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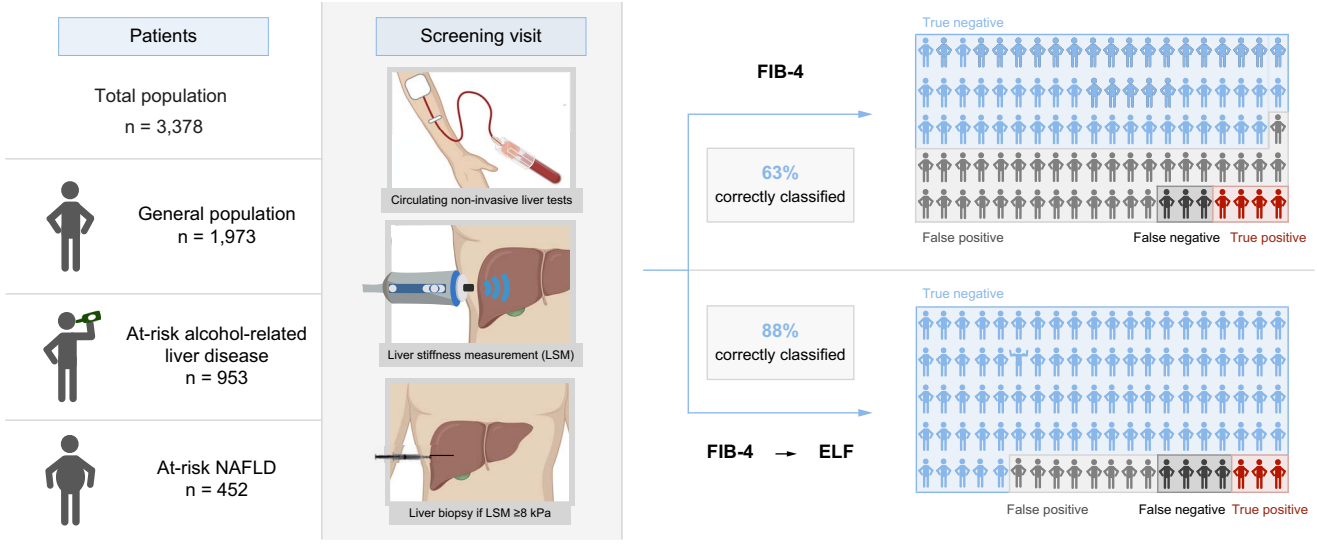
Authors

Maria Kjaergaard, Katrine Prier Lindvig, Katrine Holtz Thorhauge, …, Pere Ginés, Maja Thiele, Aleksander Krag

Correspondence

maja.thiele@rsyd.dk (M. Thiele).

Graphical abstract



Highlights

- Validated referral pathways for fatty liver disease are needed in primary care.
- FIB-4 ≥1.3 results in false positives in 35% of patients, leading to over-referrals.
- Referrals of at-risk patients can be reduced to 14% by using ELF alone.
- FIB-4 followed by ELF in indeterminate cases may further reduce referrals to 10%.
- 27% of screening positive individuals have biopsy-verified advanced fibrosis.

Impact and implications

We need referral pathways that are efficient at detecting advanced fibrosis from alcohol-related and non-alcoholic fatty liver disease in the population, but without causing futile referrals or excessive use of resources. This study indicates that a sequential test strategy of FIB-4 followed by the ELF test in indeterminate cases leads to few patients referred for confirmatory liver stiffness measurement, while retaining a high rate of detected cases, and at low direct costs. This two-step referral pathway could be used by primary care for mass, targeted, or opportunistic screening for liver fibrosis in the population.

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Using the ELF test, FIB-4 and NAFLD fibrosis score to screen the population for liver disease

Maria Kjaergaard[1,2], Katrine Prier Lindvig[1,2], Katrine Holtz Thorhauge[1,2], Peter Andersen[1], Johanne Kragh Hansen[1,2], Nanna Kastrup[3], Jane Møller Jensen[1], Camilla Dalby Hansen[1,2], Stine Johansen[1,2], Mads Israelsen[1], Nikolaj Torp[1,2], Morten Beck Trelle[2,4], Shan Shan[5], Sönke Detlefsen[2,6], Steen Antonsen[4], Jørgen Ellegaard Andersen[5,7], Isabel Graupera[8,9], Pere Ginés[8,9], Maja Thiele[1,2,*], Aleksander Krag[1,2,7]

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Background & Aims: There is a need for accurate biomarkers of fibrosis for population screening of alcohol-related and non-alcoholic fatty liver disease (ALD, NAFLD). We compared the performance of the enhanced liver fibrosis (ELF) test to the fibrosis-4 index (FIB-4) and NAFLD fibrosis score (NFS), using transient elastography as the reference standard.
Methods: We prospectively included participants from the general population, and people at risk of ALD or NAFLD. Screening positive participants (TE ≥8 kPa) were offered a liver biopsy. We measured concomitant ELF, FIB-4, and NFS using validated cut-offs: ≥9.8, ≥1.3, ≥-1.45, respectively.
Results: We included 3,378 participants (1,973 general population, 953 at risk of ALD, 452 at risk of NAFLD), with a median age of 57 years (IQR: 51-63). Two hundred-and-forty-two were screening positive (3.4% in the general population, 12%/14% who were at-risk of ALD/NAFLD, respectively). Most participants with TE <8 kPa also had ELF <9.8 (88%) despite a poor overall correlation between ELF and TE (Spearman's rho = 0.207). ELF was associated with significantly fewer false positives (11%) than FIB-4 and NFS (35% and 45%), while retaining a low rate of false negatives (<8%). A screening strategy of FIB-4 followed by ELF in indeterminate cases resulted in false positives in 8%, false negatives in 4% and the correct classification in 88% of cases. We performed a liver biopsy in 155/242 (64%) patients who screened positive, of whom 54 (35%) had advanced fibrosis (≥F3). ELF diagnosed advanced fibrosis with significantly better diagnostic accuracy than FIB-4 and NFS: AUROC 0.85 (95% CI 0.79-0.92) *vs.* 0.73 (0.64-0.81) and 0.66 (0.57-0.76), respectively.
Conclusion: The ELF test alone or combined with FIB-4 for liver fibrosis screening in the general population and at-risk groups reduces the number of futile referrals compared to FIB-4 and NFS, without overlooking true cases.
Clinical trial number: Clinicaltrials.gov number NCT03308916.

Introduction

Liver disease is the second leading cause of years of working life lost in Europe and America.[1] Cirrhosis mortality increased more than 10% between 2005 and 2015, driven by alcohol-related liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD), and is projected to increase even further.[2,3]

In the general population the prevalence of elevated liver stiffness suggestive of liver fibrosis is 5-7%, *vs.* 18-27% among individuals with risk factors for liver disease.[4] Liver fibrosis is the main predictor of overall and liver-specific mortality, which prompts the need to identify people with fibrosis at an early stage to initiate timely surveillance and interventions.[5,6] However, accurate referral pathways are lacking.[3,7]

The current non-invasive standard to diagnose liver fibrosis is liver stiffness measured by transient elastography (TE).[8] TE is well-validated, with high diagnostic and prognostic accuracy.[6,9,10] However, TE is limited by cost and availability, warranting the need for a blood-based tool that may be used as a gatekeeper in primary care. Simple blood-based algorithms such as fibrosis-4 (FIB-4) index and the NAFLD fibrosis score (NFS) are recommended in European guidelines as cheap first-line tests in primary care.[3,8] However, a substantial number of false positives and false negatives limits their use.[11,12] The enhanced liver fibrosis (ELF) test is a commercially available blood-based marker of liver fibrosis that diagnoses advanced fibrosis in ALD and NAFLD with excellent accuracy.[13,14]

Keywords: ELF test; NASH; liver stiffness; alcoholic liver disease; referral pathway; screening; fatty liver disease; liver fibrosis; non-invasive tests.
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* Corresponding author. Address: FLASH Liver Research Centre, Odense University Hospital; Kloevervaenget 10, indgang 112, 11. Sal; 5000 Odense C; Denmark. Tel.: 0045 21513074.
E-mail address: maja.thiele@rsyd.dk (M. Thiele).
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We therefore aimed to evaluate whether the ELF test could be used as a screening tool in a Danish cohort of the general population and individuals at risk of ALD or NAFLD. We used TE as a screening reference, with biopsy-verified confirmation of fibrosis stage in participants with elevated TE. Primary outcomes were the correlation between ELF and TE, and the concordance between ELF and three strata of liver stiffness: <8 kPa, 8-11.9 kPa, and ≥12 kPa. Second, we compared ELF to FIB-4 and NFS, evaluated the diagnostic accuracy of ELF for biopsy-verified advanced fibrosis, and evaluated referral strategies to detect patients with TE ≥8 kPa.

Patients and methods

Study design

This was a prospective, single-centre screening study performed at Odense University Hospital, Denmark. The study was approved by the Ethics committee of the Region of Southern Denmark (S-20170087), the Danish data protection agency (18/22692), the Danish Health Data Authority (FSEID-00005798) and is part of a larger screening study for liver disease (registered at clinicaltrials.gov: NCT03308916). All participants gave written and oral consent before inclusion and the study protocol adheres to the ethical guidelines of the 1975 Declaration of Helsinki.

Participants

We included participants, either unselected from the general population or at risk of fatty liver disease due to a history of excessive alcohol use, obesity, diabetes, and/or metabolic syndrome. The general population group was invited by a random draw of 9,500 social security numbers belonging to citizens aged 40-75 years from the Region of Southern Denmark. Study invitations were sent to their personal, secure electronic mailbox, the official digital communication route for all Danes. We included all individuals responding to the invitation. We included participants for the at-risk groups by random drawing of 4,000 social security numbers, except we asked only for a reply to the invitation if invitees complied with inclusion criteria for at-risk (below) and adjusted the inclusion age to 30-75 years. At-risk participants were further recruited through announcement of the study in community calls, social media, and municipal alcohol rehabilitation centres. Inclusion criterion for the group at risk of ALD was a history of excessive drinking (prior or current use ≥24 g/day for women, ≥36 g/day for men exceeding 5 years). Inclusion criteria for NAFLD was presence of type 2 diabetes, and/or obesity (BMI >30 kg/m^2), and/or the metabolic syndrome according to the International Diabetes Foundation.[15] We excluded participants with known chronic liver disease, severe alcohol-related hepatitis, cancer, or other debilitating diseases with an expected survival below 12 months.

Investigations at the screening visit

We performed all investigations after at least 6 h of fasting. All physical tests and questionnaires were performed on the same day. The blood tests as well as serum samples for a biobank were obtained within 1 week of the screening visit. We measured BMI and obtained a self-reported alcohol history through standardised oral and written questionnaires including the AUDIT (Alcohol Use Disorder Identification test).

Non-invasive tests

FIB-4 and NFS were calculated according to the literature (Table S1). The ELF test consists of hyaluronic acid, tissue inhibitor of metalloproteinase-1, and the N-terminal propeptide of collagen type III and was analysed using thawed biobank serum in January 2021 according to the manufacturer's instructions on an Atellica IM 1300 analyzer (Siemens Healthcare, Erlangen, Germany; supplementary CTAT table). Experienced operators performed TE and measured controlled attenuation parameter (FibroScan 502 touch, Echosens, France) according to standard operating procedures.[8] The operators were blinded to the blood sample results, including ELF, FIB-4, and NFS, but not to clinical characteristics. We considered TE ≥8 kPa as screening positive as this cut-off is the current recommendation for referral to specialist care.[8] For the blood-based fibrosis markers, we used the corresponding cut-off values based on current guidelines: ELF ≥9.8, FIB-4 ≥1.3, and NFS ≥-1.45.[8] For subgroup analyses of FIB-4 and NFS in participants aged ≥65 years, we used the age-adjusted cut-offs: FIB-4 ≥2.0 and NFS ≥0.12.[16]

Biopsy visit

Screening positive participants were invited for a liver biopsy within 3 months. Furthermore, we biopsied 15 screening negative participants to evaluate their eligibility for inclusion in a subsequent randomized trial. After repeating screening investigations, we performed an abdominal ultrasound and a percutaneous suction needle liver biopsy (17G Menghini needle; Hepafix; Germany). One experienced liver pathologist (SD) evaluated the biopsies and assigned histological fibrosis stage (0-4) according to Kleiner, and steatosis grade (0-3), lobular inflammation (0-3), and hepatocellular ballooning (0-2) according to the NASH Clinical Research Network.[17] Biopsies were considered of sufficient quality if they were ≥10 mm long and contained ≥6 portal tracts or if regeneration nodules were present. The pathologist was blinded to TE, ELF, FIB-4, and NFS. We abstained from a liver biopsy if participants had contraindications to a liver biopsy, had ultrasound evidence of certain cirrhosis, bile duct dilatation, or hepatic congestion, or if TE had decreased to <6 kPa at the biopsy visit.

Cost analysis

In a *post hoc* analysis, we estimated the direct costs related to FIB-4 and ELF testing using a micro-costing approach including direct and overhead costs, with Danish prices for the index year 2022 for FIB-4, ELF, and FibroScan (Table S2).

Statistical analysis

We reported categorical data as counts (frequency) and continuous data as median (IQR). We evaluated the correlation between TE and ELF, FIB-4, and NFS by the Spearman correlation coefficient and the variance by R-squared. Furthermore, we described ELF, FIB-4, and NFS across three strata of liver stiffness measurements: TE <8 kPa, TE 8-11.9 kPa, and TE ≥12 kPa.[18] To further test the relationships between ELF and TE, and FIB-4 and TE, we performed density estimation for their

log-transformed values. For each subgroup of participants, with liver biopsy as a reference or TE <8 for the screening negatives, we assumed that the probability density function was normally distributed and used minimum covariance determinant to obtain a robust estimate for its mean and covariance matrix.[19] We evaluated the confidence ellipses of 1.5 standard deviations in a log-log scatter plot using the robust estimates obtained as before. In the subgroup of participants with a liver biopsy, we evaluated the diagnostic accuracy of ELF, FIB-4, NFS, and TE with histological fibrosis stage as a reference by calculating the AUROC and used the DeLong test for AUROC comparisons between tests and stratified for obesity (BMI ≥30 kg/m^2). We used multivariable logistic regression with stepwise backwards elimination to evaluate potential predictors of false negative compared to true negative ELF tests, and false positive compared to true positive ELF tests, using TE ≥8 kPa as the reference. We evaluated the following potential predictors, based on a literature search: age, sex, BMI, controlled attenuation parameter, alanine aminotransferase, aspartate aminotransferase, bilirubin, gamma-glutamyltransferase, and haemoglobin A1c.[20–22] Lastly, we evaluated two referral pathways: ELF alone, or FIB-4 followed by ELF in indeterminate cases (FIB-4 from 1.30 to 2.66) compared to the current recommendation of FIB-4 alone. In *post hoc* sensitivity analyses, we tested whether age-adjusted cut-offs improved referral pathways, and whether the performance of ELF, FIB-4 and NFS differed in subgroups of participants aged <40 or ≥65 years.

In case of missing values, we performed complete case analysis. We performed all statistical analyses in STATA17 (StataCorp TX, US) and considered *p* values below 0.05 to be statistically significant.

Results

Participants

From October 2017 to November 2020, we invited 16,624 individuals and included 3,378 participants in the study: 1,973

Table 1. Baseline characteristics.

	Total population N = 3,378*	General population n = 1,973	At-risk of ALD n = 953	At-risk of NAFLD n = 452
Age	57 (51-63)	57 (52-62)	59 (51-65)	53 (46-62)
Sex, male (%)	1,663 (49)	892 (45)	613 (64)	158 (35)
BMI (kg/m^2)	27 (24-31)	27 (24-30)	27 (24-30)	33 (30-37)
Waistline (cm)	96 (86-107)	93 (83-103)	97 (88-107)	107 (99-116)
Weekly drinks	5 (1-15)	4 (1-10)	20 (6-30)	2 (1-5)
Risk factors**				
Alcohol (%)	1,145 (34)	192 (10)	953 (100)	0 (0)
Obesity (%)	1,070 (32)	453 (23)	265 (28)	352 (78)
Diabetes (%)	243 (7)	80 (4)	66 (7)	97 (21)
Metabolic syndrome (%)	1,723 (51)	847 (43)	510 (54)	366 (81)
No risk factors (%)	944 (29)	944 (48)	.	.
Fibrosis markers				
ELF test	8.9 (8.4-9.4)	8.9 (8.4-9.3)	8.9 (8.4-9.5)	9.0 (8.5-9.6)
FIB-4	1.15 (0.9-1.5)	1.16 (0.93-1.49)	1.21 (0.91-1.61)	0.99 (0.74-1.35)
NFS	-1.42 (-2.28- -0.63)	-1.52 (-2.33- -0.79)	-1.30 (-2.25- -0.47)	-1.09 (-2.03- -0.17)
TE (kPa)	4.4 (3.7-5.5)	4.3 (3.6-5.2)	4.6 (3.8-5.8)	4.9 (4.1-6.3)
TE groups				
TE <8 kPa (%)	3,136 (93)	1,906 (97)	843 (88)	387 (86)
TE 8-11.9 kPa (%)	144 (4)	49 (2)	63 (7)	32 (7)
TE ≥12 kPa (%)	98 (3)	18 (1)	47 (5)	33 (7)
Laboratory tests				
Albumin (g/L)	45 (44-47)	45 (44-47)	46 (44-47)	45 (44-47)
ALT (U/L)	25 (19-34)	24 (18-32)	26 (20-36)	29 (22-41)
AST (U/L)	25 (21-30)	24 (21-29)	26 (22-33)	25 (21-31)
Bilirubin (μmol/L)	8 (6-11)	8 (7-11)	9 (6-11)	8 (6-10)
GGT (U/L)	26 (17-44)	23 (16-37)	31 (19-60)	31 (21-51)
HbA1c (mmol/mol)	36 (34-39)	36 (34-39)	36 (33-38)	38 (34-43)
Platelets (10^9/L)	242 (208-281)	240 (208-278)	240 (206-278)	254 (214-300)
Fasting glucose (mmol/L)	5.7 (5.3-6.1)	5.6 (5.3-6.0)	5.8 (5.4-6.2)	5.9 (5.5-6.5)
Liver biopsy* **	**n =170**	**n = 44**	**n =78**	**n =48**
Fibrosis stage F0/1/2/3/4 (%)	6/58/52/28/26 (4/34/31/16/15)	0/18/17/8/1 (0/41/39/18/2)	4/19/23/12/20 (5/24/29/15/26)	2/21/12/8/5 (4/44/25/17/10)
Steatohepatitis (%)****	60 (35)	10 (23)	26 (33)	24 (50)

ALD, alcohol-related liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4; GGT, gamma-glutamyl transferase; HbA1c, haemoglobin A1C; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score; TE, transient elastography.

Reported as counts and frequencies, or medians and IQR. ELF missing in three participants due to unmeasurable tissue inhibitor of metalloproteinase-1 and N-terminal propeptide of collagen type III. FIB-4 missing in 150 participants, NFS missing in 179.32 had an unreliable TE (<10 measurements or IQR/median >30%), but these were included in the analysis.

*98.6% of included participants were Caucasians (ethnicity missing in 2,026, because recorded late in the study).

**Alcohol: prior or current alcohol overuse (>24 g/day for women and >36 g/day for men) for more than 5 years. Obesity: BMI >30. Metabolic syndrome: according to the International Diabetes Foundation.

***We performed a liver biopsy in 155 out of 242 patients with TE ≥8 (64%). We included 15 additional biopsies in patients with TE <6 kPa, due to evaluation for inclusion in randomized trials. Seven participants from the ALD group had definite signs of cirrhosis by ultrasound and did not undergo liver biopsy.

****Concomitant steatosis, lobular inflammation, and hepatocellular ballooning.

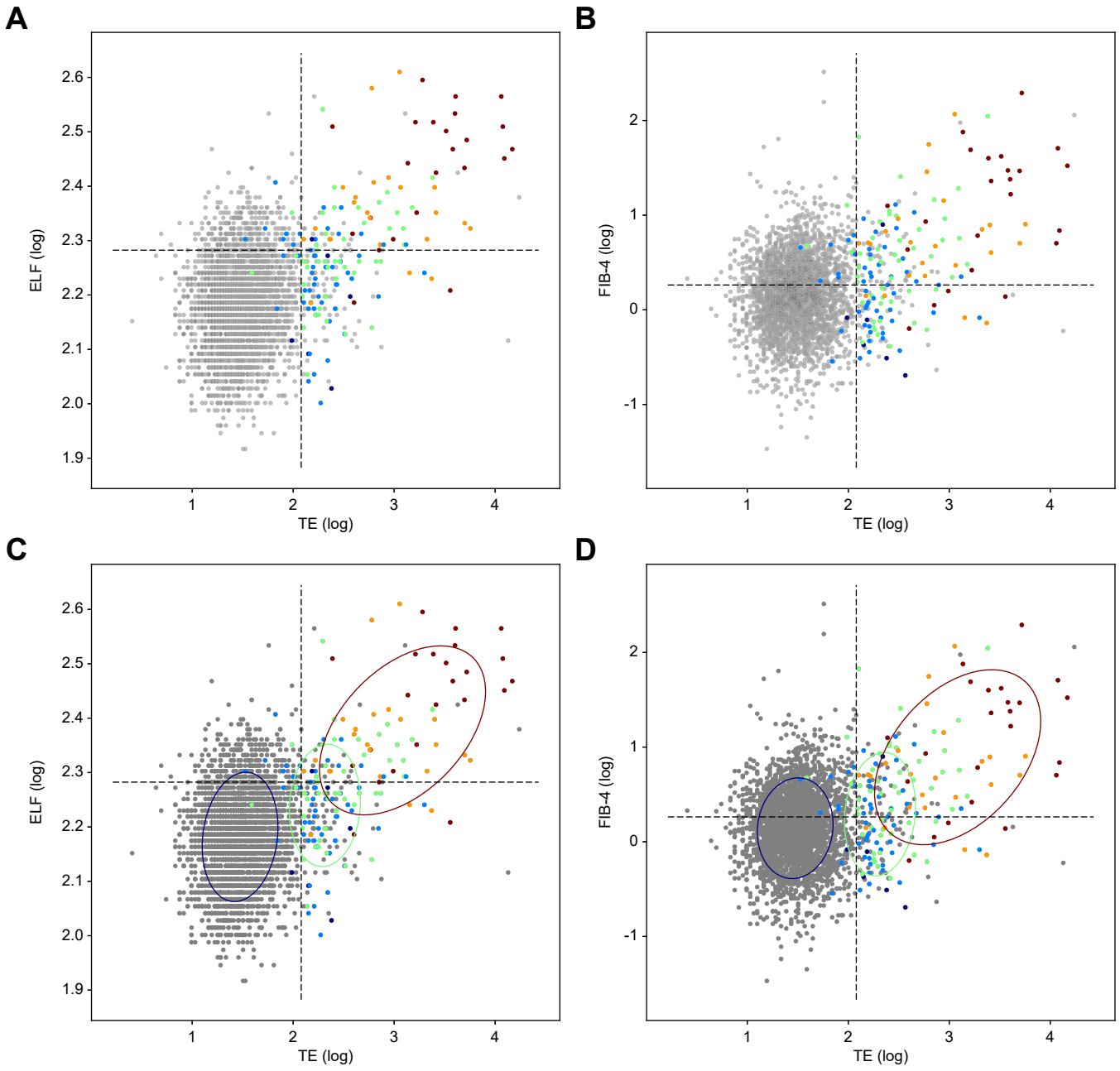

Fig. 1. Log-log scatter plots of the ELF test and FIB-4 compared to transient elastography. (A-B) Coloured dots represent screening positive participants, grey dots screening negative. F0: dark blue; F1: light blue; F2: green; F3: yellow; F4: red. (C-D) Confidence ellipses of 1.5 standard deviations for F0-1 or screening negative (blue); F2 (green); F3-4 (red). Dashed lines indicate the cut-off values of 8.0 kPa for TE, 9.8 for ELF, and 1.30 for FIB-4. ELF, enhanced liver fibrosis test; FIB-4, fibrosis-4 index. (This figure appears in color on the web.)

randomly selected from the general population, 953 at-risk of ALD, and 452 at-risk of NAFLD. TE failed in 0.26% of eligible participants (Fig. S1). Most participants were Caucasians (98.6%), and middle-aged (77% aged 40-64 years; median age 57; 51-63). A total of 49% were male, and the median BMI was 27 (24-31) kg/m^2. In the general population group, 23% were obese, and 52% had at least one risk factor for liver fibrosis. Among participants at risk of ALD, 561 (59%) had at least one metabolic risk factor (Table 1). In the general population, at-risk of ALD, and at-risk of NAFLD groups, 67 (3.4%), 110 (12%) and 65 (14%) of individuals were screening positive (TE ≥8 kPa).

We obtained a liver biopsy in 155/242 (64%) of screening positive participants, 54 of whom had biopsy-proven advanced fibrosis (≥F3; 35%, 54/155). Besides occasional post-biopsy pain, we did not observe any adverse events.

Performance of the ELF test to detect elevated liver stiffness

The ELF test exhibited a poor linear correlation with TE at the screening visit (Spearman's rho = 0.207, p <0.01; Fig. 1, Table S3; Fig. S2) and explained 12% of the linear TE variance

Table 2. Classification of participants using ELF, FIB-4, and FIB-4 followed by ELF in indeterminant cases.

TE	Total population N = 3,378		General population n = 1,973		At-risk of ALD n = 953		At-risk of NAFLD n = 452	
	ELF							
	<9.8 n = 2,890 (86)	**≥9.8 n = 485 (14)**	**<9.8 n = 1,742 (88)**	**≥9.8 n = 231 (12)**	**<9.8 n = 783 (82)**	**≥9.8 n = 169 (18)**	**<9.8 n = 365 (81)**	**≥9.8 n = 85 (19)**
<8 kPa	2,759 (82)	374 (11)	1,698 (86)	208 (11)	732 (77)	111 (12)	329 (73)	56 (12)
≥8 kPa	131 (3.9)	111 (3.3)	44 (2.2)	23 (1.2)	51 (5.4)	59 (6.2)	36 (8.0)	29 (6.4)
<12 kPa	2,860 (85)	417 (12)	1,737 (88)	221 (11)	775 (81)	130 (14)	351 (78)	66 (15)
≥12 kPa	30 (0.9)	68 (2.0)	8 (0.4)	10 (0.5)	8 (0.8)	39 (4.1)	14 (3.1)	19 (4.2)
	FIB-4							
	<1.3 n = 1,986 (62)	**≥1.3 n = 1,242 (38)**	**<1.30 n = 1,175 (62)**	**≥1.30 n = 723 (38)**	**<1.30 n = 504 (56)**	**≥1.30 n = 399 (44)**	**<1.30 n = 307 (72)**	**≥1.30 n = 120 (28)**
<8 kPa	1,895 (59)	1,114 (35)	1,143 (60)	697 (37)	474 (52)	330 (37)	278 (65)	87 (20)
≥8 kPa	91 (2.8)	128 (4.0)	32 (1.7)	26 (1.4)	30 (3.3)	69 (7.6)	29 (6.8)	33 (7.7)
<12 kPa	1,963 (61)	1,175 (36)	1,170 (62)	713 (38)	497 (55)	364 (40)	296 (69)	98 (23)
≥12 kPa	23 (0.7)	67 (2.1)	5 (0.3)	10 (0.5)	7 (0.8)	35 (3.9)	11 (2.6)	22 (5.2)
	FIB-4 → ELF*							
	Negative n = 2,893 (90)	**Positive n = 333 (10)**	**Negative n = 1,753 (92)**	**Positive n = 145 (7.6)**	**Negative n = 771 (85)**	**Positive n = 131 (15)**	**Negative n = 369 (87)**	**Positive n = 57 (13)**
<8 kPa	2,756 (85)	251 (7.8)	1,708 (90)	132 (7.0)	718 (80)	85 (9.4)	330 (77)	34 (8.0)
≥8 kPa	137 (4.3)	82 (2.5)	45 (2.4)	13 (0.7)	53 (5.9)	46 (5.1)	39 (9.2)	23 (5.4)
<12 kPa	2,862 (89)	274 (8.5)	1,746 (92)	137 (7.2)	761 (84)	99 (11)	355 (83)	38 (8.9)
≥12 kPa	31 (1.0)	59 (1.8)	7 (0.4)	8 (0.4)	10 (1.1)	32 (3.5)	14 (3.3)	19 (4.5)

ALD, alcohol-related liver disease; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4 index; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score.
Counts and frequencies based on per-protocol analyses of: valid ELF = 3,375, valid FIB-4 = 3,228, valid NFS = 3,208, valid ELF & FIB-4 = 3,226.
*Negative = FIB-4 <1.30 or FIB-4 1.30-2.66 & ELF <9.8. Positive = FIB-4 ≥2.67 or FIB-4 1.30-2.66 & ELF ≥9.8.

(R-squared 0.124). However, ELF increased significantly between the three TE strata (p <0.001; Fig. 2) and diagnosed elevated liver stiffness with moderate accuracy (AUC 0.74 for TE ≥8 kPa, 95% CI 0.71-0.78) (Table S4; Fig. S3). ELF was ≥9.8 in 14% of participants, the majority being false positives (TE <8 kPa; Table 2). In the 86% of participants with ELF <9.8, the rate of false negatives (TE ≥8 kPa) was only 4%. The proportion of false negatives was higher in the at-risk groups compared to the general population participants (Table 2). When using TE ≥12 kPa as the reference, the rate of false negatives decreased to 1%, while false positives increased to 12% (Table 2).

ELF compared to FIB-4 and NFS

FIB-4 also exhibited an overall poor correlation with TE (Spearman's rho = 0.321, R-squared 0.150, Fig. 1), but increased stepwise between the three TE strata (Fig. 2). FIB-4 resulted in false positives in more than one-third of all patients (35%), with false negatives in just 3% (Table 2). Overall, NFS exhibited the same performance characteristics as FIB-4 (Tables S3 and S4), but with 45% false positives (Table S5). As with the ELF test, at-risk groups had a higher proportion of FIB-4 and NFS false negatives compared to the general population.

The ELF test correctly classified 85% of all participants, while FIB-4 and NFS correctly classified 63% and 54%, respectively. This was largely due to ELF's superior ability to correctly classify screening negative participants (Fig. 1C *vs.* Fig. 1D, Fig. S4).

ELF, FIB-4, and NFS to diagnose advanced fibrosis in participants with a liver biopsy

In the subgroup of participants with a liver biopsy (n = 170), ELF showed a better correlation with TE (Spearman's rho = 0.526, R-squared = 0.321) than in the screening setting. ELF diagnosed advanced fibrosis (≥F3) with good accuracy (AUROC 0.85) and significantly outperformed FIB-4 and NFS, but not TE (Table 3, Fig. 3). When stratifying for obesity, ELF retained a high diagnostic accuracy (AUROC 0.85), while the accuracy decreased for TE (AUROC 0.83) and NFS (AUROC 0.65) and remained moderate for FIB-4 (AUROC 0.74; Table S6).

We performed a liver biopsy in 155 screening positive participants, while the liver stiffness of 45 screening positive participants had decreased to <6 kPa at the biopsy visit, causing us to abstain from a liver biopsy. Thus, we reached a definitive conclusion in 83% of participants (200/242), assuming that none of the 45 participants with TE <6 kPa had advanced fibrosis. Consequently, the prevalence of advanced fibrosis in screening positives was 27% (54/200).

False negative and false positive ELF results

Of the 242 screening positive participants, 131 had an ELF <9.8 and would consequently have been missed if ELF were used to select participants for referral. However, only 30 of them had TE ≥12 kPa. Furthermore, 59 of the 131 participants with false negative ELF decreased to TE <8 kPa at the biopsy visit. The median TE for ELF false negatives was 10.1 kPa (IQR 8.8-11.7) at the screening visit compared to 7.9 kPa (IQR 6.1-9.5) at the biopsy visit. Of those participants with a false negative ELF test, 8% (6/76) had advanced fibrosis on histology, compared to 61% (48/79) in true positives. Overall, false positives had characteristics more like true negatives, whereas false negatives had characteristics more like true positives (Table S7).

We observed false positive ELF results in 374 participants. The median age was higher in false positives than true negatives. Lower BMI also predicted false positives, but only in patients at risk of ALD or NAFLD (Table S8).

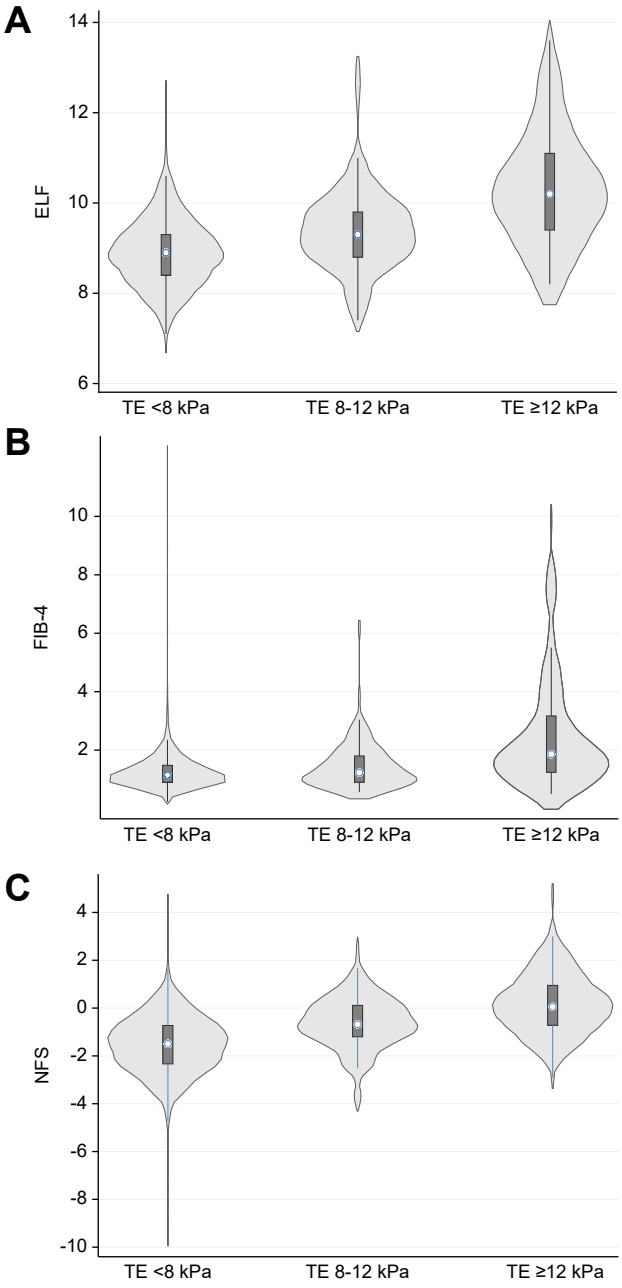

Fig. 2. Violin plots of the distribution of ELF, FIB-4, NFS according to three TE strata. The dashed lines indicate the cut-off values of 9.8 for (A) ELF, 1.30 for (B) FIB-4, and -1.45 for (C) NFS. The plots depict medians with interquartile ranges and surrounding kernel-density curves. ELF, enhanced liver fibrosis; FIB-4, fibrosis-4 index; NFS, NAFLD fibrosis score; TE, transient elastography.

Referral strategies

A referral strategy of FIB-4 followed by ELF in indeterminate cases (FIB-4 1.30-2.67) resulted in a low number of participants eligible for referral while keeping the rate of false negatives below 10% (Table 2, Fig. 4). This strategy also reduced the number of necessary ELF tests by >60% compared to ELF alone.

In contrast, a FIB-4 only referral strategy, as recommended by the EASL Clinical Practice Guidelines, resulted in 38% of the population being eligible for referral to liver specialist care due to FIB-4 ≥1.30 (Table 2), whilst NFS resulted in 45% of participants being eligible for referral (Table S5).

Age-adjusted cut-offs for FIB-4 and NFS led to higher rates of false negatives and decreased the proportion of referrals but did not change the overall conclusion that the combination strategy led to far fewer screening positives (Table S9).

Cost analyses

The referral pathway of FIB-4 followed by ELF in indeterminate cases resulted in almost half the costs compared with FIB-4 or ELF alone, due to spared TE measurements. It cost €122,548 to test 1,000 people with FIB-4 followed by ELF, compared to €208,201 for ELF alone, and €230,880 for FIB-4 alone (Table S2).

Subgroup analyses in younger and older participants

Only 3.5% of participants were younger than 40 years (n = 118), of whom 7.8% were screening positive, compared to 12% in the full at-risk cohort. One in five participants were above 65 years of age (n = 665; Fig. S5), of whom 69 were screening positives. ELF and FIB-4 were poor at detecting the young screening positive individuals, while NFS performed slightly better, but was associated with more false positives (Table S10). Among participants aged ≥65 years of age, ELF frequently gave false positive results, while FIB-4 at the age-adjusted cut-off of 2.0 was associated with fewer false positives, but more false negatives. NFS performed best of all three (Table S11).

Discussion

This large prospective study provides evidence that the ELF test can be used as an effective tool for population screening for liver fibrosis as it is associated with substantially fewer false positives than FIB-4 and NFS. However, as FIB-4 <1.30 ruled out elevated liver stiffness slightly better than ELF <9.8, a referral pathway using FIB-4 followed by ELF in indeterminate cases would retain 90% of patients in primary care, and still detect a high proportion of participants with elevated liver stiffness in need of specialist care. Direct expenditure for this sequential pathway amounted to less than half of the costs incurred by a FIB-4 only strategy.

The European guideline recommends screening at-risk individuals with FIB-4 and referring all with FIB-4 ≥1.30 for TE.[8] However, this recommendation would result in 38% of the total study population needing referral, of whom more than one-third had TE <8 kPa. Consequently, such a strategy carries a substantial risk of overdiagnosis. Our findings are in line with a recent LiverScreen consortium study, which reported a high proportion of false positives with FIB-4 (30% in the general population, 29% in at-risk individuals)[11] and false negative rates of 3–7%.

The three referral strategies – FIB-4 alone, ELF alone, and FIB-4 followed by ELF in indeterminate cases – missed 42-63% of participants with TE ≥8 kPa, and 26-34% of participants with TE ≥12 kPa, with the sequential strategy leading to the highest proportion of missed cases and FIB-4 alone the lowest. Similarly, a Swedish study combining a laboratory database of repeated FIB-4 measurements with diagnostic registries found

Table 3. Performance of ELF, FIB-4, and NFS with histology as reference standard.

	AUROC (95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	TN FN	FP TP
Significant fibrosis (≥F2)							
TE ≥8 kPa	0.83 (0.77-0.89)	90 (83-95)	50 (37-63)	74 (66-82)	76 (61-88)	32 10	32 93
ELF ≥9.8	0.76 (0.69-0.84)	70 (60-78)	64 (51-76)	76 (67-84)	56 (44-68)	41 32	23 74
FIB-4 ≥1.30	0.72 (0.63-0.80)	76 (66-83)	53 (40-66)	73 (63-81)	57 (43-69)	34 26	30 80
NFS ≥-1.45	0.56 (0.46-0.65)	88 (80-93)	14 (7-25)	63 (55-71)	41 (21-64)	9 13	55 93
Advanced fibrosis (≥F3)							
TE ≥12 kPa	0.92 (0.88-0.97)	98 (90-100)	36 (27-45)	41 (32-50)	98 (87-100)	41 1	74 51
ELF ≥10.5	0.85 (0.79-0.92)	67 (53-79)	78 (69-85)	58 (45-71)	83 (75-90)	90 18	26 36
FIB-4 ≥2.67	0.73 (0.64-0.81)	82 (69-91)	43 (34-53)	40 (31-50)	83 (72-92)	50 10	66 44
NFS ≥0.68	0.66 (0.57-0.76)	94 (85-99)	16 (10-24)	35 (27-43)	86 (65-97)	19 3	97 51

ELF, enhanced liver fibrosis test; FIB-4, fibrosis-4 index; FN, false negative; FP, false positive; NFS, NAFLD fibrosis score; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

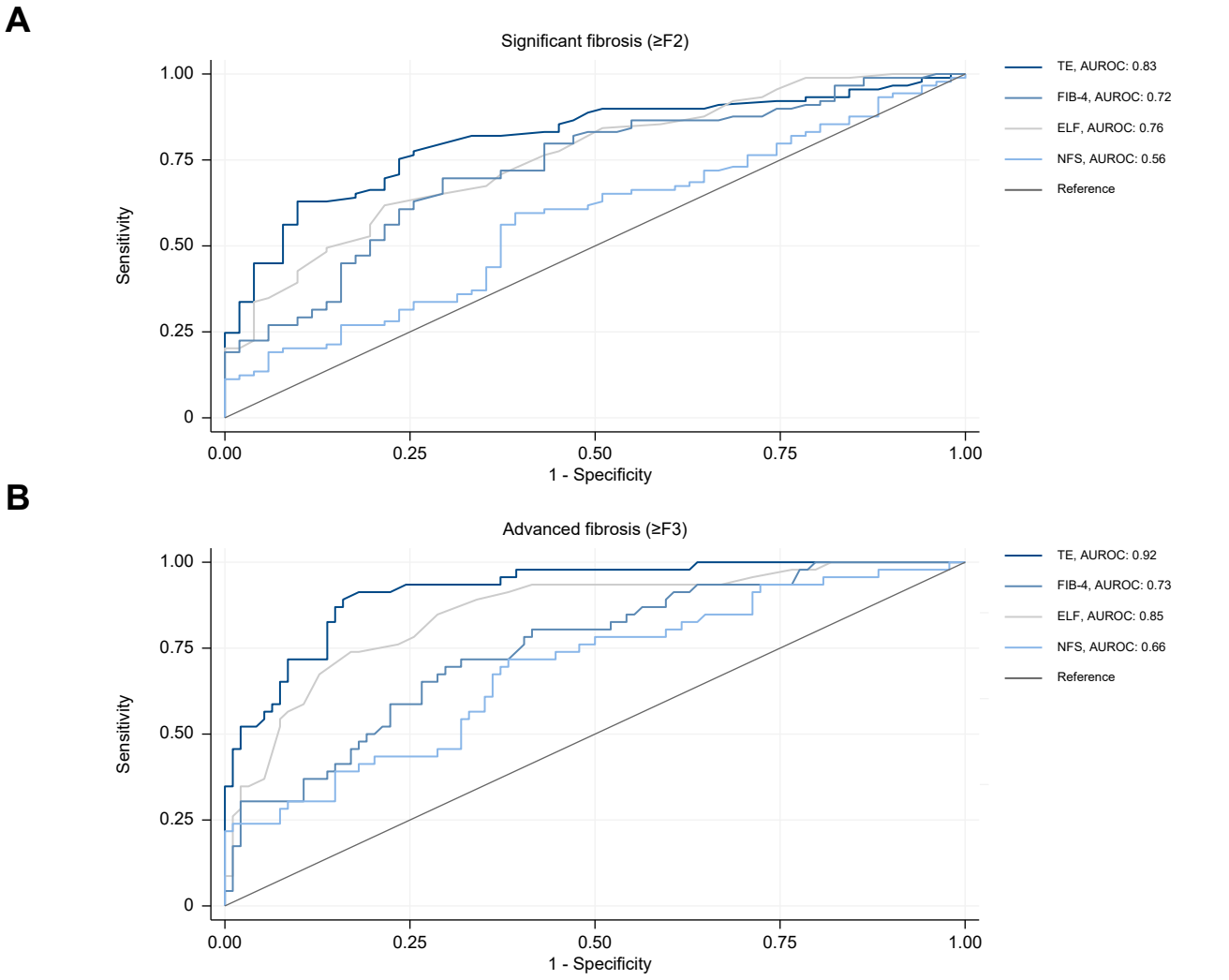

Fig. 3. Diagnostic accuracy of TE, ELF, FIB-4, and NFS. Area under the receiver operating characteristics curve for discriminative accuracy in 170 participants with a liver biopsy (155 screening positives and 15 screening negatives). (A) Significant fibrosis. (B) Advanced fibrosis. ELF, enhanced liver fibrosis; FIB-4, fibrosis-4 index; NFS, NAFLD fibrosis score; TE, transient elastography.

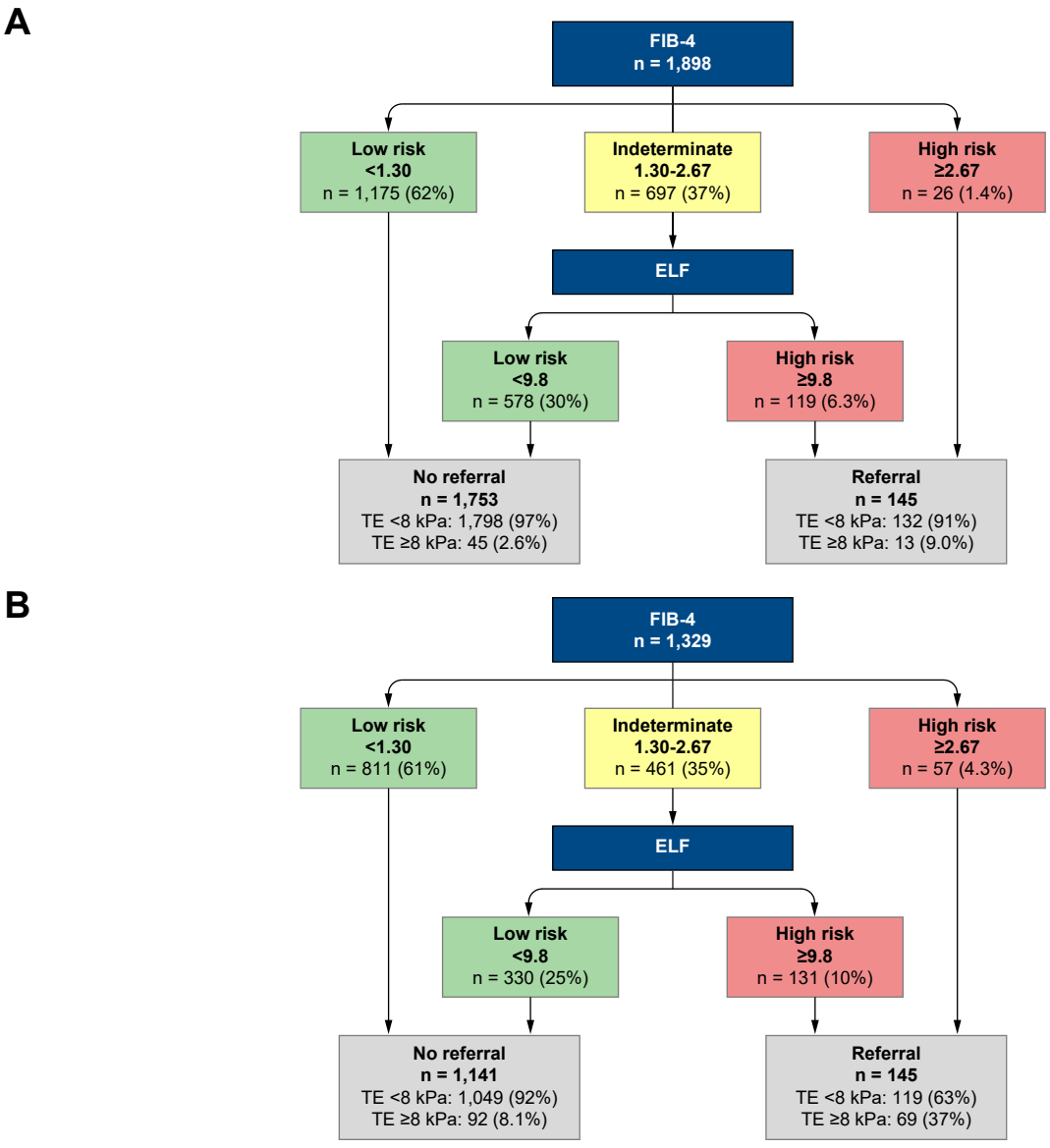

Fig. 4. Decision tree for a referral strategy of FIB-4 followed by ELF in indeterminate cases. (A) General population. (B) At-risk populations (alcohol, type 2 diabetes, obesity, the metabolic syndrome). ELF, enhanced liver fibrosis; FIB-4, fibrosis-4 index; TE, transient elastography.

that almost half of all patients who developed cirrhosis had FIB-4 <1.30 in prior measurements.[12]

This finding stresses the importance of repeating investigations every 1-5 years in people with continuing risk factors for liver fibrosis.[6,23] It also highlights the need for a continued search for more accurate biomarkers and risk stratification tools.[24,25]

In the group of participants randomly recruited from the general population, only 10% of ELF positives and 3.6% of FIB-4 positives had TE≥8 kPa, highlighting the risk of spurious results from spectrum bias in very low prevalence populations.[26] Random variation and causes of false positives are proportionally more important in low than in high prevalence populations, diminishing the diagnostic performance of any test.[27]

A UK referral pathway using FIB-4 followed by ELF in indeterminate cases reduced futile referrals for NAFLD by 81%.[7] Our study confirms the effectiveness of this referral pathway. Furthermore, we found a 27% prevalence of biopsy-verified advanced fibrosis in screening positives, confirming TE ≥8 kPa as a solid screening reference.

The sequential referral strategy was cheap but missed 42 participants with TE ≥8 kPa and 10 with TE ≥12 kPa per 1,000 screened, compared to 28 and 7 per 1,000 screened for FIB-4 alone. This warrants a full cost-benefit analysis of the different screening strategies. Health-economic studies show that sequential combinations of non-invasive tests are cost-effective in low-prevalence populations.[28,29] In a study modelled over Scandinavian healthcare costs, the ELF test alone, followed by TE in positive cases, was the most accurate and cost-effective method in a low-prevalence population of participants at risk of ALD.[29]

Almost half of screening positive participants with low ELF had normalised their TE at the biopsy visit, and only 8% had advanced fibrosis on histology, compared to 61% of those in

whom both ELF and TE were elevated. However, TE had the highest diagnostic accuracy among participants who proceeded to a liver biopsy, stressing its superior diagnostic accuracy in secondary care. Obesity is the exception, since the diagnostic accuracy of TE is lower in biopsied participants with BMI ≥30 kg/m^2, as previously shown.[30] A confirmative non-invasive test is therefore especially important in obese patients with elevated TE. Participants with a false positive ELF test were older than true negatives, which corresponds to a well-known increase in ELF with age.[14,21,31] Similar to ELF, FIB-4 also performed less well in older participants (≥65 years), but when applying the age-adjusted cut-off for FIB-4 to limit false positives, this led to an increase in false negatives, especially among at-risk individuals.[16]

Our study is strengthened by the prospective design. We performed all investigations at screening within 1 week and repeated all investigations at the biopsy visit. Furthermore, we confirmed the presence or absence of advanced fibrosis in 83% of screening positive participants, based on 64% of screening positives receiving a liver biopsy and 19% experiencing a decrease in TE to <6 kPa at the biopsy visit.

This and similar screening studies in low prevalence populations are restricted to using TE instead of liver biopsy as a reference standard. However, liver biopsy would be unethical in such low-prevalence settings, and since elevated TE is a marker of poor prognosis, we support the use of TE as a reference screening standard. Consequently, we performed liver biopsies based on elevated TE not the ELF test result. Because of this, biopsy data are not available for screening negative participants with elevated ELF. Another limitation is that this is a single-centre study only representative of Caucasians, who were mainly middle-aged, with obesity and drinking patterns representative of the Danish population. Consequently, multinational studies are needed to ensure generalisability of findings across ethnicities, age-groups, and lifestyle factors.

Use of the ELF test to screen for liver fibrosis can reduce the number of futile referrals compared to FIB-4 alone. The sequential combination of FIB-4 followed by ELF in indeterminate cases further minimizes the number of futile referrals, and saves cost, making this a promising future referral strategy.

Affiliations

[1]Centre for Liver Research, Department of Gastroenterology and Hepatology, Odense University Hospital, Denmark; [2]Department of Clinical Research, University of Southern Denmark, Denmark; [3]Danish Center for Healthcare Improvements, Aalborg University, Denmark; [4]Department of Clinical Biochemistry, Odense University Hospital, Denmark; [5]Centre for Quantum Mathematics, Department of Mathematics and Computer Science, University of Southern Denmark; [6]Department of Pathology, Odense University Hospital, Denmark; [7]Danish Institute of Advanced Study (DIAS), University of Southern Denmark, Denmark; [8]Liver Unit Hospital Clínic, Institut D'investigacions Biomédiques August Pi I Sunyer (IDIBAPS), Spain; [9]Centro de Investigación En Red de Enfermedades Hepáticas Y Digestivas (CIBEREHD) Barcelona; Faculty of Medicine and Health Sciences, University of Barcelona, Spain

Abbreviations

ALD, alcohol related fatty liver disease; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4 index; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score; TE, transient elastography.

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Conflict of interest

PA, JMJ, CDH, KHT, SJ, MI, NT, MBT, SS, SD, SA, and JEA have no conflicts of interest. MK and KPL received a speaker's fee from Siemens Healthcare. JKH received a speaker's fee from Norgine. IG received lecture fees from Gilead and Novartis. PG received independent investigator promoted research funds from Gilead, Grifols, and Mallinckrodt and has participated on Advisory Boards for which he has received honoraria for from Rallybio; Inventiv, Martin Pharmaceuticals, Novartis, Merck, Intercept, and Gilead. MT received a speaker's fee from Echosens, Siemens Healthcare, Norgine; and an advisory fee from GE Healthcare. AK reports advisory board and lecture fees from Norgine and Siemens.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

MK: Conceptualization, methodology, formal analysis, data curation, investigations, visualization, writing - original draft preparation; KL: Investigations, writing - original draft preparation; PA: Software, data curation; JKH: Investigations, writing – reviewing and editing; JMJ: Investigations, writing – reviewing and editing; CDH: Investigations, writing – reviewing and editing; KHT: Investigations, writing – reviewing and editing; SJ: Investigations, visualization, writing – reviewing and editing; MI: Writing – reviewing and editing; NT: Writing – reviewing and editing; MBT: Investigations, writing – reviewing and editing; SS: Formal analysis, writing – reviewing and editing; SD: Investigations, writing – reviewing and editing; SA: Investigations, writing – reviewing and editing; JEA: Formal analysis, writing – reviewing and editing, IG: Supervision, Funding, writing – reviewing and editing; PG: Supervision, Funding, writing – reviewing and editing; MT: Conceptualization, methodology, formal analysis, writing – original draft preparation, supervision, funding; AK: Conceptualization, methodology, supervision, funding, writing – reviewing and editing.

Data availability statement

The full study datasets are available from the authors upon request to the corresponding author and Odense Patient Data Exploratory Network (open@rsyd.dk) – a research infrastructure unit at Odense University Hospital – with reference to project ID OP_475. The study protocol, standard operating procedures and patient information are also available upon request. The data must not be processed for purposes other than statistical and scientific studies. Data are available on request for researchers who have acquired the required legal permissions from the Danish Data Protection Agency.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2023.04.002.

References

[1] Vos T, Lim S, Abbafati C, Abbas KM, Abbasi M, Abbasifard M, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 2020;396:1204–1222.

[2] GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016;388:1459–1544.

[3] Karlsen TH, Sheron N, Zelber-Sagi S, Carrieri P, Dusheiko G, Bugianesi E, et al. The EASL Lancet Liver Commission: protecting the next generation of Europeans against liver disease complications and premature mortality. The Lancet 2022;399:61–116.

[4] Gines P, Castera L, Lammert F, Graupera I, Serra-Burriel M, Allen AM, et al. Population screening for liver fibrosis: toward early diagnosis and intervention for chronic liver diseases. Hepatology 2022;75:219–228.

[5] Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver fibrosis, but No other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 2015;149:389–397 e310.

[6] Rasmussen DN, Thiele M, Johansen S, Kjærgaard M, Lindvig KP, Israelsen M, et al. Prognostic performance of 7 biomarkers compared to liver biopsy in early alcohol-related liver disease. J Hepatol 2021;75:1017–1025.

[7] Srivastava A, Gailer R, Tanwar S, Trembling P, Parkes J, Rodger A, et al. Prospective evaluation of a primary care referral pathway for patients with non-alcoholic fatty liver disease. J Hepatol 2019;71:371–378.

[8] Berzigotti A, Tsochatzis E, Boursier J, Castera L, Cazzagon N, Friedrich-Rust M, et al. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis – 2021 update. J Hepatol 2021;75:659–689.

[9] Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, et al. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology 2019;156:1717–1730.

[10] Boursier J, Vergniol J, Guillet A, Hiriart JB, Lannes A, Le Bail B, et al. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. J Hepatol 2016;65:570–578.

[11] Graupera I, Thiele M, Serra-Burriel M, Caballeria L, Roulot D, Wong GL, et al. Low accuracy of FIB-4 and NAFLD fibrosis scores for screening for liver fibrosis in the population. Clin Gastroenterol Hepatol 2022;20:2567-2576.

[12] Hagström H, Talbäck M, Andreasson A, Walldius G, Hammar N. Repeated FIB-4 measurements can help identify individuals at risk of severe liver disease. J Hepatol 2020;73:1023–1029.

[13] Sharma C, Cococcia S, Ellis N, Parkes J, Rosenberg W. Systematic review: accuracy of the enhanced liver fibrosis test for diagnosing advanced liver fibrosis and cirrhosis. J Gastroenterol Hepatol 2021;36:1788–1802.

[14] Thiele M, Madsen BS, Hansen JF, Detlefsen S, Antonsen S, Krag A. Accuracy of the enhanced liver fibrosis test vs FibroTest, elastography, and indirect markers in detection of advanced fibrosis in patients with alcoholic liver disease. Gastroenterology 2018;154:1369–1379.

[15] Alberti KG, Zimmet P, Shaw J. Metabolic syndrome–a new world-wide definition. A consensus statement from the international diabetes federation. Diabet Med 2006;23:469–480.

[16] McPherson S, Hardy T, Dufour JF, Petta S, Romero-Gomez M, Allison M, et al. Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. Am J Gastroenterol 2017;112:740–751.

[17] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.

[18] Papatheodoridi M, Hiriart JB, Lupsor-Platon M, Bronte F, Boursier J, Elshaarawy O, et al. Refining the Baveno VI elastography criteria for the definition of compensated advanced chronic liver disease. J Hepatol 2021;74:1109–1116.

[19] Hubert M, Debruyne M. Minimum covariance determinant. Wiley Interdiscip Rev Comput Stat 2010;2:36–43.

[20] Castera L, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, et al. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. Hepatology 2010;51:828–835.

[21] Fagan KJ, Pretorius CJ, Horsfall LU, Irvine KM, Wilgen U, Choi K, et al. ELF score >/=9.8 indicates advanced hepatic fibrosis and is influenced by age, steatosis and histological activity. Liver Int 2015;35:1673–1681.

[22] Tsutsumi M, Urashima S, Takase S, Ueshima Y, Tsuchishima M, Shimanaka K, et al. Characteristics of serum hyaluronate concentrations in patients with alcoholic liver disease. Alcohol Clin Exp Res 1997;21:1716–1721.

[23] Boursier J, Hagström H, Ekstedt M, Moreau C, Bonacci M, Cure S, et al. Non-invasive tests accurately stratify patients with NAFLD based on their risk of liver-related events. J Hepatol 2022;76:1013–1020.

[24] Niu L, Thiele M, Geyer PE, Rasmussen DN, Webel HE, Santos A, et al. Noninvasive proteomic biomarkers for alcohol-related liver disease. Nat Med 2022;28:1277–1287.

[25] Åberg F, Jula A, Färkkilä M, Salomaa V, Erlund I, Männistö S, et al. Comparison of various strategies to define the optimal target population for liver fibrosis screening: a population-based cohort study. United Eur Gastroenterol J 2022;10:1020–1028.

[26] Usher-Smith JA, Sharp Stephen J, Griffin SJ. The spectrum effect in tests for risk prediction, screening, and diagnosis. BMJ 2016;353.

[27] Chuang-Stein C, Tong DM. The impact and implication of regression to the mean on the design and analysis of medical investigations. Stat Methods Med Res 1997;6:115–128.

[28] Srivastava A, Jong S, Gola A, Gailer R, Morgan S, Sennett K, et al. Cost-comparison analysis of FIB-4, ELF and fibroscan in community pathways for non-alcoholic fatty liver disease. BMC Gastroenterol 2019;19:122.

[29] Asphaug L, Thiele M, Krag A, Melberg HO. Cost-effectiveness of non-invasive screening for alcohol-related liver fibrosis using real-world data from primary and secondary care patients. Hepatology 2020;71:2093–2104.

[30] Petta S, Wai-Sun Wong V, Bugianesi E, Fracanzani AL, Camma C, Hiriart JB, et al. Impact of obesity and alanine aminotransferase levels on the diagnostic accuracy for advanced liver fibrosis of noninvasive tools in patients with nonalcoholic fatty liver disease. Am J Gastroenterol 2019;114:916–928.

[31] Anstee QM, Lawitz EJ, Alkhouri N, Wong VW, Romero-Gomez M, Okanoue T, et al. Noninvasive tests accurately identify advanced fibrosis due to NASH: baseline data from the STELLAR trials. Hepatology 2019;70:1521–1530.