Noninvasive, Blood-Based Biomarkers as Screening Tools for Hepatic Fibrosis in People With Type 2 Diabetes

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Nonalcoholic fatty liver disease (NAFLD) is dramatically increasing in parallel with the pandemic of type 2 diabetes. Here, the authors aimed to assess the performance of the most commonly used noninvasive, blood-based biomarkers for liver fibrosis (FibroTest, NAFLD fibrosis score, BARD score, and FIB-4 Index) in subjects with type 2 diabetes. Liver stiffness measurement was estimated by two-dimensional shear wave elastography. Finally, the authors assessed the diagnostic role of ActiTest and NashTest 2 in liver fibrosis in the examined population.

Nonalcoholic fatty liver disease (NAFLD) (1) has been of particular interest in recent years. Based on recent epidemiological studies, it affects one-fourth of the world's population (2) and is dramatically increasing in parallel with type 2 diabetes and the metabolic syndrome. It is estimated that 70% of people with type 2 diabetes also have NAFLD (3,4), and 20–30% have the more worrying form of the disease known as nonalcoholic steatohepatitis (NASH), with lobular inflammation and hepatocyte ballooning. It is therefore clear that NAFLD has become a major issue, creating clinical and economic burden, as this population is at high risk of progressing to advanced fibrosis and cirrhosis (5) or even to hepatocellular carcinoma. Thus, it is important to recognize high-risk people with NASH and advanced fibrosis to provide them with optimal medical management.

Liver biopsy remains the gold standard for diagnosing NASH and determining the extent of fibrosis when referring to a high-risk population. Nevertheless, its invasive nature, high cost, and potential inter- and intraobserver heterogeneity make it less attractive as a diagnostic, monitoring, and screening tool at the population level (6). In the past decade, a significant number of noninvasive biomarker tests have been developed and validated to help identify people with NASH or advanced fibrosis. However, most studies have included subjects with different clinical features (e.g., BMI and sex) and comorbidities (e.g., diabetes, hypertension, and hepatitis).

Liver stiffness measurement (LSM) is also a promising surrogate biomarker of liver fibrosis stage, with two-dimensional shear wave elastography (2D SWE) being one of the elastography techniques validated for the assessment of LSM. Recent research has shown that 2D SWE has a higher degree of accuracy than point SWE in diagnosing stage F2 fibrosis (area under the curve [AUC] 0.85–0.92 vs. 0.70–0.83) (7) and moderate to high accuracy in diagnosing advanced fibrosis or cirrhosis in patients with biopsy-proven NAFLD (8).

In this study, we aimed first to assess the performance of the most commonly used noninvasive, blood-based biomarkers for the estimation of fibrosis as measured by 2D SWE specifically in a population of adults with established type 2 Diabetes. Second, we tried to investigate the prognostic value of ActiTest (9) and NashTest 2 (9) in the development of liver fibrosis. To our knowledge, there are few studies focused on people with type 2 diabetes and only one with a multiethnic cohort of

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this specific population that have assessed the performance of ActiTest and NashTest 2.

Research Design and Methods

Subjects

A total of 140 people who were followed up at the Diabetes Outpatient Clinic of the 2nd Department of Internal Medicine, Hippokration General Hospital, in Athens, Greece, consented to participate in this study. All were diagnosed with type 2 diabetes according to the American Diabetes Association criteria (10). Participants were eligible if they met the following criteria: age 20–70 years; ambulatory without a recent acute illness; alcohol consumption <30 g/day for men and <20 g/day for women; A1C <8.00% (63.9 mmol/mol); absence of nonhepatic malignancy; no use of immunosuppressive medications in the past 6 months; negative test for hepatitis B surface antigen (HBsAg); antibodies against hepatitis C (anti-HCV), anti–smooth muscle (SMA), and anti-nuclear antibodies (ANA); thyroid-stimulating hormone (TSH), immunoglobulin G (IgG), and immunoglobulin M (IgM) levels within the normal reference range; and no presence of chronic heart or renal disease that was unrelated to type 2 diabetes. Individuals with known chronic liver disease besides NAFLD or NASH-related cirrhosis; use of immunosuppressive medications currently or within the past 6 months; A1C ≥8.0%; alcohol consumption in larger quantities than those indicated in the inclusion criteria; and presence of concurrent nonhepatic malignancy or chronic respiratory, heart, or renal disease unrelated to type 2 diabetes were excluded from the study.

Each participant underwent an interview by a trained investigator, a full clinical examination, anthropometric measurements, and laboratory testing. Information regarding participants' demographic characteristics, medical history, duration of diabetes, diabetes complications, and comorbidities such as hypertension and dyslipidemia, as well as current medications, was obtained through interviews and review of their medical records.

Height, weight, and waist and hip circumference were measured without shoes or outer clothing. BMI was calculated as weight (kg)/height $(m)^2$. Blood pressure was recorded as the mean of three consecutive measurements taken 5 minutes apart and in a sitting position. Metabolic syndrome was defined using the most updated criteria of the International Diabetes Federation, including *1*) central obesity (waist circumference >94 cm in men and >80 cm in women) and *2*) at least two of the following factors: serum triglycerides >150 mg/dL or specific treatment for this lipid abnormality, serum HDL cholesterol <40 mg/dL in men and <50 mg/dL in women or specific treatment for this lipid abnormality, systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg or specific antihypertensive treatment, and fasting blood glucose (FBG) >100 mg/dL or previously diagnosed type 2 diabetes.

The study was approved by the regional ethics committee (reference number 12419/6-9-2016). Written informed consent was obtained from all patients before enrollment in the study.

Biological Parameters

After a fasting period of 12 hours, blood was collected for a complete blood count and tests for A1C, HBsAg, anti-HCV, ANA, SMA, IgG, IgM, creatinine, FBG, uric acid, serum alkaline phosphatase, AST, ALT, serum total bilirubin, γ-glutamyl transpeptidase (GGT), α2 macroglobulin, haptoglobin, apolipoprotein A1 (ApoA1), total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides, total serum protein, ferritin, TSH, albumin, γ globulin, and international normalized ratio determination in the central autoanalyzer of our hospital.

Proprietary Scores

For the prediction of hepatic steatosis (HS), we calculated SteatoTest (9), a proprietary score based on a person's serum total bilirubin, GGT, α2 macroglobulin, haptoglobin, ApoA1, FBG, triglycerides, total cholesterol, ALT, sex, age, height, and weight.

Hepatic necroinflammatory activity and NASH were determined by ActiTest (9) and NashTest 2 (9), respectively, as parts of the FibroMax diagnostic tests (BioPredictive, Paris, France). ActiTest measurement was based on serum total bilirubin, GGT, α2 macroglobulin, haptoglobin, ApoA1, and ALT; the NashTest 2 was based on α2 macroglobulin, ApoA1, haptoglobin, total bilirubin, GGT, AST, total cholesterol, and triglyceride level. Biochemical analyses were also performed at Biomedicine in Athens, Greece, and the respective scores with their equivalence to liver biopsies were provided by BioPredictive. The ActiTest was reported as a score of A0–A3 (indicating absent, minimal, significant, and severe necroinflammatory activity, respectively), and the NashTest 2 was reported as a score of N0–N3

(indicating absent, mild, moderate, and severe NASH, respectively) (Table 1).

For the prediction of advanced fibrosis, we calculated FibroTest (9), a proprietary score based on α2 macroglobulin, ApoA1, haptoglobin, total bilirubin, and GGT (Table 1).

For determination of ActiTest, NashTest 2, and FibroTest (BioPredictive algorithms), samples were blindly provided to Bioiatriki in Athens, Greece, to measure haptoglobin, α2 macroglobulin, ApoA1, total bilirubin, GGT, AST, ALT, triglycerides, and total cholesterol and calculate the above noninvasive biomarkers.

We also calculated the AST-to-platelet ratio index (APRI) (11), a noninvasive method offering information about risk of hepatic fibrosis and cirrhosis (Table 2), based on platelet count and AST level, as follows:

APRI = AST (IU/L)/AST upper limit (IU/L)/platelet count (10^9/L) × 100

Upper limit of AST was defined as 40 IU/L.

We also used the NAFLD fibrosis score (12) for the estimation of liver scarring (Table 2) based on clinical (i.e., history of impaired fasting glucose [IFG] or diabetes, BMI, and age) and biochemical (i.e., AST, ALT, platelet count, and albumin) factors as shown in the following formula:

NAFLD fibrosis score: −1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes status (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet (×10^9/L) – 0.66 × albumin (g/dL)

For the risk of advanced fibrosis (Table 2), we also calculated a BARD Score (13) by adding the selected points based on its formula:

BMI ≥28 (no = 0, yes = 1) + AST/ALT ratio ≥0.8 (no = 0, yes = 2) + diabetes (no = 0, yes = 1)

We calculated a Fibrosis-4 (Fib-4) index (14) based on age, AST, ALT, and platelet count for the detection of liver fibrosis (Table 2) based on its formula:

Fib-4 score = (age × AST)/(platelets × $\sqrt{}$ALT)

Liver Fat Content

The liver fat content was estimated with the MRI-PDFF (magnetic resonance imaging–derived proton density fat fraction) technique (15,16) using the IQ IDEAL (General Electric Healthcare, Waukesha, WI), a gradient multi-echo acquisition sequence with six echoes. The examination was performed at the MRI Section of Biomedicine. A single experienced observer measured the fat fraction value for each hepatic segment, and the total hepatic fat fraction was calculated as the average value of all segments.

The scan protocol also included a 3D LAVA (liver acquisition with volume acceleration) sequence (General Electric Healthcare). Images from that sequence were reformed on axial and coronal planes of 10-mm slices with no gap. Using the General Electric Healthcare ReportCard software, the liver volume was measured for each plane, excluding big vessels and other parenchymal lesions. From the average of each plane volume, the total liver volume was calculated in cm^3. HS was defined as the percentage of total liver fat divided by the respective liver volume. A fat fraction <5% was considered normal (no steatosis). Higher percentages were reported as grades 1–3 (G1 = mild steatosis [5–33%], G2 = moderate steatosis [34–67%],and G3 = severe steatosis [≥67%]).

2D SWE

2D SWE was performed at the Diagnostic Echotomography Center Kifissia, in Athens, Greece, by two expert

TABLE 1 Interpretation of Proprietary Scores (BioPredictive)

Score	Grade or Stage	Interpretation
ActiTest score (*a*)		
$0.00 < a < 0.17$	A0	No necroinflammatory activity
$0.18 < a < 0.52$	A1	Minimal necroinflammatory activity
$0.53 < a < 0.62$	A2	Significant necroinflammatory activity
$0.63 < a < 1.00$	A3	Severe necroinflammatory activity
NashTest 2 score (*n*)		
$0.00 < n < 0.25$	N0	No NASH
$0.26 < n < 0.50$	N1	Mild NASH
$0.51 < n < 0.75$	N2	Moderate NASH
$0.76 < n < 1.00$	N3	Severe NASH
FibroTest score (*f*)		
$0.00 < f < 0.21$	F0	No fibrosis
$0.22 < f < 0.27$	F0–F1	No fibrosis
$0.28 < f < 0.31$	F1	Minimal fibrosis
$0.32 < f < 0.48$	F1–F2	Minimal fibrosis
$0.49 < f < 0.58$	F2	Moderate fibrosis
$0.59 < f < 0.72$	F3	Advanced fibrosis
$0.73 < f < 0.74$	F3–F4	Advanced fibrosis
$0.75 < f < 1.00$	F4	Severe fibrosis (cirrhosis)

TABLE 2 Interpretation of APRI, NAFLD Fibrosis Score, BARD Score, and Fib-4 Index

Index or Score	Interpretation
APRI	
<0.5	No significant fibrosis
0.5–0.7	Some kind of liver damage
>1	Cirrhosis
NAFLD fibrosis score	
<−1.455	F0–F2
−1.455 to 0.675	Indeterminate score
>0.675	F3–F4
BARD score	
0–1	Low risk
2–4	High risk
FIB-4 index	
<0.68	No fibrosis
0.69–1.37	F0–F2
1.38–3.08	F3–F4

radiologists on the Aixplorer US system (Supersonic Imagine, Aix-en-Provence, France), with an SC6–1 curvilinear transducer. After establishing a good acoustic window, the examiners activated the elastography mode and waited for the elastogram to be stable for five consecutive frames. The stability index for SWE was used according to the manufacturer's guidelines to ensure the quality of measurements. Each SWE measurement was taken at a stability index >90%. The median value of five SWE measurements was calculated and saved for further analysis. The number of measurements taken is recommended by both European Federation of Societies for Ultrasound in Medicine and Biology and World Federation of Societies for Ultrasound in Medicine and Biology guidelines (17–19). An interquartile range-to-median ratio <30% ensured measurement reliability. All measurements were calculated in kilopascals (kPa) using Young's modulus.

Subjects with an LMS <6 kPa were characterized as normal. An LSM of 6.1–7.9 kPa indicated stage F1 fibrosis, 8.0–9.4 kPa indicated stage F2 fibrosis, 9.5–12.4 kPa indicated stage F3 fibrosis, and >12.5 kPa indicated stage F4 fibrosis.

DNA Preparation and SNP Genotyping

Testing for *PNPLA3* single nucleotide polymorphism included DNA extraction from 200 μL whole blood. Genomic DNA was extracted using a MagNa Pure LC DNA isolation kit (Roche Diagnostics,Mannheim, Germany), applying magnetic bead isolation technology on the MagNa Pure LC automated extraction instrument (Roche Diagnostics). High-purity genomic DNA was measured by a Nanodrop 1000 quantitation system, and 40 ng of the extracted DNA was amplified by conventional PCR assay (primer set rs738409_F CCC-TGC-TCA-CTT-GGA-GAA-AG and rs738409_RCTG-CAG-GCA-GGA-GAT-GTG-T) on a Bio-Rad c1000 Touch thermocycler. The 227-bp PCR product was tested using RFLP (restriction fragment–length polymorphism) digestion using the NIAIII enzyme cutting the inline image sequence according the manufacturer's reaction protocol (New England Biolabs, Hitchin, U.K.). The RFLP digestion pattern was: CC allele: 227 bp; GG allele: 112,115 bp; CG allele: 227,115,112 bp. Some of the results were confirmed by sequencing using the BigDye Terminator, v. 3.1, Cycle Sequencing Kit and analyzed on the ABI3500 automated genetic analyzer (Thermo Fisher Scientific, Waltham, MA). These techniques are complementary to identify and verify the existence or absence of the rs738409 PNPLA3 polymorphism.

Statistical Analysis

Data were analyzed using the statistical software SPSS v. 25 (IBM Corp., Armonk, NY). To describe the sample, descriptive statistics such as mean, SD, number, and percentage were performed. The differences between categorical variables were compared by Pearson χ^2, and correlations were determined using Spearman testing. Multiple linear regression analysis using the backward method was conducted to investigate whether biomarkers could identify the subjects who may benefit from LSM estimation with SWE. In regression, all variables were log-transformed. A level of significance of <0.05 was used in all instances.

Results

Of the 140 subjects recruited, 120 were included in the study; 20 subjects who did not attend for MRI-PDFF were excluded. Four of the 120 participants included did not attend for SWE or blood tests for FibroMax calculation. Table 3 shows the demographic, clinical, and biochemical characteristics of our study population. Table 4 shows participant characteristics by MRI liver steatosis grade. Only 5% (six subjects) had an AST >40 units/L, and 18.3% (22 subjects) had an ALT >38 units/L (the upper normal levels of the method performed). Table 5 shows similarly the demographic, clinical, and biological features of included subjects with type 2 diabetes and an LSM >8.0 kPa.

In our study population, eight subjects (6.7%) had diet-controlled diabetes, and 31 (25.8%) were treated with insulin plus oral medication. A total of 112 subjects (95.5%) were treated with metformin, and the most popular treatment combination was metformin plus a dipeptidyl peptidase 4 inhibitor (n = 42 [35%]). A total of 75 subjects (62.5%) were treated with statins, and 12 (10%) were treated with statins and fibrates. A total of 70 subjects (58.3%) were treated with ACE inhibitors.

The *PNPLA3* rs738409 CC/CG/GG genotype frequencies were 65 (54.2%), 42 (35%), and 13 (10.8%), respectively. HS was directly correlated with the G-allele of *PNPLA3* rs738409 (CC vs. CG/GG, P = 0.001). However, a *PNPLA3* variant was not correlated with LSM estimated with SWE (R = 0.0064, P = 0.504) or FibroTest (R = 0.081, P = 0.388).

Moderate to severe NASH (score of N2 or higher on the NashTest 2) was observed in 49 subjects (42.24%), and only three subjects (2.60%) were found to have significant to severe necroinflammatory activity (grade A2 or higher on ActiTest) (Table 3).

Most of the participants with a normal SWE (90.2%) had no evidence of necroinflammatory activity (grade A0). All participants with stage 4 fibrosis had minimal evidence of necroinflammatory activity (grade A1). However, 14.3% of participants with stage F3 fibrosis had significant necroinflammatory activity (grade A2). Finally, only 1.6% of participants with normal SWE had severe necroinflammatory activity (grade A3).

With regard to NASH, among the participants with normal SWE, only 1.8% had severe NASH (grade 3), and 80% of those with stage F2 fibrosis had moderate to severe NASH (grade 2 and 3). Finally, among the participants with the most severe stage of fibrosis per SWE, 50% had NASH grade 2, and the remaining 50% had NASH grade 3.

Table 6 shows the comparison by LSM estimated with SWE and APRI, NAFLD fibrosis score, Bard score, and Fib-4 index. FibroTest was more indicative of the LSM values estimated with SWE (P = 0.921).

LSM (via SWE) was also directly correlated with both ActiTest (R = 0.405, $P \leq 0.001$) and NashTest 2 (P = 0.299, P = 0.002). Furthermore, according to linear regression, an increase in ActiTest and NashTest 2 values increases the LSM values estimated with SWE by 5.632

TABLE 3 Demographic, Clinical, and Biological Features of Subjects With Type 2 Diabetes Included in the Study (N = 120)

Variable	Mean ± SD or *n* (%)
Age, years	60.60 ± 7.50
Sex	
Male	74 (61.7)
Female	46 (38.3)
Duration of diabetes, years	10.55 ± 7.15
BMI, kg/m^2	30.79 ± 4.54
Waist circumference, cm	
Male	107.50 ± 10.30
Female	102.00 ± 11.30
Systolic blood pressure, mmHg	126.50 ± 11.08
Diastolic blood pressure, mmHg	61.80 ± 10.08
A1C, %	6.72 ± 0.64
Glucose, mg/dL	127.98 ± 34.79
AST, units/L	21.46 ± 9.27
ALT, units/L	27.79 ± 18.21
TSH, mg/dL	1.69 ± 1.02
Total cholesterol, mg/dL	156.97 ± 37.06
HDL cholesterol, mg/dL	
Male	43.73 ± 10.30
Female	49.73 ± 11.30
LDL cholesterol, mg/dL	89.76 ± 29.88
VLDL cholesterol, mg/dL	29.18 ± 16.14
Liver fat content >5% (MRI-PDFF)	97 (80.8)
Triglycerides, mg/dL	137.63 ± 72.28
SWE LSM, kPa	
<6.0 (stage F0)	62 (53.45)
6.1–7.9 (stage F1)	38 (32.76)
8.0–9.4 (stage F2)	7 (6.03)
9.5–12.4 (stage F3)	7 (6.03)
>12.5 (stage F4)	2 (1.73)
SteatoTest grade	
S0	25 (21.55)
S0–S1	13 (11.20)
S1	16 (13.79)
S1–S2	24 (20.69)
S2	14 (12.07)
S2–S3	5 (4.31)
S3	19 (16.38)

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TABLE 3 Demographic, Clinical, and Biological Features of Subjects With Type 2 Diabetes Included in the Study (N = 120) *(continued)*

Variable	Mean ± SD or *n* (%)
NashTest 2 grade	
N0	19 (16.38)
N1	48 (41.38)
N2	33 (28.45)
N3	16 (13.79)
ActiTest grade	
A0	89 (76.7)
A1	24 (20.7)
A2	1 (0.9)
A3	2 (1.7)
FibroTest stage	
F0	48 (41.4)
F0–F1	15 (12.9)
F1	9 (7.8)
F1–F2	25 (21.6)
F2	4 (3.4)
F3	11 (9.5)
F3–F4	2 (1.7)
F4	2 (1.7)

times ($P \leq 0.001$, 95% CI 3.213–8.051) and by 3.981 times ($P \leq 0.001$, 95% CI 2.398–5.563), respectively.

Discussion

There is a complex association between NAFLD and type 2 diabetes, with each condition negatively affecting the other (3). Specifically, not only is the presence of NAFLD related to an increased risk of developing type 2 diabetes (20–22), but also subjects with type 2 diabetes and NAFLD are at risk for progressing faster to the severe forms of NAFLD such as advanced fibrosis, cirrhosis, and hepatocellular carcinoma (5,23–27).

Percutaneous liver biopsy remains the gold standard for the diagnosis of NASH and the determination of fibrosis stage. However, it is frequently avoided in clinical practice for various reasons such as cost and its invasive character with potential risks. People are also unwilling to consent for such an invasive test when there are limited U.S. Food and Drug Administration–approved treatment options for NASH (28). Furthermore, a biopsy specimen represents only 1/50,000 of liver volume, and sampling bias and underestimation of disease severity is common (29). As a result, a number of noninvasive panels have been developed and validated in order to minimize the need for liver biopsy for the diagnosis of NASH when referring to a high-risk population.

In this study, we assessed the diagnostic value of FibroTest, a well-validated panel, and nonproprietary clinical models such as the APRI, FIB-4 index, NAFLD fibrosis score, and BARD score in estimating liver fibrosis compared with 2D SWE, a promising imaging technique that has been shown as long as FibroScan, which measures the velocity of an elastic shear wave propagating through the liver, to have moderate to high accuracy in diagnosing advanced fibrosis or cirrhosis (AUC 0.85–0.92 for F2, 0.88–0.95 for F3, and 0.97 for F4) (8). FibroTest was the only proprietary score diagnostically closer to LSM estimated by 2D SWE (P = 0.921) (Table 6).

Several reasons could explain the relatively poorer performance of the above tests in subjects with type 2 diabetes. First, the pathogenetic mechanisms promoting NASH in this specific population may be different, as people with type 2 diabetes are likely to have different metabolic and biochemical variations compared with people with NAFLD without diabetes. Furthermore, many parameters used in the above panels and models such as ALT, lipid profile, and FBG can be affected by variability in glycemic control based on diet and use of antihyperglycemic medications, as well as use of lipid-lowering and blood pressure medications. Recent studies have shown that people with type 2 diabetes may respond differently to pharmacological treatment options (i.e., pioglitazone) compared with those with prediabetes (30). Moreover, NAFLD fibrosis score and BARD score use the presence of diabetes or hyperglycemia to identify individuals at high risk for liver fibrosis in a mixed population. It is questionable whether we could rely on these parameters when examining exclusively people with type 2 diabetes. FibroTest has the advantage of not including glycemia-related parameters, and this might explain its superiority over the other noninvasive panels and models.

In this study, we also showed a direct correlation between LSM (SWE) with both ActiTest (R = 0.405, $P \leq 0.001$) and NashTest 2 (R = 0.299, P = 0.002). Combining the use of both biomarkers on top of FibroTest might be an option to improve diagnostic accuracy based on the complex pathogenetic factors leading to NASH. Of note, ActiTest and NashTest 2 were both constructed and mostly validated in patients with chronic hepatitis B or C, who had a greater spectrum of fibrosis and more severe activity in the form of necroinflammation compared with patients with type 2 diabetes.

The prevalence of NAFLD in our study population was similar to the prevalence of NAFLD in the general population of people with diabetes (69% as estimated by

TABLE 4 Participant Characteristics by MRI Liver Steatosis Grade

Variable	Median (IQR) or *n* (%)			
	≤5% Steatosis (*n* = 23)	5–33% Steatosis (*n* = 64)	34–66% Steatosis (*n* = 19)	>66% Steatosis (*n* = 14)
Age, years	63 (61–67.24)	61.16 (56.80–65.64)	64.36 (55.16–68.27)	59.07 (50.61–61.85)
Sex				
Male	18 (78.3)	36 (56.3)	13 (68.4)	7 (50)
Female	5 (21.70)	28 (43.8)	6 (31.6)	7 (50)
Duration of diabetes, years	12 (6–19)	10 (5–17)	8 (3–10)	8 (1.75–10.5)
BMI, kg/m^2	26.6 (25–30.80)	30.9 (27.87–34.77)	31 (28.75–32)	33 (29.24–38.65)
Waist circumference, cm				
Male	95 (90–109.25)	109.5 (98–115.5)	111 (103–121)	120 (108–127)
Female	95 (82.5–103)	102 (96–107–25)	97 (96–100.25)	103 (100–104)
Systolic blood pressure, mmHg	120 (110–130)	130 (120–130)	125 (120–130)	120 (120–130)
Diastolic blood pressure, mmHg	65 (60–70)	60 (60–70)	60 (60–80)	60 (58.75–70)
A1C, %	6.6 (6.20–7.23)	6.6 (6.2–7.3)	6.8 (6.3–7.3)	6.79 (6.24–6.93)
Glucose, mg/dL	122 (108.75–147.75)	121 (102.5–142.75)	127 (106–144)	128 (107.75–143.75)
AST, units/L	17 (14–20)	18 (15–23)	20 (16–23)	31 (21.75–40)
ALT, units/L	14.5 (12–19.25)	20 (17–29)	24 (19–38)	48.5 (32.5–70.75)
TSH, mg/dL	1.57 (1.09–18.6)	1.41 (10.7–2.19)	1.2 (0.8–1.99)	2 (1.12–2.77)
Total cholesterol, mg/dL	147.5 (129.25–167.75)	157 (136.25–174.5)	153 (132–169)	177.5 (126–189.5)
HDL cholesterol, mg/dL				
Male	48 (37–53.75)	41.5 (35.2–49)	40 (35–49.5)	41 (35–46)
Female	65 (50–75.5)	51 (43.2–57.5)	43 (38.75–55)	44 (39–50)
LDL cholesterol, mg/dL	85 (71–97)	90.5 (76.5–108)	82 (62–94)	91.5 (63.5–114.25)
VLDL cholesterol, mg/dL	21 (15–33)	24 (18–32.75)	24 (17–48)	32.5 (19.7–41.5)
Triglycerides, mg/dL	106 (74–127)	116 (92.5–161.75)	109 (97–193)	141 (117.75–218)

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TABLE 4 Participant Characteristics by MRI Liver Steatosis Grade *(continued)*

Variable	Median (IQR) or *n* (%)			
	≤5% Steatosis (*n* = 23)	5–33% Steatosis (*n* = 64)	34–66% Steatosis (*n* = 19)	>66% Steatosis (*n* = 14)
SteatoTest grade				
S0	8 (40)	14 (22.2)	2 (10.5)	1 (7.1)
S0–S1	6 (30)	3 (4.8)	4 (21.1)	0 (0)
S1	2 (10)	12 (19)	2 (10.5)	0 (0)
S1–S2	1 (5)	14 (22.2)	4 (21.1)	5 (35.7)
S2	2 (10)	9 (14.3)	2 (10.5)	1 (7.1)
S2–S3	0 (0)	3 (4.8)	1 (5.3)	1 (7.1)
S3	0 (0)	8 (12.7)	4 (21.1)	6 (42.9)
NashTest 2 grade				
N0	8 (44.4)	9 (15.5)	2 (11.8)	0 (0)
N1	5(27.8)	29 (50)	9 (52.9)	5 (38.5)
N2	5 (27.8)	18 (31)	5 (29.4)	5 (38.5)
N3	0 (0)	2 (3.4)	1 (5.9)	3 (23.1)
ActiTest grade				
A0	18 (78.3)	53 (84.1)	14 (73.7)	4 (28.6)
A1	2 (8.7)	8 (12.7)	5 (26.3)	9 (64.3)
A2	0 (0)	0 (0)	0 (0)	1 (7.1)
A3	0 (0)	2 (3.2)	0 (0)	0 (0)
FibroTest stage				
F0	5 (25)	29 (46)	7 (36.8)	7 (50)
F0–F1	5 (25)	7 (11.1)	2 (10.5)	1 (7.1)
F1	2 (10)	2 (3.2)	3 (15.8)	2 (14.3)
F1–F2	8 (40)	13 (20.6)	3 (15.8)	1 (7.1)
F2	0 (0)	3 (4.8)	0 (0)	1 (7.1)
F3	0 (0)	6 (9.5)	3 (15.8)	2 (14.3)
F3–F4	0 (0)	2 (3.2)	0 (0)	0 (0)
F4	0 (0)	1 (1.6)	1 (5.3)	0 (0)

TABLE 5 Demographic, Clinical, and Biological Features of Included Subjects With Type 2 Diabetes and an LSM >8.0 kPa (n = 16)

Variable	Mean ± SD or n (%)
Age, years	59.60 ± 10.28
Sex	
Male	7 (43.8)
Female	9 (56.3)
Duration of diabetes, years	13.25 ± 7.87
BMI, kg/m^2	33.25 ± 2.60
Waist circumference, cm	
Male	111.71 ± 12.47
Female	106.88 ± 8.76
Systolic blood pressure, mmHg	128.44 ± 8.31
Diastolic blood pressure, mmHg	68.44 ± 13.50
A1C, %	6.83 ± 0.62
Glucose, mg/dL	125.06 ± 19.12
AST, units/L	30.44 ± 13.21
ALT, units/L	42.25 ± 24.53
TSH, mg/dL	1.64 ± 1.26
Total cholesterol, mg/dL	153.13 ± 23.34
HDL cholesterol, mg/dL	
Male	38.71 ± 9.58
Female	47.66 ± 9.93
LDL cholesterol, mg/dL	93.696 ± 27.16
VLDL cholesterol, mg/dL	35.37 ± 15.13
Liver fat content >5% (MRI PDFF)	16 (100)
Triglycerides, mg/dL	140.81 ± 65.63
NashTest2 grade	
N0	3 (18.75)
N1	3 (18.75)
N2	6 (37.50)
N3	4 (25.00)
ActiTest grade	
A0	6 (37.5)
A1	8 (50.0)
A2	1 (6.3)
A3	1 (6.3)
FibroTest stage	
F0	5 (31.3)
F0–F1	1 (6.3)
F1	2 (12.5)
F1–F2	2 (12.5)
F2	1 (6.3)
F3	3 (18.8)
F3-F4	1 (6.3)
F4	1 (6.3)
Genotype *PNPLA3*	
CC	5 (31.3)
CG	7 (43.8)
GG	4 (25)

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MRI-PDFF vs. 70%) (3,4). However, our population may behave differently compared with other ethnicities with regard to liver fat accumulation. Our most alarming finding, though, was that almost 14% of our population had unsuspected moderate to advanced fibrosis (F2 or higher), as was also shown by Lomonaco et al. (31) in a recent study.

Several limitations of our study need to be considered. First, it is a cross-sectional study. Second, the number of participants was fairly small, so our results cannot be considered as definitive and should be confirmed in large cohorts. Furthermore, all diagnostic tools we used for liver fibrosis (FibroTest, APRI, NAFLD fibrosis score, BARD score, and Fib-4 index) were originally used for populations with various liver conditions (i.e., alcoholic liver disease or hepatitis B or C) (32–34). These tools have also been validated for patients with NAFLD (35); however, these studies only included 30% of participants with type 2 diabetes, and the performance of the biomarker panels was not specifically assessed in this subgroup of participants. Bril et al. (36) recently showed that these specific noninvasive panels underperformed when applied to a large cohort of people with type 2 diabetes. The prevalence of moderate to advanced fibrosis among people with type 2 diabetes has been usually judged to be lower using blood diagnostic panels (25,37–39) than in studies based on elastography from both Europe (25,38–40) and Asia (41,42). A recent study by Lomonaco et al. (31) supported the validity of a combined blood and elastography noninvasive approach in the primary care setting. Moreover, although recent research has shown that 2D SWE has a slight superiority over FibroScan in diagnosing different stages of liver fibrosis (8), there are limited data, and further validation studies are needed to define how accurately serial measurements reflect disease progression and treatment response. Finally, because of a lack of liver biopsies in this study, our results should be

TABLE 6 2D SWE Versus Proprietary Scores in Diagnosis of Liver Fibrosis

	LSM (2D SWE)				
	F0–F1, *n* (%)	F2, *n* (%)	F3, *n* (%)	F4, *n* (%)	*P*
APRI					0.001
<0.5 (no fibrosis)	96 (88.1)	5 (4.6)	7 (6.4)	1 (0.9)	
0.5–0.7 (some liver damage)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	
0.7–1 (significant fibrosis)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	
NAFLD fibrosis score					0.408
F0–F2: <−1.455	23 (82.1)	3 (10.7)	2 (7.1)	0 (0.0)	
F2–F3: −1.455 to 0.675	63 (86.3)	4 (5.5)	5 (6.8)	1 (1.4)	
F3–F4: >0.675	11 (91.7)	0 (0.0)	0 (0.0)	1 (8.3)	
Fib-4 index					0.658
Normal: <0.68	20 (87.0)	2 (8.7)	1 (4.3)	0 (0.0)	
F0–F2: 0.97 (0.69–1.37)	55 (87.3)	2 (3.2)	5 (7.9)	1 (1.6)	
F3–F4: 1.95 (1.38–3.08)	21 (80.8)	3 (11.5)	1 (3.8)	1 (3.8)	
BARD score					0.701
Low risk (0–1)	8 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	
High risk (2–4)	89 (84.8)	7 (6.7)	7 (6.7)	2 (1.9)	
FibroTest					0.921
F0–F1/F2	78 (85.7)	6 (6.6)	5 (5.5)	2 (2.2)	
F2–F4	17 (89.5)	1 (5.3)	1 (5.3)	0 (0.0)	

interpreted with caution and should be confirmed in future studies that include histological evaluation.

Conclusion

Although well-validated biomarker panels for the diagnosis of NASH are quite promising, people with type 2 diabetes may require predictive models that have been specifically developed for them, as extrapolation of results from populations without diabetes may result in significant misclassification. Based on the complex pathogenetic factors and dynamic activity of NASH, a combination of different noninvasive biomarkers might be an option to improve diagnostic accuracy in detecting liver fibrosis in this particular population and to minimize the need for liver biopsy.

DUALITY OF INTEREST

No potential conflicts of interest relevant to this article were reported.

AUTHOR CONTRIBUTIONS

A.M. conceived of the article, wrote the manuscript, and researched data. D.L. performed statistical analysis. E.M., S.M., and D.P. reviewed and edited the manuscript. I.G. analyzed SWE data. I.T. and P.Z. performed SWE. S.R. and E.T. performed genetic analysis. H.M. reviewed the manuscript. A.T. wrote, reviewed, and edited the manuscript. A.M. and A.T. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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