World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v18.i30.3945

World J Gastroenterol 2012 August 14; 18(30): 3945-3954
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

GUIDELINES FOR CLINICAL PRACTICE

Overview and developments in noninvasive diagnosis of nonalcoholic fatty liver disease

Neven Baršić, Ivan Lerotić, Lea Smirčić-Duvnjak, Vedran Tomašić, Marko Duvnjak

Neven Baršić, Ivan Lerotić, Vedran Tomašić, Marko Duvnjak, Division of Gastroenterology and Hepatology, Department of Medicine, "Sestre milosrdnice" University Hospital Center, 10000 Zagreb, Croatia
Lea Smirčić-Duvnjak, Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, 10000 Zagreb, Croatia
Author contributions: Baršić N, Lerotić I, Smirčić-Duvnjak L and Tomašić V performed the literature search and wrote the paper; and Duvnjak M participated in drafting the outline and revised the paper.
Correspondence to: Marko Duvnjak, Professor, PhD, Division of Gastroenterology and Hepatology, Department of Medicine, "Sestre milosrdnice" University Hospital Center, Vinogradska 29, 10000 Zagreb, Croatia. marko.duvnjak1@gmail.com
Telephone: +385-1-3787549 Fax: +385-1-3787549
Received: December 8, 2011 Revised: March 1, 2012
Accepted: March 9, 2012
Published online: August 14, 2012

Abstract

High prevalence of non-alcoholic fatty liver disease (NAFLD) and very diverse outcomes that are related to disease form and severity at presentation have made the search for noninvasive diagnostic tools in NAFLD one of the areas with most intense development in hepatology today. Various methods have been investigated in the recent years, including imaging methods like ultrasound and magnetic resonance imaging, different forms of liver stiffness measurement, various biomarkers of necroinflammatory processes (acute phase reactants, cytokines, markers of apoptosis), hyaluronic acid and other biomarkers of liver fibrosis. Multicomponent tests, scoring systems and diagnostic panels were also developed with the purposes of differentiating non-alcoholic steatohepatitis from simple steatosis or discriminating between various fibrosis stages. In all of the cases, performance of noninvasive methods was compared with liver biopsy, which is still considered to be a gold standard in diagnosis, but is by itself far from a perfect comparative measure. We present here the overview of the published data on various noninvasive diagnostic tools, some of which appear to be very promising, and we address as well some of still unresolved issues in this interesting field.

Key words: Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Liver fibrosis; Liver biopsy; Biomarkers; Transient elastography; Cytokeratin-18; Oxidative stress; Insulin resistance; Hyaluronic acid

Peer reviewers: Dr. Yoshihisa Takahashi, Department of Pathology, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan; Dr. Hui-Kang Liu, PhD, Assistant Research Fellow, National Research Institute of Chinese Medicine, Li-Nung street section 2, Taipei 112, Taiwan, China; Vance Matthews, Assistant Professor, Western Australian Institute for Medical Research, Level 6, MRF Building, Rear 50 Murray Street, Perth 6000, Australia

Baršić N, Lerotić I, Smirčić-Duvnjak L, Tomašić V, Duvnjak M. Overview and developments in noninvasive diagnosis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2012; 18(30): 3945-3954 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i30/3945.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i30.3945

INTRODUCTION

Finding a means to noninvasive diagnosis of non-alcoholic fatty liver disease (NAFLD) and its entities has been the aim of many research efforts since recently, and seems to remain a very much needed goal among many clinicians and researchers in the field of hepatology. Why is it that way?

NAFLD is today considered to be the most common liver disease in adults. The prevalence of NAFLD in general population is very high, in the range of 15%-30% according to various studies, and is even increasing, due to

the rising prevalence of diabetes and obesity[1]. Spectrum of NAFLD includes two entities with very different natural course and prognosis: simple steatosis, which mostly has a benign non-progressive course and good prognosis, and non-alcoholic steatohepatitis (NASH), which demonstrates progression of fibrosis in about 30%-40% of patients and has a proven potential to eventually lead to cirrhosis and end-stage liver disease including hepatocellular carcinoma[2-4]. NASH seems to be present in a surprisingly high proportion of NAFLD patients, including 40% to 75% of cases with elevated aminotransferase levels, those data coming from recent studies using current histological definitions and including substantial number of patients[2,5,6]. In studies of liver biopsy findings from apparently healthy living liver donor candidates, the proportion of NASH among patients with newly discovered NAFLD was about 30%[7]. Even in patients with normal aminotransferase levels, proportion of NASH among NAFLD cases seems to be almost the same, and the whole spectrum of NAFLD including advanced fibrosis and cirrhosis has been observed in patients with completely normal laboratory findings[6,8,9].

Liver biopsy is still considered the gold standard in diagnosis and the only reliable tool for distinguishing NASH from simple steatosis and for grading and staging the disease, providing important information about severity of steatosis, lobular inflammation, hepatocellular ballooning, and degree of fibrosis[10]. Minimal histological criteria for NASH include steatosis, hepatocyte injury (in the form of ballooning or apoptosis) and lobular inflammation. Similarly to other chronic liver diseases, fibrosis is usually divided histologically in four stages: perisinusoidal fibrosis (F1), perisinusoidal and periportal fibrosis (F2), bridging fibrosis (F3) and cirrhosis (F4). Liver biopsy also has several negative aspects: it is invasive, unpleasant for patients, it usually includes hospitalization and a day or two lost at work, and the adequate interpretation of the specimen requires a pathologist with expertise in hepatopathology, which altogether makes it a costly and time-consuming procedure. Another significant drawback of liver biopsy, and in medical terms the most important one is its substantial sampling variability, which has been consistently proven for several chronic liver diseases including NAFLD. In a well-designed study by Ratziu *et al*[11], the negative predictive value of a single biopsy for the diagnosis of NASH was calculated to be only 74%.

Considering the mentioned high prevalence of NAFLD in the general population, and the fact that every patient with NAFLD including the one with normal aminotransferases can potentially have NASH, we come to the conclusion that it would be necessary to perform liver biopsy in about one fourth of the whole Western population. This is clearly not feasible, but is it necessary? Until recently, many have advocated against the routine use of liver biopsy for patients with NAFLD because fatty liver is still considered by many to be a benign condition-although many studies have now clearly indicated a progressive course in a proportion of patients with NASH. Another reason for avoiding biopsy and definite diagnosis was the lack of established pharmacological treatment options which would have proven efficacy in preventing progression or leading to regression of disease.

Although there are still no generally approved treatments for NASH, several treatment options have demonstrated efficacy in various clinical trials, and for example recently published results of the randomized multicenter pioglitazone *vs* vitamin E *vs* placebo for the treatment of nondiabetic patients with nonalcoholic steatohepatitis (PIVENS) trial have provided substantial evidence for the previously suggested efficacy of vitamin E (and to a lesser extent pioglitazone) in inducing histologic improvement of NASH[12]. Hopefully, we could soon expect to have several efficient treatment options available. All of this pretty much eliminates the validity of approach where NASH remains undiagnosed, which currently does happen in many clinical settings, e.g., in patients with accidental ultrasonographic finding of fatty liver and even patients with mildly elevated transaminase levels and known NAFLD risk factors who are very often not investigated any further.

The necessity of diagnosing NASH and the proportion of the population affected lead to a logical conclusion that a need for a reliable noninvasive tool in NAFLD diagnosis is highly urgent. Ideal noninvasive tool would be able to distinguish NASH from simple steatosis and allow for grading and staging of disease, which would largely facilitate screening of population at risk. Development of noninvasive tools would also enable monitoring of disease course and progression and evaluation of response to therapy, both in routine practice and in the setting of clinical trials, which is currently only possible with a follow-up liver biopsy. Another very important, and somewhat disregarded point, is that an efficient biomarker or set of biomarkers would accurately reflect the inflammatory and fibrotic processes on the level of the whole of liver parenchyma, thereby increasing the diagnostic accuracy and resolving the problem of sampling variability intrinsic to liver biopsy, which represents only about 1/50.000 part of the organ which is not homogeneously affected by disease features.

In the text below, we present the current level of knowledge and progress regarding the noninvasive diagnostic tools that have been studied in the context of NAFLD (Table 1).

ROUTINE LABORATORY TESTS

Patients with NAFLD are mostly asymptomatic and the disease is usually suspected based on either hyperechoic liver appearance on abdominal ultrasound or mild to moderate increases in liver enzyme levels. These are usually the only aberrations that can be encountered in this patient population (apart from signs of associated conditions like elevated glucose or lipid levels), and a large proportion of patients has completely normal laboratory findings. Hypoalbuminemia, prolonged prothrombin time, and hyperbilirubinemia are parameters of impaired liver function and occur only in patients who have already de-

Table 1 Overview of noninvasive methods in diagnosis of liver disease severity that have been evaluated in the context of non-alcoholic fatty liver disease

Routine laboratory tests
Liver enzymes
Parameters of liver dysfunction
Imaging methods
Ultrasound
Computed tomography
Magnetic resonance imaging
Magnetic resonance elastography
Liver stiffness measurement
Transient elastography (FibroScan)
Acoustic radiation force impulse shear wave imaging
Biomarkers of necroinflammation
Cytokeratin 18 fragments
High-sensitivity C-reactive protein
Interleukin-6
C-C chemokine ligand 2
Plasma pentraxin 3
Oxidative stress measurement
Tumor necrosis factor-α
Adiponectin
Insulin resistance measurement
Multicomponent tests for diagnosis of non-alcoholic steatohepatitis
Nash test
Non-alcoholic steatohepatitis clinical scoring system for morbid obesity
Model by Miele *et al*[61]
Biomarkers of fibrosis
Hyaluronic acid
Laminin
Type VI collagen 7S domain
Multicomponent panels for diagnosis of fibrosis
Fibrotest
Non-alcoholic fatty liver disease fibrosis score
European liver fibrosis panel/enhanced liver fibrosis panel

veloped cirrhosis. Most commonly elevated enzymes are alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT), while aspartate aminotransferase (AST) elevation is less frequent and when pronounced may indicate presence of advanced fibrosis[13]. Many studies have tried to correlate liver enzyme levels with histological severity and progression of disease, and various results have been obtained. In some cases ALT, in other AST or GGT levels demonstrated best correlation with severity of inflammation or fibrosis and their progression/regression on follow-up biopsies[4,14-16]. Equally important, the full spectrum of NAFLD including severe inflammation and fibrosis was proven to occur with almost similar frequency in patients with completely normal liver enzymes[8,9].

IMAGING METHODS

Imaging modalities frequently used in the diagnosis of NAFLD include ultrasound, computed tomography and magnetic resonance imaging (MRI). While they are all very sensitive (80%-100%) and specific in detection of steatosis, none of them can effectively distinguish simple steatosis from NASH or determine the degree of fibrosis[17]. Nevertheless, MRI is more sensitive than ultrasound in detecting lesser degrees of hepatic steatosis, and new techniques in MRI are constantly being developed that provide additional data on different tissue parameters. One of them is magnetic resonance (MR) elastography, which estimates liver tissue stiffness by imaging the propagation of induced shear waves with a modified phase-contrast MR sequence. This technique was shown to have an excellent predictive value for excluding fibrosis, while sensitivity and specificity for discriminating between mild and more severe fibrosis was around 85%[18]. A recent study investigated the performance of MR elastography in 58 patients with NAFLD and demonstrated very high accuracy with under the receiver operated curve (AUROC) of 0.93 for discriminating patients with NASH and those with simple steatosis, with a sensitivity of 94% and a specificity 73% by using a threshold of 2.74 kPa[19]. The future advances in MRI technology including hepatic flow parameters and diffusion-weighted MRI may hopefully provide more MR-based tools for liver fibrosis detection. Ultrasound has also demonstrated the potential for improvement in diagnosis of NAFLD and NASH. Apart from ultrasound-based elastography, use of ultrasound contrast agents has been studied in this scenario, and signal intensity after contrast administration was shown to be significantly lower in NASH when compared with simple steatosis and normal liver[20].

LIVER STIFFNESS MEASUREMENT

Transient elastography (FibroScan®, EchoSens, Paris, France) is a relatively novel technique which measures liver tissue elasticity by measuring the speed of propagation of probe-induced vibrations through parenchyma by ultrasound. Elasticity shows significant correlation with degree of liver fibrosis, and FibroScan is considered to produce a reliable prediction of higher degrees of liver fibrosis. The method was first assessed in population of patients with hepatitis C, and after evaluation in multiple studies it has been introduced into clinical practice. A survey performed four years ago in France showed that about a third of hepatologists was using it (mostly in evaluation of patients with hepatitis C), and the method is now gaining increasing popularity in other countries as well[21]. More recent studies have assessed transient elastography in population of patients with NAFLD, and obtained results that are similar to those from studies in hepatitis C[22-24]. A consistent increase in liver stiffness with increasing fibrosis stage was observed, and the largest study, performed by Wong *et al*[22], obtained AUROC values of 0.93 for advanced fibrosis and 0.95 for cirrhosis. When the liver stiffness cut-off was set at 7.9 kPa, negative predictive value for advanced fibrosis was excellent (97%), and could be applied to 60% of the population. On the other side, positive predictive value of having advanced fibrosis or cirrhosis was at best only 72.4%, at the 9.6 kPa cut-off. Accuracy of FibroScan in detecting significant fibrosis (defined as at least perisinusoidal and portal/periportal fibrosis) was poor, as was expected from previous experience.

Several meta-analyses have assessed performance of transient elastography in fibrosis detection, consisting

mostly of studies on hepatitis C patients. They have shown generally very good diagnostic accuracy in detecting cirrhosis and somewhat lesser precision in excluding advanced fibrosis, while they demonstrated substantial heterogeneity in diagnosis of significant fibrosis ($F \geqslant 2$)[25,26]. Importantly, variation in cut-off values of liver stiffness has been large and these values still require validation. In conclusion, due to the relatively low specificity, the value of transient elastography seems to remain in ruling out cirrhosis and advanced fibrosis in patients with low liver stiffness values, while patients with intermediate values would still require liver biopsy for correct classification, and the proportion of patients with high stiffness values who are misclassified is not negligible. It is also important to take into account that the population of patients that is usually encountered in clinical practice does not have advanced fibrosis or cirrhosis in large proportions, and diagnosis of lesser degrees of fibrosis is equally important in estimating the risk for liver-related morbidity and mortality. A large study that evaluated frequency and reasons of failure to obtain the elasticity measurement found that FibroScan was feasible in over 95% of the patients, and the only factor associated with failure was body mass index greater than 28[27]. Failure occurs due to the elastic and ultrasound wave attenuation by subcutaneous fat, and while this may not be a significant issue in other chronic liver diseases, it is an important limitation in patients with NAFLD, considering the prevalence of obesity in this population. In the study by Wong *et al*[22], measurement could not be obtained in over 10% of cases, which significantly reduced diagnostic accuracy when the 'intention-to-diagnose' analysis was performed.

Another noninvasive method of assessing tissue stiffness, acoustic radiation force impulse (ARFI) shear wave imaging, was recently assessed in a couple of studies with NAFLD patients[28,29]. This ultrasound-based technique estimates the tissue stiffness by measuring transient tissue deformations of several microns which are induced in the liver parenchyma by acoustic radiation force. In a study on 172 NAFLD patients by Palmeri *et al*[29], ARFI imaging distinguished low (Stages 0-2) from high (Stage 3-4) fibrosis stages with a sensitivity and a specificity of around 90% (AUROC of 0.90). Body mass index over 40 kg/m^2 was not a limiting factor for ARFI imaging, which overcomes part of the problems associated with FibroScan. When compared to FibroScan, ARFI imaging demonstrated similar diagnostic performance[28,30].

BIOMARKERS OF NECROINFLAMMATION

Most intense research is now being focused on biomarkers, measurable serum parameters that reflect the intensity of inflammatory processes and hepatocyte necrosis, as well as the ones that reflect extracellular matrix remodelling and collagen deposition. Ideally, an excellent biomarker would be specific for liver and accurately reflect the underlying pathogenetic processes on the level of whole organ, and thus be an even more precise indicator of the disease than liver biopsy, which is prone to sampling variability and interpretation biases as described earlier.

Cytokeratin 18 fragments

Apoptosis is an important mechanism in pathogenesis of NASH, and its initiation leads to activation of caspase family of intracellular proteases which then cleave different intracellular proteins including cytokeratin 18 (CK-18), the major intermediate filament protein in hepatocytes. By measurement of CK-18 fragments hepatocyte apoptosis can be quantified, and this method was tested as a noninvasive tool in NASH diagnosis in several studies. Initial results were very promising, as Wieckowska *et al*[31] demonstrated a striking increase in serum CK-18 fragment levels in patients with definitive NASH, as well as their high diagnostic accuracy for differentiating between NASH and simple steatosis or normal liver, with AUROC of 0.93 and positive and negative predictive value of 95.0% and 89.5%, respectively. However, this study included only 39 patients, and the larger validation study that was subsequently undertaken and included 139 patients obtained less favourable results: median CK-18 fragment levels in NASH cases were now only 335 U/L (compared to 765 U/L in the first study, and to about 200 U/L in non-NASH cases in both studies), and diagnostic performance was expectedly poorer (calculated AUROC was 0.83 and sensitivity for diagnosing NASH was at best 77%, with the specificity rising above 90% only at the highest tested cut-off value)[32]. Nevertheless, CK-18 fragment levels showed very good correlation with NASH, fibrosis and NAS (NAFLD activity score), and similar results were reported from other groups as well, supporting its potential role as a noninvasive tool in NAFLD[33-35]. Even more importantly, in the study by Diab *et al*[35,36] on 99 patients who underwent bariatric surgery, CK-18 fragment levels showed a significant decrease 6 mo postoperatively, and in another study changes in CK-18 fragment levels closely paralleled changes in NAS on follow-up biopsy. These findings indicate the potential use of CK-18 fragment levels in the follow-up of patients with NASH, including evaluation of response to therapy. This certainly requires further attention, as it could possibly lead us closer to the goal of eliminating the need for second liver biopsy and thus facilitating design and conductance of clinical trials, as well as enhancing patient follow-up in clinical practice.

High-sensitivity C-reactive protein

C-reactive protein (CRP) is an acute-phase reactant produced by the liver in many inflammatory conditions, and based on the hypothesis that NASH is associated with low-grade systemic inflammation, several studies have compared high-sensitivity CRP levels in patients with NAFLD. Two studies found that hs-CRP levels were significantly higher in cases with NASH compared to those with simple steatosis, and hs-CRP also correlated well with presence of advanced fibrosis[37,38]. However, a study performed earlier concluded that measurement of hs-CRP

was not useful in predicting the histological severity of NAFLD, as there was no relationship between the levels of hs-CRP and the grades of steatosis, necroinflammation or fibrosis[39]. Further investigation including testing of diagnostic accuracy is needed before definite conclusions can be reached about usefulness of this marker in NAFLD.

Interleukin-6 and C-C chemokine ligand 2

Interleukin-6 (IL-6) is a proinflammatory cytokine that is involved in NAFLD pathogenesis, and Wieckowska *et al*[40] demonstrated a markedly increased IL-6 expression in liver tissue of patients with NASH as compared to simple steatosis or normal liver, with a positive correlation with severity of inflammation and fibrosis. Plasma IL-6 levels that were parallely measured in this study correlated well with liver IL-6 expression. In another study, IL-6 was among several serum markers evaluated in 47 NAFLD patients and 30 controls, and it was significantly increased in patients with NAFLD as compared to controls, but not in NASH compared to simple steatosis[41]. This study also evaluated serum levels of C-C chemokine ligand 2 (CCL2), a chemokine responsible for monocyte/macrophage infiltration of liver and maintaining hepatic inflammation and fibrogenesis, and found that it was significantly elevated in patients with NASH compared to simple steatosis, but diagnostic performance of CCL2 levels was not tested. In a recent study, pharmacological inhibition of CCL2 had an effect on reduction of hepatic steatosis in a murine model, and CCL2 will presumably see some further investigation in the context of NAFLD[42].

Plasma pentraxin 3

Plasma pentraxin 3 is a novel marker of systemic inflammation from pentraxin family of acute-phase proteins that is produced by diverse cell types in response to proinflammatory cytokines[43]. Yoneda *et al*[44] have evaluated pentraxin 3 levels in 70 patients with NAFLD, and found that they were significantly higher in cases with NASH compared to non-NASH, with the AUROC value of 0.75 for NASH detection. Pentraxin 3 levels also correlated well with the stage of fibrosis. These findings should provide basis for additional evaluation of this marker in other NAFLD patient cohorts.

Oxidative stress

Oxidative stress is one of the key mechanisms in NASH pathogenesis, and several studies have measured systemic markers of oxidative stress status in NAFLD patients and compared them between cases with NASH and controls[45-47]. Different methods for measurement of oxidative stress have been used (measurement of levels of lipid peroxidation products, levels of antioxidant defence systems like vitamin E, glutathione peroxidase and superoxide dismutase activities, antioxidant capacity of the plasma and total plasma peroxide concentrations), and studies produced disparate results. Based on the current data, there is no doubt that oxidative stress is present in NASH, but the utility of its measurement as a noninvasive tool in NAFLD diagnosis probably does not have any clinical value.

Tumor necrosis factor-α and adiponectin

Tumor necrosis factor (TNF)-α and adiponectin are cytokines which have been proven to play important roles in NAFLD pathogenesis, and the serum levels of these cytokines were determined in patients with NAFLD and correlated to disease severity in multiple studies[48-52]. However, diagnostic accuracy in discerning NASH from simple steatosis and the potential for noninvasive use in diagnosis were generally not evaluated, and the data on diagnostic performance of this cytokines are not available. As of the published results, most of the studies demonstrated correlation of lower adiponectin levels with presence of NAFLD compared to healthy controls, presence of NASH compared to simple steatosis, and with histological severity of the disease, while levels of TNF-α and its soluble receptor were most often not significantly different between patients with NASH and patients with simple steatosis or controls. Thus, the potential for clinical use of this cytokines as noninvasive tools for diagnosis of NASH is questionable.

Insulin resistance

Insulin resistance state leads to increased lipolysis and free fatty acid flux to the liver, and elevated plasma glucose and insulin levels promote *de novo* fatty acid synthesis and impair β-oxidation, contributing to the development of hepatic steatosis. Although it is not clear whether insulin resistance causes hepatic steatosis or the liver fat accumulation represents the primary event leading to peripheral insulin resistance, there is no doubt that it plays an important role in the pathogenesis of NAFLD. Large population studies have shown that almost all of the NAFLD patients were insulin resistant according to the homeostasis model assessment of insulin resistance (HOMA-IR)[53]. Additionaly, the potential of insulin resistance measurement as a noninvasive diagnostic tool was also evaluated. In a study by Shimada *et al*[54], the authors tested the diagnostic performance of adiponectin, insulin resistance measured by HOMA-IR, and type IV collagen 7S in discriminating NASH from simple steatosis. While performance of each of these markers individually wasn't great, sensitivity of the combination of three markers was 94%, with a specificity of 74%.

Although insulin resistance has been usually associated with type 2 diabetes, it can also be present in type 1 diabetic patients[55]. The euglycemic insulin clamp technique which represents the gold standard for identifying type 1 diabetic patients who are insulin resistant is impractical for routine clinical use, and insulin resistance in type 1 diabetic patients was often recognized only by higher insulin requirements. Recent introduction of a validated method for estimated glucose disposal rate (eGDR) measurement based on clinical parameters has allowed its easier assessment in a clinical setting[56]. A recently pub-

lished study demonstrated that NAFLD markers were associated with insulin resistance measured by eGDR in type 1 diabetic patients. NAFLD associated markers (ALT, AST, alkaline phosphatase, GGT and ferritin) worsened in parallel with the decline in insulin sensitivity and after adjustment for covariates, ALT, AST and alkaline phosphatase were independent predictors of insulin resistance[57].

Multicomponent tests

There have been several attempts at constructing a panel of clinical and laboratory parameters that would, when combined using a formula or a scoring system, result in a value that enables distinguishing bewteen NASH and simple steatosis. The most advanced attempt was a study by the French group specialized at developing diagnostic models for various liver conditions, who constructed a complex test (NashTest) which combines 13 parameters (age, sex, height, weight, triglycerides, cholesterol, α2-macroglobulin, apolipoprotein A1, haptoglobin, GGT, ALT, AST and bilirubin) into a patented algorithm[58]. Their design and validation study included 257 patients and 383 controls, and the NashTest had AUROC of 0.79 [95% confidence interval (CI) 0.69-0.86], with sensitivity for NASH (using criteria by Kleiner *et al*[59]) of only 33% and positive predictive value of 66%. The results were somewhat better when subgroups with borderline NASH and NASH were combined, the sensitivity rising to 88% and positive predictive value to 74%. In another study, a clinical scoring system was developed based on the results of multivariate analysis in a group of morbidly obese patients that underwent intraoperative liver biopsy at bariatric surgery[60]. The proposed NASH Clinical Scoring System for Morbid Obesity included 6 clinical variables (hypertension, diabetes, AST, ALT, sleep apnea and non-black race) and was used to stratify morbidly obese into 4 groups regarding the risk for presence of NASH (low, intermediate, high and very high). In the studied group, the proportion of patients with low-risk score who had NASH was 13%, while it was 80% in those with very high-risk score. Recently, Miele *et al*[61] measured several markers of liver fibrosis in a cohort of 46 patients with NAFLD, and constructed a mathematical model based on the results of multivariate analysis that included age, hyaluronic acid and tissue inhibitor of metalloproteinase 1 levels. A specific cut-off value identified patients with NASH with 86% sensitivity, and negative and positive predictive values of 96% and 60%. This model could potentially be useful in excluding patients with negative values from liver biopsy consideration if these findings are confirmed in larger independent studies.

BIOMARKERS OF FIBROSIS

As with other chronic liver diseases, the most important indicator of severity and progression of liver damage in NAFLD is the presence and degree of liver fibrosis. Estimation of fibrosis is therefore essential in the diagnostic workup of patients with NAFLD, and it remains one of the major reasons for performing liver biopsy in this population. After a large number of studies was undertaken in hepatitis C patients that tried either to design tests and scoring systems using combinations of readily available clinical and biochemical parameters, or to find specific biomarkers of fibrosis processes that would adequately correspond to liver biopsy findings, similar attempts were made as well in populations of patients with NAFLD. Generally, while showing good accuracy in detection of advanced fibrosis or cirrhosis, all of these tests demonstrate significantly lower sensitivities in predicting the presence of mild or moderate fibrosis. The problem lies in the fact that this is exactly the group of patients that would benefit most from therapeutic interventions, before significant fibrosis has already developed, and they therefore require early diagnosis.

Hyaluronic acid and other markers of extracellular matrix turnover

Hyaluronic acid is a component of the extracellular matrix that can be measured in serum, where it partially enters through lymphatics. Serum levels are dependent on production, which increases with increased collagen synthesis, as well as degradation, which takes course in liver sinusoidal endothelial cells after binding to specific receptors. With progression of liver fibrosis, both increased production of collagen and decreased function of sinusoidal endothelial cells lead to elevation of hyaluronic acid serum levels.

Several groups have so far evaluated the potential use of hyaluronic acid levels in diagnosis of NASH-related fibrosis. Suzuki *et al*[62] investigated the potential of hyaluronic acid for use in diagnosing fibrosis in a cohort of 79 patients with NAFLD and various degrees of fibrosis. The hyaluronic acid serum levels demonstrated good correlation with the degree of hepatic fibrosis, and significant difference was noted especially when comparing mild to moderate (Stages 0-2) with severe fibrosis or cirrhosis (Stages 3-4). The calculated AUROC for severe fibrosis was 0.89 (95% CI 0.81-0.97), and at the optimal cut-off value of 46.1 ng/mL sensitivity was 85% (95% CI 62%-97%) and specificity 80% (95% CI 67%-89%). When a prevalence of severe fibrosis among NAFLD patients was assumed to be 20% (approximate of usual patient population at referral centers), the corresponding positive predictive value was 51% (95% CI 39%-68%) and negative predictive value 95% (95% CI 91%-100%). Accuracy for diagnosing mild fibrosis (Stage 1) was low and the number of patients with moderate fibrosis (Stage 2) was inadequate for valid analysis. Another study evaluated hyaluronic acid and laminin levels in 50 patients with NASH, of whom 23 had some degree of fibrosis and 27 had no fibrosis on liver biopsy[63]. Subjects with NASH and fibrosis had significantly higher hyaluronic acid and laminin levels than those without fibrosis, and AUROC and diagnostic performance of both of these markers was calculated for differentiating between presence and

absence of fibrosis, showing excellent diagnostic accuracy of hyaluronic acid at the cut-off value of 148.8 ng/mL (reported sensitivity and specificity was over 95%). In the fibrosis group, levels of hyaluronic acid significantly increased with rising fibrosis stages, however the accuracy for distinguishing different fibrosis stages was not tested due to small patient numbers. Sakugawa *et al*[64] investigated the levels of hyaluronic acid and type Ⅵ collagen 7S domain in a population of 112 patients with NAFLD, of whom 70 were classified as NASH. On regression analysis, both markers were independently associated with the presence of NASH or severe fibrosis, but demonstrated sensitivity and specificity for severe fibrosis in the range of 70%-80%. However, if both markers were negative in a given patient, severe fibrosis was highly unlikely to be present (negative predictive value 95.2%).

Although some of these results look very promising, studies are still lacking in power, and the proposed cut-off values and calculated diagnostic accuracies are quite heterogeneous, which may be due to other factors in addition to difference in sample size, like difference in measurement methods and studied populations. Another important aspect of clinical usefulness of hyaluronic acid and other serum markers of fibrosis that hasn't yet been investigated is the question of sensitivity to longitudinal changes in fibrosis of liver parenchyma, which could potentially enable noninvasive patient follow-up and evaluation of treatment effects.

Multicomponent panels

FibroTest is a copyrighted panel developed by the French group who originally conceived it for diagnosis of liver fibrosis in hepatitis C, where it has subsequently been extensively studied. It includes 5 biochemical parameters (α2-macroglobulin, apolipoprotein A1, haptoglobin, total bilirubin and GGT) that are incorporated into a patented formula. More recently, the authors of the panel conducted a study that thoroughly evaluated diagnostic performance of FibroTest in the setting of NAFLD by including 267 patients and a large number of healthy controls[65]. Fibrosis stage was determined according to Kleiner *et al*[59], and advanced fibrosis included stages F2-F4 (perisinusoidal and portal/periportal fibrosis, bridging fibrosis and cirrhosis). Mean FibroTest value steadily increased with increasing fibrosis stage, and calculated AUROC for advanced fibrosis was 0.86 (95% CI 0.77-0.91), while it was 0.92 (95% CI 0.83-0.96) for bridging fibrosis or cirrhosis (F3-F4). A FibroTest cut-off score of 0.30 had 77% sensitivity and 90% negative predictive value, and score of 0.70 had 98% specificity and 76% positive predictive value for advanced fibrosis. As expected, performance was even better in detection of F3-F4, with 92% sensitivity and 98% negative predictive value. In addition to one third of patients having the value that fell between these two cut-offs and thus a nondiagnostic test result, other causes of FibroTest failure were analyzed and included Gilbert's syndrome, acute inflammation, and abnormal apolipoprotein A1 that was related to dyslipidaemia.

Another large multicenter study by a different group was undertaken and included a total of 733 patients divided in 2 groups in an attempt to develop and validate a noninvasive scoring system that would separate NAFLD patients with and without advanced liver fibrosis[66]. The score was named NAFLD fibrosis score and included a formula with 6 variables (age, hyperglycemia, body mass index, platelet count, albumin and AST/ALT ratio), selected based on results of multivariate analysis. Biopsy was also scored according to Kleiner *et al*[59], but the diagnostic goal of advanced fibrosis included only Stages F3 and F4. AUROC values were 0.88 in estimation and 0.82 in validation set, and two cut-off points were determined similarly to the previously mentioned study. Using the low cut-off point, negative predictive value of the score was 93% in estimation group and 88% in validation group, while with the high cut-off point positive predictive value was 90% in estimation group and 82% in validation group. Only 25% of patients had score values between the cut-offs and would thus be considered as "indeterminate" and still require liver biopsy after this noninvasive test was performed.

After the European Liver Fibrosis Group developed an algorithm that included age, tissue inhibitor of matrix metalloproteinase 1, hyaluronic acid and aminoterminal peptide of pro-collagen Ⅲ and tested its performance in diagnosing significant fibrosis in a large cohort of patients with various chronic liver diseases, another study was undertaken more recently that investigated performance of this panel specifically in NAFLD patients[67,68]. The original panel was modified by excluding age and naming it enhanced liver fibrosis panel (ELF), and its diagnostic performance was tested in a cohort of 192 patients. The ELF panel had very good performance in distinguishing severe fibrosis (Stage F3-F4) with an AUROC of 0.90 (95% CI 0.84-0.96), while AUROC for detecting moderate and severe fibrosis together (F2-F4) was 0.82 (95% CI 0.75-0.88). Diagnostic accuracy varied with various cut-off points tested, and if cut-offs with 90% sensitivity and specificity for severe fibrosis detection were selected, 86% of study patients would have avoided a liver biopsy, with 76% correctly classified. The study also suggested that the addition of simple parameters, the ones included in previously mentioned NAFLD fibrosis score, could augment the diagnostic performance of the ELF panel, although additional studies with larger sample size would be required to confirm this.

CONCLUSION

Due to the very high prevalence of the disease and numerous difficulties related to establishing the diagnosis, NAFLD remains undiagnosed or incompletely defined in a large number of cases. Therefore, the search for the means to noninvasive diagnosis of different forms of NAFLD is a matter of uttermost importance. It is gaining even greater significance in the light of recent advances in the treatment of NASH, as the research efforts are finally starting to provide us with definite treatment

options. Recently published study with vitamin E and pioglitazone, as well as other current treatment trials place the necessity of establishing a correct diagnosis and not missing NASH in a whole different perspective[12]. Furthermore, given the proportion of population with fatty liver and the fact that the presence of NASH in a given patient is often not linked with elevation in liver enzymes, the number of patients in need of a screening becomes daunting. After the insight in all of the aforementioned studies, we can see that some have indeed come very close and demonstrated very good diagnostic performance of certain noninvasive tools. However, the gold standard used in almost all of the studies is liver biopsy, and the question that remains is whether we are actually able to accurately assess the performance of noninvasive methods when the gold standard by itself has significant flaws. These flaws were very clearly demonstrated in a study of sampling variability in NAFLD by Ratziu *et al*[11]. One can also pose the question: have we maybe found an excellent noninvasive tool already, but are ignorant of the fact due to our incapability to actually see "the absolute truth"? This question has been addressed in a study by Mehta *et al*[69], who calculated the AUROC for a hypothetic liver histology surrogate marker against the biopsy for a range of possible performances of both tests. The authors found that an ideal marker (99% accuracy) could in the best possible setting (sensitivity and specificity of biopsy 90%, prevalence of significant disease 40%) have an AUROC of not more than 0.90. This may mean that, unless an alternative gold standard is found, we might as well be in pursuit of something that isn't there.

REFERENCES

1 **Ratziu V**, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010; **53**: 372-384
2 **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
3 **Matteoni CA**, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419
4 **Adams LA**, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138
5 **Söderberg C**, Stål P, Askling J, Glaumann H, Lindberg G, Marmur J, Hultcrantz R. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology* 2010; **51**: 595-602
6 **Fracanzani AL**, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology* 2008; **48**: 792-798
7 **Minervini MI**, Ruppert K, Fontes P, Volpes R, Vizzini G, de Vera ME, Gruttadauria S, Miraglia R, Pipitone L, Marsh JW, Marcos A, Gridelli B, Demetris AJ. Liver biopsy findings from healthy potential living liver donors: reasons for disqualification, silent diseases and correlation with liver injury tests. *J Hepatol* 2009; **50**: 501-510
8 **Mofrad P**, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* 2003; **37**: 1286-1292
9 **Sorrentino P**, Tarantino G, Conca P, Perrella A, Terracciano ML, Vecchione R, Gargiulo G, Gennarelli N, Lobello R. Silent non-alcoholic fatty liver disease-a clinical-histological study. *J Hepatol* 2004; **41**: 751-757
10 **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
11 **Ratziu V**, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005; **128**: 1898-1906
12 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685
13 **Angulo P**, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; **30**: 1356-1362
14 **Harrison SA**, Torgerson S, Hayashi PH. The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. *Am J Gastroenterol* 2003; **98**: 2042-2047
15 **Dixon JB**, Bhathal PS, O'Brien PE. Weight loss and non-alcoholic fatty liver disease: falls in gamma-glutamyl transferase concentrations are associated with histologic improvement. *Obes Surg* 2006; **16**: 1278-1286
16 **Tahan V**, Canbakan B, Balci H, Dane F, Akin H, Can G, Hatemi I, Olgac V, Sonsuz A, Ozbay G, Yurdakul I, Senturk H. Serum gamma-glutamyltranspeptidase distinguishes nonalcoholic fatty liver disease at high risk. *Hepatogastroenterology* 2008; **55**: 1433-1438
17 **Saadeh S**, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 745-750
18 **Yin M**, Talwalkar JA, Glaser KJ, Manduca A, Grimm RC, Rossman PJ, Fidler JL, Ehman RL. Assessment of hepatic fibrosis with magnetic resonance elastography. *Clin Gastroenterol Hepatol* 2007; **5**: 1207-1213.e2
19 **Chen J**, Talwalkar JA, Yin M, Glaser KJ, Sanderson SO, Ehman RL. Early detection of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease by using MR elastography. *Radiology* 2011; **259**: 749-756
20 **Iijima H**, Moriyasu F, Tsuchiya K, Suzuki S, Yoshida M, Shimizu M, Sasaki S, Nishiguchi S, Maeyama S. Decrease in accumulation of ultrasound contrast microbubbles in nonalcoholic steatohepatitis. *Hepatol Res* 2007; **37**: 722-730
21 **Castera L**, Denis J, Babany G, Roudot-Thoraval F. Evolving practices of non-invasive markers of liver fibrosis in patients with chronic hepatitis C in France: time for new guidelines? *J Hepatol* 2007; **46**: 528-529; author reply 528-529
22 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, Kowo M, Chan AW, Merrouche W, Sung JJ, de Lédinghen V. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 454-462
23 **Yoneda M**, Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, Nozaki Y, Yonemitsu K, Higurashi T, Takahashi H, Kobayashi N, Kirikoshi H, Abe Y, Inamori M, Kubota K, Saito S, Tamano M, Hiraishi H, Maeyama S, Yamaguchi N, Togo

S, Nakajima A. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). *Dig Liver Dis* 2008; **40**: 371-378
24 **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
25 **Tsochatzis EA**, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. *J Hepatol* 2011; **54**: 650-659
26 **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
27 **Foucher J**, Castéra L, Bernard PH, Adhoute X, Laharie D, Bertet J, Couzigou P, de Lédinghen V. Prevalence and factors associated with failure of liver stiffness measurement using FibroScan in a prospective study of 2114 examinations. *Eur J Gastroenterol Hepatol* 2006; **18**: 411-412
28 **Yoneda M**, Suzuki K, Kato S, Fujita K, Nozaki Y, Hosono K, Saito S, Nakajima A. Nonalcoholic fatty liver disease: US-based acoustic radiation force impulse elastography. *Radiology* 2010; **256**: 640-647
29 **Palmeri ML**, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B, Diehl AM, Nightingale KR. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. *J Hepatol* 2011; **55**: 666-672
30 **Friedrich-Rust M**, Wunder K, Kriener S, Sotoudeh F, Richter S, Bojunga J, Herrmann E, Poynard T, Dietrich CF, Vermehren J, Zeuzem S, Sarrazin C. Liver fibrosis in viral hepatitis: noninvasive assessment with acoustic radiation force impulse imaging versus transient elastography. *Radiology* 2009; **252**: 595-604
31 **Wieckowska A**, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. *Hepatology* 2006; **44**: 27-33
32 **Feldstein AE**, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. *Hepatology* 2009; **50**: 1072-1078
33 **Yilmaz Y**, Dolar E, Ulukaya E, Akgoz S, Keskin M, Kiyici M, Aker S, Yilmaztepe A, Gurel S, Gulten M, Nak SG. Soluble forms of extracellular cytokeratin 18 may differentiate simple steatosis from nonalcoholic steatohepatitis. *World J Gastroenterol* 2007; **13**: 837-844
34 **Fitzpatrick E**, Mitry RR, Quaglia A, Hussain MJ, DeBruyne R, Dhawan A. Serum levels of CK18 M30 and leptin are useful predictors of steatohepatitis and fibrosis in paediatric NAFLD. *J Pediatr Gastroenterol Nutr* 2010; **51**: 500-506
35 **Diab DL**, Yerian L, Schauer P, Kashyap SR, Lopez R, Hazen SL, Feldstein AE. Cytokeratin 18 fragment levels as a noninvasive biomarker for nonalcoholic steatohepatitis in bariatric surgery patients. *Clin Gastroenterol Hepatol* 2008; **6**: 1249-1254
36 **Tsutsui M**, Tanaka N, Kawakubo M, Sheena Y, Horiuchi A, Komatsu M, Nagaya T, Joshita S, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Aoyama T, Tanaka E, Sano K. Serum fragmented cytokeratin 18 levels reflect the histologic activity score of nonalcoholic fatty liver disease more accurately than serum alanine aminotransferase levels. *J Clin Gastroenterol* 2010; **44**: 440-447
37 **Yoneda M**, Mawatari H, Fujita K, Iida H, Yonemitsu K, Kato S, Takahashi H, Kirikoshi H, Inamori M, Nozaki Y, Abe Y, Kubota K, Saito S, Iwasaki T, Terauchi Y, Togo S, Maeyama S, Nakajima A. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. *J Gastroenterol* 2007; **42**: 573-582
38 **Targher G**. Relationship between high-sensitivity C-reactive protein levels and liver histology in subjects with non-alcoholic fatty liver disease. *J Hepatol* 2006; **45**: 879-881; author reply 881-882
39 **Hui JM**, Farrell GC, Kench JG, George J. High sensitivity C-reactive protein values do not reliably predict the severity of histological changes in NAFLD. *Hepatology* 2004; **39**: 1458-1459
40 **Wieckowska A**, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol* 2008; **103**: 1372-1379
41 **Haukeland JW**, Damås JK, Konopski Z, Løberg EM, Haaland T, Goverud I, Torjesen PA, Birkeland K, Bjøro K, Aukrust P. Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated levels of CCL2. *J Hepatol* 2006; **44**: 1167-1174
42 **Baeck C**, Wehr A, Karlmark KR, Heymann F, Vucur M, Gassler N, Huss S, Klussmann S, Eulberg D, Luedde T, Trautwein C, Tacke F. Pharmacological inhibition of the chemokine CCL2 (MCP-1) diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. *Gut* 2012; **61**: 416-426
43 **Abderrahim-Ferkoune A**, Bezy O, Chiellini C, Maffei M, Grimaldi P, Bonino F, Moustaid-Moussa N, Pasqualini F, Mantovani A, Ailhaud G, Amri EZ. Characterization of the long pentraxin PTX3 as a TNFalpha-induced secreted protein of adipose cells. *J Lipid Res* 2003; **44**: 994-1000
44 **Yoneda M**, Uchiyama T, Kato S, Endo H, Fujita K, Yoneda K, Mawatari H, Iida H, Takahashi H, Kirikoshi H, Inamori M, Nozaki Y, Kobayashi N, Kubota K, Saito S, Maeyama S, Sagara M, Aburatani H, Kodama T, Nakajima A. Plasma Pentraxin3 is a novel marker for nonalcoholic steatohepatitis (NASH). *BMC Gastroenterol* 2008; **8**: 53
45 **Chalasani N**, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2004; **99**: 1497-1502
46 **Horoz M**, Bolukbas C, Bolukbas FF, Sabuncu T, Aslan M, Sarifakioglullari S, Gunaydin N, Erel O. Measurement of the total antioxidant response using a novel automated method in subjects with nonalcoholic steatohepatitis. *BMC Gastroenterol* 2005; **5**: 35
47 **Bonnefont-Rousselot D**, Ratziu V, Giral P, Charlotte F, Beucler I, Poynard T. Blood oxidative stress markers are unreliable markers of hepatic steatosis. *Aliment Pharmacol Ther* 2006; **23**: 91-98
48 **Targher G**, Bertolini L, Rodella S, Zoppini G, Scala L, Zenari L, Falezza G. Associations between plasma adiponectin concentrations and liver histology in patients with nonalcoholic fatty liver disease. *Clin Endocrinol* (Oxf) 2006; **64**: 679-683
49 **Hui JM**, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004; **40**: 46-54
50 **Bugianesi E**, Pagotto U, Manini R, Vanni E, Gastaldelli A, de Iasio R, Gentilcore E, Natale S, Cassader M, Rizzetto M, Pasquali R, Marchesini G. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. *J Clin Endocrinol Metab* 2005; **90**: 3498-3504
51 **Musso G**, Gambino R, Biroli G, Carello M, Fagà E, Pacini G, De Michieli F, Cassader M, Durazzo M, Rizzetto M, Pagano G. Hypoadiponectinemia predicts the severity of hepatic fibrosis and pancreatic Beta-cell dysfunction in nondiabetic nonobese patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2005; **100**: 2438-2446
52 **Abiru S**, Migita K, Maeda Y, Daikoku M, Ito M, Ohata K, Nagaoka S, Matsumoto T, Takii Y, Kusumoto K, Nakamura M, Komori A, Yano K, Yatsuhashi H, Eguchi K, Ishibashi H. Serum cytokine and soluble cytokine receptor levels in patients

with non-alcoholic steatohepatitis. *Liver Int* 2006; **26**: 39-45
53 **Chitturi S**, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002; **35**: 373-379
54 **Shimada M**, Kawahara H, Ozaki K, Fukura M, Yano H, Tsuchishima M, Tsutsumi M, Takase S. Usefulness of a combined evaluation of the serum adiponectin level, HOMA-IR, and serum type IV collagen 7S level to predict the early stage of nonalcoholic steatohepatitis. *Am J Gastroenterol* 2007; **102**: 1931-1938
55 **Kilpatrick ES**, Rigby AS, Atkin SL. Insulin resistance, the metabolic syndrome, and complication risk in type 1 diabetes: "double diabetes" in the Diabetes Control and Complications Trial. *Diabetes Care* 2007; **30**: 707-712
56 **Williams KV**, Erbey JR, Becker D, Arslanian S, Orchard TJ. Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes* 2000; **49**: 626-632
57 **Bulum T**, Kolarić B, Duvnjak L, Duvnjak M. Nonalcoholic fatty liver disease markers are associated with insulin resistance in type 1 diabetes. *Dig Dis Sci* 2011; **56**: 3655-3663
58 **Poynard T**, Ratziu V, Charlotte F, Messous D, Munteanu M, Imbert-Bismut F, Massard J, Bonyhay L, Tahiri M, Thabut D, Cadranel JF, Le Bail B, de Ledinghen V. Diagnostic value of biochemical markers (NashTest) for the prediction of non alcoholo steato hepatitis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006; **6**: 34
59 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321
60 **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
61 **Miele L**, Forgione A, La Torre G, Vero V, Cefalo C, Racco S, Vellone VG, Vecchio FM, Gasbarrini G, Rapaccini GL, Neuman MG, Grieco A. Serum levels of hyaluronic acid and tissue metalloproteinase inhibitor-1 combined with age predict the presence of nonalcoholic steatohepatitis in a pilot cohort of subjects with nonalcoholic fatty liver disease. *Transl Res* 2009; **154**: 194-201
62 **Suzuki A**, Angulo P, Lymp J, Li D, Satomura S, Lindor K. Hyaluronic acid, an accurate serum marker for severe hepatic fibrosis in patients with non-alcoholic fatty liver disease. *Liver Int* 2005; **25**: 779-786
63 **Lydatakis H**, Hager IP, Kostadelou E, Mpousmpoulas S, Pappas S, Diamantis I. Non-invasive markers to predict the liver fibrosis in non-alcoholic fatty liver disease. *Liver Int* 2006; **26**: 864-871
64 **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
65 **Ratziu V**, Massard J, Charlotte F, Messous D, Imbert-Bismut F, Bonyhay L, Tahiri M, Munteanu M, Thabut D, Cadranel JF, Le Bail B, de Ledinghen V, Poynard T. Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006; **6**: 6
66 **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
67 **Rosenberg WM**, Voelker M, Thiel R, Becka M, Burt A, Schuppan D, Hubscher S, Roskams T, Pinzani M, Arthur MJ. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004; **127**: 1704-1713
68 **Guha IN**, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, Kaye P, Burt AD, Ryder SD, Aithal GP, Day CP, Rosenberg WM. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology* 2008; **47**: 455-460
69 **Mehta SH**, Lau B, Afdhal NH, Thomas DL. Exceeding the limits of liver histology markers. *J Hepatol* 2009; **50**: 36-41

S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L