

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

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

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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.

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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, approximately 20%-30% of patients will develop non-alcoholic steatohepatitis (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 the 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 aminotransferase (ALT) and aspartate aminotransferase (AST) levels are commonly elevated in

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 ↑	X		El Bassat <i>et al</i> ^[11]	
		sFas ↑	X			
Oxidative stress	Lipid peroxidation products	Ox-LDL ↑	X		Kawanaka <i>et al</i> ^[12]	
		TBARS ↑	X			
		MDA ↑	X		Machado <i>et al</i> ^[13]	
		HNE ↑	X			
		Indicators of altered redox potential	GSH ↑	X		
			SOD ↓	X		
Chronic inflammation	Acute phase reactants	TRX ↑	X		Jarrar <i>et al</i> ^[14]	
		CRP	X			
		Ferritin	X			
	Adipocytokines	PTX3	X		Braunersreuther <i>et al</i> ^[15]	
		Leptin	X			
		Adiponectin		X		
		TNF- α	X			
		IL-1 β	X			
		IL-6		X		
		TGF- α / β 1	X			
Increased hepatocellular turnover	Extracellular matrix components	HA		X	Baranova <i>et al</i> ^[16]	
		Laminin		X		
		Type IV collagen 7S		X		
		P III NP		X		
		MMP-2/9		X		
		TIMP-1		X		

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; TNF- α : Tumor necrosis factor α ; IL: Interleukin; TGF: Transforming growth factor; HA: Hyaluronic acid; NASH: Non-alcoholic steatohepatitis; MMP: Matrix metalloproteinase; P III NP; Procollagen 3 n-terminal propeptide; TIMP: Tissue inhibitor of metalloproteinase.

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 clear that no single biochemical test is considered ideal for confirming a suspected diagnosis of NAFLD.

Imaging modalities such as ultrasonography 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 commonly used in epidemiological studies, which along with its sex-specific derivative, the lipid accumulation product, 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

Table 2 Composite diagnostic models for non-alcoholic steatohepatitis

Biomarker panel	Components	Formula	AUC	Ref.
HAIR score	Hypertension Elevated ALT (> 40 IU/L) Insulin Resistance (index > 5)	Weighted sum (0-4)	0.9	Dixon <i>et al</i> ^[52]
Gholam's model	DM II AST level	Algorithm	0.9	Gholam <i>et al</i> ^[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 <i>et al</i> ^[54]
Ulitsky's score	DM II Elevated ALT (> 40 IU/L) Elevated triglycerides (> 150 mg/dL) OSA	Weighted sum (0-5)	Not reported	Ulitsky <i>et al</i> ^[55]
NASH predictive index	Female sex Body mass index HOMA-IR AST levels ALT levels	Algorithm	0.78	Zein <i>et al</i> ^[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; NASH: Non-alcoholic steatohepatitis.

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 thiazolidinedione 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 approximately 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 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 new-onset CVD in addition to cardiac mortality^[64].

Obstructive sleep apnoea

The association between obstructive sleep apnoea 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 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 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 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 NAFLC 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 constitutes a clinico-biochemical profile consistent with the “anaemia of chronic disease”^[88,89] and compatible with a diagnosis of hepatosteatosis and/or the dys-metabolic 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 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 mutation screening of the hemochromatosis (*HFE*) gene to confirm a suspected diagnosis of 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 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 HH. In this context, the routine implementation of a similar validated diagnostic tool could assist clinicians

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 <i>et al</i> ^[96]
BAAT score	Age, BMI, ALT, triglycerides	Weighted sum	√	Ratziu <i>et al</i> ^[97]
BARD score	BMI, AST, ALT, DM II	Weighted sum	√	Harrison <i>et al</i> ^[98]
Cirrhosis discrimination score	AST, ALT, platelet count, INR	Weighted sum	×	Udell <i>et al</i> ^[99]
FIB-4 index	Age, AST, ALT, platelet count	Algorithm	√	Shah <i>et al</i> ^[100]
Fibrosis probability index	Age, AST, previous alcohol use, HOMA-IR, cholesterol	Algorithm	×	Sud <i>et al</i> ^[101]
Forns index	Age, platelet count, GGT, cholesterol	Algorithm	√	Forns <i>et al</i> ^[102]
Gholam's score	ALT, HbA1C	Weighted score	√	Gholam <i>et al</i> ^[53]
Hui model	BMI, bilirubin, albumin, platelet count	Algorithm	×	Hui <i>et al</i> ^[103]
King score	Age, AST, platelet count, INR	Algorithm	×	Cross <i>et al</i> ^[104]
Lok index	AST, ALT, platelet count, INR	Algorithm	×	Lok <i>et al</i> ^[105]
NAFLD fibrosis score	Age, BMI, platelet count, albumin, AAR, IFG/DM II	Algorithm	√	Angulo <i>et al</i> ^[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; GGT: Gamma glutamyl-transferase; INR: International normalized ratio; AAR: AST/ALT ratio.

in stratifying obese patients into meaningful subgroups based on the need for extended follow-up evaluation. This could confirm a suspected diagnosis of hepatosteatosis and/or DIOS, in addition to identifying a subgroup of patients 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 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 for reliable prediction of overall and liver-related mortality^[92]. In addition to its prognostic importance, confirmation of advanced hepatic fibrosis 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 measurements, decreased coronary blood flow 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, which causes decreased clearance of AST from the sinusoidal space resulting in gradual reversal of the AAR^[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 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^[115,116].

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 the resource-limited South African setting, comparing favourably to the NFS with superior accuracy to the AAR^[96]. 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 European Liver Fibrosis (ELF) score 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 models for the non-invasive assessment of the extent and severity of fibrotic injury should ideally reflect a dimensional pathogenic spectrum ranging from ECM deposition through 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 the predictive accuracy of these models 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 that may 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 in the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene (rs738409 C > G; I148M), 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 *PNPLA3* rs738409 SNP 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 of macrovesicular hepatosteatosis due to increased hepatic lipid accumulation and/or export associated with impaired lipidation of very low-density lipoprotein particles^[150,151]. *PNPLA3* knockout *in vivo* does not however lead to increased intra-hepatic TAG accumulation^[152] with increased lysophosphatidic acid acyltransferase activity and TAG synthesis noted for G-allele carriers, but rather supports a gain-of-function mechanism 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]. Its pathogenic role in impaired lipid homeostasis as evidenced by a peripheral decrease in serum triglyceride, total and high-density lipoprotein cholesterol^[159,160] is furthermore 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 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* rs738409 genotype along with fasting insulin and ALT levels has been shown to reliably predict the presence of undiagnosed NASH^[165]. The observation that the genotype distribution 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]. This emphasizes the need for

further research aimed at clarifying the role of ethnicity as a putative modulator of genetic risk conferred by PNPLA3 rs738409. Evidence of gene-diet interactions that may be relevant to a specific population supports the need for development of lifestyle-based intervention strategies aimed at decreasing cumulative risk for development of NAFLD and progression to NASH^[166].

GWAS have identified a number of other genetic variants as potential risk modifiers for progressive necroinflammatory injury and advanced hepatic fibrosis in NAFLD. For example, the PiS/PiZ variant of the α -1 antitrypsin (AAT) gene implicated in endoplasmic reticulum 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 HH as non-deterministic modifiers of susceptibility towards the onset and pathogenic progression of NAFLD remains incompletely elucidated. In an Italian study, Valenti *et al.*^[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, thereby 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 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 HH, only approximately 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 recognition 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 genetic subtype of iron-deficiency anaemia. It remains unclear however to what extent the association between *TMPRSS6* rs855791 and iron deficiency 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 *TMPRSS6* rs855791 in the etiology 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 to replicate its apparent dissociation from susceptibility towards DIOS considered in the context of known environmental and epistatic modulators of phenotypic expression in different 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 or 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 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 incorporating genomics could allow for the timely implementation of tailored therapeutic intervention, aimed at preventing disease onset 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 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 is 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. In resource-limited settings the establishment and ongoing development of a database resource for health outcome studies may provide a viable alternative to prospective clinical trials for validation of eligibility criteria and treatment algorithms guided partly from the genetic background.

Given the decreasing cost and growing availability of genetic testing in the clinical domain, the routine use of personalized genomic risk screening in developing nations 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 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 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. Towards this goal, a pathology-supported genetic testing approach could prove useful in developing

standardized pre-screen diagnostic algorithms to select non-responders to standard therapies and genetically uncharacterized NAFLD patients set to derive additional benefit from extended whole genome or exome sequencing^[191,192]. Next-generation sequencing could be used to validate common susceptibility variants implicated in the etiopathogenesis of NAFLD, thereby supporting the development and validation of a genomics-based screening panel to provide greater insight into the value of personalized medicine applications^[193,194]. In this context, a transdisciplinary “omics” approach has been proposed as a means of developing novel prognostic signatures incorporating relevant biochemical and genomic markers to optimize clinical diagnosis and improve risk management in patients with NAFLD^[195]. Extension of genomics-based risk profiling beyond the limited scope of single-gene testing assisted by next-generation sequencing has the potential to facilitate the detection of both known and novel genetic variations aimed at the prevention of cumulative cardio-metabolic risk across the NAFLD spectrum.

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