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Original research

Gene score to quantify systemic inflammation in patients with acutely decompensated cirrhosis

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ABSTRACT

Background and aims Quantifying systemic inflammation (SI) in acutely decompensated cirrhosis (ADC) is of major importance because SI is a driver of the most severe forms of ADC, including acute-on-chronic liver failure (ACLF). Blood biomarkers of SI already evaluated in ADC failed to appropriately assess SI in ADC. We aimed to investigate whether gene expression related to circulating immune cells could quantify SI in ADC.

Methods Standard biomarkers (white cell count, C reactive protein, cytokines) and genome-wide RNA expression (RNA-sequencing) were obtained in blood from 700 patients with ADC at the time of their hospital admission. A composite score based on standard biomarkers of SI (Chronic Liver Failure-Standard Biomarkers Composite (CLIF-SBC) score) and a gene score (CLIF-Systemic Inflammation Gene (SIG) score) composed of the 28 top differentially expressed immune cell-related genes in the comparison between high-severity and low-severity clinical phenotypes were computed. Among the 700 patients, the CLIF-SIG score was repeated once during follow-up in 375 patients, and 3 times or more in 46 patients.

Results The CLIF-SIG score was more accurate in reflecting clinical severity induced by SI than the CLIF-SBC score (area under the curve 0.803 vs 0.658). A CLIF-SIG score of 0.386 (Youden Index) was the best cut-off level discriminating patients with poor outcomes from the others, in all clinical scenarios. Sequential measurement of the CLIF-SIG score showed that 78% of patients were admitted at the peak or descending part of the SI-wave. ACLF developed during hospitalisation in 80% of patients with a CLIF-SIG score >0.386 on admission.

Conclusions In patients with ADC, the CLIF-SIG score is an accurate estimator of SI, clinical course severity and prognosis.

INTRODUCTION

The observational studies CANONIC, Predicting Acute-on-Chronic Liver Failure in Cirrhosis (PREDICT) and Prevalence, Epidemiology, Characterization and Mechanisms of ACLF in Latin America (ACLARA) have characterised the concept of acutely decompensated cirrhosis (ADC). ADC is defined as acute development of ascites, encephalopathy, gastrointestinal haemorrhage or any combination of these, requiring hospitalisation.[1–3] The CANONIC study described and defined acute-on-chronic liver failure (ACLF) as a clinical entity characterised by organ failure(s) and high short-term mortality and proposed systemic inflammation (SI) as a major pathophysiological mechanism.[1 4] The PREDICT study reported that in patients with pre-ACLF (ie, those who were free of ACLF at presentation but developed during follow-up), the development of ACLF was mainly driven by SI.[2 4–6] The ACLARA study showed that increasing percentages of Native American genetic ancestry were associated with higher severity of SI, frequency and severity of ACLF and mortality,

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Systemic inflammation is a major driver of decompensation and acute-on-chronic liver failure (ACLF).
⇒ The adequate measurement involves different pathways and biomarkers.
⇒ Standard inflammation biomarkers (eg, cytokines) show significant overlap.

WHAT THIS STUDY ADDS

⇒ The Chronic Liver Failure-Systemic Inflammation Gene (CLIF-SIG) score consists of 28 top differentially expressed immune cell-related genes between high-severity and low-severity clinical phenotypes.
⇒ The CLIF-SIG score more accurately estimates systemic inflammation than scores constructed with standard inflammation biomarkers (ie, cytokines).
⇒ CLIF-SIG score >0.386 identifies worse outcome independent of aetiology of liver disease, ancestry and precipitating event.
⇒ 80% of patients developing ACLF had a CLIF-SIG score >0.386.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study offers a tool to measure systemic inflammation and may guide future clinical trials and decisions.
⇒ Sequential measurement of the CLIF-SIG score may be useful to assess course of disease and short-term mortality.

an association that was not observed with increasing percentages of European or African-American ancestries.[3] Interestingly, patients who developed ACLF during hospitalisation (early pre-ACLF, to be differentiated from pre-ACLF who develop ACLF within 90 days (online supplemental table 1) already had very intense SI at admission.[7–9] However, traditional biomarkers of SI (ie, cytokines), as well as routine parameters such as white cell count (WCC), neutrophil-to-lymphocyte ratio and C reactive protein (CRP), frequently overlap between patients with established or developing ACLF (pre-ACLF) from other phenotypes without ACLF at hospital admission.[2] More sensitive biomarkers of SI are, therefore, needed for patient stratification and future assessment of patient' response to immunomodulators and other potential treatments,[10] since development of ACLF is a global burden of healthcare.[11]

In two recent studies,[8 12] analysis of genome-wide RNA expression in whole blood at admission for ADC identified patients with more intense SI and subsequently more severe liver disease as having upregulation of genes related to neutrophils and monocytes (innate immune cells), and downregulation of genes related to lymphocytes (in particular NK cells). Of note, transcriptomics seems useful for precision diagnosis, prognosis and treatment in a large variety of human diseases, including sepsis.[13–15] For all these reasons, we reasoned that a gene signature that captures dichotomous alterations in gene expression in circulating immune cells from patients with ADC would appropriately assess intensity of SI. We, therefore, analysed standard blood biomarkers of SI and data from whole-blood RNA sequencing (RNA-seq) in 700 patients of the PREDICT and the ACLARA studies. Using results of these analyses, we developed and validated a gene score (Chronic Liver Failure-Systemic Inflammation Gene score, CLIF-SIG score), which best estimated SI in patients with ADC.

METHODS

Patients

As indicated in protocols of the PREDICT and the ACLARA study, investigators were invited to collect blood samples for traditional biomarkers of SI and Tempus tubes for whole-blood RNA-seq on hospital admission (T1). We obtained data from 700 RNA-seq (main cohort; PREDICT study: 383 patients, ACLARA study 317 patients, see online supplemental table S2) at hospital admission (T1). In 375 patients (subcohort 1) from these 700 patients, we obtained samples at the second scheduled visit during hospitalisation (T2) with a median interval of 7 days (95% CI: 5 to 8 days) after admission. Additional RNA-seq data were obtained from 46 patients (subcohort 2) of the 375 patients (see online supplemental table S3). 23 age-matched and sex-matched healthy subjects (HS) are analysed as described in figure 1A and online supplemental figure 1A. Importantly, the 1151 whole-blood RNA-seq data (1128 RNA-seq from patients and 23 from HS) used in this study had been obtained from a single batch.

The main cohort was stratified into a high-severity group of 297 patients with ACLF at admission or developing ACLF during index hospitalisation (early pre-ACLF), and a low-severity group (403 patients who remained free of ACLF from admission to discharge). Although these two groups may include clinical different cohorts, the common denominator is the similar degree of SI in these two groups. Alcohol-related hepatitis was diagnosed according to the National Institute on Alcohol Abuse and Alcoholism (NIAAA) Consortium criteria.[16]

Patient and public involvement

Patients were not involved in the development of the research question and outcome measures.

Scores

To distinguish between the different groups, the clinical parameters were analysed using principal component analysis. The different stages of ADC, namely stable decompensated cirrhosis (SDC), unstable decompensated cirrhosis (UDC) pre-ACLF and ACLF1-2 and ACLF3, showed a wide overlap (online supplemental figure 1B).

We constructed a score based on blood levels of traditional inflammatory biomarkers; this score was named CLIF-Standard Biomarkers Composite (CLIF-SBC) score in 654 patients. To construct the score, we considered seven circulating cytokines which were highly increased in patients with ACLF (IL-6, IL-8, IL-1RA, CXCL-10, MCP-1, MIP-1β and TNF),[4 9] as well as CRP, WCC and neutrophil-to-lymphocyte ratio. Analysis of the CLIF-SBC score was also performed by stratification in training and test sets for internal validation. The CLIF-SBC score was computed with a stepwise selection method comparing patients from the high-severity and the low-severity group using logistic regression. During computation, neither cytokines nor the neutrophil-to-lymphocyte ratio remained in the model. The CLIF-SBC score was, therefore, solely based on CRP, with the known limitations (eg, hepatic synthesis) and WCC, which was available in all 700 patients.

Computation of the CLIF-SIG score was performed after randomly dividing T1 samples into training (463 patients) and test (237 patients) sets as an internal validation of the score. Using the training set, we compared whole blood transcriptome from the high-severity and the low-severity group using limma to rank the genes, thus obtaining a metric (t-statistics) related to statistical differences. Using the top gene sets from the enrichment

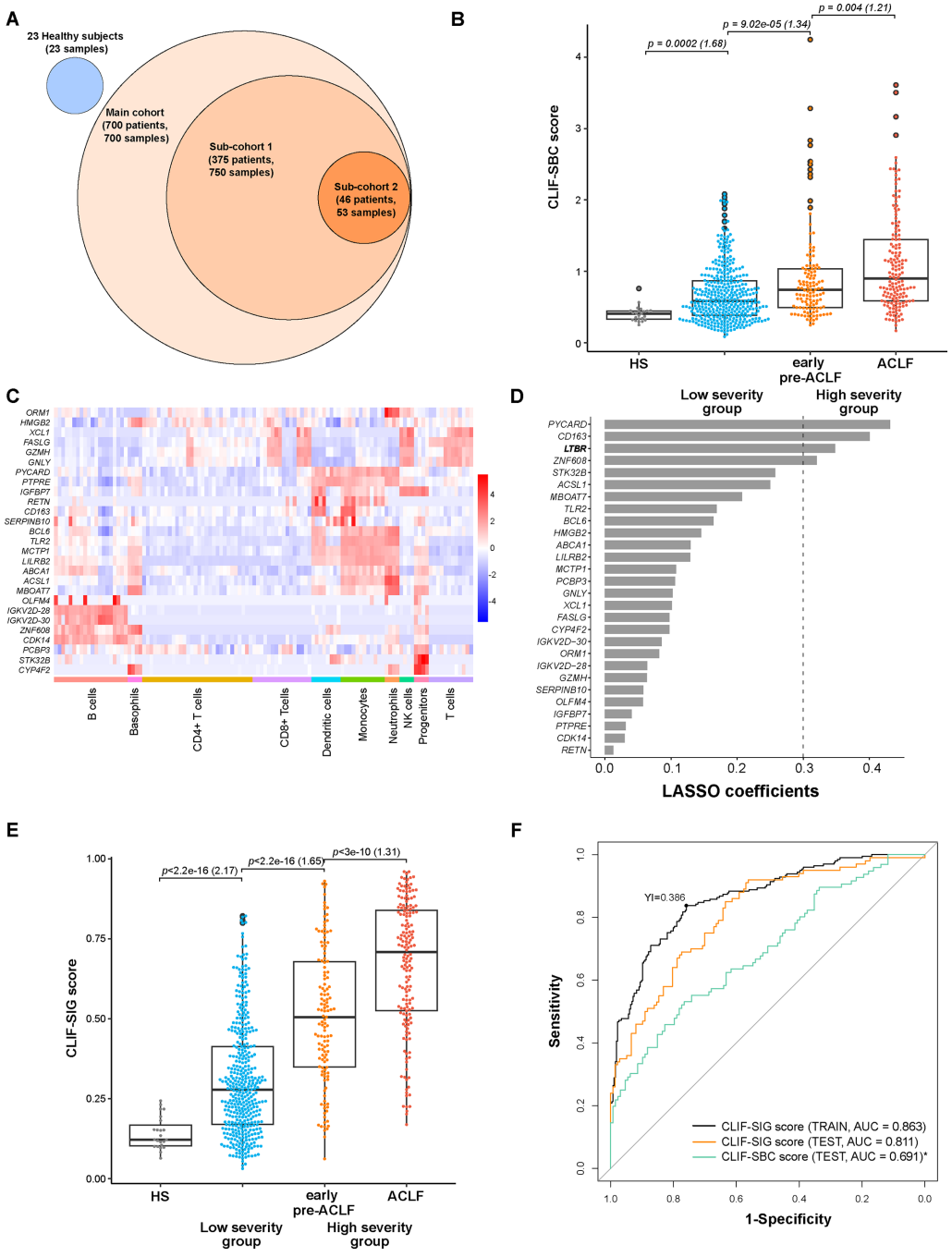


Figure 1 Design of the CLIF-SIG score. (A) Stratification of patients according to the number of CLIF-SIG scores: the main cohort includes 700 patients with ACLF-SIG score at admission; subcohort 1 includes 275 of these patients with a second CLIF-SIG score 1 week later; subcohort 2 includes 46 patients from subcohort 1 with one or two additional CLIF-SIG scores during hospitalisation. (B) Comparison of the CLIF-SBC score at baseline between HS and patients from the low-severity and the high-severity group. The high-severity group was divided into early pre-ACLF and ACLF to illustrate different score values between these subcohorts. Significance between groups was obtained using Wilcoxon-Mann-Whitney tests. Median fold-increase of each score for each comparison is represented in brackets next to each p value. (C) Cell origin of 27 of the 28 genes that compose the CLIF-SIG score inferred through their expression among gene signatures of 29 immune-cell types from healthy subjects (HS) deposited by Monaco *et al* (GSE107011).[21] The gene *LTBR*, detected in our patients, was not present in the dataset of Monaco *et al*. (D) Relative contribution of each of the 28 genes to the CLIF-SIG score. (E) Comparison of the CLIF-SIG score at baseline between HS and patients from the low-severity and the high-severity group. The high-severity group was divided into early pre-ACLF and ACLF to illustrate different score values between these subcohorts. Significance between groups was obtained using Wilcoxon-Mann-Whitney tests. Median fold-increase of each score for each comparison is represented in brackets next to each p value. (F) ROC curves differentiating between the low severity and high severity group by the CLIF-SIG score (orange) and the CLIF-SBC score (green) at baseline in the test sets; *p<0.001 between the areas under these ROC curves using Somers' D. ROC curve estimating the accuracy of the CLIF-SIG score differentiating between the low-severity and the high-severity group in the training set at baseline, and the score cut-off level differentiating between the two groups with the best Youden's index (YI<0.386) are represented in black. ACLF, acute-on-chronic liver failure; AUC, area under the curve; CLIF-SBC, CLIF-Standard Biomarkers Composite; CLIF-SIG, Chronic Liver Failure-Systemic Inflammation Gene; ROC, receiver-operating-characteristics.

analysis (GSEA) of Gene Ontology (GO) and of the blood transcription module (BTM) space (which is appropriate for the exploration of gene modules from blood immune cells),[17 18] we selected four gene sets of interest (including three BTMs and one GO gene set) that were significantly enriched in the high severity group. The BTMs were respectively named 'immune activation-generic cluster (M37.0)', 'monocyte surface signature (S4)' and 'enriched in NK cells (I) (M7.2)'; the GO gene set was named 'immunoglobulin complex (GOCC 0019814)'. As indicated, GSEA analyses revealed that there were 220 leading edge genes across the 4 gene sets which were used to compute the CLIF-SIG score. Therefore, 220 leading edge genes derived from four immunity-related gene sets differentiating between patients with early pre-ACLF or ACLF-1, ACLF-2 or ACLF-3 (high-severity group) and patients with SDC and UDC and delayed pre-ACLF (low-severity group) were selected as candidates for the CLIF-SIG score computation (online supplemental table 4), which finally included 28 genes (online supplemental figure 2, table 5). This was performed with the use of the glmnet R package[19] to compute a LASSO solution and find an optimal λ by 10-fold CV to select the genes that optimise the misclassification error when comparing patients from the above-mentioned groups (online supplemental figure 3). The resulting score was a risk probability.

CLIF-SIG score validation

We used two different external cohorts to validate the CLIF-SIG score with a total of 122 patients. The first cohort included 31 deeply phenotyped patients, 7 HS, 7 patients with acutely decompensated cirrhosis without ACLF, and 17 patients with ACLF. Results of genome-wide (microarray) analysis of whole-blood RNA expression were available in all patients at their presentation and for all HS (GSE142255).[12] In addition, we used a publicly available RNA-seq dataset from a non-cirrhotic cohort including 91 further patients with different magnitude of SI, which included 40 HS, 32 patients with infections (20 of these with sepsis) and 19 patients with septic shock (GSE154918).[20]

Additional methods

Results were expressed as median and IQR for not normally distributed data, as mean±SD for normally distributed data and as count and relative percentage for categorical data. No imputation was carried out and in data analyses, we applied an available-data-only approach. The numbers are shown in the tables.

Statistical testing was performed accordingly. We identified the immune cell subsets that preferentially express genes that compose the CLIF-SIG score by analysing the differential expression of these genes among the gene signatures of 29 fine immune cell types from HS deposited by Monaco *et al* (GSE107011).[21] A detailed description of the RNA-seq procedure is provided in online supplemental appendix.

RESULTS

Patient characteristics

Table 1 shows the characteristics of the 700 patients from the main cohort at T1, stratified into two groups by severity. The high-severity group included 297 patients; 166 had ACLF at hospital admission and 131 developed ACLF during hospitalisation (pre-ACLF). The low-severity group included 403 patients without ACLF. Patients in the high-severity group presented more frequently with ascites, encephalopathy and exogenous precipitating events (alcohol-related hepatitis and bacterial infections), worse liver and renal functions, and higher 28-day and 90-day mortality rates; demographic and etiological data were similar. Online supplemental tables 6 and 7 show clinical and laboratory features at hospital admission (T1) and 28-day and 90-day mortality rates in the 375 patients (subcohort 1) with 1 and in the 46 patients (subcohort 2) with 2 or more additional follow-up visits.

SI scores

The CLIF-SBC score (which was solely based on CRP and WCC; see Methods) was available for all patients (n=700). The CLIF-SBC score also significantly differentiated high-severity versus low-severity groups, although exhibiting greater overlapping between groups (figure 1B).

The CLIF-SIG score was composed of 28 genes of which the inferred-cell origin is provided in figure 1C, while annotation is provided in online supplemental table 5. The relative weight of each of these genes on the CLIF-SIG score is given in figure 1D. PYCARD, CD163, LTBR and ZNF608 showed a LASSO coefficient above 0.3 and thereby contribute most to the estimates of SI. Using a logistic model of the values of the CLIF-SIG score at T2 (below or above the Youden's Index (YI)) and the 28 genes as predictor variables we identified the genes that were independently associated with the fluctuation of the score above or below the YI at T2, being HMBG2, STK32B, CDK14, GZMH, PCBP3 and ORM1 (online supplemental figure 3C).

Of the 28 genes, 24 were upregulated and 4 downregulated in the high-severity group relative to the low-severity group (online supplemental figure 4). Of note, the 24 upregulated genes were related to innate myeloid cells (20 genes) or B cells (four genes), whereas the 4 downregulated genes (*XCL1, GZMH, GNLY* and *FASLG*) were related to NK cells (with an overlap with T cells; figure 1C). After the breakdown of the high-severity group, the CLIF-SIG score was markedly increased in patients with pre-ACLF relative to those of the low-severity group and was increased in patients with ACLF relative to those with pre-ACLF (figure 1E).

Comparisons between CLIF-SIG and CLIF-SBC scores

To assess the accuracy of the CLIF-SIG and CLIF-SBC scores in differentiating the high-severity from the low-severity group, we computed the median fold-increase of the following three pairwise comparisons for both scores: low-severity group versus HS, early pre-ACLF versus low-severity group, and ACLF versus early pre-ACLF. The distribution of CLIF-SIG score differentiated more clearly than the CLIF-SBC score between the high-severity and low-severity group (figure 1B, E). Figure 1F shows that the area under (AUC) ROC curves differentiating high-severity versus low-severity groups at hospital admission (T1) in the test set was significantly higher for the CLIF-SIG score (red colour) than for the CLIF-SBC score (blue colour), also implying higher accuracy of the CLIF-SIG score in estimating the magnitude of SI. Figure 1F also shows the AUC the ROC curve differentiating high versus low severity group in the training set at hospital admission (T1) and the YI (0.386, black colour), which represents the most accurate CLIF-SIG score discriminating between patients from the high-severity and the low-severity group. Consequently, YI was applied to stratify patients according to the magnitude of SI at any visit.

Online supplemental figures 5A and B show that the CLIF-SIG and the CLIF-SBC scores did not differentiate between patients with SDC and UDC in the PREDICT study cohort. Even in the patients developing ACLF after discharge (delayed pre-ACLF)

Table 1 Characteristics at presentation and deaths by 28 days and 90 days in 700 patients divided into the high-severity and the low-severity group*

Variable	Low severity group* (N=403)	High severity group (N=297)	FDR†
Characteristics at presentation			
Demographic data			
Age (year)	58.6±11.6	57.9±12.5	0.47
Female sex—no. (%)	255 (63)	205 (69)	0.17
Aetiology of cirrhosis—no. (%)			
Alcohol-related cirrhosis (ARC)	204 (51)	159 (54)	0.62
ARC+alcohol-related hepatitis†	68 (38)	81 (54)	<0.01
Hepatitis C	49 (12)	29 (10)	0.32
Hepatitis B	47 (12)	30 (11)	0.97
MASLD‡	47 (12)	32 (11)	0.91
Mixed	23 (6)	21 (7)	0.56
Complications at admission—no. (%)			
Ascites	267 (66)	222 (75)	0.01
Hepatic encephalopathy	123 (31)	149 (50)	<0.01
Gastrointestinal bleeding	95 (24)	47 (16)	0.03
Laboratory data—median (IQR)			
Total bilirubin—mg/dL	2.0 (1.2–4.2)	5.6 (1.9–13.2)	<0.01
INR	1.4 (1.8–1.7)	1.7 (1.4–2.2)	<0.01
Serum creatinine—mg/dL	0.9 (0.7–1.2)	1.4 (0.9–2.1)	<0.01
Biomarkers of systemic inflammation and scores—median (IQR)			
White cell count ($\times 10^9$/L)	5.9 (4.2–8.5)	7.5 (5.3–11.0)	<0.01
C reactive protein—mg/L	16.9 (7.3–38.3)	29.4 (15–64.8)	<0.01
Neutrophile-to-lymphocyte ratio	3.9 (2.4–6.2)	6.2 (3.9–9.5)	<0.01
IL-1RA—pg/mL	3.3 (1.7–7.4)	4.99 (2.3–10.9)	<0.01
IL-6—pg/mL	11.5 (5.6–23.2)	21.1 (9.2–49.6)	<0.01
IL-8—pg/mL	3.4 (1.4–7.7)	4.7 (2.1–12.0)	<0.01
CXCL-10—pg/mL	203.2 (118.5–362.1)	253.5 (123.5–434.6)	0.03
MCP-1—pg/mL	167.1 (123.9–231.8)	227.5 (137.0–357.4)	<0.01
MIP-1β—pg/mL	15.3 (10.9–20.7)	17.62 (12.6–26.6)	<0.01
TNF-α—pg/mL	24.0 (14.8–47.7)	36.4 (23–63.8)	<0.01
Treatments—no. (%)			
Antibiotics§	271 (68)	201 (69)	0.93
Vasopressors	41 (10)	36 (12)	0.53
Diuretics	250 (63)	131 (45)	<0.01
Betablockers	179 (45)	102 (35)	0.01
Albumin	104 (26)	89 (30)	0.30
Scores and bacterial infections and ACLF prevalence and grades			
Mean CLIF-SIG score	0.3±0.2	0.6±0.2	<0.01
MELD-Na score—median (IQR)	18 (15–22)	26 (22–30)	<0.01
CLIF-SBC score—median (IQR)	0.6 (0.4–0.9)	0.8 (0.7–1.2)	<0.01
Prevalence of bacterial infections—no. (%)	92 (23)	123 (41)	0.03
Prevalence of ACLF—no. (%)	–	166 (56)	–
ACLF-1—no. (%)	–	97 (33)	–
ACLF-2—no. (%)	–	53 (18)	–
ACLF-3—no. (%)	–	16 (5)	–
Deaths—no. (%)			
By 28 days	4 (1)	101 (34)	<0.01
By 90 days	50 (12)	151 (52)	<0.01
Liver transplant—no. (%)			
By 28 days	6 (1)	14 (5)	<0.01
By 90 days	21 (5)	26 (9)	<0.01

*Low-severity group included patients who presented without ACLF and remained free of ACLF from admission to discharge; the high-severity group included patients who presented with ACLF or developed ACLF during index hospitalisation (early pre-ACLF).
†Significance levels between groups were assessed using $\chi 2$ test for categorical variables, t-test for normally-distributed variables and U-Mann-Whitney test for non-normal variables.
‡Missing data for NIAA criteria in 12% and 6% of the patients from the low-severity and the high-severity group, respectively.
§Orally non-absorbable or poorly absorbable oral antibiotics included
ACLF, acute-on-chronic liver failure; CLIF-SBC, Chronic Liver Failure-Standard Biomarkers Composite; CLIF-SIG, CLIF-Systemic Inflammation Gene; FDR, false discovery rate; INR, international normalised ratio; MASLD, metabolic-associated steatotic liver disease; MELD-Na, Model for End-Stage Liver Disease-sodium; NIAAA, National Institute on Alcohol Abuse and Alcoholism.

Table 2 AUCs assessing the discriminative accuracy of the CLIF-SIG score and other variables in differentiating between the high-severity and the low-severity group in patients of the test set

Variable	Patients considered	AUC	AUC vs AUC for the CLIF-SIG score in the test set†
CLIF-SIG score	237 patients at presentation	0.811	
MELD-Na score	237 patients at presentation	0.787	0.471
CLIF-SBC score	224 patients at presentation	0.691	<0.001
WCC	235 patients at presentation	0.65	0.036
CRP	225 patients at presentation	0.664	0.048
IL-6	234 patients at presentation	0.637	0.015
CLIF-SIG score	Sex (female, n=79)	0.7752	0.567
CLIF-SIG score	Sex (male, n=158)	0.8329	0.607
CLIF-SIG score	Alcohol-related cirrhosis (n=113)	0.8478	0.417
CLIF-SIG score	Cirrhosis related to hepatitis C (n=14)	0.6875	0.44
CLIF-SIG score	Cirrhosis related to MASLD (n=17)	0.6944	0.415
CLIF-SIG score	Alcohol-related hepatitis at presentation (n=54)	0.8049	0.927
CLIF-SIG score	Bacterial infection at presentation (n=92)	0.7579	0.355
CLIF-SIG score	Gastrointestinal haemorrhage at presentation (n=44)	0.8095	0.984
CLIF-SIG score	PREDICT study cohort (n=139)	0.7882	0.652
CLIF-SIG score	ACLARA study cohort (n=98)	0.8494	0.429
CLIF-SIG score	Native American ethnicity (n=12)‡	0.8	0.961
CLIF-SIG score	African American ethnicity (n=20)‡	0.810	0.99
CLIF-SIG score	European ethnicity (n=189)‡	0.783	0.528

*Test set included 237 patients.
†Each AUC shown in the table was compared with the AUC of the CLIF-SIG score (0.811) with the use of the DeLong's test in patients from the test set.
‡Patients from the ACLARA study
ACLARA, Prevalence, Epidemiology, Characterization and Mechanisms of ACLF in Latin America; AUC, area under the curve; CLIF-SIG, Chronic Liver Failure-Systemic Inflammation Gene; CRP, C reactive protein; MASLD, metabolic-associated steatotic liver disease; MELD-Na, Model for End-Stage Liver Disease-sodium; PREDICT, Predicting Acute-on-Chronic Liver Failure in Cirrhosis; SBC, Standard Biomarkers Composite; WCC, white cell count.

the CLIF-SIG scores were similar to those of the SDC and UDC patients. Of note, the CLIF-SIG score clearly separated these less severe three phenotypes from patients with ACLF at admission or those with early pre-ACLF.

Utility of CLIF-SIG and CLIF SBC scores

Interestingly, the CLIF-SIG score was significantly correlated with MELD-Na score (R^2=0.54) and also with the CLIF-SBC score (R^2=0.57) (online supplemental figure 5C). Importantly, CLIF-SBC did not correlate with MELD-Na score (R^2=0.34) (online supplemental figure 5D). The combination of CLIF-SIG with MELD-Na did not significantly improve the AUC (online supplemental figure 5E), while the combination with CLIF-SBC score revealed an even lower AUC than SIG-Score alone (data not shown). Also, the correlation between the changes (delta) of CLIF-SIG and CLIF-SCB was weak (R^2=0.36), although highly statistical significance of p=1.819e−10 (online supplemental figure 5F).

Table 2 shows that the CLIF-SIG score (AUC=0.811) was more accurate than the MELD-Na score (AUC=0.787), although not statistically significant (online supplemental figure 5E). Yet, CLIF-SIG score was significantly more accurate than the CLIF-SBC score (AUC=0.691) and individual markers of SI, in discriminating between the high-severity and the low-severity group. This accuracy was not influenced by sex, aetiology of cirrhosis, bacterial infections, association with alcoholic-related hepatitis or gastrointestinal haemorrhage, geographical regions (Europe or Latin America), or ethnicities among Latin American patients (table 2).

To render the CLIF-SIG score more clinically relevant, we calculated the best cut-off using the YI to discriminate between low-severity and high-severity groups; the best cut-off was for YI of 0.386. It was previously shown that patients who present with more than one precipitant exhibited more marked SI than patients who present with only one precipitant.[22] In the present study, the CLIF-SIG score was significantly higher in patients with more than one precipitant than in patients with only one precipitant (figure 2A, B). These findings confirm the ability of the CLIF-SIG score to monitor the intensity of SI. In addition, the CLIF-SIG score discriminated the outcome in these patients (figure 2C–F).

An important finding of the ACLARA study was that Native American patients had more intense SI than European American and African American patients.[3] Applying the CLIF-SIG score to patients of the ACLARA study enrolled in the present study, we found that the score was higher in Native American patients than in the two other ethnic groups (online supplemental figure 6A,B), a finding that provides additional evidence for the ability of the CLIF-SIG score to distinguish the 'more inflamed' from the 'less inflamed' patients. In addition, online supplemental figure 6C clearly shows that the severity of SI clustered the patients and not the aetiology. In addition, the CLIF-SIG score had a similar AUC in the different aetiologies, at least in the training cohort (online supplemental figure 6D, upper panel). In the validation cohort, the numbers may not give enough for HCV and metabolic-associated steatotic liver disease (online supplemental figure 6D, lower panel).

The CLIF-SIG score during follow-up

SI in decompensated cirrhosis likely occurs in the form of repeated inflammatory waves (ascending, intermediate-top and descending recovery phases), each wave having a

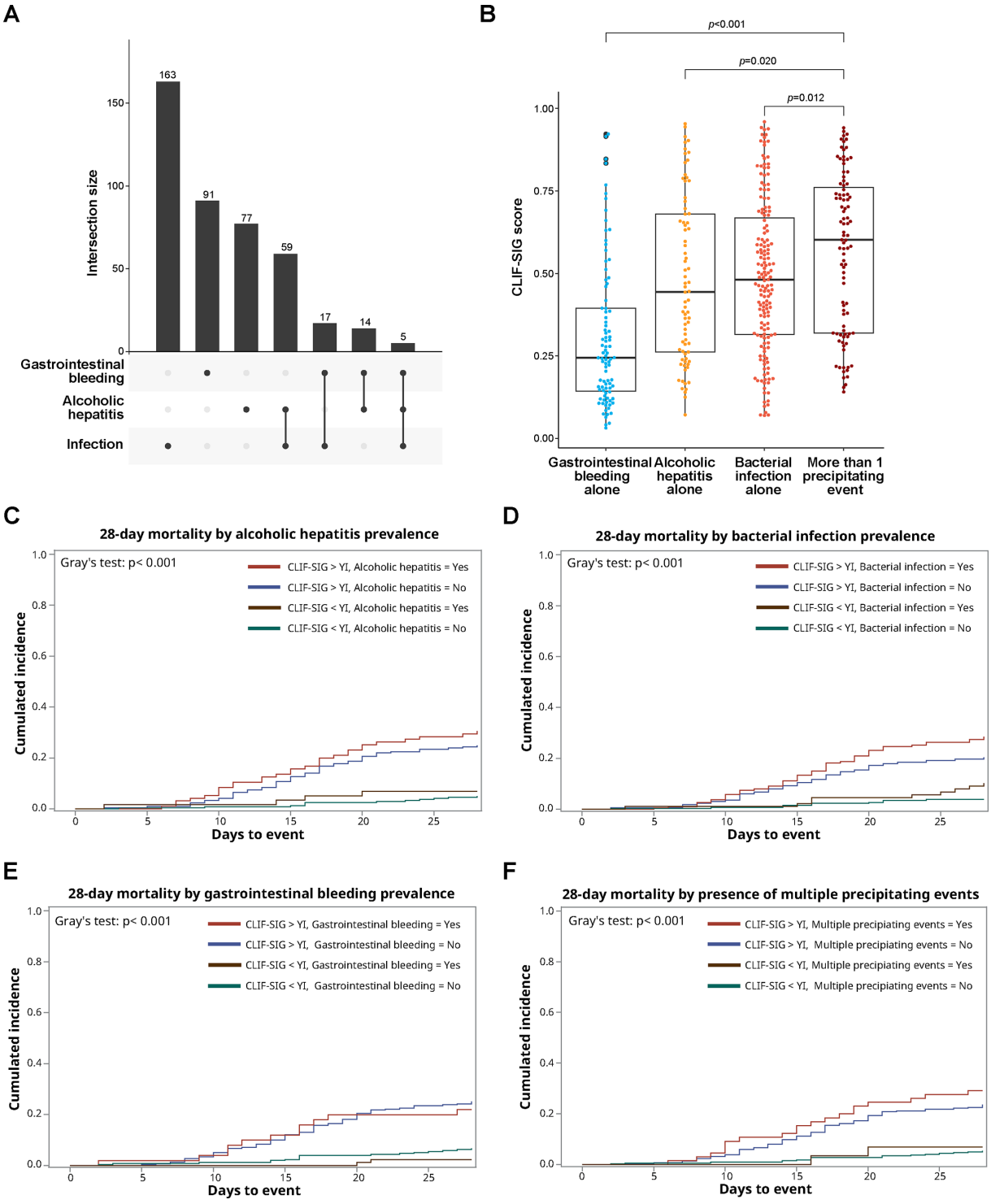
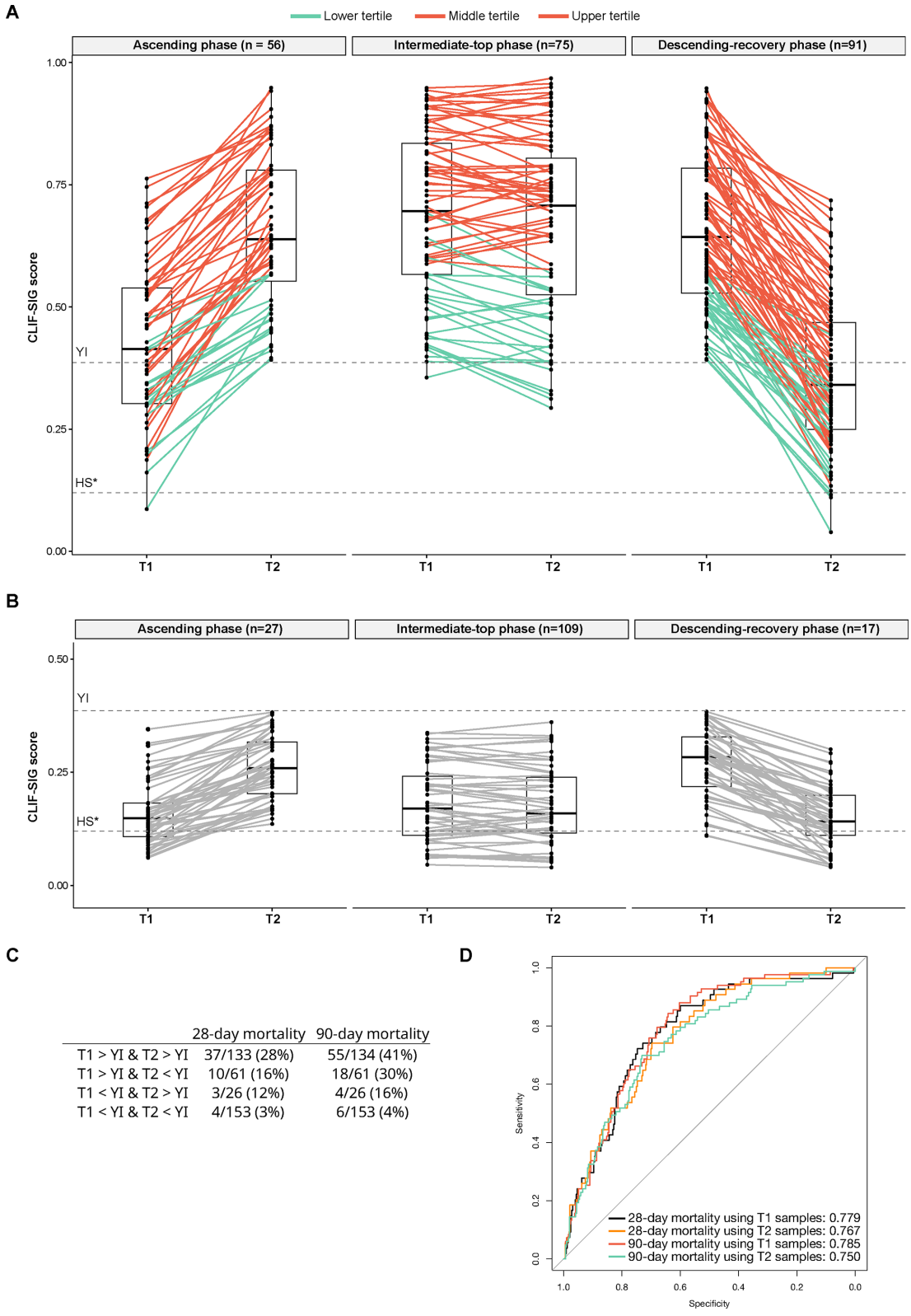


Figure 2 (A) Interaction plot showing the number of patients with only one precipitating event and the number of patients with a combination of precipitating events. (B) Levels of CLIF-SIG score between the patients with different precipitating events. Significance between groups was obtained using Wilcoxon-Mann-Whitney tests. Cumulative incidence curves for 28-day mortality stratifying the patients according to CLIF-SIG score levels using the Youden Index (YI) (0.386) as cut-off and the prevalence at inclusion of alcohol-related hepatitis (C), bacterial infection (D), gastrointestinal bleeding (E) and presence of one or multiple precipitating events (F). Significant differences between groups were assessed using Gray's test. CLIF-SIG, Chronic Liver Failure-Systemic Inflammation Gene.

duration of approximately 3 weeks.[23] Based on two sequential CLIF-SIG scores at hospital admission (T1) and during follow-up (T2) with an average interval of 7 days in 357 patients (subcohort 1), we captured in the initial ascending phase in 83 patients (22%), the intermediate-top phase in 184 (49%) and the descending recovery phase in 108 (29%) (online supplemental figure 7A).

Figure 3A includes patients with high SI, as defined by a CLIF-SIG score above YI at admission or during follow-up. We considered the 148 patients with scores in the middle and the



	28-day mortality	90-day mortality
T1 > YI & T2 > YI	37/133 (28%)	55/134 (41%)
T1 > YI & T2 < YI	10/61 (16%)	18/61 (30%)
T1 < YI & T2 > YI	3/26 (12%)	4/26 (16%)
T1 < YI & T2 < YI	4/153 (3%)	6/153 (4%)

Figure 3 Assessing the CLIF-SIG score. Individual changes of the CLIF-SIG score in the 375 patients with two RNA-seq stratified according to the phase of the inflammatory response captured by each patient (only one phase per patient was detected). T1 and T2 represent baseline and second visit values, respectively. (A) Red and green colours indicate patients with severe and those with intense systemic inflammation, respectively, as estimated at T2 in patients capturing the initial ascending phase and at T1 in patients capturing the intermediate-top and the descending recovery phases. (B) Grey colour indicates patients with moderate systemic inflammation (CLIF-SIG score below 0.386 at T1 and T2). There was a small group of patients who developed AD in the setting of normal CLIF-SIG score at T1 and T2. (C) 28 days and 90 days mortality in the cohorts of patients with different combinations of the CLIF-SIG above or below YI at baseline (T1) or follow-up (T2). (D) AUC values of the CLIF-SIG-score at baseline (T1) or follow-up (T2) for 28-day and 90-day mortality. *Healthy subjects. AUC, area under the curve; CLIF-SIG, Chronic Liver Failure-Systemic Inflammation Gene; HS, healthy subjects; YI, Youden's Index.

Table 3 Characteristics at presentation, clinical course and deaths by 28 days and 90 days in the 375 patients from subcohort 1 according to the magnitude of inflammation*

Variable	Moderate inflammation (N=153)	Intense inflammation (N=74)	Severe inflammation (N=148)	FDR†
Characteristics at presentation				
Age—year	57.6±12.3	56.5±14.0	55.4±13.1	0.37
Female sex—no. (%)	58 (38)	22 (30)	54 (36)	0.48
Alcohol-related cirrhosis (ARC)—no. (%)	66 (43)	31 (42)	83 (56)	0.05
ARC+Alcohol-related hepatitis‡—no. (%)	16 (27)	11 (38)	48 (65)	< 0.01
Hepatitis C—no. (%)	20 (13)	12 (16)	14 (9)	0.34
Hepatitis B—no. (%)	26 (17)	9 (12)	16 (11)	0.29
MASLD—no. (%)	17 (11)	7 (9)	25 (17)	0.22
Mixed—no. (%)	11(7)	5 (7)	3 (2)	<0.01
Complications—no. (%)				
Ascites	76 (50)	45 (61)	104 (70)	<0.01
Hepatic encephalopathy	49 (32)	35 (47)	83 (56)	<0.01
Gastrointestinal bleeding	65 (42)	13 (18)	31 (21)	<0.01
Laboratory data and biomarkers of systemic inflammation—median (IQR)				
Total bilirubin—mg/dL	1.6 (0.8–2.9)	2.5 (1.2–6.3)	7.7 (2.1–16.9)	<0.01
INR	1.4 (1.2–1.6)	1.5 (1.2–1.7)	1.8 (1.4–2.3)	<0.01
Serum creatinine—mg/dL	0.8 (0.7–1.1)	1.1 (0.7–1.9)	1.2 (0.7–1.9)	<0.01
White cell count ($\times 10^9$/L)	5.2 (3.7–7.4)	6 (4.6–8.1)	8.4 (6.6–11.9)	<0.01
C reactive protein—mg/L	13 (5.6–28.9)	25.9 (10.8–49.1)	36.8 (16–59.3)	<0.01
Neutrophil-to-lymphocyte ratio	3.3 (2.1–4.9)	4.3 (3.3–7.4)	6.5 (4.13–10.3)	<0.01
IL-1RA—pg/mL	2.7 (1.6–5.4)	4.6 (1.9–8.7)	7.1 (3–15.2)	<0.01
IL-6—pg/mL	8.3 (4.3–18.8)	15.7 (7.1–31.1)	19.4 (8.6–59.1)	<0.01
IL-8—pg/mL	3.2 (1.4–6.7)	4.5 (2.04–10.5)	6 (2.3–16.3)	<0.01
CXCL-10—pg/mL	197.6 (104.7–330.1)	258.6 (169–399.7)	276.8 (133.6–507.9)	<0.01
MCP-1—pg/mL	173.9 (131.9–255.8)	227.3 (129.2–308.5)	230.9 (142.3–387.4)	0.02
MIP-1β—pg/mL	14.9 (10.4–20)	16.7 (12.3–29.13)	18.1 (12.5–26.4)	<0.01
TNF-α—pg/mL	25.5 (15–47.3)	35.2 (20.4–57.1)	38.2 (22.6–78.3)	<0.01
Scores and bacterial infections and ACLF prevalence and grades				
Mean CLIF-SIG score	0.2±0.1	0.4±0.1	0.7±0.2	<0.01
CLIF-SBC score—median (IQR)	0.5 (0.3–0.7)	0.7 (0.5–0.9)	1 (0.7–1.4)	<0.01
MELD-Na score—median (IQR)	16.5±5.1	21.4±6.4	26.3±6.6	<0.01
Prevalence of ACLF—no. (%)	10 (7)	27 (36)	91 (61)	<0.01
ACLF-1—no. (%)	10 (7)	17 (23)	49 (33)	<0.01
ACLF-2—no. (%)	0 (0)	8 (11)	31 (21)	<0.01
ACLF-3—no. (%)	0 (0)	2 (3)	11 (7)	<0.01
Prevalence of bacterial infections—no. (%)	28 (18)	22 (30)	60 (41)	<0.01
Clinical course—no. (%)				
Bacterial infections at any time	40 (26)	35 (47)	80 (54)	<0.01
Good clinical course§	37 (93)	20 (57)	50 (62)	<0.01
Poor clinical course¶	3 (7)	15 (43)	30 (38)	<0.01
No resolution of infections	2 (5)	8 (23)	26 (33)	<0.01
Septic shock	3 (7.5)	12 (34)	26 (33)	<0.01
ACLF clinical course				
Good clinical course**	124 (90%)	47 (65%)	75 (52%)	<0.01
Poor clinical course††	13 (10%)	25 (35%)	69 (48%)	<0.01
Deaths—no. (%)				
By 28 days	4 (3)	11 (15)	39 (27)	<0.01
By 90 days	6 (4)	20 (27)	57 (39)	<0.01

*Criteria defining moderate, intense and severe inflammation are described in the Results section and legend to figure 3.
†Significance levels between groups were assessed using $\chi 2$ test for categorical variables, ANOVA for normally distributed variables and Kruskal-Wallis test for non-normal variables.
‡Missing data for NIAA criteria in 9%, 6% and 11% of the patients from the moderate, intense and severe severity groups, respectively.
§Infections resolved at discharge.
¶Infections not resolved at discharge of septic shock development during hospitalisation.
**No ACLF during the hospitalisation or at discharge.
††ACLF-1, ACLF-2 or ACLF-3 at discharge.
ACLF, acute-on-chronic liver failure; ANOVA, analysis of variance; CLIF-SBC, Chronic Liver Failure-Standard Biomarkers Composite; FDR, false discovery rate; INR, international normalised ration; MASLD, metabolic-associated steatotic liver disease; MELD-Na, Model for End-Stage Liver Disease-sodium; NIAAA, National Institute on Alcohol Abuse and Alcholism; SIG, Systemic Inflammation Gene.

highest thirds (red colour) as having severe SI and the 74 patients with scores in the lowest third (green colour) as having only intense SI. The CLIF-SIG scores of 153 patients remained below YI having moderate SI (figure 3B). The overall mortality of these patients was 3% at 28 days, 4% at 90 days and 10% (15/153) at 180 days. The main reported causes of death were hypovolaemic

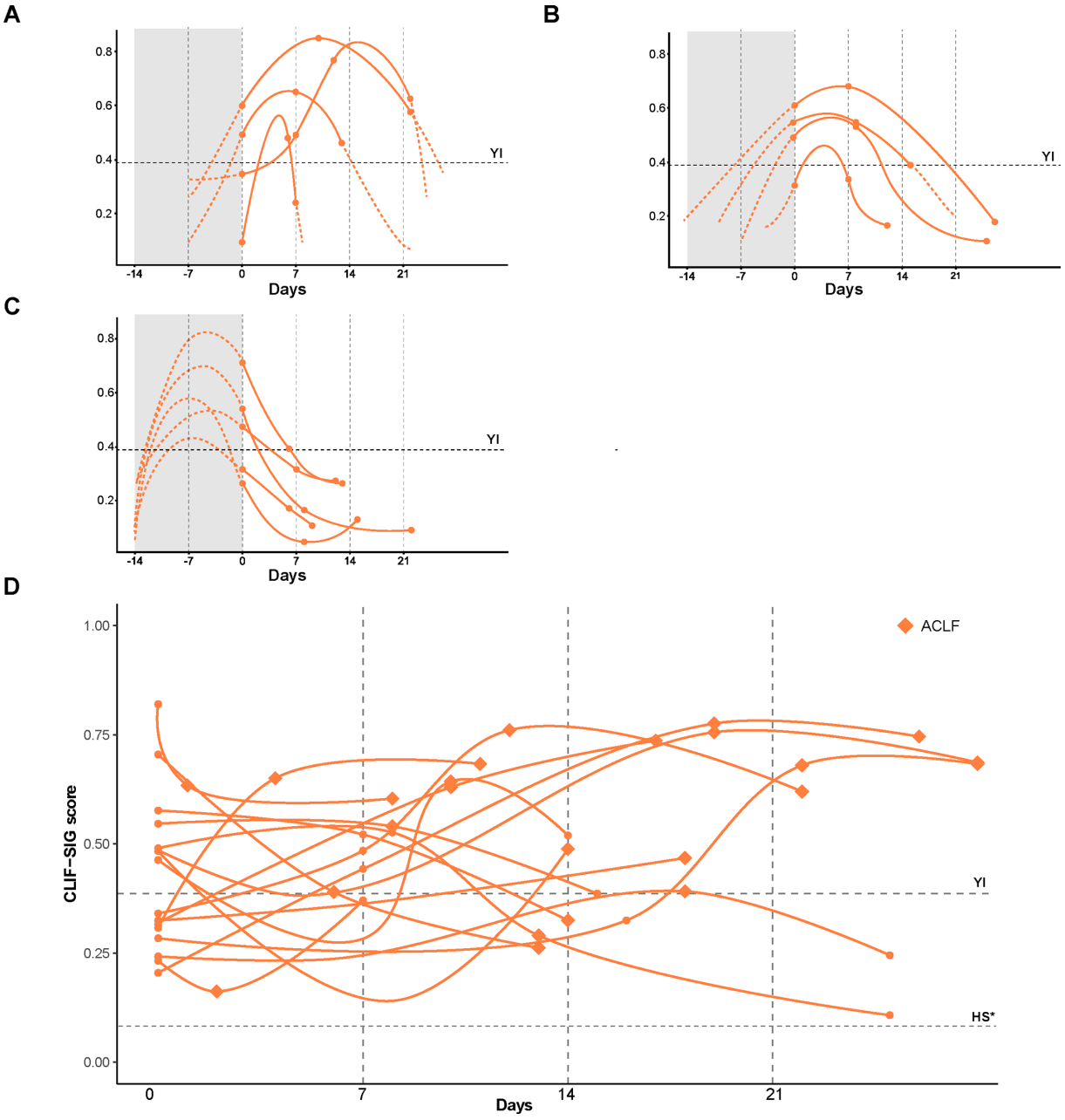


Figure 4 Assessing the CLIF-SIG score. (A–C) Representative examples of the time course of the inflammatory response estimated by sequential measurements of the CLIF-SIG score in patients included in subcohort 2. The horizontal discontinued line represents the Youden's index (YI) differentiating between moderate and severe systemic inflammation. The shadowed area represents days before hospital admission. (A) The three phases of the systemic inflammatory reaction were totally or partially captured within the first 3 weeks after hospital admission in only four patients. (B, C) Representative examples of systemic inflammation responses in patients lacking the initial ascending phase or the initial ascending phase plus the intermediate-top phase plus ascending development phase, which developed prior to hospital admission and are estimated by the discontinued segment of the curve. (D) Systemic inflammation responses during hospitalisation captured in 15 patients from subcohort 2 who developed ACLF. The horizontal discontinued line represents the YI differentiating between moderate and severe systemic inflammation. The vertical discontinued lines correspond to the first 3 weeks after baseline. Most patients developing ACLF showed a CLIF-SIG score consistently above 0.386 during hospitalisation. The diamonds represent time points of ACLF detection. ACLF, acute-on-chronic liver failure; CLIF-SIG, Chronic Liver Failure-Systemic Inflammation Gene; HS, healthy subjects.

shock (2 until 90 days; 3 additional patients 91–180 days) and development of ACLF (3 until 90 days; additional patients 91–180 days). There were 11 patients (2.6%) with CLIF-SIG score similar to HSs indicating that AD is an exception in the absence of SI.

Table 3 shows that stratification according to CLIF-SIG score of these patients with follow-up (subcohort 1) identifies clearcut differences in the prevalence of major complications, standard laboratory data, biomarkers of SI, CLIF-C organ failure, MELD-Na scores and CLIF-SBC scores, and prevalence and severity of bacterial infections and ACLF at admission. The stratification also disclosed a highly significant association between magnitude of SI and the clinical course of bacterial infections or ACLF. Not surprisingly,

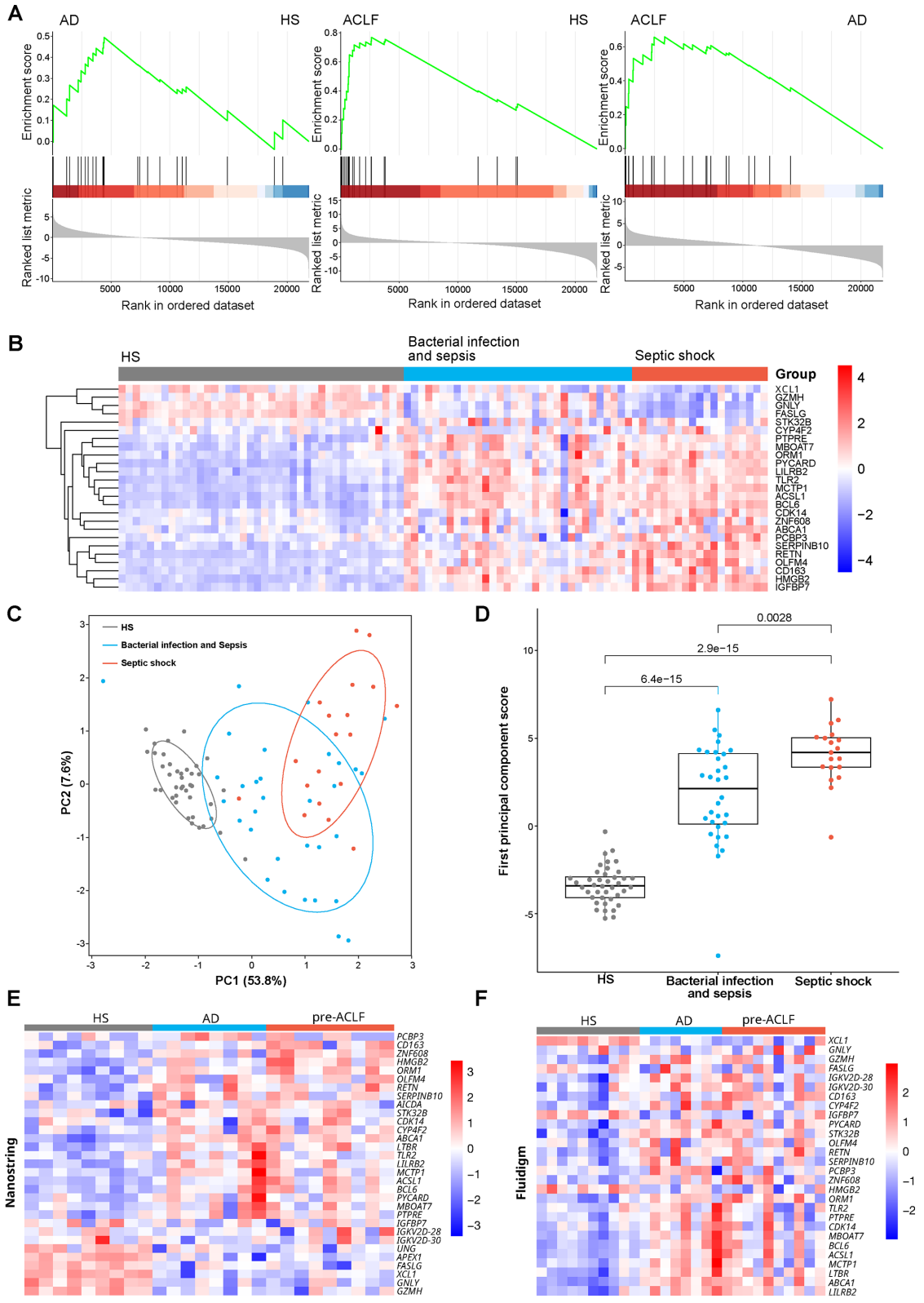


Figure 5 External validation of CLIF-SIG score genes. (A) Enrichment plots of whole-blood CLIF-SIG score genes used as a gene set in three comparisons: AD without ACLF (n=7) vs healthy subjects (HS, n=7) (left), ACLF (n=17) vs HS (middle), and ACLF vs AD without ACLF (right). Whole-blood RNA expression data from patients and HS were obtained from references (Weiss *et al*,[12] Front Immunol). (B–D) Results of analyses were obtained using a publicly available RNA-seq dataset.[15] The data set was composed of three groups: HS (n=40), non-cirrhotic patients with bacterial infections (n=32, including 20 with sepsis and non-cirrhotic patients with septic shock (n=19). (B) Heatmap showing expression of CLIF-SIG score genes across the three groups. (C) Principal component analysis (PCA) plot across the three groups. (D) Box plots discriminating HS and patients with and without septic shock by the first principal component score. Significance between groups was obtained using Wilcoxon-Mann-Whitney tests. (E) Heatmap showing expression of CLIF-SIG score genes across the three groups by Nanostring. (F) Heatmap showing expression of CLIF-SIG score genes across the three groups by Fluidigm. ACLF, acute-on-chronic liver failure; CLIF-SIG, Chronic Liver Failure-Systemic Inflammation Gene.

28-day and 90-day mortality rates increased in parallel with magnitude of SI.

To further address the fluctuation of the CLIF-SIG score over time, we analysed again the data of the subcohort one with at least one follow-up measurement. Indeed, we found that the patients who had at both visits CLIF-SIG scores above the YI had a 41% mortality rate at 90 days, the patients with CLIF-SIG score>YI only at baseline a 30% mortality rate at 90 days, and the patients with a CLIF-SIG score>YI only at follow-up had a 16% mortality rate at 90 days, while 4% in patients with a SIG-SIG score consistently below the YI at both time points (figure 3C). Yet, the prognostic value of the CLIF-SIG-score at the second time point had a similar prognostic value as at baseline, as shown by similar AUC values for the 28-day and 90-day mortality (figure 3D). In addition, we observe similar predictive power for the high-intensity group as shown in online supplemental figure 7B.

Next, we intended to draw the full course of SI and AD. We observed patients, who were admitted during the ascending phase and the SI-boost was captured during hospitalisation (figure 4A), patients admitted at the peak of their SI (figure 4B), and patients admitted during descending phase (figure 4C).

The longitudinal assessments of CLIF-SIG indicate that hospital admission and thereby the onset of clinical AD may be delayed in 78% of patients. This delay was independent of SI severity.

Interestingly, ACLF develops in the setting of intense or severe sustained SI. 15 patients developed ACLF during hospitalisation (pre-ACLF, orange colour, figure 4D). ACLF (diamonds in figure 4D) was developed mostly when CLIF-SIG score >0.386 (80% of patients).

Validation of genes included in the CLIF-SIG score

We first leveraged the results of genome-wide (microarray) analysis of whole-blood RNA expression in HS, patients with SDC, patients with AD without ACLF and patients with ACLF using GSEA plots rank.[12] The GSEA plots (figure 5A) rank all the genes according to their differences across two groups. Every time a gene of interest (black lines) is found in the ranked list the enrichment score increases. Therefore, a skewed curve can be translated as a significant difference in expression of genes of interest when comparing two groups of patients. Using CLIF-SIG score genes as a gene set, we found that this gene set was significantly enriched among the most upregulated genes in three comparisons (figure 5A): ADC without ACLF vs HS (left); ACLF versus HS (middle) and ACLF versus ADC without ACLF (right). Next, we used an external cohort composed of three groups: HS, patients without cirrhosis with bacterial infection and patients without cirrhosis with septic shock.[20] Figure 5B, which shows the individual expression of CLIF-SIG score genes, indicates that the expression of the four genes related to NK cells was lower, while the expression of genes related to myeloid cells was higher in patients with bacterial infections relative to HS. These alterations were even more marked in patients with septic shock than in HS. It is noteworthy that the four genes related to NK cells were downregulated with increasing severity as observed in patients with acutely decompensated cirrhosis. Figure 5C, D illustrates the ability of the CLIF-SIG score to differentiate between the three study groups. Collectively, these results provide an external validation of the use of CLIF-SIG score genes as a tool that positively correlates with the intensity of SI in populations of patients with and without cirrhosis.

Finally, to validate the clinical applicability, we measured a subset of patients (n=10 HS, n=8 ADC with low intensity, n=10 pre-ACLF) using two different technological platforms (ie, Fluidigm and Nanostring) available in tertiary hospitals (figure 5E, F). The heatmaps show that patients clustered according to their SI intensity in both platforms. In addition, we observed a very good correlation of RNA-sequencing data with the levels of the respective genes assessed by Fluidigm and Nanostring (online supplemental figure 8).

DISCUSSION

The availability of accurate biomarkers of SI in ADC is an unmet medical need regarding patient stratification and assessment of patients' responses to immunomodulators and other potential treatments. In this study, we have now revealed that the CLIF-SIG score was more accurate than standard inflammatory markers (eg, CLIF-SBC score, WCC or blood levels of CRP or IL-6) in discriminating the most severe patients from the other patients. These findings indicate that alterations in gene expression in circulating immune cells are more closely associated with severity than are elevated WCCs or elevated levels of blood inflammatory proteins. Therefore, our study shows that we now have a tool that specifically assesses SI and may be used to assess the effects of immunomodulators for ADC, and measure the effect of a specific drug. Especially, CLIF-SIG score identifies patients developing ACLF during hospitalisation, and therefore, has the potential to stratify those for interventional measures aiming to prevent the development of ACLF. Interestingly, we have validated the genes that compose the CLIF-SIG score with the use of two external cohorts: one including patients with AD without and with ACLF, the other comprising patients with SI due to sepsis and septic shock. Finally, we could validate the gene signature in two different clinically available platforms Fluidigm and Nanostring.

Another important finding of our study is that the CLIF-SIG score was not more accurate than the MELD-Na score in discriminating the most severe patients from the other patients. The similarity between the two scores is probably related to the fact that each score is measuring indirectly (CLIF-SIG score) or directly (MELD-Na score) organ system dysfunctions which underpin severity. Nevertheless, we showed that while the MELD-Na score can be used to assess organ system functions, it cannot be used to determine the intensity of SI (see online supplemental figure 5D); findings indicating that usage of MELD-Na and CLIF-SIG scores are not interchangeable.

To further support a pathophysiological role of genes that compose the CLIF-SIG score, we collected information about their biological functions (online supplemental table 5). We identified functions that are relevant for decompensated cirrhosis; this was the case, for example, for genes such as *PYCARD, CD163, GNLY, GZMH* and *XCL1* among others. Immune cell *PYCARD* encodes a protein which plays a crucial role in the assembly of inflammasome machinery that leads to the activation of inflammatory caspases resulting in the release of proinflammatory cytokines, such as interleukin IL-1β and IL-18.[24] Inflammasome activation has been shown to be associated with ACLF development.[25] *CD163* codes for a membrane protein expressed exclusively on monocytes and macrophages. When it meets proinflammatory stimuli, for example, bacterial cues, CD163 is enzymatically cleaved from the membrane of immune cells, resulting in soluble CD163. In patients with acutely decompensated cirrhosis, elevated blood levels of soluble CD163 are independent predictors of short-term and long-term

mortality.[26] *GNLY* encodes granulysin, an antimicrobial peptide which is expressed in NK cells and cytotoxic T cells. Similarly, *GZMH*, which is expressed in NK, is translated into granzyme A, an antimicrobial serine protease. The fact that *GNLY* and *GZMH* were downregulated in our high-severity group suggests that defective production of granulysin and granzyme A contributes to the susceptibility to infection that characterises patients of the high-severity group.[27] *XCL1* encodes lymphotactin, which is a chemokine attracting lymphocytes. *XCL1* downregulation in our patients of the high-severity group may result in decreased lymphocyte recruitment at infected sites. However, our results obtained in blood should be interpreted with great caution since changes in gene expression in circulating immune cells may differ from changes in the expression of the same genes in tissue-resident immune cells located, for example, in the peritoneum border[28] or in the liver.[29] We believe that further genes of this gene set are relevant and deserve further investigation in the future.

Our results indicate that SI in patients with ADC is not a steady condition. In most patients, it consists of an acute inflammatory response characterised, as in sepsis, by an initial ascending phase, an intermediate-top phase and a descending recovery phase.[23 30] Sequential measurement of the CLIF-SIG score at admission and 1 week later in 375 patients allowed us to identify three major characteristics of the SI response in ADC. First, while the duration of the inflammatory response is a matter of days for sepsis in the general population,[30] it is a matter of weeks in ADC. Second, in a large proportion of patients, the systemic inflammatory response frequently starts 1–2 weeks before hospital admission, which explains why ACLF is already present at the time of hospital admission in most patients. Third, we were able to stratify every patient into one of three groups using the CLIF-SIG score and YI as cut-off level by discriminating moderate versus severe SI. These groups show clearcut differences in clinical features, laboratory data, standard biomarkers of SI, MELD-Na score, prevalence and clinical course of bacterial infections and ACLF, and prognosis. This finding supports the critical importance of SI as a pathophysiological mechanism of most clinical features associated with ADC. This highlights the clinical practice applicability of sequential measurements of the CLIF-SIG score at hospital admission and 1 week later. Finally, the application of 3–4 sequential CLIF-SIG score measurements during hospitalisation allowed for a more complete estimate of the profile of systemic inflammatory response. It provided strong evidence that ACLF develops within a context of sustained intense or severe SI since it occurred with extremely high frequency in patients with CLIF-SIG scores persistently above YI.

This study has several limitations. Neither the PREDICT nor the ACLARA study was specifically designed to develop a gene score assessing the intensity of SI. This study distinguished between the early pre-ACLF (ACLF develops within hospitalisation) and late pre-ACLF (after discharge but within 90 days), which is another difference to the original PREDICT definition. Nevertheless, the combination of a large number of RNA-seq data obtained in a single batch with the granularity of clinical and laboratory features collected in the two studies allowed us to develop a robust and reproducible gene score. Another limitation is that in this study, due to the small numbers of individual treatments, we could not focus on the severity of each precipitant and response to treatment, but this may be done in future clinical trials. Although the external validation demonstrated that the gene signature was different in patients with different levels of SI, even in absence of cirrhosis, we could not calculate the score, since few gene levels were missing, and clinical outcomes for non-ADC patients were not available. Finally, our RNA-seq-inferred CLIF-SIG score cannot provide immediate clinical applicability given the lack of ready availability of components of the score at bedside and costs. Because of these limitations, we have launched a programme to develop tools for rapid estimation of the CLIF-SIG score in clinical practice.

In summary, the CLIF-SIG score is a biomarker specifically designed to estimate the magnitude of SI and to predict clinical course severity and prognosis in patients with ADC. As any prognostic score, it is more accurate when applied sequentially at admission and 1 week later.[31 32] Sequential application of the CLIF-SIG score for longer periods allows for estimation of the entire course of the SI response and is likely the best method to explore the potential efficacy of new treatments.

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Competing interests JT has received speaking and/or consulting fees from Versantis, Gore, Boehringer-Ingelheim, Falk, Grifols, Genfit and CSL Behring; PA received lecture fees from BioMarin, Grifols and has served on the advisory board for Biovie and BioMarin; WB received consulting fees from Versantis and Flagship Pioneering; PC received grant support from Grifols SA and Octapharma SA, lecture fees from Grifols SA, Octapharma SA, Takeda SA and CSL Behring SA and has served on the data safety advisory board for Grifols SA, Takeda SA and CSL Behring SA; MM received speaking fees/consulting: Biosidus, Gador, Gilead Sciences, Intercept, Knight, Roche; JFer received lecture fees from Grifols SA; TG received a research grant from Gilead and has served on the advisory board for GoLiver Therapeutics and Cellaïon; RJ had research collaborations with Yaqrit and Takeda, is the inventor of OPA, which has been patented by UCL and licensed to Mallinckrodt Pharma and is the founder of Yaqrit Limited, a spin-off company from University College London and Thoeris; RMor received consulting fees from Genfit and CSL Behring; SP received consulting fees from the Plasma Protein Therapeutics Association, Boehringer Ingelheim and lecture fees from Grifols, Ferring and MEDSCAPE; WL has received consultancy fees from CSL Behring, MRM Health, Cook Medical and Boston-Scientific; AZ received speaking and consulting fees from Gore, Falk, CSL Behring and Grifols. The remaining authors disclosed no conflicts.

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Correction: *Gene score to quantify systemic inflammation in patients with acutely decompensated cirrhosis*

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