Review

FIB-4 First in diagnostic algorithm of MAFLD at the era of "Global Metabodemic "

Yoshio Sumida [1,*], Masashi Yoneda [1], Katsutoshi Tokushige [2], Miwa Kawanaka [3], Hideki Fujii [4], Masato Yoneda [5], Kento Imajo [5], Hirokazu Takahashi [6], Yuichiro Eguchi [7], Masafumi Ono [8], Yuichi Nozaki [9], Hideyuki Hyogo [10], Masahiro Koseki [11], Yuichi Yoshida [12], Takumi Kawaguchi [13], Yoshihiro Kamada [14], Takeshi Okanoue [15], Atsushi Nakajima [5] and Japan Study Group of NAFLD (JSG-NAFLD) [†]

[1] Division of Hepatology and Pancreatology, Department of Internal Medicine, Aichi Medical University, Nagakute, Aichi 480-1195, Japan; yoneda@aichi-med-u.ac.jp

[2] Department of Internal Medicine, Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo 162-8666, Japan; tokushige.ige@twmu.ac.jp

[3] Department of General Internal Medicine2, Kawasaki Medical School, Okayama 700-8505, Japan; m.kawanaka@med.kawasaki-m.ac.jp

[4] Department of Hepatology, Graduate School of Medicine, Osaka City University, Osaka 558-8585, Japan; rolahideki@med.osaka-cu.ac.jp

[5] Department of Gastroenterology and Hepatology, Yokohama City University Graduate School of Medicine, Yokohama 236-0004, Japan; yoneda-ycu@umin.ac.jp (M.Y.); kento318@yokohama-cu.ac.jp (K.I.); nakajima-tky@umin.ac.jp (A.N.)

[6] Department of Metabolism and Endocrinology, Faculty of Medicine, Saga University, Saga 840-8502, Japan; takahas2@cc.saga-u.ac.jp

[7] Loco Medical General Institute, Saga 840-8502, Japan; eguchiyu@cc.saga-u.ac.jp

[8] Division of Gastroenterology and Hepatology, Department of Internal Medicine, Tokyo Women's Medical University Medical Center East, Tokyo 116-8567, Japan; ono.masafumi@twmu.ac.jp

[9] Department of Gastroenterology, National Center for Global Health and Medicine, Tokyo 162-8655, Japan; ynozaki@hosp.ncgm.go.jp

[10] Department of Gastroenterology, JA Hiroshima General Hospital, Hiroshima 738-8503, Japan; hidehyogo@ae.auone-net.jp

[11] Division of Cardiovascular Medicine, Department of Medicine, Osaka University Graduate School of Medicine, Suita Osaka 565-0871, Japan; koseki@cardiology.med.osaka-u.ac.jp

[12] Department of Gastroenterology and Hepatology, Suita Municipal Hospital, Osaka, 564-8567, Japan; yu1yoshida@gmail.com

[13] Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume 830-0011, Japan; takumi@med.kurume-u.ac.jp

[14] Department of Molecular Biochemistry & Clinical Investigation, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan; ykamada@gh.med.osaka-u.ac.jp

[15] Hepatology Center, Saiseikai Suita Hospital, Osaka 564-0013, Japan; okanoue@suita.saiseikai.or.jp

[*] Correspondence: sumida@koto.kpu-m.ac.jp; Tel.: +81-561-62-3311; Fax: +81-561-62-1508

Abstract

The prevalence of obesity or metabolic syndrome is increasing worldwide ("Globally metabodemic"). Approximately 25% of adult general population is suffering from nonalcoholic fatty liver disease (NAFLD) which has become serious health problem. Hepatic fibrosis is the most significant determinant of all cause and liver -related mortality in NAFLD. Noninvasive test (NIT) should be urgently required to evaluate hepatic fibrosis in NAFLD. Fibrosis-4 (FIB-4) index is the 1st triaging tool for excluding advanced fibrosis because of its accuracy, simplicity, and cheapness especially for general physicians or endocrinologists, although FIB-4 index has several drawbacks. Accumulating evidence has suggested that vibration controlled transient elastography (VCTE) and the enhanced liver fibrosis (ELF) test may become useful as the 2nd step after triaging by FIB-4 index. The leading cause of mortality in NAFLD is cardiovascular disease (CVD), extrahepatic malignancy, and liver-related diseases. NAFLD often complicates chronic kidney disease (CKD), resulting in increased simultaneous liver kidney transplantation (SLKT). FIB-4 index could be a predictor of not only liver-related mortality and incident hepatocullar carcinoma (HCC) but also prevalent and incident CKD, CVD, and extrahepatic malignancy. Although NITs as milestones for evaluating treatment efficacy have never been established, FIB- 4 index is expected to reflect histological hepatic fibrosis after treatment in several longitudinal studies. We here review the role of FIB-4 index as 1st step NIT in management of NAFLD.

Key words: hepatic fibrosis; hepatocullar carcinoma; vibration controlled transient elastography nonalcoholic fatty liver disease, type 2 diabetes; metabolic dysfunction associated fatty liver disease; cardiovascular disease

1. Introduction

Obesity associated disease is the most serious health problem worldwide (so called "Metabodemic") [1]. In adult population, 25% of general population are estimated to be suffering from nonalcoholic fatty liver disease (NAFLD) [2]. Life-style related diseases such as obesity, type 2 diabetes (T2D), dyslipidemia, and hypertension are closely associated with NAFLD. The nomenclature of NAFLD should be updated to metabolic dysfunction associated fatty liver disease (MAFLD) [3]. Global experts suggest that the term "MAFLD" is more appropriate than NAFLD. NAFLD has been diagnosed after exclusion of other liver diseases [3], while MAFLD can coexist with other liver diseases. Therefore, MAFLD plus hepatitis B virus (HBV) inactive carrier, MAFLD plus alcoholic liver disease (ALD), MAFLD plus autoimmune hepatitis (AIH) or MAFLD plus drug induced liver injury (DILI) are plausible as final diagnosis in clinical practice. Hepatic fibrosis is the most important risk factor for not only incident HCC but also liver-related mortality in NAFLD [4]. Liver biopsy is now the gold standard for evaluating hepatic fibrosis, but it has several drawbacks such as hemorrhage risk, invasiveness, cost, observers' variability and patients' unwillingness. Considering a large population of NAFLD, non-invasive tests (NITs) without performing liver biopsy are urgently required [5]. The American Association for the Study of Liver Disease (AASLD) practice guidance 2018 recommend use of NAFLD fibrosis score (NFS), fibrosis-4 (FIB-4) index, vibration-controlled transient elastography (VCTE), and magnetic resonance elastography (MRE) [6]. However, all institutions do not have these innovative imaging modalities such as VCTE or MRE. FIB-4 index consisting four parameters such as age, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and platelet is a simple, cheap, and accurate parameter [7,8]. We here review the role of FIB-4 index for evaluation of hepatic fibrosis, incident comorbidities, carcinogenesis (HCC and extrahepatic malignancy), over-all/liver-related mortality or morbidity, treatment efficacy in the management of NAFLD.

2.   Which fibrosis stage should we pick up in NAFLD ?

Globally, fibrosis stages in NAFLD can be classified into F0, F1, F2, F3, and F4 [9,10]. F3 or F4 were defined as advanced fibrosis. Currently, NAFLD patients with advanced fibrosis should be detected for HCC surveillance considering cost-benefit balance [11,12]. Considering exponential increase in liver-related mortality in NAFLD patients with $\geq$ F2 compared with those with F0/1 (hazard ratio [HR] 9.57, 95% confidence interval [CI] 1.67-54.93) [4], we wonder which fibrosis stage (F2, F3, or F4) we should mine among a huge population of NAFLD. A variety of NITs for identifying advanced fibrosis in NAFLD have been established (Table 1). Vilar-Gomez et al. reported that NFS and the FIB-4 index are useful screening tools for determining the stage of liver fibrosis to be routinely applied in clinical practice [13]. Thus, the FIB-4 index and NFS are now recommended for excluding advanced fibrosis in the AASLD practice guidance 2018 [4].

The enhanced liver fibrosis (ELF) test is a non-invasive blood test that measures three direct markers of fibrosis: hyaluronic acid (HA), procollagen III amino-terminal peptide (PIIINP), and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1)[14]. According to a two step algorithm from EU [15], ELF test can be applied to the intermediate group of FIB-4 index (1.3-3.25). If NAFLD patients have an ELF score of 10.35 or above, they are likely to have advanced fibrosis. ELF can reduce unnecessary liver biopsies. Recently, the usefulness of ELF test was validated also in Japanese NAFLD population [16]. Combinations or sequential procedures using VCTE complement the diagnostic performance of ELF test for the identification of advanced fibrosis. On the view of economic cost, the combination of FIB-4 index plus ELF test is superior to the combination of FIB-4 index plus VCTE [17]. In the two step algorithm for identifying severe fibrosis in NAFLD, FIB-4 index has been established as the 1st step, while ELF score, VCTE, or MRE may be diagnostic modalities as the 2nd step.

Table 1. A variety of non-invasive tests (NITs) for identifying severe fibrosis (F3/4) in NAFLD

Index	Formula	Strengths	Weaknesses
FIB-4 index	(age [years] × AST [U/L]/(platelet count [10^9/L] × $\sqrt{}$ALT [U/L]) https://www.eapharma.co.jp/ medicalexpert/product/livact/f ib-4/calculator.html	・Simple (only four parameters) ・Accurate ・Validated globally	・Requires an intermediate group ・Overpredict in old patients ・Inferior in patients with T2D?
NAFLD fibrosis score	$-1.675 + 0.037$ × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glucose/diabetes (yes=1, no=0) + 0.99 × AST/ALT ratio - 0.013 × platelet count (×10^9/L) - 0.66 × albumin (g/dL) http://nafldscore.com/	・Validated globally ・Accurate	・Complex (six parameters) ・Requires an intermediate group ・Overpredict in old patients
APRI	AST to platelet ratio index	・Simple (only two parameters)	・conflicting results
BARD	BMI > 28 kg/m^2 = 1 point AST/ALT ratio > 0.8 = 2 points	・very Simple	・conflicting results

	Diabetes = 1 point		
CA-fibrosis index	1.5 × type IV collagen 7s (ng/ml) + 0.0264 × AST (IU/l)	・Simple (only two parameters)	・Only available in Japan ・No external validation studies
ELF test	−7.412 + (In [HA] × 0.681) + (In [P3NP] × 0.775) + (In [TIMP1] × 0.494)	・Accurate ・Validated globally	・High cost ? (three parameters)

NAFLD: nonalcoholic fatty liver disease, APRI: AST to platelet ratio index, BARD: BMI, AST/ALT ratio, and diabetes , ELF: enhanced liver fibrosis, FIB-4: fibrosis-4, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BMI: body mass index, HA: hyaluronic acid, PIIINP: aminoterminal propeptide of type III procollagen. TIMP-1: tissue inhibitor of matrix metalloproteinase type 1, CA: type IV collagen 7s and AST

3. The usefulness of FIB-4 index to evaluating severe fibrosis in NAFLD

The FIB-4 index is a score based on readily available blood tests that are routinely measured (age, AST, ALT, and platelet count). FIB-4 index is originally developed for evaluating hepatic fibrosis in patients with HIV/HCV co-infection [18]. In NAFLD, the first report by Shah and colleagues in a study of 541 NAFLD patients found that FIB-4 index had better diagnostic accuracy for estimation of liver fibrosis among various serum markers [7]. FIB-4 index has been suggested as a prescreening strategy to improve the efficiency of referral for specialized liver care, prioritizing patients who are at higher risk of significant liver disease. First of all, diagnostic accuracy is superior to other simple NITs such as NFS, AST to platelet ratio index (APRI), and BARD (BMI, AST/ALT ratio, diabetes) score [7,8,19-23] (Table 1). FIB-4 index could differentiate between nonalcoholic steatohepatitis (NASH) and nonalcoholic fatty liver (NAFL), even when NASH patients with mild or no fibrosis [24]. FIB-4 index has several advantages. First, calculation of FIB-4 index requires only four parameters, age, AST, ALT and platelet count, while calculating formula of NFS is slightly complex [25] (Table 1). Second, FIB-4 index is available even in NAFLD patients with normal ALT levels [26,27]. A meta-analysis proved that 25% NAFLD patients and 19% NASH patients possess the normal ALT value [26]. Another strength of FIB-4 index is the availability of free online calculators (https://www.eapharma.co.jp/medicalexpert/product/livact/fib-4/calculator.html). At Aichi Medical University, we introduced a calculation formula of FIB-4 index on the inspection request screen of the electronic medical chart (Figure 1). NAFLD patients with high FIB-4 index are more frequently referred from general physicians or endocrinologists to hepatologists than before.
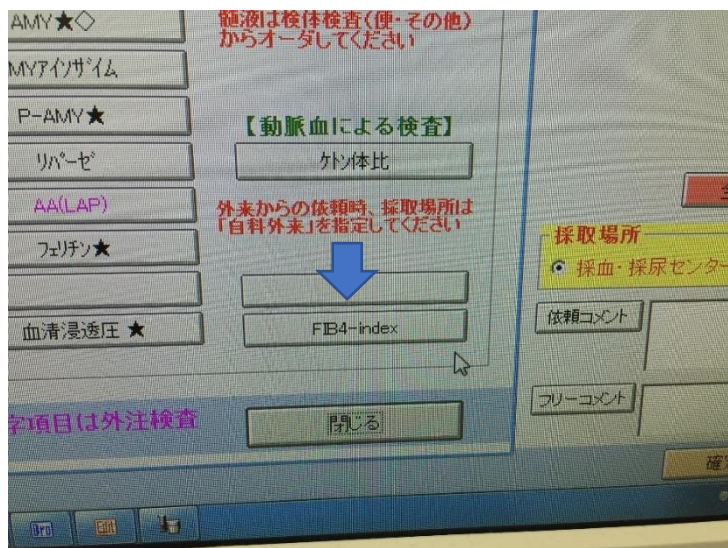
Figure 1. FIB-4 index on the inspection request screen of the electronic medical chart in Aichi Medical University

4. The compassion between FIB-4 index and VCTE

To assess liver fibrosis, several noninvasive US-based elastography techniques have been developed. These methods include VCTE (FibroScan; Echosens, Paris, France), acoustic radiation force impulse (ARFI) imaging and shear wave elastography (SWE) [28]. US-based VCTE performed with the FibroScan (Echosens) is the most thoroughly validated and commonly used elastography method worldwide. A systematic review and meta-analysis of VCTE in patients with NAFLD by Kwok et al. indicated that VCTE is good for the diagnosis of F3 (85% sensitivity and 82% specificity) and excellent for F4 (92% sensitivity and 92% sensitivity). However, it has a slightly lower accuracy for diagnosing F2 (79% sensitivity and 75% specificity)[29]. VCTE has several limitations. VCTE is limited to referral centers due to high equipment cost and had substantial failure rate, especially in obese patients. VCTE has a better diagnostic accuracy for advanced fibrosis than both FIB-4 index and NFS only in nonobese and/or low ALT patients [30]. However, liver stiffness measurement (LSM) by VCTE is influenced by not only hepatic fibrosis but also a various factors, including steatosis, inflammation, congestion, and cholestasis. LSM has also intra- or inter- observers' variability. The two step algorhythm using FIB-4 index as 1st step followed by VCTE as 2nd step have been proposed in US, Canada, and Asia [31-35]. Optimal cuttoff value of LSM for identifying advanced fibrosis should be disscussed.

5. FIB-4 index and carcinogenesis

In HCV, increased risk for HCC persists up to 10 years after HCV eradication in patients with baseline cirrhosis or high FIB-4 index [36]. In hepatis virus infected patients, a meta-analysis confirmed prognostic values of the FIB-4 index for overall survival and recurrence-free survival in HCC [37]. In NAFLD, Kanwal et al. showed that an FIB-4 index > 2.67 is associated with an increased risk of HCC not only in those with known cirrhosis but also in those without a prior diagnosis of cirrhosis [38]. It is noteworthy whether FIB-4 index can be a predictor of incident malignancy in NAFLD, including HCC. NAFLD patients had higher risk of HCC, colon cancer, and breast cancer compared with non-NAFLD population [39]. NAFLD patients with FIB-4 index >1.45 had higher risk of all cancer incidence compared to those with FIB-4 index <1.45 (HR: 13.99, 95% CI: 3.00-65.23) [39]. In another study, FIB-4 index and NFS can predict HCC development and extra-cancer incidence, although a number of NAFLD patients involved in this study is small (n=123) [40]. In Japan, the FIB-

4 index was useful for predicting liver-related diseases but had limitations in predicting extrahepatic malignancies [41]. The relationship between NITs and extrahepatic cancer should be explored further.

It remains to be solved whether hepatic fibrosis could accelerate carcinogenesis in extrahepatic organs.

6. FIB-4 index and mortality

NAFLD patients with higher FIB-4 index are associated with increased liver disease and overall mortality [42-45] **(Table 2)**. When NITs are applied to the general population, NITs did not become better predictor of severe liver disease than expected [40]. In NAFLD with diabetes, FIB-4 index, NFS, and APRI cannot predict liver-related mortality and morbidity [47]. In Japan, liver related mortality is extremely low in US-diagnosed NAFLD patients (9/4,073) [48]. The main cause of mortality is cardiovascular events and extrahepatic malignancies in that study. NFS can stratify risk of cardiovascular events and extrahepatic malignancies [48]. FIB-4 index is also associated with all-cause mortality of systemic chronic diseases such as rheumatoid arthritis [49], microscopic polyangiitis, granulomatosis with polyangiitis [50] and chronic obstructive pulmonary disease [51]. The underlying mechanisms of these relationships remain unknown.

Table 2. NITs predicting for over-all mortality/morbidity, liver-related mortality/morbidity, liver related event, CVD, mortality, and extrahepatic cancer incidence in NAFLD

Subjects	N	Nation	Dx	Observation period	Over-all Mortality /morbidity	Liver-related mortality/morbidity	Liver event	HCC	CVD mortality	Extrahepatic cancer
NAFLD [45]	646	Sweden	biopsy	19.9 ±8.7 yr	FIB-4 ○ NFS ○		FIB-4○ NFS○			
viral hepatitis negative adults [44]	14,841	US	General population	median 19.3 yr (IRQ, 17.5-21.1) yr	APRI ○ FIB-4 ○ NFS ○ Forns score○	APRI ○ FIB-4 ○ NFS ○ Forns score○			FIB-4 ○	APRI ○
NAFLD [40]	153	Israel	biopsy	100mo (mean)	FIB-4 ○ NFS ○ APRI ×			FIB-4○ NFS○ APRI○		FIB-4 ○ NFS ○ APRI ○
NAFLD [52]	180	China	US	6.6 (range 0.5-14.8) yr	NFS ◎ FIB-4○ APRI× BARD×					
NAFLD [41]	646	Japan	biopsy		FIB-4 ○			FIB-4○		FIB-4×
NAFLD [48]	4,073	Japan	US						NFS○	NFS○
NAFLD with diabetes [47]	284	Australia.	US	51.4 (range 6.1-146). mo		NFS × FIB-4 × APRI ×				
NAFLD [43]	11,154	US	US	14.5 yr	FIB-4 ○ NFS ○ APRI ○				FIB-4○ NFS○ APRI○	
NASH [53]	148	Canada	biopsy	Median: 5 years (IQR: 3-8)	FIB-4 ○ NFS ○ APRI ○					

NAFLD [42]	153	US	biopsy	Median 104.8 (range, 3-317) mo		NFS ◎ FIB-4 ○ APRI ○					

HCC: hepatocellular carcinoma, CVD: cardiovascular disease, FIB-4 : fibrosis-4, NFS: NAFLD fibrosis score, APRI : AST to platelet ratio index, IQR: interquartile range.

Forns score=$7.811 - 3.131 \log(\text{platelet count } [10^9/\text{L}]) + 0.781 \log(\text{GGT [IU/L]}) + 3.467 \log(\text{age [years]}) - 0.014$ total cholesterol (mg/dL)

7. FIB-4 index and risk of cardiovascular disease

The leading cause of mortality in NAFLD patients is cardiovascular disease (CVD), followed by extrahepatic cancer and liver related diseases [54]. NAFLD is an independent risk factor of coronary sclerosis [55], atrial fibrillation (AF) [56], coronary artery disease (CAD) and left ventricular dysfunction [57,58]. In daily clinical practice, we should pay attention to CVD event and control other risk factors, such as hypertension, dyslipidemia, and type 2 diabetes (T2D). FIB-4 index appears to be associated with high risk of CVD mortality [43]. Over a median follow-up time of 41.4 months (3044.4 patient-years) in 898 consecutive outpatients (mean age, 56.4 ± 12.7 years; 37.5% women), 58 cardiovascular events (1.9%/year) were registered. The rate of cardiovascular events was higher in patients with (n = 643, 2.1%/year) vs without NAFLD (n = 255, 1.0%/year) (p = .066). In multivariable Cox proportional regression analysis, NAFLD increased risk for cardiovascular events (HR, 2.41; 95% CI, 1.06-5.47; p = .036), after adjustment for metabolic syndrome. Among patients with NAFLD, male sex, previous cardiovascular events, metabolic syndrome and FIB-4 index ≥ 2.67 (HR, 4.02; 95% CI, 1.21-13.38; p = .023) were independently associated with risk of incident cardiovascular events [59]. A post hoc analysis of SAKURA AF Registry study showed that higher FIB-4 index ≥ 2.51 is independently associated with risks of CVD events and all-cause mortality in patients with AF [60]. Highest levels of NIT such as NFS, FIB-4 index, APRI, gamma-glutamyltransferase (GGT) to platelet ratio (GPR) and Forns score were associated with all-cause mortality and cardiovascular mortality [61]. In Japan, FIB-4 index is well correlated with coronary atherosclerosis (coronary artery calcium [CAC] score >100) and subjects with higher FIB-4 index were prone to receive percutaneous coronary intervention [62]. In 665 Korean NAFLD subjects, the NFS and FIB-4 index were associated with coronary atherosclerosis (CAC score >100) [63]. In patients with coronary artery disease (CAD), highest NITs of hepatic fibrosis are associated with increased risks of all-cause and cardiovascular mortality [64]. FIB-4 index is also associated with all-cause mortality in patients with heart failure (HF) [65]. Among 96,373 participants over 6.9 years, 3844 incident congestive heart failure (CHF) events occurred. FIB-4 between 1.45 and 3.25 and FIB-4 > 3.25 were associated with incident CHF (HR [95% CI], 1.17 [1.07-1.27] and 1.65 [1.43-1.92], respectively) [66]. This results suggest that hepatic fibrosis (mild to severe) is associated with incident HF in the general population.

8. FIB-4 index and risk of chronic kidney disease

NAFLD often complicates chronic kidney disease (CKD), resulting in growing indication for simultaneous liver kidney transplantation (SLKT) [67]. Risk of kidney graft loss was over 1.5-fold higher in recipients with NASH-cirrhosis than those with other etiologies [67]. A meta-analysis by Musso from Italy showed that NAFLD was associated with an increased risk of prevalent (OR 2.12,

95% CI 1.69-2.66) and incident (HR 1.79, 95% CI 1.65-1.95) CKD. Advanced fibrosis was associated with a higher prevalence (OR 5.20, 95% CI 3.14-8.61) and incidence (HR 3.29, 95% CI 2.30-4.71) of CKD than non-advanced fibrosis [68]. A variety of common drug pipelines exists for NASH and CKD [69, 70]. In a cross sectional study based on 755 patients with US-based diagnosed NAFLD, high FIB-4 index ($\geq$ 1.10) is associated with an increased risk of prevalent CKD. The area under the receiver operating characteristic curve (AUROC) was the greatest for FIB-4 index (0.750), followed by NFS (0.710), AAR (0.594), APRI (0.587), and BARD score (0.561). In an analysis of the National Health and Nutrition Examination Survey (NHANES) conducted in the USA between 1988 and 1994, FIB-4 index is the better predictor of an increased risk of prevalent CKD compared with NFS, BARD, and APRI score [71].

Annual rate of incident CKD in NAFLD patients is estimated to be about 1.2% [72]. Five factors of baseline low eGFR level (60-75 mL/min), aging, T2D, hypertension, and elevated GGT, increase the risk of the development of CKD [72]. High FIB-4 index is a significant risk factor for incident CVD, and patients with increased FIB-4 index showed larger reduction in eGFR compared with those with decreased FIB-4 index [73]. The association of PNPLA3 genotype with incident CVD is conflicting [73-75].

9.   Distribution of FIB-4 index in NAFLD population

The distribution of FIB-4 index in a healthy general population remains unknown, while some reports showed the distribution of FIB-4 index in NAFLD population. A total of 1,370 NAFLD patients (78.5%) exhibited a low cut-off index (COI) (<1.30), 357 (20.5%), exhibited an indeterminate COI (1.30-2.67), and 18 (1.0%) exhibited a high COI (>2.67) [76]. Among 5, 410 Japanese NAFLD patients who diagnosed by health checkups, 87.4% exhibited low COI (<1.45), 12.1% exhibited an indeterminate COI (1.45-3.26), and 0.5% exhibited a high COI (>3.26) [77]. On data of 576 NAFLD with biopsy proven NAFLD from JSG-NAFLD, 336 (58.3%) exhibited low COI (<1.45), 31.4% exhibited an indeterminate COI (1.45-3.26), and 59 (10.2%) exhibited a high COI (>3.26) [8]. Distribution of FIB-4 index in NAFLD depends on population age, ethnics, and selection bias (population-based , hospital-based or biopsy proven). We are now planning to clarify the distribution of FIB-4 index in a healthy general population undergoing health checkup or non-biased NAFLD population.

10. Drawbacks of FIB-4 index

FIB-4 index is a simple, reliable, and cheap parameter. Because FIB-4 index shows high negative positive value (NPV) for detecting advanced fibrosis, FIB-4 index is useful to exclude advanced hepatic fibrosis. However, the FIB-4 index has also several drawbacks [78]. First, FIB-4 index requires an intermediate group. NAFLD patients classified into that group have to receive other NITs or liver biopsies. After exclusion of no or mild fibrosis, 2nd step diagnosis should be applied to the intermediate group. In Europe, ELF test is usually applied to this intermediate group [15]. In US or Asia, VCTE has been inducted as 2nd step. Second, positive predictive value (PPV) for identifying advanced fibrosis is not so high, so FIB-4 index cannot help us to pick up advanced fibrosis. Third, low cutoff points of FIB-4 index are variable according to ethnics. Low cut-off value of FIB-4 index was generally accepted as 1.3 in western countries [7,79], while 1.45 in Asia [8,33,35]. Fourth, it is concerned that FIB-4 index may overpredict fibrosis in older patients [80,81], because its formula includes age. On the basis of data in JSG-NAFLD, the new proposed low cutoff points are 1.88 in 60–69 years, and 1.95 in $\geq$ 70 years [81]. Mcpherson and colleagues also suggested that 2.0 of low COI in 65 years or older [80]. On data of 1008 patients with NAFLD from nine centers across eight countries (The Gut and Obesity in Asia (GOASIA) Workgroup), NITs such as APRI, NFS, and FIB-4 index had a lower specificity in elderly (AUROC 0.62-0.65) [82]. Female (OR: 3.21; 95% CI 1.37-7.54] and hypertension (OR 3.68; 95%CI 1.11-12.23) were predicting factors for advanced fibrosis in the elderly [82].

Over-referral and under-referral are tradeoff relationship (**Table 3**). The problem of over-referral includes increased unnecessary liver biopsies, overwork of hepatologists, and high healthcare costs

[83]. Over-referral has merit such as burden for GP, early identification of HCC, resulting in improving all-over survival. The selection of over-referral or under-referral depends on hospital human resource, physicians ' or hepatologists' commitment for NASH.

Table 3. The tradeoff relationship between over-referral and under-referral for NAFLD

	Over-referral	Under-referral
FIB-4 index LCO	1.3	1.45
GP	Work ↓	Work ↑
Hepatologists	Work ↑	Work ↓
Unnecessary liver biopsy	May increase	May reduce
HCC early detection	Possible ?	May delay diagnosis?
Heath economic costs	High ?	Low ?

Fifth, FIB-4 index has limitations in a certain population of NAFLD patients. FIB-4 index showed significantly lower AUROCs for advanced fibrosis in obese NAFLD than in nonobese NAFLD [84]. Moreover, we found that FIB-4 index might be inferior in NAFLD patients with T2D compared to those without T2D [85]. In a study from Australia, NITs such as FIB-4 index, NFS, and APRI did not predict liver related events in 284 pts with NAFLD and diabetes [86]. Although its precise mechanism underlying inferiority of these NITs in T2D patients remains unknown, platelet count tends to be higher in NAFLD patients with T2D compared to those without T2D. FIB-4 index in NAFLD patients with T2D is also lower than in those without T2D at the same fibrosis stages. FIB-4 index had reasonable specificity (69.9%), but poor sensitivity for detecting advanced fibrosis (72.6%) in T2D [87]. Type IV collagen 7s is the best predictor in Japanese NAFLD patients with T2D [85]. The combination of type IV collagen 7s and AST (CA index) is also useful for detecting severe fibrosis [88].

Sixth, Shah S and colleagues feel that a low cut-off of 1.3 may be inappropriate, as it would include patients with F2 fibrosis [89]. They propose lowering the cut-off of FIB-4 index to 1.0 in order to capture F2 patients. F2 fibrosis confers an increased mortality of liver-related diseases compared with no fibrosis (F0) (HR: 2.52) [4]. "Active NASH" which requires intensive treatment is defined as NASH with NAFLD activity score (NAS) $\geq$ 4 and $\geq$ F2. Inclusion criteria in a variety of drug pipelines include NASH with NAS $\geq$ 4 and $\geq$ F2 [90,91]. FAST (FibroScan–AST) score, consisting of three parameters including FibroScan-based controlled attenuation parameter (CAP), FibroScan -based LSM, and AST, can predicts "active NASH" [92-94]. "Active NASH" patients had better receive intensive treatments for preventing progression to advanced stage. FAST score was designed to isolate "active NASH" patients with elevated NAS $\geq$ 4 and significant fibrosis ($\geq$ F2) who could benefit from early interventions with anti-steatohepatitis and/or antifibrotic agents.

Although several problems of FIB-4 index remain to be solved, FIB-4 index is believed to be enough as 1st triaging tool to exclude hepatic fibrosis, especially for general physicians or endocrinologists.

$$\text{FAST score} = \frac{e - 1.65 + 1.07 \times \ln(LSM) + 2.66 \times 10^{-8} \times CAP^3 - 63.3 \times AST^{-1}}{1 + e - 1.65 + 1.07 \times \ln(LSM) + 2.66 \times 10^{-8} \times CAP^3 - 63.3 \times AST^{-1}}$$

11. Two step diagnostic algorithm using FIB-4 index as 1st step

Globally, two step diagnostic algorithm using FIB-4 index as 1st step are generally accepted [79]. Assessment of the potential impact of implementing a FIB-4 first strategy to triage patients using a clinical referral pathway for suspected NAFLD was performed at a tertiary liver center in Canada [79]. FIB-4 first strategy would decrease costs and decrease unnecessary referrals as well as increase

access to screening in non-specialized facilities. It remains unknown which parameters are the most appropriate as 2nd step among a variety of NITs, including ELF test [15], Mac-2 binding protein glycated isomer (M2BPGi) [95-97], type IV collagen 7S [88,96], ProC3 [98], and autotaxin [99,100].

12. FIB-4 index as milestones of treatment in NAFLD

Hard endpoints of treatments such as over-all or liver-related mortality are difficult to evaluate. The gold standard to evaluate NASH treatment efficacy is now histological finding by liver biopsy. The primary endpoints are 1) NASH resolution without worsening fibrosis, or 2) fibrosis improvement more than 1 stage without worsening NASH. However, repeated biopsy is also difficult to perform, because of risk, patients' unwillingness, cost, and diagnostic variability. NITs monitoring treatment efficacy are urgently needed to avoid repeated liver biopsies for evaluation of treatment efficacy. Hepatic steatosis has been evaluated by innovative imaging modalities such as VCTE-based CAP, magnetic resonance imaging-proton density fat fraction (MRI-PDFF), or ultrasound-guided attenuation parameter (UGAP) [101-103]. However, it remains unknown that reduction in hepatic fat content can really result in amelioration of hepatic fibrosis in NAFLD. It also remains unknown whether NITs evaluating hepatic fibrosis in cross-sectional studies can reflect hepatic fibrosis also in longitudinal studies. Accumulating evidence has suggested that improvement in ABC (<u>A</u>LT, <u>b</u>ody weight, and A1<u>c</u>) related to ameliorating hepatic fibrosis [91] (Table 1). It is expected that FIB-4 index can become alternative to liver biopsies for evaluating treatment efficacy [104,105]. Finally, reduction in ALT, body weight, HbA1c, APRI and FIB-4 index may become milestones for ameliorate hepatic fibrosis in these longitudinal studies (Table 4).

Table 4 NITs or parameters for monitoring treatment efficacy in NASH/NAFLD

Author	Subjects	Outcomes	Parameter correlated with pathological improvement
Hamaguchi [106]	NAFLD (n=39)	Hepatic fibrosis	△HbA1c reduction
Seko [107]	NASH (n=52)	NAS Hepatic fibrosis	△ALT reduction ≥30% from baseline
Hoofnagle [108]	NASH (n=139) without DM PIVENS trial	NAS Hepatic fibrosis	△ALT reduction ≥30% from baseline or post-treatment ALT≤ 40 IU/L
Vilar-Gomez [109]	NASH (n=261)	NASH resolution w/o worsening fibrosis	△BW reduction, T2D (-), ALT normalization, younger age, NAS<5
Vuppalanchi [110]	Adult NASH (n=231) Pediatric NAFLD (n=152)	Histological improvement	△CK18 reduction (inferior to △ALT reduction)
Siddiqui [104]	NAFLD (n=292)	Hepatic fibrosis	△**FIB-4 index**, △NFS, △APRI
Jayakumar [111]	NASH, stage 2-3 (n=54) Selonsertib (Phase 2)	Hepatic fibrosis	△MRE
		Hepatic steatosis	MRI-PDFF ≥ 25% reduction

Chalasani [105]	NASH (n=200) FLINT trial (Phase2） Placebo vs OCA 72wk	Hepatic fibrosis	⊿**FIB-4 index** ○ ⊿APRI useful ○ ⊿NFS ×
Loomba [112]		NAS ≥ 2 points reduction without worsening fibrosis	OCA(+), pretreatment NAS>5, TG≤ 154 mg/dL, INR≤1, AST ≤49 IU/L, ⊿ ALT at 24wk (>17 IU/L)

NAFLD nonalcoholic fatty liver disease, NASH nonalcoholic steatohepatitis, DM diabetes mellitus, HbA1c glycated hemoglobin, NAS: NAFLD activity score, ALT alanine aminotransferase, BW body weight, CK18 cytokeratin 18, FIB-4 fibrosis-4, NFS NAFLD fibrosis score, APRI AST to platelet ration, MRE magnetic resonance elastography, MRI-PDFF magnetic resonance imaging-proton density fat fraction, OCA obeticholic acid, NAS NAFLD activity score, TG triglyceride, INR international normalized ratio, AST aspartate aminotransferase

13. NAFLD and coronavirus induced disease-19

Coronavirus induced disease-19 (COVID-19) due to severe acute respiratory syndrome coronavirus 2, which has become a global pandemic. Chinese studies have suggested that MAFLD was associated with severity of COVID-19 [113-115]. According to the fact that patients with FIB-4 ≥ 2.67 more frequently required mechanical ventilation (37.8% vs 18.3%; p=0 .009), hepatic fibrosis may affect natural history of COVID-19 [116]. Because comorbidities such as T2D, obesity, and hypertension are well known to be associated with severity of COVID-19 [117-119], it remains to be resolved whether MAFLD with hepatic fibrosis is independently related to clinical course of COVID-19.

14. Conclusions

In 2030, a number of Japanese NAFLD patients with advanced fibrosis are estimated to reach one million people. In China, about eight people will be suffering from advanced fibrosis [120]. Early identification of advanced fibrosis can result in early detection of HCC or early intervention for NASH patients. FIB-4 index is positioned as the 1st triaging tool for excluding advanced fibrosis due to its simplicity, low cost, and a predictor of liver-related or over-all mortality. It remains unknown which NITs are the most appropriate as the 2nd step in two step algorithm on the view of cost-benefit balance [121]. The ELF test, VCTE, MRE, and other hepatic fibrosis markers are expected. FIB-4 index can predict incident CVD, CKD, and extrahepatic cancer. FIB-4 index can also be used to evaluate treatment efficacy, although validation studies are required. Interface between primary care and second care is essential for stratifying high risk of HCC/hepatic decompensation to improve survival in a large population of NAFLD. It is concerned that primary care clinicians underestimate the prevalence of NAFLD and under-recognize the clinical spectrum of NAFLD [122].

Medicine, Aichi Medical University (Nagakute, Japan): Satoshi Kimoto. Department of Gastroenterology and Hepatology, Yokohama City University Graduate School of Medicine (Yokohama, Japan): Yuji Ogawa, Yasushi Honda, and Takaomi Kessoku. Department of Hepatology, Gifu Municipal Hospital (Gifu, Japan): Hideki Hayashi, Yoichi Nishigaki, Takafumi Uchiki, Yusuke Suzuki and Eiichi Tomita. Department of Internal Medicine, Himeji Red Cross Hospital (Himeji, Japan): Toshifumi Tada. Department of Gastroenterology and Metabolism, Graduate School of Biomedical and Health Sciences, Hiroshima University (Hiroshima, Japan): Takashi Nakahara. Department of Internal Medicine II, Shimane University Faculty of Medicine (Izumo, Japan): Hiroshi Tobita and Shuichi Satoh. Department of Medicine, Division of Gastroenterology and Hematology/Oncology, Asahikawa Medical University (Asahikawa, Japan): Koji Sawada. Department of Gastroenterology and Hepatology, Kochi Medical School (Kochi, Japan): Kensuke Munekage and Tsunehiro Ochi. Department of Internal Medicine, Institute of Gastroenterology, Tokyo Women's Medical University (Tokyo, Japan): Tomomi Kogiso. Department of Gastroenterology and Neurology, Faculty of Medicine, Kagawa University (Kagawa, Japan): Tomohiro Morishita. Department of Gastroenterology, Fukushima Medical University School of Medicine (Fukushima, Japan): Atsushi Takahashi. Second Department of Internal Medicine, Osaka Medical College (Osaka, Japan): Shinya Fukunishi. Department of Hepatology, Hamamatsu University School of Medicine (Hamamatsu, Japan): Kazuhito Kawata. Department of General Internal Medicine, Fukui-ken Saiseikai Hospital (Fukui, Japan): Kazuo Notsumata. Division of Gastroenterology and Hepatology, Department of Internal Medicine, National Defense Medical College (Tokorozawa, Japan): Kengo Tomita. Department of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University (Toyoake, Japan): Naoto Kawabe. Department of Gastroenterology, JA Hiroshima General Hospital (Hiroshima, Japan): Michihiro Nonaka. Division of Gastroenterology, Department of Internal Medicine, Nippon Medical School Musashikosugi Hospital (Kawasaki, Japan): Taeang Arai. Tokyo Medical University Ibaraki Medical Center, Ibaraki, Japan: Tadashi Ikegami. Department of Gastroenterology and Hepatology, Ogaki Municipal Hospital (Ogaki, Japan): Hidenori Toyoda and Takashi Kumada.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

AASLD	American Association for the study of Liver Diseases
AF	atrial fibrillation
AFP	α-Fetoprotein
AFP-L3	lens culinaris-agglutinin-reactive fraction of AFP
AGA	American Gastroenterology Association
AIM	apoptosis inhibitor of macrophage
AST	aspartate aminotransferase
ALD	alcoholic liver disease
ALT	alanine aminotransferase
APRI	AST to platelet ratio index
ARFI	acoustic radiation force impulse
AUROC	area under receiver operating characteristics curve
BMI	body mass index
HA	hyaluronic acid
PIIINP	aminoterminal propeptide of type III procollagen
TIMP-1	tissue inhibitor of matrix metalloproteinase type 1
CAC	coronary artery calcium
CAD	coronary artery disease

CAP	controlled attenuation parameter
CHF	congestive heart failure
CKD	chronic kidney disease
COI	cutoff index
COVID-19	coronavirus induced disease-19
CVD	cardiovascular disease
CI	confidence interval
CT	computed tomography
DILI	drug induced liver injury
eGFR	estimated glomerular filtration rate
ELF	enhanced liver fibrosis
ELISA	enzyme linked immunosolvent assay
FAST	FibroScan–AST
FIB-4	Fibrosis-4
GGT	gamma glutamyltransferas
HBV	hepatitis B virus
HCV	hepatitis C virus
HCC	hepatocellular carcinoma
HF	heart failure
HIV	human immunodeficiency virus
HR	hazard ratio
LSM	liver stiffness measurement
MAFLD	metabolism associated fatty liver disease
M2BPGi	Mac-2 binding protein glycosylation isomer
MRE	magnetic resonance elastography
MRI	magnetic resonance imaging
NAFL	nonalcoholic fatty liver
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NFS	NAFLD fibrosis score
NIT	noninvasive test
NPV	negative predictive value
OCA	obeticholic acid
OR	odds ratio
PDFF	proton density fat fraction
PIIINP	aminoterminal propeptide of type III procollagen
PNPLA3	patatin-like phospholipase domain-containing protein 3
PPV	positive predictive value
SLKT	simultaneous liver kidney transplantation

TIMP-1	tissue inhibitor of matrix metalloproteinase type 1,
T2D	type 2 diabetes
UCAP	ultrasound-guided attenuation parameter
US	ultrasonography
VCTE	vibration-controlled transient elastography

References

[1] GBD 2015 Obesity Collaborators, Afshin A, Forouzanfar MH, et al. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med*. **2017**, 377, 13-27.

[2] Younossi, Z., Anstee, Q. M., Marietti, M., Hardy, T., Henry, L., Eslam, M., George, J., Bugianesi, E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol*. **2018**, 15, 11-20.

[3] Eslam M, Newsome PN, Sarin SK Anstee, Q. M., Targher, G., Romero-Gomez, M., Zelber-Sagi, S., Wai-Sun Wong, V., Dufour, J. F., Schattenberg, J. M.,, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* **2020**, 73, 202.

[4] Dulai PS, Singh S, Patel J, Soni, M., Prokop, L. J., Younossi, Z., Sebastiani, G., Ekstedt, M., Hagstrom, H., Nasr, P., et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. *Hepatology*. **2017**, 65, 1557-65.

[5] Eguchi Y, Wong G, Akhtar O, Sumida Y. Non-invasive diagnosis of nonalcoholic steatohepatitis (NASH) and advanced fibrosis in Japan: a targeted literature review. *Hepatol Res*, **2020**, in press

[6] Chalasani, N.; Younossi, Z.; Lavine, J.E.; Charlton, M.; Cusi, K.; Rinella, M.; Harrison, S. A.; Brunt, E. M.; Sanyal, A. J. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* **2018**, *67*, 328–357.

[7] Shah, A. G., Lydecker, A., Murray, K., Tetri, B. N., Contos, M. J., Sanyal, A. J., & Nash Clinical Research Network. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. **2009**, 7, 1104-12

[8] Sumida Y, Yoneda M, Hyogo H, Itoh, Y., Ono, M., Fujii, H., Eguchi, Y., Suzuki, Y., Aoki, N., Kanemasa, K.,et al ; Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD). Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol*. **2012**, 12, 2.

[9] Brunt, E. M., Janney, C. G., Di Bisceglie, A. M., Neuschwander-Tetri, B. A., Bacon, B. R. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol*. **1999**, 94, 2467-2474.

[10] Kleiner, D. E., Brunt, E. M., Van Natta, M., Behling, C., Contos, M. J., Cummings, O. W., Ferrell, L. D., Liu, Y. C., Torbenson, M. S., Unalp-Arida, A., et al.;Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*. **2005**, 41, 1313-1321.

[11] Loomba R, Lim JK, Patton H, El-Serag HB. AGA Clinical Practice Update on Screening and Surveillance for Hepatocellular Carcinoma in Patients With Nonalcoholic Fatty Liver Disease: Expert Review. *Gastroenterology* **2020**, 158, 1822.

[12] Sumida, Y.; Yoneda, M.; Seko, Y.; Ishiba, H.; Hara, T.; Toyoda, H.; Yasuda, S.; Kumada, T.; Hayashi, H.; Kobayashi, T.; et al ; Japan Study Group of NAFLD (JSG-NAFLD). Surveillance of Hepatocellular Carcinoma in Nonalcoholic Fatty Liver Disease. *Diagnostics* **2020**, *10*, 579.

[13] Vilar-Gomez, E., Chalasani, N. Non-invasive assessment of non-alcoholic fatty liver disease: Clinical prediction rules and blood-based biomarkers. *J Hepatol,* **2018**, *68*, 305–315.

[14] Vali, Y., Lee, J., Boursier, J., Spijker, R., Löffler, J., Verheij, J., Brosnan, M. J., Böcskei, Z., Anstee, Q. M., Bossuyt, P. M., Zafarmand, M. H., LITMUS systematic review team. Enhanced liver fibrosis test for the non-invasive diagnosis of fibrosis in patients with NAFLD: A systematic review and meta-analysis. *J Hepatol*. **2020**, 73, 252-262.

[15] Srivastava A, Gailer R, Tanwar S, Trembling, P., Parkes, J., Rodger, A., Suri, D., Thorburn, D., Sennett, K., Morgan, S., et al. Prospective evaluation of a primary care referral pathway for patients with non-alcoholic fatty liver disease. *J Hepatol*. **2019**, 71, 371-8.

[16] Inadomi C, Takahashi H, Ogawa Y, Inadomi, C., Takahashi, H., Ogawa, Y., Oeda, S., Imajo, K., Kubotsu, Y., Tanaka, K., Kessoku, T., Okada, M., Isoda, H., et al. Accuracy of the Enhanced Liver Fibrosis test, and combination of the Enhanced Liver Fibrosis and non-invasive tests for the diagnosis of advanced liver fibrosis in patients with non-alcoholic fatty liver disease. *Hepatol Res*, **2020**, 50, 682-692.

[17] Crossan C, Majumdar A, Srivastava A, Thorburn, D., Rosenberg, W., Pinzani, M., Longworth, L.,

Tsochatzis, E. A. Referral pathways for patients with NAFLD based on non-invasive fibrosis tests: Diagnostic accuracy and cost analysis. *Liver Int*. **2019**, 39, 2052-60.

[18] Sterling, R. K., Lissen, E., Clumeck, N., Sola, R., Correa, M. C., Montaner, J., S Sulkowski, M., Torriani, F. J., Dieterich, D. T., Thomas, D. L., Messinger, D., Nelson, M., APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology*. **2006**, 43, 1317-1325.

[19] Xiao, G., Zhu, S., Xiao, X., Yan, L., Yang, J., Wu, G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: A meta-analysis. *Hepatology*, **2017**, *66*, 1486–1501.

[20] Peleg, N., Issachar, A., Sneh-Arbib, O., Shlomai, A. AST to Platelet Ratio Index and fibrosis 4 calculator scores for non-invasive assessment of hepatic fibrosis in patients with non-alcoholic fatty liver disease. *Dig Liver Dis* **2017**, *49*, 1133–1138.

[21] Patel, Y. A., Gifford, E. J., Glass, L. M., Turner, M. J., Han, B., Moylan, C. A., Choi, S., Suzuki, A., Provenzale, D., Hunt, C. M. Identifying Nonalcoholic Fatty Liver Disease Advanced Fibrosis in the Veterans Health Administration. *Dig Dis Sci* **2018**, *63*, 2259–2266.

[22] McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut.* **2010**, 59, 1265-9.

[23] de Carli MA, de Carli LA, Correa MB, Junqueira G Jr, Tovo CV, Coral GP. Performance of noninvasive scores for the diagnosis of advanced liver fibrosis in morbidly obese with nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol*. **2020**, 32, 420-425.

[24 Kobayashi, N., Kumada, T., Toyoda, H., Tada, T., Ito, T., Kage, M., Okanoue, T., Kudo, M. Ability of Cytokeratin-18 Fragments and FIB-4 Index to Diagnose Overall and Mild Fibrosis Nonalcoholic Steatohepatitis in Japanese Nonalcoholic Fatty Liver Disease Patients. *Dig Dis* , **2017**, *35*, 521–530.

[25] Angulo, P., Hui, J. M., Marchesini, G., Bugianesi, E., George, J., Farrell, G. C., Enders, F., Saksena, S., Burt, A. D., Bida, J. P., et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology*. **2007**, 45, 846-854.

[26] McPherson S, Anstee QM, Henderson E, Day CP, Burt AD. Are simple noninvasive scoring systems for fibrosis reliable in patients with NAFLD and normal ALT levels? *Eur J Gastroenterol Hepatol*. **2013**, 25, 652-658.

[26] Ma, X., Liu, S., Zhang, J., Dong, M., Wang, Y., Wang, M., & Xin, Y.   Proportion of NAFLD patients with normal ALT value in overall NAFLD patients: a systematic review and meta-analysis. *BMC Gastroenterol*. **2020**, 20, 10.

[27] Yoneda, M., Imajo, K., Eguchi, Y., Fujii, H., Sumida, Y., Hyogo, H., Ono, M., Suzuki, Y., Kawaguchi, T., Aoki, N., et al. ; Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD). Noninvasive scoring systems in patients with nonalcoholic fatty liver disease with normal alanine aminotransferase levels. *J Gastroenterol* **2013**, *48*, 1051–1060.

[28] Honda, Y., Yoneda, M., Imajo, K., Nakajima, A. Elastography Techniques for the Assessment of Liver Fibrosis in Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci*. **2020**, 21, 4039.

[29] Kwok R, Tse YK, Wong GL, Ha, Y., Lee, A. U., Ngu, M. C., Chan, H. L., Wong, V. W. Systematic review with meta-analysis: non-invasive assessment of non-alcoholic fatty liver disease--the role of transient elastography and plasma cytokeratin-18 fragments. *Aliment Pharmacol Ther* **2014**, 39, 254-269.

[30] Petta, S., Wai-Sun Wong, V., Bugianesi, E., Fracanzani, A. L., Cammà, C., Hiriart, J. B., Lai-Hung Wong, G., Vergniol, J., Wing-Hung Chan, A., et al. Impact of Obesity and Alanine Aminotransferase Levels on the Diagnostic Accuracy for Advanced Liver Fibrosis of Noninvasive Tools in Patients With Nonalcoholic Fatty Liver Disease. *Am J Gastroenterol* **2019**, *114*, 916–928.

[31] Castera, L.; Friedrich-Rust, M.; Loomba, R. Noninvasive Assessment of Liver Disease in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology*. **2019**, 156, 1264-1281.

[32] Davyduke, T.; Tandon, P.; Al-Karaghouli, M.; Abraldes, J. G.;   Ma, M. M. Impact of Implementing a "FIB-4 First" Strategy on a Pathway for Patients With NAFLD Referred From Primary Care. *Hepatol Commun*. **2019**, 3, 1322-1333.

[33] Sumida, Y.; Shima, T.; Mitsumoto, Y.; Katayama, T., Umemura, A., Yamaguchi, K., Itoh, Y., Yoneda, M., Okanoue, T. Epidemiology, Pathogenesis, and Diagnostic Strategy of Diabetic Liver Disease in Japan. *Int. J. Mol. Sci.* **2020**, *21*, 4337.

[34] Yoneda, M.; Imajo, K.; Takahashi, H.; et al. Clinical strategy of diagnosing and following patients with nonalcoholic fatty liver disease based on invasive and noninvasive methods. *J. Gastroenterol.* **2018**, *53*, 181.

[35] Chan, W.K.; Treeprasertsuk, S.; Goh, G.B.; Fan, J.G.; Song, M.J.; Charatcharoenwitthaya, P.; Duseja, A.; Dan, Y.Y.; Imajo, K.; Nakajima, A.; et al. Optimizing Use of Nonalcoholic Fatty Liver Disease Fibrosis Score, Fibrosis-

4 Score, and Liver Stiffness Measurement to Identify Patients with Advanced Fibrosis. *Clin. Gastroenterol. Hepatol.* **2019**, *17*, 2570–2580.

[36] Ioannou GN, Beste LA, Green PK, Singal, A. G., Tapper, E. B., Waljee, A. K., Sterling, R. K., Feld, J. J., Kaplan, D. E., Taddei, T. H., et al. Increased Risk for Hepatocellular Carcinoma Persists Up to 10 Years After HCV Eradication in Patients With Baseline Cirrhosis or High FIB-4 Scores. *Gastroenterology*. 2019, 157, 1264-1278.

[37] Zhang, Y., Wang, R., Yang, X. FIB-4 index serves as a noninvasive prognostic biomarker in patients with hepatocellular carcinoma: A meta-analysis. *Medicine (Baltimore)*. 2018, 97, e13696.

[38] Kanwal, F.; Kramer, J.R.; Mapakshi, S.; Natarajan, Y.; Chayanupatkul, M.; Richardson, P. A.; Li, L., Desiderio, R.; Thrift, A. P.; Asch, S. M.; Chu, J.; et al. Risk of Hepatocellular Cancer in Patients with Non-Alcoholic Fatty Liver Disease. *Gastroenterology* **2018**, *155*, 1828.

[39] Kim GA, Lee HC, Choe J, Kim, M. J., Lee, M. J., Chang, H. S., Bae, I. Y., Kim, H. K., An, J., Shim, J. H., Kim, K. M., et al. Association between non-alcoholic fatty liver disease and cancer incidence rate. *J Hepatol*. **2017**, S0168-8278(17)32294-8.

[40] Peleg, N., Sneh Arbib, O., Issachar, A., Cohen-Naftaly, M., Braun, M., Shlomai, A. Noninvasive scoring systems predict hepatic and extra-hepatic cancers in patients with nonalcoholic fatty liver disease. *PLoS One*. **2018**, 13, e0202393.

[41] Ito T, Ishigami M, Ishizu Y, Kuzuya, T., Honda, T., Hayashi, K., Nishimura, D., Toyoda, H., Kumada, T., Goto, H., et al. Utility and limitations of noninvasive fibrosis markers for predicting prognosis in biopsy-proven Japanese non-alcoholic fatty liver disease patients. *J Gastroenterol Hepatol*. **2019**, 34, 207-214.

[42] Angulo P, Bugianesi E, Bjornsson ES, Charatcharoenwitthaya, P., Mills, P. R., Barrera, F., Haflidadottir, S., Day, C. P., George, J. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology*. **2013**, 145, 782-9.e4.

[43] Kim D, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. *Hepatology*. **2013**, 57, 1357-1365.

[44] Unalp-Arida A, Ruhl CE. Liver fibrosis scores predict liver disease mortality in the United States population. *Hepatology*. 2017, 66, 84-95.

[45] Hagström H, Nasr P, Ekstedt M, Stål P, Hultcrantz R, Kechagias S. Accuracy of Noninvasive Scoring Systems in Assessing Risk of Death and Liver-Related Endpoints in Patients With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol*. 2019, 17, 1148-1156.

[46] Hagström H, Talbäck M, Andreasson A, Walldius G, Hammar N. Ability of Noninvasive Scoring Systems to Identify Individuals in the Population at Risk for Severe Liver Disease. *Gastroenterology*. **2020**, 158, 200-214.

[47] Bertot LC, Jeffrey GP, de Boer B, MacQuillan, G., Garas, G., Chin, J., Huang, Y., Adams, L. A. Diabetes impacts prediction of cirrhosis and prognosis by non-invasive fibrosis models in non-alcoholic fatty liver disease. *Liver Int*. **2018**, 38, 1793-1802.

[48] Tada, T., Kumada, T., Toyoda, H., Mizuno, K., Sone, Y., Akita, T., Tanaka, J. Progression of liver fibrosis is associated with non-liver-related mortality in patients with nonalcoholic fatty liver disease. *Hepatol Commun*, **2017**, *1*, 899–910.

[49] Kim, S. U., Kim, B. K., Park, J. Y., Kim, D. Y., Ahn, S. H., Park, Y. B., Han, K. H., Lee, S. W. Fibrosis-4 index at diagnosis can predict all-cause mortality in patients with rheumatoid arthritis: A retrospective monocentric study. *Mod Rheumatol*. **2020**, 30, 70-77.

[50] Park, H. J., Park, J. Y., Jung, S. M., Song, J. J., Park, Y. B., & Lee, S. W. Fibrosis-4 index at diagnosis is associated with all-cause mortality in patients with microscopic polyangiitis and granulomatosis with polyangiitis. *BMC Gastroenterol*, **2019**, *19*, 90.

[51] Yong, S. H., Leem, A. Y., Kim, Y. S., Park, M. S., Chang, J., Kim, S. U., Jung, J. Y. Hepatic Fibrosis Assessed Using Fibrosis-4 Index Is Predictive of All-Cause Mortality in Patients with Chronic Obstructive Pulmonary Disease. *Int J Chron Obstruct Pulmon Dis*. **2020**, 15, 831-839.

[52] Xun, Y. H., Guo, J. C., Lou, G. Q., Jiang, Y. M., Zhuang, Z. J., Zhu, M. F., Luo, Y., Ma, X. J., Liu, J., Bian, D. X., Shi, J. P. Non-alcoholic fatty liver disease (NAFLD) fibrosis score predicts 6.6-year overall mortality of Chinese patients with NAFLD. *Clin Exp Pharmacol Physiol*. **2014**, 41, 643-649.

[53] Sebastiani, G., Alshaalan, R., Wong, P., Rubino, M., Salman, A., Metrakos, P., Deschenes, M., Ghali, P. Prognostic Value of Non-Invasive Fibrosis and Steatosis Tools, Hepatic Venous Pressure Gradient (HVPG) and Histology in Nonalcoholic Steatohepatitis. *PLoS One*. **2015**, 10, e0128774.

[54] Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *J Hepatol* **2008**, 49, 608–612.

[55] Lee SB, Park GM, Lee JY, Lee, B. U., Park, J. H., Kim, B. G., Jung, S. W., Jeong, I. D., Bang, S. J., Shin, J. W., et al. Association between non-alcoholic fatty liver disease and subclinical coronary atherosclerosis: An observational cohort study. *J Hepatol*. **2018**, 68, 1018-24.

[56] Käräjämäki A.J., Pätsi O.P., Savolainen M., Kesäniemi, Y. A., Huikuri, H., & Ukkola, O. Non-Alcoholic Fatty Liver Disease as a Predictor of Atrial Fibrillation in Middle-Aged Population (OPERA Study). *PLoS ONE*. **2015**, 10, e0142937.

[57] Mantovani A, Pernigo M, Bergamini C, Bonapace, S., Lipari, P., Pichiri, I., Bertolini, L., Valbusa, F., Barbieri, E., Zoppini, G., et al. Nonalcoholic Fatty Liver Disease Is Independently Associated with Early Left Ventricular Diastolic Dysfunction in Patients with Type 2 Diabetes. *PLoS One*. **2015**, 10, e0135329.

[58] Chung GE, Lee JH, Lee H, Kim, M. K., Yim, J. Y., Choi, S. Y., Kim, Y. J., Yoon, J. H., Kim, D. Nonalcoholic fatty liver disease and advanced fibrosis are associated with left ventricular diastolic dysfunction. *Atherosclerosis*. **2018**, 272, 137-44.

[59] Baratta, F., Pastori, D., Angelico, F., Balla, A., Paganini, A. M., Cocomello, N., Ferro, D., Violi, F., Sanyal, A. J., Del Ben, M. Nonalcoholic Fatty Liver Disease and Fibrosis Associated With Increased Risk of Cardiovascular Events in a Prospective Study. *Clin Gastroenterol Hepatol*. **2019**, S1542-3565(19)31506-X.

[60] Saito, Y., Okumura, Y., Nagashima, K., Fukamachi, D., Yokoyama, K., Matsumoto, N., Tachibana, E., Kuronuma, K., Oiwa, K., Matsumoto, M., et al. Impact of the Fibrosis-4 Index on Risk Stratification of Cardiovascular Events and Mortality in Patients with Atrial Fibrillation: Findings from a Japanese Multicenter Registry. *J Clin Med*. **2020**, 9, 584.

[61] Chen, Q., Li, Q., Li, D., Chen, X., Liu, Z., Hu, G., Wang, J., Ling, W. Association between liver fibrosis scores and the risk of mortality among patients with coronary artery disease. *Atherosclerosis*, **2000**, *299*, 45–52.

[62] Ishiba H, Sumida Y, Kataoka S, Kuroda, M., Akabame, S., Tomiyasu, K., Tanaka, M., Arai, M., Taketani, H., Seko, Y., et al. Association of coronary artery calcification with liver fibrosis in Japanese patients with non-alcoholic fatty liver disease. *Hepatol Res*. **2016**, 46, 1107-1117.

[63] Song, D. S., Chang, U. I., Kang, S. G., Song, S. W., Yang, J. M. Noninvasive Serum Fibrosis Markers are Associated with Coronary Artery Calcification in Patients with Nonalcoholic Fatty Liver Disease. *Gut Liver*. **2019**, 13, 658-668.

[64] Chen, Q., Li, Q., Li, D., Chen, X., Liu, Z., Hu, G., Wang, J., Ling, W. Association between liver fibrosis scores and the risk of mortality among patients with coronary artery disease. *Atherosclerosis*, **2020**, *299*, 45–52.

[65] Sato, Y., Yoshihisa, A., Kanno, Y., Watanabe, S., Yokokawa, T., Abe, S., Misaka, T., Sato, T., Suzuki, S., Oikawa, M., et al. Liver stiffness assessed by Fibrosis-4 index predicts mortality in patients with heart failure. *Open Heart*. **2017**,4, e000598.

[66] So-Armah, K. A., Lim, J. K., Lo Re, V., Tate, J. P., Chang, C. H., Butt, A. A., Gibert, C. L., Rimland, D., Marconi, V. C., Goetz, M. B., et al. Veterans Aging Cohort Study Project Team. FIB-4 stage of liver fibrosis predicts incident heart failure among HIV-infected and uninfected patients. *Hepatology* **2017**, *66*, 1286–1295.

[67] Singal, A. K., Hasanin, M., Kaif, M., Wiesner, R., Kuo, Y. F. Nonalcoholic Steatohepatitis is the Most Rapidly Growing Indication for Simultaneous Liver Kidney Transplantation in the United States. *Transplantation*. 2016, 100, 607-612.

[68] Musso, G., Gambino, R., Tabibian, J. H., Ekstedt, M., Kechagias, S., Hamaguchi, M., Hultcrantz, R., Hagström, H., Yoon, S. K., Charatcharoenwitthaya, P., et al. Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. *PLoS Med*. **2014**, 11, e1001680.

[69] Sumida, Y., Yoneda, M., Toyoda, H., Yasuda, S., Tada, T., Hayashi, H., Nishigaki, Y., Suzuki, Y., Naiki, T., Morishita, A., et al. Japan Study Group Of JSG-NAFLD. Common Drug Pipelines for the Treatment of Diabetic Nephropathy and Hepatopathy: Can We Kill Two Birds with One Stone? *Int J Mol Sci*. **2020**, 21, E4939.

[70] Musso, G., Cassader, M., Cohney, S., De Michieli, F., Pinach, S., Saba, F., Gambino, R. Fatty Liver and Chronic Kidney Disease: Novel Mechanistic Insights and Therapeutic Opportunities. *Diabetes Care*. **2016**, 39, 1830-1845.

[71] Wijarnpreecha, K., Thongprayoon, C., Scribani, M., Ungprasert, P., Cheungpasitporn, W. Noninvasive fibrosis markers and chronic kidney disease among adults with nonalcoholic fatty liver in USA. *Eur J Gastroenterol Hepatol*. **2018**, 30, 404-410.

[72] Arase, Y., Suzuki, F., Kobayashi, M., Suzuki, Y., Kawamura, Y., Matsumoto, N., Akuta, N., Kobayashi, M., Sezaki, H., Saito, S., et al. The development of chronic kidney disease in Japanese patients with non-alcoholic fatty liver disease. *Intern Med*. **2011**, 50, 1081-1087.

[73] Seko Y, Yano K, Takahashi A, Okishio, S., Kataoka, S., Okuda, K., Mizuno, N., Takemura, M., Taketani, H., Umemura, A., et al. FIB-4 Index and Diabetes Mellitus Are Associated with Chronic Kidney Disease in Japanese Patients with Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci*. **2019**, 21, 171.

[74] Mantovani, A., Taliento, A., Zusi, C., Baselli, G., Prati, D., Granata, S., Zaza, G., Colecchia, A., Maffeis, C., Byrne, C. D., et al. PNPLA3 I148M gene variant and chronic kidney disease in type 2 diabetic patients with NAFLD: Clinical and experimental findings. *Liver Int*. **2020**, 40, 1130-1141.

[75] Sun, D. Q., Zheng, K. I., Xu, G., Ma, H. L., Zhang, H. Y., Pan, X. Y., Zhu, P. W., Wang, X. D., Targher, G., Byrne, C. D., Chen, Y. P., Yuan, W. J., Zheng, M. H. PNPLA3 rs738409 is associated with renal glomerular and tubular injury in NAFLD patients with persistently normal ALT levels. *Liver Int*. **2020**, 40, 107-119.

[76] Wada T, Zeniya M. Background of the FIB-4 index in Japanese non-alcoholic fatty liver disease. *Intern Med*. **2015**, 54, 127-132.

[77] Sumida Y, Sakuragi S, Hibino S, Furutani M. Diatribution of FIB4 index in Japanese Nonalcoholic fatty liver disease population: a multi-center study. Ningendock Int **2015**, 2, 32-34.

[78] Sumida, Y., Shima, T., Mitsumoto, Y., Katayama, T., Umemura, A., Yamaguchi, K., Itoh, Y., Yoneda, M., & Okanoue, T. *Int J Mol Sci*. **2020**, 21, 4337.

[79] Davyduke, T., Tandon, P., Al-Karaghouli, M., Abraldes, J. G.; Ma, M. M. Impact of Implementing a "FIB-4 First" Strategy on a Pathway for Patients With NAFLD Referred From Primary Care. *Hepatol Commun*, **2019**, *3*, 1322–1333.

[80] McPherson S, Hardy T, Dufour JF, Petta, S., Romero-Gomez, M., Allison, M., Oliveira, C. P., Francque, S., Van Gaal, L., Schattenberg, J. et al. Age as a Confounding Factor for the Accurate Non-Invasive Diagnosis of Advanced NAFLD Fibrosis. *Am J Gastroenterol*. **2017**, 112, 740-751.

[81] Ishiba H, Sumida Y, Tanaka S, Yoneda, M., Hyogo, H., Ono, M., Fujii, H., Eguchi, Y., Suzuki, Y., Yoneda, M., et al; Japan Study Group of Non-Alcoholic Fatty Liver Disease (JSG-NAFLD). The novel cutoff points for the FIB4 index categorized by age increase the diagnostic accuracy in NAFLD: a multi-center study. *J Gastroenterol*. **2018**, 53, 1216-1224.

[82] Pitisuttithum, P., Chan, W. K., Piyachaturawat, P., Imajo, K., Nakajima, A., Seki, Y., Kasama, K., Kakizaki, S., Fan, J. G., Song, M. J., et al;   Predictors of advanced fibrosis in elderly patients with biopsy-confirmed nonalcoholic fatty liver disease: the GOASIA study. *BMC Gastroenterol*. **2020**. 20, 88.

[83] Blond, E., Disse, E., Cuerq, C., Drai, J., Valette, P. J., Laville, M., Thivolet, C., Simon, C., Caussy, C. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease in severely obese people: do they lead to over-referral?. *Diabetologia*, **2017**, *60*, 1218–1222.

[84] Joo, S. K., Kim, W., Kim, D., Kim, J. H., Oh, S., Lee, K. L., Chang, M. S., Jung, Y. J., So, Y. H., Lee, M. S., et al. Steatosis severity affects the diagnostic performances of noninvasive fibrosis tests in nonalcoholic fatty liver disease. *Liver Int* **2018**, *38*, 331–341.

[85] Ishiba Y, Sumida Y, Tanaka S, Yoneda, M., Hyogo, H., Ono, M., Fujii, H., Eguchi, Y., Suzuki, Y., Yoneda, M., et al; Japan Study Group of Non-Alcoholic Fatty Liver Disease (JSG-NAFLD). Type IV collagen 7S is the most accurate test for identifying advanced fibrosis in non-alcoholic fatty liver disease with type 2 diabetes. *Hep Commun* (submitted)

[86] Bertot LC, Jeffrey GP, de Boer B, MacQuillan, G., Garas, G., Chin, J., Huang, Y., Adams, L. A.. Diabetes impacts prediction of cirrhosis and prognosis by non-invasive fibrosis models in non-alcoholic fatty liver disease. *Liver Int*. **2018**, 38, 1793-1802.

[87] Singh A, Gosai F, Siddiqui MT, Gupta M, Lopez R, Lawitz E, Poordad F, Carey W, McCullough A, Alkhouri N. Accuracy of Noninvasive Fibrosis Scores to Detect Advanced Fibrosis in Patients With Type-2 Diabetes With Biopsy-proven Nonalcoholic Fatty Liver Disease. *J Clin Gastroentero 2020*, in press.

[88] Okanoue, T., Ebise, H., Kai, T., Mizuno, M., Shima, T., Ichihara, J., Aoki, M. A simple scoring system using type IV collagen 7S and aspartate aminotransferase for diagnosing nonalcoholic steatohepatitis and related fibrosis. *J Gastroenterol*. **2018**, 53, 129-139.

[89] Shah S, Dhami-Shah H, Kamble S, Shukla A. FIB-4 cut-off of 1.3 may be inappropriate in a primary care referral pathway for patients with non-alcoholic fatty liver disease. *J Hepatol*. **2020** Apr 7:S0168-8278(20)30017-9.

[90] Sumida, Y., Yoneda, M., Ogawa, Y., Yoneda, M., Okanoue, T., Nakajima, A.; Japan Study Group of NAFLD (JSG-NAFLD). Current and new pharmacotherapy options for non-alcoholic steatohepatitis. *Expert Opinion On Pharmacotherapy*, 2020 Apr 1:1-15.

[91] Sumida Y, Yoneda M. Current and future pharmacological therapies for NAFLD/NASH. *J Gastroenterol*.

2018, 53, 362-76.

[92] Newsome PN, Sasso M, Deeks JJ, Paredes, A., Boursier, J., Chan, W. K., Yilmaz, Y., Czernichow, S., Zheng, M. H., Wong, V. W., et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol*. **2020**, 5, 362-373.

[93] Oeda S, Takahashi H, Imajo K, Seko, Y., Kobayashi, T., Ogawa, Y., Moriguchi, M., Yoneda, M., Anzai, K., Irie, H., et al. Diagnostic accuracy of FibroScan-AST score to identify non-alcoholic steatohepatitis with significant activity and fibrosis in Japanese patients with non-alcoholic fatty liver disease: Comparison between M and XL probes. *Hepatol Res*, in press.

[94] Noureddin, N., Alkhouri, N., Brown, K. A., Noureddin, M. Driving NASH forward using the FAST score but obey the traffic lights. *Hepatology*, *in press.*

[95] Abe, M., Miyake, T., Kuno, A., Imai, Y., Sawai, Y., Hino, K., Hara, Y., Hige, S., Sakamoto, M., Yamada, G., Kage, M., Korenaga, M., Hiasa, Y., Mizokami, M., & Narimatsu, H. (2015). Association between Wisteria floribunda agglutinin-positive Mac-2 binding protein and the fibrosis stage of non-alcoholic fatty liver disease. *Journal of gastroenterology*, **2015**, 50, 776–784.

[96] Ogawa Y, Honda Y, Kessoku T, Tomeno, W., Imajo, K., Yoneda, M., Kawanaka, M., Kirikoshi, H., Ono, M., Taguri, M., et al. Wisteria floribunda agglutinin-positive Mac-2-binding protein and type 4 collagen 7S: useful markers for the diagnosis of significant fibrosis in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol*. **2018**, 33, 1795-1803.

[97] Kim M, Jun DW, Park H, Kang BK, Sumida Y. Sequential Combination of FIB-4 Followed by M2BPGi Enhanced Diagnostic Performance for Advanced Hepatic Fibrosis in an Average Risk Population. *J Clin Med*. **2020**, 9, pii: E1119.

[98] Daniels, S. J., Leeming, D. J., Eslam, M., Hashem, A. M., Nielsen, M. J., Krag, A., Karsdal, M. A., Grove, J. I., Neil Guha, I., Kawaguchi, T., et al. ADAPT: An Algorithm Incorporating PRO-C3 Accurately Identifies Patients With NAFLD and Advanced Fibrosis. *Hepatology*. **2019**, 69, 1075-1086.

[99] Fujimori N, Umemura T, Kimura T, Tanaka N, Sugiura A, Yamazaki T, Joshita S, Komatsu M, Usami Y, Sano K, et al. Serum autotaxin levels are correlated with hepatic fibrosis and ballooning in patients with non-alcoholic fatty liver disease. *World J Gastroenterol*. **2018**, 24, 1239-1249.

[100] Honda Y, Imajo K, Kobayashi T, Kessoku T, Ogawa Y, Tomeno W, Yoneda M, Kobayashi N, Saito S, Nakajima A. Autotaxin is a valuable biomarker for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. *Hepatol Res*. **2019**, 49, 1136-1146.

[101] Eddowes PJ, Sasso M, Allison M, Tsochatzis, E., Anstee, Q. M., Sheridan, D., Guha, I. N., Cobbold, J. F., Deeks, J. J., Paradis, et al. Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology*. **2019**, 156, 1717-1730.

[102] Gu, J., Liu, S., Du, S., Zhang, Q., Xiao, J., Dong, Q., Xin, Y. Diagnostic value of MRI-PDFF for hepatic steatosis in patients with non-alcoholic fatty liver disease: a meta-analysis. *Eur Radiol*. **2019**, 29, 3564-3573.

[103] Tada T, Kumada T, Toyoda H, Kobayashi, N., Sone, Y., Oguri, T., Kamiyama, N. Utility of Attenuation Coefficient Measurement Using an Ultrasound-Guided Attenuation Parameter for Evaluation of Hepatic Steatosis: Comparison With MRI-Determined Proton Density Fat Fraction. AJR *Am J Roentgenol*. **2019**, 212, 332-41.

[104] Siddiqui, M. S., Yamada, G., Vuppalanchi, R., Van Natta, M., Loomba, R., Guy, C., Brandman, D., Tonascia, J., Chalasani, N., Neuschwander-Tetri, B., et al; Diagnostic Accuracy of Noninvasive Fibrosis Models to Detect Change in Fibrosis Stage. *Clin Gastroenterol Hepatol*. **2019**, 17, 1877-1885.

[105] Chalasani, N., Abdelmalek, M. F., Loomba, R., Kowdley, K. V., McCullough, A. J., Dasarathy, S., Neuschwander-Tetri, B. A., Terrault, N., Ferguson, B., Shringarpure, R., et al. Relationship between three commonly used non-invasive fibrosis biomarkers and improvement in fibrosis stage in patients with non-alcoholic steatohepatitis. *Liver Int*. **2019**, 39, 924-932.

[106] Hamaguchi, E., Takamura, T., Sakurai, M., Mizukoshi, E., Zen, Y., Takeshita, Y., Kurita, S., Arai, K., Yamashita, T., Sasaki, M., et al. Histological course of nonalcoholic fatty liver disease in Japanese patients: tight glycemic control, rather than weight reduction, ameliorates liver fibrosis. *Diabetes Care*, **2010**, *33*, 284–286.

[107] Seko, Y., Sumida, Y., Tanaka, S., Mori, K., Taketani, H., Ishiba, H., Hara, T., Okajima, A., Yamaguchi, K., Moriguchi, M., et al.; Serum alanine aminotransferase predicts the histological course of non-alcoholic steatohepatitis in Japanese patients. *Hepatol Res*. **2015**, 45, E53-61

[108] Hoofnagle, J. H., Van Natta, M. L., Kleiner, D. E., Clark, J. M., Kowdley, K. V., Loomba, R., Neuschwander-Tetri, B. A., Sanyal, A. J., Tonascia, J., Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN). Vitamin E and changes in serum alanine aminotransferase levels in patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther.* **2013**, 38, 134-43.

[109] Vilar-Gomez, E., Yasells-Garcia, A., Martinez-Perez, Y., Calzadilla-Bertot, L., Torres-Gonzalez, A., Gra-Oramas, B., Gonzalez-Fabian, L., Villa-Jimenez, O., Friedman, S. L., Diago, M., et al; Development and validation of a noninvasive prediction model for nonalcoholic steatohepatitis resolution after lifestyle intervention. *Hepatology*. **2016**, 63, 1875-87.

[110] Vuppalanchi, R., Jain, A. K., Deppe, R., Yates, K., Comerford, M., Masuoka, H. C., Neuschwander-Tetri, B. A., Loomba, R., Brunt, E. M., Kleiner, D. E., et al.; Relationship between changes in serum levels of keratin 18 and changes in liver histology in children and adults with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol.* **2014**, 12, 2121-30.e1-2.

[111] Jayakumar, S., Middleton, M. S., Lawitz, E. J., Mantry, P. S., Caldwell, S. H., Arnold, H., Mae Diehl, A., Ghalib, R., Elkhashab, M., Abdelmalek, M. F., et al. Longitudinal correlations between MRE, MRI-PDFF, and liver histology in patients with non-alcoholic steatohepatitis: Analysis of data from a phase II trial of selonsertib. *J Hepatol*. **2019**, 70, :133-141.

[112] Loomba, R., Sanyal, A. J., Kowdley, K. V., Terrault, N., Chalasani, N. P., Abdelmalek, M. F., McCullough, A. J., Shringarpure, R., Ferguson, B., Lee, L., et al; Factors Associated With Histologic Response in Adult Patients With Nonalcoholic Steatohepatitis. *Gastroenterology*. **2019**, 156, 88-95

[113] Ji D, Qin E, Xu J, Zhang, D., Cheng, G., Wang, Y., Lau, G.. Non-alcoholic fatty liver diseases in patients with COVID-19: a retrospective study. *J Hepatol*. **2020**, 73, 451-453.

[114] Zhou, Y. J., Zheng, K. I., Wang, X. B., Sun, Q. F., Pan, K. H., Wang, T. Y., Ma, H. L., Chen, Y. P., George, J., & Zheng, M. H. Metabolic-associated fatty liver disease is associated with severity of COVID-19. *Liver Int* **2020**, in press

[115] Gao, F., Zheng, K. I., Wang, X. B., Yan, H. D., Sun, Q. F., Pan, K. H., Wang, T. Y., Chen, Y. P., George, J., Zheng, M. H. Metabolic associated fatty liver disease increases coronavirus disease 2019 disease severity in nondiabetic patients. *J Gastroenterol Hepatol*. **2020**, in press

[116] Ibáñez-Samaniego, L., Bighelli, F., Usón, C., Caravaca, C., Carrillo, C. F., Romero, M., Barreales, M., Perelló, C., Madejón, A., Marcos, A. C., et al. Elevation of liver fibrosis index FIB-4 is associated with poor clinical outcomes in patients with COVID-19. *J Infect Dis*. **2020**, in press.

[117] Kumar A, Arora A, Sharma P, Anikhindi, S. A., Bansal, N., Singla, V., Khare, S., Srivastava, A. et al. Is diabetes mellitus associated with mortality and severity of COVID-19? A meta-analysis. *Diabetes Metab Syndr*. **2020**, 14, 535-545.

[118] Yang, J.; Hu, J.; Zhu, C. Obesity aggravates COVID-19: a systematic review and meta-analysis. *J Med Virol*. **2020**, in press.

[119] Pranata, R.; Lim, M. A.; Huang, I.; Raharjo, S. B.; Lukito, A. A. Hypertension is associated with increased mortality and severity of disease in COVID-19 pneumonia: A systematic review, meta-analysis and meta-regression. *J Renin Angiotensin Aldosterone Syst*. **2020**, 21, 1470320320926899.

[120] Estes, C., Anstee, Q. M., Arias-Loste, M. T., Bantel, H., Bellentani, S., Caballeria, J., Colombo, M., Craxi, A., Crespo, J., Day, C. P., et al . Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. *J Hepatol*. **2018**, 69, 896-904.

[121] Srivastava A, Jong S, Gola A, Srivastava, A., Jong, S., Gola, A., Gailer, R., Morgan, S., Sennett, K., Tanwar, S., Pizzo, E., O'Beirne, J., Tsochatzis, E, et al. Cost-comparison analysis of FIB-4, ELF and fibroscan in community pathways for non-alcoholic fatty liver disease. *BMC Gastroenterol*. **2019**, 19, 122.

[122] Patel, P. J., Banh, X., Horsfall, L. U., Hayward, K. L., Hossain, F., Johnson, T., Stuart, K. A., Brown, N. N., Saad, N., Clouston, A., et al; Underappreciation of non-alcoholic fatty liver disease by primary care clinicians: limited awareness of surrogate markers of fibrosis. *Intern Med J*. **2018**, 48, 144-151.