World Journal of Gastroenterology

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DOI: 10.3748/wjg.v21.i11.3223

World J Gastroenterol 2015 March 21; 21(11): 3223-3231
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Biomarkers in nonalcoholic fatty liver disease-the emperor has no clothes?

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Author contributions: Sanal MG conceived the issues which formed the content of the manuscript and wrote the manuscript.
Supported by IIP fellowship (2013-2014), Albert Einstein College of Medicine, New York, through the generosity of the Gruss Lipper Family Foundation.
Conflict-of-interest: The author does not have any conflict of interest associated with this manuscript.

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Received: November 22, 2014
Peer-review started: November 23, 2014
First decision: December 26, 2014
Revised: January 16, 2015
Accepted: February 11, 2015
Article in press: February 11, 2015
Published online: March 21, 2015

Abstract

Fatty liver is present in over ten percentage of the world population and it is a growing public health problem. Nonalcoholic fatty liver disease (NAFLD) is not a single disease, but encompasses a spectrum of diseases of different etiologies. It is difficult to find highly specific and sensitive diagnostic biomarkers when a disease is very complex. Therefore, we should aim to find relevant prognostic markers rather than accurate diagnostic markers which will help to minimize the frequency of liver biopsies to evaluate disease progression. There are several biomarker panels commercially available, however, there is no clear evidence that more sophisticated panels are better compared to simple criteria such as, presence of diabetes over five years, metabolic syndrome, obesity, obstructive sleep apnea, aspartate transaminase/alanine transaminase (ALT) ratio > 0.8 or ferritin levels > 1.5 times normal in patients with over six month history of raised ALT and/or ultrasonological evidence of fat in the liver. Currently the biomarker panels are not a replacement for a liver biopsy. However the need and benefit of liver biopsy in NAFLD is questionable because there is no convincing evidence that biopsy and detailed staging of NAFLD improves the management of NAFLD and benefits the patient. After all there is no evidence based treatment for NAFLD other than management of lifestyle and components of "metabolic syndrome".

Key words: Nonalcoholic fatty liver disease; Biomarkers; Fibrosis; Cirrhosis; Steatohepatitis; Liver biopsy

Core tip: Nonalcoholic fatty liver disease (NAFLD) is not a single disease, but encompasses a spectrum of diseases and this makes it very difficult to find highly specific and sensitive biomarkers. We should therefore aim to find relevant prognostic markers rather than accurate diagnostic markers which will help to minimize the frequency of liver biopsies to evaluate disease progression. There is no evidence that biopsy and detailed staging of NAFLD is important in the NAFLD management and benefits patients. Finally, there is no evidence based treatment for NAFLD other than management of 'metabolic syndrome' by pharmacological or non-pharmacological (lifestyle management/surgical) approaches.

Sanal MG. Biomarkers in nonalcoholic fatty liver disease-the emperor has no clothes? *World J Gastroenterol* 2015; 21(11): 3223-3231 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i11/3223.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i11.3223

INTRODUCTION

Fatty liver can be a sign of an underlying disorder but by itself it is not a disease. Nonalcoholic fatty liver disease (NAFLD) is not a single disease but encompasses a spectrum of diseases. No wonder that efforts to find a highly specific and sensitive biomarker for NAFLD have not become successful. About a quarter of fatty livers develop liver inflammation [nonalcoholic steatohepatitis (NASH)] and over a quarter of NASH patients develop severe fibrosis. We need biomarkers for the excess fat in liver, inflammation and fibrosis of liver. It is less likely that we could find liver specific proteins/molecules which can be used in commercial settings for identifying fat in liver. While there are several markers for inflammation, but it is difficult to find markers which are liver specific but superior to classic liver enzymes such as alanine transaminase (ALT). Similarly, it is difficult to find biomolecules which are specific for fibrosis of liver. We should therefore aim to find relevant prognostic markers rather than accurate diagnostic markers which will help to minimize the frequency of liver biopsies to evaluate disease progression. Despite several years of research, there is no clear evidence in the literature that any of the sophisticated algorithms or proprietary biomarker panels are good enough to avoid a liver biopsy compared to simple criteria such as, presence of diabetes over five years, metabolic syndrome, obesity, obstructive sleep apnea, aspartate transaminase (AST)/ALT ratio > 0.8 or ferritin levels > 1.5 times normal in patients with over six months history of raised ALT and/or ultrasonological evidence of fat in liver. Therefore, "more" is not necessarily 'the better' when it comes to the number of biomarkers, accuracy of diagnosis and staging of NAFLD. Moreover, the performance of biomarkers depends on the etiology of NAFLD and the stage of the disease and compromising their reliability. After all there is no evidence based treatment for NAFLD other than management of lifestyle and components of "metabolic syndrome". There is no convincing evidence that biopsy and detailed staging of NAFLD improves the management of NAFLD and benefits the patients. Appropriate combination of lifestyle adjustments, pharmacological and non-pharmacological (such as bariatric surgery) intervention to improve the underlying cause of NAFLD such as diabetes should be undertaken in all cases of NAFLD with diabetes over five years, metabolic syndrome, obesity, obstructive sleep apnea, AST/ALT ratio > 0.8 or ferritin levels > 1.5 times normal in patients with over six months history of raised ALT and/or ultrasonological evidence of fat in liver.

It is important to detect the development of inflammation in fatty liver because greater than a quarter of these patients develop fibrosis which is associated with a high mortality rate. Detection of inflammation requires microscopic examination of liver biopsy specimens. The diagnosis of nonalcoholic steatohepatitis (inflamed fatty liver) is therefore histological[1-3]. However, liver biopsy is an invasive procedure which involves some serious patient risk and suffers from sampling errors[3]. In association with liver biopsy, various studies have reported mortality as high as 2% in the literature[4]. Though liver biopsy is recommended for therapeutic decisions, clinical practice guidelines for NAFLD have been modified therefore to include noninvasive tests for diagnosis of NASH. The European Association for the Study of the Liver had a special topic conference in NAFLD which showed a renewed interest on noninvasive biomarkers[5]. The prospect of imaging techniques [such as real-time elastography, acoustic radiation force impulse elastography, magnetic resonance spectroscopy and certain magnetic resonance imaging (MRI) based techniques] are currently more promising when compared to the prospect of biomarkers in the evaluation of fibrosis. Many of the non-invasive diagnosis techniques now employed for NAFLD were actually developed for managing chronic hepatitis C. The most important criteria to be evaluated in hepatitis C virus (HCV) and NAFLD are inflammation and progression of fibrosis, the two most important turning points in the course of fatty liver disease progression.

While there are several markers for inflammation only liver enzymes are specific to liver and even few are sufficiently sensitive enough to be a serum biomarker for clinical use. For example cytokeratin-18 (CK-18) is a relatively useful marker to differentiate non-alcoholic steatohepatitis (NASH) from fatty liver without inflammation. However its plasma levels are altered in several inflammatory conditions involving apoptotic response such as chronic viral hepatitis, chronic lung and renal diseases. Therefore, CK-18 is not definitive enough for routine diagnostic use as a marker for staging NASH[6].

This review will focus on the limitations of biomarkers and diagnostic panels presently available in the diagnosis and management of NAFLD. Although tremendous advances are presently being made in non-invasive imaging methods and other non-biomarker based methods inclusive of ultrasound based methods such as transient ultrasound elastography, Doppler analysis, acoustic radiation force impulse (ARFI), real-time elastography, tissue strain imaging, supersonic shear imaging, magnetic resonance based techniques such as MRI, diffusion-weighted MRI, magnetic resonance spectroscopy, X-ray based imaging techniques such as computed tomography (CT) and radioisotope

based imaging techniques such as positron emission tomography and single photon emission computed tomography (SPECT), however they are beyond the scope of this review.

There exists a plethora of panels and scoring systems and plenty of redundancy exists among these tests. We will only consider some of these panels or scoring systems as detailed discussion about these all is also beyond the scope of this review. There are already many good reviews on biomarkers and diagnostic panels used in NAFLD, NASH and fibrosis[7-12].

MicroRNAs are implicated in pathogenesis of NAFLD, however more research is required to confirm and validate their usefulness as diagnostic or prognostic markers to qualify them for clinical use[13].

QUESTION OF BECOMING BETTER THAN THE GOLD STANDARD

An important fact to note is that when we decide the quality of a non-invasive test or biomarker, all non-invasive tests or biomarkers are compared against the "gold standard" and for NASH diagnosis it is liver biopsy. It is well documented that liver biopsy suffers from sample variability and inter-observer variability[3]. It is possible that in a proportion of samples where liver biopsy results were inaccurate but the biomarkers were correct, the comparative performance of bio-marker will be reported inferior despite the reality that they gave superior results.

MARKERS OF INFLAMMATION

Pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), are raised in plasma in NASH patients compared to patients who suffer from fatty liver without inflammation. There are several reports showing strong association between IL-6 and non-alcoholic steatohepatitis (NASH)[14]. However, IL-6 is raised in several inflammatory conditions including insulin resistance and triggers fibrosis in multiple organs[15] IL-6 is not only involved in inflammation and infection responses but also it has anti-inflammatory action, besides, it is also involved in the regulation of metabolic, regenerative, and neural processes[16]. TNF-α level is increased several fold in NASH, however it is also increased in several inflammatory diseases, cancer and infections. Obesity is characterized by increased plasma levels of TNF-α, IL-6 and acute phase reactant proteins like C-reactive protein (CRP). It may be noted that about 70% of adults age twenty years and over are overweight or obese according to Center for Disease Control and Prevention, United States[17]. Pentraxin-related protein (PTX3), also known as TNF-inducible gene 14 (TSG-14) protein is rapidly induced in many cell types, in particular by mononuclear phagocytes, fibroblasts and endothelial cells in response to inflammatory signals such as TNF-α[18]. To be useful, IL-6 and TNF-α, should be sufficiently specific and should be able to distinguish between a fatty liver without inflammation from one with inflammation. The same is true for markers such as CRP, adiponectin, resistin, leptin, visfatin or retinol-binding protein 4 and PTX3. Ferritin is an intracellular protein that binds to iron and releases it in a controlled fashion present in all cells. Ferritin level increases in response to infection and inflammation. Serum ferritin is an independent predictor of advanced hepatic fibrosis among patients with NAFLD[19]. Both inflammation and accumulated fat in liver creates oxidative stress. Partially oxidized fat causes cellular damage and is known to attract leukocytes resulting in inflammatory response. Measurement of oxidative stress therefore is an indirect predictor of inflammation. However, both obesity and diabetes are independently associated with oxidative stress and inflammation[20]. Accumulated fat in liver will undergo slow oxidation inside hepatocytes, generating free radicals which will initiate a cascade of free radical reactions. Several of the stable intermediates and final products of these reactions can be quantified. Products of free radical–mediated oxidation of linoleic acid (9- and 13-hydroxy octadecadienoic acid and 9-13-oxo-octadecadienoic acid) measured in plasma were significantly elevated in NASH patients with reference to patients with fatty liver without inflammation or patients with normal biopsies[21]. Several compounds such as oxidized low density lipoproteins, malonaldehyde, thiobarbituric acid reactive substances (TBARS) or compounds arising from oxidized tyrosine are useful markers of oxidative stress. However they are of limited use in clinical diagnosis or management of NASH[22,23].

The human body has an anti-free radical regimen to counteract oxidative/nitrosative stress which is depleted during chronic free radical stress conditions such as NASH. The degree of depletion of antioxidant components of mammalian systems, such as glutathione (which is considered the main regulator of redox balance) is a reasonable surrogate measure for oxidative stress[24]. However, oxidative/nitrosative stress is now recognized to be a common characteristic of many acute and chronic diseases in addition to the normal aging process[25].

MARKERS OF REPAIR AND REMODELING RESPONSE

Chronic inflammation results in cell death (apoptosis and necrosis) which in turn induces repair and remodeling responses. Liver has enormous regeneration potential[3,7,9] and during this process several biomolecules are released into the bloodstream mainly from damaged/dying cells, tissue matrix, infiltrated immune cells and possibly from regenerating cells. This includes, liver enzymes and other proteins such as aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT), α2

macroglobulin (an inhibitor of fibrinolysis), haptoglobin (a protein which binds to free hemoglobin), apolipoprotein A1 (component of high density lipoprotein), tissue inhibitor of metalloproteinase 1 (TIMP1), Chitinase-3-like protein 1 (CHI3L1 also known as YKL-40, is a secreted glycoprotein) and constituents of extracellular matrix such as hyaluronic acid (HA) , laminin ,type Ⅳ collagen 7S domain, Pro-collagen Ⅲ (PⅢNP), procollagen Ⅰ carboxyl terminal peptide (PICP), procollagen Ⅳ C peptide, procollagen Ⅳ N peptide (7-S collagen), cytokeratin 18 (CK-18 or KRT18- a type Ⅰ cytokeratin present in glandular epithelia of the digestive, respiratory and urogenital tracts *etc.*)[7-12].

PRIMARY ETIOLOGICAL MARKERS OF NAFLD AND INDIRECT MARKERS ASSOCIATED WITH DECLINING LIVER FUNCTION AND HEALTH

Type 2 diabetes mellitus and adipose tissue dysfunction results in deposition of fat in liver[3]. Insulin resistance, dyslipidemia and obesity are therefore markers of fatty liver disease. Similarly, dysfunction of other organ systems may result in liver pathology. Liver is a key organ in maintaining good health and liver damage results in secondary damage to other organ systems. Liver damage is associated with changes in platelet values, renal and nervous system pathology. NAFLD is associated with cardiovascular risk and events associated with primary arterial hypertension[1-3].

MICRORNAS AS BIOMARKERS IN NAFLD

Recently, certain microRNAs were implicated in NAFLD, however, the available data is not sufficient to suggest their diagnostic use as markers of steatosis, inflammation or fibrosis. miR-122 and miR-34a levels were positively correlated with disease severity from simple steatosis to steatohepatitis. In both chronic hepatitis C (CHC) and NAFLD patients serum levels of miR-122 and miR-34a correlated with serum lipids, liver enzymes levels, and fibrosis stage and inflammation activity[26]. In a recent study, serum levels of circulating miRNAs, miR-21, miR-34a, miR-122 and miR-451 were found associated with nonalcoholic fatty liver disease and the serum level of miR-122 was correlated with the severity of liver steatosis[27]. Over-expressed microRNA-27a and 27b influence fat accumulation and cell proliferation during rat hepatic stellate cell activation but corresponding data from human studies are not presently available or corroborative[28]. In another rat study, Venugopal *et al*[29], reported that liver fibrosis is associated with a down regulation of miRNA-150 and miRNA-194 in hepatic stellate cells and their overexpression causes decreased stellate cell activation. In a study by Alisi *et al*[30] in rats, the miRNAs analysis showed the significant down regulation of three miRNAs, (miR-122, miR-451 and miR-27) and the up regulation of three (miR-200a, miR-200b and miR-429) in high fat diet (standard diet with high fructose and high fat diet combined with high fructose).

NONALCOHOLIC STEATOHEPATITIS DIAGNOSTIC PANELS: THE MORE PARAMETERS THE BETTER?

Although, there exists a variety of scoring systems and panels for evaluating the progression of fatty liver to NASH and cirrhosis exists, none of these markers can be a replacement for liver biopsy. Some of these panels depend on a dozen or more variables to derive the scores while others depend only on three or four parameters (Table 1). Despite the difference in the number of factors and the complexity of the mathematics involved in the biomarker panel development, the difference in efficiency and accuracy in diagnosing and/or staging inflammation and fibrosis that is associated with fatty liver disease is not very much different between these tests (see below).

Brief review on the biomarkers/panels in NAFLD

In a paper published in 2001 Dixon *et al*[31] found that: (1) a raised index of Insulin; Resistance (OR = 9.3); (2) systemic hypertension (OR = 5.2); and (3) raised alanine aminotransferase (OR = 8.6) were independent predictors of NASH. A combination of any two or all three of these predictors showed a sensitivity of 0.8 and specificity of 0.89 for NASH. The accuracy of the test was found by receiver operating characteristic (ROC) analysis. They reported an area under the curve (AUC) equal to 0.90 for the combination of these three predictors[31].

A composite index for distinguishing steatosis from NASH was formulated by Palekar *et al*[32] which included the risk factors, age > 50 years, female gender, AST 45 IU/L, BMI 30 kg/m^2, AST/ALT ratio $\geqslant$ 0.80, and HA $\geqslant$ 55 mcg/L, and its accuracy was determined by ROC analysis to be 0.763. The presence of three or more risk factors had sensitivity and specificity of 73.7% and 65.7% respectively[32].

A commercial panel, the "NashTest" from BioPredictive a French company, combines α2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, GGT, fasting blood glucose (FBS), triglycerides (TG), cholesterol, ALT and AST, with parameters adjusted for patient's age, gender, weight and height[33]. According to Thierry Poynard, the inventor of this patented test, the accuracy of NashTest was determined by ROC analysis. The AUC of the "NashTest" for diagnosing NASH in the training and validation groups were, 0.79 and 0.79 (P = 0.94) respectively[34]. Therefore the test result for "NashTest" was not quite as impressive; and we need several independent and international studies to prove the usefulness of the "NashTest".

The "FibroTest" (in the United States it is marketed as "FibroSure") is a hepatic damage score that is

Table 1 Some of these panels depend on a dozen or more variables to derive the scores while others depend only on three or four parameters

Noninvasive test	Parameters	Disease	AUC	Ref.
APRI	AST, platelet count	Fibrosis, cirrhosis in mixed patient population	0.82	Adler *et al*[40], *Hepatology* (2008)
Enhanced liver fibrosis (ELF) test	Hyaluronic acid, tissue inhibitor of matrix metalloproteinase-1, amino terminal propeptide of procollagen type Ⅲ	NAFLD in children	0.92 to 0.99	Nobili *et al*[50], *Gastroenterology* (2009)
		Chronic liver disease	0.80	Nobili *et al*[50], *Gastroenterology* (2009)
HAIR	Hypertension, ALT, IR index	NAFLD	0.90	Dixon *et al*[31], *Gastroenterology* (2001)
NashTest A commercial panel from Biopredictive, France	Alpha2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, GGT, fasting glucose, triglycerides, cholesterol, ALT, AST, age, gender, weight, height	NAFLD	0.79	Poynard *et al*[34], *BMC Gastroenterology* (2006)
FIB-4	Platelets, ALT, AST and age	HCV fibrosis	0.76	Vallet-Pichard *et al*[38], *Hepatology* 2006
		NAFLD fibrosis	0.80	Shah *et al*[39], *Clinical Gastroenterology and Hepatology* (2009)
FibroTest/FibroSure A commercial panel from Biopredictive, France	α2-macroglobulin , apolipoprotein A1, haptoglobin, total bilirubin, GGT	NAFLD fibrosis	0.86	Ratziu *et al*[37], *BMC gastroenterology* (2006)
FibroQ	Age, AST, platelet Count, PT-INR	HCV fibrosis (F2-4)	0.783	Hsieh *et al*[41], *Chang Gung Med J* (2009)
Lok index	Platelet count, PT-INR, AST, ALT	HCV fibrosis	0.78	Lok *et al*[42], *Hepatology* (2005)
Forns Score	Age, platelet count, GGT, cholesterol	HCV fibrosis	0.86	Forns X *et al*, *Hepatology* (2002)
		Fibrosis from all causes	0.76	Adler *et al*[40], *Hepatology* (2008)
BARD Score	Body-mass index, AST/ALT ratio, type 2 diabetes mellitus	NAFLD fibrosis	0.67	Ruffillo *et al*[47], *Journal of Hepatology* (2011)
NAFLD fibrosis score	Age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT ratio	NAFLD fibrosis	0.82	Angulo *et al*[45], *Hepatology* (2007)
			0.68	Ruffillo *et al*[47], *Journal of Hepatology* (2011)
Fibrometer	Platelets, prothrombin index, aspartate aminotransferase, α2-macroglobulin (A2M), hyaluronate, urea, and age	Viral and alcoholic chronic liver diseases fibrosis	0.883	Calès *et al*[43], *Hepatology* (2005)
		NAFLD	0.943	Calès *et al*[44], *Journal of Hepatology* (2009)

NashTest, FibroTest/FibroSure. SteatoTest and FibroMax are products from Biopredictive, France. FibroMax is the combination of NashTest, FibroTest/FibroSure and SteatoTest. AUC: Area under the curve; APRI: AST-to-platelet ratio index; AST: Aspartate transaminase; ALT: Alanine transaminase; NAFLD: Nonalcoholic fatty liver disease; GGT: Gamma-glutamyl transpeptidase; HCV: Hepatitis C virus.

useful in a variety of diseases involving the liver. It is derived from age, gender and five serum markers[33]. The markers are α-2-macroglobulin, haptoglobin, apolipoprotein a1 (APOA1), GGT, total bilirubin. ALT is used in another sub-test called ActiTest, for measuring necro inflammatory activity in patients with chronic hepatitis C or B. The patented formula for calculating the FibroTest score logistic regression coefficient is the following or a variant which can be found in the public domain[35].

The "FibroTest" is well standardized, reproducible and commercially available. According to a report by Imbert-Bismut *et al*[36] the impact of parameter analytical variability on Fibrotest and Actitest results was less than 10% and intra-patient reproducibility was within acceptable limits[36]. FibroTest was evaluated in two groups, group 1 from a reference center and group 2 was a multicenter study. The ROCs for the diagnosis of advanced fibrosis (F2F3F4): 0.86 in group 1 and 0.75 in group 2[37].

Biopredictive also offers the "SteatoTest" which combines α 2-macroglobulin, haptoglobin, APOA1, total bilirubin, GGT, fasting glucose, triglycerides, cholesterol and ALT, parameters adjusted for patient's age, gender, weight and height according to the company's website. Fibromax is the combination of FibroTest, SteatoTest and NashTest, available from the same company, "Biopredictive"[33].

FIB-4 is "an inexpensive and accurate marker of fibrosis in HCV infection in comparison with liver biopsy and Fibrotest" according to Vallet-Pichard *et al*[38] in a paper published in 2006. FIB-4, depends common clinical parameters-platelets, ALT, AST and age. According to the authors, "FIB-4 value < 1.45 or > 3.25 (64.6% of the cases) was concordant with the FibroTest results in 92.1% and 76%, respectively" and AUC was 0.76. A 2009 study by Shah *et al*[39] compared the performance of the FIB4 index with six other non-invasive markers of fibrosis in patients with NAFLD. They found that the FIB4 index is superior to the other noninvasive markers of fibrosis in patients with NAFLD [the AUC was greatest for FIB4 (AUC = 0.802)]. The authors however highlighted the need for even better noninvasive markers for NAFLD.

The AST-to-platelet ratio index (APRI) was developed as a simple, easy to use method in clinics to predict, severe fibrosis or cirrhosis in both HCV mono-infected and co-infected (HCV and HIV) patients. According to a meta-analysis of twenty two studies with 4,266 subjects, the summary AUCs of the APRI for significant fibrosis and cirrhosis were 0.76 and 0.82, respectively. For significant fibrosis, an APRI threshold of 0.5 was 81% sensitive and 50% specific. The Forns Index is mathematically derived from four simple parameters, age, GGT, cholesterol and platelet count. This index is best studied in HCV related fibrosis and it is useful with AUC of 0.750 and 0.760 respectively for the prediction of significant fibrosis (F/S2-4) in HCV and fibrosis from all causes. Comparable values for FibroTest are AUC of 0.794 and 0.800 respectively[40].

AST level, platelet count and prothrombin time (PT) international normalized ratio (INR) and the at onset are the variables considered in "FibroQ", another test for predicting fibrosis in HCV developed by a group in Taiwan in 2009. According to these investigators, FibroQ performed better than APRI, but was similar to ALT/AST ratio, in the prediction of significant fibrosis (it was possible to distinguish between patients with or without fibrosis in 77% of the patient population)[41].

Lok *et al*[42] proposed another simple formula for predicting fibrosis in chronic hepatitis C (CHC). The Lok index was based on platelet count, PT-INR, serum AST and ALT levels. Lok *et al*[42] studied a cohort of 1141 patients with CHC and reported an AUROC of 0.78-0.81 to detect cirrhosis. Calès *et al*[43] in 2005 reported a test which they named the "Fibrometer" to characterize different fibrosis parameters in viral and alcoholic chronic liver diseases. This test is based on the values platelets, prothrombin index, aspartate aminotransferase, α2-macroglobulin, hyaluronate, urea, and age. The AUC for Fibrometer was 0.883 compared with 0.808 for the Fibrotest. Recently the same group used Fibrometer to measure fibrosis in NAFLD. They found that it was superior to NAFLD fibrosis score (NFSA) and APRI. AUC for Fibrometer was 0.943 and for NFSA and APRI the values were 0.884 and 0.866, respectively[44]. The NAFLD fibrosis score was introduced by Angulo *et al*[45] in 2007 and includes routine clinical/lab variables such as age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT ratio. This scoring was efficient in predicting fibrosis and had an AUC of 0.82 in the validation group. Harrison *et al*[46] proposed an index, referred to as the BRAD score, which included- body-mass index (BMI), AST/ALT ratio (AAR), and presence of type 2 diabetes mellitus. They scored these variables as follows-BMI $\geqslant$ 28 kg/m^2 = 1 point, BMI < 28 kg/m^2 = 0 point; AST/ALT ratio $\geqslant$ 0.8 = 2 points, AST/ALT ratio < 0.8 = 0 points; freshly recognized or preexisting diabetes = 1 point. A total of 2-4 points meant significant fibrosis[46]. Ruffillo *et al*[47] evaluated the diagnostic accuracy of this score in NAFLD patients and concluded that this score is useful in identifying patients without severe fibrosis.

A total of 2411 patients with compensated CLD (HCV = 75.1%, HBV = 10.5%, NASH = 7.9%, HIV/HCV = 6.5%) were evaluated by APRI, Forns index, Lok index, AST/ALT ratio, Fib-4, platelets and Fibrotest-Fibrosure against liver biopsy, in a multicenter study. This study concluded that the diagnostic performance is better for significant fibrosis for CHC compared with NAFLD patients, but accuracy was relatively poor among CHC patients with ALT[48].

Enhanced liver fibrosis (ELF) is a modified version of the original European Liver Fibrosis panel[49]. The original panel includes hyaluronic acid, tissue inhibitor of matrix metalloproteinases-1, amino terminal propeptide of procollagen type Ⅲ (which are involved in the synthesis and degradation of extracellular matrix) and age. Later the parameter "age" was removed from the panel establishing the enhanced liver fibrosis (ELF) test[49,50]. The test was effective in predicting NAFLD in children (AUC ranging from 0.92 to 0.99, from fibrosis stage 1 to stage 3)[50].

HOW IMPORTANT IS EXACT STAGE INFORMATION IN THE MANAGEMENT OF NAFLD?

It seems, rather than patient management, exact stage information is more important in academic research and in clinical trials (especially where different drugs are being tried on a limited number of patients). There may be only subtle differences between some of these drugs, which can only be better identified if there exists a good scoring system to evaluate the progress or regression of steatohepatitis.

The appearance and persistence of inflammation is an important turning point in the history of fatty liver disease. The presence of inflammation in "fatty liver" needs to be taken quite seriously because it can progress to fibrosis depending on the patient's genome and epigenome over time[1-4]. The major deficiency of most of the panels is the inability to identify this critical point effectively. Current panels are not reliable in distinguishing fatty liver disease from NASH accurately, although they are good at deciding fibrosis. Identification of fatty liver disease is important because of the associated liver, cardiovascular and cerebrovascular risk[3,51]. However when it comes to the disease staging, it is hitherto not clear whether accurate staging of the disease has a role in the management and what is its implication in practice.

The usefulness of accurate staging and grading of steatosis, inflammation and fibrosis in the management of NAFLD is controversial because of the following reasons. Firstly, pharmacological treatment is not warranted for simple fatty liver (fatty liver without inflammation). Secondly, there are no approved drugs for NASH[5]. Finally, to date, anti-fibrotic treatment

of fibrosis represents, an unsuccessful area, by and large, for drug development[52]. Currently therapy for NAFLD aims at achieving good control of diabetes, hypertension and body mass in diabetic, hypertensive and overweight/obese NASH patients through pharmacological, surgical or non-pharmacological methods such as lifestyle modification. There is no clear "evidence-based treatment" for NAFLD[53,54]. A literature search, didn't reveal to date, any definite guidelines from professional organizations other than what is described (vide-supra) for management of inflammation and fibrosis associated with NAFLD in a stage specific manner. It is therefore difficult to decide the usefulness of staging information on steatosis, inflammation and fibrosis in the currently available treatment methods for NAFLD. This implies, as far as treatment and benefit to the patients is concerned, small differences in efficiency (calculated often in terms of AUC by ROC analysis) between sophisticated, proprietary and costly/commercial tests and scoring algorithms versus simple, inexpensive, easily available non-proprietary tests and scoring systems may be insignificant (Table 1). A simple criteria such as presence of diabetes over five years, metabolic syndrome, obesity, obstructive sleep apnea, AST/ALT ratio > 0.8 or ferritin levels > 1.5 times normal in patients with over six months history of raised ALT and/or ultrasonological evidence of fat in liver would identify patients who need special care and personalized treatment depending on the comorbidities and etiology of NAFLD.

CONCLUSION

Despite the extensive research, development and investment in the field of biomarkers for NAFLD, it is doubtful how much benefit this has brought to the patients. Commercial panels and scoring systems have not improved upon the simpler, widely available, cost effective tests and clinical parameters and they offer little benefit in the management of NAFLD. The performance to date of biomarkers depends very much on the patient, the etiology of NAFLD and the stages of the disease and cannot be considered as a replacement for liver biopsy. Biomarkers, therefore, should serve as a tool to optimize the selection of patients with NAFLD for liver biopsy. There is no clear evidence that liver biopsy and detailed staging of the disease significantly influences the management decisions and benefits the patient. After all, there is no "evidence based medicine" for NAFLD except the management of associated morbidities such as components of the "metabolic syndrome" or (the largely symptomatic management) of cirrhosis.

ACKNOWLEDGMENTS

The manuscript was edited by Mr. Alan Alfieri and proof read by both A.Alfieri and R.Khan.

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P- Reviewer: Fierbinteanu-Braticevici C, Gong ZJ, Roncucci L
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Liu XM

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ISSN 1007-9327