Fecal Cytolysin does not predict disease severity in acutely decompensated cirrhosis and acute-on-chronic liver failure

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Abstract

Background: Cirrhosis with acute decompensation (AD) and acute-on-chronic liver disease (ACLF) are characterized by high morbidity and mortality. Cytolysin, a toxin from *Enterococcus faecalis* (*E. faecalis*), is associated with mortality in alcohol-associated hepatitis (AH). It is unclear whether cytolysin also contributes to disease in AD and ACLF.

Methods: We studied the role of fecal cytolysin in 78 patients with AD/ACLF.

Results: Fecal cytolysin and *E. faecalis* abundance do not predict chronic liver failure (CLIF-C) AD and ACLF scores in AD and ACLF patients, respectively. Presence of fecal cytolysin is not associated with other liver disease markers, including Fibrosis-4 (FIB-4) index, 'Age, serum

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Bilirubin, INR, and serum Creatinine' (ABIC) score, Child-Pugh score, Model for End-Stage Liver Disease (MELD) or MELD-Na scores in AD and/or ACLF patients (including in patients with alcohol-associated etiology only).

Conclusions: In conclusion, fecal cytolysin does not predict disease severity in AD and ACLF patients, including patients with alcohol-associated cirrhosis with AD or ACLF. The predictive value of fecal cytolysin positivity for mortality appears to be restricted to AH.

Keywords

Background

Intestinal dysbiosis, or an imbalance of beneficial and potentially pathogenic microbes [1, 2], is present in several hepatic diseases [3, 4]. Besides fungal [5-8] and viral dysbiosis[9, 10], bacterial dysbiosis plays a central role in the pathogenesis of liver disease [11, 12]. Particularly liver cirrhosis is associated with dysbiosis and increased gut permeability [13, 14]. We recently found that cytolysin, a toxin from *Enterococcus faecalis* (*E. faecalis*), directly lyses hepatocytes in ethanol-induced liver disease (ALD) models [15]. Cytolysin-positive *E. faecalis* is a significant predictor for mortality in alcohol-associated hepatitis (AH) with 89% 180-day mortality in cytolysin-positive AH patients compared with only 3.8% in cytolysin-negative AH patients [15]. It is unclear whether its predictive value is confined to ALD, or AH specifically. Cytolysin-positive *E. faecalis* does not associate with disease severity in nonalcoholic fatty liver disease (NAFLD) [16]. The role of cytolysin-positive *E. faecalis* is unknown in other liver diseases, including cirrhosis with acute decompensation (AD) or acute-on-chronic liver failure (ACLF). The pathophysiology of ACLF is still incompletely understood and it is a difficult-to-treat condition with a very high transplant-free 90-day mortality up to 50% [17]. AH is one of the most common triggers for the development of ACLF and, together with bacterial infection, is present in almost all patients who develop ACLF [18, 19].

We therefore aimed to investigate the role and predictive value of cytolysin in cirrhosis with AD and ACLF. We hypothesized that cytolysin predicts disease severity in AD and ACLF.

Material and methods

Patients

Fecal samples were collected when patients were evaluated for a transjugular intrahepatic portosystemic shunt (TIPS). Inclusion criteria were over 18 years of age with clinical signs of liver cirrhosis and an indication for TIPS insertion. Exclusion criteria were the presence of systemic infection, severe hepatic encephalopathy of unknown cause, severe hyperbilirubinemia, pulmonary hypertension or pregnancy as previously described [20].

Ethics approval and consent to participate

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the human research and ethical committee of the University of Bonn (121/14). Written informed consent was obtained from all patients.

Bacterial DNA extraction and real-time quantitative PCR

Human stool samples were stored at −80°C, DNA from fecal samples was extracted and qPCR performed as described [15]. Primer sequences: *E. faecalis* forward: 5′-CGCTTCTTTCCTCCCGAGT-3′, reverse: 5'-GCCATGCGGCATAAACTG-3'; *E. faecalis* cylLL forward: 5′-CTGTTGCGGCGACAGCT-3′, reverse: 5′-CCACCAACCCAGCCACAA-3′; *E. faecalis* cylLS forward: 5'-GTAAAATAAGTAAAATCAAGAAAACTATTACTC-3', reverse: 5′-CAAAAGAAGGACCAACAAGTTCTAATT-3′ [15, 16].

Statistical analysis

Results are expressed as median and interquartile range in brackets for each continuous outcome and as number and percentage for factor variables, if not stated otherwise. Area under the curve (AUC), best threshold to maximize the Youden Index, sensitivity, specificity, accuracy, precision, positive predictive value, negative predictive value, and *p* value between two AUCs of receiver operating characteristic (ROC) curves per Delong method were calculated using the pROC library in R. Kaplan-Meier curves to visualize survival were created employing the survival library in R. Mean decrease Gini score and mean decrease accuracy were calculated with the randomForest library, and odds ratios with the questionr library in R. Continuous variables were compared using the Mann-Whitney test. Categorical variables were compared using the Pearson's Chi-squared test. A value of $p < 0.05$ was considered to be statistically significant. Statistical analyses were performed using R statistical software, R version 1.3.1093, 2020 the R Foundation for Statistical Computing

Results

Study population with cirrhosis and acute decompensation or acute-on-chronic liver failure

The study population consisted of 78 patients with acutely decompensated cirrhosis (AD, n=63) or acute-on-chronic liver failure (ACLF, n=15) (Table 1). All subjects were enrolled when they received a transjugular intrahepatic portosystemic shunt (TIPS) to treat complications of portal hypertension, most frequently for ascites (47.4%) and variceal bleeding (26.3%) (Table 1). Half of the subjects were male (52.6%), the average age was 56.5 years. 57.7% of all subjects had alcohol-associated liver disease (ALD). Liver cirrhosis was cryptogenic in 9%, due to Hepatitis C virus (HCV) in 6.4%, and mixed in 6.4%. The ACLF patients had a significantly higher Model for End-Stage Liver Disease (MELD) score (medians 16.2 vs 10.2), MELD-Na score (18.2 vs 10.2), European Foundation for the study of chronic liver failure (CLIF-C) organ failure score (8 vs 6), higher grade of hepatic encephalopathy, significantly more frequently renal failure (46.7% vs 0%), circulatory

failure (33.3% vs 1.6%), and respiratory failure (13.3% vs 0%) than AD patients. The CLIF-C AD score median was 48.08 in the AD group and the CLIF-C ACLF score median was 43.83 in the ACLF group. The white blood count (WBC) and serum neutrophils were significantly higher in the ACLF group compared with the AD group (7.55 vs 5.39 leukocytes/mL and 5.47 vs 3.67 neutrophils/mL, respectively). Both cohorts had a similar rate of antibiotic use but ACLF patients had a significantly higher use of rifaximin (40.0% vs 12.7%). However, the groups did not significantly differ regarding fecal cytolysin positivity (ACLF 6.67% vs AD 15.9%) (Table 1). Only one of the 78 patients died within 90 days after enrollment.

Presence of fecal cytolysin does not predict liver disease severity in cirrhosis with acute decompensation or acute-on-chronic liver failure per CLIF-C AD and ACLF scores

Since fecal cytolysin positivity predicts mortality in patients with alcohol-associated hepatitis (AH) [15, 21], we aimed to examine its role in patients with cirrhosis and AD or ACLF. To determine disease severity in patients with acutely decompensated cirrhosis, the CLIF-C AD score was employed, which has a significantly better performance for predicting mortality in AD patients than Child-Pugh score (CPS), MELD, and MELD-Na scores [22]. Similarly, disease severity in patients with ACLF was quantified using the CLIF-C ACLF score, which also has better predictive value in ACLF patients than CPS, MELD, and MELD-Na [23, 24]. To create binary disease measures for the AD and ACLF groups, "High CLIF-C AD" and "Low CLIF-C AD" as well as "High CLIF-C ACLF" and "Low CLIF-C ACLF" were designated using the respective medians as cutoffs (Table 1). In our cohort, fecal cytolysin had a lower performance than all other tested liver disease markers (including Fibrosis-4 index [FIB-4], 'Age, serum Bilirubin, INR, and serum Creatinine' [ABIC] score, CPS, MELD, MELD-Na) in patients with AD with a low area under the curve (AUC) of 0.53 and low best Youden Index of 1.06 compared with the best performer CPS with an AUC of 0.75 and best Youden Index 1.47 (Figure 1A, Table 2). The performance of fecal cytolysin was similar to fecal *E. faecalis* abundance (Figure 1A, Table 2). Likewise, its predictive performance was poor in the ACLF group with an AUC of 0.56 and best Youden Index of 1.12, whereas the ABIC score had a high AUC of 0.83 and best Youden Index of 1.58 (Figure 1B, Table 3).

Next, we used the best threshold that maximized the Youden Index in AD (Table 2) and calculated the odds ratios (ORs) for a high CLIF-C AD score for values exceeding the best threshold for the respective marker. All ORs for high liver disease markers – but not for cytolysin positivity or high *E. faecalis* abundance – were significant per simple logistic regression: cytolysin positivity OR=1.56 (95% confidence interval 0.40-6.69; p=0.53), high *E. faecalis* OR=2.06 (0.66-6.95; p=0.22), high FIB-4 OR=0.21 (0.07-0.62; p=0.006), high ABIC OR=9.92 (2.39-68.18; p=0.005), high CPS OR=8.36 (2.63-30.85; p<0.001), high MELD OR=6.68 (2.16-23.91; p=0.002), and high MELD-Na OR=6.68 (2.16-23.91; p=0.002) (Figure 2A). As high MELD and high MELD-Na had the same values for OR and significance, the MELD/MELD-Na score was only used once for multiple logistic regression. After multiple logistic regression using all markers, high FIB-4 OR=0.05 (95% confidence interval 0.003-0.33; p=0.009), high ABIC OR=37.73 (3.98-1033.18; p=0.007), and high MELD/MELD-Na OR=15.88 (1.88-364.24; p=0.026)

retained significance, whereas cytolysin positivity OR=0.83 (0.10-6.40; *p*=0.86), high *E. faecalis* OR=1.99 (0.26-19.15; *p*=0.52), and high CPS OR=3.19 (0.58-20.22; *p*=0.19) were not significant (Figure 2B). Odds ratios for high CLIF-C ACLF for the ACLF group using the aforementioned markers could not be calculated or were not significant (not shown).

Consistent with the findings above, presence of fecal cytolysin had a low feature importance for high CLIF-C AD and ACLF scores per random forest analysis with a very low mean decrease Gini score and mean decrease accuracy in relation to the other markers in AD (Figure 3A-B) and ACLF (Figure 3C-D).

Other liver disease markers do not depend on fecal cytolysin positivity in cirrhosis with acute decompensation or acute-on-chronic liver failure

Next, we performed simple linear regression with fecal cytolysin as the independent variable and the liver disease markers FIB-4, ABIC, CPS, MELD, and MELD-Na as the dependent outcome variables. None of the liver disease markers were significantly associated with presence of fecal cytolysin in the combined AD and ACLF cohort (Table 4) nor for the AD (Supplementary Table S1) or ACLF cohorts separately (Supplementary Table S2). As fecal cytolysin positivity predicts mortality in patients with AH [15, 21], we further assessed the predictive value of fecal cytolysin in patients with AD or ACLF of our cohort in whom liver disease was alcohol-associated. However, also in this subgroup, none of the liver disease markers were significantly associated with fecal cytolysin (Supplementary Table S3). The cytolysin positivity in that subgroup was 13.3%, similar to 14.1% overall (Table 1). Finally, various liver disease indicators (e.g. MELD score, CPS, but also laboratory markers ALT, AST, GGT, or AP) were not significantly different between fecal cytolysin positive vs fecal cytolysin negative subjects in the combined AD and ACLF cohort (Figure 4), the AD cohort only (Supplementary Figure S1), or in the alcohol-associated combined AD and ACLF cohort (Supplementary Figure S2). Rifaximin use was significantly higher in the ACLF group compared with the AD group (Table 1). To account for possible confounding by Rifaximin, we excluded all patients that were given Rifaximin and evaluated for an association between fecal cytolysin and the various liver disease markers, and again none of the disease measures depended on fecal cytolysin (not shown).

Discussion

We have shown previously that fecal cytolysin positivity predicts mortality in patients with AH [15, 21]. We therefore aimed to investigate its role in patients with cirrhosis and AD or ACLF. However, we found that fecal cytolysin does not predict disease severity in acutely decompensated cirrhosis or ACLF. We have also demonstrated previously that cytolysin positivity is not associated with disease severity in NAFLD [16], which indicates that cytolysin might only confer risk in ALD. However, even if we select subjects with alcohol-associated cirrhosis and AD or ACLF in the current cohort, no liver disease markers were associated with presence of fecal cytolysin. This further supports the idea that cytolysin positivity indicates a higher risk for worse outcome in AH specifically. Even though AH is a major trigger of AD and ACLF, the colonization with *E. faecalis* associated with fecal cytolysin does not appear to be an important mechanism in AD and ACLF. This study shows

once again that AD and ACLF are multifactorial, and besides AH as one of the triggers, other infections are also relevant inducers of AD and ACLF.

Strengths of this study include that this is a well-characterized AD and ACLF cohort. Moreover, the predictive value of fecal cytolysin for disease severity in this cohort was evaluated comprehensively from various angles and the results have been consistent throughout the different methodologies. Limitations of the study include that the studied cohort had overall mild disease with only one dead subject in the first 90 days after enrollment, which appears low in the context of cirrhosis with AD or ACLF, which oftentimes has transplant-free 90-day mortality up to 50% [17]. The low cytolysin positivity of only 14.1% of the overall study population (compared with up to 30% in AH) [15] likely did not contribute to the relatively low mortality, as cytolysin did not associate with any liver disease severity measures in our study cohort. It is possible that higher rifaximin use in the ACLF group might have contributed to a better survival in that group. Rifaximin is known to kill *E. faecalis* [25] and reduces its translocation into the blood stream [26]. However, even after exclusion of Rifaximin-exposed subjects, there was no association between fecal cytolysin and liver disease measures in our cohort.

Conclusion

In conclusion, fecal cytolysin positivity does not indicate higher disease severity in patients with AD and ACLF. Fecal cytolysin appears to exclusively predict worse outcome in AH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Financial support:

Abbreviations:

ACE	angiotensin-converting enzyme
ACLF	acute-on-chronic liver failure

AD	acute decompensation
ALT	alanine aminotransferase
AP	alkaline phosphatase
AST	aspartate aminotransferase
AT2	angiotensin 2
Ca	calcium
CLIF-C	European Foundation for the study of chronic liver failure
CRP	C-reactive protein
FIB-4	Fibrosis-4 Index
GGT	gamma-glutamyltransferase
HCV	hepatitis C virus
HE	hepatic encephalopathy
INR	international normalized ratio
MELD	model for end-stage liver disease
MELD-Na	sodium-adjusted model for end-stage liver disease
NASH	nonalcoholic steatohepatitis
OF	organ failure
PBC	primary biliary cholangitis
PSC	primary sclerosing cholangitis
SBP	spontaneous bacterial peritonitis
TIPS	transjugular intrahepatic portosystemic shunt
WBC	white blood count

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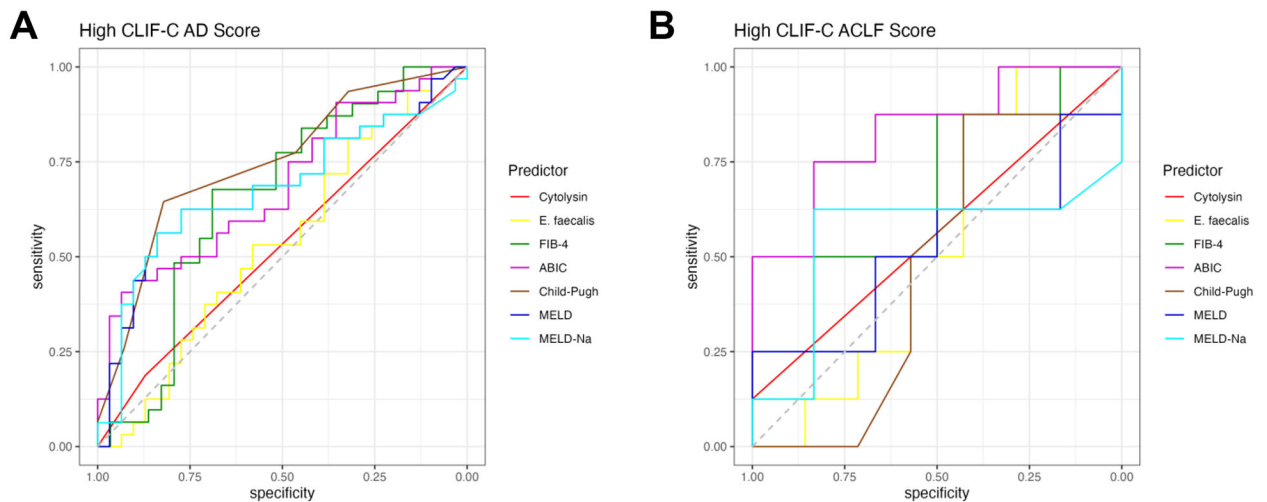
Figure 1. Presence of fecal cytolysin does not predict liver disease severity in cirrhosis with acute decompensation or acute-on-chronic liver failure per CLIF-C AD and ACLF scores.
(**A**) ROC curves of liver disease markers for high CLIF-C AD score in AD patients (Cytolysin n=63, *E. faecalis* n=63, FIB-4 n=60, ABIC n=63, Child-Pugh=59, MELD score n=63, and MELD-Na score n=63). (**B**) ROC curves of liver disease markers for high CLIF-C ACLF score in ACLF patients (Cytolysin n=15, E. faecalis n=15, FIB-4 n=14, ABIC n=14, Child-Pugh=15, MELD score n=14, and MELD-Na score n=14). ABIC, 'Age, serum bilirubin, INR, and serum creatinine score'; ACLF, acute-on-chronic liver failure; AD, acute decompensation; CLIF-C, European Foundation for the study of chronic liver failure; FIB-4, Fibrosis-4 Index; INR, international normalized ratio; MELD, model for end-stage liver disease; MELD-Na, sodium-adjusted model for end-stage liver disease; ROC, receiver operating characteristic.

A

Odds ratio for high CLIF-C AD Score per simple logistic regression

B

Odds ratio for high CLIF-C AD Score per multiple logistic regression

Figure 2. Cytolysin positivity does not confer higher odds ratio for high CLIF-C AD score.
(A-B) Odds ratios for high CLIF-C AD score per **(A)** simple logistic regression and **(B)** multiple logistic regression were calculated for cytolysin positivity and various liver disease markers for values that exceeded the best thresholds per Table 2 (95% confidence interval indicated by black brackets for each marker). Cytolysin n=63, *E. faecalis* n=63, FIB-4 n=60, ABIC n=63, Child-Pugh=59, MELD score n=63, and MELD-Na score n=63. ABIC, 'Age, serum bilirubin, INR, and serum creatinine score'; AD, acute decompensation; CLIF-C, European Foundation for the study of chronic liver failure; FIB-4, Fibrosis-4 Index; INR, international normalized ratio; MELD, model for end-stage liver disease; MELD-Na, sodium-adjusted model for end-stage liver disease. Significance is indicated by *P<0.05, **P<0.01, and ***P<0.001.

A

B

C

D
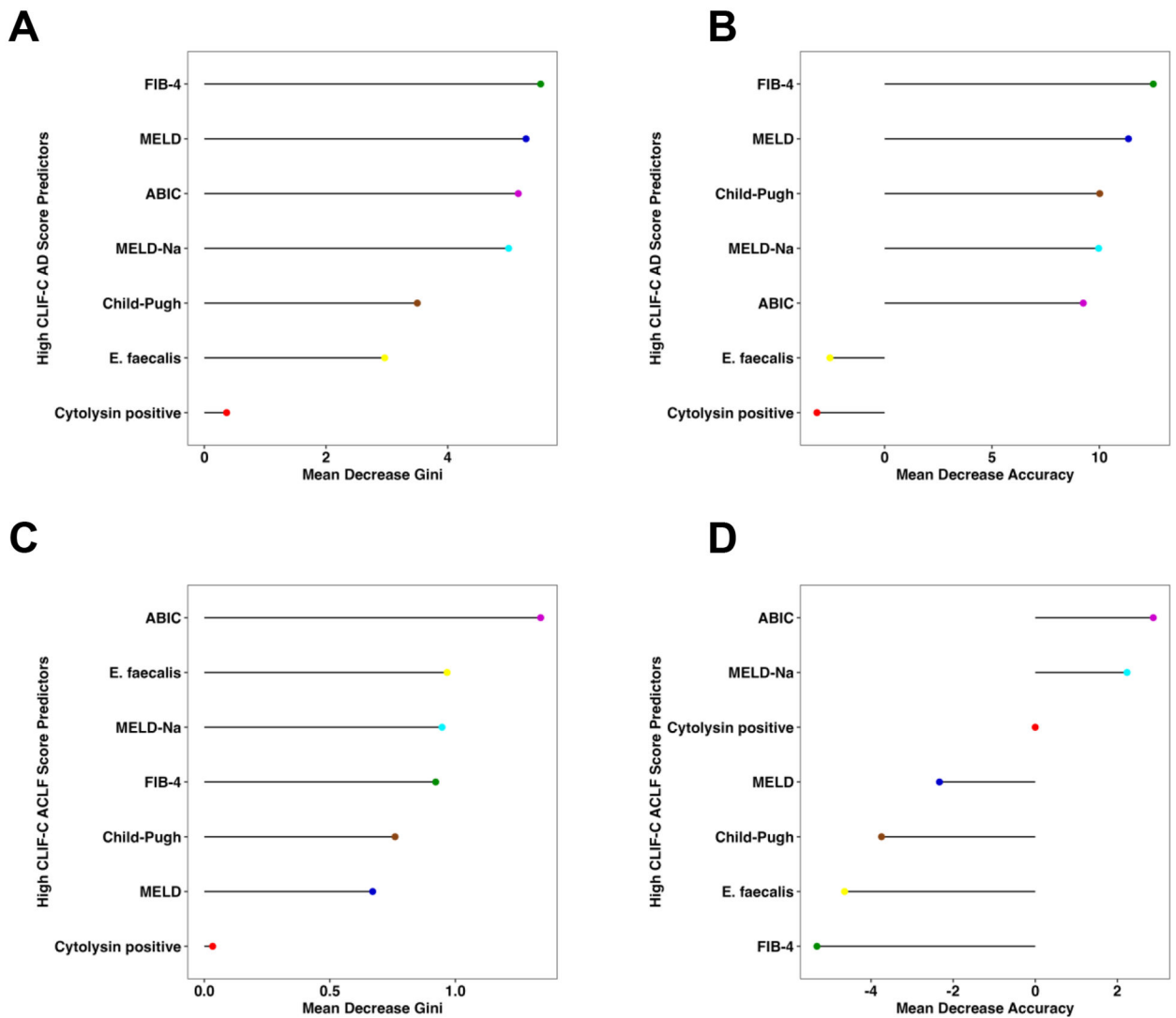
Figure 3. Fecal cytolysin is not an important feature for liver disease severity in cirrhosis with acute decompensation and acute-on-chronic liver failure per random forest analysis.

(**A**) Mean decrease Gini score and (**B**) mean decrease accuracy by random forest analysis were quantitated for presence of cytolysin and multiple liver disease markers to determine their respective feature importance for high CLIF-C AD score (Cytolysin n=63, *E. faecalis* n=63, FIB-4 n=60, ABIC n=63, Child-Pugh=59, MELD score n=63, and MELD-Na score n=63). (**C**) Mean decrease Gini score and (**D**) mean decrease accuracy for high CLIF-C AD score (Cytolysin n=15, E. faecalis n=15, FIB-4 n=14, ABIC n=14, Child-Pugh=15, MELD score n=14, and MELD-Na score n=14). ABIC, 'Age, serum bilirubin, INR, and serum creatinine score'; ACLF, acute-on-chronic liver failure; AD, acute decompensation; CLIF-C, European Foundation for the study of chronic liver failure; FIB-4, Fibrosis-4 Index; INR, international normalized ratio; MELD, model for end-stage liver disease; MELD-Na, sodium-adjusted model for end-stage liver disease.
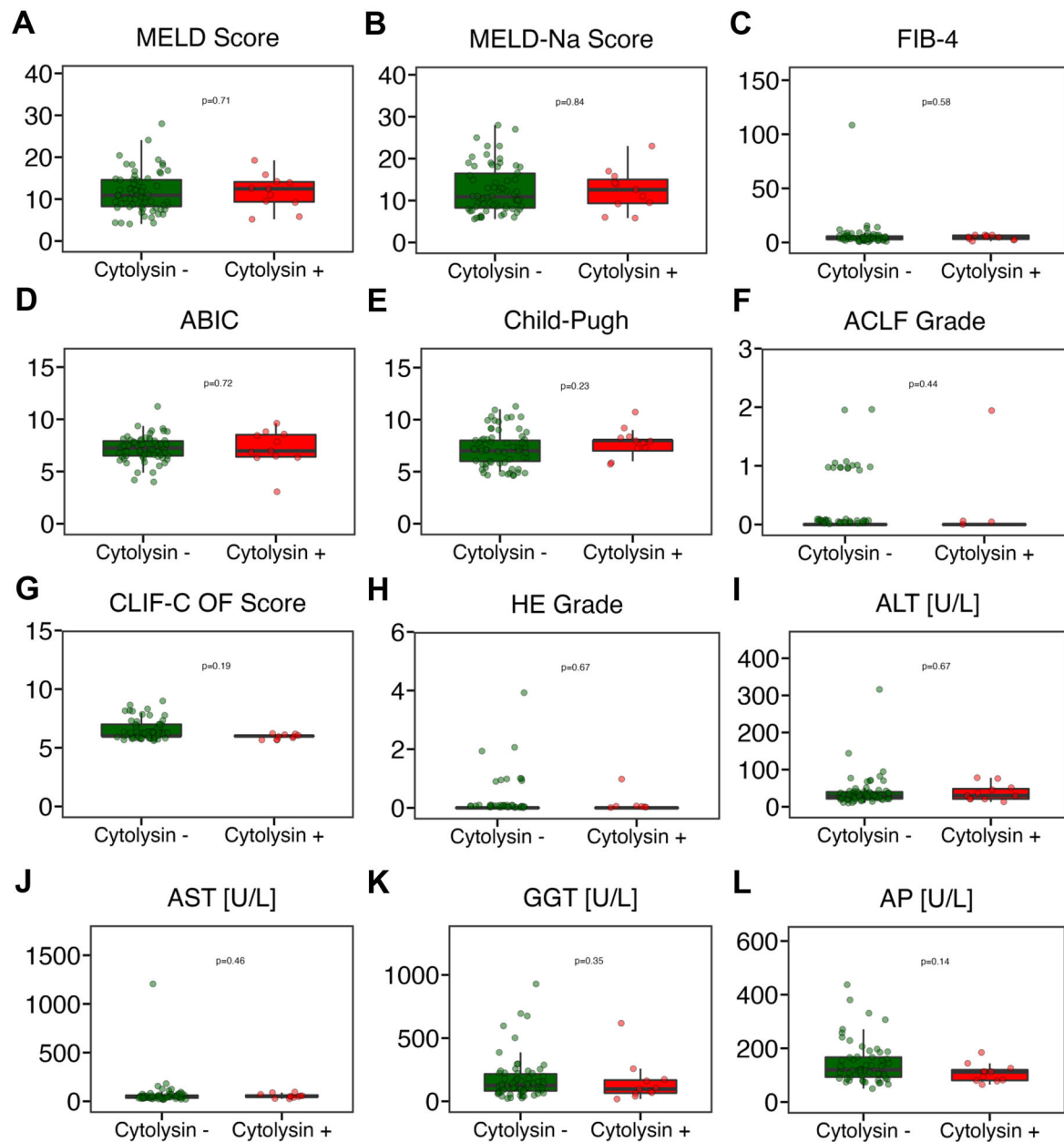
Figure 4. Fecal cytolysin-positive patients with cirrhosis and acute decompensation or acute-on-chronic liver disease do not have more severe liver disease than cytolysin-negative patients.
(**A**) MELD score (n=77). (**B**) MELD-Na score (n=77). (**C**) FIB-4 (n=74). (**D**) ABIC score (n=77). (**E**) Child-Pugh score (n=74). (**F**) ACLF grade (n=78). (**G**) CLIF-C organ failure score (n=78). (**H**) Hepatic encephalopathy (n=78). (**I**) ALT (n=78). (**J**) AST (n=75). (**K**) GGT (n=77). (**L**) AP (n=68). ABIC, 'Age, serum bilirubin, INR, and serum creatinine score'; ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CLIF-C, European Foundation for the study of chronic liver failure; FIB-4, Fibrosis-4 Index; GGT, gamma-glutamyltransferase; HE, hepatic encephalopathy; INR, international normalized ratio; MELD, model for end-stage

liver disease; MELD-Na, sodium-adjusted model for end-stage liver disease; OF, organ failure. *P* value of equal or less than 0.05 was considered as statistically significant.
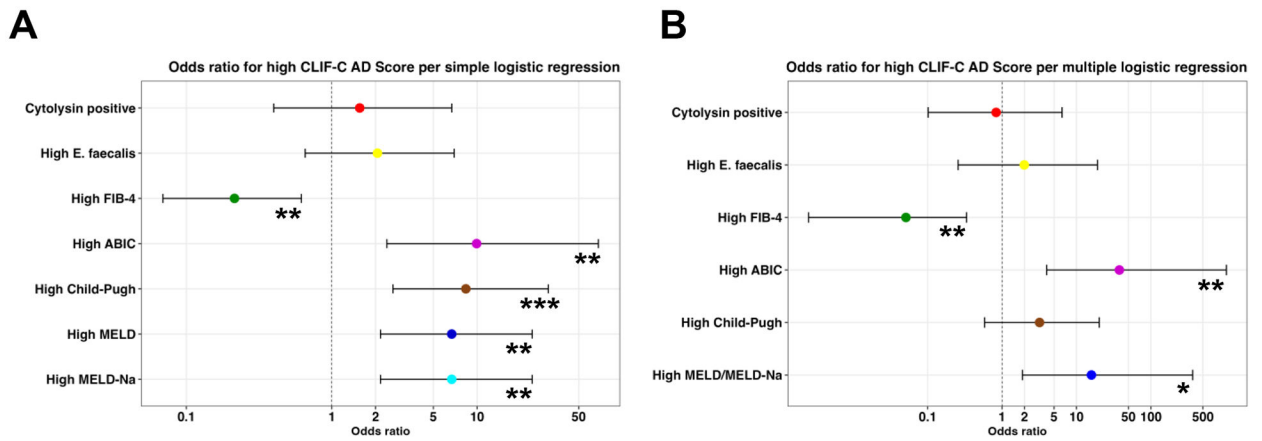
Table 1.

Baseline demographic and laboratory data of the study population.

	N	ALL SUBJECTS	ACUTE DECOMPEN- SATION	ACLF	P VALUE
		N=78	*N=63*	*N=15*	
Gender:	78				0.353
Male		41 (52.6%)	31 (49.2%)	10 (66.7%)	
Age	78	56.5 [51.0;65.0]	56.0 [51.0;65.0]	57.0 [48.5;69.5]	0.770
Etiology:	78				0.127
Alcohol-associated		45 (57.7%)	36 (57.1%)	9 (60.0%)	
Budd-Chiari-Syndrome		2 (2.56%)	0 (0.00%)	2 (13.3%)	
Cryptogenic		7 (8.97%)	6 (9.52%)	1 (6.67%)	
Drug-Induced Liver Injury		2 (2.56%)	1 (1.59%)	1 (6.67%)	
HCV		5 (6.41%)	4 (6.35%)	1 (6.67%)	
Hepatic Sinusoidal Obstruction Syndrome		2 (2.56%)	1 (1.59%)	1 (6.67%)	
Mixed		5 (6.41%)	5 (7.94%)	0 (0.00%)	
NASH		1 (1.28%)	1 (1.59%)	0 (0.00%)	
Other		7 (8.97%)	7 (11.1%)	0 (0.00%)	
PBC/PSC		2 (2.56%)	2 (3.17%)	0 (0.00%)	
Child-Pugh	74	7.00 [6.00;8.00]	7.00 [6.00;8.00]	8.00 [6.00;9.50]	0.292
MELD	77	11.0 [8.53;14.6]	10.2 [7.99;12.7]	16.2 [12.6;17.8]	**0.001**
MELD-Na	77	11.0 [8.53;16.0]	10.2 [7.99;13.6]	18.2 [15.9;22.0]	**<0.001**
CLIF-C OF Score	78	6.00 [6.00;7.00]	6.00 [6.00;6.00]	8.00 [7.50;8.50]	**<0.001**
ACLF Grade:	78				**<0.001**
0		63 (80.8%)	63 (100%)	0 (0.00%)	
1		12 (15.4%)	0 (0.00%)	12 (80.0%)	
2		3 (3.85%)	0 (0.00%)	3 (20.0%)	
CLIF-C AD Score	63	48.08 [43.52;51.42]	48.08 [43.52;51.42]	-	
CLIF-C ACLF Score	15	43.83 [38.80;46.18]	-	43.83 [38.80;46.18]	
HE Grade:	78				**0.003**
0		68 (87.2%)	59 (93.7%)	9 (60.0%)	
1		7 (8.97%)	3 (4.76%)	4 (26.7%)	
2		2 (2.56%)	1 (1.59%)	1 (6.67%)	
3		0 (0.00%)	0 (0.00%)	0 (0.00%)	
4		1 (1.28%)	0 (0.00%)	1 (6.67%)	
Ascites Grade:	78				0.568
0		22 (28.2%)	17 (27.0%)	5 (33.3%)	
1		20 (25.6%)	18 (28.6%)	2 (13.3%)	
2		33 (42.3%)	25 (39.7%)	8 (53.3%)	
3		3 (3.85%)	3 (4.76%)	0 (0.00%)	
SBP:	78				0.826
In past		6 (7.69%)	5 (7.94%)	1 (6.67%)	

	N	ALL SUBJECTS	ACUTE DECOMPEN-SATION	ACLF	P VALUE
No		67 (85.9%)	53 (84.1%)	14 (93.3%)	
Yes		5 (6.41%)	5 (7.94%)	0 (0.00%)	
Liver Failure:	78				0.192
Yes		1 (1.28%)	0 (0.00%)	1 (6.67%)	
Renal Failure:	78				**<0.001**
Yes		7 (8.97%)	0 (0.00%)	7 (46.7%)	
Cerebral Failure:	78				0.350
Yes		2 (2.56%)	1 (1.59%)	1 (6.67%)	
Coagulation Failure:	78				-
Yes		0 (0.00%)	0 (0.00%)	0 (0.00%)	
Circulatory Failure:	78				**0.001**
Yes		6 (7.69%)	1 (1.59%)	5 (33.3%)	
Respiratory Failure:	78				**0.035**
Yes		2 (2.56%)	0 (0.00%)	2 (13.3%)	
TIPS indication:	57				0.499
Ascites		27 (47.4%)	23 (51.1%)	4 (33.3%)	
Ascites + Hydropic decompen-sation		4 (7.02%)	2 (4.44%)	2 (16.7%)	
Ascites + Pleural effusion		1 (1.75%)	1 (2.22%)	0 (0.00%)	
Ascites + Portal vein thrombosis		1 (1.75%)	1 (2.22%)	0 (0.00%)	
Hydropic decompen-sation		1 (1.75%)	1 (2.22%)	0 (0.00%)	
Other		1 (1.75%)	0 (0.00%)	1 (8.33%)	
Portal vein thrombosis		2 (3.51%)	2 (4.44%)	0 (0.00%)	
Variceal bleeding		15 (26.3%)	11 (24.4%)	4 (33.3%)	
Variceal bleeding + Ascites		4 (7.02%)	3 (6.67%)	1 (8.33%)	
Variceal bleeding + Pleural effusion		1 (1.75%)	1 (2.22%)	0 (0.00%)	
Liver Transplantation	77	2 (2.60%)	2 (3.23%)	0 (0.00%)	1.000
90-day Survival:	78				1.000
Alive		77 (98.7%)	62 (98.4%)	15 (100%)	
WBC [/mL]	78	6.17 [4.52;8.78]	5.39 [4.20;7.94]	7.55 [6.38;11.6]	**0.019**
Neutrophils [/mL]	57	4.05 [3.04;6.41]	3.67 [2.79;5.65]	5.47 [4.56;7.22]	**0.045**
CRP [mg/dL]	67	1.00 [0.57;2.70]	0.96 [0.53;2.70]	1.38 [0.78;2.10]	0.601
Hemoglobin [g/dL]	78	10.6 [9.10;12.2]	10.5 [9.35;12.2]	11.1 [8.35;12.2]	0.800
Platelets [10^9/L]	77	134 [90.0;184]	134 [90.8;195]	116 [65.5;158]	0.348
ALT [U/L]	78	29.5 [21.0;40.8]	29.0 [21.0;39.0]	30.0 [22.5;44.0]	0.590
AST [U/L]	75	47.0 [34.5;62.0]	47.0 [35.0;61.0]	47.5 [36.0;71.2]	0.892
GGT [U/L]	77	120 [75.0;199]	115 [73.0;180]	150 [102;240]	0.232
AP [U/L]	68	118 [88.0;162]	117 [88.0;157]	133 [106;169]	0.553
Bilirubin [mg/dL]	78	1.14 [0.82;1.93]	1.10 [0.78;1.66]	1.58 [0.97;2.50]	0.085
Albumin [g/dL]	71	3.10 [2.72;3.90]	3.06 [2.76;3.69]	3.61 [2.65;4.02]	0.508
INR	78	1.20 [1.10;1.30]	1.20 [1.10;1.30]	1.20 [1.10;1.30]	0.781
Sodium [mmol/L]	78	138 [136;141]	139 [136;141]	138 [132;142]	0.554

	N	ALL SUBJECTS	ACUTE DECOMPEN-SATION	ACLF	P VALUE
Creatinine [mg/L]	77	0.99 [0.79;1.38]	0.96 [0.78;1.30]	1.56 [0.82;2.30]	0.082
Dialysis	78	1 (1.28%)	0 (0.00%)	1 (6.67%)	0.192
Anticoagulation	58	48 (82.8%)	37 (80.4%)	11 (91.7%)	0.670
Statins	58	4 (6.90%)	1 (2.17%)	3 (25.0%)	**0.025**
Beta-Blockers	58	32 (55.2%)	29 (63.0%)	3 (25.0%)	**0.042**
Spirono-lactone	58	38 (65.5%)	31 (67.4%)	7 (58.3%)	0.734
Furosemide	58	11 (19.0%)	10 (21.7%)	1 (8.33%)	0.429
ACE Inhibitors	58	4 (6.90%)	4 (8.70%)	0 (0.00%)	0.571
AT2 Inhibitors	58	2 (3.45%)	1 (2.17%)	1 (8.33%)	0.374
Ca Inhibitors	58	3 (5.17%)	1 (2.17%)	2 (16.7%)	0.106
Antibiotics	78	31 (39.7%)	22 (34.9%)	9 (60.0%)	0.136
Rifaximin	78	14 (17.9%)	8 (12.7%)	6 (40.0%)	**0.023**
Lactulose	58	34 (58.6%)	28 (60.9%)	6 (50.0%)	0.527
Terlipressin	57	7 (12.3%)	5 (11.1%)	2 (16.7%)	0.630
Human Albumin	58	27 (46.6%)	21 (45.7%)	6 (50.0%)	1.000
Vaso-pressors	78	7 (8.97%)	2 (3.17%)	5 (33.3%)	**0.002**
Cytolysin:	**78**				**0.681**
Positive		**11 (14.1%)**	**10 (15.9%)**	**1 (6.67%)**	

Values are presented as median and interquartile range in brackets. The number of subjects for which data were available is indicated in the second column. Continuous variables were compared using the Mann-Whitney test. Categorical variables were compared using the Pearson's Chi-squared test. Level for statistical significance is $P<0.05$. Significant *p* values are indicated in bold. ACE, angiotensin-converting enzyme, ACLF, acute-on-chronic liver failure; AD, acute decompensation; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; AT2, angiotensin 2; Ca, calcium; CLIF-C, European Foundation for the study of chronic liver failure; CRP, C-reactive protein; FIB-4, Fibrosis-4 Index; GGT, gamma-glutamyltransferase; HCV, hepatitis C virus; HE, hepatic encephalopathy; INR, international normalized ratio; MELD, model for end-stage liver disease; MELD-Na, sodium-adjusted model for end-stage liver disease; NASH, nonalcoholic steatohepatitis; OF, organ failure; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; SBP, spontaneous bacterial peritonitis; TIPS, transjugular intrahepatic portosystemic shunt; WBC, white blood count.

Table 2.

High CLIF-C AD Score Predictors

Marker	AUC	Threshold	Youden	Sens	Spec	Acc	Prec	PPV	NPV	*p* value vs Cyt. AUC
Cytolysin	**0.53**	positive	1.06	0.19	0.87	0.52	0.60	0.60	0.51	1.000
E. faecalis	**0.54**	0.51	1.14	0.81	0.32	0.57	0.55	0.55	0.62	0.899
FIB-4	**0.66**	3.99	1.37	0.68	0.69	0.68	0.70	0.70	0.67	0.167
ABIC	**0.69**	7.95	1.34	0.41	0.94	0.67	0.87	0.87	0.60	0.061
Child-Pugh	**0.75**	7.50	1.47	0.65	0.82	0.73	0.80	0.80	0.68	**0.003**
MELD	**0.68**	11.64	1.40	0.56	0.84	0.70	0.78	0.78	0.65	0.059
MELD-Na	**0.68**	11.81	1.40	0.56	0.84	0.70	0.78	0.78	0.65	0.074

The best threshold was determined to maximize the youden index (= sensitivity + specificity) for each marker. Cytolysin n=63, *E. faecalis* n=63, FIB-4 n=60, ABIC n=63, Child-Pugh=59, MELD score n=63, and MELD-Na score n=63. ABIC, 'Age, serum bilirubin, INR, and serum creatinine score'; acc, accuracy; AD, acute decompensation; AUC, area under the curve; CLIF-C, European Foundation for the study of chronic liver failure; Cyt, cytolysin; FIB-4, Fibrosis-4 Index; INR, international normalized ratio; MELD, model for end-stage liver disease; MELD-Na, sodium-adjusted model for end-stage liver disease; NPV, negative predictive value; PPV, positive predictive value; pre, precision; sens, sensitivity; spec, specificity.

Table 3.

High CLIF-C ACLF Score Predictors

Marker	AUC	Threshold	Youden	Sens	Spec	Acc	Prec	PPV	NPV	*p* value vs Cyt. AUC
Cytolysin	**0.56**	positive	1.12	0.12	1.00	0.53	1.00	1.00	0.50	1.000
E. faecalis	**0.54**	7.69	1.30	0.88	0.43	0.67	0.64	0.64	0.75	0.536
FIB-4	**0.65**	3.10	1.38	0.88	0.50	0.71	0.70	0.70	0.75	0.646
ABIC	**0.83**	7.42	1.58	0.75	0.83	0.79	0.86	0.86	0.71	0.833
Child-Pugh	**0.47**	9.50	1.30	0.88	0.43	0.67	0.64	0.64	0.75	0.473
MELD	**0.52**	21.22	1.25	0.25	1.00	0.57	1.00	1.00	0.50	0.521
MELD-Na	**0.52**	17.35	1.46	0.62	0.83	0.71	0.83	0.83	0.62	0.552

The best threshold was determined to maximize the youden index (= sensitivity + specificity) for each marker. Cytolysin n=15, *E. faecalis* n=15, FIB-4 n=14, ABIC n=14, Child-Pugh=15, MELD score n=14, and MELD-Na score n=14. ABIC, 'Age, serum bilirubin, INR, and serum creatinine score'; acc, accuracy; ACLF, acute-on-chronic liver failure; AUC, area under the curve; CLIF-C, European Foundation for the study of chronic liver failure; Cyt, cytolysin; FIB-4, Fibrosis-4 Index; INR, international normalized ratio; MELD, model for end-stage liver disease; MELD-Na, sodium-adjusted model for end-stage liver disease; NPV, negative predictive value; PPV, positive predictive value; pre, precision; sens, sensitivity; spec, specificity.

Table 4.

Simple linear regression of fecal cytolysin with liver disease markers for combined cohort

Outcome	Variable	Estimate	2.5 %	97.5 %	*p* value
FIB-4	Cytolysin	−1.68	−9.82	6.46	0.68
ABIC	Cytolysin	0.03	−0.81	0.88	0.93
Child-Pugh	Cytolysin	0.58	−0.52	1.68	0.30
MELD	Cytolysin	0.11	−2.95	3.16	0.95
MELD-Na	Cytolysin	−0.02	−3.63	3.59	0.99

Cytolysin n=78, FIB-4 n=74, ABIC n=77, Child-Pugh=74, MELD score n=77, and MELD-Na score n=77. ABIC, 'Age, serum bilirubin, INR, and serum creatinine score'; FIB-4, Fibrosis-4 Index; INR, international normalized ratio; MELD, model for end-stage liver disease; MELD-Na, sodium-adjusted model for end-stage liver disease.