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Assessment of intestinal inflammation via fecal calprotectin for early prediction of adverse outcomes in advanced chronic liver disease

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Abstract

Background and aims: Intestinal inflammation assessed by fecal calprotectin (F-CAL) in advanced chronic liver disease (ACLD) may represent an early sign of intestinal barrier dysfunction. We aimed to explore the usefulness of F-CAL testing in ACLD in the prediction of adverse outcomes (AO, death, or LT) and refinement of prognostic stratification.

Patients and methods: We explored the RH7 cirrhosis registry comprising consecutive hospitalized patients and a control group with data on disease phenotype, demographics, anthropometrics, prognostic indices, and medication. The F-CAL was evaluated on admission and reported in multiples of the upper limit of normal or terciles. Predictive power was tested in the Cox model for AO over 180 days. Additional risk refinement by F-CAL was tested for both groups.

Results: We enrolled 263 cases in the study group with a median age of 57.2 years, M/F ratio 167/96, with alcohol, metabolic dysfunction-associated steatotic liver disease, MetALD, and viral etiologies in 72.2%, 9.1, 8.0, 3.4%. The median F-CAL was 3.92 × ULN. The control group comprised 108 cases. The adjusted Cox model confirmed F-CAL (hazard ratio [HR] = 1.05, $p < 0.001$) and F-CAL terciles (HR = 1.413, $p = 0.009$) as independent predictors of AO. F-CAL terciles had higher predictive accuracy in CLIF-C-AD<50 (HR = 2.49, $p = 0.013$) and Child stages A and B (HR = 1.706, $p = 0.025$), in whom high F-CAL (cut-off >11 × ULN) could identify patients having 2–3 times higher risk of AO. This approach has been validated in the control group.

Conclusion: Among hospitalized patients with ACLD, F-CAL values were independently proportional to the risk of AO, particularly in early disease stages when high F-CAL values could refine prognostic stratification.

KEYWORDS
advanced chronic liver disease, fecal calprotectin, liver cirrhosis, liver transplantation, metabolic dysfunction associated steatotic liver disease

INTRODUCTION

Advanced chronic liver disease (ACLD) poses a significant burden on healthcare systems in Europe.[1] In Central Europe, a stable high prevalence of end-stage liver disease has a substantial share of premature mortality.[2] Alleviating this burden remains a major unmet need of public health policies. One of the pathogenetic factors involved in the progression of ACLD is bacterial translocation (BT). It is defined as the passage of viable bacteria from the lumen of the digestive tract into mesenteric lymph nodes, extraintestinal organs, and circulation.[3] There is evidence that BT increases the risk of acute infections[4] while chronic BT with activation of immunocompetent cells gradually leads to immune system exhaustion.[5] There are several described mechanisms of BT,[6] including a change in intestinal microbiota (dysbiosis), intestinal blood congestion in portal hypertension, and bacterial overgrowth, all facilitating the passage of bacteria and their components into structures of the intestinal wall.[6] The local inflammatory response serves as a gatekeeper for translocated elements reaching the bloodstream. Intestinal inflammation may thus represent an early pathogenetic link between the disruption of the intestinal barrier, BT, and its downstream effects.[7,8]

Fecal calprotectin (F-CAL) is a calcium- and zinc-binding complex making 45%–60% of human neutrophil granulocytes, activated macrophages, and monocyte proteins. Fecal calprotectin is a sensitive marker of intestinal inflammation and is used in clinical practice in the differential diagnosis of chronic diarrhea and monitoring of inflammatory bowel diseases (IBD).[9,10] In ACLD, there is evidence for increased calprotectin concentrations in serum, stool, and ascites.[11] It has been demonstrated that F-CAL is increased in patients with cirrhosis, particularly in those with hepatic encephalopathy,[12] spontaneous bacterial peritonitis,[13] or other infections.[14] Several studies have also suggested its serum component as a valid marker of short- to mid-term survival.[14,15] Despite the initial reports and the convenience of use, F-CAL has yet to gain sufficient attention from liver specialists. To bring more evidence to the field, we seized the opportunity offered by the RH7 cirrhosis registry[16] and aimed to explore the usefulness of testing F-CAL in the management of ACLD patients. We aimed to prospectively analyze F-CAL in hospitalized patients, explore its predictive potential as a surrogate marker of adverse outcomes, refine the definition of at-risk patients, and validate it in an independent control group.

Key summary

Summarize the established knowledge on this subject

- Fecal calprotectin (F-CAL) is an established marker of intestinal inflammation.
- F-CAL is substantially increased in patients with cirrhosis compared with healthy subjects. In some studies, it correlated with stages of hepatic encephalopathy, and markers of disease severity (Child-Pugh-Turcotte score and MELD).
- In a recent report of 30 advanced chronic liver disease (ACLD) patients, F-CAL was directly proportional to liver disease severity, significantly higher in non-survivors, and was associated with the onset of sepsis.
- However, F-CAL has not gained much attention from liver specialists due to insufficient evidence for its usefulness in clinical practice.

What are the significant and/or new findings of this study?

- From our RH7 cirrhosis registry, we explored data from 263 patients having a single F-CAL essay at baseline and a 180-day follow-up.
- We bring evidence that F-CAL is an independent predictor of adverse outcomes (AO) defined as liver transplantation or death, and the probability of AO is directly proportional to F-CAL values.
- The predictive power of F-CAL was most relevant in the subgroup of patients with early disease stage: CLIF-C AD <50, Child-Pugh-Turcotte stages A and B, MELD ≤15, or no refractory ascites.
- In early disease stages, high F-CAL values (>11) identified additional high-risk patients having a window of opportunity for upfront treatment strategies. This approach was validated in an independent control group.

PATIENTS AND METHODS

The RH7 cirrhosis registry was established in 2014 and is prospectively registering all consecutive adult patients hospitalized at the Department of Hepatology, Gastroenterology and Transplantation (HEGITO, Center1) for acute or chronic ACLD complications or consideration of liver transplantation.[17,18] The registry data include main ACLD complications leading to index hospitalization (e.g., acute alcohol-associated hepatitis, bleeding into the digestive tract, encephalopathy, ascites ...), and other diagnosed conditions, such as infection. RH7 data also include basic demographics and anthropometrics, blood parameters, and prognostic indices (CLIF-C AD score, MELD-Na score, Child-Pugh-Turcotte stage). The qualified nurse captures data into the registry from the patient's source documentation and the medical information system. Since 2019, patients from other centers have been added to the registry. For this study, we included patients hospitalized at the University Hospital Center (Center 2). For all included cases, we also recorded treatment status with a beta-adrenergic blocker, lactulose, or rifaximin on admission and discharge.

We started investigating F-CAL at Center 2 on 1 January 2019, and in the Center 1 (RH7 registry) on 17 August 2021. We included patients registered until 4 April 2023, ensuring a 180-day follow-up.

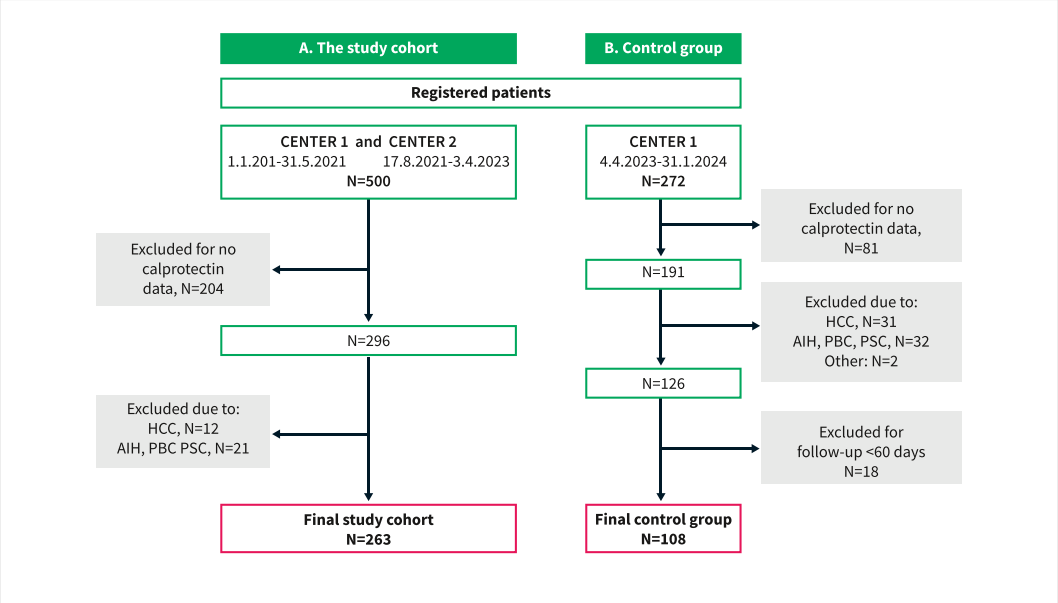

FIGURE 1 Flowchart of the included advanced chronic liver disease patients from the RH7 registry, (a) the study cohort, (b) control group.

For the control group, we included patients registered after the completion of the study cohort starting from 5 April 2023 until 31 January 2024, ensuring a minimum of 60-day follow-up.

We excluded patients with missing data on calprotectin, patients with hepatocellular carcinoma, autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cholangitis, and those with severe systemic disease of any organ system and life expectancy inferior to 6 months. Figure 1 displays the flowchart of patient recruitment.

Grading of ascites was defined according to the Child-Pugh-Turcotte scoring system. Hepatic encephalopathy (HE) was graded according to the West-Haven classification. Patients with no overt encephalopathy (HE stage 0) and a number connection test (NCT) time≥ 120 s were also considered as having HE. We defined early stages of liver disease using the CLIF-C AD score cut-off <50 from the PREDICT study,[19] MELD-Na score ≤15,[20] Child-Pugh-Turcotte (CPT) score stages A and B from the Padua model,[21] and by the absence of refractory ascites.

Stool samples for calprotectin essay were collected during the first days of hospital stay, and patients and physicians were instructed not to take/prescribe non-steroidal anti-inflammatory drugs before the sampling. The stool was collected in a Buhlman Calex tube and sent to the laboratory as soon as possible. Standardized automated calprotectin essays of routine practice were used in both hospitals. Center 1 used the essay Alegria® Calprotectin by Orgentech GmbH, Mainz, Germany, while Center 2 the Calprolab ELISA (ALP) CALPO170, by CALPRO AS, Lysaker, Norway. Different upper limits of normal were defined for stool calprotectin in the two centers, 50 μg/g in Center 1 and 80 μg/g in Center 2. For more convenience, F-CAL values in the results section are reported in multiples of the upper limit of normal (ULN).

After the index hospitalization, patients enrolled in the registry continued to be clinically monitored in the respective facilities or local hospitals according to the plan at discharge. During follow-up, we recorded the occurrence of the first event, which could be liver transplantation or death, or if such an event did not occur, the follow-up was censored at 180 days in the study cohort, and at 120 days in the control group. Adverse outcomes were defined as death from any cause or liver transplantation within the first 180 days (study group) or 120 days (control group). The survival status of the registry patients was verified in the national registry of deceased inhabitants. Liver transplantation is currently performed in a single center in Slovakia, which is the center involved in this study (Center 1).

Statistical analysis

Parameters that did not show a normal distribution are presented as medians and 25–75 percentiles, categorical variables as numbers, and percentages of the total (Table 1). Numerical values of F-CAL in multiples of the ULN were compared by the Spearman rank correlation, values between two categories by Mann-Whitney test, and more categories by Kruskal-Wallis test and Jonckheere-Terpstra test for trend (Figure 2). The predictive potential of F-CAL was determined in the Cox model in a univariate model and two multivariate models (Table 2). The former was adjusted for possible confounding factors and the latter copied the Padua model[21] with CLIF-C AD score, C reactive protein, and Child-Pugh-Turcotte score. Adjusted survival probability curves from the former model were plotted for F-CAL terciles (Figure 3). We also tested the former model on the above-mentioned subgroups of early disease (Table S1). In search for predictive F-CAL cut-offs for 90-day and 180-day adverse outcomes, we used the receiver operating characteristic (ROC) analysis by DeLong et al. We explored the most convenient and the pre-defined forced specificity cut-offs (Table 3). To bring the results of our study closer to clinical practice, we explored the rate of adverse outcomes in validated disease

TABLE 1 Summary statistics and characteristics of the study cohort and control group of advanced chronic liver disease patients.

Parameter		Study cohort N = 263 Median [25–75P] or N (%)	Control group N = 108 Median [25–75P] or N (%)
Age, years		57.22 [46.52, 64.16]	55.33 [45.80, 62.84]
Sex, *n* (%)	Male	167 (63.5)	69 (63.9)
	Female	96 (36.5)	39 (36.1)
Body mass index, kg%m^2		25.95 [23.39, 30.42]	27.00 [23.86, 30.48]
Tricipital skinfold, mm		14.00 [9.00, 20.00]	10.50 [6.75, 17.70]
Midarm circumference, cm		26.00 [23.00, 29.25]	26.50 [24.00, 30.00]
Handgrip, kg		23.93 [16.57, 32.50]	24.24 [17.04, 33.80]
Cirrhosis etiology, *n* (%)	Alcohol	190 (72.2)	67 (62.6)
	Cryptogenic	11 (4.2)	9 (8.4)
	MASLD	24 (9.1)	6 (5.6)
	MET-ALD	21 (8.0)	17 (15.9)
	Secondary biliary	8 (3.0)	2 (1.9)
	Viral hepatitis	9 (3.4)	6 (5.6)
Principal cause for admission, *n* (%)	Hepatic encephalopathy	23 (9.9)	0 (0)
	Variceal bleeding	33 (14.2)	8 (7.4)
	Acute alcoholic hepatitis	42 (18.1)	35 (32.4)
	Elective variceal ligation	16 (6.9)	7 (6.5)
	Ascites	97 (41.8)	29 (26.9)
	Evaluation	21 (9.1)	29 (26.9)
Number connection test, s		85.00 [64.00, 130.00]	91.00 [72.0, 127.50]
Hepatic encephalopathy, *n* (%)	NCT >120s, and or WH stage >0	152 (57.8)	45 (41.7)
Infection during hospital stay, *n* (%)		64 (24.3)	30 (27.8)
Refractory ascites, *n* (%)		84 (32.3)	27 (25.0)
Child-Pugh-Turcotte stage, *n* (%)	A	10 (3.9)	12 (11.2)
	B	114 (44.7)	45 (42.1)
	C	131 (51.4)	50 (46.7)
CLIF-C AD score		52.25 [46.97, 59.37]	53.22 [46.81, 61.20]
MELD-Na score		21.00 [16.00, 26.00]	21.00 [14.50, 27.00]
Serum albumin, mg/L		28.90 [24.89, 32.00]	31.00 [26.75, 35.00]
White blood cells, ×10.9/L		7.60 [4.79, 12.02]	7.60 [4.88, 10.35]
Neutrophil/lymphocyte ratio		4.71 [2.76, 8.55]	4.30 [2.83, 7.89]
C reactive protein, mg/L		17.76 [8.10, 42.96]	12.48 [5.78, 35.30]
Beta-adrenergic blocker therapy (admission/discharge), *n* (%)		139 (53.1)/150 (59.1)	
	Dose equivalent (admission/discharge)	1.0/1.0 [0.5–1.00]	
Lactulose therapy (admission/discharge), *n* (%)		70 (33.8)/128 (50)	
Rifaximin therapy (admission/discharge), *n* (%)		95 (36.1)/149 (58.2)	
Fecal calprotectin, multiples of ULN		**3.92 [1.15, 11.78]**	**3.83 [1.67, 13.54]**
Length of hospital stay, days		9.00 [6.00, 15.00]	9.00 [6.00, 15.00]

TABLE 1 (Continued)

Parameter		Study cohort N = 263 Median [25–75P] or N (%)	Control group N = 108 Median [25–75P] or N (%)
Mortality, *n* (%)	In hospital	31 (11.8)	7 (6.5)
	30 days	43 (16.3)	13 (12.0)
	90 days	73 (27.8)	25 (23.1)
	180 days	99 (37.6)	
Event during follow-up, *n* (%)		180 days	60–120 days
	None	164 (62.4)	82 (75.9)
	Liver transplantation	17 (6.5)	2 (1.9)
	Death	82 (31.2)	24 (22.2)

Note: Calprotectin concentration - the main topic of the paper, is highlighted and marked in bold.

Abbreviations: ALD, alcohol-associated liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; NCT, number connection test; West-Haven (WH).

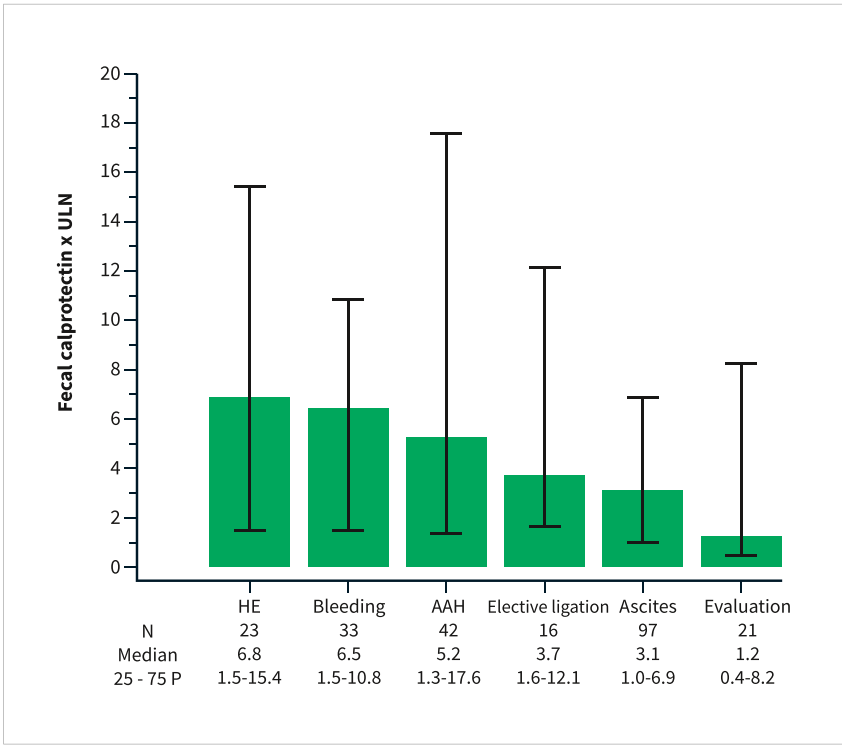

FIGURE 2 Comparison of fecal calprotectin concentration in multiples of the upper limit of normal among different causes of baseline hospital admission. AAH, acute alcoholic hepatitis; HE, overt hepatic encephalopathy; Kruskal–Wallis test, $p = 0.082$, Jonckheere–Terpstra trend test, $p = 0.005$.

stratification categories defined by the CLIF-C AD score <50, the CPT stages, and added a new category with a specific F-CAL cut-off (Table 4). We identified two distinct risk groups according to the 180-day mortality threshold of 15%: low-risk and high-risk groups. Kaplan Meier curves for the risk groups were plotted and compared by the log-rank test (Figure 4).

Statistically significant differences were defined by the probability of the null hypothesis being inferior to 0.05. The analysis was carried out using the software package MedCalc v. 20 (www.medcalc.org), R statistics (www.r-foundation.com), R-commander, and EZR package.[22]

Ethical considerations

This non-interventional study was conducted according to the proceedings of the Declaration of Helsinki. The subjects signed informed consent when registering for the registry. The protocol was approved

TABLE 2 Univariate and multivariate analysis for predictors of adverse outcomes in the study cohort over 6 months of follow-up.

	Univariate		Multivariate, $N = 254$		The Padua model + F-CAL[b]	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Age, years[a]	1.015 (0.9978–1.033)	0.087				
Female sex, *n* (%)[a]	1.042 (0.693–1.565)	0.844				
Body mass index, kg%m^2[a]	1.036 (1.004–1.068)	0.028				
Tricipital skinfold, mm[a]	0.989 (0.967–1.013)	0.384				
Midarm circumference, cm[a]	1.002 (0.959–1.046)	0.938				
Handgrip, kg[a]	0.955 (0.936–0.974)	<0.001				
Hepatic encephalopathy (NCT >120 s, WH stage >0), *n* (%)[a]	2.771 (1.76–4.364)	<0.001				
Infection during hospital stay, *n* (%)	1.604 (1.045–2.464)	0.031				
Refractory ascites, *n* (%)[a]	1.722 (1.151–2.578)	0.008				
CLIF-C AD score[a]	1.085 (1.066–1.106)	<0.001	1.068 (1.046–1.091)	<0.001	1.071 (1.049–1.094)	<0.001
MELD-Na score	1.094 (1.062–1.127)	<0.001				
Child-Pugh-Turcotte (CPT) score[a]	1.294 (1.174–1.426)	<0.001			1.123 (1.007–1.254)	0.038
Serum albumin, mg/L[a]	0.905 (0.872–0.939)	<0.001	0.951 (0.912–0.991)	0.017		
White blood cells, ×10.9/L	1.049 (1.026–1.073)	<0.001				
Neutrophil/lymphocyte ratio[a]	1.057 (1.038–1.075)	<0.001				
C reactive protein, mg/L[a]	1.007 (1.002–1.011)	0.003				
Beta-adrenergic blocker therapy on discharge, *n* (%)[a]	0.619 (0.41–0.936)	0.023				
Lactulose therapy on discharge, *n* (%)	2.557 (1.653–3.957)	<0.001				
Rifaximin therapy on discharge, *n* (%)	2.259 (1.427–3.578)	<0.001				
Fecal calprotectin, multiples of ULN[a]	1.05 (1.022–1.079)	<0.001	1.05 (1.02–1.081)	<0.001	1.041 (1.013–1.069)	0.004
Fecal calprotectin terciles	1.414 (1.105–1.811)	0.006				
C-index			0.742 (0.695–0.789)		0.739 (0.693–0.786)	

Abbreviations: HR, hazard ratio; NCT, number connection test; ULN, upper limit of normal; West-Haven (WH).

[a]Variables included in the multivariate model.

[b]F-CAL, fecal calprotectin in multiples of the ULN.

by the local ethics committee in both participating institutions, and RH7 is a registered trial in the clinicaltrials.gov (NCT04767945). Ethical committees approved the data registry, data processing, data storage, and publishing of the results under the condition of complete anonymity of study participants. Data are available from the corresponding author upon request.

RESULTS

The summary statistics and characteristics of the study cohort and control group are displayed in Table 1. The study group comprised 263 patients with a median age of 57.2 years and a male to female ratio of 167/96. The main ACLD etiology was alcohol-associated liver disease (ALD) in 72.2%, followed by metabolic dysfunction-associated steatotic liver disease (MASLD) in 9.1%, combined etiology with MASLD and alcohol consumption (Met-ALD) in 8.0%, and viral hepatitis in 3.4%. The main cause of hospitalization was ascites in 41.8%, followed by acute alcohol-associated hepatitis in 18.2%, variceal bleeding in 14.2%, and hepatic encephalopathy in 9.9%. Overall, 9.1% of patients were hospitalized for pretransplant evaluation having no such complication at baseline. On admission, 84 patients (32.3%) fulfilled the criteria for refractory ascites, 152 (57.8%) for hepatic encephalopathy (overt or covert), and 64 (24.3%) cases were diagnosed with infection during the hospital stay. The median MELD score was 21, and the CLIF-C AD score 52.3. The median length of hospital stay was 9 days, 17 patients underwent liver transplantation within 180 days. During follow-up, adverse outcomes (death or liver transplantation) occurred in 37.6% of cases. The median F-CAL concentration was 3.92 × ULN. The control group comprised 108 cases with a median F-CAL of 3.83 × ULN.

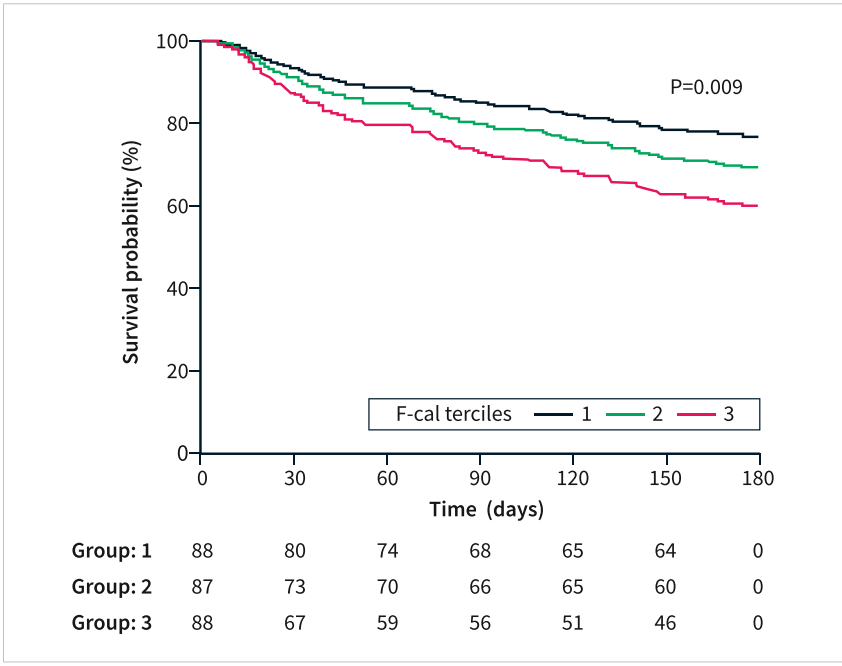

FIGURE 3 Probability of adverse outcomes plotted by terciles of baseline fecal calprotectin derived from the Cox model in the study group ($N = 263$). For F-CAL terciles HR = 1.413 (95% CI 1.09–1.832, $p = 0.009$). The final model was adjusted for CLIF-C AD score (HR = 1.075, 95% CI 1.053–1.097, $p < 0.001$), and hepatic encephalopathy (HR = 1.79, 95% CI 1.119–2.885, $p = 0.015$). Harrell's C-index = 0.736 (95% CI 0.687–0.786). F-CAL, fecal calprotectin. hazard ratio (HR)

F-CAL and disease phenotype

Patients hospitalized for overt hepatic encephalopathy, bleeding, and acute alcoholic hepatitis had the highest F-CAL values with medians exceeding 5 × ULN. The lowest F-CAL values were observed among patients hospitalized for pre-transplant evaluation or ascites (Figure 2). There was a statistically significant downward trend in the F-CAL values among the groups, but differences between the groups were not statistically significant ($p = 0.08$). F-CAL values were lower in patients with refractory ascites (2.92, 25–75P 1.05–6.19 vs. 5.1, 1.22–13.85, $p = 0.029$) and higher in patients with diagnosed infection during hospitalization (7.93, 25–75P 3.28–17.75 vs. 3.01, 0.91–7.76, $p < 0.001$). F-CAL values correlated with serum CRP (rho = 0.154, $p = 0.012$), MELD (rho = 0.141, $p = 0.022$), but not with white blood cell count (rho = 0.113, $p = 0.068$), neutrophil to lymphocyte ratio (rho = 0.078, $p = 0.207$), CLIF-C AD score (rho = 0.091, $p = 0.14$), or the CPT score (rho = 0.044, $p = 0.489$).

F-CAL and prediction of adverse outcomes

Results of the univariate analyses for relevant covariates from the Cox model are displayed in Table 2. The first stepwise multivariate model yielded three independent covariates: CLIF-C AD score (hazard ratio [HR] = 1.068 95% CI 1.046–1.091), serum albumin (HR = 0.951, 0.912–1.991), and numerical F-CAL (1.05, 1.02–1.081). Results of the same model with F-CAL terciles (Figure 3) yielded three independent covariates: CLIF-C AD score (HR = 1.075 95% CI 1.053–1.097), hepatic encephalopathy (HR = 1.79, 1.119–2.885), and F-CAL terciles (1.413, 1.09–1.832). We also tested the Padua model including CLIF-C AD score, CPT score, CRP, and F-CAL yielding three independent covariates: CLIF-C AD score (HR = 1.071 95% CI 1.049–1.094), CPT score (HR = 1.123, 1.007–1.254), and numerical F-CAL (1.041, 1.013–1.069). All parameters of the multivariate models and survival probability plots for F-CAL terciles are displayed in Table 2 and Figure 3.

In the control group, the model yielded two independent covariates: third F-CAL tercile HR = 2.257 (95% CI 1.04–4.899, $p = 0.04$), and CLIF-C AD score (HR = 1.021, 95% CI 1.003–1.041, $p \leq 0.027$). The survival probability plot for F-CAL terciles (1 and 2 vs. 3) is displayed in Figure S1.

F-CAL prediction of adverse outcomes at 90 and 180 days from the ROC analysis showed the area under the receiver operating curve (AUROC) = 0.607 (95% CI 0.545–0.666) and 0.601 (0.539–0.661). Cut-offs for 90 days and 180 days were >18.3 and 3.2 × ULN, with sensitivity, specificity, positive and negative predictive values at 26, 93.7, 61.3, 76.7 (90-days) and 64.7, 54.3, 46, and 71.8 (180-days) (Table 3).

F-CAL as an early marker

To explore F-CAL as an early prognostic marker, we used the former Cox model in several subgroups. F-CAL terciles were independent predictors of adverse outcomes in subgroups with CLIF-C AD score

TABLE 3 Prognostic performance of fecal calprotectin among 263 advanced chronic liver disease patients and subgroups.[a]

	N	Prevalence	Youden index	AUROC	95% CI	Cut-off	Sensitivity	Specificity	PPV	NPV	Fixed specificity	Sensitivity	Cut-off
All patients	263												
Mortality 90 days		27.80%	0.197	0.607	0.545–0.666	>18.3	26	93.7	61.3	76.7	80	32.900	>12.5
Mortality 180 days		37.60%	0.189	0.601	0.539–0.661	>3.2	64.7	54.3	46	71.8	80	33.330	>11.7
MELD ≤15	60												
Mortality 90 days		15.00%	0.418	0.735	0.605–0.841	>8.9	55.6	83.3	41.7	91.7	80	55.600	>7.8
Mortality 180 days		25.00%	0.467	0.773	0.646–0.871	>8.0	60	86.7	60	86.7	80	60.000	>6.8
CLIF-C AD score <50	104												
Mortality 90 days		7.69%	0.487	0.758	0.664–0.836	>14.3	62.5	86.46	27.8	96.5	80	62.500	>12.3
Mortality 180 days		17.30%	0.517	0.755	0.661–0.834	>3.2	88.9	62.8	33.3	96.4	80	50.000	**>11.1**
CLIF-C AD score ≥50	159												
Mortality 90 days		40.90%	0.167	0.586	0.506–0.664	>17.6	23.1	93.6	71.4	63.8	80	26.150	>13.6
Mortality 180 days		50.90%	0.152	0.553	0.472–0.631	>8.6	38.3	76.9	63.3	54.5	80	28.400	>12.2
Child ≤9	124												
Mortality 90 days		16.90%	0.336	0.676	0.586–0.757	>5.3	71.4	62.1	27.8	91.4	80	52.400	>9.6
Mortality 180 days		28.20%	0.239	0.635	0.544–0.720	>7.8	48.57	75.3	43.6	78.8	80	40.000	>9.6
No refractory ascites	179												
Mortality 90 days		24.00%	0.353	0.709	0.636–0.774	>3	86.1	49.3	34.9	91.8	80	41.860	>13.6
Mortality 180 days		32.40%	0.33	0.691	0.608–0.774	>3.2	79.3	53.7	45.1	84.4	80	39.660	>13.1

Note: The best cut-off value of fecal calprotectin used later in analyses and suggested for clinical practice, is highlighted in bold.
Abbreviations: AUROC, area under the receiver operating curve; NPV, negative predictive value; PPV, positive predictive value.
[a]ROC function according to DeLong et al.

TABLE 4 Probability of adverse outcomes (%) over 180-day of follow-up, green fields: low risk, orange fields: high risk.

	Child-Pugh-Turcotte A		Child-Pugh-Turcotte B		Child-Pugh-Turcotte C	
	F-CAL ≤11	F-CAL >11	F-CAL ≤11	F-CAL >11	F-CAL ≤11	F-CAL >11
(A) Study cohort, *N* = 263						
CLIF-C AD <50	9.1		13.6	43.75	9.1	18.2
CLIF-C AD ≥50			35	43.8	52	75
(B) Control group, *N* = 108						
CLIF-C AD <50	0	0	13.3	20	0	50
CLIF-C AD ≥50		50	31.6	33.3	19.23	46.7

Note: F-CAL, fecal calprotectin in multiples of the upper limit of normal. Percentages lower than 15% are marked as low risk of adverse events (green), and those above 15% as high risk (red).

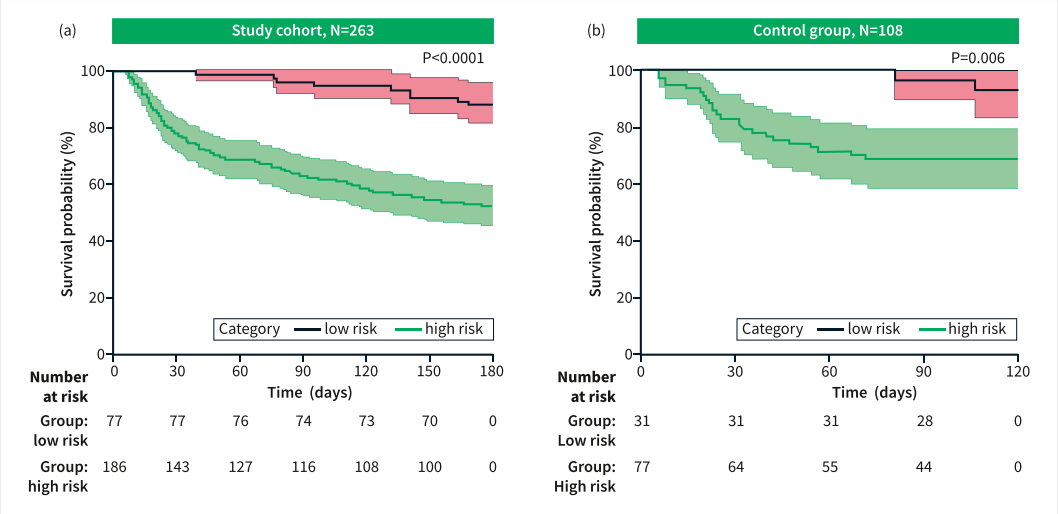

FIGURE 4 Comparison of Kaplan–Meier curves for the probability of LT-free survival between high- and low-risk groups of advanced chronic liver disease patients. (a) The study cohort, chi-squared = 30.618, $p < 0.0001$, HR = 3.2133 (95% CI 2.125–4.859), (b) control group, chi-squared = 7.476, $p = 0.006$, HR = 3.1462 (95% CI = 1.383–7.155). HR, hazard ratio.

<50 (F-CAL terciles HR = 2.485 95% CI 1.214–5.086, $p = 0.013$), and MELD score≤15 (F-CAL terciles HR = 2.789, 1.262–6.164, $p = 0.011$). In contrast, F-CAL terciles did not predict the outcomes in cases with CLIF-C AD≥50 and MELD score >15. F-CAL terciles were also independent predictors of adverse outcomes in subgroups with CPT ≤9 (F-CAL terciles HR = 1.706, 1.069–2.721, $p = 0.025$), and without refractory ascites (F-CAL terciles HR = 1.763, 1.218–2.552, $p = 0.003$). In contrast, F-CAL terciles did not predict the outcomes in cases with CPT score >9 or refractory ascites. Details of the models are displayed in Table S1.

F-CAL prediction of adverse outcomes from the ROC analysis in the subgroups is displayed in Table 3. Generally, the predictive power of F-CAL was higher in the subgroups: MELD ≤15 (AUROC = 0.773, 95% CI 646–0.871), CLIF-C AD score <50 (AUROC = 0.755, 95% CI 0.661–0.834), and no refractory ascites (AUROC = 0.691, 95% 0.608–0.774) compared with the entire cohort (AUROC = 0.601, 95% CI 0.539–0.661).

F-CAL in clinical practice

Finally, we explored the rates of adverse outcomes by prognostic stratification categories validated in clinical practice: CLIF-C AD score (<50, ≥50), and CPT stages (A, B, C). To these, we added a new category of a specific F-CAL cut-off >11 × ULN derived from Table 3. Results for 12 theoretical categories are displayed in Table 4A. The risk of adverse outcomes lower than 15% was considered low-risk, and the remaining was considered high-risk. The pattern was compared to the control group (Table 4B). Overall, 77 (29.3%) patients in the study group and 31 (28.7%) in the control group were identified as being low-risk. Kaplan–Meier curves for the overall probability of survival without adverse outcomes are compared between high-risk and low-risk groups in Figure 4. The differences between the groups were statistically significant (A. Study group, HR = 3.21, 95% CI 2.125–4.859, $p < 0.0001$; and B. Control group, HR = 3.146, 95% CI 1.383–7.155, $p = 0.006$).

DISCUSSION

The principal findings

Among 263 hospitalized ACLD patients from two centers, we confirmed intestinal inflammation by an increased F-CAL of 3.9 times the ULN. The highest F-CAL values were observed in patients with hepatic encephalopathy, bleeding, and alcoholic hepatitis. During follow-up, the risk of adverse outcomes was independently and directly proportional to F-CAL values or terciles. Notably, F-CAL more accurately predicted the outcomes in early disease stages defined by low CLIF-C AD, MELD, CPT, or no refractory ascites. Thus, high F-CAL values with the identified cut-off could serve in clinical practice for refinement of their risk stratification. This approach was validated in a control group.

Serum calprotectin in ACLD

Calprotectin testing in cirrhosis has been proposed as a marker of neutrophil activation. Plasma calprotectin was first reported as a prognostic marker independent of the severity of liver disease in patients with alcohol-induced cirrhosis by Homann et al.[14] The same group reported that its prognostic significance was only relevant for alcohol-induced cirrhosis but not for other etiologies (2003).[23] In a recent report by Matiollo et al.,[15] serum calprotectin predicted survival among 200 hospitalized decompensated patients with a cut-off >580 ng/mL, a value more than 5,8 times higher than that reported in

the controls (98 ng/mL). In the subgroup of patients with very severe disease, such as acute-on-chronic liver failure, the predictive power of calprotectin was lost. However, it appears that serum calprotectin is a less specific marker of intestinal inflammation compared to F-CAL.[11,24]

F-CAL in hepatic encephalopathy

Fecal calprotectin was first explored as a diagnostic marker of hepatic encephalopathy by Gundling et al. who investigated 61 patients with cirrhosis and 42 controls.[25] In cases with a mean MELD score of 14 (compared to 21 in our study), authors reported higher F-CAL in patients with cirrhosis compared to controls with a mean value of 65.8 mg/kg (1.4 × ULN). They found significant correlations between F-CAL and stages of HE according to West-Haven and critical flicker frequency, MELD or Child-Pugh-Turcotte score, and plasma ammonia. The authors also identified an F-CAL cut-off at 164 mg/kg having a high specificity and sensitivity in differentiating HE grade 0–1 from 2 to 3. Considering the reported threshold for "explicitly elevated" F-CAL of 48 mg/kg, the cut-off was 3.41 × ULN, comparable to the median of 3.9 × ULN in our study. Similar results were reported by Alempijevic et al.[26] in a cohort of 60 patients with mainly alcoholic cirrhosis with a mean MELD score of 14.7. The upper limit of normal was 50 µg/g and the median was 121 µg/g (2.4 × ULN). Here, the authors observed a significant difference between HE stages 0 and 2 or 1 and 2. The authors did not find significant correlations between F-CAL and MELD, Child-Pugh-Turcotte stages, ammonium ion, or the number connection test. In our study, patients admitted to the hospital for overt hepatic encephalopathy had a trend for the highest F-CAL values, but the difference from other causes was not statistically significant. We also found a weak but significant correlation between F-CAL and MELD but not with CLIF-C AD or CPT scores. Although F-CAL has been suggested as a clinically convenient marker of HE, the latest systematic review concludes that its role in diagnosing HE remains unclear.[11]

F-CAL and systemic infection

Intestinal inflammation is believed to represent a frontline between the intestinal barrier and immune system activation. In compensated cirrhosis, there is evidence of a biological equilibrium between the intestinal barrier and immune system activation.[27] In contrast, it appears that in the decompensated stage the equilibrium is shifted toward immune system activation.[27] In the study investigating stool cytokines in ACLD, F-CAL reflected nonspecific immune system activation in comparison with other stool cytokines. A recent study exploring F-CAL in 5 healthy controls and 30 patients with liver cirrhosis (10 compensated, 10 end-stage, and 10 ACLF) reported a progressive increase in F-CAL with disease severity. The F-CAL≥200 µg/g was associated with incident sepsis and non-survivors had significantly higher mean F-CAL (494 µg/g) than survivors (200 µg/g). The authors concluded that F-CAL might be a marker of imminent sepsis, but larger studies are needed for validation.[28,29] Our study adds evidence in support of the hypothesis that F-CAL is a marker of BT and imminent systemic infection. First, we observed the highest values of F-CAL in cases hospitalized for hepatic encephalopathy, bleeding, or alcoholic hepatitis, which are conditions known to be associated with BT. In a recent study, overt hepatic encephalopathy was directly linked to bacterial DNA translocation and 6-month mortality among 294 patients with cirrhosis.[30] Second, we found a positive correlation of F-CAL with CRP. The absence of a significant correlation with white blood cells or neutrophil to lymphocyte ratio could be explained by lower specificity for bacterial infection and the possible impact of non-infectious related inflammatory response in alcoholic hepatitis. Third, patients diagnosed with infection during the initial hospital stay had F-CAL values significantly higher than patients without infection.

F-CAL and ascites

Patients with refractory ascites are expected to have higher stages of portal hypertension, so our observation of decreased F-CAL values might come as a surprise. Although we do not have an explanation for the findings or evidence from the literature, we may speculate that calprotectin from the stool may translocate to ascites. There is evidence of increased calprotectin levels in ascites with an identified diagnostic threshold for spontaneous bacterial peritonitis[13] but no data on the correlation of F-CAL in stool and ascites.

F-CAL in clinical practice

The advantage of F-CAL could lie in the established availability of use and higher specificity in early stages with otherwise negative established prognostic markers. We confirmed that its prognostic power was particularly relevant among patients with lower CLIF-C AD, a subgroup of seemingly stable patients. In patients with low-risk CLIF-C AD <50 and CPT stage B, 16 of 60 patients (26.6%) and CPT stage C, 11 of 33 (33.3%) had high F-CAL and the risk of adverse outcomes was 2 to 3 times higher compared with patients in the same risk category and low F-CAL. Adding a new risk category (F-CAL >11 × ULN) allowed us to refine the clinical risk stratification by re-assigning low-risk patients with high F-CAL to high-risk. This approach may offer a window of opportunity for studying targeted interventions such as microbiome manipulation, intestinal barrier restoration, and improvement of portal hypertension or intestinal motility. When ACLD complications are already present, that is, a systemic infection, severe liver dysfunction, or refractory ascites, the prognosis is driven by the complications and liver dysfunction, and the window of opportunity appears closed.

Limitations

Our study has several limitations. First, for the study purposes, we only used a single F-CAL measurement. There is evidence from the IBD literature that F-CAL concentrations change dynamically over time.[31] Our F-CAL values demonstrated a substantial variance with a median value of 3.9 × ULN but 25th and 75th percentiles at 1.15 and 11.78 (Figure 2), making it more difficult to observe statistically significant differences. Second, our RH7 registry works as a real-life mixed cohort with some cases not having F-CAL measurement. To explore a possible bias, we conducted an additional analysis of the 157 patients from Center 1 with missing F-CAL data. It showed that 90-day LT-free survival was identical to 296 patients having the F-CAL essays (76.4 vs. 73.3, $p = 0.343$). The registry operates with the two predefined endpoints and unlike major studies in the field,[19,21] it is not designed to evaluate prospective occurrence of ACLF, sepsis, or hepatic decompensation. Our registry data do not record the exact cause of death and a more precise deciphering between sepsis-related, liver-related, or other causes was not available. Limited data from the Center 2 indicate that all observed deaths were related to liver or systemic infection. Third, we included the control group of patients with a limited size and follow-up, allowing for a limited statistical power to validate the findings observed for the study group. However, it allowed us to test the proof of concept that F-CAL values independently predict adverse outcomes and could refine the risk stratification. Fourth, our registry data do not allow a mechanistic explanation of our observations and we need more evidence on the correlation of F-CAL with histological signs of intestinal inflammation, its phenotype, the state of the intestinal barrier, or a synchronous detection of BT. Although we corrected our model for the treatment with beta-adrenergic blockers, we did not have the means of measuring their real effect on portal hypertension. We acknowledge that it remains an unmet need and future studies should aim to decipher the mechanisms of calprotectin release and assess the effect of various strategies for improving intestinal inflammation on portal hypertension and the risk of adverse outcomes.

Our study has several strengths. We explored the usefulness of F-CAL in the routine clinical practice of two centers using different essays. Its values in multiples of the ULN enabled us to analyze them in one cohort. Our study also has sufficient statistical power allowing the assessment of F-CAL as an early marker of disease outcomes in different disease stages and phenotypes. The results of our study have been confirmed in an independent control group.

CONCLUSION

Our study of 263 patients confirms that hospitalized ACLD patients have evidence of intestinal inflammation by the increased levels of F-CAL, with the highest values in patients with overt hepatic encephalopathy, bleeding, and alcoholic hepatitis. Adverse outcomes occurring within 180 days were independently and directly proportional to F-CAL values. In early disease stages with CLIF-C AD <50, high F-CAL refines the prognostic stratification in identifying roughly one-third of cases with 2 to 3 times the likelihood of adverse outcomes. This approach was validated in an independent control group of 108 patients. The results of our study provide more evidence for the use of F-CAL in clinical care for ACLD patients. However, external validation is warranted and the exact mechanisms of calprotectin release and its association with BT are yet to be deciphered.

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CONFLICT OF INTEREST STATEMENT

All authors declare having no conflicts of interest regarding the present study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Rose TC, Pennington A, Kypridemos C, Chen T, Subhani M, Hanefeld J, et al. Analysis of the burden and economic impact of digestive diseases and investigation of research gaps and priorities in the field of digestive health in the European Region—White Book 2: Executive summary. United Eur Gastroenterol J. 2022;10(7):657–62. https://doi.org/10.1002/ueg2.12298
2. Sepanlou SG, Safiri S, Bisignano C, Ikuta KS, Merat S, Saberifiroozi M, et al. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Gastroenterol Hepatol. 2020;5(3):245–66. https://doi.org/10.1016/s2468-1253(19)30349-8
3. Berg RD, Garlington AW. Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in a gnotobiotic mouse model. Infect Immun. 1979;23(2):403–11. https://doi.org/10.1128/iai.23.2.403-411.1979
4. Wiest R, Albillos A, Trauner M, Bajaj JS, Jalan R. Targeting the gut-liver axis in liver disease. J Hepatol. 2017;67(5):1084–103. https://doi.org/10.1016/j.jhep.2017.05.007
5. Pabst O, Hornef MW, Schaap FG, Cerovic V, Clavel T, Bruns T. Gut-liver axis: barriers and functional circuits. Nat Rev Gastroenterol Hepatol. 2023;20(7):447–61. https://doi.org/10.1038/s41575-023-00771-6
6. Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. J Hepatol. 2014;60(1):197–209. https://doi.org/10.1016/j.jhep.2013.07.044
7. Wang L, Llorente C, Hartmann P, Yang AM, Chen P, Schnabl B. Methods to determine intestinal permeability and bacterial

translocation during liver disease. J Immunol Methods. 2015;421:44–53. https://doi.org/10.1016/j.jim.2014.12.015
8. Skladany L, Koller T, Kukla M, Soltys K. Gut microbiome and liver diseases from the perspective of 3PM: the predictive, preventive, and personalized medicine. Adv Predictive, Prev Personalised Med. 2023;17:141–75. https://doi.org/10.1007/978-3-031-34884-6_9
9. Pouillon L, Peyrin-Biroulet L. It is time to revise the STRIDE guidelines determining therapeutic goals for treat-to-target in inflammatory bowel disease. J Crohns Colitis. 2018;12(4):509. https://doi.org/10.1093/ecco-jcc/jjx174
10. Ananthakrishnan AN, Adler J, Chachu KA, Nguyen NH, Siddique SM, Weiss JM, et al. AGA clinical practice guideline on the role of biomarkers for the management of Crohn's disease. Gastroenterology. 2023;165(6):1367–99. https://doi.org/10.1053/j.gastro.2023.09.029
11. Fortuny M, Sarrias M, Torner M, Iborra I, Clos A, Ardèvol A, et al. Systematic review of the role of calprotectin in cirrhosis. Eur J Clin Invest. 2024;54(2):e14111. https://doi.org/10.1111/eci.14111
12. Bender MH, Hartmann L, Anemüller JR, Schepp W, Gundling F. Calprotectin as diagnostic marker for hepatic encephalopathy and spontaneous bacterial peritonitis in cirrhosis. Clin Lab. 2020;66(06/2020):951–9. https://doi.org/10.7754/clin.lab.2019.190934
13. Weil D, Heurgue-Berlot A, Monnet E, Chassagne S, Cervoni J, Feron T, et al. Accuracy of calprotectin using the Quantum Blue Reader for the diagnosis of spontaneous bacterial peritonitis in liver cirrhosis. Hepatol Res. 2019;49(1):72–81. https://doi.org/10.1111/hepr.13239
14. Homann C, Garred P, Graudal N, Hasselqvist P, Christiansen M, Fagerhol MK, et al. Plasma calprotectin: a new prognostic marker of survival in alcohol-induced cirrhosis. Hepatology. 1995;21(4):979–85. https://doi.org/10.1016/0270-9139(95)90244-9
15. Matiollo C, Rateke ECM, Moura EQA, Andrigueti M, Augustinho FC, Zocche TL, et al. Elevated calprotectin levels are associated with mortality in patients with acute decompensation of liver cirrhosis. World J Hepatol. 2022;14(11):1964–76. https://doi.org/10.4254/wjh.v14.i11.1964
16. Skladany L, Molcan P, Vnencakova J, Vrbova P, Kukla M, Laffers L, et al. Frailty in nonalcoholic fatty liver cirrhosis: a comparison with alcoholic cirrhosis, risk patterns, and impact on prognosis. Can J Gastroenterol Hepatol. 2021;2021:5576531. https://doi.org/10.1155/2021/5576531
17. Skladany L, Drotarova Z, Vnencakova J, Jancekova D, Molcan P, Koller T. Applicability and prognostic value of frailty assessment tools among hospitalized patients with advanced chronic liver disease. Croat Med J. 2021;62(1):8–16. https://doi.org/10.3325/cmj.2021.62.8
18. Skladany L, Vnencakova J, Laffers L, Skvarkova B, Hrubá E, Molcan P, et al. Adherence to oral nutritional supplements after being discharged from the hospital is low but improves outcome in patients with advanced chronic liver disease. Patient Prefer Adherence. 2021;14:2559–72. https://doi.org/10.2147/ppa.s283034
19. Trebicka J, Fernandez J, Papp M, Caraceni P, Laleman W, Gambino C, et al. The PREDICT study uncovers three clinical courses of acutely decompensated cirrhosis that have distinct pathophysiology. J Hepatol. 2020;73(4):842–54. https://doi.org/10.1016/j.jhep.2020.06.013
20. Mahmud N. Selection for liver transplantation: indications and evaluation. Curr Hepatol Rep. 2020;19(3):203–12. https://doi.org/10.1007/s11901-020-00527-9
21. Zanetto A, Pelizzaro F, Campello E, Bulato C, Balcar L, Gu W, et al. Severity of systemic inflammation is the main predictor of ACLF and bleeding in individuals with acutely decompensated cirrhosis. J Hepatol. 2023;78(2):301–11. https://doi.org/10.1016/j.jhep.2022.09.005
22. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2012;48(3):452–8. https://doi.org/10.1038/bmt.2012.244
23. Homann C, Christensen E, Schlichting P, Philipsen EK, Graudal NA, Garred P. Ascites fluid and plasma calprotectin concentrations in liver disease. Scand J Gastroenterol. 2003;38(4):415–20. https://doi.org/10.1080/00365520310000870
24. Serrano E, Bastard JP, Trystram L, Fellahi S, Soula HA, Thenet S, et al. Serum versus fecal calprotectin levels in patients with severe obesity before and 6 months after Roux-Y-Gastric Bypass: report of the prospective leaky-gut study. Obes Surg. 2023;33(12):4017–25. https://doi.org/10.1007/s11695-023-06911-w
25. Gundling F, Schmidtler F, Hapfelmeier A, Schulte B, Schmidt T, Pehl C, et al. Fecal calprotectin is a useful screening parameter for hepatic encephalopathy and spontaneous bacterial peritonitis in cirrhosis. Liver Int. 2011;31(9):1406–15. https://doi.org/10.1111/j.1478-3231.2011.02577.x
26. Alempijević T, Stulic M, Popovic D, Culafic D, Dragasevic S, Milosavljevic T. The role of fecal calprotectin in assessment of hepatic encephalopathy in patients with liver cirrhosis. Acta Gastroenterol Belg. 2014;LXXVII:302–5.
27. Riva A, Gray EH, Azarian S, Zamalloa A, McPhail MJ, Vincent RP, et al. Faecal cytokine profiling as a marker of intestinal inflammation in acutely decompensated cirrhosis. JHEP Rep. 2020;2(6):100151. https://doi.org/10.1016/j.jhepr.2020.100151
28. Jothimani D, Paramasivam R, Manoharan M, Ramachandran H, Muthusamy S, Simon E, et al. Fecal calprotectin in patients with liver cirrhosis. Indian J Gastroenterol. 2023;42(6):818–23. https://doi.org/10.1007/s12664-023-01450-9
29. Jena A, Sharma V, Dutta U. Fecal calprotectin in cirrhosis—does "ESR" of the intestine measure the liver dysfunction too? Indian J Gastroenterol. 2023;42(6):754–6. https://doi.org/10.1007/s12664-023-01481-2
30. Thanapirom K, Suksawatamnuay S, Wejnaruemarn S, Thaimai P, Siripon N, Ananchuensook P, et al. Bacterial DNA translocation is associated with overt hepatic encephalopathy and mortality in patients with cirrhosis. Clin Transl Gastroenterol. 2024;15(5):e00697. https://doi.org/10.14309/CTG.0000000000000697
31. Rendek Z, Falk M, Grodzinsky E, Kechagias S, Hjortswang H. Oral omeprazole and diclofenac intake is associated with increased faecal calprotectin levels: a randomised open-label clinical trial. Eur J Gastroenterol Hepatol. 2023;35(1):52–8. https://doi.org/10.1097/meg.0000000000002473

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