

Supplementary materials

Supplementary Table 1. Description of the tested algorithms

Single-parameter algorithms	
Based on the MANPOWER cut-offs	Increased ALT vs. normal ALT
	Increased AST vs. normal AST
	Increased GGT vs. normal GGT
Based on the statistical distribution of enzyme levels (spectrum of disease severity)	Q4 ALT vs. remaining categories (based on quartiles)
	Q4 AST vs. remaining categories (based on quartiles)
	Q4 GGT vs. remaining categories (based on quartiles)
Multi-parameter algorithms	
Based on the MANPOWER cut-offs	Algorithm 1: Increased ALT AND increased AST AND increased GGT vs. remaining categories
	Algorithm 2: Increased ALT OR increased AST OR increased GGT vs. remaining categories
	Algorithm 3: Increased ALT OR increased AST AND increased GGT vs. remaining categories
	Algorithm 4: ALT/AST increased ratio >1 AND GGT increased vs. remaining categories
	Algorithm 5: (Increased ALT and increased AST) OR (increased ALT and increased GGT) OR (increased AST and increased GGT) vs. remaining categories
Based on the statistical distribution of enzyme levels (spectrum of disease severity)	Algorithm 1a: Q4 ALT AND Q4 AST AND Q4 GGT vs. remaining categories
	Algorithm 2a: Q4 ALT OR Q4 AST OR Q4 GGT vs. remaining categories
	Algorithm 3a: (Q4 ALT OR Q4 AST) AND Q4 GGT vs. remaining categories
	Algorithm 5a: (Q4 ALT and Q4 AST) OR (Q4 ALT and Q4 GGT) OR (Q4 AST and Q4 GGT) vs. remaining categories

ALT, alanine transferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; Q, quartile.

Supplementary Table 2. Diagnostic accuracy of the main algorithms based on enzymes levels, using physician-diagnosed NASH as the reference standard

Algorithms	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Increased ALT vs. normal ALT (MANPOWER cut-offs)	93.7	44.3	39.7	94.7	58.2
Increased AST vs. normal AST (MANPOWER cut-offs)	75.7	59.5	42.3	86.2	64.1
Increased GGT vs. normal GGT (MANPOWER cut-offs)	78.1	46.9	36.6	84.5	55.7
Increased ALT OR increased AST OR increased GGT vs. remaining categories (MANPOWER cut-offs; Algorithm 2)	98.0	34.8	35.8	97.2	47.8
Q4 ALT OR Q4 AST OR Q4 GGT vs. remaining categories (Algorithm 2a)	75.6	71.0	50.5	88.1	72.3
Increased ALT OR increased AST OR abnormal GGT vs. remaining categories (ROC curve; Algorithm 2)	89.9	56.3	44.7	93.4	65.8

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; NASH, non-alcoholic steatohepatitis; NPV, negative predicative value; PPV, positive predicative value; Q, quartile; ROC, receiver operating characteristic.

Supplementary Table 3. Enzyme levels at baseline in the overall population, stratified by gender

	Female patients			Male patients		
	N=1,767			N=1,076		
Enzyme, U/L	ALT n=1,767	AST n=1,767	GGT n=1,767	ALT n=1,076	AST n=1,076	GGT n=792
Mean (SD)	46.74 (30.38)	41.62 (24.65)	48.1 (35.76)	55.96 (36.65)	46.96 (27)	57.65 (39.83)
Median (IQR)	40 (28–58)	38 (26–51.4)	39 (26–56)	48 (33–71)	42 (29.4–60)	48.35 (33–68.7)
Minimum–maximum	0.2–437	0.12–400	0.4–344.3	0.4–345.1	0.2–266	5.5–400

ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range; GGT, gamma-glutamyl transferase; SD, standard deviation.

Supplementary Table 4. Mean change in liver enzyme levels (unadjusted and adjusted) from baseline to W12

		Overall population	Based on the statistical distribution (disease severity spectrum)				Based MANPOWER cut-offs		Based on physician diagnosis	
			Very normal (minimum–Q1)	Normal (Q1–Median)	Increased (Median–Q3)	Very increased (Q3–Max)	Increased	Normal	NASH	No NASH
ALT, U/L	Baseline values, mean (SD)	50.2 (33.2)	20.4 (7.3)	37.1 (4.3)	53.4 (5.9)	94.4 (37.5)	64.9 (32.9)	24.3 (8.8)	72.6 (38.6)	42.8 (27.4)
	N (at W12)	2,697	717	704	630	646	1,270	1,427	703	1,994
	Change from baseline, mean (SD)*	–12.3 (21.6)	–6.2 (11.4)	–10 (21.5)	–15.1 (19)	–19 (29.1)	–16.9 (26.9)	–8.2 (14.2)	–24.2 (28.1)	–8.2 (16.9)
	Adjusted mean change from baseline		–6.81 p≤0.001			–24.2 p≤0.001	–12.5 p≤0.001	–8.67 p≤0.001	–10.65 p≤0.001	–11.97 p≤0.001
AST, U/L	Baseline values, mean (SD)	43.6 (25.7)	21.9 (9.7)	35.5 (12.9)	48.3 (11.5)	71.9 (30.8)	54 (25.2)	25.3 (13.4)	58.8 (27.8)	38.6 (22.8)
	N (at W12)	2,696	703	657	689	647	789	1,907	703	1,993
	Change from baseline, mean (SD)*	–10.6 (17)	–6.5 (11.2)	–8.5 (15.8)	–12.5 (15.7)	–15.1 (22.3)	–14 (21.4)	–9.2 (14.5)	–19.1 (22.6)	–7.6 (13.2)
	Adjusted mean change from baseline		–6.88 p≤0.001			–21.2 p≤0.001	–13.7 p≤0.001	–6.94 p≤0.001	–12.4 p≤0.001	–9.69 p≤0.001
GGT, U/L	Baseline values, mean (SD)	51.7 (37.6))	35.4 (26.2)	41.6 (26.3)	56.8 (34)	73 (47.9)	59.5 (40.6)	36.4 (24.5)	71.4 (49.4)	44 (28.4)
	N (at W12)	1,936	482	479	476	499	1,282	654	567	1,369
	Change from baseline, mean (SD)*	–10.6 (24.9)	–7.4 (13.9)	–7.2 (11.2)	–8.9 (16.1)	–18.7 (42.5)	–13.4 (32.4)	–7.9 (14.2)	–19 (33.1)	–6.9 (19.3)
	Adjusted mean change from baseline		–2.31 p≤0.001			–25.7 p≤0.001	–13.8 p≤0.001	–0.78 p=0.54	–12.5 p=0.0017	–8.11 p=0.0017

*Adjusted for age, BMI, number of comorbidities, presence of diabetes, obesity, hypertension and NASH (multiple definitions were included in the model, e.g., physician-diagnosed [yes/no], severity spectrum, MANPOWER cut-offs and algorithm of the disease). ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range; GGT, gamma-glutamyl transferase; NASH, non-alcoholic steatohepatitis; Q, quartile; SD, standard deviation; W, week.

Supplementary Table 5. Mean change in liver enzyme levels (unadjusted and adjusted) from baseline to W24

		Overall population	Based on the statistical distribution (disease severity spectrum)				Based MANPOWER cut-offs		Based on physician diagnosis	
			Very normal (minimum–Q1)	Normal (Q1–median)	Increased (median–Q3)	Very increased (Q3–maximum)	Increased	Normal	NASH	No NASH
ALT, U/L	Baseline values, mean (SD)	50.2 (33.2)	20.4 (7.3)	37.1 (4.3)	53.4 (5.9)	94.4 (37.5)	64.9 (32.9)	24.3 (8.8)	72.6 (38.6)	42.8 (27.4)
	N (at W24)	2,764	722	718	666	658	669	2,095	709	2,055
	Change from baseline, mean (SD)*	–20.4 (27.8)	–13.5 (23.9)	–17.4 (22)	–23.6 (25.5)	–28.2 (36.3)	–25.9 (35)	–18.7 (24.9)	–37.2 (35.3)	–14.6 (22)
Adjusted mean change from baseline			–13.82 p≤0.001			–39.1 p≤0.001	–22.97 p≤0.001	–15.18 p≤0.001	–19.26 p≤0.001	–21.74 p≤0.001
AST, U/L	Baseline values, mean (SD)	43.6 (25.7)	21.9 (9.7)	35.5 (12.9)	48.3 (11.5)	71.9 (30.8)	54 (25.2)	25.3 (13.4)	58.8 (27.8)	38.6 (22.8)
	N (at W24)	2,761	659	786	660	656	297	2,464	708	2,053
	Change from baseline, mean (SD)*	–16.9 (20.9)	–12.5 (15.8)	–16 (21)	–18.2 (22)	–20.8 (23.1)	–18.5 (23.9)	–16.7 (20.5)	–28.5 (26.1)	–12.8 (17)
Adjusted mean change from baseline			–11.99 p≤0.001			–32.8 p≤0.001	–23.1 p≤0.001	–11.1 p≤0.001	–19.3 p≤0.001	–16.56 p≤0.001
GGT, U/L	Baseline values, mean (SD)	51.7 (37.6)	35.4 (26.2)	41.6 (26.3)	56.8 (34))	73 (47.9)	59.5 (40.6)	36.4 (24.5)	71.4 (49.4)	44 (28.4)
	N (at W24)	1,964	485	520	481	478	736	1,228	557	1,407
	Change from baseline, mean (SD)*	–17.1 (31.1)	–13.2 (21)	–13.8 (23.5)	–19.4 (32.8)	–21.9 (41.7)	–20.4 (38.1)	–15 (25.5)	–29.2 (40.6)	–12.1 (24.6)
Adjusted mean change from baseline			–6.16 p≤0.001			–42.8 p≤0.001	–24 p≤0.001	–4.6 p≤0.001	–21.6 p≤0.001	–15.9 p≤0.001

*Adjusted for age, BMI, number of comorbidities, presence of diabetes, obesity, hypertension and NASH (multiple definitions were included in the model, e.g., physician-diagnosed [yes/no], severity spectrum, MANPOWER cut-offs and algorithm of the disease).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range; GGT, gamma-glutamyl transferase; NASH, non-alcoholic steatohepatitis; Q, quartile; SD, standard deviation; W, week.

Supplementary Table 6. Changes in diffuse liver hyperechogenicity at W12 and W24 in the overall population and stratified by physician-diagnosed NASH

Changes from baseline	Overall population (n=2,375)		Physician-diagnosed NASH			
			NASH (n=633)		No NASH (n=1,742)	
W	12	24	12	24	12	24
USG abnormality improved, %	69.6	76.84	69.83	74.25	69.52	77.78
No USG improvement, %	26.11	17.14	27.65	20.06	25.55	16.07
Missing, %	4.29	6.02	2.53	5.69	4.94	6.14

NASH, non-alcoholic steatohepatitis; USG, ultrasonography; W, week.

Supplementary Table 7. Changes in diffuse liver hyperechogenicity at W12 and W24, stratified by ALT, AST and GGT liver enzyme levels

Changes from baseline	Based on the statistical distribution of ALT (disease severity spectrum)								Based on MANPOWER ALT cut-offs			
	Very normal (n=564)		Normal (n=591)		Increased (n=604)		Very increased (n=616)		Increased (n=1,513)		Normal (n=844)	
W	12	24	12	24	12	24	12	24	12	24	12	24
USG abnormality improved, %	65.25	73.76	71.57	80.71	65.89	79.64	75.32	73.21	71.26	77.99	66.59	74.76
No USG improvement, %	30.14	19.86	22.5	12.86	30.79	15.73	21.27	20.13	25.15	16.66	27.84	18.01
Missing, %	4.61	6.38	5.92	6.43	3.31	4.64	3.41	6.66	3.59	5.36	5.57	7.23
	Based on the statistical distribution of AST (disease severity spectrum)								Based on MANPOWER AST cut-offs			
	Very normal (n=613)		Normal (n=565)		Increased (n=639)		Very increased (n=558)		Increased (n=1,141)		Normal (n=1,234)	
W	12	24	12	24	12	24	12	24	12	24	12	24
USG abnormality improved, %	64.27	72.59	69.03	74.16	67.45	81.06	78.49	79.39	73.09	79.75	66.37	74.15
No USG improvement, %	30.18	20.55	26.02	18.41	28.64	13.93	18.82	15.77	23.49	15.16	28.53	18.96
Missing, %	5.55	6.85	4.96	7.43	3.91	5.01	2.69	4.84	3.42	5.08	5.11	6.89
	Based on the statistical distribution of GGT (disease severity spectrum)								Based on MANPOWER GGT cut-offs			
	Very normal (n=407)		Normal (n=465)		Increased (n=456)		Very increased (n=457)		Increased (n=1,063)		Normal (n=722)	
W	12	24	12	24	12	24	12	24	12	24	12	24
USG abnormality improved, %	61.18	70.27	75.05	80.43	71.71	79.82	65.86	73.52	69.71	77.42	67.17	74.38
No USG improvement, %	30.22	24.32	21.29	16.13	25.44	15.57	30.42	19.47	26.72	17.4	26.73	20.64
Missing, %	8.6	5.41	3.66	3.44	2.85	4.61	3.72	7	3.57	5.17	6.09	4.99

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; USG, ultrasonography; W, week.

Supplementary Table 8. Diagnostic accuracy of the main algorithms based on enzymes levels, using physician-diagnosed NASH as the reference standard

	Adjusted OR (95% CI)	p value
Age, years		
)		
18–50	1.33 (1.06, 1.67)	0.014
>50–65	1 (reference)	
Number of comorbidities		
2	1.07 (0.76, 1.49)	0.06258
3	1.87 (1.13, 3.10)	0.0102
4	16.4 (0.68, 3.94)	0.4897
1	1 (reference)	
Diabetes		
Yes	1.61 (0.99, 2.59)	0.0515
No	1 (reference)	
Obesity		
Yes	1.24 (0.86, 1.79)	0.2429
No	1 (reference)	
Hypertension		
Yes	0.86 (0.63, 1.19)	0.373
No	1 (reference)	

CI, confidence interval; NASH, non-alcoholic steatohepatitis; OR, odds ratio.