Review

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

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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 hepatocellular 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; hepatocellular 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 ≥ 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>
<thead>
<tr>
<th>Index</th>
<th>Formula</th>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIB-4 index</td>
<td>(age [years] × AST [U/L]/(platelet count ×10^9/L) × √ALT [U/L])</td>
<td>• Simple (only four parameters)</td>
<td>• Requires an intermediate group</td>
</tr>
<tr>
<td></td>
<td>[9] <a href="https://www.eapharma.co.jp/medicalexpert/product/livact/fib-4/calculator.html">https://www.eapharma.co.jp/medicalexpert/product/livact/fib-4/calculator.html</a></td>
<td>• Accurate</td>
<td>• Overpredict in old patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Validated globally</td>
<td>• Inferior in patients with T2D</td>
</tr>
<tr>
<td>NAFLD fibrosis score</td>
<td>−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) [9] <a href="http://nafldscore.com/">http://nafldscore.com/</a></td>
<td>• Validated globally</td>
<td>• Complex (six parameters)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Accurate</td>
<td>• Requires an intermediate group</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Overpredict in old patients</td>
</tr>
<tr>
<td>APRI</td>
<td>AST to platelet ratio index</td>
<td>• Simple (only two parameters)</td>
<td>• conflicting results</td>
</tr>
<tr>
<td>BARD</td>
<td>BMI &gt; 28 kg/m^2 = 1 point, AST/ALT ratio &gt; 0.8 = 2 points</td>
<td>• very Simple</td>
<td>• conflicting results</td>
</tr>
</tbody>
</table>

Table 1. A variety of non-invasive tests (NITs) for identifying severe fibrosis (F3/4) in NAFLD
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 |
| ELF test | −7.412 + (ln [HA] × 0.681) + (ln [P3NP] × 0.775) + (ln [TIMP1] × 0.494) | • Accurate | • No external validation studies |
|           |                                                   | • Validated globally | • High cost? |

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% specificity). 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 algorithm 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 discussed.

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 hepatitis 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 a 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

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>US biopsy</th>
<th>Median</th>
<th>NFS ◎ FIB-4 ○ APRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forns score = 7.811 − 3.131 log(platelet count [10⁹/L]) + 0.781 log(GGT [IU/L]) + 3.467 log(age [years]) − 0.014 total cholesterol (mg/dL)</td>
<td></td>
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</tbody>
</table>

**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 [77,79], 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 ≥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
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

<table>
<thead>
<tr>
<th></th>
<th>Over-referral</th>
<th>Under-referral</th>
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<tbody>
<tr>
<td>FIB-4 index LCO</td>
<td>1.3</td>
<td>1.45</td>
</tr>
<tr>
<td>GP</td>
<td>Work ↓</td>
<td>Work ↑</td>
</tr>
<tr>
<td>Hepatologists</td>
<td>Work ↑</td>
<td>Work ↓</td>
</tr>
<tr>
<td>Unnecessary liver biopsy</td>
<td>May increase</td>
<td>May reduce</td>
</tr>
<tr>
<td>HCC early detection</td>
<td>Possible ?</td>
<td>May delay diagnosis?</td>
</tr>
<tr>
<td>Heath economic costs</td>
<td>High ?</td>
<td>Low ?</td>
</tr>
</tbody>
</table>

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) ≥ 4 and ≥ F2. Inclusion criteria in a variety of drug pipelines include NASH with NAS ≥ 4 and ≥ 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 ≥ 4 and significant fibrosis (≥ 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.

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

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 (ALT, body weight, and A1c) 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>
<thead>
<tr>
<th>Author</th>
<th>Subjects</th>
<th>Outcomes</th>
<th>Parameter correlated with pathological improvement</th>
</tr>
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<tbody>
<tr>
<td>Hamaguchi [106]</td>
<td>NAFLD (n=39)</td>
<td>Hepatic fibrosis</td>
<td>ΔHbA1c reduction</td>
</tr>
<tr>
<td>Seko [107]</td>
<td>NASH (n=52)</td>
<td>NAS Hepatic fibrosis</td>
<td>ΔALT reduction &gt;30% from baseline</td>
</tr>
<tr>
<td>Hoofnagle [108]</td>
<td>NASH (n=139) without DM PIVENS trial</td>
<td>NAS Hepatic fibrosis</td>
<td>ΔALT reduction &gt;30% from baseline or post-treatment ALT&lt;40 IU/L</td>
</tr>
<tr>
<td>Vilar-Gomez [109]</td>
<td>NASH (n=261)</td>
<td>NASH resolution w/o worsening fibrosis</td>
<td>ΔBW reduction, T2D (-), ALT normalization, younger age, NAS&lt;5</td>
</tr>
<tr>
<td>Vuppalanchi [110]</td>
<td>Adult NASH (n=231) Pediatric NAFLD (n=152)</td>
<td>Histological improvement</td>
<td>ΔCK18 reduction (inferior to ΔALT reduction)</td>
</tr>
<tr>
<td>Siddiqui [104]</td>
<td>NAFLD (n=292)</td>
<td>Hepatic fibrosis</td>
<td>ΔFIB-4 index, ΔNFS, ΔAPRI</td>
</tr>
<tr>
<td>Jayakumar [111]</td>
<td>NASH, stage 2-3 (n=54) Selonsertib (Phase 2)</td>
<td>Hepatic fibrosis</td>
<td>ΔMRE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hepatic steatosis</td>
<td>MRI-PDFF ≥25% reduction</td>
</tr>
<tr>
<td>Chalasani [105]</td>
<td>NASH (n=200) FLINT trial (Phase2) Placebo vs OCA 72wk</td>
<td>Hepatic fibrosis</td>
<td>ΔFIB-4 index ○</td>
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<tr>
<td>Loomba [112]</td>
<td>NAS ≥ 2 points reduction without worsening fibrosis</td>
<td>OCA(+), pretreatment NAS&gt;5, TG≤154 mg/dL, INR&lt;1, AST&lt;49 IU/L, ALT at 24wk (&gt;17 IU/L)</td>
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</table>

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].

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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
PIINP 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 glutamyltransferase
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
M2BP  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
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>TIMP-1</td>
<td>tissue inhibitor of matrix metalloproteinase type 1,</td>
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<tr>
<td>T2D</td>
<td>type 2 diabetes</td>
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<tr>
<td>UCAP</td>
<td>ultrasound-guided attenuation parameter</td>
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<tr>
<td>US</td>
<td>ultrasonography</td>
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<tr>
<td>VCTE</td>
<td>vibration-controlled transient elastography</td>
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</table>
References


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.


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


[119] Pranata R; Lim MA; Huang I; Raharjo SB; Lukito AA; 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.

