🗶 | Cross *et al*[104] |
| **Lok index** | AST, ALT, platelet count, INR | Algorithm | 🗶 | Lok *et al*[105] |
| **NAFLD fibrosis score** | Age, BMI, platelet count, albumin, AST/ALT ratio, IFG/DM II | Algorithm | ✓ | Angulo *et al*[106] |

NAFLD: Non-alcoholic fatty liver disease; APRI: Aspartate aminotransferase to platelet ratio index; AST: Aspartate aminotransferase; BMI: Body mass index; ALT: Alanine aminotransferase; DM II: Diabetes mellitus type II; HOMA-IR: Homeostatic Measurement Assessment of Insulin Resistance; IFG: Impaired fasting glucose.