Noninvasive evaluation of nonalcoholic fatty liver disease (NAFLD)

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Introduction

- The increasing prevalence of NAFLD and the severe outcomes of NASH may call for effective methods to identify NAFLD.

- Although biopsy is the gold standard, it is limited by its sampling bias, poor acceptability, and severe complications, such as mortality, bleeding, and pain.

- Therefore, noninvasive methods are urgently needed to avoid biopsy for diagnosing NAFLD.

NAFLD Presentation

Symptoms

- Usually asymptomatic; majority discovered by chance
- Fatigue frequently present

Often an "incidental finding"

- Incidental abnormal LFTs
- Incidental "bright liver" on imaging
- Incidental hepatomegaly

Common scenarios

- Statin monitoring
- "Annual reviews" in T2D/lipid/ hypertension clinics
- Medical insurance/occupational health checks

De Alwis. Dig Dis. 2016;34:19.

Pragmatic First Steps in Suspected NAFLD

1. Risk Identification

- Metabolic syndrome or other high prevalence group

2. History

- Alcohol intake (< 14/21 units/wk)
- No known preexisting liver disease

3. Investigations

- Liver biochemistry (ALT, AST, etc)
- Exclude/identify other liver diseases:
  - Negative HBV & HCV serology
  - Negative autoantibodies (ANA, AMA, SMA, LKM1, ANCA)
  - Negative coeliac serology
  - Normal immunoglobulins, ferritin, A1AT, Cu2+, etc
- Liver ultrasound: increased echogenicity (steatosis)

Chalasani. Hepatology. 2018;67:328.

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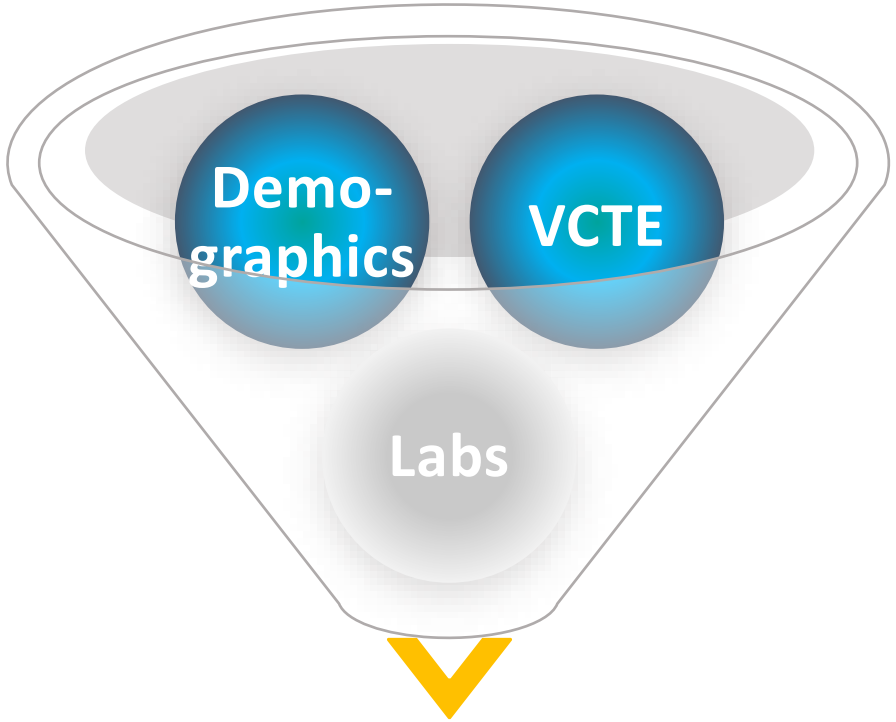
Liver Enzymes: Inadequate in Assessing NAFLD/NASH

- ALT can be normal in > 50% of individuals with NASH, 80% of individuals with NAFLD[1,2]
- ALT can be elevated in > 50% of individuals with NAFLD but without NASH
- In NAFLD, ALT is neither indicative nor predictive of NASH or fibrosis stage[3]:
  - Normal ALT does not preclude NASH/progressive disease
  - Elevated ALT cannot predict NASH or fibrosis
  - **ALT and AST not sensitive for NAFLD/NASH**

1. Browning. Hepatology. 2004;40:1387. 2. Dyson. Frontline Gastroenterol. 2014;5:211. 3. Mofrad. Hepatology. 2003;37:1286.

Noninvasive Tests

- Proper use of noninvasive tests can aid risk stratification



Enrich for NASH and F2/3 Fibrosis

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Advantages of the Current Noninvasive Methods

- The current noninvasive methods is useful for assessing NAFLD, including steatosis, NASH, and NAFLD-related fibrosis, and explores the advantages and disadvantages of measurement tools.

- In clinical practice, we analyze potential noninvasive biomarkers
  - for tracking disease processes
  - monitoring treatment effects
  - explore effective algorithms consisting of imaging and non-imaging biomarkers for advanced fibrosis.

Diagnosis of NAFLD

- Serum biomarkers and biomarker panels
    - Fatty liver index (FLI)
    - Hepatic steatosis index (HSI)
    - Steato Test
    - NAFLD screening score
- Imaging
    - US
    - Computed tomography (CT)
    - Controlled attenuation parameter (CAP)
    - Magnetic resonance based techniques

Serum biomarkers and biomarker panels

Fatty liver index (FLI)

- The FLI is a prevalent biomarker panel consisting of
    - body mass index (BMI)
    - waist circumference
    - Triglycerides
    - gamma-glutamyl transferase
- For identifying NAFLD, with a total score varying between 0 and 100
- AUROC of FLI for identifying NAFLD is 0.84 , a low cutoff of 30 is used to rule out NAFLD (the negative likelihood ratio 0.2), and a high cutoff of 60 rule is used with a positive likelihood ratio of 4.3.
- FLI poorly distinguishes moderate-to-severe steatosis from mild steatosis.

Hepatic steatosis index (HSI)

- The HSI is a biomarker panel consisting of BMI, diabetes, and the alanine transaminase (ALT)/ aspartate transaminase (AST) ratio.

- AUROC of 0.79 and 0.82 in the derivation and validation groups, and the two cutoffs, 30 and 36, achieved a > 90% sensitivity and specificity.

- HSI accuracy decreases in obese children, with an AUROC of 0.67, sensitivity of 67%, and specificity of 62%.

HSI poorly distinguishes moderate to severe steatosis from mild steatosis.

SteatoTest

- The SteatoTest is a biomarker panel consisting of 10 biochemical tests, age, gender, and BMI.

- AUROC of 0.8 for identifying a > 5% liver fat content in patients with chronic liver diseases.

- Further studies are needed to validate the SteatoTest for differentiating individuals with NAFLD from healthy people.

NAFL screening score

- The NAFL screening score is a model with age, fasting blood glucose, BMI, triglyceride, ALT/AST, and uric acid.

- In a study of 48,489 patients, the NAFL screening score had different cutoffs for males and females, with a cutoff of 32 yielding an AUROC of 0.83 for males and a cutoff of 29 yielding an AUROC of 0.86 for females.

- NAFLD ridge score include six lab markers, with an AUROC of 0.87-0.88. The low cutoff of 0.24 achieved a sensitivity of 92% and negative predictive value (NPV) of 95%, and the high cutoff of 0.44 achieved a 90% specificity with a corresponding positive predictive value (PPV) of 84%.

Imaging

- **Ultrasound (US)**
  - the first-line imaging test used in clinical practice
  - a typical appearance of a hyper-echogenic liver.
  - sensitivity of 85% and specificity of 94% for moderate-to-severe steatosis
- US was incapable of detecting steatosis of less than 20% or steatosis in individuals with morbid obesity.
- The accuracy of US for hepatic steatosis assessment is affected by the presence of severe fibrosis and intra- and inter-observer variability.
- To detect NAFLD at early stage, the computed-assisted US hepatic/renal ratio (H/R) and US hepatic attenuation rate are used to assess steatosis quantitatively.

Computed tomography (CT)

- Non-enhanced CT has been used to evaluate the severity of fatty liver since 1970, hepatic attenuation is inversely associated with the hepatic fat content.

- Normal liver has an attenuation value of 50-65 HU, and 8-10 HU higher than that of the spleen. However, the attenuation may decrease to less than 40 HU when fatty infiltration occurs.

- Non-enhanced CT outperforms US in evaluating the severity of fatty liver, achieving a specificity of 100% and sensitivity of 82% for diagnosing higher (>30%) degrees of hepatic steatosis.

Computed tomography (CT)

- Contrast-enhanced CT images are another CT model that can reduce the radiation exposure of non-enhanced CT.

- More suitable for severe hepatic steatosis using paraspinal or intercostal muscle because its sensitivity for mild to moderate hepatic steatosis is only 25%.
- CT may also be used for hepatic fat quantification, such as dual-energy CT and hepatic attenuation measurement.

- Although CT is more effective, it is also limited by insufficient accuracy for mild-to-moderate hepatic steatosis and radiation exposure, especially in children.

Controlled attenuation parameter (CAP)

- CAP, a parameter based on ultrasonic signals, is measured by the FibroScan® with an M probe (3.5 MHz), with a result of 100-400 dB/m.

- CAP with an M probe is reported to have an AUROC of 0.82 for differentiating any degree of steatosis vs no steatosis.

- The cutoff of 248 dB/m yields a sensitivity of 69% and specificity of 82%.

Magnetic resonance based techniques

- MRI determines steatosis by signal intensity differences on opposed-phase or fat saturation MRI.

- MRI-derived proton density fat fraction (MRI-PDFF) is a robust, noninvasive MRI-based methods for assessing hepatic steatosis.

- MRI-visible protons that combine with fat in the liver to quantify steatosis by dividing all protons in the liver.

Magnetic resonance based techniques

- MRI-PDFF was significantly associated with the histological steatosis grade according to the NASH-CRN grade ($\rho = 0.69$, $P < 0.001$), independent of age, sex, other NASH parameters, and NASH diagnosis.

- MRI-PDFF is superior to other imaging tools for the assessment of hepatic steatosis, and its performance is not affected by obesity.

- MRI-PDFF is also regarded as a robust noninvasive method to monitor the treatment effect.

Magnetic resonance based techniques

- H-MRS is another MR-based technique that directly measures the chemical compositions of the liver.

- used for measurement of intrahepatocellular lipid (IHCL) through calculating PDFF.
- a high correlation with biopsy in steatosis assessment and a sensitivity of 80% for diagnosis of liver fat content ≥ 5%.

- a good accuracy to detect small amounts of liver fat.
- H-MRS had a specificity of 100% and sensitivity of 79% with a PDFF cutoff value of 3%, a specificity of 94% and sensitivity of 87% with a PDFF cut-off value of 2%.

Diagnosis of NASH

- NASH is characterized by steatosis, ballooning, and inflammation, with/without fibrosis, which accelerates disease progression.

- Early detection of NASH is conducive to the prevention of NASH-related fibrosis.

- Noninvasive biomarkers for NASH include
  - Simple serum biomarkers
  - Biomarker panels
  - Imaging.

Serum Biomarkers

Cytokeratin-18 (CK18)

- CK18 is an intermediate filament protein.
- It is cleaved during the period of cell death, containing CK18 M30 and CK18 M65.
- A meta-analysis of 25 studies reported that M30 and M65 withAUROCs of 0.82, the sensitivity and specificity were 75% and 77%.
- Combining metabolic syndrome, ALT, and CK18 in a morbidly obese population could achieve an AUROC of 0.88 compared with CK18 alone, with an AUROC of 0.74.

- The triple combination of adiponectin, CK18, and interleukin (IL)-6 achieved an AUROC of 0.90, a specificity of 85.7%, and a sensitivity of 84.5%.

- The difference in the accuracy of CK18 in assessing NASH with different stages of fibrosis.

- AUROC of 0.93 for NASH with fibrosis stages 3-4 and 0.63-0.78 for NASH with fibrosis stages 0-2, which may indicate that CK18 can predict the disease severity in NASH patients.

Inflammatory markers

- CXCL10 is a proinflammatory cytokine involved in diabetes and obesity.
- CXCL10 exhibited a moderate accuracy for differentiating NASH from simple steatosis.
- Tumor necrosis factor-α (TNF-α) and IL-8 are common inflammatory markers, which also exhibit a moderate performance with a sensitivity and specificity of 72% and 76%.
- When combining these two markers with pyroglutamate, the panel could achieve a sensitivity of 91% and specificity of 87% .

Adipocytokines and hormones

- Fibroblast growth factor 21 (FGF21) secreted by the liver is another potential biomarker for NASH.

- FGF21 had an AUROC of 0.62, and had a > 90% sensitivity and specificity for diagnosing NASH, but the PPV and NPV were (0.59-0.78) and (0.49-0.60), respectively.

  FGF21 was combined with CK18, to improve the PPV to 82% and the NPV to 74%.

- Adiponectin was reported to be decrease in NASH patients , which had an AUROC of 0.71 for diagnosing NASH. AUROC could reach to 0.90 when adiponectin was combined with CK18 M65 and IL-8.

- Other adipocytokines, such as leptin and resistin, may be potential markers for diagnosing NASH

Other Serum Biomarkers

- Serum iron is a common protein associated with oxygen radicals, which contribute to necroinflammation and fibrosis.

- Serum iron was higher in individuals with NASH than in those with simple steatosis.
- In a Japanese study, serum ferritin exhibited a moderate performance for diagnosing NASH.

- the combined serum ferritin with type IV collagen 7S and fasting insulin, which could be used to predict NASH with an AUROC of 0.78-0.85.

Biomarker panel

NASHTest

- The NASHTest combines 13 parameters to diagnose NASH in three categories, namely, NASH, Borderline NASH, and No-NASH, according to Kleiner's criteria.

- A study with 257 people found that the NASHTest achieved an AUROC of 0.79 for NASH, 0.69 for borderline NASH, and 0.77-0.83 for no-NASH.

NASH ClinLipMet score

- The NASH Clin score is a biomarker panel combining AST, fasting insulin, and the PNPLA3 genotype at rs738409, which achieved an AUROC of 0.78 for diagnosing NASH.

- To improve the accuracy, metabolic syndrome-based factors to the NASH Clin score, which was named the ‘NASH ClinLipMet score that improve the AUROC to 0.87 and the sensitivity to 75%.

- More suitable for research because the measurement of fasting insulin and PNPLA3 genotype is costly and complex in clinical practice.

Other biomarker panels

- Simple biomarker panel with the parameters of BMI, ALT, and triglycerides.
- AUROC of 0.80-0.82 in the training and validation cohorts and only included 180 morbidly obese patients after bariatric surgery.

- A clinical score with ALT, gamma-glutamyl transpeptidase, C-reactive protein, and ApoB/ApoA1 ratios.

- The cutoff of 3.8 gave a sensitivity of 90% and a specificity of 87% for distinguishing NASH from NAFLD,

Imaging for NASH

- NASH consists of various parameters; it is difficult to use routine imaging techniques (ultrasonography, CT, or MRI) to distinguish between NASH and simple steatosis.

- Elastography was investigated to distinguish NASH and simple steatosis.

- The cutoff of 2.74 kPa of magnetic resonance elastography (MRE) had an AUROC of 0.93, but the study had several limitations.

- Vibration-controlled transient elastography (VCTE) was performed in South Korean patients with an AUROC of 0.75 and a sensitivity of 86% for diagnosing NASH, but the specificity was only 58%.

- liver iron accumulation (LIC), measured by the MR signal decay values, is significantly related to NAFLD disease severity or fibrosis progression.

- The MRI-based technology assessing LIC was found to have an AUROC of 0.91 for assessing NASH, with a sensitivity of 83% and specificity of 80%[103] .

- Multipara metric MRI technology was used to quantify hepatic steatosis, iron accumulation and fibrosis by 1H-MRS, a T2* map and a T1 relaxation time map, respectively.
- The technology is regarded as a promising imaging biomarker in small studies[108] but awaits independent confirmation from larger trials.

New biomarkers

- Many potential biomarkers involving NASH are under study.
- Circulating microRNAs are potentially regarded as attractive biomarkers for NAFLD disease severity due to their stability.

- A meta-analysis found that miR-34a was reported to have a moderate AUROC of 0.78. MiR-122 had a pooled AUROC of 0.64-0.70 for differentiating NASH and simple steatosis.

- The combination of miR-122, -192, and -21 with CK18-Asp396 achieved an AUROC of 0.83 for diagnosing NASH, while the optimal cutoff gave a moderate sensitivity and specificity.

New biomarkers

- Other new methods have been investigated, such as breath volatile organic compounds (VOCs). Breath VOCs are closely related to oxidative stress, inflammation, and liver diseases.

- A panel consisting of three exhaled compounds, 1-propanol, 3-methyl-butanonitrile, and n-tridecane, had an AUROC of 0.77, PPV of 81%, and NPV of 82% for differentiating NASH and non-NASH.

- Some studies have focused on omic markers.
- The production of lipidomic, proteomic, metabolomics, and microbiome markers was elevated in NASH patients, but more studies with larger validation groups in the future are needed to confirm these findings.

Clinical implication

- Noninvasive biomarkers for NASH are an attractive field.
- CK18 is regarded as a popular biomarker for NASH, but the accuracy varies in current studies.
- Biomarker panels perform well in diagnosing NASH, but most of them are not validated in an independent group.
- Although other noninvasive biomarkers, such as imaging and gene biomarkers, are reported to be relatively high in accuracy, effective methods should be available, simple, inexpensive, and accurate in the clinic.
- Serum biomarkers (e.g., CK18) are less accurate for diagnosing NASH with mild fibrosis, which could lead to higher rates of misdiagnosis.
- To improve the diagnosis of early NASH, biomarker panels or the combination of serum biomarkers with imaging may contribute to ruling in or ruling out NASH with early fibrosis.

Diagnosis of NAFLD Related Fibrosis

According to NASH-CRN Recommendation, fibrosis is categorized into;

- Non-fibrosis or mild fibrosis (Metavir = F0-1)
- Significant fibrosis (SF, Metavir ≥ F2)
- Advanced Fibrosis (AF, Metavir ≥ F3)
- Cirrhosis (Metavir = F4)

It is urgent to identify early fibrosis through effective noninvasive methods.

Proprietary Biomarkers of fibrosis

1. Procollagen of type III collagen(PIIINP)
2. Precursor C3-protein (PRO-C3)
3. Hyaluronic acid (HA)
4. TIMP1

1. **Procollagen of type III collagen(PIIINP)**
    - Common fibrosis marker during fibrogenesis
    - Has a good performance for diagnosing SF (AUROC, 0.81)

Proprietary Biomarkers of fibrosis

2. Precursor C3-protein (PRO-C3)

- Marker of the N-terminal propeptide of type III collagen
- Has an AUROC of 0.75-0.83 for diagnosing AF and 0.76 for cirrhosis

3. Hyaluronic acide (HA)

- Important element of extracellular matrix
- Has an AUROCs of 0.87 for SF, 0.89 for AF and 0.92 for Cirrhosis

4. TIMP1

- fibrosis biomarker reflecting tissue matrix remodeling
- moderate performance for diagnosing SF (AUROC 0.74)

Proprietary Biomarkers of fibrosis

Enhanced Liver Fibrosis (ELF) Test

- ELF test is a commercial tool that combines three circulating matrix turnover components, including HA, PIIINP, and TIMP-1, with age.
- Using a cutoff of 9.8, the ELF test identified AF with a PPV of 72% and NPV of 97%.
- Another model consisting of PRO-C3, age, platelets, and the presence of diabetes can achieve an AUROC of 0.86-0.87 and an NPV of 0.97 for identifying AF.

Nonproprietary Biomarkers of fibrosis or biomarkers panels

1. AST-to-platelet ratio index (APRI)
2. FIB-4 (Fibrosis-4 index)
3. NAFLD fibrosis score (NFS)
4. BARD score

1. APRI

- The APRI is a simpler calculation for diagnosing fibrosis severity in chronic hepatitis C. A recent meta-analysis reported that the APRI had an AUROC of 0.70 for SF, 0.75 for AF, and 0.75 for cirrhosis.

- Additionally, the pooled sensitivity of the APRI was relatively low, with a range of 0.33-0.73 for different cutoffs.

2. FIB-4

- FIB-4 is a common biomarker panel used for assessing fibrosis severity and includes age, platelet count, AST, and ALT. FIB-4 was primarily devised to assess the liver fibrosis severity in hepatitis C patients who were also infected with human immunodeficiency virus.
- An AUROC value of 0.75 for SF, 0.80 for AF, and 0.85 for cirrhosis was reported in NAFLD patients. Two cutoffs were used for a higher PPV and NPV.
- For instance, using a cutoff of 1.3 for FIB-4, the panel predicted AF with an 85% sensitivity, 65% specificity, 36% PPV, and 95% NPV.
- On the other hand, using a cutoff of 3.25, FIB-4 predicted AF with a 26% sensitivity, 98% specificity, 75% PPV, and 85% NPV.

2. FIB-4

- The two cutoffs may improve the PPV and NPV, avoiding unnecessary biopsy, while the specificity of FIB-4 was 0.35 for assessing AF in elderly individuals ≥ 65 years of age, which contributed to a high false positive rate.

3. NAFLD fibrosis score (NFS)

- The NFS is the most common noninvasive biomarker panel for assessing fibrosis severity; the panel consists of age, BMI, hyperglycemia, AST/ALT ratio, platelets, and albumin.
- A multicenter study reported a low cutoff of -1.455 for AF with a PPV of 51%-56% and NPV of 88%-93%, and a high cutoff of 0.676 yielded a PPV of 82%-90% and NPV of 80%-85%.
- Using this model, 75% of biopsies could be spared with 90% correct prediction
- The NFS was widely validated in different races, with a high AUROC and NPV. However, a low cutoff of 0.12 for NFS assessing fibrosis is recommended for the elderly due to a high false positive rate.
- The NFS and FIB-4 are recommended to identify those at low or high risk for AF or cirrhosis in clinical guidelines.

4. BARD score

- The BARD score was an easily calculated score system for assessing fibrosis severity, containing the parameters of BMI, aldosterone renin activity ratio, and the presence of type 2 diabetes mellitus.
- A score of 2-4 increased the risk of AF by 17-fold, with an AUROC of 0.81 and NPV of 96%, but a low PPV of 43%.

- In addition, a meta-analysis reported that the BARD score had a pooled AUROC of 0.64 for SF, 0.73 for AF, and 0.70 for cirrhosis in NAFLD patients.
- Even so, the BARD score was a valuable model for predicting SF due to its ease and lack of indeterminate results in clinical application.

Imaging

1. VCTE

- VCTE is the first FDA-approved elastographic modality performed by FibroScan employing US-based technology.
- This technology measures the velocity of a 50 MHz shear wave that is emitted by a probe in the intercostal space into the liver. The velocity is positively related to liver stiffness with a range of 1.5 to 75 kPa. A higher shear wave value indicates higher liver stiffness.
- However, technical failure was found to be a common phenomenon during the operation, ranging from 6.7% to 27.0%, and was related to a high BMI.
- The “M” probe was the most prevalent probe measuring shear wave velocity, with an AUROC of 0.83 for SF, 0.87 for AF, and 0.92 for cirrhosis.

2. Shear wave elastography (SWE)

- SWE is a new method integrated into conventional US for assessing fibrosis.
- Can measure the shear wave velocity and provide a 2-D, real-time, color map of liver elasticity, but it should be conducted under apnea, and the region of the color map should be large vessel-free and at least 15 mm below the capsule.
- SWE reportedly has a high diagnostic performance for fibrosis assessment in chronic hepatitis patients.
- In NAFLD patients, SWE yielded an AUROC value of 0.86 for SF, 0.89 for AF, and 0.88 for cirrhosis, respectively.

2. Shear wave elastography (SWE)

- SWE was better than FibroScan and acoustic radiation force impulse (ARFI).
- No specific regulations are recommended by the manufacturer for assessing the quality of measurement; thus, some studies assessed the failure rate of SWE with reliability criteria of FibroScan .
- As with the ARFI, the accuracy of SWE is affected by interobserver variation and food intake.
- These measurements are recommended to be performed by very experienced radiologists in patients with fasting for at least 2 hours.

3. ARFI

- ARFI elastography is an alternative tool for fibrosis assessment integrated intoconventional US.
- It uses short-term acoustic pulses to produce shear waves with the results expressed in m/s.
- ARFI should be operated under apnea, and the region of interest should be a vessel-free region.
- AUROC of 0.77 for SF, 0.84 for AF, and 0.84 for cirrhosis.
- Its accuracy was affected by the presence of severe steatosis.
- Further studies are needed to explore the optimal cutoffs of ARFI at different levels of steatosis.

4. MRE

- MRE is a noninvasive MRI-based method measuring liver stiffness by using a modified phase-contrast method.
- MRE can assess the entire liver with a high success rate. It is not affected by steatosis and may be applied in patients with obesity, ascites, or bowel interposition between the liver and anterior abdominal wall.

4. MRE

- The available MRE model contains 2D-MRE (shear wave frequency 60Hz) and 3D-MRE (shear wave frequency 40Hz). 2D-MRE is more frequently used for assessing liver fibrosis in NAFLD patients.
- 3D-MRE had a better performance (AUROC, 0.98) for detecting AF than 2D-MRE (AUROC, 0.92), and the NPVs of 2D-MRE and 3D-MRE were 0.98 and 1.0, respectively.

4. MRE

- , MRE was superior to FibroScan, ARFI, and common biomarker panels for discriminating dichotomized fibrosis stages in NAFLD patients.
- Considering the higher accuracy of MRE in diagnosing fibrosis, it is increasingly regarded as a promising surrogate biomarker for monitoring fibrosis progression and endpoints of fibrosis therapy.
- It cannot be applied to individuals with hepatic iron overload due to the interfering signal intensity.
- the cost of MRE and its dependence on MRI facilities limit its wide application.

New Biomarkers

- Serum DNA methylation has been investigated as a potential biomarker for assessing fibrosis.
- The plasma DNA methylation of PPARγ promoter was reported to have a good performance for diagnosing AF (AUROC, 0.91), and the cutoff of 0.81 gave a PPV of 91% and NPV of 87%.
- In addition, the DNA methylation at the PPARγ promoter is superior to the NFS in diagnostic performance and avoids using two cutoffs, but it should be validated in more independent groups.

Clinical Implication

- Biomarker panels are cheap, feasible, reproducible, and have a good NPV for fibrosis, but they are limited by its low PPV .
- MRE shows excellent accuracy for fibrosis severity but may only be used in some drug studies due to its high cost and unavailability.
- Transient elastography together with biomarker panels would be widely used for assessing fibrosis, but the efficiency should be evaluated in more independent groups.
- It is recommended to combine serum biomarkers or clinical rules with imaging tools to diagnose fibrosis, which could reduce unnecessary diagnostic liver biopsies.

NONINVASIVE BIOMARKERS FOR DISEASE PROGRESSION AND THERAPY

Tracking disease progression

- NAFLD significantly increases the risk of liver disease-related morbidity, mortality, and liver transplantation.
- Fibrosis, but not simple steatosis and NASH, increased the risk of mortality in NAFLD patients in a retrospective study with a mean followup period of 20 years.
- One stage of fibrosis progression takes 14.3 years and 7.1 years in individuals with simple steatosis and NASH patients, respectively.
- In addition, most NAFLD cases are asymptomatic until the disease has progressed to cirrhosis, and repeated biopsy is impractical.
- Therefore, there is a need to apply useful noninvasive biomarkers to monitor disease progression.

Tracking disease progression

- Baseline liver histology, APRI, FIB-4, and NFS for predicting clinical outcomes had AUROCs of 0.85, 0.89, 0.89 and 0.79, respectively.
- Another study reported that FibroScan had an accuracy of 0.73 for predicting all-course mortality.
- Further studies are needed to determine more effective noninvasive biomarkers for the progression of NASH to NASH-related fibrosis and the progression of NASH-related fibrosis to adverse clinical outcomes.

Monitoring responses to therapies

- In terms of NAFLD treatment, it is impractical to observe the primary endpoint of mortality due to long-term follow-up.
- FDA recommends that histological improvement be confirmed when the resolution of NASH is obtained without the worsening of fibrosis or when fibrosis is improved without the worsening of NASH.
- However, repeated biopsy hinders the development of drugs; thus, there is a need to investigate noninvasive surrogates replacing biopsy.
- MRI-PDFF was usually employed to evaluate the liver fat content change in clinical trials of NASH patients.
- A study of 113 NASH patients treated with obeticholic acid found that MRI-PDFF had an AUROC of 0.81 for reduced histological steatosis grade.
- In contrast, a recent phase II trial of selonsertib found that MRI-PDFF had an AUROC of 0.70 for reduced histological steatosis grade, and the optimal cutoff was 0% with a PPV of 39% and NPV of 92%.
- Whether the change in MRI-PDFF could be regarded as an effective surrogate endpoint for NASH treatment should be further evaluated.

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Monitoring responses to therapies

- Liver function has been regularly regarded as a noninvasive biomarker for assessing the monitoring treatment effect, while ALT concentrations in about two-thirds of patients is normal, and NASH patients usually exhibit spontaneous changes in liver function.

- ALT change is usually accompanied by a steatosis change, which is regarded as an effective noninvasive endpoint substituting the histological changes in NASH.

- The change in liver stiffness measurement (LSM) measured by MRE was evaluated to investigate the antifibrosis effect in NAFLD.

- MRE had an AUROC of 0.62, PPV of 39%, and NPV of 92% for fibrosis improvement.

CONCLUSION

- the extensive development of noninvasive methods in the NAFLD field, from serum biomarkers and imaging to omics.

- US and H-MRI have a relatively high accuracy for diagnosing NAFLD, and US is prevalently used in clinical practice and research due to its availability and low cost.

- There are currently no effective noninvasive biomarkers recommended for diagnosing NASH.

- Future studies are needed to investigate more efficient noninvasive biomarkers for distinguishing NASH from simple steatosis.

- VCTE is the FDA-approved elastographic model for assessing fibrosis severity, and it could further improve the diagnostic performance when combined with biomarker panels.

- Furthermore, effective algorithms consisting of imaging and nonimaging biomarkers should be applied to clinical practice to reduce unnecessary biopsies .

- In addition, there is a need to investigate the cost-effectiveness of noninvasive evaluations in diagnosing NAFLD, tracking disease progression, and monitoring responses to the therapies.

Thank you for your kind attention