Diagnostic evaluation of Fibrotest panel as a Biomarkers for the prediction of fatty liver disease: A Review Article

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Abstract
Non-alcoholic fatty liver disease (NAFLD) is a potentially progressive disorder, representing a wide spectrum of disease from simple steatosis and different degrees of fibrosis to non-alcoholic steatohepatitis (NASH), and eventually cirrhosis. With a global prevalence of approximately 25%, NAFLD is now the leading cause of chronic liver disease worldwide and a growing challenge to public health. Haptoglobin, alpha2macroglobulin, apolipoprotein A1, play main roles in liver diseases, which can be analysed via blood tests which It is considered non-invasive method and replacement of liver fibro scan or biopsy. There are several drawbacks in using liver biopsy and Fibroscan for this purpose. Therefore, the needing for noninvasive diagnostic methods is urgent. this review would aim to focused on the crucial biomarkers related to the fibrosis stages progression.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a major public health concern because of its increased prevalence worldwide with a global prevalence of approximately 25% and potentially severe sequelae[1] . NAFLD is a hepatic manifestation of metabolic syndrome and a risk factor for type 2 diabetes mellitus, dyslipidemia, and hypertension[2] . NAFLD encompasses a broad spectrum of liver disorders , from simple steatosis and different degrees of fibrosis to non-alcoholic steatohepatitis (NASH), and eventually cirrhosis[3] .The hallmark of NAFLD is triglyceride (TG) accumulation in the cytoplasm of hepatocytes. This arises from an imbalance between lipid acquisition (ie, fatty acid uptake and de novo lipogenesis [DNL]) and removal (ie, mitochondrial fatty acid oxidation and de novo lipogenesis [DNL]) and removal (ie, mitochondrial fatty acid oxidation [FAO] and export as a component of very low-density lipoprotein [VLDL] particles)[3].

2. Pathogenesis

Major advances in our understanding of the pathogenesis have revealed the complexity of the disease. The 'two-hit' hypothesis has now been superseded by a 'multi-hit' model incorporating multiple interlocking processes, including lipotoxicity, innate immune activation and the microbiome on a background of genetic and environmental factors[4] . A detailed description is summarised in Fig 1 .

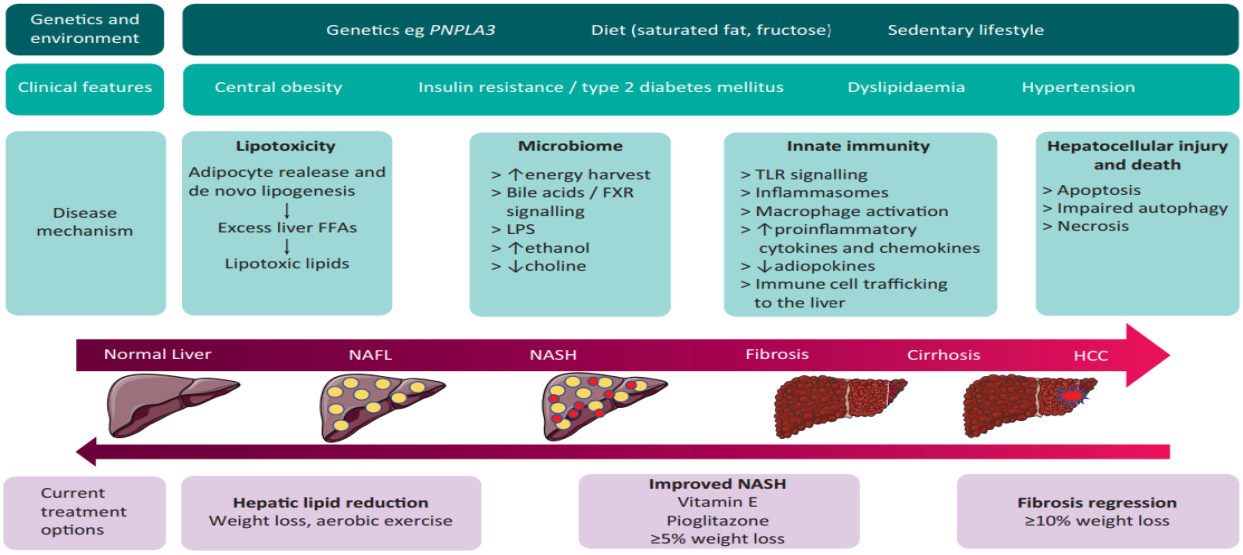

Fig 1. Pathophysiology and treatment options in NAFLD. DM = diabetes mellitus; FXR = farsinoid X receptor; HCC = hepatocellular carcinoma; LPS = lipopolysaccharide; NAFLD = non-alcoholic fatty liver; NASH = non-alcoholic steatohepatitis; PNPLA3 = patatin-like phospholipase domain-containing protein 3; TLR = toll-like receptor[4]

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3. Fibrosis stages

The most important predictor of adverse outcomes in NAFLD is the presence of fibrosis, rather than histological features of NASH[5] . There is a small increase in all-cause mortality even at very early fibrosis, which rises on a linear scale with progressive fibrosis stage. Early (F1) fibrosis is not associated with a significant increase in liver-related mortality, but notably this rises exponentially with increasing stage such that mortality rates from liver disease with bridging fibrosis (F3) and cirrhosis (F4) are 7.92 (per 1000 person years follow-up vs stage 0 fibrosis) and 23.3 respectively[6].

4. Diagnosis

Nonalcoholic Fatty Liver Disease (NAFLD) is currently reliably and increasingly being diagnosed and classified by non-invasive methods in comparison with standard invasive methods .NAFLD patients are mostly asymptomatic; although sixty six percent of NAFLD patients have normal liver enzymes however, asymptomatic liver enzyme elevation gives a clue to the diagnosis[7]. According to the latest guidelines established by the American Association for the Study of Liver Diseases (AASLD), NAFLD is diagnosed when the following 4 criteria are met: (1) fatty change of the liver is observed by imaging or histologically; (2) no marked alcohol drinking habit is present (3) no presence of other factors inducing fatty change of the liver; and (4) no concomitant factors causing chronic liver disease are present[8]. However, before labelling a person as NAFLD extensive detail history (alcohol abuse, drug intake) must be taken and serological studies (viral and autoimmune hepatitis, alpha-1-antitrypsin deficiency, hemochromatosis and Wilson's disease) should be done. crucial step in the management of NAFLD patients is the identification of advanced fibrosis , Fibrosis have stronger impact on outcome than inflammation. Authors have advocated, noninvasive scoring systems for fibrosis and inflammation as more accurate measure of global liver fibrosis severity than liver biopsy which samples only 0.02% of entire organ

Invasive method Liver biopsy

Despite the introduction of possible diagnostic techniques over the past 50 years, liver biopsy is still the gold standard approach for diagnosing liver fibrosis since it uses a tiny amount of liver tissue and can identify other harmful processes like necrosis, inflammation, and steatosis. The liver biopsy limitation is very invasive. Furthermore, poor tissue quality and sample quality make biopsy non-reproducible and rely on the pathologist's experience, which causes interobserver variations. The risk associated with liver biopsy includes pain (84%), hypertension, bleeding (0.5%), and injury to the biliary system, as well as hemorrhagic complications (0.05%) and bacteremia (0.08%) [9]. Due to the limits of liver biopsy, the need for noninvasive diagnostic methods is urgent.

Non-invasive methods in NFLD diagnosis

1. Transient Elastography (Fibroscan)

non-invasive diagnostic device used to assess liver fibrosis, by measures the velocity of a low frequency (50 Hz) elastic shear wave propagating through the liver which is directly related to tissue stiffness, the stiffer the tissue, the faster the shear wave propagates. waves emitted by a probe to detect the transmission velocity at a depth of 2.5 cm to 6.5 cm beneath the skin. Its results are expressed in kilopascals (kPa) with a range from 2.5 to 75 kPa; a normal value is around 5 kPa[10]. When this method is used to diagnose significant fibrosis (METAVIR system: F ≥ 2) or liver cirrhosis (F4), the cutoff values are >7 kPa in the case of significant fibrosis (F2 to F4) and >11 kPa to 14 kPa in the case of liver cirrhosis. the interpretation of the findings should be carefully considered in the presence of limiting factors such as impossibility of obtaining reliable liver stiffness measurements in around 20% of cases, mainly comprising obese patients[10]. Also, transient elastography can fail for people with pre-hepatic portal hypertension [11].

2. Serum Biomarkers

Since liver biopsy is invasive, expensive and prone to sampling error, and The accuracy of grading and staging is dependent on their own experience of the pathologist. several clinical prediction rules and blood-based biomarkers have been developed as attractive and affordable alternatives for identification of patients at high risk of advanced fibrosis.

3. Galectini3 binding protein (G3BP):

also known as (Mac-2 binding protein or 90K, gene name LGALS3BP) Glycoproteins are a class of proteins containing glycans linked to amino acid side chains[12] , expressed by hematopoietic and epithelial cells but is also present in many other tissues including colon, duodenum, stomach, and lung . Furthermore, Gal-3BP is detectable in many body fluids like semen, saliva, urine, tears , human milk , and plasma, where it is associated with microparticles[13].the role for estimating liver fibrosis, Bekki et al. [14] found that hepatic stellate cells (HSCs) are the source of M2BPGi in subpopulations of liver-derived cells such as HSCs, Kupffer cells, endothelial cells, biliary epithelial cells, and hepatocytes. An in vitro study found that the addition of exogenous M2BPGi enhances Mac-2 (galectin 3) expression by Kupffer cells. Furthermore, in cocultures of HSCs and Kupffer cells, alpha-SMA expression by HSCs is increased, which is reduced by Mac2 depletion from Kupffer cells. These findings suggest that M2BPGi is a juxtacrine-acting messenger sent by HSCs to Kupffer cells during liver fibrosis and that it plays an important role in the progression of fibrosis (Fig. 1). Thus, M2BPGi levels reflect the activation of HSCs during the progression

of liver fibrosis, in contrast to the amount of collagen. This may explain the rapid decrease of M2BPGi levels after patients with hepatitis C achieve a sustained virus response (SVR). An immunohistochemical analysis of cirrhotic human liver found that Mac-2 (galectin 3) and M2BPGi are expressed by CD68-positive cells, which are likely Kupffer cells. Therefore, M2BPGi may interact with Mac-2-positive cells to induce biological activity. Mac-2 is involved in diverse functions such as cell adhesion, growth regulation, cytokine production, T cell apoptosis, and immune responses. For example, Kianoush et al. [15] suggest that Mac-2 induces M2 polarization of macrophages, and other studies indicate that Mac-2 may stimulate cancer progression[16] . Thus, the biological activities of M2BPGi mediated by Mac2 may explain the high incidence of the development of HCC development in patients with high M2BPGi levels. other studies showed increased expression of Gal-3BP in different phenotypes of metabolic syndrome, including NAFLD, obesity, and silent atherosclerosis[17]

4. Alpha 2 Macroglobulin (α2M)

is a major extracellular glycoprotein in the blood of 725 kDa that consists of four identical subunits of 185 kDa that are linked in pairs by disulfide bonds, synthesized in hepatocytes and stellate cells. It functions as an antiprotease, which can inactivate a great variety of proteinases, like trypsin, chymotrypsin, elastase or matrix metalloproteinases [MMPs]. Human A2M is carrier protein as it able to bind a very wide range of cytokines, growth factors, especially TGF-ß1, TNF-alpha and IL-1ß and hormones[18]. In patients with NAFLD, alpha 2 macroglobulins engaging in hepatocyte-mediated fibrogenic response[19].Some studies show that A2M was increasing in the inflammatory or injured liver, and inhibits catabolism of matrix proteins, and thus causes liver fibrosis[20].

5. ApolipoproteiniA1

Is a 28 kDa glycoprotein that is the major protein component of high density lipoprotein (HDL) particles. HDL particles play a central role in the reverse transport of cholesterol from peripheral tissues to the liver. The main sites for apoA-I synthesis are the liver and intestine, with the liver being the major contributor to plasma apoA-I and HDL levels[21] , it enables efflux of fat molecules by accepting fats from within cells and transport elsewhere, including back to LDL particles or the liver for excretion.It has been shown that APOA1 levels were significantly decreased in NAFLD patients[22], which increase the risk of NAFLD developing [23]. In chronic liver disease, the decrease in ApoA1 according to the progression of liver fibrosis was first described in 1986 [50,51], and has been successively included in multivariate biomarkers, mostly combined with A2M and haptoglobin, for the surveillance of chronic liver diseases including NAFLD and NASH with or without T2DM [32–38,52]. In liver fibrosis, decreased serum ApoA1 is observed without hepatic insufficiency, the ApoA1 being trapped by the collagenization of the endothelial cells [51] before advanced fibrosis and before the hepatic insufficiency. In patients with cirrhosis, ApoA1 and HDL3 levels were significantly lower in patients who developed severe infection [53]. This sensitivity of ApoA1 is an advantage for its inclusion in multivariate biomarkers because of its prognostic value

6. Haptoglobin

is an abundant plasma protein produced mostly in hepatic cells containing 0.4–2.6% of total blood proteins, consists of two α- and two β-subunits[24].Plays a critical role for the elimination of free hemoglobin(Hb) and the neutralization of oxidative damage. Haptoglobin (Hp) binds to free hemoglobin in blood which released during red blood cell turnover, intravascular, and extravascular hemolysis through high-affinity binding to the macrophage scavenger receptor CD163, at the same time preventing loss of iron through the kidneys and protecting the kidneys from damage by hemoglobin. The haptoglobin-hemoglobin complex is then processed by the reticuloendothelial system (mostly spleen)[25]. In addition to this antioxidant function, haptoglobin has a role in the immune response and during the acute phase response. Haptoglobin can act either as an anti-inflammatory modulator or as a pro-inflammatory activator suppressing T cell proliferation and regulating the balance between T helper cells Th1 and Th2. aptoglobin is a marker of inflammation, its level increasing during infections, injuries, and malignancies[26].In patients at risk of NAFLD, haptoglobin is increased in obese patients with T2DM but decreased in patients who progress to advanced fibrosis[27]

7. Gamma Glutamyl transferase

Gamma-glutamyl transferase (GGT) is an enzyme located on the external surface of membranes of various cells, the molecular weight has been reported to vary between 38 to 72 kDa for the large and 20 to 66 kDa for the small GGT subunit. The large subunit has an intracellular N-terminal sequence, a transmembrane hydrophobic domain and an extracellular domain and is responsible for GGT anchorage on the cellular membrane surface whereas the small subunit harbors the enzyme active center [28]. GGT is present in all cells with the exception of erythrocytes. GGT activity was reported to be particularly high in tissues with secretory and absorptive function such as kidney, biliary system, intestine and epididymis and the enzyme activity is greatest in the ductal luminal surface of these tissues[29]. Abundant GGT activity has been demonstrated in the proximal tubule of the kidney. GGT activity is particularly intensive in biliary pole of hepatocytes and cholangiocytes[30] .GGT is produced as a single polypeptide chain which undergoes autoproteolytic cleavage into the large and small subunits. Human GGT is encoded by a

multigene family of at least 7 different genes located in the chromosome 22; nevertheless only 1 of these genes produces complete and functional GGT Between 50% and 77% of GGT activity is genetically determined[31] .

GGT functions are not entirely known. The localization of GGT in tissues with transport function has led to the suggestion that GGT is involved in the transport of amino acids via the "gamma-glutamyl cycle" . Cleavage of glutathione—the main thiol antioxidant in humans—is the most important physiological function of GGT. Glutathione has profound cellular functions including protection from oxidative stress, redox signaling, detoxification of xenobiotics, cellular proliferation, fibrogenesis, nitric oxide metabolism, storage and transport of cysteine, sulphur metabolism and apoptosis [32]. Thus GGT contributes in maintaining the physiological concentrations of glutathione in cytoplasm and cellular defense against oxidative stress. Other functions of GGT include involvement in the metabolism of leukotrienes, xenobiotics and glutaminase action (cleavage of amide bond of amino acid glutamine to produce glutamate and ammonium) [29] . Several cross-sectional and prospective studies showed that plasma γ-GT is associated with metabolic syndrome risk factors and with markers of inflammation [33].In this line, recent studies suggest that plasma γ-GT is a significant predictor of NAFLD , It can rise by 2 to 3 times in 50 percent of the patients with NAFLD[34].and that more specifically elevated γ-GT levels are associated with a more severe histological spectrum of NAFLD, namely the presence of NASH and fibrosis [35].being this test also included in non-invasive scores for hepatic fibrosis assessment[36].

8. Total bilirubin

bilirubin is the end product of heme catabolism and originates primarily from the breakdown of erythrocyte hemoglobin in the reticuloendothelial system. It is included both conjugated (direct) and unconjugated (indirect) bilirubin. Bilirubin is a poorly soluble in water and when circulating in blood is mostly bound to serum albumin. The liver is responsible for clearance the blood of unconjugated bilirubin by conjugating it (modified to make it water-soluble) through an enzyme named UDPglucuronosyltransferase[37]. Bilirubin, and particularly unconjugated bilirubin, has been found to possess potential antagonizing oxidative stress and inflammatory properties by acting as antioxidant and cytoprotectant *in vitro* and *in vivo*[38] . It has been recognized that unconjugated bilirubin contains hepatic anti-fibrogenic. So, Unconjugated hyperbilirubinemia is inversely associated with the histopathological severity of liver damage in non-alcoholic fatty liver disease[39]. several previous studies have been performed to examine the association between bilirubin levels and the risk of NAFLD[40], [41]. Chang et al., found that serum bilirubin levels are inversely associated with the prevalence of NAFLD independent of known metabolic risk factors[42].

9. Fibro test:

(Fibro Sure in USA) was patented since 2001 by APHP (Assistance publique - Hopitaux de Paris), the Parisian public hospital system. It is is a multi-marker panel, with acceptable performance for detecting fibrosis in patients with non-alcoholic fatty liver disease (NAFLD).It is based on age, gender, serum haptoglobin, α2 macroglobulin, apolipoprotein A1, γGT and bilirubin, calculated be the following equation [43][44]

z= 4.467x logo [alpha2 macroglobulin (g/1)]-1.357x logto [haptoglobin (g/1)]
+1.017x log IyGT (IU/L)]+0.0281x[age (years)]
+1.737x log [bilirubin (u mol/L)}]
-1.184x [apolipoprotein Al (g/1)]+0.301x sex (female = 0, male = 1) -5.54

FT scores range from zero to 1.00, with higher scores indicating a greater probability of significant lesions. The predetermined FT conversion for the METAVIR fibrosis stage scoring system (scoring system for histological staging of liver fibrosis is the most used system in clinical practice today on a scale of F0-F4) is 0.00–0.27 for F0;.0.27–0.48 for F1;.0.48–0.58 for F2;.0.58–0.74 for F3;.0.74 for F4 [43][45][46] . as shown in table. Where a stage of F2 or above indicates significant fibrosis, which implies that a patient's fibrosis has progressed and clinical treatment is urgent. A stage of F4 indicates advanced fibrosis or precirrhosis

FibroTest	METAVIR score	Knodell score	Ishak score
0.75–1.00	F4	F4	F6
0.73–0.74	F3–F4	F3–F4	F5
0.59–0.72	F3	F3	F4
0.49–0.58	F2	F1–F3	F3
0.32–0.48	F1–F2	F1	F2–F3
0.28–0.31	F1	F1	F2
0.22–0.27	F0–F1	F0–F1	F1
0.00–0.21	F0	F0	F0

Conversion between FibroTest and fibrosis stages using METAVIR, Knodell and Ishak fibrosis scoring

systems .Data taken from[47].

10. Implications and contribution to the knowledge Gap

There is evidence that liver fibrosis stage is the most potent prognostic factor for NAFLD patients. Any progress in the fibrosis stage (from less than F2 to F3 and F4) is associated with a higher risk of long-term outcomes and an increase in liver-related mortality [48].

On the other hand, Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of conditions characterized histologically by excessive accumulation of hepatic fat in the absence of alcohol consumption. Two main histological patterns of NAFLD are described: fatty liver alone and steatohepatitis (NASH). NAFLD is an increasingly recognized cause of liver-related morbidity and mortality [49]. Although the majority of patients do not develop complications, 28% may develop serious liver sequelae, including end-stage liver disease and hepatocellular carcinoma [50]. Those at highest risk include patients with significant hepatic necro-inflammation and fibrosis [51].

There are several drawbacks in using liver biopsy and Fibroscan for this purpose. This procedure is invasive, costly, and prone to complications.

Since 2003, two panels of simple biochemical markers, named FibroTest (FT) were developed. The fibrosis index includes α2-macroglobulin (A2M), apolipoprotein A1, haptoglobin, total bilirubin, and γ-glutamyl- transpeptidase (GGT). A few accuracy studies have evaluated the performance of fibrosis index in comparison to other blood tests in detecting different stages of fibrosis [52]. Therefore, there is a needing for further well-designed comparisons in the intended use population. These biomarkers might be useful tool as first-line procedures as they give an immediate result after a quick and easy-to-perform examination [53]

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