Machine Learning Models of Non-invasive Tests to Predict MASH and Fibrosis Stage Based on MAESTRO-NAFLD-1 and MAESTRO-NASH Liver Biopsies

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INTRODUCTION

- Metabolic dysfunction-associated steatohepatitis (MASH), the more advanced form of metabolic dysfunction-associated steatotic liver disease (MASLD), is a progressive liver disease characterized by 5% or greater hepatic steatosis with hepatic inflammation, hepatocellular ballooning, and fibrosis of varying severity (ie, none [F0], mild [F1], moderate [F2], advanced [F3], and cirrhosis [F4])[1-2]
- Once MASH progresses to clinically meaningful fibrosis (stages F2 and F3), the risk of adverse clinical outcomes including hepatocellular cancer markedly increases.[3-5]
- Although, liver biopsy is a reliable approach for identifying the presence of steatohepatitis and fibrosis in patients with MASH; it is limited by cost, infrequent real-world use, sampling error, and procedure-related morbidity (and very rarely mortality)[6]
- For these reasons, non-invasive tests (NITs) that can replace serial liver biopsies for identifying and following patients with MASH who are at risk of developing advanced fibrosis are urgently needed[7-9]
  - Although NITs have emerged, the relative importance and diagnostic accuracy of these tools in contemporary trial populations remains limited.
- We aimed to develop machine learning (ML) methods to predict fibrosis stage and/or MASH with F2/F3 fibrosis using non-invasive biomarkers.

METHODS

- Data from 1970 biopsy-confirmed patients who screened for two phase 3 resmetirom trials (MAESTRO-NASH, MAESTRO-NAFLD-1) were retrospectively analyzed
- Screening criteria required adults (aged ≥18 years) with three metabolic risk factors consistent with MASH (diabetes/insulin resistance, hypertension, obesity/overweight, dyslipidemia), as defined by a modified International Diabetes Federation metabolic syndrome framework, and evidence of hepatic steatosis and fibrosis from recent assessments
  - Eligibility was supported by either VCTE performed within 3 months before screening or a liver biopsy obtained within 6 months and a screening MRI proton density fat fraction (MRI-PDFF) showing ≥8% liver fat content
- Our analysis focused on classification of two separate outcomes at baseline based on thirty-seven predictors (including clinical, laboratory, and imaging measures):
  - Outcome 1: Fibrosis stage (F0/F1 vs. F2/F3 vs. F4)—a classification exercise with 3 categories
  - Outcome 2: MASH with F2/F3 fibrosis, with MASH defined as a baseline NAS ≥4 and moderate-to-advanced noncirrhotic fibrosis
- Models using random forest were developed, selected for accuracy and stability, in two settings: full predictor models (using all 37 variables including MRE/MRI derived variables) and "lean" models (using a subset of between 20 and 24 predictors routinely available in clinical practice) (**Table 1**)
- Performance was evaluated with 4-fold cross-validation via area under the curve (AUC)

TABLE 1. Variables included in each of the prediction models

	Variable	Variable Short Name	Prediction Model (Full, LM)			
			Full	LM 1	LM 2	LM 3
1	Age	AGE	X	X	X	X
2	Sex	SEX	X	X	X	X
3	Race	RACE	X	X	X	X
4	Body Mass Index	BMI	X	X	X	X
5	Alcohol Use	ALCOHOL	X	X	X	X
6	Tobacco Use	TOBACCO	X	X	X	X
7	Hypertension	HTN	X			
8	Type 2 Diabetes	T2D	X	X	X	X
9	VCTE	VCTE	X	X	X	
10	CAP score	CAP	X	X	X	
11	Enhanced Liver Fibrosis Test[a]	ELF	X		X	X
12	Hyaluronic Acid	HA	X		X	X
13	Procollagen 3 N-Terminal Propeptide	P3NP	X		X	X
14	Tissue Inhibitor of Metalloproteinase 1	TIMP1	X		X	X
15	Sex Hormone Binding Globulin	SHBG	X			
16	M30 (CK18)	M30	X			
17	Adiponectin	APN	X			
18	Alkaline Phosphatase	ALP	X	X	X	X
19	Aspartate Aminotransferase	AST	X	X	X	X
20	Alanine Aminotransferase	ALT	X	X	X	X
21	Gamma Glutamyl Transferase	GGT	X	X	X	X
22	Thyroxine, Free	FT4	X	X	X	X
23	MRI-PDFF	MRIPDFF	X			
24	MRE Result, Average	MRE	X			
25	MRI Liver Volume	LIVERVOL	X			
26	MRI Spleen Volume	SPLNVOL	X			
27	PRO-C3	PROC3	X			
28	FAST Score (based on VCTE, CAP, AST)	FAST	X	X	X	
29	MAST Score (based on MRE, MRI-PDFF, AST)	MAST	X			
30	FIB-4 Score	FIB4	X	X	X	X
31	Reverse-T3	RT3	X			
32	Free-T3	T3FR	X	X	X	X
33	Free T3/Reverse T3 – Ratio	T3FR_RT3	X			
34	International normalized ratio	INR	X	X	X	X
35	Total Bilirubin	BILI	X	X	X	X
36	Platelets	PLAT	X	X	X	X
37	Steatosis-Associated Fibrosis Estimator Score	SAFE	X	X	X	X

RESULTS

TABLE 2. Analysis cohorts

Variable	Baseline Fibrosis Stage				
	F0 N=243	F1 N=313	F2 N=592	F3 N=651	F4 N=171
Age, years	54.7 (12.4)	53.0 (12.9)	55.2 (11.8)	58.5 (9.9)	61.6 (8.6)
Female, n (%)	126 (51.9%)	139 (44.4%)	320 (54.1%)	362 (55.6%)	109 (63.7%)
Body Mass Index, kg/m²	37.8 (8.1)	36.6 (6.7)	36.0 (6.8)	35.2 (6.6)	35.3 (6.6)
T2DM, n (%) Present	84 (34.6%)	111 (35.5%)	339 (57.3%)	445 (68.4%)	97 (56.7%)
Fibroscan – VCTE, kPa	11.3 (6.3)	10.7 (5.9)	11.3 (5.6)	14.3 (6.5)	24.2 (13.3)
Fibroscan – CAP Score, dB/m	346.7 (35.6)	345.6 (38.3)	345.4 (38.2)	344.9 (39.6)	327.6 (52.8)
Enhanced Liver Fibrosis Test	9.3 (0.8)	9.4 (0.9)	9.5 (0.8)	10.0 (0.9)	10.6 (1.0)
PRO-C3, ng/mL	13.1 (4.7)	15.2 (6.2)	17.3 (7.1)	20.3 (11.3)	25.3 (15.2)
Aspartate Aminotransferase, U/L	24.0 (13.5)	29.5 (15.2)	37.6 (24.5)	41.8 (22.5)	40.0 (21.1)
Alanine Aminotransferase, U/L	35.4 (26.3)	47.1 (31.6)	54.2 (36.6)	54.4 (30.5)	43.9 (28.7)
Gamma-Glutamyl Transferase, U/L	48.7 (50.2)	60.2 (71.6)	68.3 (74.9)	92.7 (111.8)	104.7 (115.5)
MRI-PDFF, %	19.1 (8.2)	19.5 (7.8)	18.0 (7.2)	16.4 (6.5)	10.7 (5.6)
MRE Result, kPa	2.5 (0.5)	2.7 (0.6)	3.1 (0.7)	4.0 (1.0)	5.5 (1.8)
FAST Score	0.1 (0.1)	0.2 (0.1)	0.2 (0.1)	0.3 (0.2)	0.3 (0.2)
FIB-4 Score	0.9 (0.4)	1.0 (0.5)	1.2 (0.6)	1.6 (0.7)	2.3 (1.2)

- 1970 patients were included from the MAESTRO-NASH and MAESTRO-NAFLD-1 trials (**Table 2**)
- Most patients (71.8%) had moderate to advanced fibrosis, and nearly three-quarters (74.7%) had a baseline NAS ≥4.
- Random forest models to predict three classes of fibrosis stages (F0/F1 vs. F2/F3 vs. F4) resulted in one-vs-rest cross-validation mean AUCs for the full data model (0.76, 0.75, and 0.94, respectively) and best performing Lean Model 1 (0.83, 0.78, and 0.89, respectively) (**Table 3**)
  - In a model predicting MASH with significant (i.e., stage 2/3) liver fibrosis, the full and lean models achieved AUCs of 0.75 and 0.79, respectively
  - Full model showed high sensitivity (82.3%) and NPV (98.1%) for F4
- Lean Model 1 performed as well as the Full Model in sensitivity (72.5% vs 65.3%), specificity (73.7% vs 71.1%), PPV (78.3% vs 74.6%), and NPV (67.4% vs 61.2%)

TABLE 3. Cross-validation AUC mean (SD) for fibrosis stage (one-vs-rest) and MASH with F2/F3 fibrosis (yes/no) predictions

Model	Fibrosis Stage (3-class prediction)			At Risk MASH
	F0/F1 vs. Rest	F2/3 vs. Rest	F4 vs. Rest	Yes vs. No
Full Model	0.76 (0.068)	0.75 (0.055)	0.94 (0.032)	0.75 (0.050)
Lean Model 1	0.83 (0.021)	0.78 (0.021)	0.89 (0.026)	0.79 (0.020)
Lean Model 2	0.71 (0.043)	0.69 (0.037)	0.91 (0.029)	0.66 (0.037)
Lean Model 3	0.68 (0.044)	0.64 (0.038)	0.85 (0.037)	0.62 (0.034)

- The most important factors contributing to fibrosis stage predictions included liver stiffness measures (MRE; magnetic resonance elastography, VCTE; transient elastography) and MASH composite algorithms (FAST, SAFE, FIB-4). (**Figure 1**)

FIGURE 1A Random forest–based variable importance for fibrosis stage prediction: full model

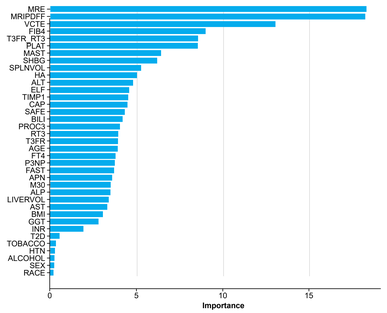


FIGURE 1B Random forest–based variable importance for fibrosis stage prediction: Lean Model 1

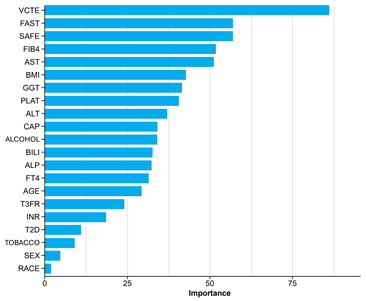
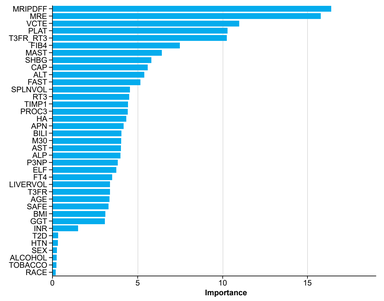


FIGURE 1C Random forest–based variable importance for MASH with F2/F3 fibrosis: full model



- MRE was the most influential biomarker for prediction of fibrosis stage in the full model, followed by VCTE and platelets/FIB-4. (**Figure 2**)
- Reduced platelet count only showed prediction for F4.
- Higher VCTE and FIB-4 values shifted predictions toward advanced categories, particularly F4, while SAFE and FAST scores displayed discriminative patterns in the intermediate range, favoring F2/F3.
- SHAP values and accumulated local effects (ALE) plots were used to assess predictor contributions at both the individual patient and population levels (**Figures 3** and **4**).

FIGURE 2 Accumulated local efffects plots for key variables

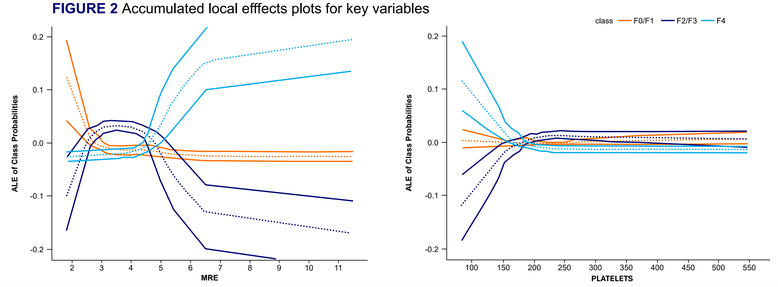


FIGURE 3 SHAP value explaining individual patient predictions

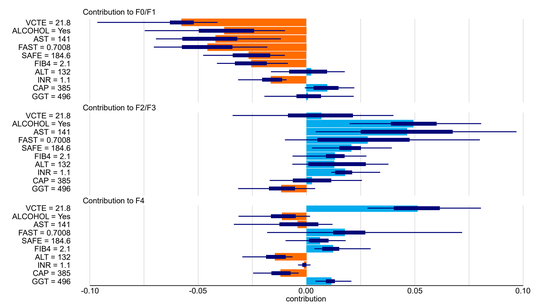
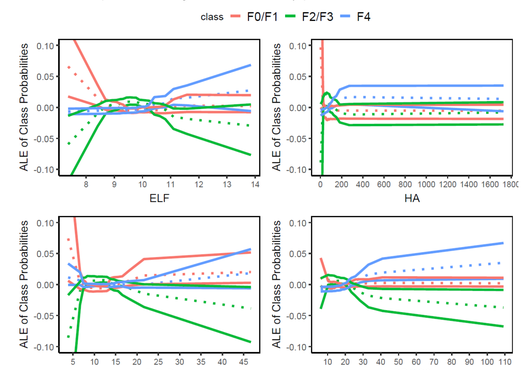


FIGURE 4 ALE plots for marginal effects of key predictors



CONCLUSIONS

- **ML models using non-invasive biomarkers, with routinely available clinical predictors, accurately predict fibrosis stage and MASH with F2/F3 fibrosis**
- **ML models that included MRI-PDFF/MRE measurements discriminated cirrhosis (F4) particularly well (F4 vs. rest AUC of 0.94)**
- **Full model showed high sensitivity and NPV for F4/cirrhosis**
- **Lean Model 1 performed as well as the Full Model in sensitivity, specificity, PPV, and NPV**
  - **Lean Models 2 and 3 showed lower performance across outcomes, underscoring that Lean Model 1 offers the best balance of clinical practicality and predictive accuracy**
- **The findings support potential of ML models of non-invasive biomarkers to identify patients with MASH and moderate to advanced liver fibrosis for clinical trials or clinical practice.**

ABBREVIATIONS

ALE, Accumulated Local Effects; AUC, area under the curve; FAST, FibroScan-AST; FIB-4, Fibrosis-4; MASH, metabolic dysfunction-associated steatohepatitis; ML, machine learning; MRI, magnetic resonance imaging; MRI-PDFF, MRI proton density fat fraction; MRE, magnetic resonance elastography; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NPV, negative predictive value; PPV, positive predictive value; SAFE, Steatosis-Associated Fibrosis Estimator; SHAP, shapley additive explanations; VCTE, vibration controlled transient elastography.

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