Plasma ammonia levels predict hospitalisation with liver-related complications and mortality in clinically stable outpatients with cirrhosis

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Definitions of liver-related complications:

Variceal bleeding was defined as bleeding from an oesophageal, gastric, or ectopic varix at the time of endoscopy, or the presence of large varices with blood in the lumen and no other recognisable cause of bleeding.[1]

New onset or worsening ascites was defined as the development or acute progression to grade 2 to 3 ascites, according to the International Ascites Club Classification, within ≤4 weeks.[2] Patients with chronic refractory ascites who presented to hospital frequently for therapeutic paracentesis due to rapid reaccumulation of large ascites were not included in this definition.

Acute hepatic encephalopathy (HE) was defined by the acute development of a change in mental status in a patient with previous normal consciousness and no evidence of an acute neurologic disease.[3] Events were recorded regardless of whether this was the first episode of HE or a new acute episode. Patients with persistent hepatic encephalopathy were not included in this definition.

Although bacterial infections are not an organ specific complication of cirrhosis, they are considered as such in this study due to their high incidence in cirrhotic patients and their associations with the multifaceted disruptions in innate and adaptive immune responses collectively recognised in cirrhosis-associated immune dysfunction.[4] Episodes of infection were defined as clinical signs of infection [fever ≥38°C, leucocytosis (white blood cell count ≥12,000/mm^3) or requiring antibiotic therapy] or positive confirmatory test such as polymorphonuclear count in ascitic/pleural fluid ≥250/mm^3, infiltrates on chest x-ray or positive cultures (urinary, blood, sputum, cutaneous, stool).

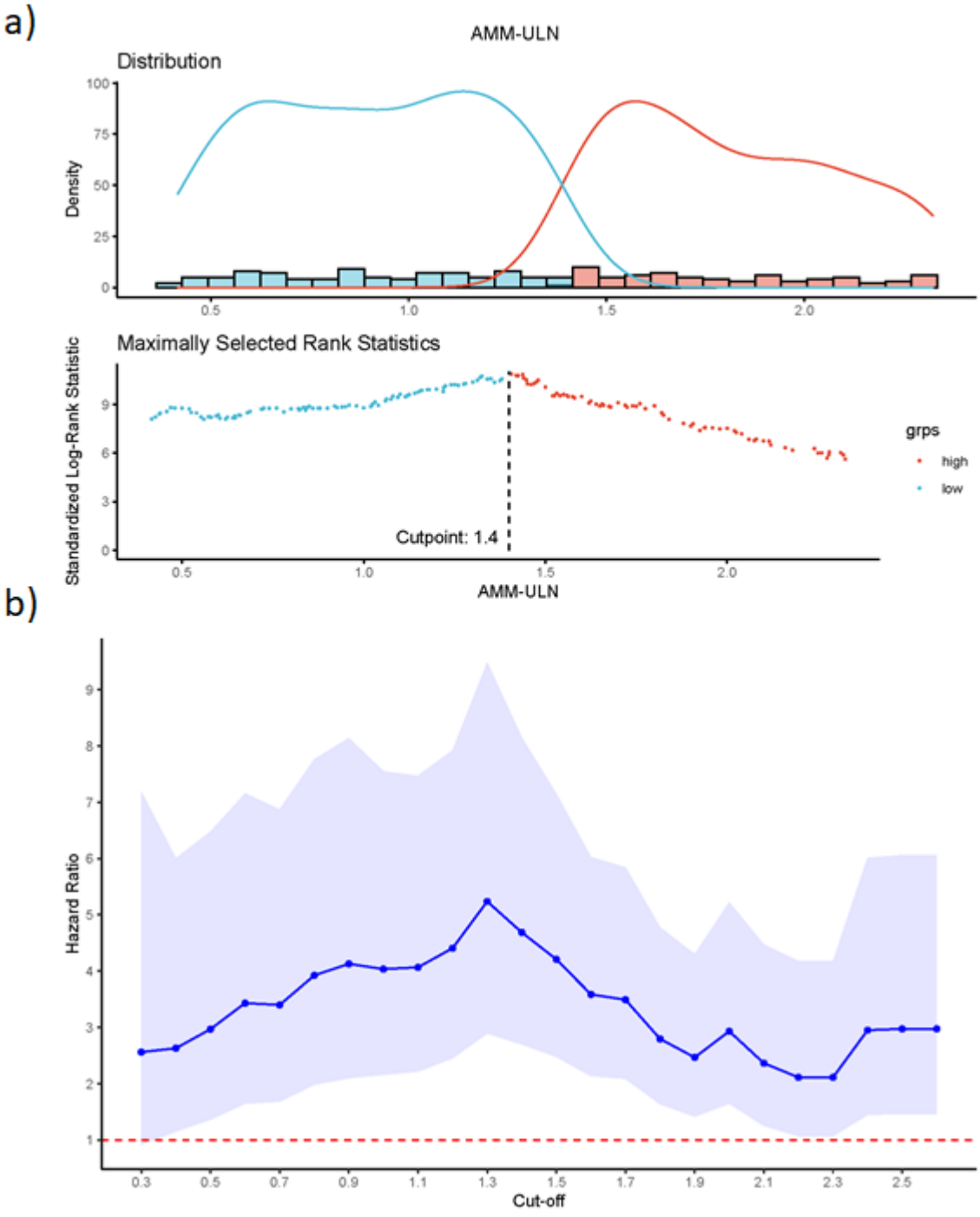

Fig. S1: Defining the optimal cut-off of AMM-ULN a) maximally selected rank statistics in the training set for the primary outcome, development of liver-related complications b) hazard ratio with confidence intervals for each cut-off.

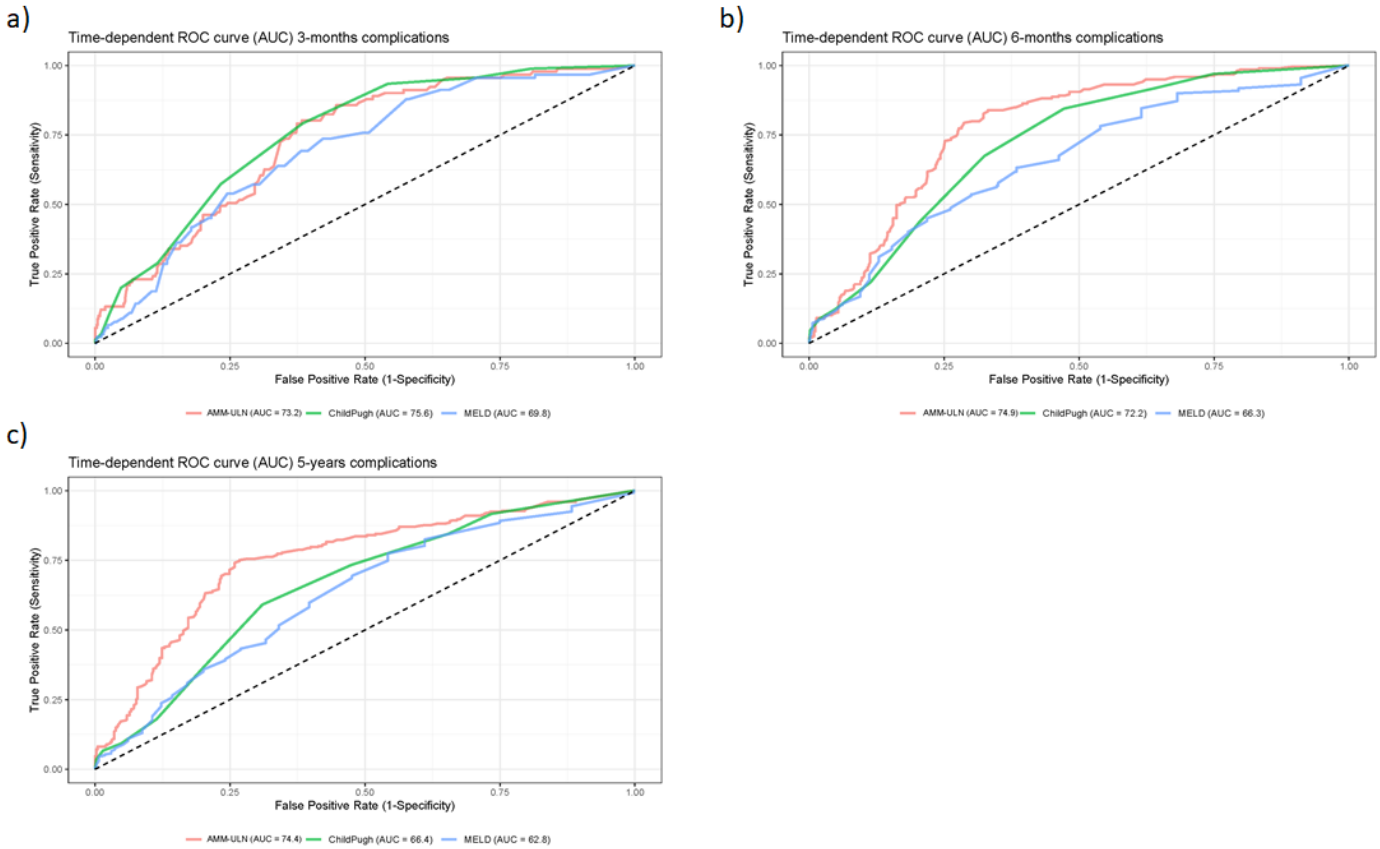

Fig. S2: Time-dependent ROC curve of AMM-ULN, MELD and CP score for the development of liver-related complications. AMM-ULN performed similarly to MELD and CP scores at 3-months (a). However, AMM-ULN demonstrated better predictive accuracy than the MELD score for liver-related complications at (b) 6-months (74.9 vs. 66.3, p=0.015) and (c) both the MELD (74.4 vs. 62.8, p<0.001) and CP (74.4 vs. 66.4, p=0.014) scores at 5-years.

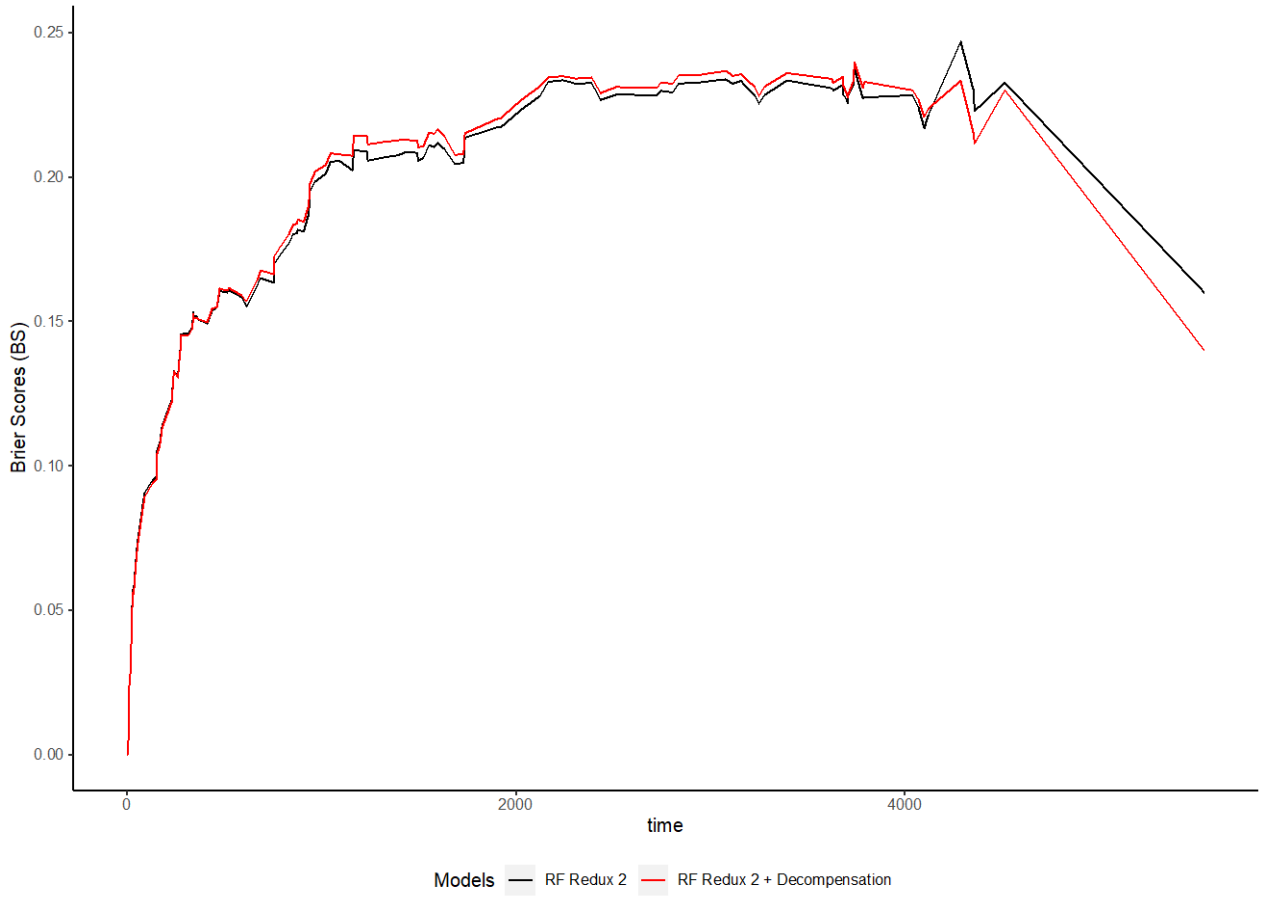

Fig. S3: Brier score comparison of predictive models for random forest (RF) Redux 2 and random forest (RF) Redux 2 plus prior decompensation showing a brier score of 0.216 and 0.218, respectively.

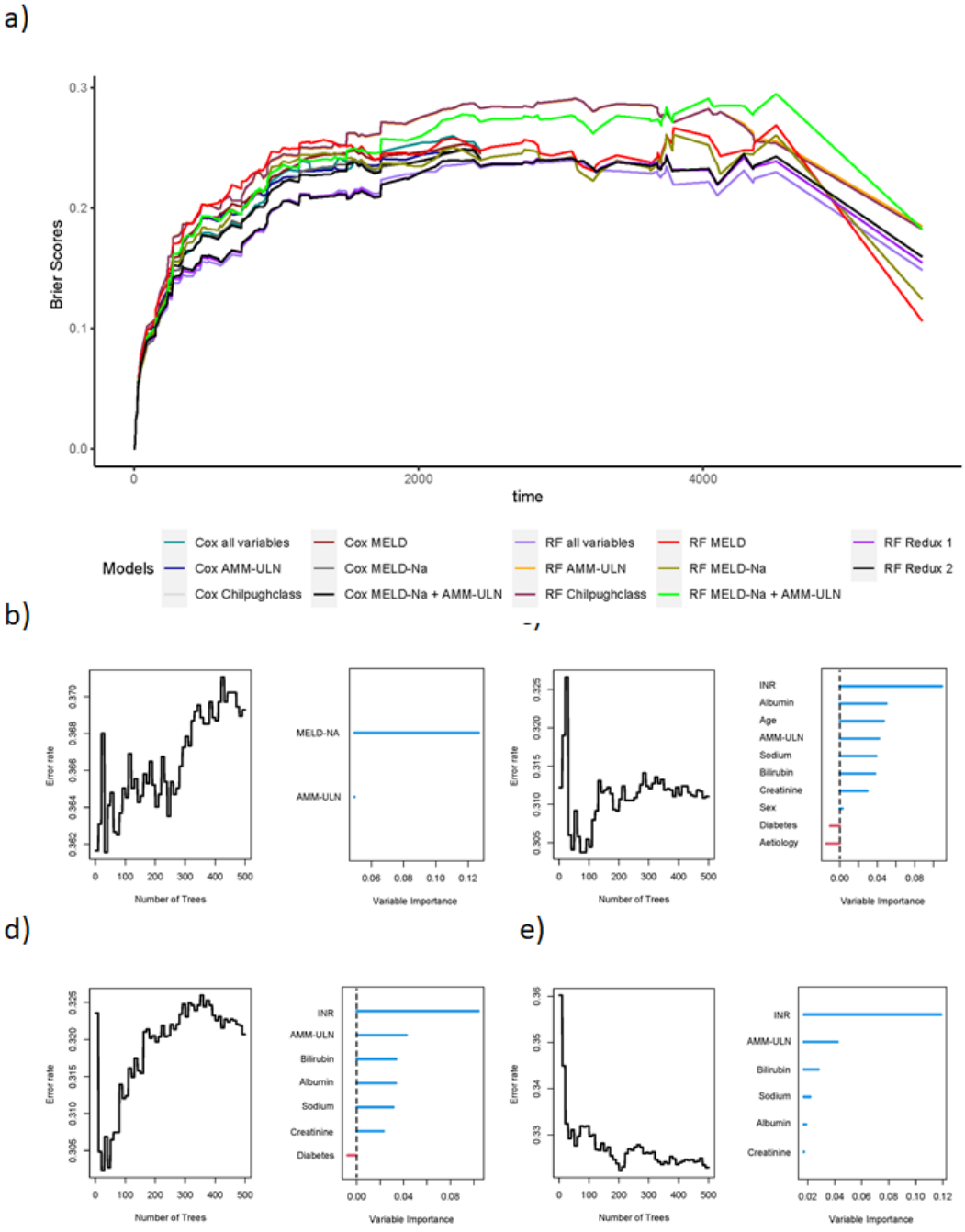

Fig. S4: Brier score comparison of predictive models in the external validation set demonstrating (a) that the random forest (RF) model continued to demonstrate less prediction error than cox regression-derived models over time. AMM-ULN maintained a high variable importance (VIMP) in c) RF with all variables, d) RF Redux 1 and e) RF Redux 2. This is not the case with b) RF with MELD-Na plus AMM-ULN, but error rate is higher than the other models and does not stabilise with 500 trees, indicating poor performance.

Table S1: Comparison of distributions of ammonia (AMM-ULN) measurements with clinical variables.

Parameter	AMM-ULN (mean, SD / r)	p value
Demographic		
Age	-0.03	0.41
Sex		0.362
- Male	1.4 (0.9)	
- Female	1.4 (0.7)	
Disease Aetiology		
ALD	1.4 (0.9)	<0.002
NAFLD	1.6 (0.8)	
Viral hepatitis	1.2 (0.8)	
Autoimmune	1.4 (0.7)	
Other	1.4 (0.8)	
Comorbidities		
Diabetes		<0.001
- Yes	1.5 (0.9)	
- No	1.3 (0.8)	
Co-prescribed medications		
Non-selective β-blockers		0.019
- Yes	1.5 (0.8)	
- No	1.3 (0.9)	
Lactulose		<0.001
- Yes	1.6 (1.0)	
- No	1.2 (0.8)	
Rifaximin		<0.001
- Yes	1.8 (1.0)	
- No	1.2 (0.8)	
Laboratory Parameters		
ALP (iU/mL)	0.07	0.077
AST (iU/mL)	0.07	0.05
ALT (iU/mL)	0.07	0.157
Albumin (g/dL)	-0.32	<0.001
Bilirubin (mg/dL)	0.08	0.024
Creatinine (mg/dL)	0.12	0.001
INR	0.17	<0.001
WBC ($x10^9$/L)	-0.10	0.01
Platelets ($x10^9$/L)	-0.21	<0.001
Sodium (mmol/L)	-0.06	0.1
Disease Severity		
Portal hypertension		<0.001
- No	0.7	
- Yes	1.4	
Decompensated cirrhosis		<0.001
- No	0.9 (0.7)	
- Yes	1.6 (0.8)	
Child Pugh Class		
A	0.9 (0.7)	<0.001
B	1.5 (0.8)	
C	1.6 (0.8)	

MELD-Na	0.25	<0.001
MELD	0.22	<0.001

Analysis of variance (ANOVA) and t-test were performed to compare ammonia distributions between categorical groups, Pearson rank correlations were used to calculate the correlation with continuous clinical variables. **Abbreviations:** AMM-ULN, ammonia level corrected to the upper limit of normal; SD, standard deviation; r, Pearson rank correlation coefficient.

Table S2: Multivariable competing risk frailty models with markers of muscle and nutritional status for the prediction of liver-related complications

	Multivariable models		
	HR	**95% CI**	**p value**
Estimated dry BMI and AMM-ULN			
AMM-ULN	2.42	2.16-2.71	<0.001
Handgrip (male) and AMM-ULN			
AMM-ULN	2.46	2.08-2.92	<0.001
Handgrip (female) and AMM-ULN			
Handgrip	0.95	0.89-1.01	0.123
AMM-ULN	2.30	1.79-2.97	<0.001
Median arm circumference			
AMM-ULN	2.48	2.12-2.87	<0.001
Triceps skin fold thickness			
AMM-ULN	2.51	2.17-2.91	<0.001
Global nutrition score			
AMM-ULN	2.51	2.20-2.87	<0.001

Analysis of nutritional assessment data variables from the KCH cohort (n=447) within multivariable competing risk frailty prognostic models. Variable selection was performed using stepwise forward-backward selection; markers of muscle and nutritional status did not significantly improve the models. Median arm circumference and triceps skin fold thickness categorised and grouped (<5th, 5-10th, 10-25th, 25-50th, 50-75th, 75-90th, 90-95th and >95th centile). Nutritional status score was a categorical variable (well nourished, moderately malnourished, severely malnourished).

Table S3: Univariable and multivariable competing risk frailty modelling for mortality.

	Univariable model			Multivariable model		
	HR	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Demographic						
Age	1.02	1.00-1.04	0.038	1.03	1.005 - 1.05	0.017
Sex	1.09	0.26-2.29	0.650			
Disease Aetiology						
ALD	Ref.	Ref.	Ref.			
NAFLD	1.32	0.74-2.38	0.350			
Viral Hepatitis	0.69	0.42-1.13	0.140			
Autoimmune liver disease	1.15	0.61-2.15	0.660			
Other	1.20	0.59-2.47	0.620			
Comorbidities						
Diabetes mellitus	1.78	1.23-2.59	0.002	1.51	0.94 - 2.44	0.089
Co-prescribed medications						
Non-selective β-blockers	1.08	0.73-1.58	0.710			
Lactulose	1.21	0.80 - 1.84	0.360	0.65	0.39 - 1.08	0.095
Rifaximin	1.88	1.09 - 3.25	0.023			
Laboratory Parameters						
ALP (iU/mL)	1.0003	0.99-1.002	0.740			
AST (iU/mL)	1.01	1.001-1.009	0.005			
ALT (iU/mL)	1.01	1.001-1.01	0.030			
Albumin (g/dL)	0.45	0.33-0.61	<0.001	0.48	0.29 - 0.79	0.004
Bilirubin (mg/dL)	1.04	1.01-1.08	0.020			
Creatinine (mg/dL)	1.52	1.09-2.12	0.013	1.63	1.15 - 2.32	0.006
INR	1.58	1.08-2.30	0.017			
WBC (x10^9/L)	0.99	0.92-1.09	0.960			
Platelets (x10^9/L)	0.99	0.99-0.99	0.051			
Sodium (mmol/L)	0.97	0.95-0.99	0.015			
AMM-ULN	1.73	1.48-2.02	<0.001	1.45	1.20 - 1.76	<0.001
Disease Severity Scores						
Child Pugh class - A - B - C	 Ref. 2.42 4.44	 Ref. 1.39-4.2 2.36-8.36	 Ref. <0.001 <0.001			
MELD Score	1.06	1.03-1.09	<0.001			
Decompensated cirrhosis	2.69	1.97-3.66	<0.001	1.95	1.04-3.66	0.004

Variable selection for the multivariable competing risk frailty model was performed using stepwise forward-backward selection with estimated HR including only original variables within the final model. **Abbreviations:** HR, hazard ratio; CI, confidence interval; ALD, alcohol-related liver disease;

NAFLD, non-alcoholic fatty liver disease; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; WBC, white blood cell count; MELD, model for end-stage liver disease score.

Table S4: Description of patient characteristics according to AMM-ULN-stratified risk-group.

Parameter	High risk (n=328)	Low risk (n=426)	p-value
Demographic			
Age (mean, SD)	56 (10)	56 (12)	0.722
Sex (no. male, %)	205 (63)	293 (69)	0.084
Disease Aetiology [n (%)]			
ALD	137 (42)	162 (38)	0.063
NAFLD	55 (17)	51 (12)	
Viral Hepatitis	64 (20)	118 (28)	
Autoimmune liver disease	49 (15)	61 (14)	
Other	23 (7)	33 (8)	
Comorbidities [n (%)]			
Diabetes mellitus	136 (42)	127 (30)	0.001
Co-prescribed medications [n (%)]			
Non-selective β-blockers	169 (52)	186 (44)	0.037
Lactulose	138 (42)	128 (30)	<0.001
Rifaximin	109 (33)	67 (16)	<0.001
Laboratory Parameters (mean, SD)			
ALP (iU/mL)	183 (136)	171 (166)	0.300
AST (iU/mL)	69 (58)	59 (48)	0.013
ALT (iU/mL)	51 (46)	43 (34)	0.024
Albumin (g/dL)	3.1 (0.6)	3.5 (0.7)	<0.001
Bilirubin (mg/dL)	3.9 (5)	3 (4)	0.006
Creatinine (mg/dL)	1 (0.6)	0.9 (0.3)	0.009
INR	1.6 (0.5)	1.4 (0.4)	<0.001
WBC	5.1 (2.5)	5.4 (2.8)	0.087
Platelets (x10^9/L)	98 (53)	127 (80)	<0.001
Sodium	135.5 (5)	136.3 (5)	0.030
AMM-ULN	2.1 (0.8)	0.9 (0.4)	<0.001
Disease Severity Scores (mean, SD)			
Child Pugh Score	8.8 (2)	7.6 (2)	<0.001
MELD Score	15 (7)	13 (6)	<0.001
MELD-Na	17 (6)	14 (7)	<0.001
Complications [n (%)]			
Transplant	159 (49)	200 (47)	0.732
Liver-related complication	189 (58)	71 (17)	<0.001
Type of Complication [n (%)]			
Ascites	40 (12)	16 (4)	<0.001
Variceal Bleeding	17 (5)	11 (3)	
Infection	91 (28)	32 (8)	
Hepatic Encephalopathy	39 (12)	13 (3)	
Mortality [n (%)]	76 (23)	44 (10)	<0.001

Patients stratified according to AMM-ULN into high-risk (AMM-ULN >1.4) and low-risk (AMM-ULN was $\leq$1.4). **Abbreviations:** HR, hazard ratio; CI, confidence interval; ALD, alcohol-related liver disease; NAFLD, non-alcoholic fatty liver disease; ALP, alkaline phosphatase; AST, aspartate

aminotransferase; ALT, alanine aminotransferase; WBC, white blood cell count; MELD, model for end-stage liver disease score.

Table S5: Description of patient characteristics in the derivation and validation groups.

Parameter	Derivation Cohort (n=754)	Validation cohort (n=130)	Total (n=884)	p value
Demographic				
Age (mean, SD)	56 (11)	58 (10)	56 (11)	0.118
Sex (no. male, %)	498 (66)	97 (75)	595 (67)	0.068
Disease Aetiology [n (%)]				
ALD	300 (40)	68 (52)	368 (42)	<0.001
NAFLD	106 (14)	5 (4)	111 (13)	
Viral Hepatitis	182 (24)	49 (38)	231 (26)	
Autoimmune liver disease	110 (15)	8 (6)	118 (13)	
Other	56 (7)	0 (0)	56 (6)	
Co-morbidity [n (%)]				
Diabetes mellitus	263 (35)	51 (39)	314 (36)	0.397
Laboratory parameters (mean, SD)				
AST (iU/mL)	63 (53)	62 (47)	63 (52)	0.830
ALT (iU/mL)	47 (40)	56 (46)	48 (41)	0.030
Albumin (g/dL)	3.3 (0.7)	3.9 (0.7)	3.4 (0.7)	<0.001
Bilirubin (mg/dL)	3.4 (4.4)	1.8 (1.5)	3.1 (4.2)	<0.001
Creatinine (mg/dL)	0.9 (0.5)	0.9 (0.2)	0.9 (0.4)	0.665
INR	1.5 (0.4)	1.2 (0.3)	1.4 (0.4)	<0.001
WBC (x10^9/L)	5.3 (2.7)	5.6 (2.4)	5.2 (2.7)	0.282
Sodium (mmol/L)	136 (5)	139 (3)	137 (5)	<0.001
AMM-ULN	1.4 (0.8)	0.8 (0.5)	1.3 (0.8)	<0.001
Disease Severity Scores				
Child Pugh Score	8.1 (2.2)	6.4 (1.6)	7.9 (2.2)	<0.001
Child Pugh Group - A - B - C	 197 (26) 346 (46) 207 (28)	 76 (58) 48 (37) 6 (5)	 273 (31) 394 (45) 213 (24)	<0.001
MELD Score	14 (6)	11 (4)	13 (6)	<0.001
MELD-Na Score	15 (7)	11 (4)	15 (7)	<0.001
Complications [n (%)]				
Transplant	359 (48)	11 (9)	370 (42)	<0.001
Liver-related complication	260 (35)	72 (55)	332 (38)	<0.001
Type of Complication [n (%)]				
Ascites	56 (7)	23 (18)	15 (7)	<0.001
Variceal Bleeding	28 (4)	13 (10)	41 (5)	
Infection	123 (16)	15 (12)	138 (16)	
Hepatic Encephalopathy	52 (7)	21 (16)	73 (8)	
Mortality [n (%)]	120 (16)	56 (43)	176 (20)	<0.001

Comparisons were performed between patient cohorts using ANOVA and Kruskal Wallis tests for normally and non-normally distributed data respectively and Chi-Square (χ^2) for categorical data.

Abbreviations: HR, hazard ratio; CI, confidence interval; ALD, alcohol-related liver disease; NAFLD, non-alcoholic fatty liver disease; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; WBC, white blood cell count; MELD, model for end-stage liver disease score

Supplementary references

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