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Validation of the Blood Test MACK-3 for the Noninvasive Diagnosis of Fibrotic Nonalcoholic Steatohepatitis: An International Study With 1924 Patients

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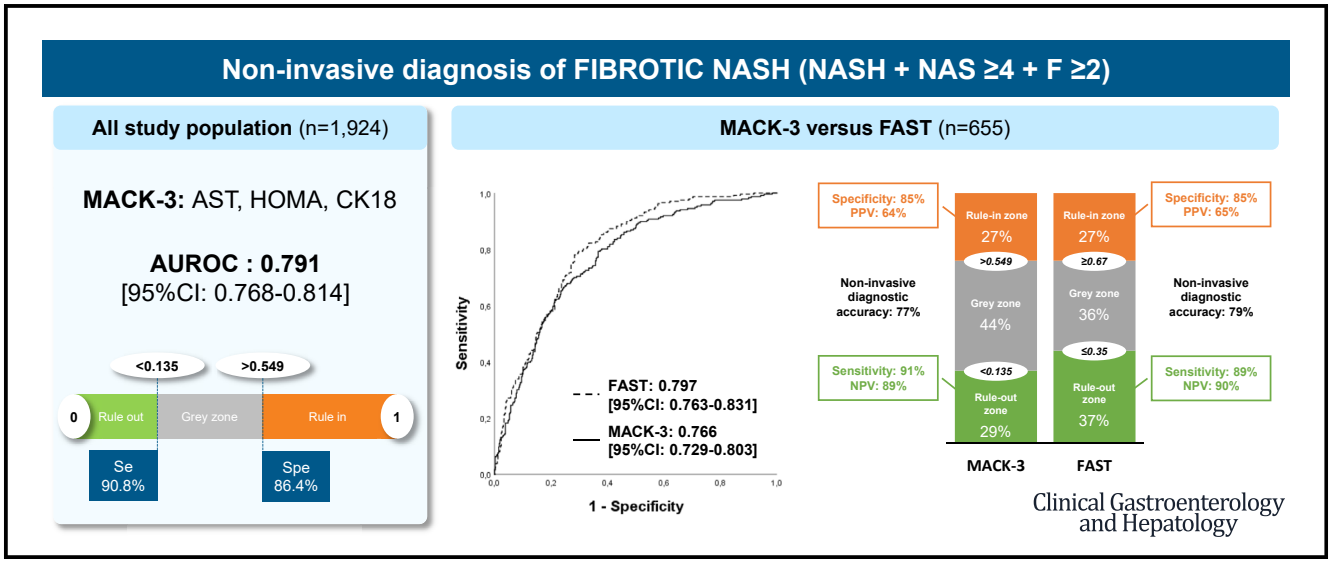

Abbreviations used in this paper: AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic; BMI, body mass index; CK18, cytokeratin 18; CRN, Clinical Research Network; FAST, FibroScan–aspartate aminotransferase score; HOMA, Homeostasis Model Assessment; MAST, MRI-based; NAFLD, nonalcoholic fatty liver disease; NAS, Nonalcoholic Fatty Liver Disease Activity Score; NASH, nonalcoholic steatohepatitis; NIDA, noninvasive diagnostic accuracy.

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BACKGROUND & AIMS: **Drug development in nonalcoholic steatohepatitis (NASH) is hampered by a high screening failure rate that reaches 60% to 80% in therapeutic trials, mainly because of the absence of fibrotic NASH on baseline liver histology. MACK-3, a blood test including 3 biomarkers (aspartate aminotransferase, homeostasis model assessment, and cytokeratin 18), recently was developed for the noninvasive diagnosis of fibrotic NASH. We aimed to validate the diagnostic accuracy of this noninvasive test in an international multicenter study.**

METHODS: **A total of 1924 patients with biopsy-proven nonalcoholic fatty liver disease from 10 centers in Asia, Australia, and Europe were included. The blood test MACK-3 was calculated for all patients. FibroScan–aspartate aminotransferase score (FAST), an elastography-based test for fibrotic NASH, also was available in a subset of 655 patients. Fibrotic NASH was defined as the presence of NASH on liver biopsy with a Nonalcoholic Fatty Liver Disease Activity Score of 4 or higher and fibrosis stage of F2 or higher according to the NASH Clinical Research Network scoring system.**

RESULTS: **The area under the receiver operating characteristic of MACK-3 for fibrotic NASH was 0.791 (95% CI 0.768–0.814). Sensitivity at the previously published MACK-3 threshold of less than 0.135 was 91% and specificity at a greater than 0.549 threshold was 85%. The MACK-3 area under the receiver operating characteristic was not affected by age, sex, diabetes, or body mass index. MACK-3 and FAST results were well correlated (Spearman correlation coefficient, 0.781; $P < .001$). Except for an 8% higher rate of patients included in the grey zone, MACK-3 provided similar accuracy to that of FAST. Both tests included 27% of patients in their rule-in zone, with 85% specificity and 35% false positives (screen failure rate).**

CONCLUSIONS: **The blood test MACK-3 is an accurate tool to improve patient selection in NASH therapeutic trials.**

Keywords: NAFLD; NASH; Fibrotic NASH; Blood Test; FAST.

In parallel with the global obesity pandemic, nonalcoholic fatty liver disease (NAFLD) has become the main cause of chronic liver disease worldwide.[1] Nonalcoholic steatohepatitis (NASH) is the aggressive form of NAFLD, with liver inflammation that promotes the progressive accumulation of fibrosis in the liver parenchyma. NASH has risen to become the leading cause of cirrhosis and hepatocellular carcinoma in some regions,[2,3] and the second for liver transplantation in Europe and the United States.[4,5] There is no approved pharmacologic treatment for NASH, but extensive research is underway to find new therapeutic agents able to halt progression to cirrhosis and hepatocellular carcinoma and enable the ultimate goal of reducing the worldwide burden of NASH.[6] Experience from phase 2b and 3 therapeutic trials has shown a dramatically high rate of screening failure, reaching 60% to 80%, resulting from biopsy results not meeting the inclusion and exclusion histologic criteria. In this context, noninvasive liver tests represent a very attractive option to better select patients, limit screening failures, and therefore reduce unnecessary, invasive, and costly procedures. Therapeutic trials include patients in need of an intervention, namely those with fibrotic NASH, a composite histologic criterion combining NASH + a NAFLD Activity Score (NAS) of 4 or higher and fibrosis stage of F2 or higher. Most of the noninvasive tests currently available in clinical practice are mainly intended for advanced liver fibrosis of F3 or higher and show limited accuracy for earlier stages of the disease, such as fibrotic NASH.[7,8] In addition to considerably improving patient selection for therapeutic trials, any new test that can better target fibrotic NASH would be a very useful tool for identifying patients who would benefit from NASH therapeutics once they become available in clinical practice.

MACK-3 is such a blood-based test developed specifically for the noninvasive diagnosis of fibrotic NASH.[8] This new test combines 3 biomarkers associated with, respectively, liver inflammation (aspartate aminotransferase [AST]), insulin resistance (Homeostasis Model Assessment [HOMA]), and apoptosis (cytokeratin 18 [CK18]). In its development study, MACK-3 showed very good diagnostic accuracy for fibrotic NASH with an area under the receiver operating characteristic (AUROC) curve of 0.85.[8] The aim of the present study was to validate the MACK-3 in a large international multicenter cohort of biopsy-proven NAFLD patients.

Patients and Methods

Patients

The study population was obtained by pooling the data from 10 cohorts. The patients from the Angers (France) and Antwerp (Belgium) cohorts for the present

work were enrolled after the end of recruitment for the MACK-3 development study. Thus, these 2 cohorts are independent of the originally published study.[8] The cohorts from Helsinki (Finland), Kuala Lumpur (Malaysia), Wenzhou (China), Turin (Italy), and Sydney (Australia) came from previously published works.[9–12] The last 3 were local cohorts of patients with biobanked samples from centers in Bern (Switzerland), Grenoble (France), and Perth (Australia). Each cohort received approval from its local ethics committee, and all patients gave written informed consent before their inclusion.

All 10 cohorts included adult patients who underwent a liver biopsy for suspected NAFLD, after exclusion of concomitant steatosis-inducing drugs (such as corticosteroids, tamoxifen, amiodarone, or methotrexate), excessive alcohol consumption (>30 g/d in men or >20 g/d in women), chronic hepatitis B or C infection, and other causes of chronic liver disease. Patients were not included if they had a history of liver-related complications (ascites, variceal bleeding, jaundice, encephalopathy, hepatocellular carcinoma) or biopsy specimen length less than 10 mm. More information on the cohorts of the 10 investigating centers is presented in Supplementary Table 1.

What You Need To Know

Background

Drug development for nonalcoholic steatohepatitis (NASH) is hampered by a high 60-80% screening failure rate, mainly because of the absence of fibrotic NASH on the baseline liver biopsy. MACK-3, a blood test combining aspartate aminotransferase (AST), Homeostasis Model Assessment (HOMA) and cytokeratin-18, has been recently developed specifically for the noninvasive diagnosis of fibrotic NASH.

Findings

Coming from a large multicentric international cohort including 1,924 patients, our results validate the good accuracy of MACK-3 for the noninvasive diagnosis of fibrotic NASH. MACK-3 decreases the screen failure rate in a similar rate than FibroScan–aspartate aminotransferase score (FAST), to only 35%.

Implications for patient care

MACK-3 allows better identification of candidates for NASH therapeutic trials.

Histology

Pathologic examination of liver biopsy specimens was performed in each center by a senior expert pathologist who specialized in hepatology and was blinded for patient data. Steatosis, lobular inflammation, ballooning, and fibrosis were evaluated semiquantitatively using the NASH–Clinical Research Network (CRN) scoring system in all centers,[13] except in Bern (65 patients), where the steatosis activity fibrosis score was used.[14] NASH was defined as a grade of 1 or higher in each component of steatosis, lobular inflammation, and hepatocellular ballooning. The NAS (range, 0–8) corresponded to the sum of the steatosis, lobular inflammation, and ballooning grades. Fibrotic NASH, the primary diagnostic target of the study, was defined as the presence of NASH with NAS of 4 or higher and F2 or higher.

Blood Tests

Blood markers were measured in the local laboratories of the investigating centers. The serum CK18 levels were measured on frozen (-80°C) samples using the M30-Apoptosense enzyme-linked immunosorbent assay kit from PEVIVA (Bromma, Sweden), except in the Wenzhou center where the kit from Suzhou Herui Biomed Technology Co, Ltd (China) was used. HOMA was calculated as follows: fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5. The blood fibrosis test MACK-3 (AST, HOMA, CK18) was calculated as previously published.[8] The thresholds for ruling out and ruling in fibrotic NASH were less than 0.135 and greater than 0.549, respectively.[8]

Liver Elastography

When available, liver elastography (FibroScan; Echosens, Paris, France) data were collected to calculate the FibroScan-AST score (FAST), an elastography-based score recently developed for the noninvasive diagnosis of fibrotic NASH.[15] FAST is a combination of serum AST with the 2 FibroScan results: liver stiffness as measured by vibration-controlled transient elastography and the controlled attenuation parameter, the latter being a surrogate of liver steatosis. In each center, FibroScan examinations were performed in fasting conditions, by experienced operators blinded to patient data, and according to the manufacturer's recommendations. FAST was calculated as per the previously published formula. The thresholds for ruling out and ruling in fibrotic NASH were 0.35 or less and 0.67 or greater.[15]

Statistical Analysis

Continuous variables were expressed as means ± SD and compared using the Mann–Whitney or the Kruskal–Wallis tests. Categoric variables were expressed as percentages and compared using the chi-squared or Fisher tests. Correlations between quantitative variables were determined using the Spearman correlation coefficient. The diagnostic accuracies of noninvasive tests were evaluated using the AUROC curve, presented with their 95% CI and compared with the DeLong test.[16] We also calculated the noninvasive diagnostic accuracy (NIDA), which corresponds to the diagnostic accuracy of a noninvasive test when used with 2 thresholds (rule-out, rule-in). In this situation, the diagnosis relies on the noninvasive test only when its result is in the rule-out or the rule-in

zone. Indeed, when the test result is in the grey zone between the 2 thresholds, the diagnosis remains undetermined, with further second-line testing required. NIDA thus corresponds to the rate of correctly classified patients by the noninvasive test in the pooled rule-out and rule-in zones. NIDA was calculated as follows: ([true negatives in the rule-out zone] + [true positives in the rule-in zone])/(all patients included in both rule-out and rule-in zones). Statistical analyses were performed using SPSS version 25.0 software (IBM, Armonk, NY).

Results

Patients

The characteristics of the 1924 patients included in the present study are summarized in Table 1. The mean age was 49.2 ± 13.0 years, 55.4% of the patients were male, the mean body mass index (BMI) was 32.4 ± 7.5 kg/m^2, and 36.5% had type 2 diabetes mellitus. The

Table 1. Patient Characteristics

	All (n = 1924)	No fibrotic NASH (n = 1480)	Fibrotic NASH (n = 444)	*P*
Age, *y*	49.2 ± 13.0	48.0 ± 12.6	53.3 ± 13.5	<.001
Male sex, n (%)	1065 (55.4)	831 (56.1)	234 (52.7)	.211
BMI, *kg/m^2*	32.4 ± 7.5	32.4 ± 7.8	32.4 ± 6.2	.095
Diabetes, n (%)	703 (36.5)	465 (31.4)	238 (53.6)	<.001
Bariatric patients, n (%)	335 (17.4)	323 (21.8)	12 (2.7)	<.001
Steatosis grade, n (%)				<.001
0	234 (12.2)	234 (15.8)	0 (0.0)	
1	717 (37.3)	629 (42.5)	88 (19.8)	
2	603 (31.1)	412 (27.8)	191 (43.0)	
3	370 (19.2)	205 (13.9)	165 (37.2)	
Ballooning, n (%)				<.001
0	661 (35.6)	661 (45.4)	0 (0.0)	
1	788 (42.4)	625 (43.0)	163 (40.3)	
2	410 (22.1)	169 (11.6)	241 (59.7)	
Lobular inflammation, n (%)				<.001
0	602 (32.4)	602 (41.4)	0 (0.0)	
1	958 (51.5)	712 (48.9)	246 (60.9)	
2	288 (15.5)	138 (9.5)	150 (37.1)	
3	11 (0.6)	3 (0.2)	8 (2.0)	
NAFLD Activity Score	3.3 ± 1.9	2.7 ± 1.7	5.2 ± 1.0	<.001
NASH, n (%)	1001 (53.8)	597 (41.0)	404 (100.0)	<.001
Fibrosis stage, n (%)				<.001
0	678 (35.2)	678 (45.8)	0 (0.0)	
1	604 (31.4)	604 (40.8)	0 (0.0)	
2	287 (14.9)	90 (6.1)	197 (44.4)	
3	241 (12.5)	59 (4.0)	182 (41.0)	
4	114 (5.9)	49 (3.3)	65 (14.6)	
AST, *IU/L*	44 ± 35	39 ± 31	61 ± 42	<.001
ALT, *IU/L*	66 ± 58	60 ± 54	87 ± 67	<.001
GGT, *IU/L*	93 ± 130	79 ± 117	142 ± 158	<.001
Alkaline phosphatase, *IU/L*	84 ± 35	82 ± 35	93 ± 35	<.001
Albumin, *g/L*	43.2 ± 4.7	43.2 ± 4.8	43.2 ± 4.5	.950
Platelets, *G/L*	244 ± 67	248 ± 67	229 ± 66	<.001
HOMA	6.7 ± 13.2	5.7 ± 12.5	10.1 ± 15.0	<.001
CK18, *IU/L*	346 ± 449	274 ± 319	587 ± 678	<.001
FIB-4	1.28 ± 1.03	1.14 ± 0.92	1.75 ± 1.21	<.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CK18, cytokeratin 18; FIB-4, Fibrosis-4; GGT, γ-glutamyltransferase; HOMA, Homeostasis Model Assessment; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

mean biopsy length was 21 ± 10 mm and 84.3% of the biopsy specimens were at least 15 mm in length. The prevalence of NASH was 53.8%, significant fibrosis was 33.4%, and fibrotic NASH was 23.1%. As expected, patients with fibrotic NASH were older, were more likely to have diabetes, and more elevated liver tests. Patients with fibrotic NASH had significantly higher levels of AST, HOMA, and CK18, thus confirming the relevance of their combination in MACK-3. The characteristics of the patients included in each of the 10 investigating centers are summarized in Supplementary Table 2.

Accuracy of MACK-3

MACK-3 showed significant correlation with all individual liver lesions (Supplementary Table 3), and its results increased progressively as a function of steatosis, lobular inflammation, and ballooning grades, as well as across NAS and fibrosis stages (Supplementary Figures 1–5). The AUROC curve of MACK-3 for the diagnosis of fibrotic NASH was 0.791 (95% CI 0.768–0.814) (Figure 1), and was similar across the 10 investigating centers (Table 2). The less than 0.135 rule-out threshold of MACK-3 provided an excellent 90.8% sensitivity for fibrotic NASH, and the specificity was 86.4% at the greater than 0.549 rule-in threshold. As expected, the negative and positive predictive values of MACK-3 were influenced by the prevalence of the diagnostic target: the increasing prevalence of fibrotic NASH observed across the 10 investigating centers was associated with a linear increase in the positive predictive value, while the negative predictive value decreased only slightly (Supplementary Figure 6). Importantly, 86% of the misclassified patients from the rule-out zone showed only borderline misclassification (ie, only 1 NAS point or only 1 fibrosis stage discrepancy) (Supplementary Table 4). In the rule-in zone, misclassification was borderline in 67% of the cases.

Sensitivity Analysis

Sensitivity analysis showed that the MACK-3 AUROC curve was not influenced by age, sex, diabetes, or the biopsy length (Table 3). Accuracy was higher in patients with a BMI of 35 kg/m^2 or higher, but half of these patients (48.6%) came from the bariatric surgery setting, which corresponds to a particular population with only a 3.6% prevalence of fibrotic NASH despite the highly increased BMI risk factor. The AUROC curve of MACK-3 was 0.937 (95% CI 0.884–0.990) in patients with a BMI of 35 kg/m^2 or higher coming from bariatric surgery, whereas it was 0.793 (95% CI 0.744–0.842) in patients with a BMI of 35 kg/m^2 or higher outside bariatric surgery. Thus, the results in this latter group of patients were similar to those of patients with a BMI less than 30 kg/m^2 or those with a BMI of 30 to 35 kg/m^2 (Table 3). The accuracy of MACK-3 in the non-bariatric group (n = 1589 patients) is presented in Table 2 (last line of Table 2).

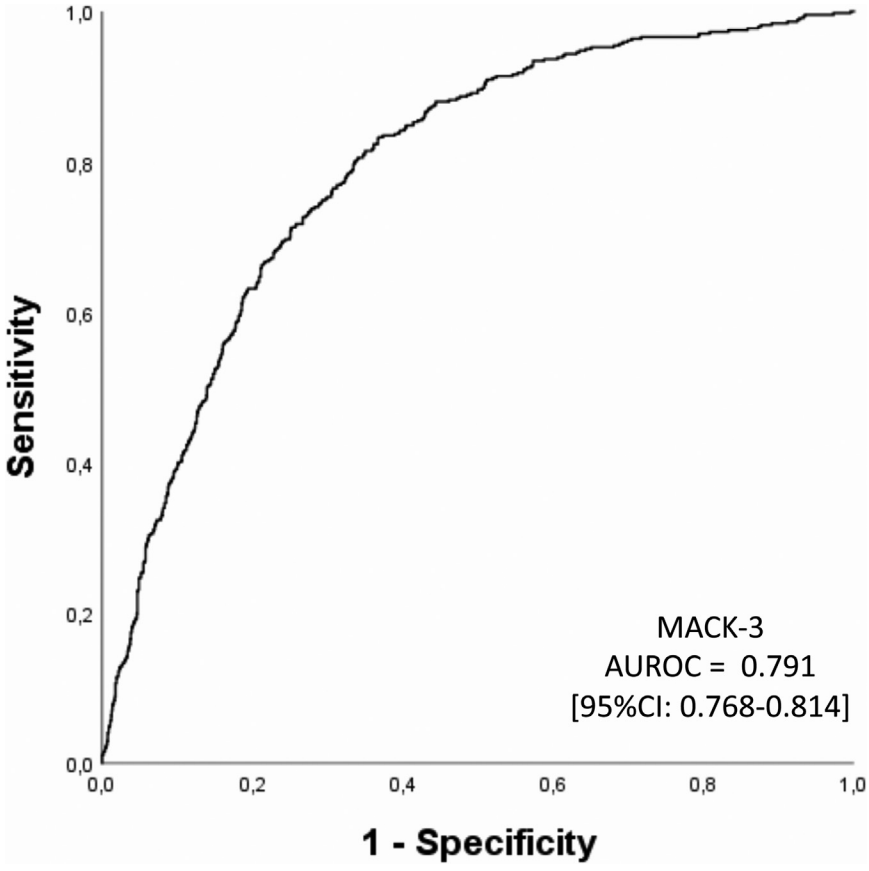

Figure 1. Area under the receiver operating characteristic (AUROC) curve of MACK-3 for the diagnosis of fibrotic nonalcoholic steatohepatitis in the whole study set.

Table 2. Diagnostic Accuracy of MACK-3 Across the 10 Investigating Centers

				Rule-out zone (MACK-3, <0.135)					Rule-in zone (MACK-3, >0.549)						
Center	Patients, n	Fibrotic NASH, %	AUROC curve (95% CI)	Patients, %[a]	Se, %	Spe, %	-LR	+LR	Patients, %[a]	Se, %	Spe, %	-LR	+LR	Grey zone patients, %[a]	NIDA, %
Helsinki	356	5.9	0.926 (0.887–0.966)	61.0	100.0	64.8	0.00	2.84	7.3	47.6	95.2	0.55	9.97	31.7	93.4
Wenzhou	440	13.9	0.721 (0.653–0.790)	49.3	77.0	53.6	0.43	1.66	20.0	42.6	83.6	0.69	2.61	30.7	75.1
Sydney	128	16.4	0.826 (0.749–0.902)	31.3	100.0	37.4	0.00	1.60	24.2	57.1	82.2	0.52	3.22	44.5	73.2
Kuala Lumpur	193	21.8	0.801 (0.734–0.868)	16.6	100.0	21.2	0.00	1.27	37.3	73.8	72.8	0.36	2.72	46.1	60.6
Antwerp	211	23.2	0.836 (0.777–0.895)	35.1	93.9	43.8	0.14	1.67	19.9	49.0	88.9	0.57	4.41	45.0	81.9
Torino	101	25.7	0.741 (0.631–0.851)	48.5	80.8	58.7	0.33	1.95	15.8	26.9	88.0	0.83	2.24	35.6	78.5
Perth	65	27.7	0.801 (0.690–0.913)	29.2	100.0	40.4	0.00	1.68	33.8	55.6	74.5	0.60	2.18	36.9	70.7
Angers	236	42.4	0.798 (0.742–0.855)	23.3	95.0	36.8	0.14	1.50	29.2	50.0	86.0	0.58	3.58	47.5	80.6
Grenoble	129	51.2	0.763 (0.682–0.845)	38.0	80.3	57.1	0.34	1.87	16.3	30.3	98.4	0.71	19.09	45.7	80.0
Bern	65	61.5	0.781 (0.665–0.897)	9.2	97.5	20.0	0.13	1.22	41.5	57.5	84.0	0.51	3.59	49.2	84.8
All	1,924	23.1	0.791 (0.768–0.814)	39.4	90.8	48.4	0.19	1.76	21.5	48.0	86.4	0.60	3.53	39.1	79.4
All (no bariatric)[b]	1,589	27.2	0.761 (0.735–0.786)	33.5	90.5	42.4	0.22	1.57	25.2	47.9	83.3	0.63	2.87	41.3	74.9

AUROC, area under the receiver operating characteristic; NASH, nonalcoholic steatohepatitis; NIDA, noninvasive diagnostic effectiveness (rate of correct classification among the patients noninvasively diagnosed, ie, those included in pooled rule-out and rule-in zones); Se, sensitivity; Spe, specificity; -LR, negative likelihood ratio; +LR, positive likelihood ratio.

[a]Rate of patients included in the test interval.

[b]After exclusion of the 335 patients who underwent bariatric surgery.

Table 3. Accuracy of MACK-3 for Fibrotic NASH: Sensitivity Analysis

	Fibrotic NASH, %	AUROC curve (95% CI)	Sensitivity, %[a]	Specificity, %[b]
Sex				
Female	24.4	0.810 (0.778–0.843)	90.0	88.1
Male	22.0	0.777 (0.745–0.809)	91.5	85.1
Age, *y*				
<40	17.1	0.815 (0.768–0.863)	94.9	80.4
40–59	19.5	0.787 (0.752–0.823)	85.9	89.1
≥60	37.5	0.788 (0.746–0.830)	94.6	86.7
Bariatric				
No	27.2	0.761 (0.735–0.786)	90.5	83.3
Yes	3.6	0.938 (0.887–0.989)	100.0	97.5
BMI, kg/m^2				
<30	18.8	0.758 (0.718–0.798)	86.4	86.9
30–35	34.7	0.716 (0.666–0.765)	89.9	77.9
>35	20.8	0.880 (0.850–0.910)	98.4	90.4
Diabetes				
No	16.9	0.775 (0.742–0.809)	86.4	88.4
Yes	33.9	0.781 (0.746–0.816)	94.5	82.2
Biopsy specimen length, *mm*				
<20	18.7	0.783 (0.744–0.822)	91.3	81.9
≥20	38.6	0.755 (0.717–0.793)	89.8	85.2

AUROC, area under the receiver operating characteristic; BMI, body mass index; NASH, nonalcoholic steatohepatitis.
[a]Sensitivity at the <0.135 threshold of MACK-3.
[b]Specificity at the >0.549 threshold of MACK-3.

Comparison Between MACK-3 and FibroScan–Aspartate Aminotransferase

The FAST score was available in 655 patients whose characteristics are summarized in Supplementary Table 5. MACK-3 and FAST were well correlated, with a Spearman correlation coefficient of 0.781 ($P < .001$) (Figure 2). The AUROC curve of MACK-3 (0.766 [95% CI 0.729–0.803]) and FAST (0.797 [95% CI 0.763–0.831]) were close, but significantly different ($P = .033$) (Figure 3). This translated to less patients included in the grey zone with FAST compared with MACK-3 (36% vs 44%; $P < .001$) (Figure 4). Within the rule-out zone, the accuracy of MACK-3 and FAST were similar, with sensitivity and negative predictive values of approximately 90%. In terms of patient selection for clinical trials,

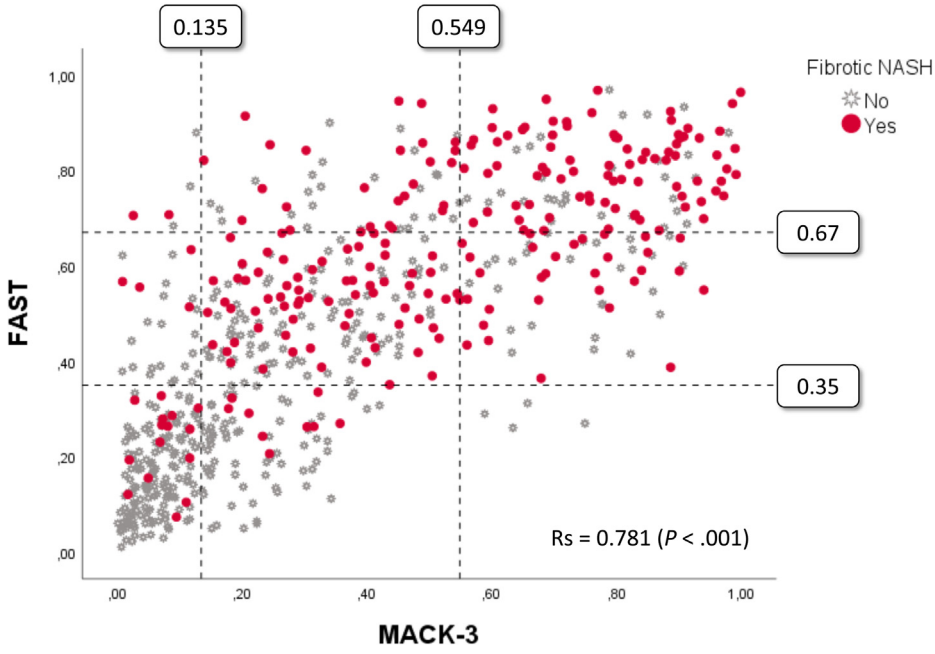

Figure 2. Correlation between MACK-3 and FibroScan–aspartate aminotransferase score (FAST) in the 655 patients who underwent both tests. NASH, nonalcoholic steatohepatitis; RS, Spearman correlation coefficient.

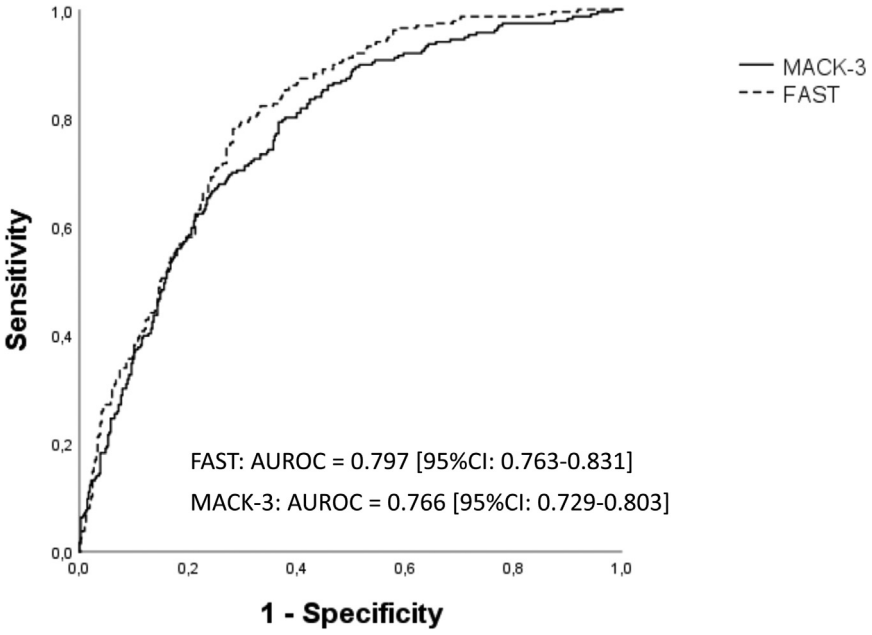

Figure 3. Area under the receiver operating characteristic (AUROC) curve of MACK-3 and FibroScan–aspartate aminotransferase score (FAST) for the diagnosis of fibrotic nonalcoholic steatohepatitis in the 655 patients who underwent both tests.

MACK-3 and FAST both included 27% of the patients in their rule-in zone. Within the rule-in zone, the 2 tests showed the same accuracy with 85% specificity and 65% positive predictive value (35% screen failure rate). Finally, NIDA was similar between MACK-3 (77%) and FAST (79%). Supplementary Figure 7 shows there were very few discrepant results between MACK-3 and FAST (ie, patients with FAST $\leq$0.35 and MACK-3 $>$0.549, or patients with FAST $\geq$0.67 and MACK-3 $<$0.135). Interestingly, the positive predictive value increased to 73% when both FAST and MACK-3 agreed for fibrotic NASH.

Discussion

Therapeutics in the setting of NASH is a highly active field of research with numerous ongoing clinical trials. All of these efforts, however, are hampered by a dramatically high screening failure rate, reaching 60% to 80% in some instances. Therefore, there is an urgent need to develop new tools that are able to target fibrotic NASH accurately, the histologic criterion that usually defines the indication for patient inclusion in NASH therapeutic trials. Because they have the potential to be accessible to all

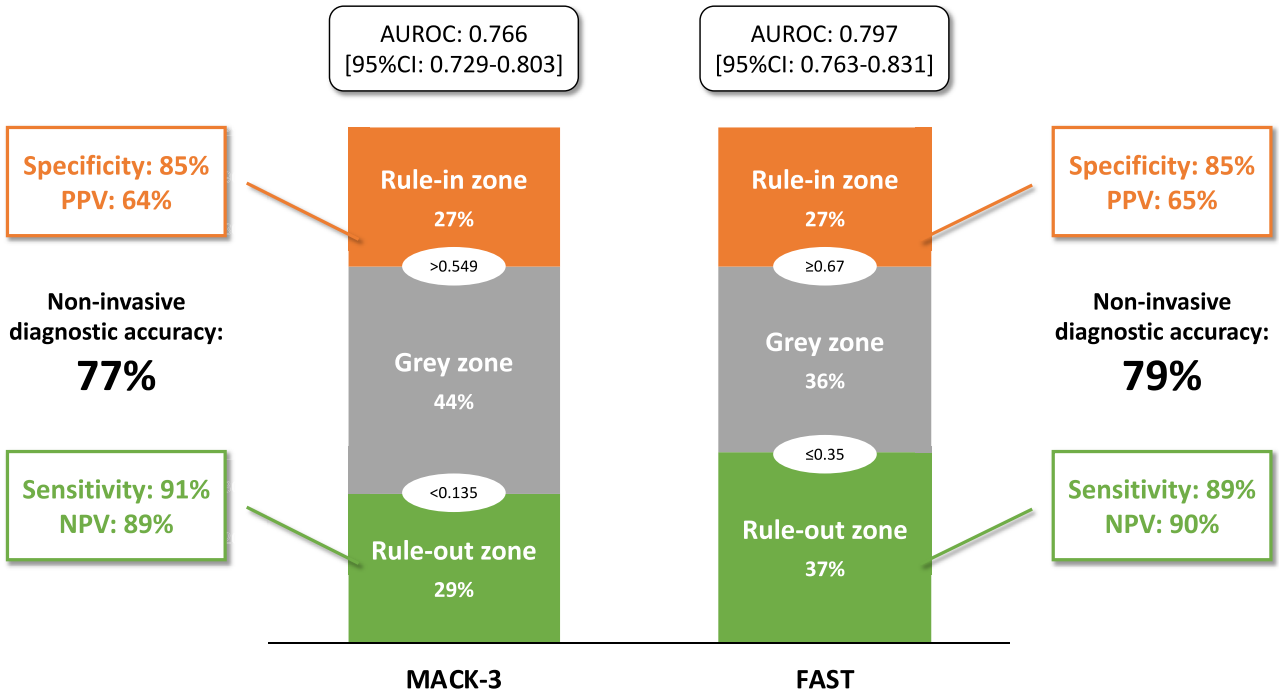

Figure 4. Accuracy of MACK-3 and FibroScan–aspartate aminotransferase score (FAST) for the noninvasive diagnosis of fibrotic nonalcoholic steatohepatitis (NASH) in the 655 patients who underwentboth tests. AUROC curve, area under the receiver operating characteristic; NPV, negative predictive value; PPV, positive predictive value.

physicians, blood tests appear as an attractive option to facilitate the identification and referral to liver clinics of candidates for therapeutic trials. In this context, our study validates the good accuracy of the blood test MACK-3 for the noninvasive diagnosis of fibrotic NASH, and the relevance of its rule-out and rule-in thresholds ($<$0.135 and $>$0.549) as determined in the development study.[8]

A range of currently available noninvasive tests can diagnose advanced liver fibrosis accurately, but they perform less well for earlier stages of the disease. Therefore, several novel noninvasive tests recently have been developed specifically to diagnose fibrotic NASH. These include blood tests such as MACK-3 and NIS4, and elastography-based tests such as FAST and MAST (MRI-based) score. FAST and MAST have shown promising diagnostic accuracy,[15,17] but the need for specific elastography devices limits their availability, which is especially tangible with MAST that requires magnetic resonance elastography technology. On the other hand, blood-based tests offer an opportunity for more generalized use, which is a crucial advantage given the very large number of at-risk patients to evaluate. NIS4 (combination of hemoglobin A1c, α2 macroglobulin, YKL-40, and the micro-RNA 34a-5p) shows good accuracy for fibrotic NASH with an AUROC curve of 0.80 (95% CI 0.77–0.84).[18] However, the deployment of this test is limited by the micro-RNA assay that requires sophisticated and expensive technology with currently limited availability. In this context, MACK-3 is a very attractive option for the first-line evaluation of fibrotic NASH in at-risk patients with metabolic factors. Indeed, the accuracy of MACK-3 is comparable with that of NIS4, with an AUROC curve of 0.791 (95% CI 0.768–0.814) in our validation study. Moreover, MACK-3 combines simple biomarkers that already are available (AST and HOMA index) or easily implementable in biological platforms (CK18). These advantages should enable the widespread use of MACK-3 in clinical practice.

Fibrotic NASH is difficult to diagnose because it results from the combination of 4 different liver lesions (steatosis, lobular inflammation, ballooning, and fibrosis). We therefore evaluated how MACK-3 behaved according to each liver lesion. Our results show that MACK-3 is a sensitive test: it increased significantly from the first grade of steatosis, lobular inflammation, and ballooning, and from the first stage of liver fibrosis. Except for an 8% higher rate of patients included in the grey zone, MACK-3 provided similar accuracy to that of FAST (Figure 4). Importantly, when considering patient selection for therapeutic trials, it is noteworthy that MACK-3 included the same rate of patients as FAST in its rule-in zone (27%), and that the accuracy within this zone was similar between the 2 tests with only 35% false positives. The FibroScan device and FAST score are now widely available in liver clinics, and MACK-3 has the potential to be implemented in biological platforms. Therefore, these tests provide physicians with 2 different solutions they can use according to their local resources, with comparable accuracy for fibrotic NASH.

A limitation of the study was the lack of a central reading of liver biopsy specimens, with histologic diagnosis of fibrotic NASH relying on the local expert reading at each of the 10 investigating centers. It should be emphasized, however, that previous works have shown that the reliability of histologic diagnosis remains suboptimal even with a central reading.[19] Knowing these limitations, our study nevertheless is of interest in showing that MACK-3 and FAST target groups of patients enriched in fibrotic NASH, which is very relevant in the current context of high screening failure rates observed in NASH therapeutic trials. We also acknowledge that CK18 is currently not widely available, but this biomarker could be implemented in biology platforms to overcome this limitation and allow wide dissemination of MACK-3 in clinical practice. Finally, because our study was designed to validate the MACK-3 in the same context it initially was developed, further studies now are required to validate its accuracy in less-specialized settings in which it is assumed to be used in clinical practice.

In conclusion, MACK-3 is an accurate blood test for the noninvasive diagnosis of fibrotic NASH. Currently, it represents an attractive option to improve patient selection in ongoing NASH therapeutic trials. In the near future, when drugs for NASH have been approved for clinical practice, MACK-3 could help physicians identify patients in need of pharmacologic therapy for their liver disease.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2023.03.032.

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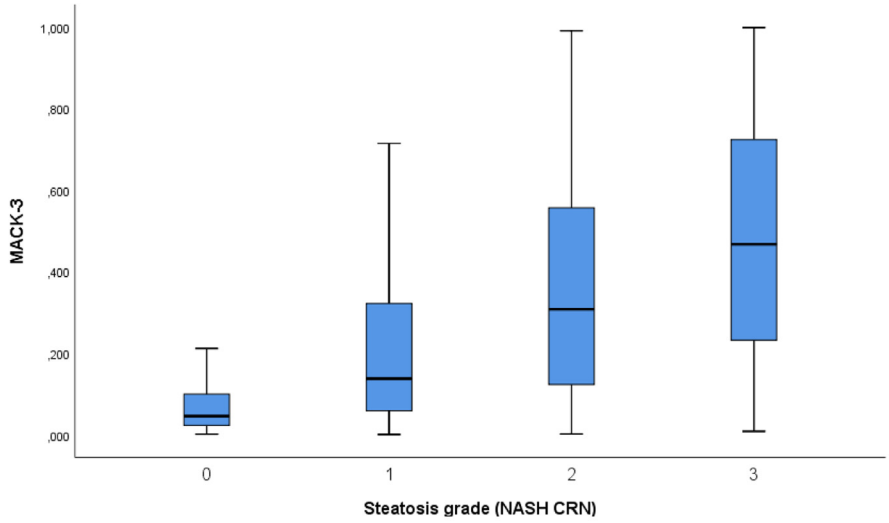
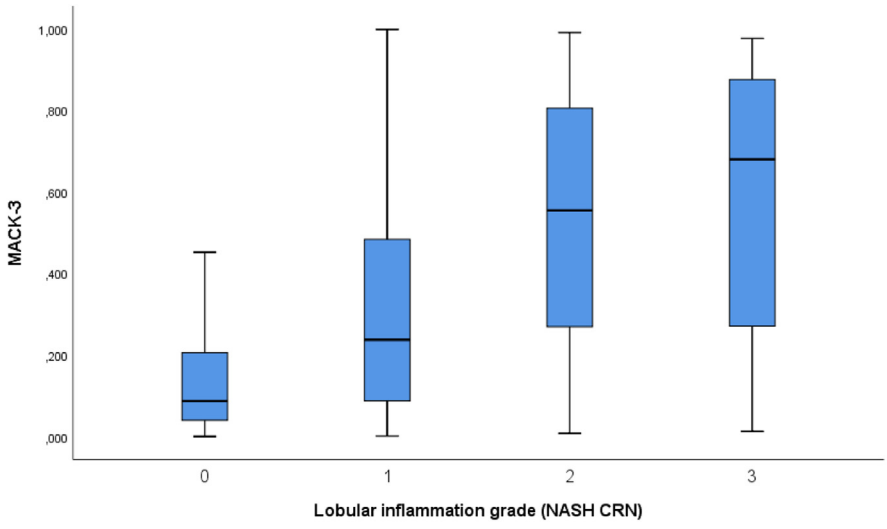
Conflicts of interest
The authors disclose no conflicts.



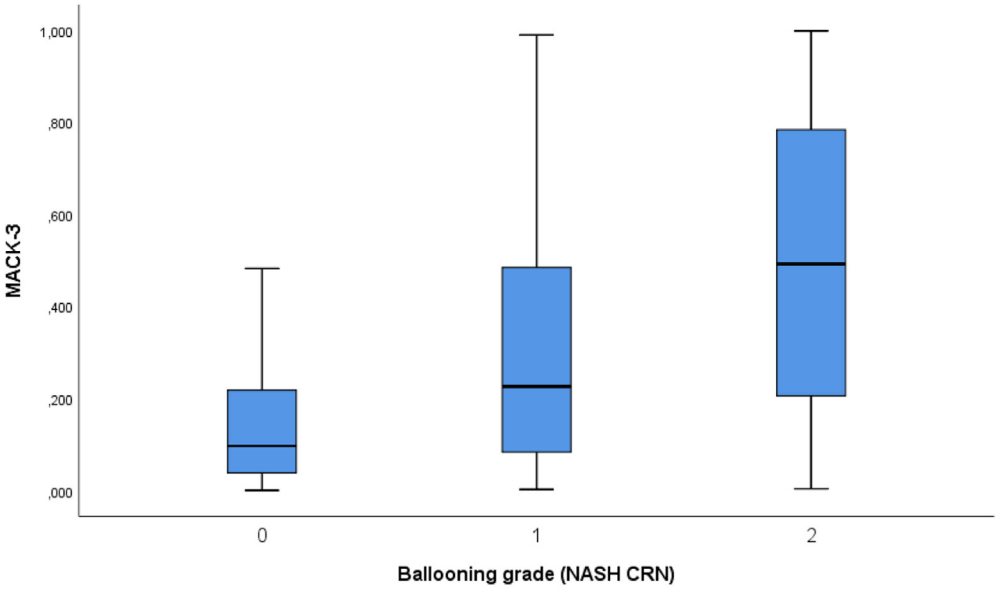
Grade	MACK-3	Comparison (*P*)		
		Vs grade 1	Vs grade 2	Vs grade 3
0	0.101 ± 0.164	< .001	< .001	< .001
1	0.235 ± 0.242	-	< .001	< .001
2	0.364 ± 0.273	-	-	< .001
3	0.478 ± 0.283	-	-	-

Supplementary Figure 1. MACK-3 results as a function of histologic grades of steatosis. NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network.



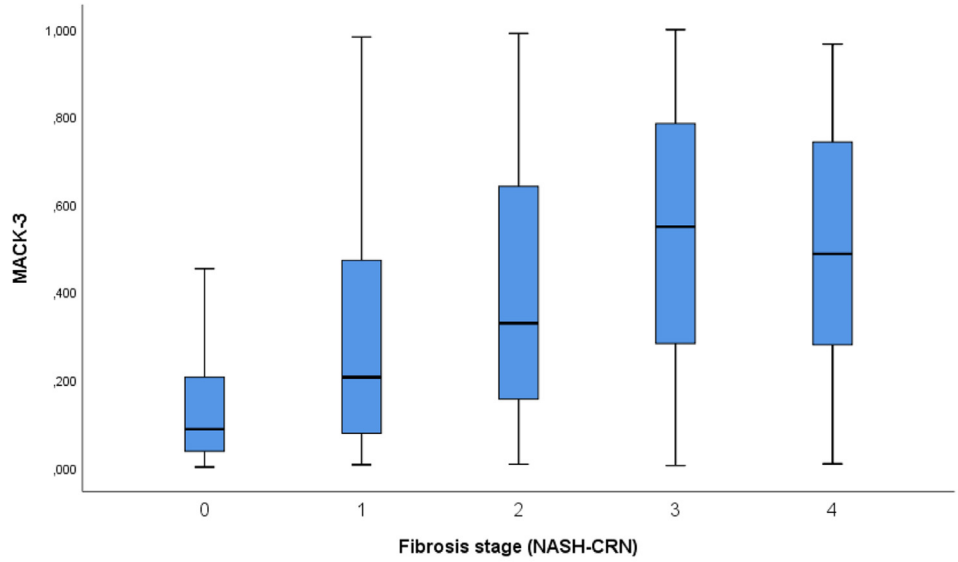
Grade	MACK-3	Comparison (*P*)		
		vs grade 1	vs grade 2	vs grade 3
0	0.159 ± 0.181	< .001	< .001	< .001
1	0.314 ± 0.265	-	< .001	.030
2	0.535 ± 0.298	-	-	.657
3	0.567 ± 0.371	-	-	-

Supplementary Figure 2. MACK-3 results as a function of histologic grades of lobular inflammation. NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network.



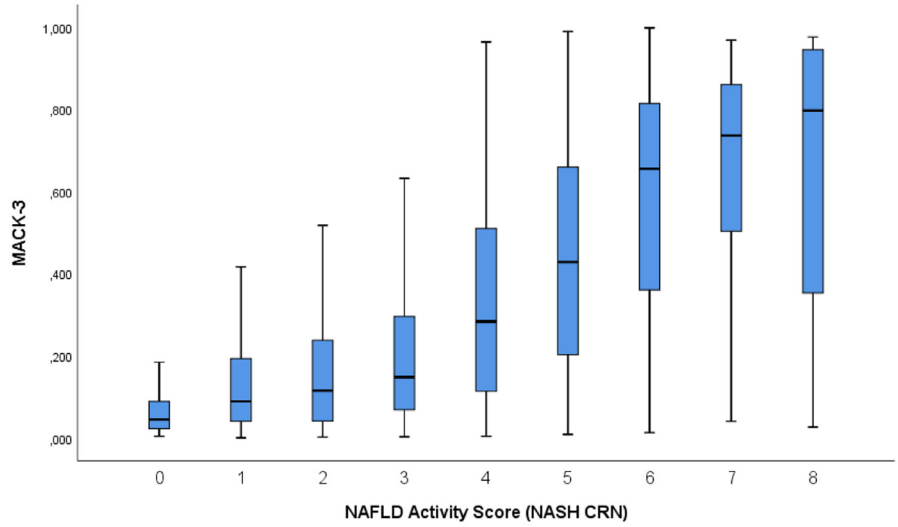
Grade	MACK-3	Comparison (*P*)	
		vs grade 1	vs grade 2
0	0.167 ± 0.187	< .001	< .001
1	0.312 ± 0.265	-	< .001
2	0.488 ± 0.306	-	-

Supplementary Figure 3. MACK-3 results as a function of histologic grades of ballooning. NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network.



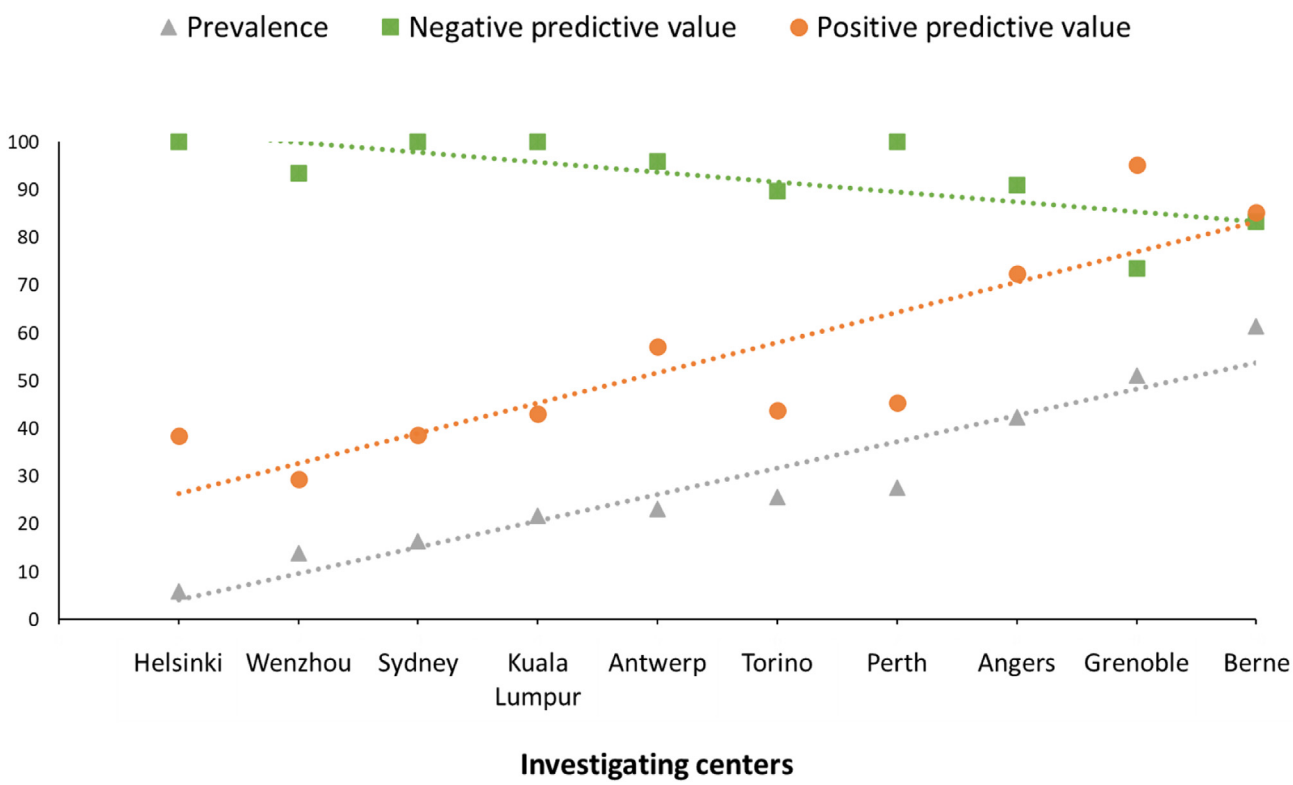
Stage	MACK-3	Comparison (*P*)			
		vs stage 1	vs stage 2	vs stage 3	vs stage 4
0	0.160 ± 0.184	< .001	< .001	< .001	< .001
1	0.299 ± 0.267	-	< .001	< .001	< .001
2	0.403 ± 0.291	-	-	< .001	.001
3	0.521 ± 0.279	-	-	-	.570
4	0.504 ± 0.265	-	-	-	-

Supplementary Figure 4. MACK-3 results as a function of histologic stages of fibrosis. NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network.

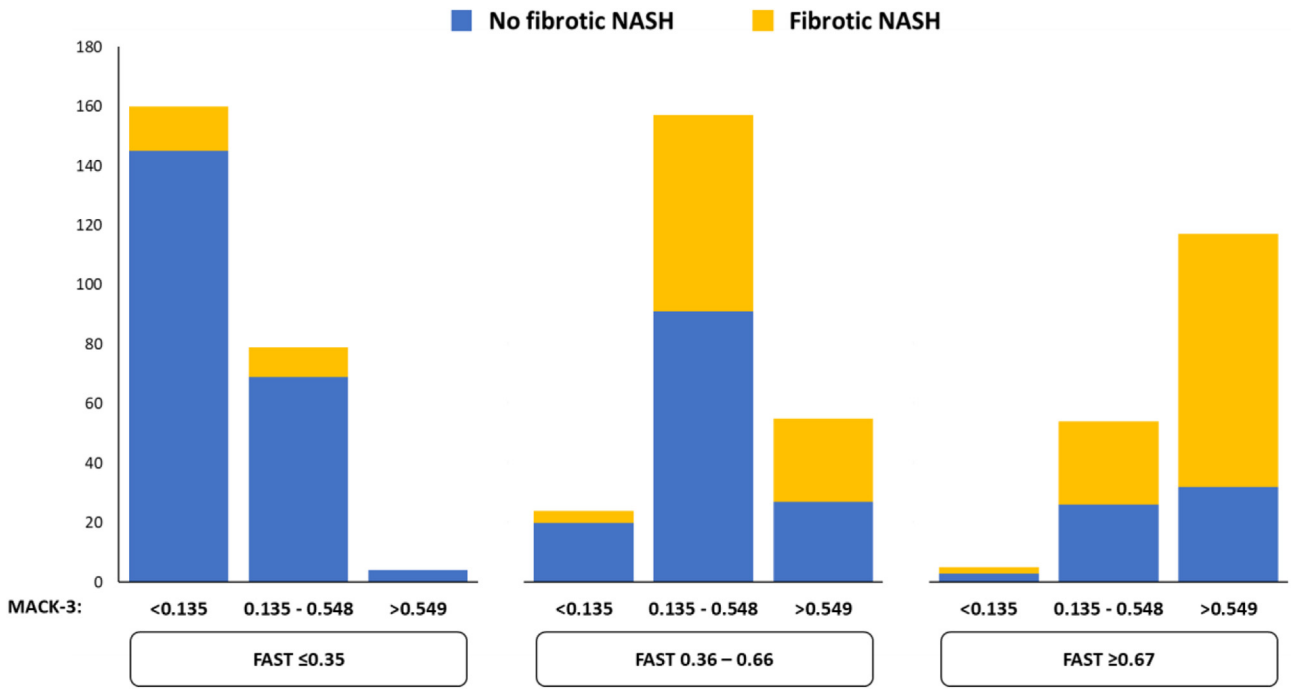


NAS	MACK-3	Comparison (*P*)							
		vs NAS 1	vs NAS 2	vs NAS 3	vs NAS 4	vs NAS 5	vs NAS 6	vs NAS 7	vs NAS 8
0	0.072 ± 0.080	< .001	< .001	< .001	< .001	< .001	< .001	< .001	.027
1	0.156 ± 0.178	-	.128	< .001	< .001	< .001	< .001	< .001	.053
2	0.186 ± 0.202	-	-	.007	< .001	< .001	< .001	< .001	.058
3	0.221 ± 0.213	-	-	-	< .001	< .001	< .001	< .001	.082
4	0.333 ± 0.257	-	-	-	-	< .001	< .001	< .001	.129
5	0.449 ± 0.278	-	-	-	-	-	< .001	< .001	.217
6	0.592 ± 0.270	-	-	-	-	-	-	.332	.461
7	0.628 ± 0.284	-	-	-	-	-	-	-	.600
8	0.649 ± 0.435	-	-	-	-	-	-	-	-

Supplementary Figure 5. MACK-3 results as a function of the Nonalcoholic Fatty Liver Disease (NAFLD) Activity Score (NAS). NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network.



Supplementary Figure 6. Negative and positive predictive values of MACK-3 as a function of the prevalence of fibrotic nonalcoholic steatohepatitis (NASH) observed across the 10 investigating centers.



Supplementary Figure 7. Fibrotic nonalcoholic steatohepatitis (NASH) as a function of FibroScan–aspartate aminotransferase score (FAST) and MACK-3 results.

Supplementary Table 1. Study Cohorts

	Angers	Antwerp	Bern	Helsinki	Grenoble	Kuala Lumpur	Perth	Sydney	Torino	Wenzhou
Enrolment dates (first and last inclusion)	May 2016–July 2020	September 2012–December 2020	September 2017–September 2019	April 2007–March 2020	November 2014–February 2019	November 2012–May 2015	February 2010–January 2020	January 1999–March 2011	September 2008–October 2018	December 2016–December 2018
Study design	Prospective cross-sectional single-center	Prospective cross-sectional single-center	Retrospective cross-sectional single-center	Prospective cross-sectional single-center	Prospective cross-sectional single-center	Prospective cross-sectional single-center	Prospective cross-sectional single-center	Prospective cross-sectional single-center	Prospective cross-sectional single-center	Prospective cross-sectional single-center
Center description	Hepatology tertiary care	Hepatology tertiary care	Hepatology tertiary care	Obesity clinic tertiary care	Hepatology tertiary care	Hepatology tertiary care	Hepatology tertiary care	Hepatology tertiary care	Hepatology tertiary care	Hepatology tertiary care
Eligibility criteria	Liver biopsy scheduled for the evaluation of NAFLD	Liver biopsy scheduled for the evaluation of NAFLD	Liver biopsy scheduled for the evaluation of NAFLD	Patients with suspected NAFLD undergoing weight-loss surgery	Liver biopsy scheduled for the evaluation of NAFLD	NAFLD patients screened and enrolled for a clinical trial	Liver biopsy scheduled for the evaluation of NAFLD	Liver biopsy scheduled for the evaluation of NAFLD	Liver biopsy scheduled for the evaluation of NAFLD	Liver biopsy scheduled for the evaluation of NAFLD
Reason to send a patient for a liver biopsy	Abnormal liver function tests, hyperferritinemia, metabolic syndrome, abnormal noninvasive tests of liver fibrosis	Abnormal liver tests, hyperferritinemia, metabolic syndrome, abnormal noninvasive tests of liver fibrosis	Abnormal liver function tests, hyperferritinemia, metabolic syndrome, abnormal noninvasive tests of liver fibrosis	Routine liver biopsy during weight-loss surgery to exclude advanced liver disease in patients with obesity and metabolic risk factors	Abnormal liver function tests, hyperferritinemia, metabolic syndrome, abnormal noninvasive tests of liver fibrosis	Abnormal liver function tests, metabolic syndrome, abnormal noninvasive tests of liver fibrosis	Abnormal liver function tests, hyperferritinemia, abnormal noninvasive tests of liver fibrosis	Abnormal liver function tests, hyperferritinemia, abnormal noninvasive tests of liver fibrosis	Abnormal liver function tests, hyperferritinemia, metabolic syndrome, abnormal noninvasive tests of liver fibrosis	CAP, US, CT, or MRI showing fatty liver disease; and/or abnormal ALT levels but <5 ULN; no alcohol drinking history or daily alcohol intake <20 g for males or daily intake alcohol <10 g for females
Liver biopsy reading	Read by expert pathologist	Read by expert pathologist	Read by expert pathologist	Read by expert pathologist	Read by expert pathologist	Read by expert pathologist	Read by expert pathologist	Read by expert pathologist	Read by expert pathologist	Read by expert pathologist

ALT, alanine aminotransferase; CAP, controlled attenuation parameter; CT, computed tomography; NAFLD, nonalcoholic fatty liver disease; ULN, upper limit of normal; US, ultrasound.

Supplementary Table 2. Patient Characteristics in Each of the 10 Investigating Centers

	Angers	Antwerp	Bern	Helsinki	Grenoble	Kuala Lumpur	Perth	Sydney	Torino	Wenzhou
Patients, n	236	211	65	356	129	193	65	128	101	440
Age, *y*	57.5 ± 11.9	49.6 ± 14.3	55.4 ± 14.3	50.7 ± 9.5	54.5 ± 11.3	49.9 ± 11.3	54.0 ± 13.1	48.4 ± 12.2	44.4 ± 12.9	41.3 ± 11.8
Male sex, n (%)	146 (61.9)	97 (46.0)	43 (66.2)	100 (28.1)	77 (59.7)	96 (49.7)	26 (40.0)	79 (61.7)	73 (72.3)	328 (74.5)
BMI, kg/m^2	33.9 ± 6.8	34.9 ± 7.4	31.3 ± 5.0	40.4 ± 7.2	31.1 ± 5.2	29.9 ± 4.5	35.8 ± 6.8	30.7 ± 5.1	28.1 ± 4.0	26.6 ± 3.3
Diabetes, n (%)	111 (47.0)	58 (27.5)	27 (41.5)	155 (43.5)	57 (44.2)	90 (46.6)	35 (53.8)	31 (24.2)	17 (16.8)	122 (27.7)
Bariatric patients, n (%)	6 (2.5)	25 (11.8)	0 (0.0)	304 (85.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Steatosis grade, n (%)										
0	17 (7.2)	26 (12.3)	2 (3.1)	135 (37.9)	2 (1.6)	3 (1.6)	1 (1.5)	3 (2.3)	1 (1.0)	44 (10.0)
1	106 (44.9)	86 (40.8)	19 (29.2)	139 (39.0)	29 (22.5)	46 (23.8)	18 (27.7)	65 (50.8)	50 (49.5)	159 (36.1)
2	61 (25.8)	71 (33.6)	19 (29.2)	52 (14.6)	41 (31.8)	100 (51.8)	30 (46.2)	43 (33.6)	31 (30.7)	155 (35.2)
3	52 (22.0)	28 (13.3)	25 (38.5)	30 (8.4)	57 (44.2)	44 (22.8)	16 (24.6)	17 (13.3)	19 (18.8)	82 (18.6)
Ballooning, n (%)										
0	63 (26.7)	18 (8.5)	–	305 (85.7)	19 (14.7)	59 (30.6)	20 (30.8)	70 (54.7)	20 (19.8)	87 (19.8)
1	98 (41.5)	127 (60.2)	–	35 (9.8)	64 (49.6)	88 (45.6)	31 (47.7)	39 (30.5)	44 (43.6)	262 (59.5)
2	75 (31.8)	66 (31.3)	–	16 (4.5)	46 (35.7)	46 (23.8)	14 (21.5)	19 (14.8)	37 (36.6)	91 (20.7)
Lobular inflammation, n (%)										
0	48 (20.3)	67 (31.8)	–	299 (84.0)	40 (31.0)	3 (1.6)	18 (27.7)	45 (35.2)	24 (23.8)	58 (13.2)
1	161 (68.2)	126 (59.7)	–	47 (13.2)	70 (54.3)	105 (54.4)	37 (56.9)	70 (54.7)	68 (67.3)	274 (62.3)
2	27 (11.4)	18 (8.5)	–	10 (2.8)	18 (14.0)	82 (42.5)	10 (15.4)	13 (10.2)	9 (8.9)	101 (23.0)
3	0 (0.0)	0 (0.0)	–	0 (0.0)	1 (0.8)	3 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	7 (1.6)
NAFLD Activity Score	3.6 ± 1.7	3.5 ± 1.6	2.0 ± 1.0[a]	1.3 ± 1.6	4.2 ± 1.8	4.3 ± 1.4	3.7 ± 1.5	2.9 ± 1.6	3.7 ± 1.3	3.8 ± 1.6
NASH, n (%)	155 (65.7)	135 (64.0)	54 (83.1)	46 (12.9)	85 (65.9)	132 (68.4)	37 (56.9)	52 (40.6)	61 (60.4)	298 (67.7)
Fibrosis stage, n (%)										
0	39 (16.5)	75 (35.5)	5 (7.7)	199 (55.9)	17 (13.2)	66 (34.2)	16 (24.6)	37 (28.9)	34 (33.7)	190 (43.2)
1	47 (19.9)	61 (28.9)	15 (23.1)	116 (32.6)	23 (17.8)	81 (42.0)	20 (30.8)	52 (40.6)	17 (16.8)	172 (39.1)
2	68 (28.8)	25 (11.8)	19 (29.2)	18 (5.1)	43 (33.3)	15 (7.8)	4 (6.2)	17 (13.3)	18 (17.8)	60 (13.6)
3	62 (26.3)	28 (13.3)	23 (35.4)	14 (3.9)	23 (17.8)	25 (13.0)	14 (21.5)	12 (9.4)	25 (24.8)	15 (3.4)
4	20 (8.5)	22 (10.4)	3 (4.6)	9 (2.5)	23 (17.8)	6 (3.1)	11 (16.9)	10 (7.8)	7 (6.9)	3 (0.7)
Fibrotic NASH, n (%)	100 (42.4)	49 (23.2)	40 (61.5)	21 (5.9)	66 (51.2)	42 (21.8)	18 (27.7)	21 (16.4)	26 (25.7)	61 (13.9)
AST, *IU/L*	45 ± 39	38 ± 29	63 ± 42	33 ± 14	42 ± 22	48 ± 26	54 ± 37	49 ± 24	44 ± 32	50 ± 50
ALT, *IU/L*	62 ± 46	58 ± 48	82 ± 84	39 ± 25	71 ± 35	79 ± 45	80 ± 76	74 ± 41	72 ± 44	79 ± 84
GGT, *IU/L*	118 ± 175	80 ± 117	162 ± 197	54 ± 100	151 ± 190	96 ± 80	134 ± 184	110 ± 102	104 ± 117	77 ± 98
Alkaline phosphatase, *IU/L*	82 ± 29	89 ± 36	86 ± 28	70 ± 31	90 ± 35	85 ± 29	93 ± 36	100 ± 36	83 ± 38	88 ± 38

Supplementary Table 2. Continued

	Angers	Antwerp	Bern	Helsinki	Grenoble	Kuala Lumpur	Perth	Sydney	Torino	Wenzhou
Albumin, *g/L*	43.3 ± 3.5	43.0 ± 5.5	38.5 ± 3.8	38.6 ± 3.7	45.2 ± 4.1	43.1 ± 3.4	41.8 ± 3.5	44.6 ± 3.4	45.2 ± 4.1	46.2 ± 3.6
Platelets, *G/L*	222 ± 65	245 ± 76	224 ± 62	252 ± 62	223 ± 64	275 ± 67	222 ± 66	253 ± 68	231 ± 74	246 ± 61
HOMA	9.8 ± 19.7	10.6 ± 23.2	8.8 ± 16.5	3.9 ± 3.2	5.6 ± 10.1	7.8 ± 7.2	11.8 ± 26.5	5.5 ± 5.7	3.5 ± 2.7	5.4 ± 7.8
CK18, *IU/L*	386 ± 472	410 ± 447	364 ± 277	218 ± 162	424 ± 466	516 ± 461	503 ± 985	313 ± 348	345 ± 484	285 ± 461
FIB-4	1.67 ± 1.04	1.28 ± 1.34	2.11 ± 1.75	1.23 ± 0.82	1.40 ± 0.94	1.07 ± 0.62	1.89 ± 1.74	1.28 ± 0.85	1.15 ± 0.96	1.02 ± 0.79

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CK18, cytokeratin 18; FIB-4, Fibrosis-4; GGT, γ-glutamyltransferase; HOMA, Homeostasis Model Assessment; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

[a]According to the steatosis, activity fibrosis scoring system.

Supplementary Table 3. Correlation of MACK-3 Results With Individual Liver Lesions

	MACK-3	
	Rs	*P*
Steatosis grade	0.469	<.001
Lobular inflammation grade	0.434	<.001
Ballooning grade	0.417	<.001
NAFLD Activity Score	0.566	<.001
Fibrosis stage	0.479	<.001

NAFLD, nonalcoholic fatty liver disease; Rs, Spearman correlation coefficient.

Supplementary Table 4. Contingency Tables for Patients Ruled Out (<0.135), Ruled In (>0.549), or in the Grey Zone (0.135–0.549) With MACK-3

	Fibrosis	NAS								
MACK-3	stage	0	1	2	3	4	5	6	7	8
<0.135	0	13.7%; 103[a]	14.0%; 105[a]	9.0%; 68[a]	12.9%; 97[a]	4.7%; 35[a]	2.1%; 16[a]	0.3%; 2[a]	0.1%; 1[a]	0.0%; 0[a]
	1	4.5%; 34[a]	5.2%; 39[a]	4.7%; 35[a]	7.3%; 55[a]	5.9%; 44[a]	2.3%; 17[a]	0.7%; 5[a]	0.0%; 0[a]	0.0%; 0[a]
	2	0.3%; 2[a]	0.3%; 2[a]	1.5%; 11[a]	1.5%; 11[a]	2.5%; 19[b]	1.5%; 11[b]	0.1%; 1[b]	0.3%; 2[b]	0.1%; 1[b]
	3	0.1%; 1[a]	0.3%; 2[a]	0.4%; 3[a]	0.8%; 6[a]	0.9%; 7[b]	0.5%; 4[c]	0.3%; 2[c]	0.0%; 0[c]	0.0%; 0[c]
	4	0.1%; 1[a]	0.5%; 4[a]	0.3%; 2[a]	0.3%; 2[a]	0.1%; 1[b]	0.1%; 1[c]	0.0%; 0[c]	0.0%; 0[c]	0.0%; 0[c]
0.135–0.549	0	2.1%; 15[c]	5.0%; 36[c]	4.9%; 35[c]	6.8%; 49[c]	6.0%; 43[c]	3.3%; 24[c]	0.8%; 6[c]	0.1%; 1[c]	0.0%; 0[c]
	1	0.4%; 3[c]	2.6%; 19[c]	3.9%; 28[c]	7.1%; 51[b]	9.6%; 69[b]	6.9%; 50[b]	2.6%; 19[b]	0.4%; 3[b]	0.0%; 0[b]
	2	0.0%; 0[c]	1.1%; 8[c]	1.1% 8[c]	3.2%; 23[b]	4.3%; 31[a]	5.0%; 36[a]	2.6%; 19[a]	0.8%; 6[a]	0.0%; 0[a]
	3	0.3%; 2[c]	0.3%; 2[c]	1.3%; 9[c]	2.1%; 15[b]	3.8%; 27[a]	2.8%; 20[a]	1.4%; 10[a]	0.4%; 3[a]	0.0%; 0[a]
	4	0.4%; 3[c]	1.1%; 8[c]	0.3%; 2[c]	1.4%; 10[b]	1.1%; 8[a]	1.5%; 11[a]	1.0%; 7[a]	0.1%; 1[a]	0.0%; 0[a]
>0.549	0	0.0%; 0[c]	1.0%; 4[c]	0.0%; 0[c]	1.8%; 7[c]	3.4%; 13[c]	2.1%; 8[c]	1.0%; 4[c]	0.3%; 1[c]	0.0%; 0[c]
	1	0.0%; 0[c]	1.0%; 4[c]	2.3%; 9[c]	3.1%; 12[b]	5.9%; 23[b]	8.8%; 34[b]	7.0%; 27[b]	2.1%; 8[b]	0.3%; 1[b]
	2	0.0%; 0[c]	0.0%; 0[c]	0.5%; 2[c]	1.6%; 6[b]	2.3%; 9[a]	7.8%; 30[a]	5.9%; 23[a]	1.6%; 6[a]	0.3%; 1[a]
	3	0.0%; 0[c]	0.3%; 1[c]	0.3%; 1[c]	2.6%; 10[b]	4.9%; 19[a]	6.5%; 25[a]	8.8%; 34[a]	3.6%; 14[a]	0.3%; 1[a]
	4	0.0%; 0[c]	0.8%; 3[c]	1.3%; 5[c]	1.0%; 4[b]	3.9%; 15[a]	1.3%; 5[a]	4.4%; 17[a]	0.3%; 1[a]	0.0%; 0[a]

NAS, Nonalcoholic Fatty Liver Disease Activity Score.
[a]Well classified for fibrotic nonalcoholic steatohepatitis.
[b]Borderline misclassification for fibrotic NASH with a discrepancy of only 1 Nonalcoholic Fatty Liver Disease Activity Score point or 1 fibrosis stage.
[c]Misclassified for fibrotic nonalcoholic steatohepatitis.

Supplementary Table 5. Characteristics of the Patients for Whom FAST Score Was Available

	All (n = 1924)	FAST available (n = 655)	FAST not available (n = 1269)	*P*
Age, *y*	49.2 ± 13.0	51.4 ± 14.2	48.1 ± 12.2	<.001
Male sex, n (%)	1065 (55.4)	418 (63.8)	647 (51.0)	<.001
BMI, kg/m^2	32.4 ± 7.5	31.3 ± 6.5	33.0 ± 7.8	<.001
Bariatric patients, n (%)	335 (17.4)	8 (1.2)	327 (25.8)	<.001
Diabetes, n (%)	703 (36.5)	254 (38.8)	449 (35.4)	.148
Steatosis grade, n (%)				<.001
0	234 (12.2)	37 (5.6)	197 (15.5)	
1	717 (37.3)	249 (38.0)	468 (36.9)	
2	603 (31.1)	210 (32.1)	393 (31.0)	
3	370 (19.2)	159 (24.3)	211 (16.6)	
Ballooning, n (%)				<.001
0	661 (35.6)	111 (18.7)	550 (43.5)	
1	788 (42.4)	320 (53.9)	468 (37.0)	
2	410 (22.1)	163 (27.4)	247 (19.5)	
Lobular inflammation, n (%)				<.001
0	602 (32.4)	114 (19.2)	488 (38.6)	
1	958 (51.5)	383 (64.5)	575 (45.5)	
2	288 (15.5)	93 (15.7)	195 (15.4)	
3	11 (0.6)	4 (0.7)	7 (0.6)	
NAFLD Activity Score	3.3 ± 1.9	3.8 ± 1.6	3.0 ± 1.9	<.001
NASH, n (%)	1001 (53.8)	406 (68.4)	595 (47.0)	<.001
Fibrosis stage, n (%)				<.001
0	678 (35.2)	151 (23.1)	527 (41.5)	
1	604 (31.4)	175 (26.7)	429 (33.8)	
2	287 (14.9)	152 (23.2)	135 (10.6)	
3	241 (12.5)	129 (19.7)	112 (8.8)	
4	114 (5.9)	48 (7.3)	66 (5.2)	
Fibrotic NASH, n (%)	444 (23.1)	238 (36.3)	206 (16.2)	<.001
AST, *IU/L*	44 ± 35	48 ± 40	42 ± 32	<.001
ALT, *IU/L*	66 ± 58	71 ± 60	64 ± 57	<.001
GGT, *IU/L*	93 ± 130	112 ± 165	84 ± 107	<.001
Alkaline phosphatase, *IU/L*	84 ± 35	86 ± 38	83 ± 33	.044
Albumin, *g/L*	43.2 ± 4.7	44.1 ± 4.6	42.7 ± 4.7	<.001
Platelets, *G/L*	244 ± 67	232 ± 64	250 ± 68	<.001
HOMA	6.7 ± 13.2	8.7 ± 18.3	5.7 ± 9.5	<.001
CK18, *IU/L*	346 ± 449	400 ± 563	318 ± 375	<.001
FIB-4	1.28 ± 1.03	1.45 ± 1.11	1.20 ± 0.97	<.001
MACK-3	0.306 ± 0.279	0.360 ± 0.284	0.277 ± 0.272	<.001
Liver stiffness, *kPa*	–	10.6 ± 8.9	–	–

NOTE. Patients with a FAST score available came from the following centers: Angers (n = 220), Antwerp (n = 107), Berne (n = 61), Grenoble (n = 80), Perth (n = 24), and Wenzhou (n = 163).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CK18, cytokeratin 18; FAST, FibroScan–aspartate aminotransferase score; FIB-4, Fibrosis-4; GGT, γ-glutamyltransferase; HOMA, Homeostasis Model Assessment; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.