Non-invasive methods for steatosis and fibrosis detection

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Introduction

- The diagnostic assessment of liver injury is an important step in the management of patients with chronic liver disease (CLD).
- Although liver biopsy is the reference standard for the assessment of necroinflammation and fibrosis, there was a development of several non-invasive tests (NITs) as alternatives to liver biopsy.
- Such non-invasive approaches mostly include biological (serum biomarker algorithms) or physical (imaging assessment of tissue stiffness) assessments.

Estimated prevalence NAFLD

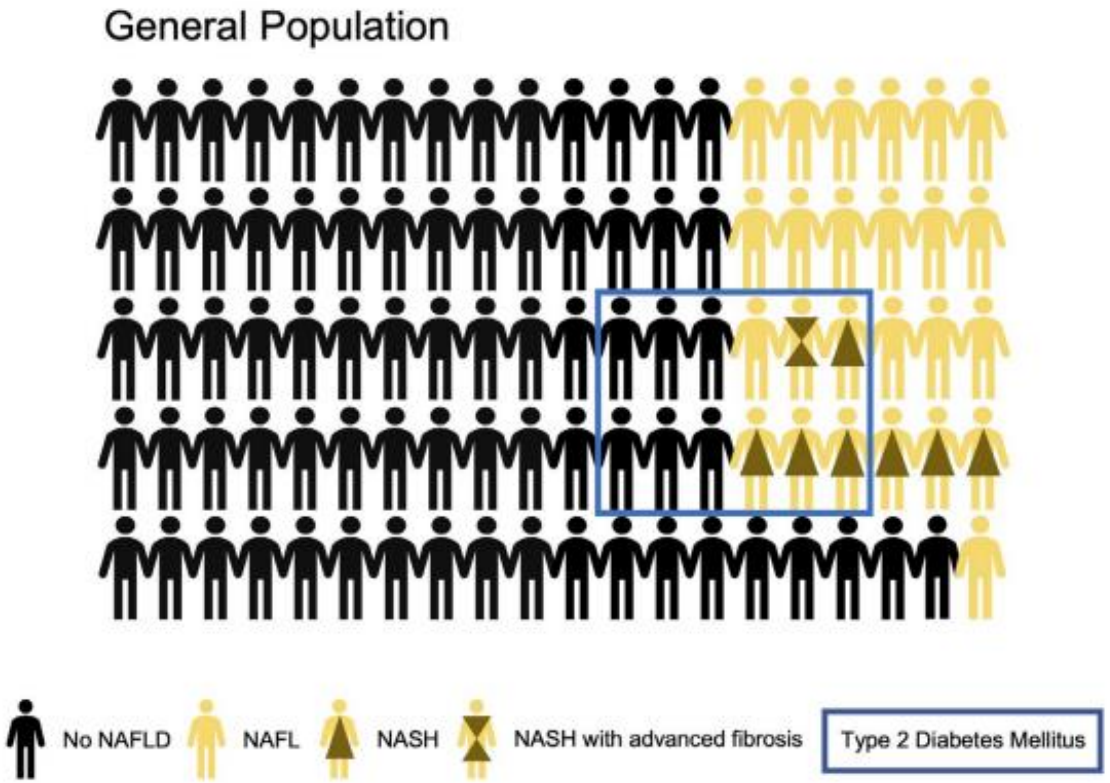

FIG. 1. Schematic representation of the proportions of patients with NAFL, NASH, and advanced fibrosis in the general population and among patients with T2DM. In the general population, approximately 25% of patients have NAFLD; among those, up to 30% have NASH, of whom up to 20% have developed or will develop advanced liver fibrosis (stage 3-4 fibrosis). T2DM represents approximately 10% of the U.S. population. It is estimated that 40%-70% of patients with T2DM have underlying NAFLD, and among those ≈37% have NASH and ≈17% will develop advanced fibrosis.

Vieira et al. 2021

1. NAFLD

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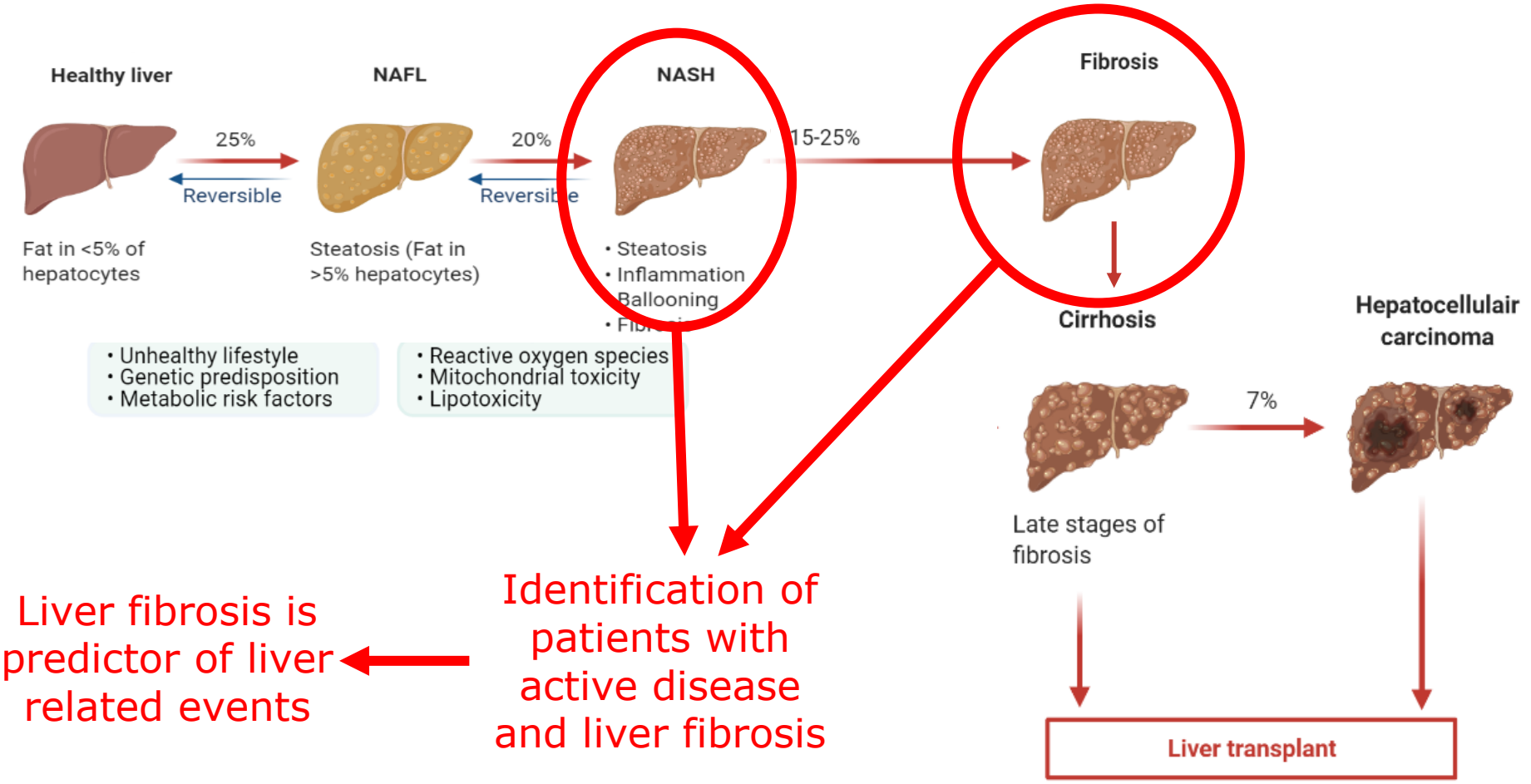
NAFLD spectrum



Identification of patients with active disease and liver fibrosis

Liver fibrosis is predictor of liver related events

L.Heyens et al 2021

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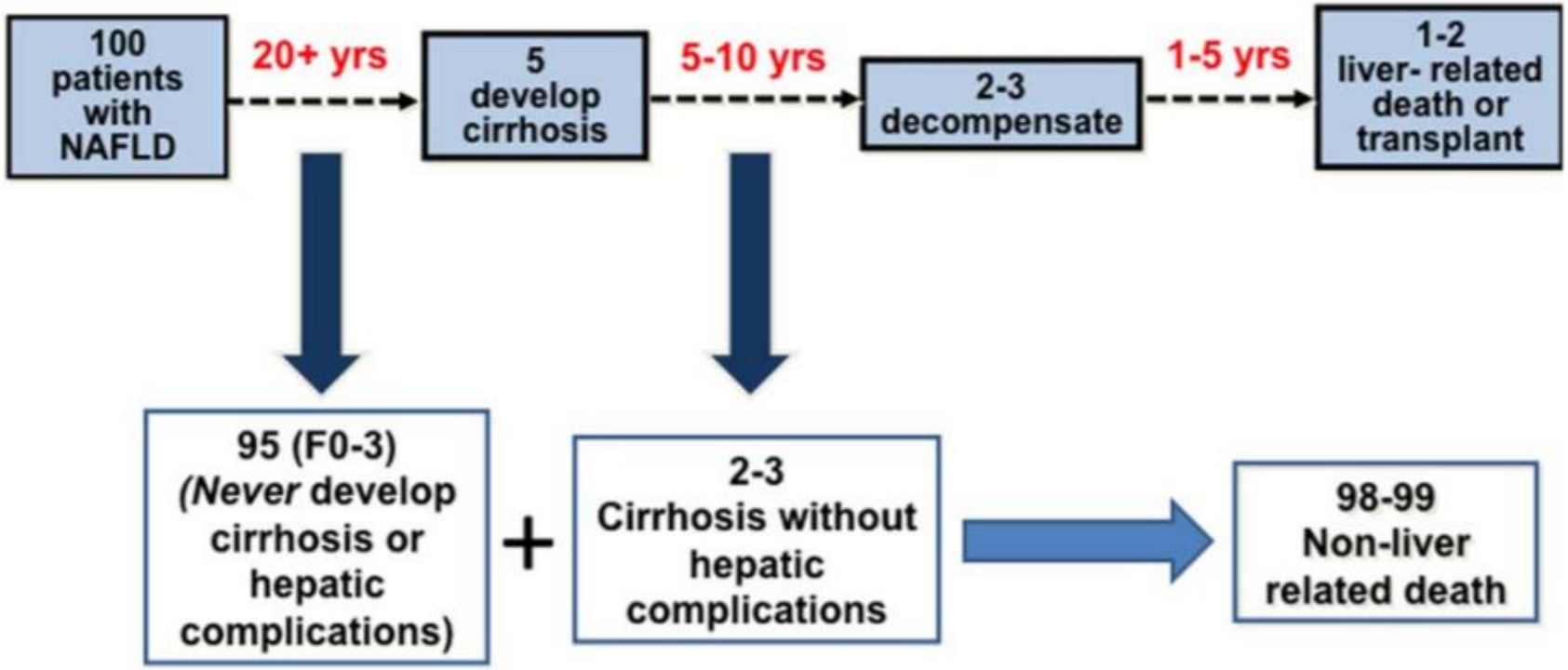
Progression to cirrhosis



Rinella et al. 2016

2. Diagnostic methods

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2. Diagnostic methods

2.1 Liver biopsy

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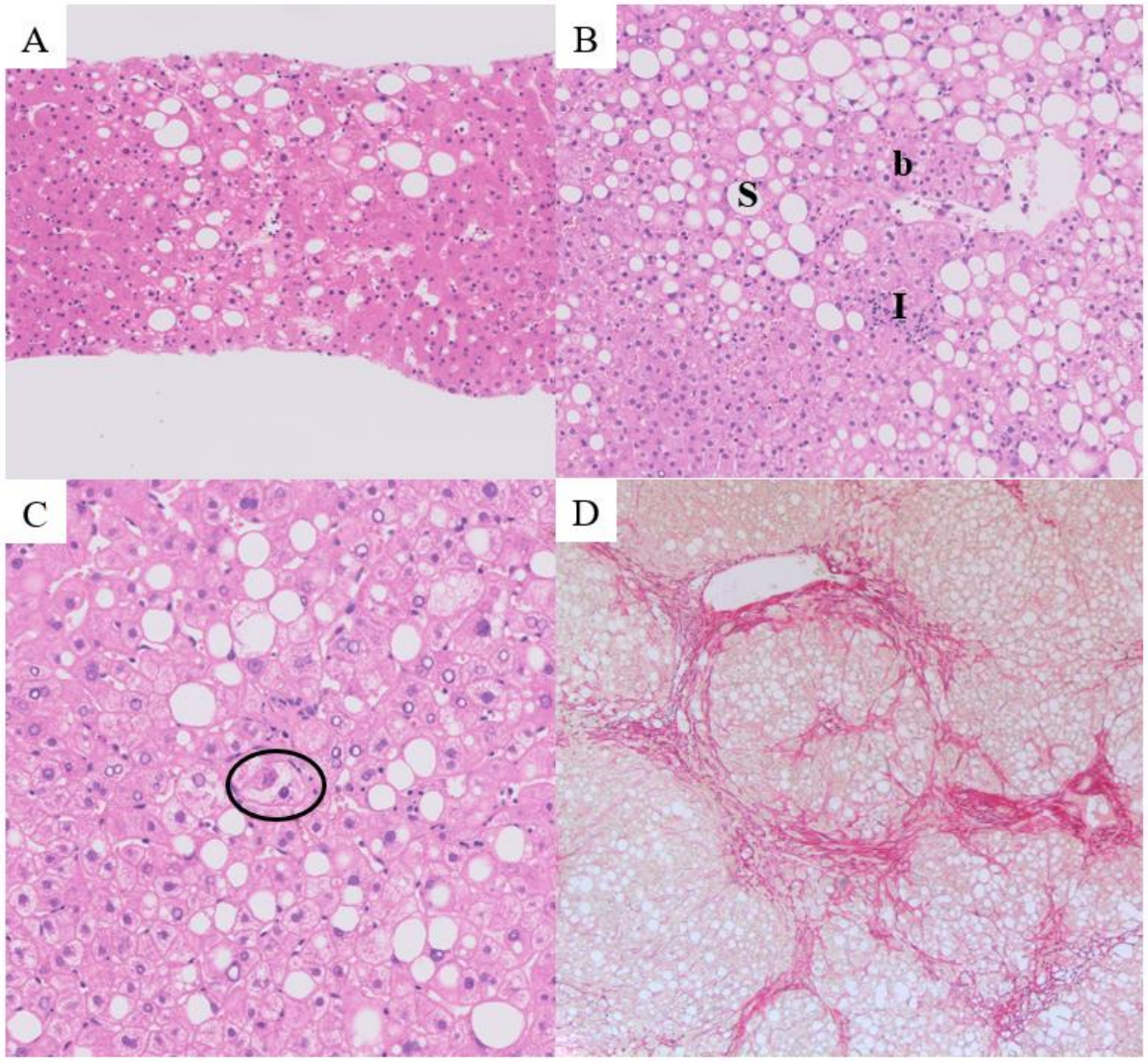
Histological features



A: Steatosis without inflammation (hematoxylin-eosin (H&E) staining 10x).

B: Steatohepatitis (NASH) with marked steatosis (S), ballooning (b), and inflammation (I) (H&E staining 20x).

C: Group of hepatocytes with ballooning (H&E staining 20x).

D: Fibrosis formation corresponding to an NAFLD activity score of 6 and F3 fibrosis (Sirius staining 20x).

Heyens et al. 2021

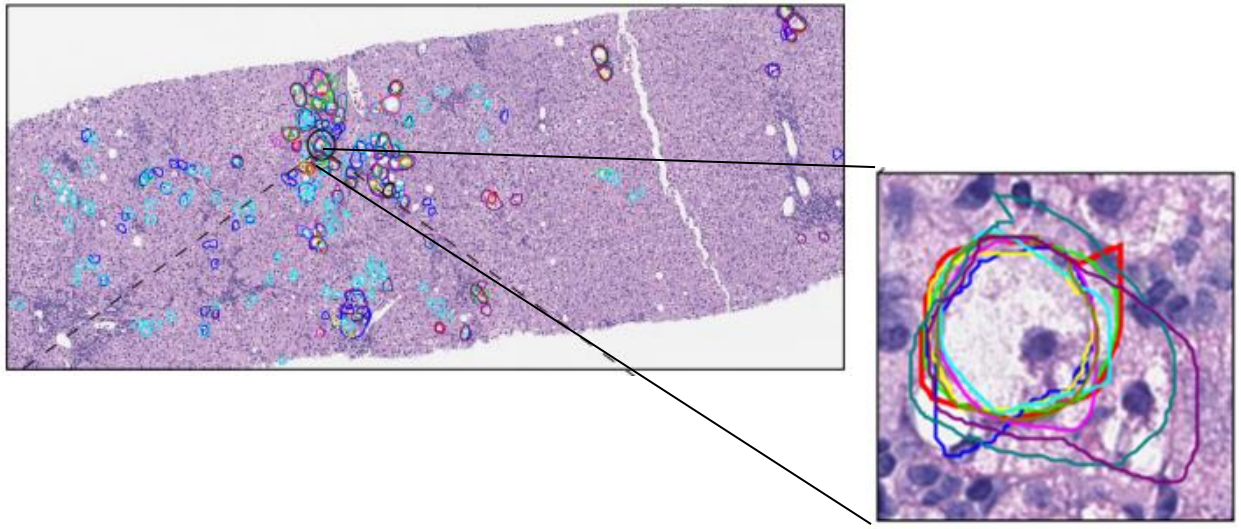
Thanks to Prof. Dr. Ann Driessen (Department of Pathology, Antwerp University Hospital, Antwerp University, Belgium) and Dr. P. Van Eyken (Department of Pathological Dissection, East Limburg Hospital, Genk, Belgium) for the images.

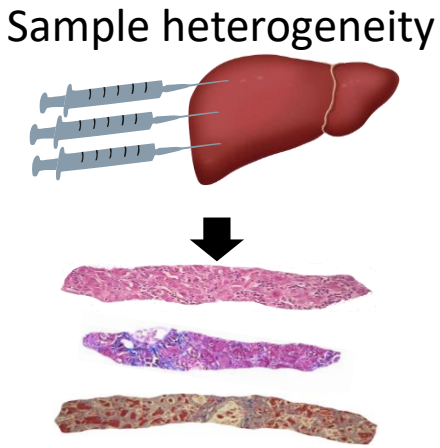
Liver biopsy

Pro's and cons

☑ To identify cause of fibrosis / cirrhosis → e.g. AI, PBC…

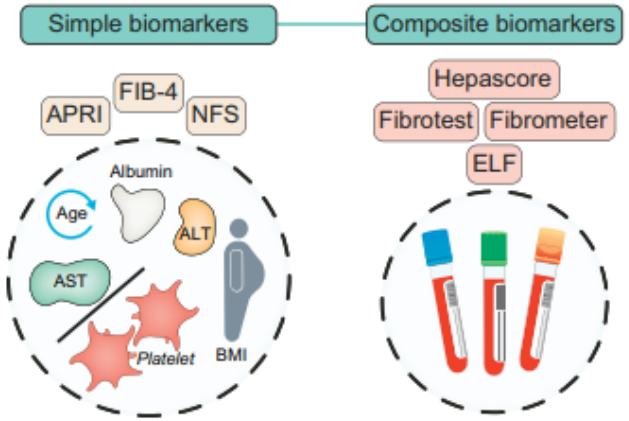
☒ Costly
☒ Possibility of complications
☒ Invasive → repetition is not preferred
☒ Sampling error → ballooning with NASH
☒ Misclassification bias
☒ Sample heterogeneity

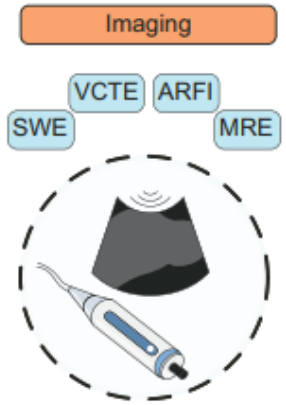




Brunt et al. 2022
Afdhal et al. 2004
Rosenberg et al. 2004

Established NITs





Cost of patented tests	Local resources /cost	VCTE: provider experience crucial (>500 examinations); MRE: cost, requires MR infrastructure/radiologist
NFS is etiology specific; other biomarkers validated in several etiologies	Validation in specific etiology	VCTE: validated in multiple etiologies of CLD Other techniques: mostly validated in HCV and NAFLD
Composite (patented) biomarkers performed in validated laboratories	Quality check	Pivotal for VCTE (>10 validated measures, IQR <30%); other elastographic techniques ?
Acute hepatitis, Gilbert's, cholestasis, hemolysis, HIV infection	Risk factors for error	VCTE: steatosis, inflammation, cholestasis, congestion, food intake; other elastographic techniques?

Combine whenever possible → explain discordance → consider liver biopsy

Risk factors

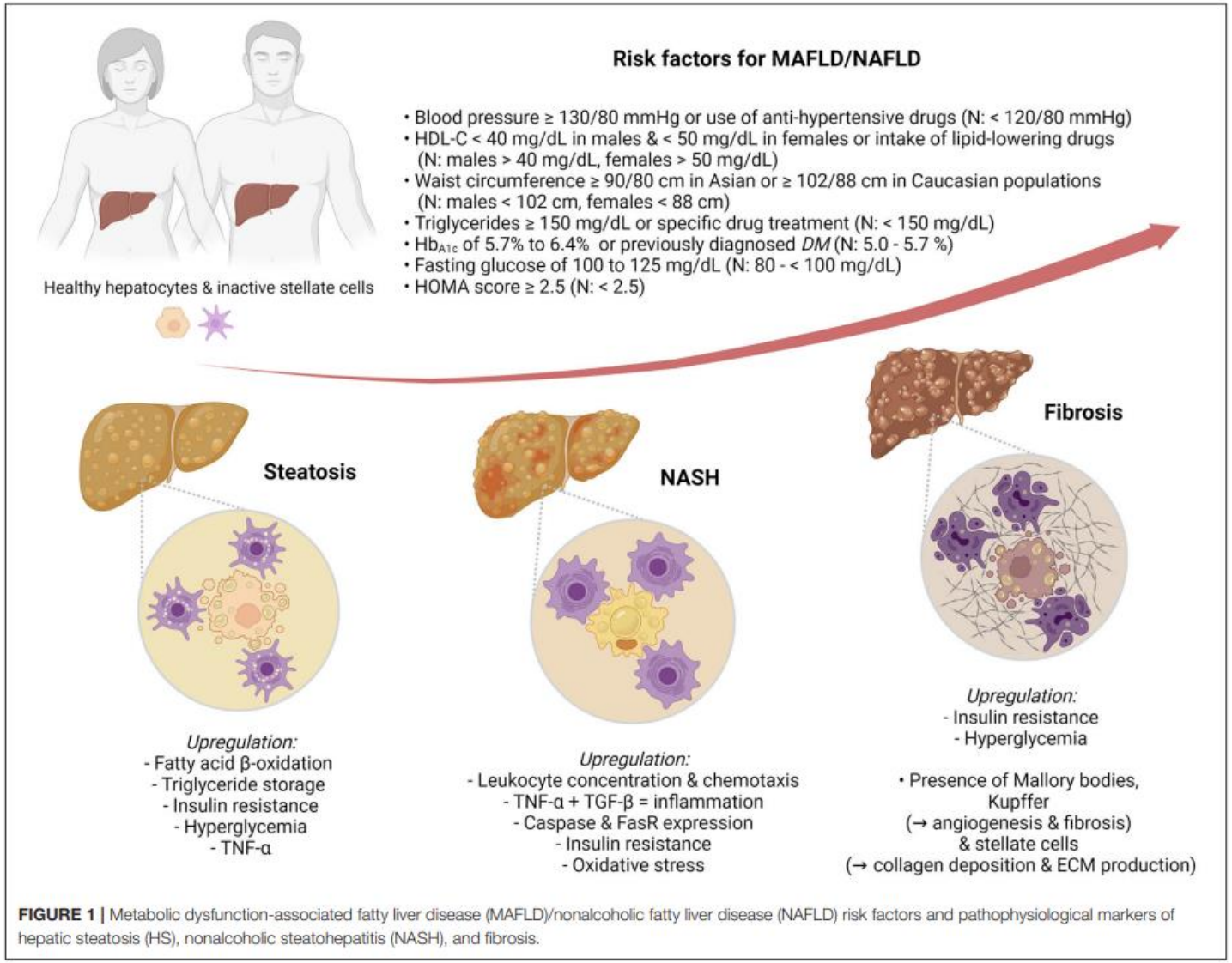
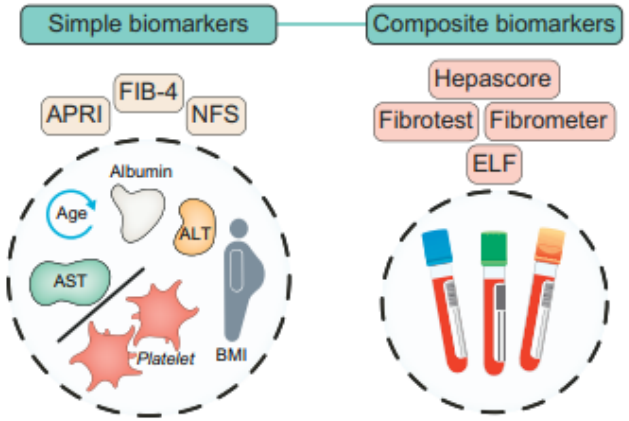
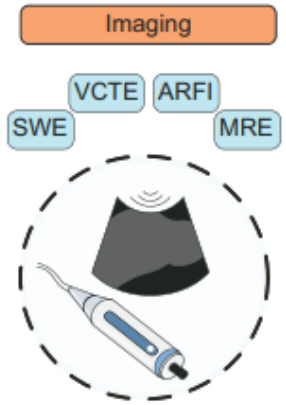


FIGURE 1 | Metabolic dysfunction-associated fatty liver disease (MAFLD)/nonalcoholic fatty liver disease (NAFLD) risk factors and pathophysiological markers of hepatic steatosis (HS), nonalcoholic steatohepatitis (NASH), and fibrosis.

N. De los Angeles Segura-Azuara et al 2022

Established NITs





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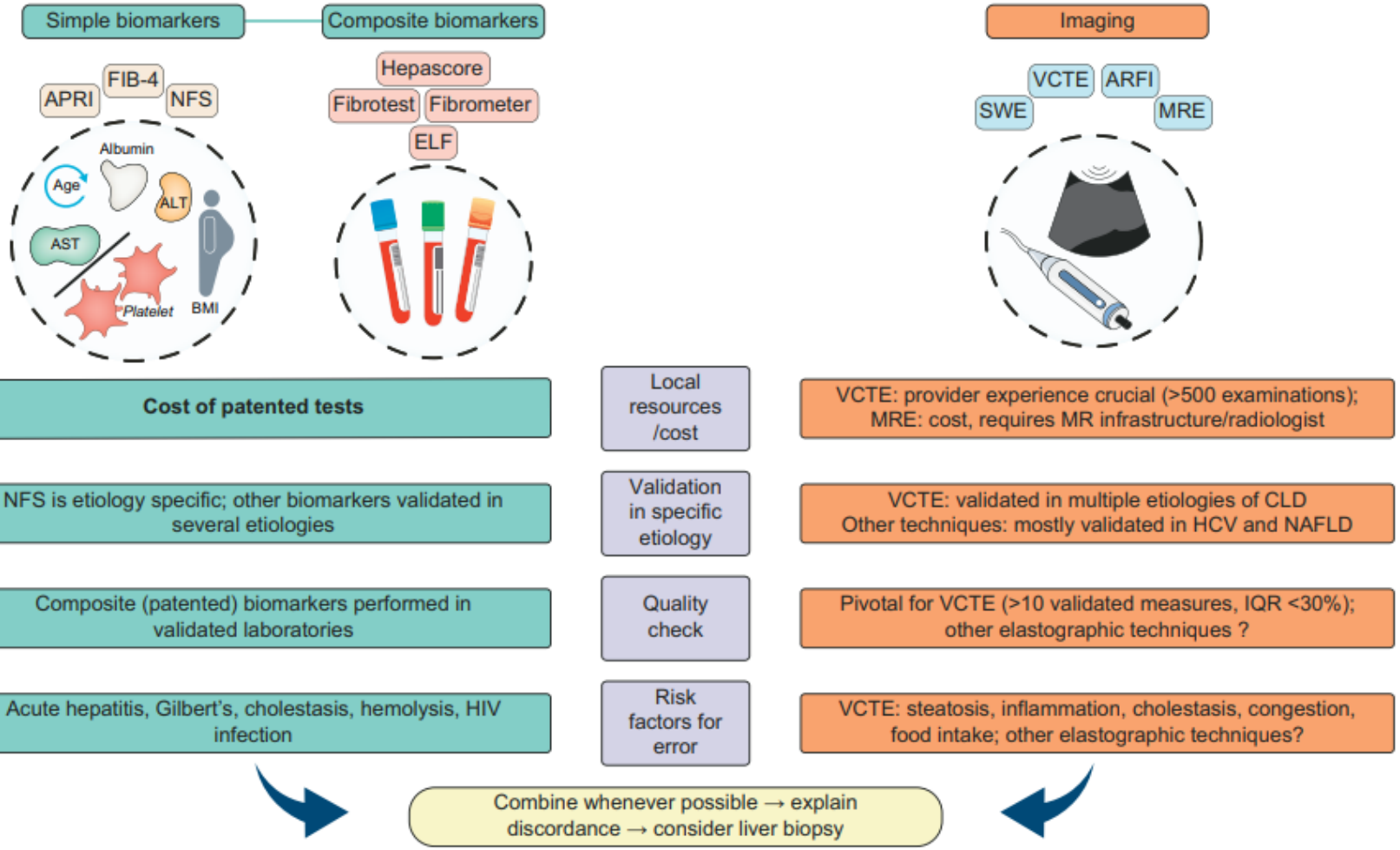
Performance NITs



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Concepts to keep in mind

Table 1. Common measures for evaluating the diagnostic accuracy of non-invasive fibrosis tests.

Measures	
Sensitivity	Probability that a patient with the condition (*e.g.* advanced fibrosis) tests positive
Specificity	Probability that a patient without the condition tests negative
Positive predictive value	Probability that a patient who tests positive has the condition
Negative predictive value	Probability that a patient who tests negative does not have the condition
Area under the receiver operating curve	The diagnostic ability of a binary classifier at a specific cut-off, *i.e.* the probability that this classifier will correctly rank a randomly chosen person with the disease higher than a randomly chosen person without the disease
Positive likelihood ratio	How many times more likely positive index test results are in the diseased group compared to the non-diseased group. Estimated as sensitivity/(1−specificity)
Negative likelihood ratio	How many times less likely negative index test results are in the diseased group compared to the non-diseased group. Estimated as (1−sensitivity)/specificity

		Disease		Predictive Value	
		⊕	⊖		
Test	⊕	**A** True Positive (TP)	**B** False Positive (FP)	Positive Predictive Value (PPV) $\frac{TP}{TP+FP} = \frac{A}{A+B}$	Total Positive Results (A + B)
	⊖	**C** False Negative (FN)	**D** True Negative (TN)	Negative Predictive Value (NPV) $\frac{TN}{FN+TN} = \frac{D}{C+D}$	Total Negative Results (C + D)
Sensitivity & Specificity		Sensitivity $\frac{TP}{TP+FN} = \frac{A}{A+C}$	Specificity $\frac{TN}{FP+TN} = \frac{D}{B+D}$		
		All diseased patients (A + C)	All non-diseased patients (B + D)		

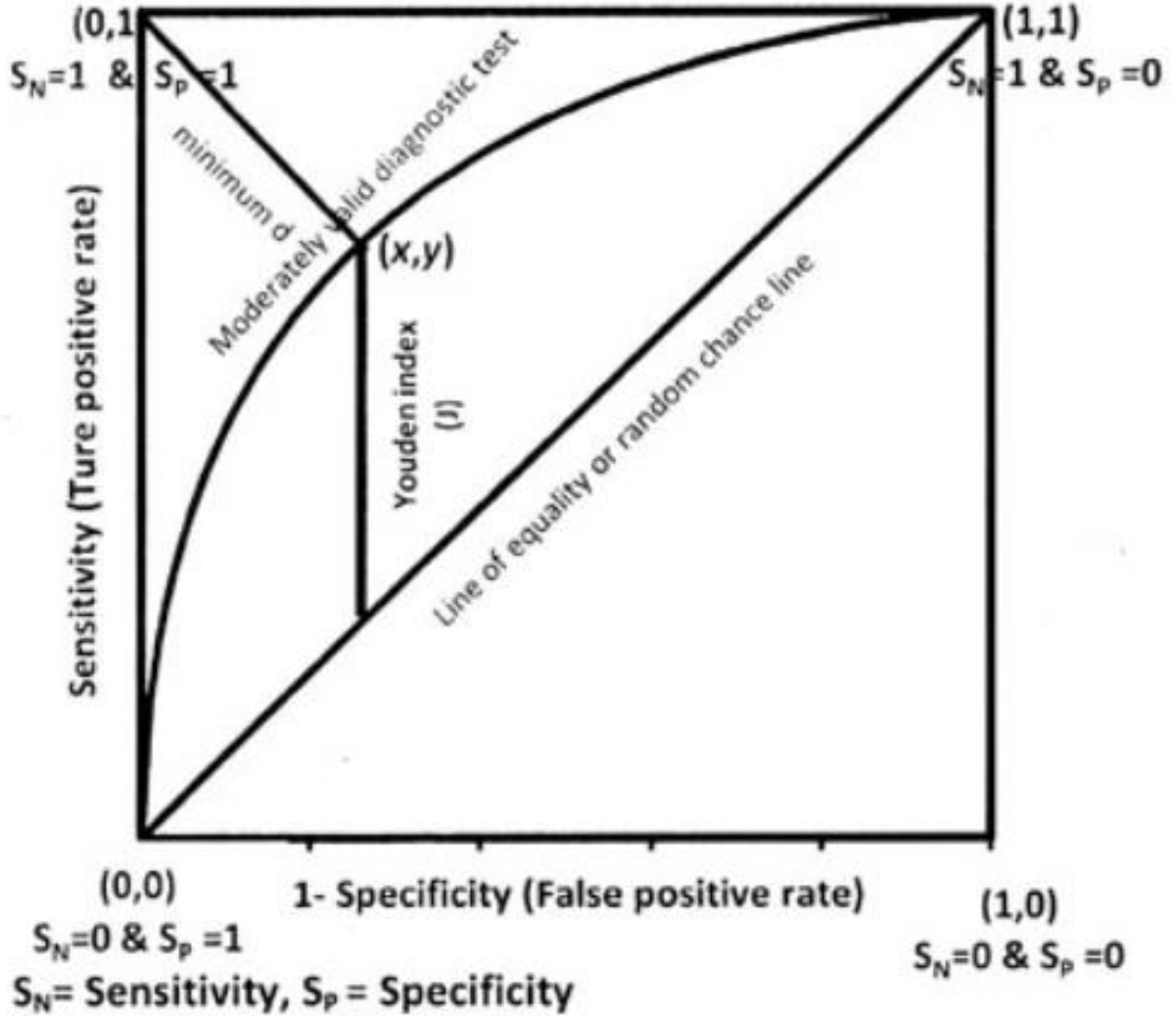

Fig. 1 *ROC curve and its components.*

2. Diagnostic methods

2.2 NITs for steatosis

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Diagnosing steatosis

Blood tests

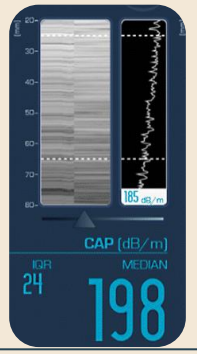
Markers

Waist circumference. BMI. GGT. lipid profile. insulin…

Tests

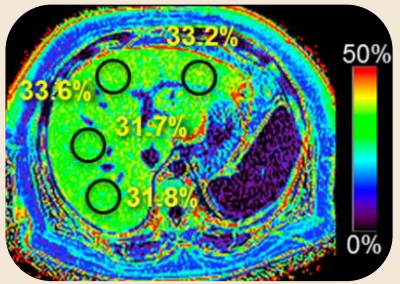
FLI, HIS, NAFLD liver fat score, ANI

Imaging



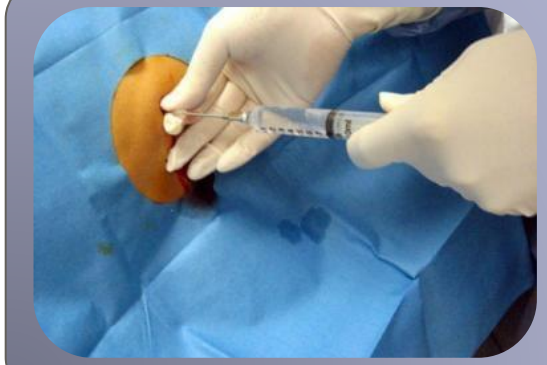
CAP

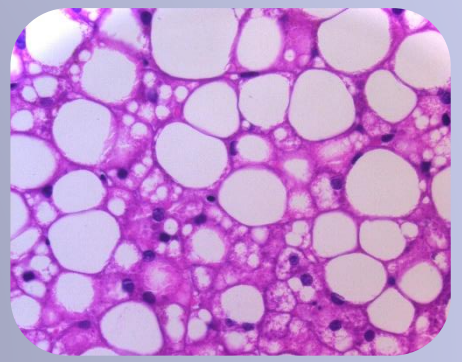
Ultrasound



MRI-PDFF

Liver biopsy





Availability

Cost

Heyens et al. 2021
Caussy et al. 2019

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Diagnosing liver steatosis – blood based biomarkers

NAFLD screening tool	Patient data required
TyG Index	Triglycerides, glucose
Fatty Liver Index (FLI)	Body mass index, waist circumference, triglycerides, GGT
Hepatic Steatosis Index (HSI)	Body mass index, gender, AST, ALT, presence or absence of type 2 diabetes
Non-Alcoholic Fatty Liver Disease - Liver Fat Score (NAFLD-LFS)	Presence of absence of metabolic syndrome or type 2 diabetes, insulin levels, AST, ALT
NAFLD Ridge Score	ALT, HDL-cholesterol, triglycerides, HbA1c, WBC, presence of hypertension
Lipid accumulation product	Waist circumference, triglycerides

Diagnosing steatosis

Blood tests

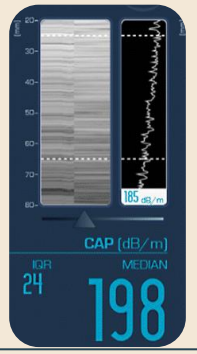
Markers

Waist circumference. BMI. GGT. lipid profile. insulin…

Tests

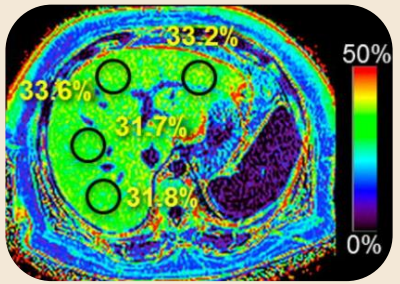
FLI, HIS, NAFLD liver fat score, ANI

Imaging



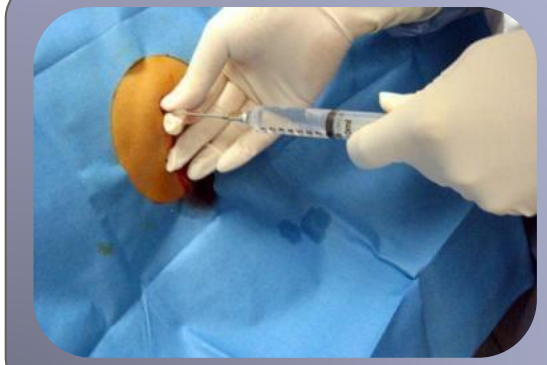
CAP

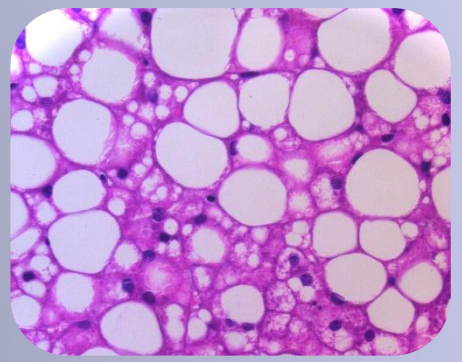
Ultrasound



MRI-PDFF

Liver biopsy





Availability

Cost

Heyens et al. 2021
Caussy et al. 2019

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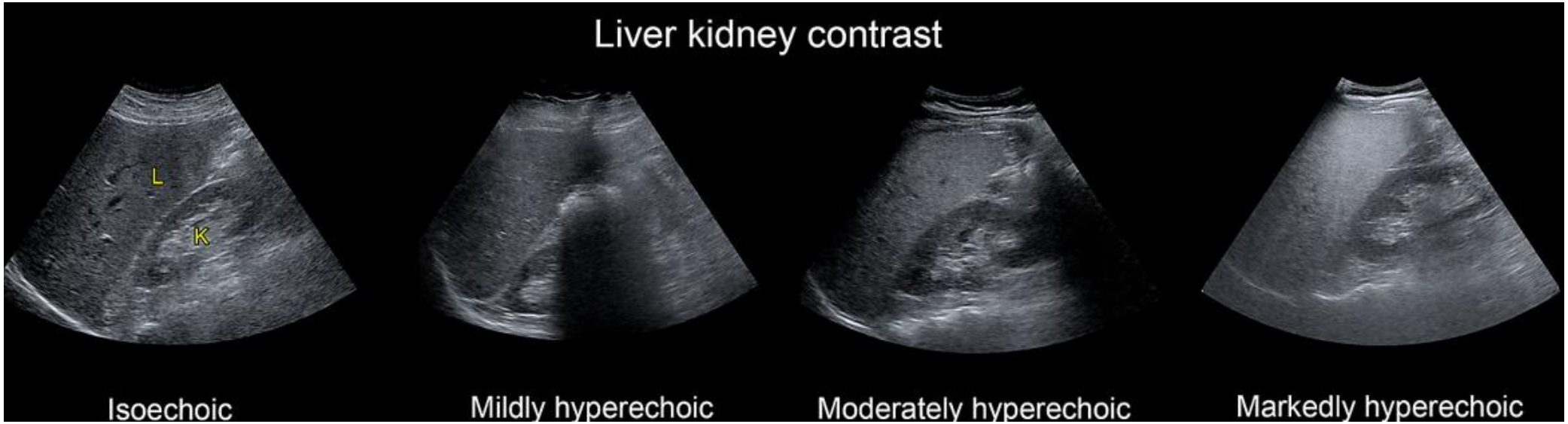
1. Standard ultrasonography

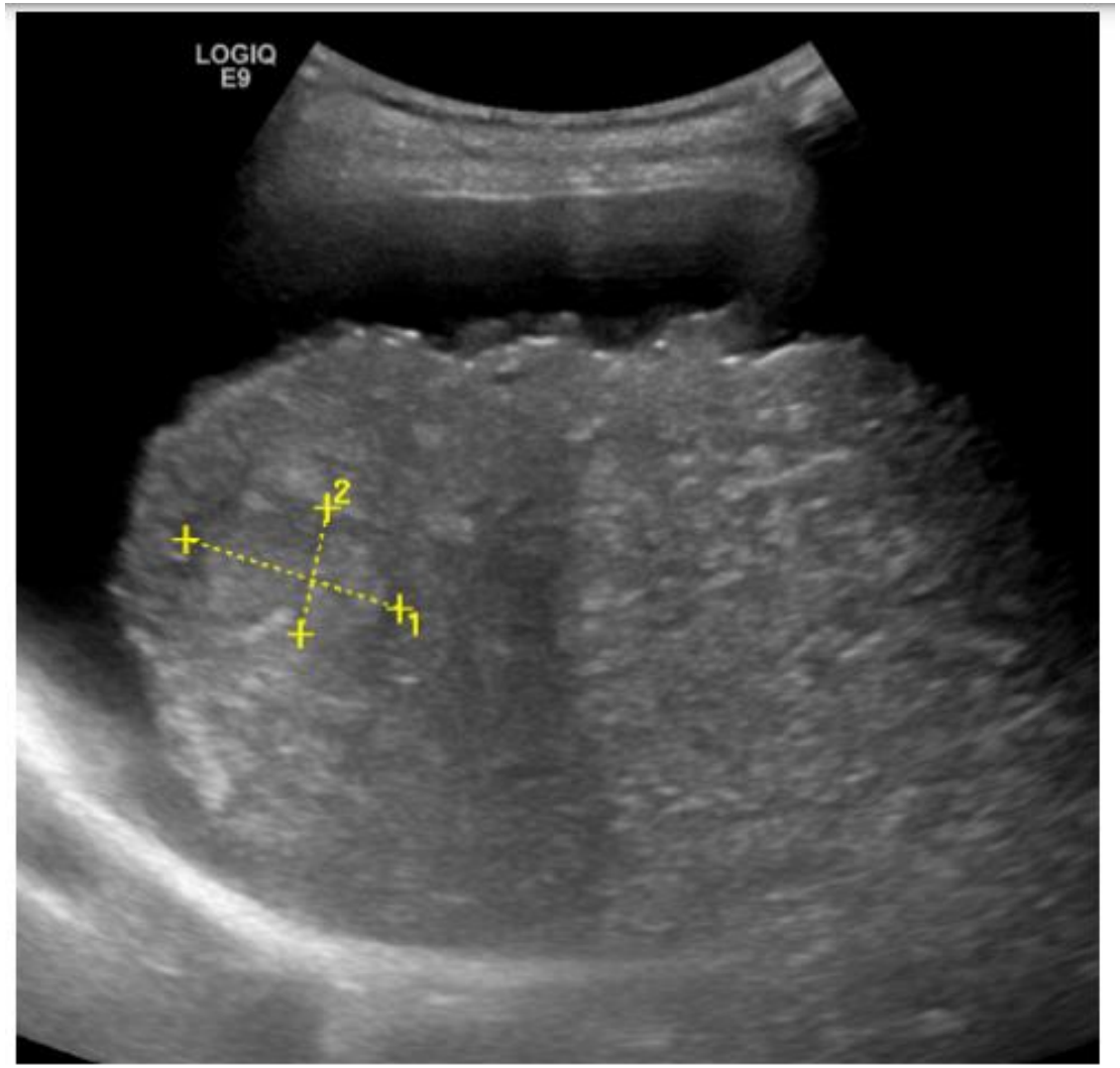
- Diagnose and grade steatosis
  - mild (increased echogenicity compared to the right kidney)
  - moderate (obliteration of the portal triads in the affected liver)
  - severe (attenuation of the hepatic parenchyma with non-visualization of the right hemi diaphragm)



C. Wong et al 2018

1. Standard ultrasonography

- Also used to:
  - Detect cirrhosis
  - Screening for HCC



C. Wong et al 2018
Petzold 2022

1. Standard ultrasonography

- ☑ Widely available
- ☑ Inexpensive
- ☑ Well tolerated
- ☑ Safe modality

- ☒ Subjectivity and operator dependence *limit its usefulness for accurately grading steatosis.*
- ☒ Can be false negative with steatosis of 6–10%, morbid obesity, and concomitant renal disease
- ☒ Obesity can impair visualization of liver

In the absence of more available or widely validated method, ultrasonography is the method currently recommended for diagnosis of steatosis by both the AASLD and EASL

C. Wong et al 2018
D. Huang et al. 2022

2. Controlled attenuation parameter (CAP)

- Not free
- Company: EchoSens (incorporated into FibroScan® device)
- **Method:**
  - Providing a numerical value (dB/m) that correlates with steatosis grading.
- **Differentiates:** mild, moderate, and severe steatosis
- **AUROC**: 0.82, 0.86, and 0.88 at cut off 248, 268, and 280 dB/m respectively.
- **Cut-off**: varies and no consensus has been reached
- **Aiming at:**

2. Controlled attenuation parameter (CAP)

☑ Many studies have assessed this technique
☑ Easy to perform
☑ Relatively widely available
☑ Accurate
☑ Reproducible

☒ However, they are limited by small sample size
☒ Limited among patients with morbid or severe obesity
☒ Limited with ascites
☒ Adjustments are needed based on BMI and diabetes

3. MRI-PDFF

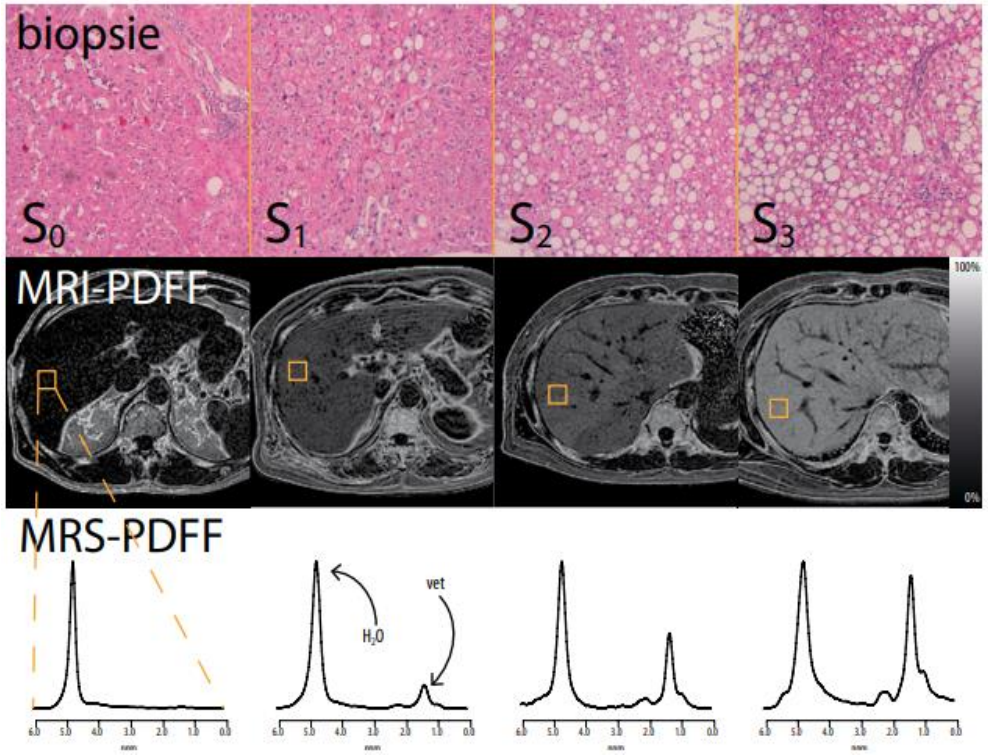
- Not free
- Method: depends on the ability of MRI technique to separate water and fat signals in any tissue including liver parenchyma, based on chemical shift encoded MRI

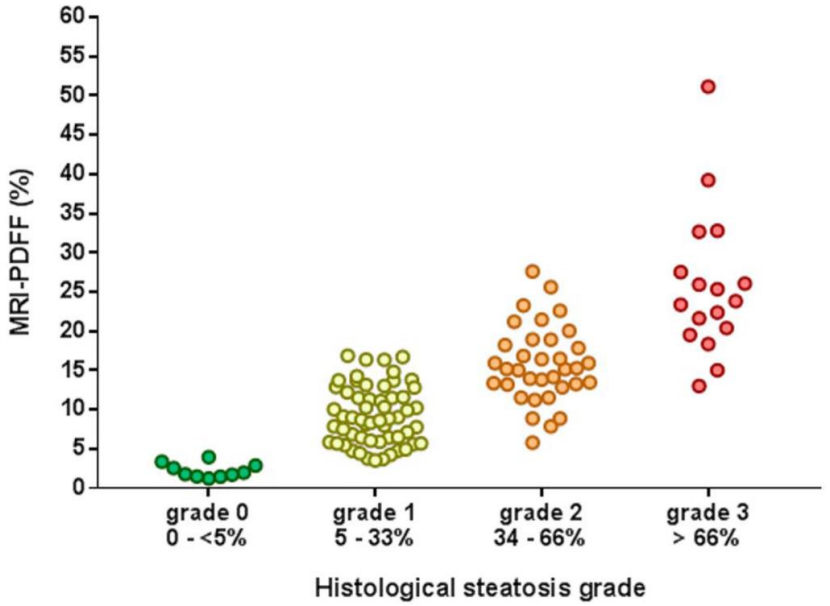
$$\text{MRI-PDFF} = \frac{fat}{fat+water} \times 100\,\%$$

- AUROC: 0.99 (for quantifying hepatic fat)
- Aiming at:

Park et al 2017

3. MRI-PDFF





Comparison of liver biopsies (top row) with MRI images (middle row) and proton MR spectra (bottom row) of the 'proton density fat fraction' (PDFF). Each row shows hepatic fatty degeneration in ascending grade (S0-S3). In the liver biopsies, one assesses the number of hepatocytes containing a fat vacuole (white recess). At MRI-PDFF the signal intensity of the liver increases (the scale goes from 0-100%), while with MRS-PDFF the signal peak of the liver fat - on the right side of the graph -increases.

Runge et al. 2016

3. MRI-PDFF

☑ Superior to CAP for quantifying hepatic fat with AUROC of 0.99 vs. 0.85 (P=0.009)

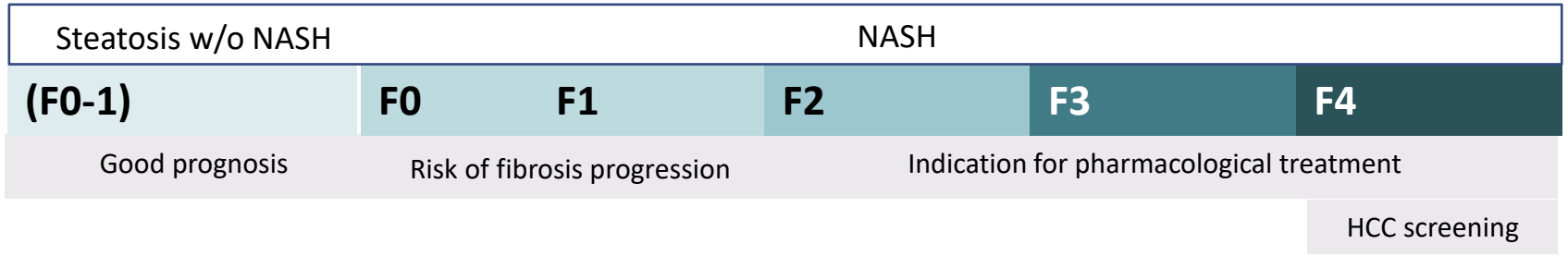
☑ Excellent concordance to liver biopsy based quantification of liver fat

☒ Expensive

☒ Lack of widespread availability

☒ Expert needed for assessment

Altamirano et al. 2020



STEATOSIS DIAGNOSIS, QUANTIFICATION

MRI PDFF
US DEVICES
CAP
Blood based biomarkers

2. Diagnostic methods

2.3 Fibrosis

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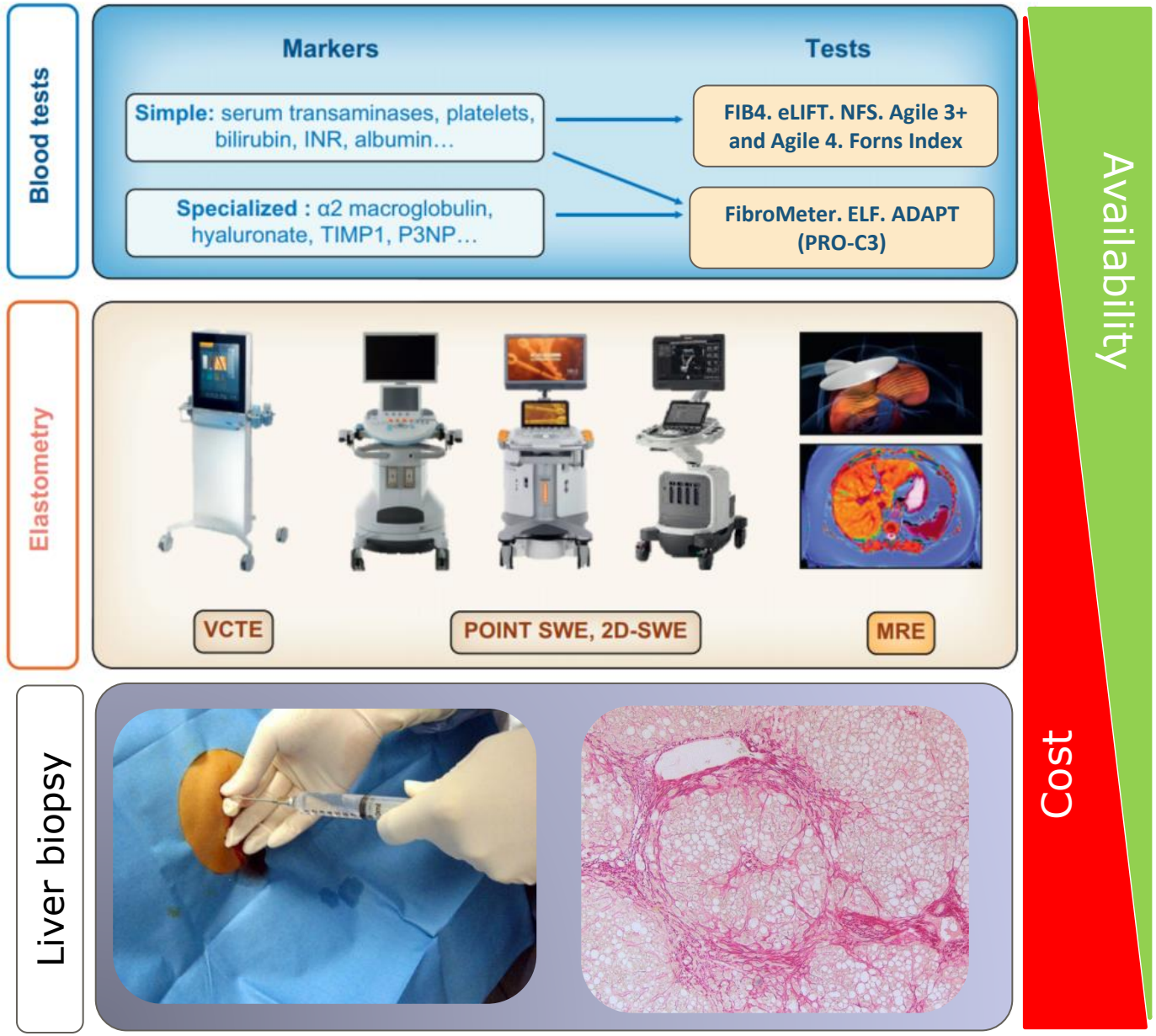
Diagnosing liver fibrosis



Heyens et al. 2021

Boursier et al. 2020

Diagnosis of fibrosis - blood-based biomarkers

1. NAFLD Fibrosis Score (NFS)
2. Fibrosis-4 (FIB-4) and subtypes
3. AST to Platelet Ratio Index (APRI)
4. BARD Score
5. FibroTest® (FT) (FibroSURE in the United States)
6. Enhanced liver fibrosis (ELF)
7. eLIFT
8. Forns Index
9. FibroMeter
10. Agile 3+ and Agile 4

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NFS and FIB-4

- Based on
  - NFS : the combination of 6 variables (age, BMI, AST/ALT ratio, platelet count, hyperglycaemia and albumin)
  - FIB-4: the combination of age, AST, ALT and platelet count.

- Use 2 cut-offs to rule-out or rule-in advanced fibrosis:
  - one with high sensitivity (1.3 for FIB-4, and -1.455 for NFS)
  - another with high specificity (3.25 for FIB-4 and 0.676 for NFS).

Advantages of NFS and FIB-4

- i) They are both based on simple variables widely available in clinical practice.
- ii) Their results can be easily obtained at bedside on free online calculators.
- iii) Their overall diagnostic accuracy for advanced fibrosis, as reported by a recent meta-analysis (n = 36 studies in 9,074 patients), is good with AUROCs of 0.80 for FIB-4 and 0.78 for NFS.
- iv) Both can **exclude the presence of advanced fibrosis with high NPV** (>90%).

Xiao et al, 2017

Disadvantages of NFS and FIB-4

- i) Their **PPV** for confirming advanced fibrosis is **modest** (<70%) with the risk of false positive results.
- ii) About one-third of patients fall in-between the upper and lower cut-off values giving an undetermined result.
- iii) Older **age** has been suggested to affect their diagnostic accuracy. Therefore higher cut-offs have been proposed for ruling out advanced fibrosis in patients older than 65 years (2.0 for FIB-4, and 0.12 for NFS) but they need to be externally validated.
- iv) Preliminary evidence suggests lower performance of NFS in obese patients and in diabetic patients, where FIB-4 could be preferred.

Xiao et al, 2017; McPherson et al, 2017; Petta et al, 2019; Joo et al, 2018; Bril et al, 2020; Bertot et al, 2018

FIB-4

FREE

☒Inclusion of age might lead to a falsely worse score in the elderly population and thus increase the false-positive rate

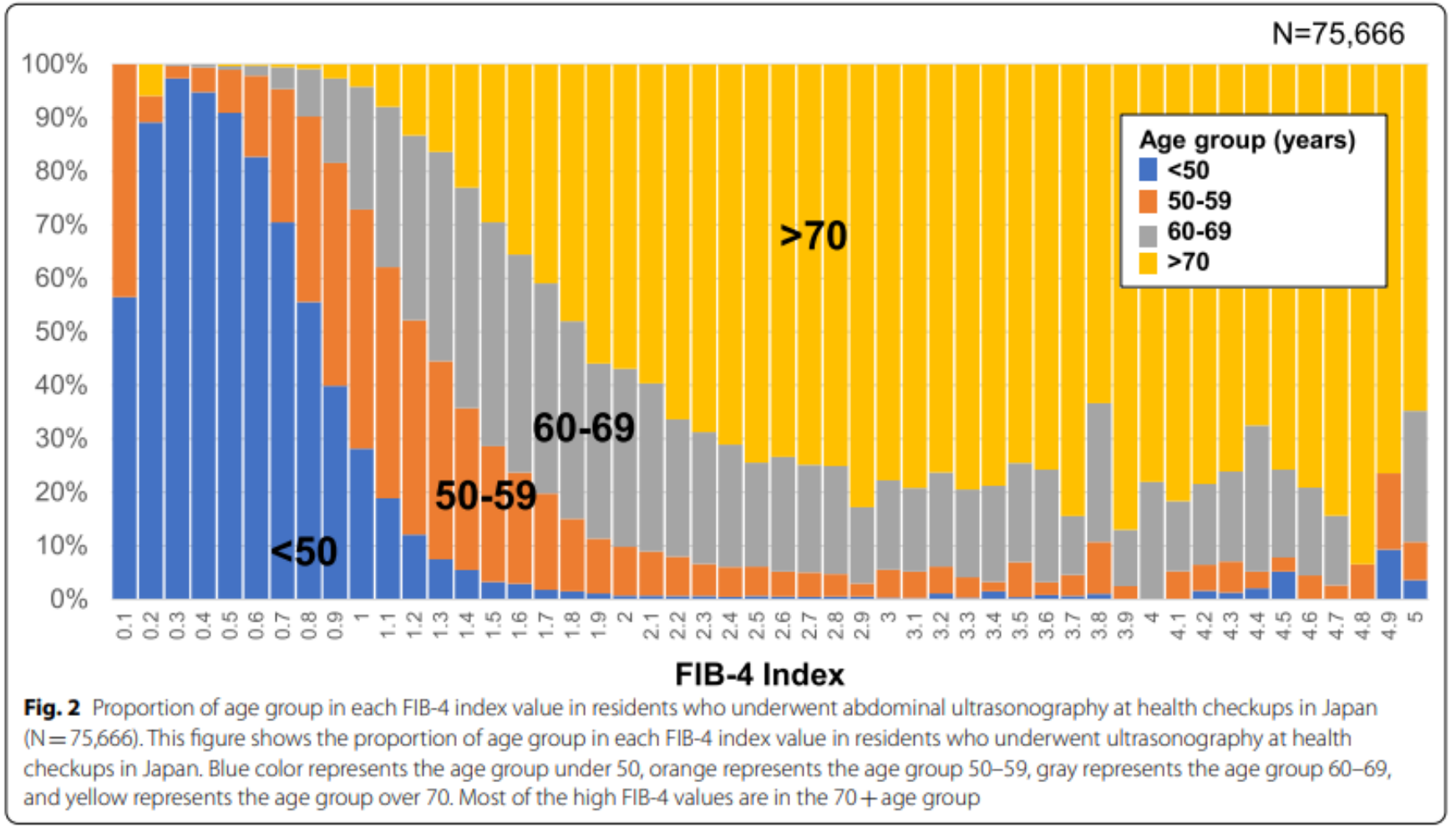

Fig. 2 Proportion of age group in each FIB-4 index value in residents who underwent abdominal ultrasonography at health checkups in Japan (N = 75,666). This figure shows the proportion of age group in each FIB-4 index value in residents who underwent ultrasonography at health checkups in Japan. Blue color represents the age group under 50, orange represents the age group 50–59, gray represents the age group 60–69, and yellow represents the age group over 70. Most of the high FIB-4 values are in the 70 + age group

Sugiyama et al. 2022

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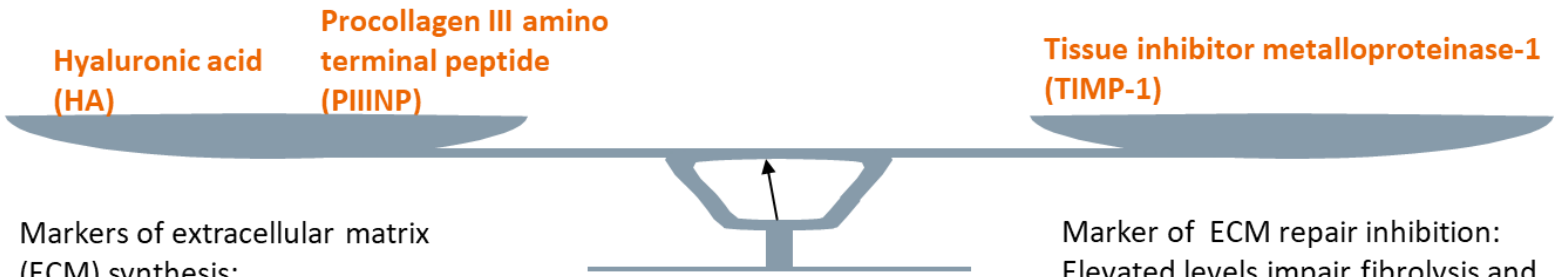
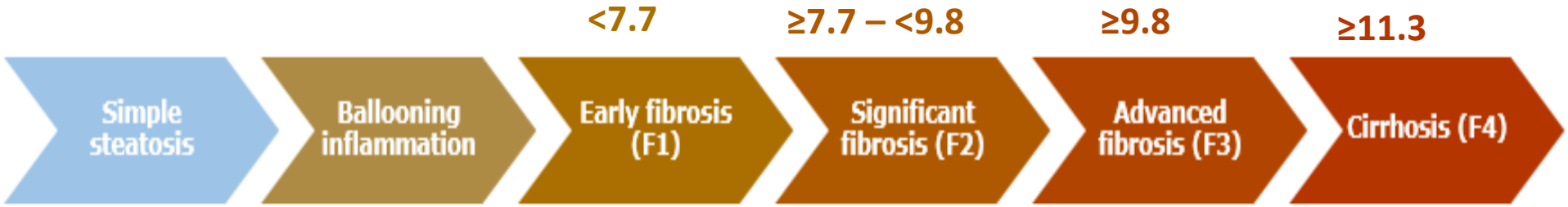
Enhanced liver fibrosis test

- Available via Siemens



- Formula: 2.278 + 0.851 ln (CHA) + 0.751 ln (CPIIINP) + 0.394 ln (CTIMP-1)
- Auroc: 0.85 for stage F2 and 0.90 for stage F3 with NASH
- Aiming at

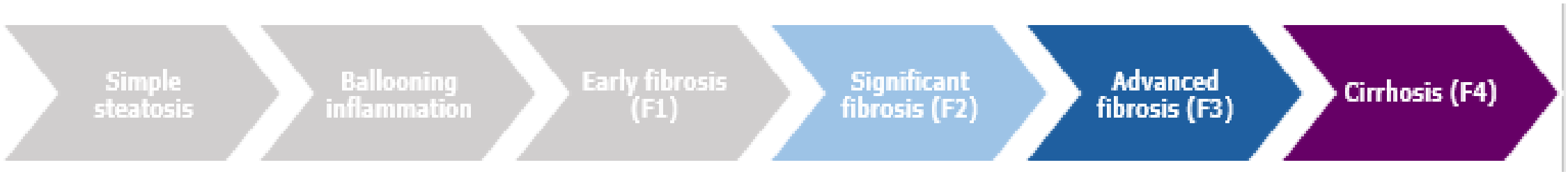


Score shown is for the test run on the ADVIA Centaur XP system.

Arpino V. et al. 2015
Rosenberg WM. et al. 2004

FibroMeter

- Patented
- Formula: age, ALT level, AST level, body weight, ferritin level, glucose level, and platelet counts
- AUROC: 0.94, 0.93, and 0.9 for significant fibrosis, advanced fibrosis, and cirrhosis, respectively
- Aiming at



Calès et al. 2008
Calès et al. 2010

FibroMeter and ELF

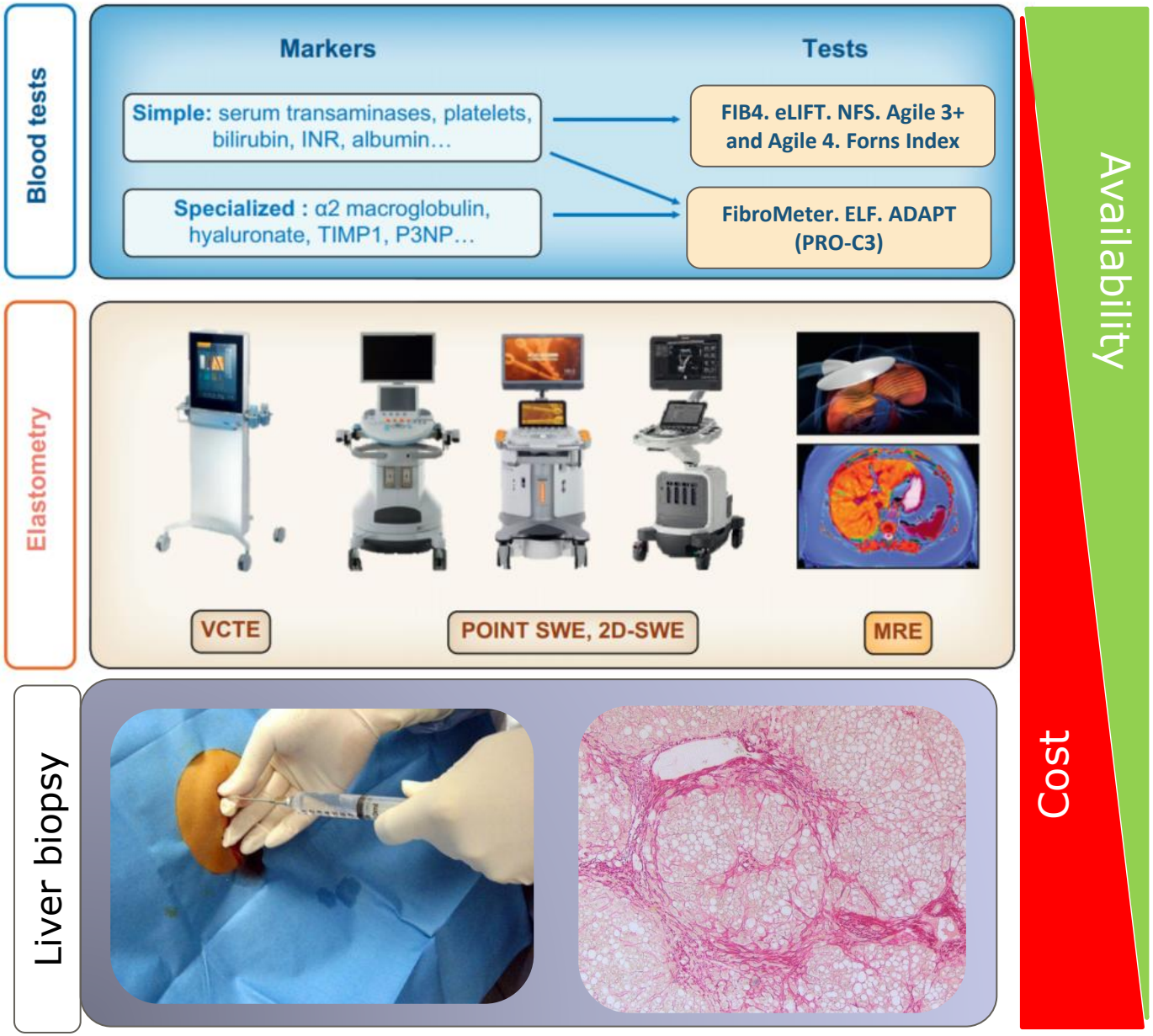
- The 2 most validated patented serum fibrosis biomarkers
  - ELFTM has been evaluated in an independent meta-analysis (n = 11 studies in 4,452 patients) with an AUROC of 0.83 for detecting advanced fibrosis.

- Overall, diagnostic **accuracy** of patented serum fibrosis tests for staging fibrosis is **at least similar, if not higher, than that of FIB-4 and NFS.**

- But their widespread application in clinical practice is limited by cost and availability.

Vali et al, 2020;Anstee et al, 2019; Guillaume er al, 2019

Diagnosing liver fibrosis



Heyens et al. 2021
Boursier et al. 2020

Transient Elastography

- **TE is the most widely available device for LSM** with the largest amount of data in the NAFLD setting.
- The use of both M and XL probes reduces the failure rate to less than 5% of cases.
- Use the same LSM cut-offs for M probe in non-obese and XL probe in obese patients.
- TE has a **high NPV** (above 90%) to rule-out advanced fibrosis but a modest PPV in NAFLD compared to viral hepatitis; LSM more often leads to false positive results in NAFLD.

Eddowes et al, 2019; Vuppalanchi et al, 2018

pS and 2D shear wave elastography

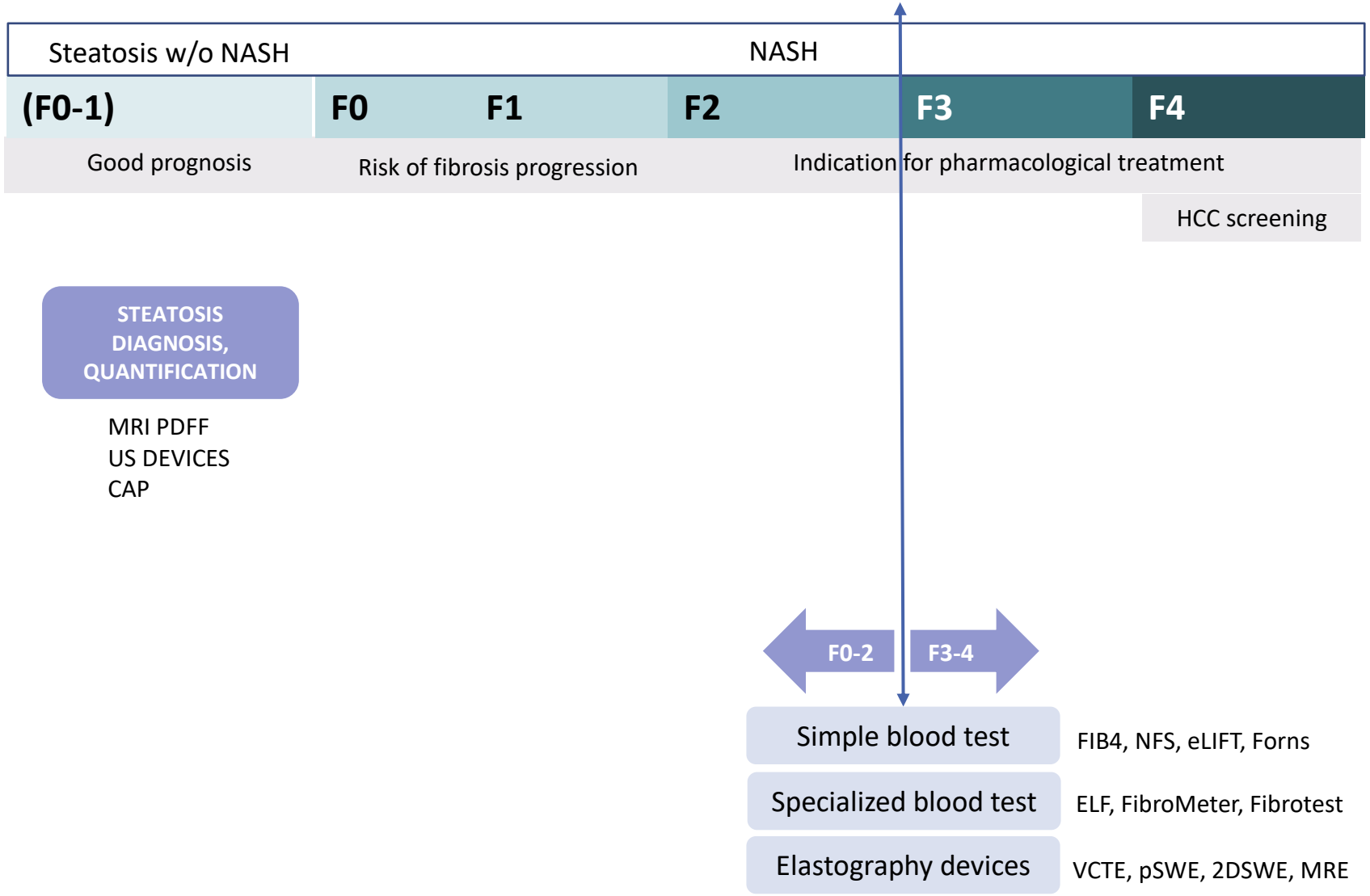
- 2 recent meta-analyses suggest performance for detecting advanced fibrosis in keeping with those reported for FibroScan®.
- However, they are **less available** in liver clinics and **data in patients with NAFLD remain limited.**

Herrmann et al, 2018: Jiang,et al, 2018; Cassinotto et al, 2016

MR Elastography (MRE)

- The **most accurate non-invasive method for detecting advanced fibrosis.**
  - In a recent individual patient data meta-analysis, based on 3 studies in 230 patients, comparing MRE to TE, MRE **outperformed TE for detecting advanced fibrosis** (AUC 0.94 vs. 0.83, respectively, p = 0.001).
  - However, the amount of data in NAFLD remains limited.
  - In addition, given its cost and limited availability, MRE cannot be recommended in clinical practice and is more suited to clinical trials.

Hsu et al, 2019


Steatosis w/o NASH
NASH
(F0-1)
F0
F1
F2
F3
F4
Good prognosis
Risk of fibrosis progression
Indication for pharmacological treatment
HCC screening
STEATOSIS DIAGNOSIS, QUANTIFICATION
MRI PDFF
US DEVICES
CAP
F0-2
F3-4
Simple blood test
FIB4, NFS, eLIFT, Forns
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ELF, FibroMeter, Fibrotest
Elastography devices
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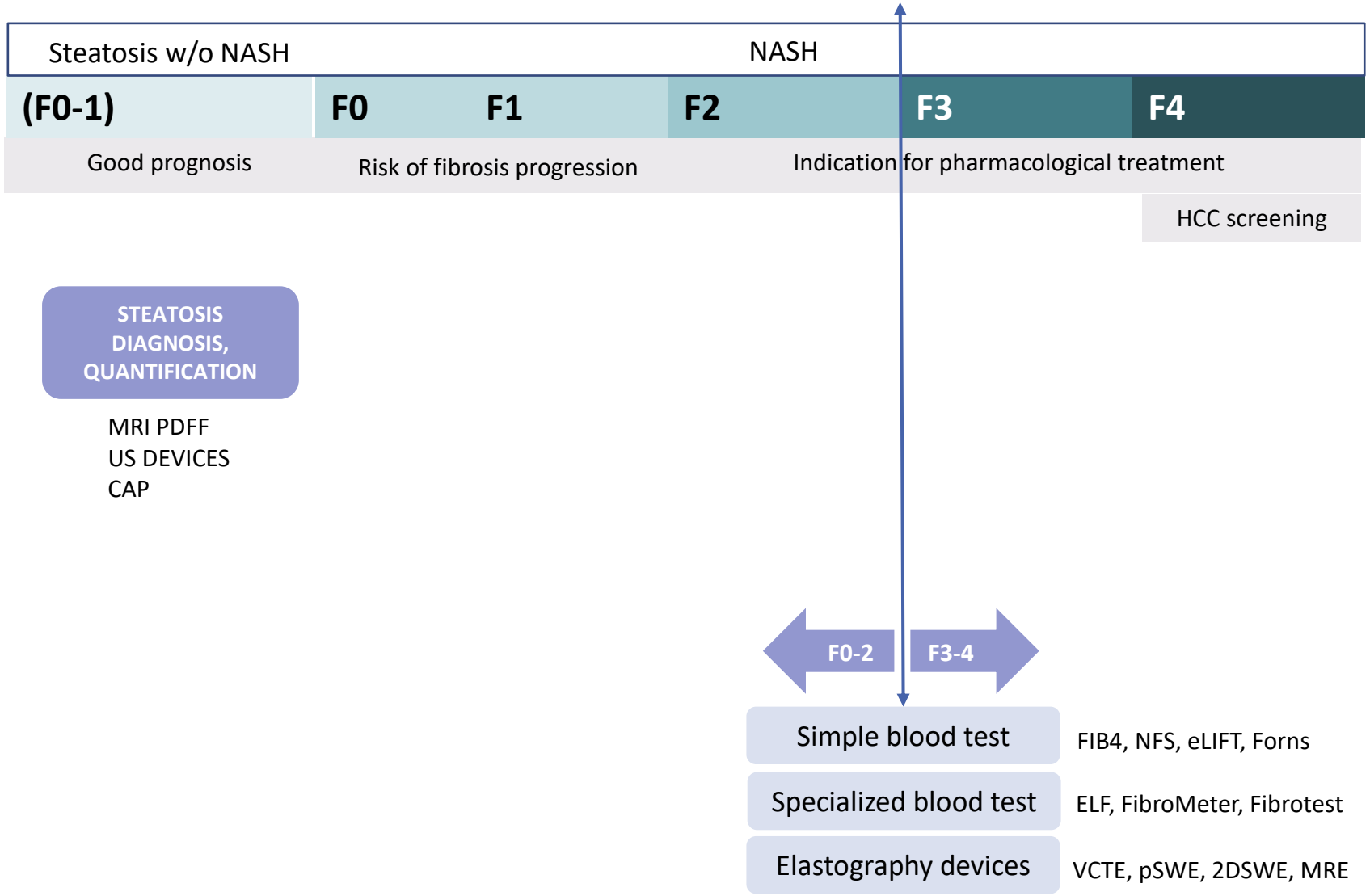
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2. Diagnostic methods

2.4 Fibrotic NASH

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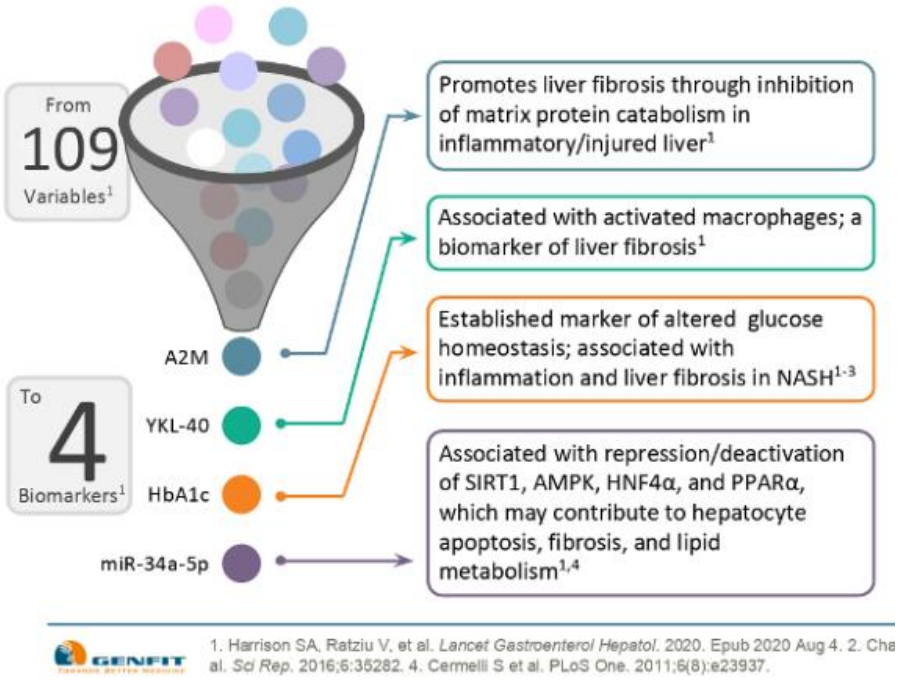
Diagnosing Fibrotic NASH

1. NIS4
2. MACK3
3. FAST
4. MAST

1. NIS4

- Proprietary
- Formula: algorithm comprising of 4 NASH associated biomarkers → miR-34a-5p, α2 macroglobulin, YKL-40 and glycated haemoglobin



$$NIS4_{score} = \frac{e^{\gamma}}{(1+e^{\gamma})}$$

$$\text{Where } \gamma = \beta_0 + \beta_1 \times (\text{miR-34a-5p log [copies/µL]}) + \beta_2 \times (\text{A2M [g/L]}) + \beta_3 \times (\text{YKL40 [ng/mL]}) + \beta_4 \times (HbA_{1c}\ [\%])$$

- Aiming at: NAS ≥ 4 and F ≥ 2

Harrison et al. 2020

1. NIS4

- Cut off:
  - <0.36 = no risk at NASH (NPV 77.9%, sens 81.5%, spec 63%
  - >0.63 = rule in NASH (PPV 79.2%, sens 87.1%, spec 50.7%
- **AUROC: 0.80**

☑ No adjustment needed for age, sex, BMI or aminotransferase concentrations

☑ Rule in and/or rule out at-risk NASH (NAS ≥ 4 and F ≥ 2) in patients with at least one metabolic risk factor

☒ Proprietary

☒ Lack of patients with cirrhosis in the discovery cohort

☒ **Further validation is necessary**

Harrison et al. 2020

2. MACK-3

- Site: https://gilles-hunault.leria-info.univ-angers.fr/wstat/mack3-calculator.php
- Formula: fasting glucose, fasting insulin, aspartate aminotransferase (AST) and **cytokeratin 18 (CK18)**
- Cut off: cut-offs ≤0.134 and ≥0.550 to predict absence and presence of fibrotic NASH
- Aiming at: NAS ≥ 4 and F ≥ 2

☑ Proposed to only use in patients with MetS or AST ≥35 UI/L to suppress costs (prevalence of NASH in the other group is only 0.7%)

☒ Needs to be validated in longitudinal cohort to determine prognostic value

☒ External validation in Asian cohorts

J. Boursier et al.2018
Gao et al. 2020
Chuah et al. 2019

3. FAST

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

- Cut off: 0.35 (rule-out) – 0.67 (rule-in)
- AUROC: 0.81
- Aiming at: NAS ≥ 4 and F ≥ 2

☑ Low cost compared to MRI-based techniques

☒ In some of the validation cohorts, patients with a high BMI were excluded → performance bias of the FAST score

☒ Moderate performance in low prevalence populations

☒ 39% had a score in the grey zone

Newsome et al. 2020
Woreta et al. 2022

4. MAST

- MRI-AST (MAST)
- Formula: MAST = -12.17 + 7.07 log MRE + 0.037 PDFF + 3.55 log AST
- Cut off:
  - 0.242 (sens 75%, spec 90%, PPV 50% NPV 96,5%

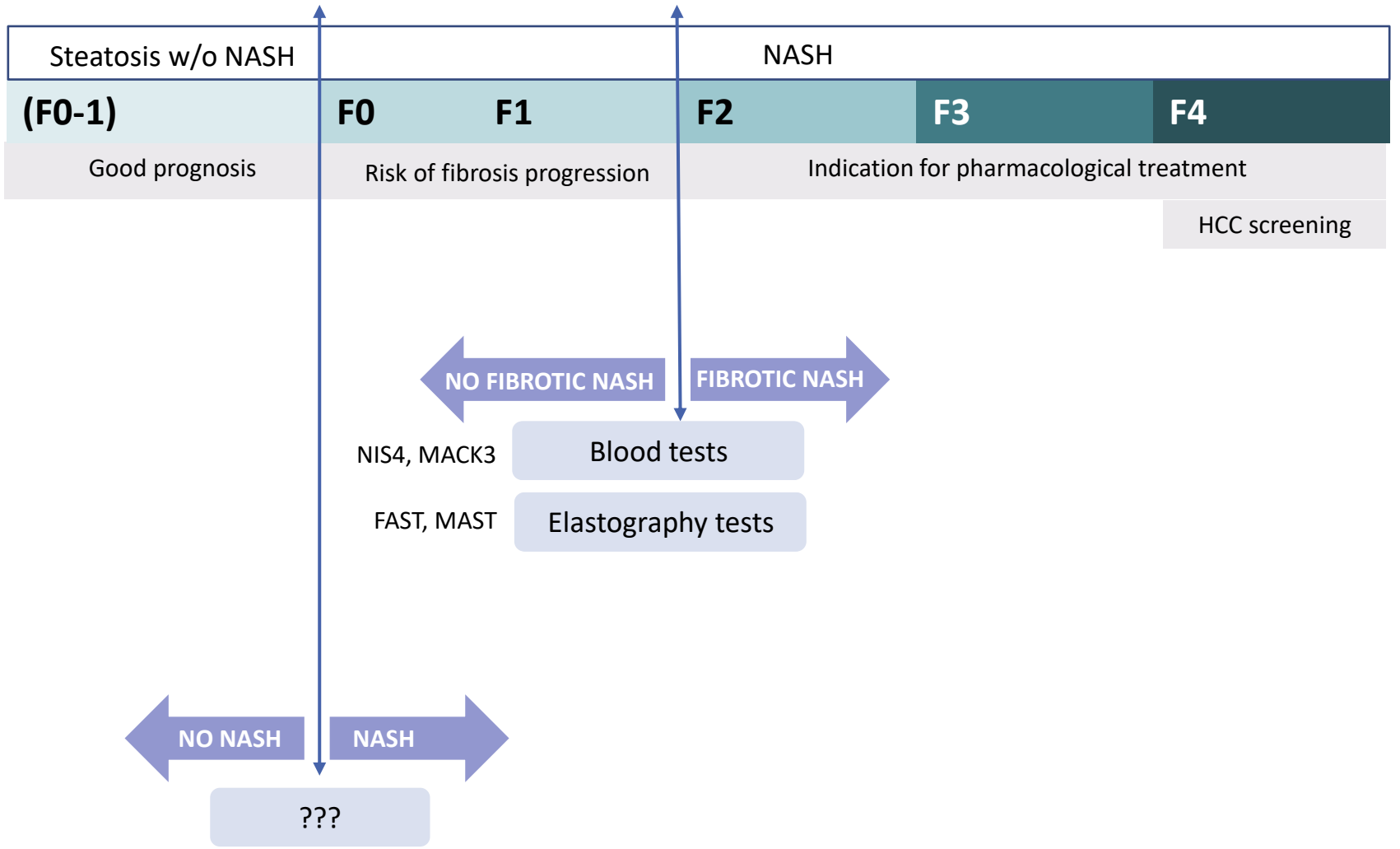
  OR
  - 0.165 (sens 90%, spec 72.2% PPV 29.4% NPV 98.1%)
- AUROC: 0.93
- Aiming at: NAS ≥ 4 and F ≥ 2

☑ **Outperforms FIB4, NFS and FAST**

☒ MRI needed to calculate MRE and MRI-PDFF

☒ Only validated in one external cohort

Kim et al. 2022
Noureddin et al. 2022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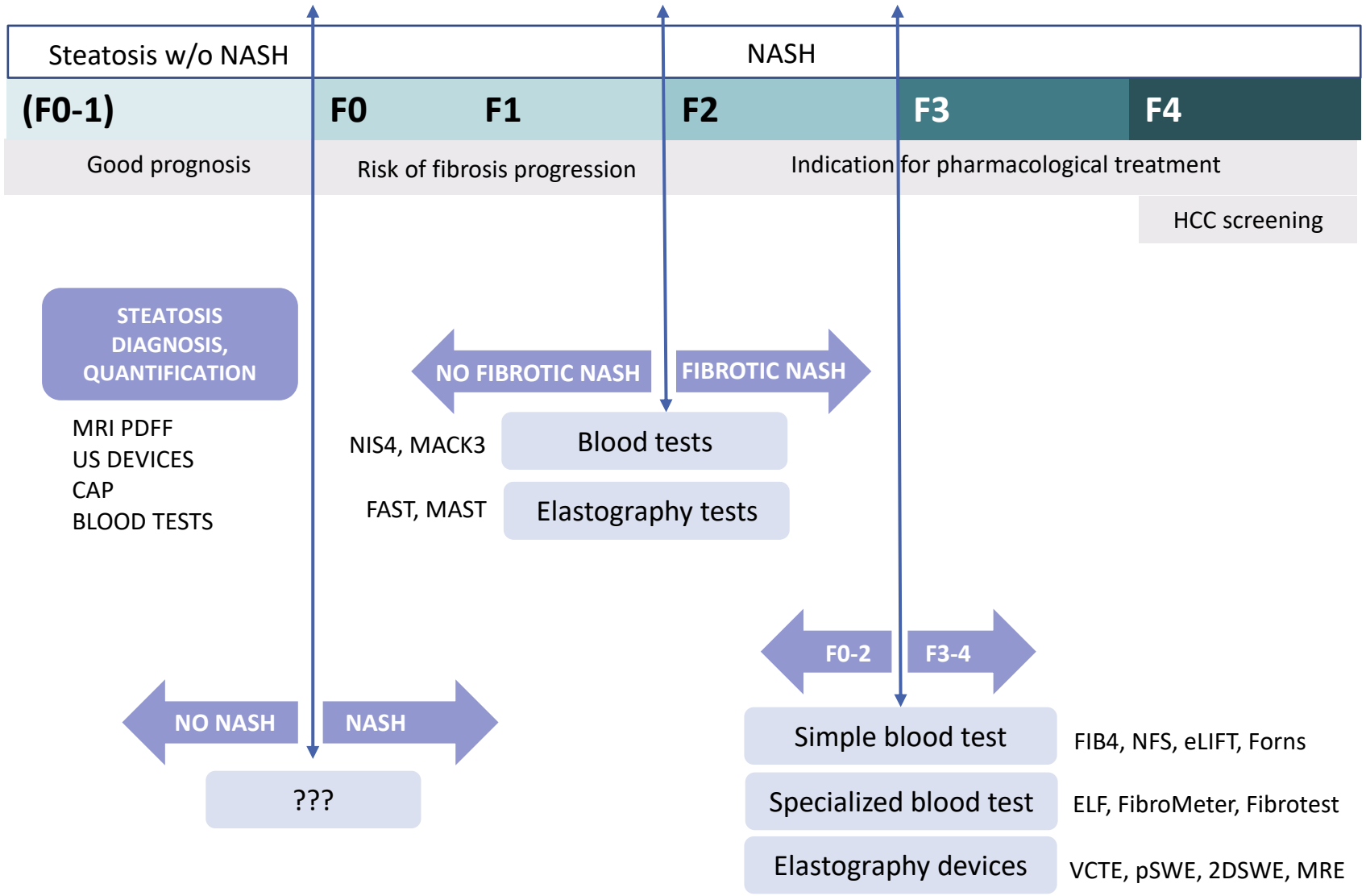
NIS4, MACK3
Blood tests
FAST, MAST
Elastography tests
NO NASH
NASH
???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3. Prognostic value of NIT

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Prognostic value NIT

- **NFS and FIB-4** predicted the occurrence of **liver events**
- **APRI** value >1.5 significantly predicted the occurrence of **HCC**
- Good accuracy of both tests in predicting **liver-related events** and **overall mortality**
- **ELF : liver related events**

Angulo et al, 2013
Kawamura et al, 2012
Sebastiani 2015, Hagström 2019, Onnerhag 2019

Are VS. et al. 2021

Dynamic changes in FIB-4 and LSM on long-term outcomes

- **Progression of FIB-4** from a low- or intermediate-to a high-risk group was associated with an increased risk of severe liver disease (adjusted hazard ratio 7.99 and 8.64, respectively): mean time 2.4 years.(Hagström et al, 2021)

- **Changes in LSM** were independently associated with hepatic decompensation, HCC, overall mortality, and liver-related mortality. LSM at baseline and within 1 year from the last follow-up (median time 37 months) (Petta et al, 2021)

- It seems reasonable to **repeat NITs** every 3 years in patients with early stage disease and every year in patients with advanced stage liver disease.

Hagstrom et al, 2020
Petra et al, 2021

4. Screening for fibrosis with NIT

Screening populations

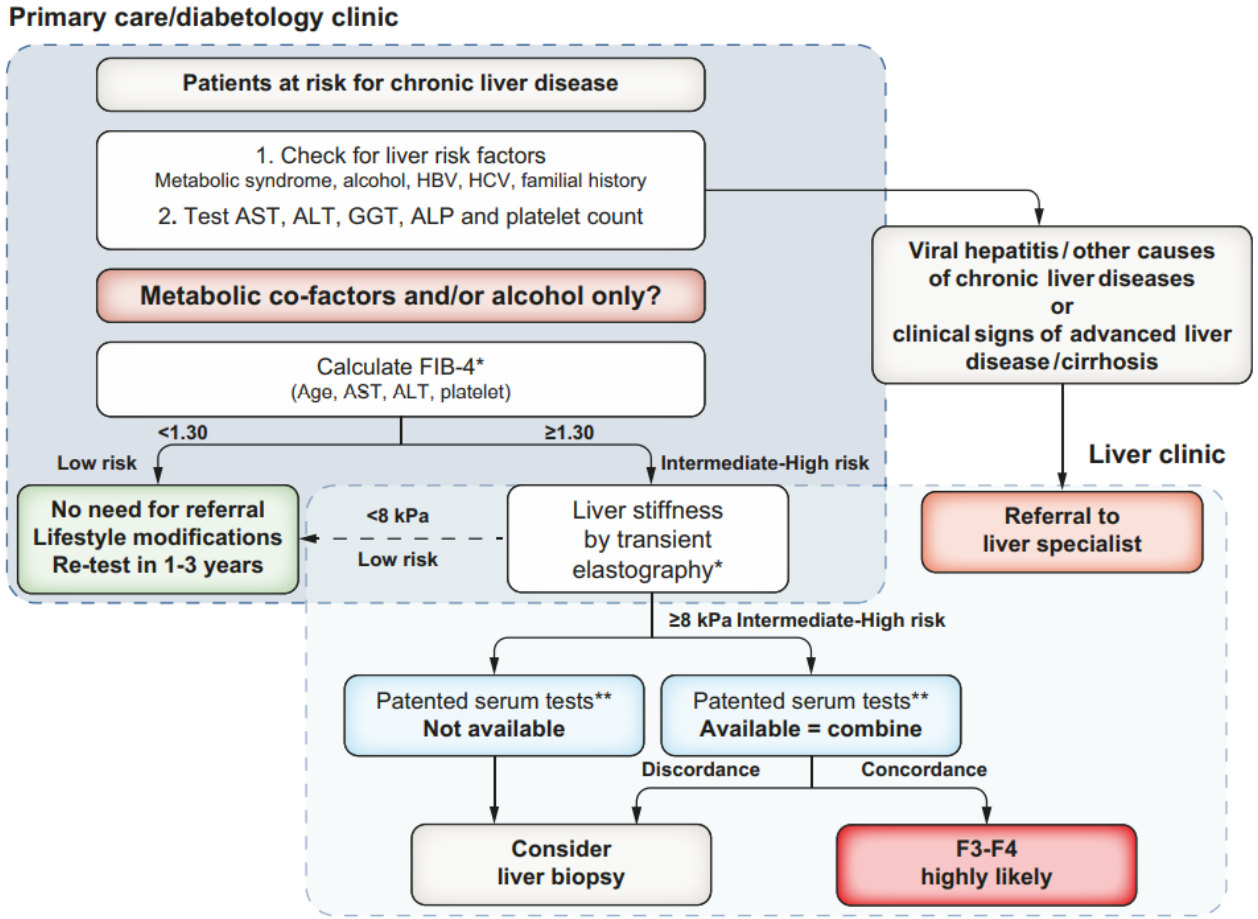

Fig. 1. Proposed use of NITs in patients observed in primary care or outside the liver clinic. As shown, FIB-4 can be used in patients with metabolic co-factors and/or alcoholic liver disease to identify patients requiring referral to the specialist liver clinic. *Transient elastography or FIB-4 may be performed before or after referral to liver specialist according to local availability and pathways. **Cut-offs to use: ELF™ 9.8 (NAFLD/ALD); FibroMeter 0.45 (NAFLD), Fibrotest 0.48 (NAFLD). ALD, alcohol-related liver disease; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4; NAFLD, non-alcoholic fatty liver disease.

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Screening populations

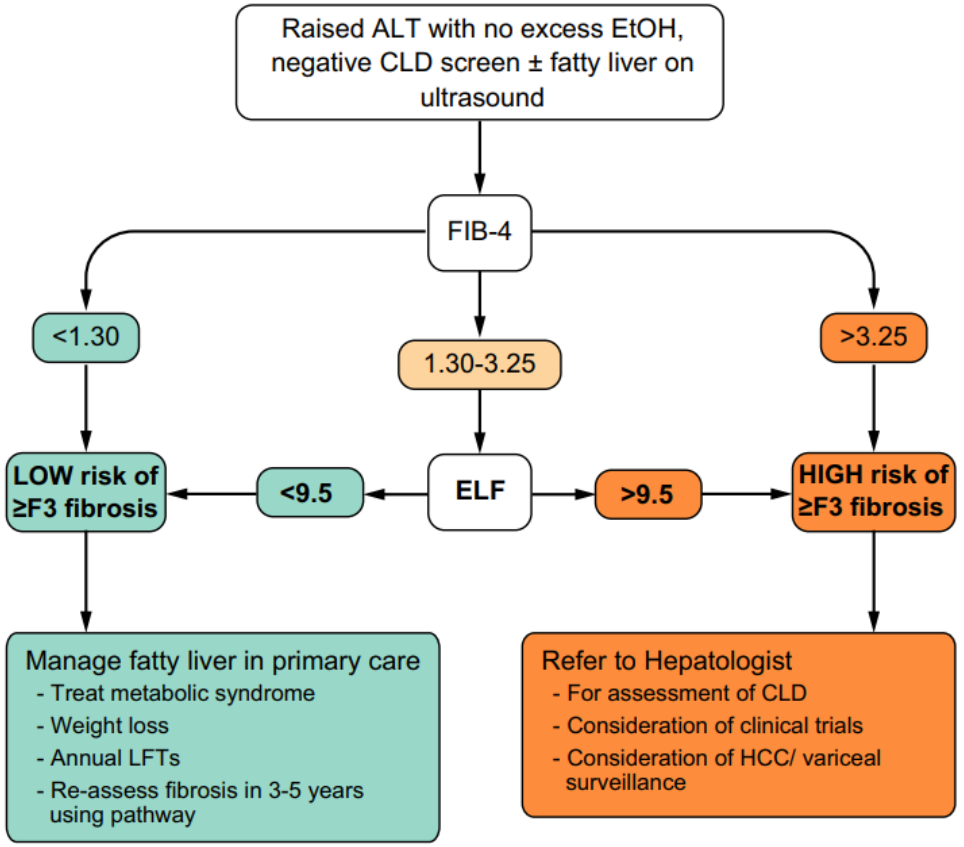

Fig. 1. The Camden and Islington NAFLD pathway. CLD, chronic liver disease; ELF, enhanced liver fibrosis; EtOH, ethanol; FIB-4, Fibrosis-4; HCC, hepatocellular carcinoma; LFTs, liver function tests; NAFLD, non-alcoholic fatty liver disease. (This figure appears in colour on the web.)

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5. The future

New NITs

Name
miR-34a-Sp;YKL-40
CK-18
Type 4 collagen
type IV collagen 7S levels
CK-18 M30. adiponectin; resistin
CK-18 M30; Golgi protein 73; thyroxine, and zinc
PNPLA3 genotype rs738409;
A2 macroglobulin
Haptoglobin
Hyaluronic acid
Procollagen III amino terminal peptide
Tissue inhibitor metalloproteinase-1 (TIMP-1)
Metabolomics: MASEF
Proteomics: ADAMTSL2

The ideal NIT

Box 1. Ideal biomarkers of fibrosis in chronic liver disease.

- Easy to perform
- Cost-effective
- Readily available
- Provides early diagnosis
- High diagnostic accuracy
- Correlates with extracellular matrix deposition
- Validated independently of manufacturer across different etiology of liver disease
- Follows longitudinal change in fibrosis progression/regression
- Tissue specific
- Provides prognosis
- Not influenced by physiologic variation (for example, due to age, gender, diet, bodyhabitus, exercise, diurnal variation)
- Reproducible characteristics across diagnostic platforms
- Minimal variation across multiethnic populations
- Avoids further invasive or other complex diagnostic testing

Collaborations

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Leen Heyens
Dr. Wouter Robaeys

Antwerp University:
Prof. Dr. Sven Francque
Prof. Dr. C van Steenkiste

Maastricht University:
Dr. Ger Koek

Ziekenhuis Oost-Limburg:
Dr. Mathieu Struyve

Leuven University:
Prof. Dr. Frederik Nevens

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Heyens et al. BVTG

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