Journal of Hepatology

The PREDICT study uncovers three clinical courses of acutely decompensated cirrhosis that have distinct pathophysiology

--Manuscript Draft--

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Abstract:	Background/Aims:Acute decompensation (AD) of cirrhosis is defined by the acute development of ascites, gastrointestinal hemorrhage, hepatic encephalopathy, i nfection or any combination of these, requiring hospitalization. The presence of organ failure(s) in patients with AD defines acute-on-chronic liver failure (ACLF), while their absence defines AD. We designed the PREDICT study, a European, prospective, observational study, to characterize the clinical course of AD and predict ACLF . Methods:A total of 1071 patients with AD were enrolled to collect detailed pre-specified information on the 3-month period prior to enrollment, and clinical and laboratory data at enrollment. Patients were then closely followed-up for 3 months. The 12-month outcomes (liver transplantation, and death) were also recorded. Results:Three groups of patients were identified: Pre-ACLF patients (n=218), who developed ACLF and had 3-month and 1-year mortality rates of 53.7% and 67.4%, respectively. Unstable decompensated cirrhosis (UDC) patients (n = 233) required ≥1 readmission but not developing ACLF and had 21.0% and 35.6% mortality rates. Stable decompensated cirrhosis (SDC) patients (n = 620) who were neither readmitted, nor developed ACLF and showed a 1-year mortality of only 9.5%. The 3 groups differed significantly in the grade and course of systemic inflammation (high-grade at enrollment with aggravation during follow-up in pre-ACLF; low-grade at enrollment with subsequent steady-course in UDC; and low-grade at enrollment with subsequent improvement in the SDC) and prevalence of surrogates of severe portal hypertension throughout the study (high in UDC versus low in pre-ACLF and SDC). Conclusions:Acute decompensation without ACLF is a heterogeneous condition with three different clinical courses and two major pathophysiological mechanisms: systemic inflammation and portal hypertension. Prediction of ACLF development remains a major future task.(ClinicalTrials.gov number, NCT03056612)
Response to Reviewers:	POINT-BY-POINT RESPONSES Dear Editors and Reviewer, As indicated below, the new manuscript has been submitted to an intense remodeling, in order to take into consideration all suggestions of the reviewers. Now the main data on the prediction analysis are included in the main body of the manuscript. The supplementary material contains also three tables (Tables S2, S3 and S4) dealing with missing values, and the Univariate and Multivariate analysis for the CLIF-C ACLF-D score. The revised manuscript underlines in red font color the changes introduced in response to the Reviewer's criticisms of Reviewer 3. The result section contains all the original data included in the previous manuscript, non-essential parts of the material and methods were removed and the discussion was reduced. We are most grateful to the Reviewer (i.e., Reviewer #3) for his/her valuable criticisms. Yours sincerely Jonel Trebicka

POINT-BY-POINT RESPONSES TO REVIEWER 3:
We thank the referee for the kind re-assessment of our work and the critical input on the statistical review. We hope that those changes satisfactorily address all issues raised by the reviewer.

Point 1 of the reviewer 3.
Using available-cases analysis is notoriously suboptimal for building prediction models, and approaches such as multiple imputation or weighting may have better properties. I did not see any reason why they could not be used here.
Answer to Point 1.
Thank you very much for this useful comment. We apologize for not understanding what the reviewer suggested in the previous version of the manuscript. Indeed, we initially decided not to apply any imputation strategy because the proportion of missing data related to the study variables collected at enrollment and used in multivariate analyses was minimal and similarly distributed among the three main prognostic groups of the study. We have included in the revised manuscript a new table in the supplementary material (Table S2) showing that there were no missing data in most potential predictors of ACLF development at enrollment, except for serum albumin and C-reactive protein concentration, whose values were not available in 4.6%, 9.0% and 7.9% of patients and in 20.2%, 13.3% and 11% of patients, in Pre-ACLF, UDC and SDC respectively (Table S2). According to the reviewer's suggestion, were carried out a multiple imputation based on a mixed model including all potential predictors significantly associated with ACLF in the univariate analysis (page 15 on the revised manuscript).

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Developing and validation risk predictions models is not an easy task. As I said before, the use of a Fine-Gray model is correct, but how the model was developed and validated is not sufficiently described in the manuscript. In the methods, there is a mere reference to a derivation and validation sets, without explaining how they were created and used. This does not comply with TRIPOD guidelines, contrary to what is written in the rebuttal letter. Without precise methods, it is impossible to evaluate the study findings. Prognostic performance of the new score is no more presented in the main article, which is not acceptable, whereas it is for a decision tree-based model that we discover in the results, without any mention in the methods. Again, we do not know what was done, and this makes it difficult to draw any conclusion.

Answer to point 2.
Thank you for these remarks. We apologize for not having answered clearly to this point. Initially, the main objective of the paper involved only the definition of the three prognostic groups. As already pointed out, the derivation and validation of the CLIF-C ACLF-D score, as well as the decision-tree model were included in the article following the suggestions and requests of the other reviewers. In the present manuscript TRIPOD recommendations are followed both in the methodology and in the process of data analysis and the checklist is included as supplementary material (see below). Yet the focus is again the detailed description of the three groups of AD. We hope that this sufficiently addresses the raised point.

The new version of the manuscript contains a detailed explanation of the statistical method used. To include this, we have modified the whole article reducing the extension of the contents included in the initial manuscript to provide space for these new text passages. Now the Material and Method section of the revised manuscript contains the following new information explaining the methodology used:
"For the prediction of ACLF development during the 90-day follow-up period, the CLIF-Consortium ACLF development score was fitted according to the TRIPOD recommendations (please see TRIPOD checklist). There were no missing data in most potential predictors of ACLF development at enrollment, except for serum albumin and plasma CRP levels, whose values were not available, respectively, in 5%, 9% and 8% of patients of the pre-ACLF, UDC, and SDC groups and in 20%, 13% and 11% of

patients of the three groups (Table S2). Therefore, for multivariate analysis we assumed that these missing values could be considered at random and carried out a multiple imputation based on a mixed model including all potential predictors significantly associated with ACLF in the univariate analysis [13].
We used the proportional-hazards model for competing risks (CR) proposed by Fine and Gray to identify the best subset of independent predictors associated with the onset of ACLF and to develop a new predictive score [the CLIF-C ACLF-Development score (CLIF-C- ACLF-D score)] [14]. The CR model allows to account for liver transplantation and mortality as events “competing” with ACLF. The initial model included the most relevant characteristics at enrollment found to be significantly associated (both clinically and statistically) with ACLF development at 3 months in the univariate analysis (Table S3). In the final CLIF-C ACLF-D score model, the best subset of independent predictors was selected based on a stepwise forward procedure with p-in<0.05 and p-out<0.10 for the change in model log-likelihood (Table S4). The coefficients estimated for each predictor were used as relative weights to compute the score.
Because the PREDICT study is the sole thorough investigation on the factors leading to develop ACLF, there were no other cohort that could serve for external validation. As a result, we had to carry out a random split-sample derivation and validation processes for the new score. The subset of patients used to derive the score included 2/3 of patients (n=707) randomly selected from each patient group. The internal score validation was performed on the remaining 1/3 of patients (n=364) and compared the predictive ability of the CLIF-C ACLF-D score with those of the CLIF-C AD, MELD, MELD-sodium and Child-Pugh scores by estimating the corresponding Harrel’ C-indexes and 95% confident intervals (CIs) both in the derivation and validation sets.
As a complementary tool to predict ACLF development, a decision tree model was fitted using the 980 patients with information about the development of ACLF. Patients, who died or were transplanted without presenting ACLF before 3 months were excluded. The clinical variables selected for the model were the independent predictors of ACLF development obtained in the multivariate analysis for the CLIF-C ACLF-D score. The decision tree algorithm selected the most relevant of these clinical variables, their position within the Decision Tree and their optimal cut-off values. The model was fitted using R software (version 3.6.3) rpart package with settings minsplit=10 and maxdepth=5. Also, the complexity parameter was set by default to 0.01. Model parameters were estimated using the function tune.rpart from the R package e1071, to select the best decision tree model, according to accuracy, sensitivity and specificity. A decision-tree plot was generated based on the model fitted. A 10-Fold cross validation was used to reduce over-fitting and to assess the discrimination ability of the model, by estimating the corresponding sensitivity and specificity of the model and compute the Area Under the ROC curve (AUC).”

Point 3 of the reviewer 3.
With all those analyses poorly presented, it is even not possible to disentangle what was pre-specified and what was done post-hoc.

Answer to point 3.
We apologize for this inconvenience. The present manuscript expands the description of the analysis approach and the different statistical methods used (see Answer to Point 2) and also on the result section.
The extension of the result section dealing with the prediction models has been expanded as follows:
“The CLIF-C ACLF-D score was developed to predict, at the time of hospital admission, the probability for a patient with AD to develop ACLF during the following 3 months. The initial model was fitted including all the main characteristics at enrollment found to be associated with the development of ACLF in the univariate analysis (Table S2). Patients age (years), presence of ascites, WBC count (x109/L), serum albumin (g/dL), serum bilirubin (mg/dL), and serum creatinine (mg/dL) at study enrollment were subsequently identified as the best subset of independent predictors in the final model (Table S3) and their coefficients were used as relative weight to compute the corresponding score. The equation for CLIF-C ACLF-D score is as follows:
CLIF-C ACLF-D score = ((0.03*Age) + (0.45*Ascites) + (0.26*ln(WBC)) – (0.37*Albumin) + (0.57*ln(Bilirubin)) + (1.72*ln(Creatinine)) +3*10.
The prognostic accuracy of CLIF-C ACLF-D score (Fig. 5A) was higher than those of

CLIF-C AD, MELD, MELD-Na and Child-Pugh scores in the derivation set. In the validation set, the CLIF-C ACLF-D showed a similar accuracy but smaller differences with regards to the other scores. Therefore, we were unable to design a new score to predict ACLF development more accurately than the traditional clinical scores.
The most relevant clinical variable selected by the Decision Tree model was creatinine, with a threshold of 1.3 mg/dL (Fig. 5B). Bilirubin, albumin, age and WBC were also selected to subsequently discriminate the patients. The terminal nodes with a probability of ACLF higher than 0.5, so classifying the patients as ACLF development included 14.1% of the patients. The model achieved a discriminating ability (AUC) of 0.76 (0.72-0.79), with high specificity (95%) but low sensitivity (38%), indicating an important misclassification among those patients who actually developed ACLF."
In addition, the revised version of the manuscript contains a new figure with two panels (Fig. 5) showing the results obtained in the prediction analysis. The legend of the figure shows a detailed explanation of the Decision Tree model as follows:
"Figure 5. Panel A. Comparison between the predictive ability of the CLIF-C ACLF-D score with those of the CLIF-C AD, MELD, MELD-sodium and Child-Pugh scores using Harrel' C-indexes and 95% confident intervals (Cis) both in the derivation and validation sets. Panel B. Decision Tree plot for the prediction of ACLF development during the 90-day follow-up period after enrollment. Each node shows the percentage of patients classified and their probability of ACLF development within the 90-day follow-up period after enrollment (also represented by the colors and color intensity). The blue color represents a probability of ACLF development >0.5. The green color represents a probability of ACLF development ACLF <0.5. The intensity of the color represents the estimated probability value. The upper node (root node) represents the entire population of patients (980 patients, 100%) included in the analysis and its corresponding probability of ACLF development before entering the model (0.22). Each node includes the estimated probability of subsequent subsets of patients."
We hope now the Reviewer will find the paper much clearer.

Point 4 of the reviewer 3.
Concerning the three "clinical courses", the wording is better, but the analysis still confusing. There are too many analyses and too much emphasis of these "groups", especially compared to the risk prediction model. If the focus is on these groups, then all the part on the risk prediction models could be removed. With the space constraints, choices have to be made, but a consistent and correctly described piece of research should be presented. The authors agreed that some analyses were prone to immortal time bias, but the figure 3 remains. Since the "groups" are defined by looking at the 3 first months of follow-up, no survival or cumulative incidence curves should be drawn for these group before 90 days. This is not correct. And there are two problems: 1) it is evident that individuals in the SDC "group" cannot dies or develop ACLF in this period, so the curve is not a scientific result. We know the answer by definition. So, it is totally useless. 2) patients do not belong to these groups since inclusion, but only when the event that classifies them in one of the groups occurs (or not for SDC). This is a well-known issue of immortal time bias. Some approaches have been proposed, such as landmark analyses or Mantel-Byar approach, for instance. In any case, the comparison of these groups cannot be performed as if they were defined at inclusion.

Answer to point 4.
We thank the referee for the important comment. Indeed, the main objective of the manuscript was to report new concepts on the clinical course and pathophysiology of acutely decompensated cirrhosis derived from the observation of the 1071 patients included in the study. These patients were stratified into three groups based on the development of ACLF during follow-up and the stability of the clinical course, estimated by the requirement of new hospital admissions during the 90-day follow-up period. Because ACLF is associated with high mortality and patients with stable clinical course (not requiring readmission), by definition, did not die during the 90-day follow-up period, the stratification process delineated three groups different prognosis. We were always aware of this feature. Consequently, the cumulative mortality curves included in the previous manuscript (Figure 3) did not attempt to prove differences in survival between the three groups, which were obviously determined by the stratification method, but just to show the morphology of the survival curves. The p value in the figure was related to the different survival between group 1 (pre-ACLF) and group 2 (UDC), both associated with high follow-up mortality. The reviewer is right to suggest

	that this figure was insufficiently explained and could induced confusion. Accordingly, this figure has been deleted in the new version of the manuscript. Additionally, the new text clearly indicates that differences in mortality rate between groups were determined by the stratification criteria. The new figure 3 consists in two panels. Figure 3 panel A is just descriptive, showing the timing of ACLF development during the 90-day follow-up period. It clearly shows that ACLF development in patients with pre-ACLF started to occur within the first days after hospitalization. This observation is essential for the design of future prophylactic treatments in these patients, which should be given soon after admission to hospital. Figure 3 panel B shows the cumulative incidence of death in the three groups of patients, using the 90th day after enrollment as landmark. This figure was previously included in the supplementary material but now it has been moved to the main body of the article as proposed by the reviewer. It shows that the different survival rates between groups detected within the first 90 days of follow-up were maintained within the period between the 90th day (landmark) and 1 year after enrollment. The following sentences were added to the text in relation with this new figure: “Fig.3A visualizes the cumulative rate of weekly occurrence of ACLF during the first 90 days after enrollment of patients with pre-ACLF. Fig. 3B shows that using the 90th day after enrollment as a landmark, the cumulative incidence of death one year after enrollment was also higher among patients assigned to the pre-ACLF group than among those assigned to one of the other two groups.” We hope that the raised issues are addressed properly in the revised manuscript.

Dear Professor Valla,
Dear Dominique,

Enclosed please find the point-by-point letter answering the criticisms and suggestions raised by Reviewer 3 to our article entitled **"The PREDICT study Uncovers Three Clinical Courses in Acutely Decompensated Cirrhosis with Distinct Pathophysiology".**

We have greatly modified the manuscript according to the suggestions of the third reviewer, taking care at the same time to keep changes, which were previously suggested and had received agreement by reviewers 1 and 2.

As indicated, the new manuscript has been submitted to an intense remodeling. We have taken into consideration all the suggestion of reviewer 3. In particular, we have carefully checked our compliance to TRIPOD guidelines and included to the submitted documents a completed check-list, as recommended by these guidelines. Now the main data on the prediction analysis are included in the main body of the manuscript. The supplementary material contains also three new tables (Tables S2, S3 and S4) dealing with missing values, and the Univariate and Multivariate analysis for the CLIF-C ACLF-D score. The revised manuscript underlines (red color) the changes introduced in response to the criticisms of Reviewer 3. The result section contains all the original data included in the previous versions of the manuscript. Therefore, non-essential parts of the material and methods and discussion were reduced in order to respect the space limitation and the length of the manuscript has not increased with respect to the last revised version of the manuscript.

We are most grateful to reviewer 3 for his/her valuable criticisms. I hope you will find now the article suitable for publication in the most prestigious **Journal of Hepatology**.

Sincerely yours

Jonel Trebicka on behalf of the authors

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Revised Submission Checklist

This form must be completed and submitted for all revised manuscripts. Without this form the manuscript will be returned to the corresponding author for completion.

Corresponding Author:	Jonel Trebicka
Manuscript Number:	JHEPAT-D-20-00507-R3

Below, provide the page number(s) or figure legend(s) where the information can be located. Please make sure that all the information requested below is present in the manuscript.

		Completed, or reported on page(s) or figure legend(s):
1) Submission		
a)	Title page: COI, Financial support, Authors' contributions, keywords.	1-6
b)	Structured abstract and lay summary	7, 8
c)	All tables and figures included, numbered correctly, with legends (p value and statistical test)	33-40
d)	Supplementary data included in a single, separate word file	Pages 1-12
e)	A detailed point by point response to reviewers comments and changes highlighted in text	1-9
f)	All authors to complete and upload an ICMJE conflict of interest form.	Y
g)	Graphical abstract	Y
2) Materials and methods		
a)	Completed the CTAT form for all reagents and resource to be added to supplementary material	Completed
b)	Identify the source and authentication of cell lines	N/A
c)	Identify animal species, number of animals used, strain, sex and age.	N/A
d)	For animal studies include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
e)	For qPCR data provide information according to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines	N/A
3) Human subjects		
a)	Identify the committee(s) approving the study protocol.	Page 11 and study protocol
b)	Include a statement confirming that informed consent was obtained from all subjects.	Page 11

c)	For randomized studies report the clinical trial registration number (at ClinicalTrials.gov or equivalent).	8
d)	For phase II and III randomized controlled trials:	
I.	Please refer to the CONSORT statement and submit the CONSORT checklist with your submission.	N/A
II.	Include all version of the study protocol and statistical plan (to be published as supplementary information)	N/A
e)	Identify the inclusion/exclusion criteria in the selection process for the patients included in the study	Page 11, 12

4) Statistics

a)	State what statistical tests were completed and why	Page 13-17
b)	Explain the sample size and how this size provides an adequate power to detect a pre-specified effect size.	Page 13-17

5) Data deposition (Provide accession codes for deposited data)

a)	When using public databases:	
I.	Identify the source and include a valid link	N/A
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b)	Data deposition in a public repository is mandatory for:	
I.	Protein, DNA and RNA sequences	N/A
II.	Microarray data	N/A
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CTAT methods

Tables for a "Complete, Transparent, Accurate and Timely account" (CTAT) are now mandatory for all revised submissions. The aim is to enhance the reproducibility of methods.

- Only include the parts relevant to your study
- Refer to the CTAT in the main text as 'Supplementary CTAT Table'
- Do not add subheadings
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If the CTAT form is not relevant to your study, please outline the reasons why:

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1.1 Antibodies

Name	Citation	Supplier	Cat no.	Clone no.
N/A				

1.2 Cell lines

Name	Citation	Supplier	Cat no.	Passage no.	Authentication test method
N/A					

1.3 Organisms

Name	Citation	Supplier	Strain	Sex	Age	Overall n number
N/A						

1.4 Sequence based reagents

Name	Sequence	Supplier
N/A		

1.5 Biological samples

Description	Source	Identifier
N/A		

1.6 Deposited data

Name of repository	Identifier	Link
N/A		

1.7 Software

Software name	Manufacturer	Version
SAS	SAS Institute Inc, Cary, NC	9.4
SPSS	IBM, Chicago, IL	19.0

1.8 Other (e.g. drugs, proteins, vectors etc.)

N/A		

1.9 Please provide the details of the corresponding methods author for the manuscript:

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2.0 Please confirm for randomised controlled trials all versions of the clinical protocol are included in the submission. These will be published online as supplementary information.

No randomization. Observational cohort study design. Study protocol has been introduced in supplementary materials and methods

POINT-BY-POINT RESPONSES

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Yours sincerely

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Developing and validation risk predictions models is not an easy task. As I said before, the use of a Fine-Gray model is correct, but how the model was developed and validated is not sufficiently described in the manuscript. In the methods, there is a mere reference to a derivation and validation sets, without explaining how they were created and used. This does not comply with TRIPOD guidelines, contrary to what is written in the rebuttal letter. Without precise methods, it is impossible to evaluate the study findings. Prognostic performance of the new score is no more presented in the main article, which is not acceptable, whereas it is for a decision tree-based model that we discover in the results, without any mention in the methods. Again, we do not know what was done, and this makes it difficult to draw any conclusion.

Answer to point 2.

Thank you for these remarks. We apologize for not having answered clearly to this point. Initially, the main objective of the paper involved only the definition of the three prognostic groups. As already pointed out, the derivation and validation of the *CLIF-C ACLF-D* score, as well as the decision-tree model were included in the article following the suggestions and requests of the other reviewers. In the present manuscript TRIPOD recommendations are followed both in the methodology and in the process of data analysis and the checklist is included as supplementary material (see below). Yet the focus is again the detailed description of the three groups of AD. We hope that this sufficiently addresses the raised point.

TRIPOD Checklist: Prediction Model Development

Section/Topic	Item	Checklist Item	Page
Title and abstract			
Title	1	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	8
Introduction			
Background and objectives	3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	9
	3b	Specify the objectives, including whether the study describes the development or validation of the model or both.	10
Methods			
Source of data	4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	11-13
	4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	11
Participants	5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	11
	5b	Describe eligibility criteria for participants.	11
	5c	Give details of treatments received, if relevant.	n.a.
Outcome	6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	14
	6b	Report any actions to blind assessment of the outcome to be predicted.	
Predictors	7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	15-17
	7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	n.a.
Sample size	8	Explain how the study size was arrived at.	15-17
Missing data	9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	15
Statistical analysis methods	10a	Describe how predictors were handled in the analyses.	15-17
	10b	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	15-17
	10d	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	15-17
Risk groups	11	Provide details on how risk groups were created, if done.	n.a.
Results			
Participants	13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	18-19
	13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	19-20
Model development	14a	Specify the number of participants and outcome events in each analysis.	24-25
	14b	If done, report the unadjusted association between each candidate predictor and outcome.	24-25
Model specification	15a	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	24-25
	15b	Explain how to the use the prediction model.	24-25
Model performance	16	Report performance measures (with CIs) for the prediction model.	24-25
Discussion			
Limitations	18	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	27
Interpretation	19b	Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence.	27
Implications	20	Discuss the potential clinical use of the model and implications for future research.	29
Other information			
Supplementary information	21	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Tables S2-S4
Funding	22	Give the source of funding and the role of the funders for the present study.	5

The new version of the manuscript contains a detailed explanation of the statistical method used. To include this, we have modified the whole article reducing the extension of the contents included in the initial manuscript to provide space for these new text passages. Now the Material and Method section of the revised manuscript contains the following new information explaining the methodology used:

"For the prediction of ACLF development during the 90-day follow-up period, the CLIF-Consortium ACLF development score was fitted according to the TRIPOD recommendations (please see TRIPOD checklist). There were no missing data in most potential predictors of ACLF development at enrollment, except for serum albumin and plasma CRP levels, whose values were not available, respectively, in 5%, 9% and 8% of patients of the pre-ACLF, UDC, and SDC groups and in 20%, 13% and 11% of patients of the three groups **(Table S2)**. Therefore, for multivariate analysis we assumed that these missing values could be considered at random and carried out a multiple imputation based on a mixed model including all potential predictors significantly associated with ACLF in the univariate analysis [13].

We used the proportional-hazards model for competing risks (CR) proposed by Fine and Gray to identify the best subset of independent predictors associated with the onset of ACLF and to develop a new predictive score [the CLIF-C ACLF-Development score (CLIF-C- ACLF-D score)] [14]. The CR model allows to account for liver transplantation and mortality as events "competing" with ACLF. The initial model included the most relevant characteristics at enrollment found to be significantly associated (both clinically and statistically) with ACLF development at 3 months in the univariate analysis (**Table S3**). In the final CLIF-C ACLF-D score model, the best subset of independent predictors was selected based on a stepwise forward procedure with p-in<0.05 and p-out<0.10 for the change in model log-likelihood (**Table S4**). The coefficients estimated for each predictor were used as relative weights to compute the score.

Because the PREDICT study is the sole thorough investigation on the factors leading to develop ACLF, there were no other cohort that could serve for external validation. As a result, we had to carry out a random split-sample derivation and validation processes for the new score. The subset of patients

used to derive the score included 2/3 of patients (n=707) randomly selected from each patient group. The internal score validation was performed on the remaining 1/3 of patients (n=364) and compared the predictive ability of the CLIF-C ACLF-D score with those of the CLIF-C AD, MELD, MELD-sodium and Child-Pugh scores by estimating the corresponding Harrel' C-indexes and 95% confident intervals (CIs) both in the derivation and validation sets.

As a complementary tool to predict ACLF development, a decision tree model was fitted using the 980 patients with information about the development of ACLF. Patients, who died or were transplanted without presenting ACLF before 3 months were excluded. The clinical variables selected for the model were the independent predictors of ACLF development obtained in the multivariate analysis for the CLIF-C ACLF-D score. The decision tree algorithm selected the most relevant of these clinical variables, their position within the Decision Tree and their optimal cut-off values. The model was fitted using R software (version 3.6.3) rpart package with settings minsplit=10 and maxdepth=5. Also, the complexity parameter was set by default to 0.01. Model parameters were estimated using the function tune.rpart from the R package e1071, to select the best decision tree model, according to accuracy, sensitivity and specificity. A decision-tree plot was generated based on the model fitted. A 10-Fold cross validation was used to reduce over-fitting and to assess the discrimination ability of the model, by estimating the corresponding sensitivity and specificity of the model and compute the Area Under the ROC curve (AUC)."

Point 3 of the reviewer 3.

With all those analyses poorly presented, it is even not possible to disentangle what was pre-specified and what was done post-hoc.

Answer to point 3.

We apologize for this inconvenience. The present manuscript expands the description of the analysis approach and the different statistical methods used (see Answer to Point 2) and also on the result section.

The extension of the result section dealing with the prediction models has been expanded as follows:

"The CLIF-C ACLF-D score was developed to predict, at the time of hospital admission, the probability for a patient with AD to develop ACLF during the following 3 months. The initial model was fitted including all the main characteristics at enrollment found to be associated with the development of ACLF in the univariate analysis (**Table S2**). Patients age (years), presence of ascites, WBC count ($x10^9$/L), serum albumin (g/dL), serum bilirubin (mg/dL), and serum creatinine (mg/dL) at study enrollment were subsequently identified as the best subset of independent predictors in the final model (**Table S3**) and their coefficients were used as relative weight to compute the corresponding score. The equation for CLIF-C ACLF-D score is as follows:

CLIF-C ACLF-D score = ((0.03*Age) + (0.45*Ascites) + (0.26*ln(WBC)) – (0.37*Albumin) + (0.57*ln(Bilirubin)) + (1.72*ln(Creatinine)) +3*10.

The prognostic accuracy of CLIF-C ACLF-D score (**Fig. 5A**) was higher than those of CLIF-C AD, MELD, MELD-Na and Child-Pugh scores in the derivation set. In the validation set, the CLIF-C ACLF-D showed a similar accuracy but smaller differences with regards to the other scores. Therefore, we were unable to design a new score to predict ACLF development more accurately than the traditional clinical scores.

The most relevant clinical variable selected by the Decision Tree model was creatinine, with a threshold of 1.3 mg/dL (Fig. 5B). Bilirubin, albumin, age and WBC were also selected to subsequently discriminate the patients. The terminal nodes with a probability of ACLF higher than 0.5, so classifying the patients as ACLF development included 14.1% of the patients. The model

achieved a discriminating ability (AUC) of 0.76 (0.72-0.79), with high specificity (95%) but low sensitivity (38%), indicating an important misclassification among those patients who actually developed ACLF.*"*

In addition, the revised version of the manuscript contains a new figure with two panels **(Fig. 5)** showing the results obtained in the prediction analysis. The legend of the figure shows a detailed explanation of the Decision Tree model as follows:

"Figure 5. *Panel A*. Comparison between the predictive ability of the CLIF-C ACLF-D score with those of the CLIF-C AD, MELD, MELD-sodium and Child-Pugh scores using Harrel' C-indexes and 95% confident intervals (Cis) both in the derivation and validation sets. Panel B. Decision Tree plot for the prediction of ACLF development during the 90-day follow-up period after enrollment. Each node shows the percentage of patients classified and their probability of ACLF development within the 90-day follow-up period after enrollment (also represented by the colors and color intensity). The blue color represents a probability of ACLF development >0.5. The green color represents a probability of ACLF development ACLF <0.5. The intensity of the color represents the estimated probability value. The upper node (root node) represents the entire population of patients (980 patients, 100%) included in the analysis and its corresponding probability of ACLF development before entering the model (0.22). Each node includes the estimated probability of subsequent subsets of patients."

We hope now the Reviewer will find the paper much clearer.

Point 4 of the reviewer 3.

Concerning the three "clinical courses", the wording is better, but the analysis still confusing. There are too many analyses and too much emphasis of these "groups", especially compared to the risk prediction model. If the focus is on these groups, then all the part on the risk prediction models could be removed. With the space constraints, choices have to be made, but a consistent and correctly described piece of research should be presented. The authors agreed that some analyses were prone to immortal time bias, but the figure 3 remains. Since the "groups" are defined by looking at the 3 first months of follow-up, no survival or cumulative incidence curves should be drawn for these group before 90 days. This is not correct. And there are two problems: 1) it is evident that individuals in the SDC "group" cannot dies or develop ACLF in this period, so the curve is not a scientific result. We know the answer by definition. So, it is totally useless. 2) patients do not belong to these groups since inclusion, but only when the event that classifies them in one of the groups occurs (or not for SDC). This is a well-known issue of immortal time bias. Some approaches have been proposed, such as landmark analyses or Mantel-Byar approach, for instance. In any case, the comparison of these groups cannot be performed as if they were defined at inclusion.

Answer to point 4.

We thank the referee for the important comment. Indeed, the main objective of the manuscript was to report new concepts on the clinical course and pathophysiology of acutely decompensated cirrhosis derived from the observation of the 1071 patients included in the study. These patients were stratified into three groups based on the development of ACLF during follow-up and the stability of the clinical course, estimated by the requirement of new hospital admissions during the 90-day follow-up period. Because ACLF is associated with high mortality and patients with stable clinical course (not requiring readmission), by definition, did not die during the 90-day follow-up period, the stratification process delineated three groups different prognosis. We were always aware of this feature. Consequently, the cumulative mortality

curves included in the previous manuscript (**Figure 3**) did not attempt to prove differences in survival between the three groups, which were obviously determined by the stratification method, but just to show the morphology of the survival curves. The p value in the figure was related to the different survival between group 1 (pre-ACLF) and group 2 (UDC), both associated with high follow-up mortality. The reviewer is right to suggest that this figure was insufficiently explained and could induced confusion. Accordingly, this **figure has been deleted in the new version of the manuscript**. Additionally, the new text clearly indicates that differences in mortality rate between groups were determined by the stratification criteria.

The new **figure 3** consists in two panels**. Figure 3 panel A** is just descriptive, showing the timing of ACLF development during the 90-day follow-up period. It clearly shows that ACLF development in patients with pre-ACLF started to occur within the first days after hospitalization. This observation is essential for the design of future prophylactic treatments in these patients, which should be given soon after admission to hospital. **Figure 3 panel B** shows the cumulative incidence of death in the three groups of patients, using the 90th day after enrollment as landmark. This figure was previously included in the supplementary material but now it has been moved to the main body of the article as proposed by the reviewer. It shows that the different survival rates between groups detected within the first 90 days of follow-up were maintained within the period between the 90th day (landmark) and 1 year after enrollment. The following sentences were added to the text in relation with this new figure:

"Fig.3A visualizes the cumulative rate of weekly occurrence of ACLF during the first 90 days after enrollment of patients with pre-ACLF. **Fig. 3B** shows that using the 90th day after enrollment as a landmark, the cumulative incidence of death one year after enrollment was also higher among patients assigned to the pre-ACLF group than among those assigned to one of the other two groups."

We hope that the raised issues are addressed properly in the revised manuscript.

PATTERNS OF ACUTE DECOMPENSATION IN CIRRHOSIS

Title page

TITLE: The PREDICT study uncovers three clinical courses of acutely decompensated cirrhosis that have distinct pathophysiology

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Short title: PATTERNS OF ACUTE DECOMPENSATION IN CIRRHOSIS

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Keywords: Chronic liver disease, Non-elective admission, acute complications, Outcome, Risk factors.

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Abbreviations: ACLF (acute-on-chronic liver failure), CLIF-C (European Foundation for the study of chronic liver failure consortium), AD (acute decompensation), MELD (Model of End-Stage Liver Disease), INR (international normalized ratio), WBC (white blood cell count), HR (hazard ratio), 95% CI (95 % confidence interval),

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Conflict of Interest: None of the authors have conflicts of interest for the reported study.

Author contributions: JT, JF, WL, JC, RJ, RM, PG, PA, VA: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, funding recipient, administrative, technical and material support, study supervision; EG, AA, AC, CP, MP, CS, AC, AM, FA: acquisition of data, analysis of data, technical and material support; TT, MB, PA, CA, FEU, CJ, MST, TG, AA, WL, ES, RB, MJ, CS, TR, JA, PG, WB, SZ, CR, TB, AS, LLG, MC, OR, RS, HZ, AC, GSP, AdG, HG, FS, CT, OCÖ, FS, SR, RA, MRG, HVV, CF, MM, MP, PC, SP, IG, MP, VV, RM, ZV, MB, EB: acquisition of data, interpretation of data, critical revision of the manuscript regarding important intellectual content

Tables and Figures: 3 Tables, 5 Figures

Word count (max. **6,000 words,** inclusive of abstract, main text, references, figure legends): **6,623**

Lay summary:

Patients with acutely decompensated cirrhosis, who do not have organ failures (i.e., no acute-on-chronic liver failure [ACLF]) are considered as having acute decompensation (AD). The present study describes for the first time three different clinical courses of patients with AD after hospital admission. The first clinical course (pre-ACLF) includes patients who develop ACLF and has high probability of death. These patients are characterized by high-grade systemic inflammation. The second clinical course (unstable decompensated cirrhosis) includes patients requiring frequent hospitalizations unrelated with ACLF, show low-grade systemic inflammation but suffer characteristically from complications related to severe portal hypertension. They present lower risk of mortality than patients with pre-ACLF. Finally, the third clinical course (stable decompensated cirrhosis), includes two-third of all patients admitted hospital with AD. They do not present severe systemic inflammation or frequent complications related with portal hypertension, rarely require hospital admissions and present an extremely low 1-year mortality risk.

Abstract

Background & Aims:

Acute decompensation (AD) of cirrhosis is defined by the acute development of ascites, gastrointestinal hemorrhage, hepatic encephalopathy, ~~i~~nfection or any combination of these, requiring hospitalization. The presence of organ failure(s) in patients with AD defines acute-on-chronic liver failure (ACLF), while their absence defines AD. We designed the PREDICT study, a European, prospective, observational study, to characterize the clinical course of AD and predict ACLF.

Methods:

A total of 1071 patients with AD were enrolled to collect detailed pre-specified information on the 3-month period prior to enrollment, and clinical and laboratory data at enrollment. Patients were then closely followed-up for 3 months. The 12-month outcomes (liver transplantation, and death) were also recorded.

Results:

Three groups of patients were identified: Pre-ACLF patients (n=218), who developed ACLF and had 3-month and 1-year mortality rates of 53.7% and 67.4%, respectively. Unstable decompensated cirrhosis (UDC) patients (n = 233) required ≥1 readmission but not developing ACLF and had 21.0% and 35.6% mortality rates. Stable decompensated cirrhosis (SDC) patients (n = 620) who were neither readmitted, nor developed ACLF and showed a 1-year mortality of only 9.5%. The 3 groups differed significantly in the grade and course of systemic inflammation (high-grade at enrollment with aggravation during follow-up in pre-ACLF; low-grade at enrollment with subsequent steady-course in UDC; and low-grade at enrollment with subsequent improvement in the SDC) and prevalence of surrogates of severe portal hypertension throughout the study (high in UDC versus low in pre-ACLF and SDC).

Conclusions:

Acute decompensation without ACLF is a heterogeneous condition with three different clinical courses and two major pathophysiological mechanisms: systemic inflammation and portal hypertension. Prediction of ACLF development remains a major future task.

(ClinicalTrials.gov number, NCT03056612)

Word count (max. 275): 270

Acute decompensation (AD) of cirrhosis defines the acute development of ascites, hepatic encephalopathy, gastrointestinal hemorrhage or bacterial infections or any combination of these [1-3]. AD is an extremely relevant feature during the clinical course of cirrhosis. The first episode of AD signals the transition from compensated to decompensated cirrhosis [4]. Decompensated cirrhosis is characterized by recurrent episodes of AD. Finally, recent data from the CANONIC study have shown that AD has two distinct clinical presentations, depending on the presence or absence of organ failures and the grade of systemic inflammation [5-8]. The presence of both organ failures and high-grade systemic inflammation is the hallmark of acute-on-chronic liver failure (ACLF), a syndrome associated with very high 28-day mortality rate, while AD is associated with moderate systemic inflammation and low 28-day mortality rate. Systemic inflammation in AD and ACLF frequently develops in association with exogenous precipitating events (mainly bacterial infections or acute alcoholic liver injury). However, it might also be secondary to translocation of intestinal bacterial immunogenic material to the systemic circulation [9,10]. Systemic inflammation may induce organ dysfunction/failure by a direct immunopathological effect on peripheral organs and on the other hand by mitochondrial dysfunction, both identified in decompensated cirrhosis with and without ACLF [8].

The CANONIC study was specifically designed to characterize ACLF but did not provide detailed information on the clinical context prior to and after ACLF and AD development. Yet, the CANONIC study showed that patients with AD had very low mortality rate (~2%) at 28 days but a substantial mortality rate (10%) at 90 days, suggesting a heterogeneity of clinical course in AD patients. Detailed information on this period is an unmet medical need for the rational management of patients with AD and the prevention of ACLF development.

To answer these questions, we designed the PREDICT study (PREDICTing Acute-on-Chronic Liver Failure), the second prospective large-scale observational investigation performed by the European Association for the Study of the Liver (EASL)-Chronic Liver Failure (CLIF) Consortium. It included 1071 patients with cirrhosis hospitalized for the treatment of an episode of AD without ACLF. The current article reports the results of the first study derived from this investigation, which was aimed to characterize the clinical course and pathophysiology of AD, and to predict development of ACLF.

Methods

Study oversight

The PREDICT study is a European, multicenter, prospective, observational study performed in 48 hospitals. Each hospital had liver unit, specific ward(s) for liver patients and intensive care facilities, and all of them had access to a liver transplantation program. The study protocol (available with the full text of this article) was approved by the institutional review board (IRB) at each participating center. Patients were screened and enrolled from March 2017 to July 2018. Written informed consent was obtained from patients or their legal surrogates before enrollment. An investigator was responsible for enrolling patients in the study at each center, ensuring adherence to the protocol, and completing the electronic case-report form (eCRF). Data were continuously monitored on-line by the Data Management Center of the EF-Clif. All authors had access to the study data and reviewed and approved the final manuscript.

Patients

A total of 1219 patients non-electively admitted for the treatment of an episode of AD were eligible. One-hundred and forty-eight patients had exclusion criteria **(Table S1)** and 1071 patients were analyzed. Among these, 218 developed AD for the first time, and the remaining 853 had a prior history of AD. The diagnosis of cirrhosis was based on previous liver biopsy findings or a composite of clinical signs and findings provided by laboratory test results, endoscopy and ultrasonography. Diagnostic criteria for AD upon hospitalization were based on development of ascites, hepatic encephalopathy, gastrointestinal hemorrhage, infection, or any combination of these. Importantly, in none of the enrolled patients was AD due to an isolated bacterial infection. Diagnosis of ACLF during follow-up was performed

according to the CANONIC study criteria [7]. Organ failure and organ dysfunction were defined according to the CLIF consortium (CLIF-C) Organ Failure (OF) score [11].

Study Design

Pre-specified clinical data, standard laboratory data and biological samples for biobanking were obtained at enrollment and sequentially during the follow up visits **(Fig. 1)**. The eCRF was designed to collect granularity in the clinical data and the detailed queries answered remaining issues in case of inconsistencies. This manuscript analyzes only clinical and standard laboratory data.

Data Obtained at Enrollment

Two categories of pre-specified information were obtained at enrollment. The first category included general characteristic and demographic data, specific data related to the AD episode at enrollment, results of physical examination and standard laboratory analysis, including differential white-cell blood count (WBC) and C-reactive protein (CRP) levels, as markers of systemic inflammation. Cultures were routinely performed in patients with suspected bacterial infections.

The second category of pre-specified data is related to the past medical history and include: a) the timepoint of the onset of decompensated cirrhosis (as defined by the first episode of AD); b) the complications of AD occurring within the last 3 months prior to enrollment; c) treatment of complications (including prior trans-jugular portosystemic shunt stent (TIPS) and its indication); and d) any hospitalization during the last 3 months prior to enrollment. Data regarding onset of decompensated cirrhosis could be obtained in 612 patients. Data regarding the occurrence of ascites, gastrointestinal hemorrhage, hepatic encephalopathy, bacterial infections and

hospitalizations within the last 3-month period prior to enrollment were obtained in 860, 796, 793, 791 and 831 patients, respectively.

Data Obtained During Follow-up

After enrollment, patients were prospectively followed up for a period of 3 months. The scheme of visits and collection of data and samples at enrollment and during the 3-month follow-up period after enrollment is indicated in **Fig. 1**. Finally, data on liver transplantation or death and causes of death were prospectively collected 3, 6 and 12 months after enrollment in all patients.

Defining the 6-Month Observational Period

Of note, according to the pattern of data collection described earlier, we defined a 6-month observational period, which included the 3-month period prior to enrollment, the enrollment visit and the 3-month follow-up period after enrollment **(Fig. 1)**.

Amendment to the initial study protocol

During the first eight months of the study, 720 patients were consecutively enrolled, and used for prevalence calculations. Subsequently, since the number of patients developing ACLF was low, we amended the study protocol to enroll only high-risk patients. After IRB approval of this amendment, the last 351 patients were enrolled in the study.

Statistical analysis

Patients stratification

Patients stratification was performed based on the clinical course during the 3-month follow-up period for several reasons: 1. The main objective of the study was the characterization of the clinical course after enrollment; 2. A preliminary analysis of an incomplete set of consecutive patients included in the PREDICT study showed that AD consisted in a single complication (either ascites, encephalopathy or gastrointestinal hemorrhage) in only 50% of patients. The remaining patients had 2 or 3 simultaneous complications, making extremely complex the stratification based on complications at enrollment. 3) By contrast, stratification of patients based on ACLF development (yes or no) and clinical course profile (unstable versus stable, among ACLF-free patients) during the 3-month follow-up was more simple and appropriated for the main objective of the study.

Therefore, for data analysis, our patients were stratified for data analysis into three groups: 1) Pre-ACLF group: patients who developed ACLF within 90-day after enrollment; 2) Unstable decompensated cirrhosis (UDC) group: patients who experienced at least one hospital readmission, but without ACLF development within 90-day follow-up period; and 3) Stable decompensated cirrhosis (SDC) group: patients without ACLF development or readmissions within 90-day follow-up period.

Because bacterial infections are major precipitants of AD and ACLF, and systemic inflammation the hallmark of these complications, infections and systemic inflammation were considered in detail in the characterization process of these groups.

Data analysis

Discrete variables were summarized as counts (percentages) and continuous variables as mean ± standard deviation (SD). Non-normally distributed variables are summarized by the median (interquartile range [IQR]) and were log-transformed for

some statistical analyses and for graphical comparisons. In univariate statistical comparisons, the chi-square test was used for categorical variables, whereas the Student's *t*-test or analysis of variance were used for normal-distributed continuous variables and the Wilcoxon signed-rank test or the Kruskal-Wallis test for continuous variables not normally distributed. In all statistical analyses, significance was set at $p<0.05$.

Tools to predict ACLF development

For the prediction of ACLF development during the 90-day follow-up period, the CLIF-Consortium ACLF development score was fitted according to the recommendations for "Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis" (TRIPOD; please see TRIPOD checklist). There were no missing data in most potential predictors of ACLF development at enrollment, except for serum albumin and plasma CRP levels, whose values were not available, respectively, in 5%, 9% and 8% of patients of the pre-ACLF, UDC, and SDC groups and in 20%, 13% and 11% of patients of the three groups **(Table S2)**. Therefore, for multivariate analysis we assumed that these missing values could be considered at random and carried out a multiple imputation based on a mixed model including all potential predictors significantly associated with ACLF in the univariate analysis [13].

We used the proportional-hazards model for competing risks (CR) proposed by Fine and Gray to identify the best subset of independent predictors associated with the onset of ACLF and to develop a new predictive score [the CLIF-C ACLF-Development score (CLIF-C- ACLF-D score)] [14]. The CR model allows to account for liver transplantation and mortality as events "competing" with ACLF. The initial model included the most relevant characteristics at enrollment found to be

significantly associated (both clinically and statistically) with ACLF development at 3 months in the univariate analysis (**Table S3**). In the final CLIF-C ACLF-D score model, the best subset of independent predictors was selected based on a stepwise forward procedure with p-in<0.05 and p-out<0.10 for the change in model log-likelihood (**Table S4**). The coefficients estimated for each predictor were used as relative weights to compute the score.

Because the PREDICT study is the sole thorough investigation on the factors leading to develop ACLF, there were no other cohort that could serve for external validation. As a result, we had to carry out a random split-sample derivation and validation processes for the new score. The subset of patients used to derive the score included 2/3 of patients (n=707) randomly selected from each patient group. The internal score validation was performed on the remaining 1/3 of patients (n=364) and compared the predictive ability of the CLIF-C ACLF-D score with those of the CLIF-C AD, MELD, MELD-sodium and Child-Pugh scores by estimating the corresponding Harrel' C-indexes and 95% confident intervals (CIs) both in the derivation and validation sets.

As a complementary tool to predict ACLF development, a decision tree model was fitted using the 980 patients with information about the development of ACLF. Patients, who died or were transplanted without presenting ACLF before 3 months were excluded. The clinical variables selected for the model were the independent predictors of ACLF development obtained in the multivariate analysis for the CLIF-C ACLF-D score. The decision tree algorithm selected the most relevant of these clinical variables, their position within the Decision Tree and their optimal cut-off values. The model was fitted using R software (version 3.6.3) rpart package with settings minsplit=10 and maxdepth=5. Also, the complexity parameter was set by default to 0.01. Model parameters were estimated using the function tune.rpart from

the R package e1071, to select the best decision tree model, according to accuracy, sensitivity and specificity. A decision-tree plot was generated based on the model fitted. A 10-Fold cross validation was used to reduce over-fitting and to assess the discrimination ability of the model, by estimating the corresponding sensitivity and specificity of the model and compute the area under the receiver-operating-characteristic curve (AUC).

Results

Heterogeneity of the clinical course of AD

Clinical course of patients with AD

As expected, the Pre-ACLF group, which included 218 patients who developed ACLF during the 3-month follow-up period after enrollment, showed the highest 3-month and 1-year mortality rates (53.7% and 67.4%, respectively) (**Table 1).** 22 patients with Pre-ACLF were transplanted after ACLF episode within the 3-month follow-up period. The 233 patients included in the UDC group, who did not develop ACLF, but who died or required at least one hospital readmission within the 3-month follow-up period, showed a 3-month and 1-year mortality rates of 21.0% and 35.6%, respectively; 177 of these patients required one readmission, 32 patients two readmissions, and 17 patients three or more readmissions. 14 patients with UDC were transplanted after readmission for an AD episode within the 3-month follow-up period. Finally, the 620 patients included in the SDC group, who by definition did not develop ACLF nor required hospital readmissions or died during the 3-month follow-up period after enrollment, showed very low mortality (9.5%) within the 1-year follow-up period after enrollment. Among the 720 patients consecutively enrolled during the first 8 months after the onset of the study, 425 (59%) were in SDC group. 28 patients with SDC were transplanted from the waitlist without ACLF or new episode of AD within the 3-month follow-up period.

The clinical course of patients with pre-ACLF was characterized by a huge density of bacterial infections, episodes of ACLF and death, which are summarized as events (**Fig. 2**). A total of 120 patients (55% of this group) developed ACLF during the first hospitalization and 98 developed the syndrome from first discharge to the end of the 3-month follow-up period. The bacterial infection density curve preceded chronologically the ACLF density curve, and both curves preceded the mortality

density curve, supporting a cause to effect relationship between the three events. The extreme proximity between the bacterial infection and ACLF density curves reflects that ACLF is a hyperacute process with a very short time period between precipitating events and the onset of the syndrome. **Fig.3A** visualizes the cumulative rate of weekly occurrence of ACLF during the first 90 days after enrollment of patients with pre-ACLF. **Fig. 3B** shows that using the 90th day after enrollment as a landmark, the cumulative incidence of death one year after enrollment was also higher among patients assigned to the pre-ACLF group than among those assigned to one of the other two groups. The density of events in the UDC group was remarkably lower than the density of events in the pre-ACLF group. Although this feature was mainly due to the lack of ACLF episodes in the UDC group, the density of bacterial infections and deaths were also lower. Finally, although the density of bacterial infections at first presentation in the SDC group was as high as in the UDC group, it was remarkably lower during the rest of the 3-month follow-up period.

There were no significant differences between the three groups of patients regarding the etiology of cirrhosis (**Table 1**), prevalence of active alcoholism (26.6%, 23.2% and 27.6%, respectively) or presence of hepatocellular carcinoma (within Milan criteria) at enrollment (5.4%, 6.5% and 3%, respectively). Moreover, there was no between-group difference in the number of patients with alcohol cessation (52 [23.9%] patients for pre-ACLF, 46 [19.7%] for UDC, and 146 [23.6%] for SDC; p = 0.456) and the number of those receiving HCV-therapy (4 [1.9%] patients for pre-ACLF, 3 [1.3%] for UDC, and 14 [2.3%] for SDC; p = 0.650).

Duration of the decompensated phase of cirrhosis

The time-course density curves of liver transplantation or death in the 234 patients developing these events are shown in **Fig. 4A**. Time zero in this figure

represents the onset of decompensated cirrhosis. Therefore, this analysis estimates the between-group differences in the length of the entire phase of decompensated cirrhosis. The pre-ACLF density curve preceded the UDC density curve, and both curves preceded the SDC density curve. These findings clearly indicate that ACLF development in patients with pre-ACLF significantly reduced the duration of the decompensated phase of the disease. Confirming these observations, the median time from the onset of decompensated cirrhosis to death or liver transplantation was 12 months (IQR 5.2-25.8) in patients with pre-ACLF, 14 months (9.6-24.3) in patients with UDC (p = 0.01 versus patients with pre-ACLF), and 20 months (11.4-41.3) in patients with SDC (p = 0.04 versus patients with UDC). These findings are confirmed by comparing individual values of the time period between the onset of decompensated cirrhosis and liver transplantation, death or end of follow-up between the three groups (**Fig. 4B**). Considering the between-group differences in mortality, this distinct duration of the decompensation phase of the disease would have been even more marked with a follow-up of mortality for more than 1 year.

Prevalence and severity of bacterial infections

Table 2 provides information about infections during the 3 months before enrollment, at enrollment and during the 3 months after enrollment. Overall, 178 (22.4%) out of the 796 patients with data developed at least one bacterial infection during the three-month period prior to enrollment. The number of patients with infections at enrollment and during the three-month follow-up period in the 1071 patients included in the analysis were 29.3% (n=314) and 24% (n=257), respectively. These 571 patients with infections at enrollment or during follow-up (53.3%) presented a total of 674 infections.

Considering bacterial infections**, Table 2** shows that the distinctive features of patients with pre-ACLF relative to patients of the two other groups included a higher proportion of patients with at least one infectious episode during the 6-month observational period (see also **Fig. 4C**); higher proportion of patients with sepsis at enrollment and during follow-up; higher proportion of patients with pneumonia during follow-up; and higher proportion of patients receiving therapeutic antibiotics; all these differences being significant. During follow-up, the proportion of patients with community-acquired infection was significantly lower among patients with pre-ACLF than among those of the two other groups (**Table 2**). These findings are consistent with higher prevalence and severity of bacterial infections in the pre-ACLF group. At any time, the proportion of patients with infections caused by multi-drug-resistant bacteria was significantly higher between the pre-ACLF group and the UDC group (**Table 2**).

Clinical features prior to enrollment

Patients with pre-ACLF and UDC, who showed by definition significantly greater clinical course instability during the first 3 months after enrollment compared to patients with SDC, also had greater clinical course instability within the 3-month period prior to enrollment, as indicated by the significantly higher frequency of bacterial infections, ascites or hepatic encephalopathy and, consequently, hospital admissions, presented by these groups of patients (**Tables 1 and 2**).

Clinical features and laboratory data at enrollment and during follow-up

Markers of systemic inflammation across groups

The WBC count and the C-reactive protein levels (CRP) were significantly higher at enrollment in patients with pre-ACLF than in patients from the other two

groups (**Table 1**). In contrast, there were no significant differences in these biomarkers between patients with UDC and SDC.

We compared the CRP levels and WBC measured at enrollment in patients with SDC, UDC and pre-ACLF, with those measured at the time of follow-up diagnosis of ACLF in 176 patients from the pre-ACLF group (including 103 patients with ACLF-1, 52 with ACLF-2, and 21 with ACLF-3), and those measured in a control group of 34 patients with compensated cirrhosis (no prior history of AD) (**Fig. 4D**) previously described [5, 6]. Of note, the last two groups were included to facilitate the comparison of systemic inflammation throughout the whole spectrum of cirrhosis. There was a progressive increase in the grade of systemic inflammation across the different groups.

We also performed within-group comparisons of the levels of inflammatory markers measured at enrollment versus those measured during follow-up (**Table 3**). The follow-up time point was the time of diagnosis of ACLF for the pre-ACLF group, while for the other two groups of patients, it was the last measurement prior to liver transplantation, or death, or the end of the three-month follow-up period. Within each group, there was a close relationship between changes in inflammatory markers and the clinical course (**Table 3**). Progression of AD to ACLF in the pre-ACLF group occurred in the setting of a significant increase in WBC count and serum concentration of CRP. In patients with UDC there were no significant changes in WBC count and a small, but significant decrease in CRP, suggesting minor improvement of systemic inflammation. Finally, patients with SDC had a significant reduction in WBC and PCR.

Association between systemic inflammation and complications that define AD

In order to assess the association between systemic inflammation and the three major complications that define AD, we explored 134 patients who had no prior history of AD and were enrolled only for ascites (n=99), encephalopathy (n=14) or gastrointestinal hemorrhage (n=21). The median (IQR) levels of plasma CRP was remarkably higher (p <0.002) in patients with ascites (23.4 [12.5-38.0]) than in those with encephalopathy (11.0 [4.4-21.6]) and gastrointestinal hemorrhage (5.0 [3.0-22.4]) (**Fig. 4E**).

Organ function and scores

The prevalence of liver failure, liver dysfunction and renal dysfunction (as defined by the CLIF-C OF score [11]) at enrollment was significantly higher among patients with pre-ACLF group than among those with UDC and SDC (**Table 1**). Moreover, laboratory measurements estimating liver and renal function at enrollment were significantly more impaired among patients with pre-ACLF than among those with UDC and SDC, suggesting that a significant deterioration of organ function existed prior to enrollment in patients with pre-ACLF.

CLIF-C AD and MELD-Na scores significantly worsened during the progression of pre-ACLF to ACLF and improved in patients with SDC (**Table 3**). Scores also improved in patients with UDC, although to a lesser extent than in patients with SDC.

Increased prevalence of features suggesting severe portal hypertension in patients with UDC

Whereas severe systemic inflammation and organ failure or dysfunction were the most prominent features in patients from the pre-ACLF group, surrogates of severe portal hypertension were the hallmark of patients with UDC. First, the

prevalence of circulatory dysfunction at enrollment (**Table 1**) and of gastrointestinal hemorrhage within the 6-month observational period (32% versus 22% [*p* = 0.01] and 25% [*p* = 0.03], respectively) were significantly higher among patients with UDC than among those with pre-ACLF and those with SDC. Second, the percentage of patients who received TIPS during this period was also higher in the UDC group than in the other two groups (14.2% versus 8.3% [*p* = 0.04] and 10.2% [*p* = 0.1], respectively). Finally, the prevalence of hypovolemic shock as the main cause of death was 6- and 3-times higher in patients with UDC group (16.9%) than in those with pre-ACLF (2.7%; *p* <0.001) and SDC (5.1%; *p* <0.001). **Fig. 4F** shows, that the percentage of patients with at least one subrogate of severe portal hypertension, was significantly higher in patients with UDC group (44.6%) compared to patients with pre-ACLF (27.1%) and SDC (31.3%).

Tools to predict development of ACLF

The CLIF-C ACLF-D score was developed to predict, at the time of hospital admission, the probability for a patient with AD to develop ACLF during the following 3 months. The initial model was fitted including all the main characteristics at enrollment found to be associated with the development of ACLF in the univariate analysis (**Table S2**). Patients age (years), presence of ascites, WBC count (x10^9/L), serum albumin (g/dL), serum bilirubin (mg/dL), and serum creatinine (mg/dL) at study enrollment were subsequently identified as the best subset of independent predictors in the final model (**Table S3**) and their coefficients were used as relative weight to compute the corresponding score. The equation for CLIF-C ACLF-D score is as follows:

CLIF-C ACLF-D score = ((0.03*Age) + (0.45*Ascites) + (0.26*ln(WBC)) – (0.37*Albumin) + (0.57*ln(Bilirubin)) + (1.72*ln(Creatinine)) +3*10.

The prognostic accuracy of CLIF-C ACLF-D score (**Fig. 5A**) was higher than those of CLIF-C AD, MELD, MELD-Na and Child-Pugh scores in the derivation set. In the validation set, the CLIF-C ACLF-D showed a similar accuracy but smaller differences with regards to the other scores. Therefore, we were unable to design a new score to predict ACLF development more accurately than the traditional clinical scores.

The most relevant clinical variable selected by the Decision Tree model was creatinine, with a threshold of 1.3 mg/dL (Fig. 5B). Bilirubin, albumin, age and WBC were also selected to subsequently discriminate the patients. The terminal nodes with a probability of ACLF higher than 0.5, so classifying the patients as ACLF development included 14.1% of the patients. The model achieved a discriminating ability (AUC) of 0.76 (0.72-0.79), with high specificity (95%) but low sensitivity (38%), indicating an important misclassification among those patients who actually developed ACLF.

Discussion

The most outstanding finding of the current study was the identification of three different clinical courses with distinct pathophysiology and prognosis in patients hospitalized for the treatment of an episode of AD. These three clinical courses were unrelated to the etiology of cirrhosis, and in patients with alcoholic cirrhosis to active alcoholism, indicating that they were largely dependent on other mechanisms.

The three distinct types of clinical courses coincided with specific changes in the grade of systemic inflammation. Patients with pre-ACLF showed significantly higher grade of systemic inflammation at enrollment than patients with UDC and SDC. By contrast, there was no significant difference in systemic inflammation between patients with UDC and SDC. Moreover, whereas the levels of inflammatory markers increased significantly during follow-up accompanying the progression of AD to ACLF in patients with pre-ACLF, they decreased intensely in patients with SDC, while they did not show clear changes in patients with UDC. Therefore, a distinct progression of systemic inflammation is likely a major pathogenetic mechanism underlying the three clinical courses of patients with AD. This finding is a key feature in the new comprehensive hypothesis for AD presented in the current article.

Thus, patients with SDC developed the index episode of AD in the context of moderate systemic inflammation. In addition, systemic inflammation decreased rapidly and remained at low intensity during the 3-month follow-up. Probably due to this, all patients recovered from the index episode of AD, most presented a long-term relatively benign clinical course and only 9.5% died within the 1-year follow-up. Around half of the few patients who died within the 1-year follow-up period reproduced at the end of their life the clinical course of the pre-ACLF group and developed multiorgan failure. In contrast, in only 5% of cases who died was hypovolemic shock reported as the main cause of death.

On the contrary, patients with pre-ACLF developed AD in the context of more intense systemic inflammation, which further increased with ACLF development during follow-up. These patients differed significantly from patients with SDC in many other features reported at enrollment, clearly supporting that they were in a pre-ACLF stage. They presented significantly higher prevalence of liver failure, liver dysfunction, renal dysfunction, ascites, encephalopathy and bacterial infections and significantly worse prognostic scores than patients with SDC and UDC.

The median time between the onset of decompensated cirrhosis to liver transplantation or death, which covers the complete phase of clinically decompensated cirrhosis, was remarkably shorter in patients of the pre-ACLF group (12 months) than in those with SDC (20 months), indicating an accelerated clinical course of the decompensated phase of the disease towards death in patients with pre-ACLF.

Finally, the clinical course during the first three-month period prior to admission, as estimated by the prevalence of ascites, encephalopathy and bacterial infections, was significantly more unstable in patients with pre-ACLF group than in those with SDC. This finding suggests that patients with pre-ACLF were already more severely ill than patients with SDC months before reaching the pre-ACLF status. We presume that the intensity of systemic inflammation during this period was probably sufficient to induce this frequent development of complications requiring hospital admission, but not enough to reach the critical threshold beyond which ACLF develops [15]. Therefore, Pre-ACLF should be suspected in patients hospitalized for AD with prior unstable clinical course, very high levels of inflammatory markers and liver failure or liver or kidney dysfunction. Unfortunately, we were unable to design new specific tools that improve the accuracy of the CLIF-C AD and MELD-Sodium scores for predicting ACLF development.

Patients with UDC shared many characteristics with patients with pre-ACLF and SDC. Like patients with pre-ACLF, they presented clinical course instability within the 3-month periods prior to and after enrollment. However, they did not present severe systemic inflammation at enrollment or clear increase of systemic inflammation level during follow-up. This probably explains the lack of development of ACLF in this group of patients. A second important finding in patients with UDC was their significantly higher prevalence of features suggesting severe portal hypertension. This finding supports that the second major pathophysiological mechanism of AD is likely related to changes in portal hypertension.

Therefore, the most severe course of AD corresponds to patients with pre-ACLF who develop rapid progression of systemic inflammation leading to ACLF development and death. The second course in severity corresponds to patients with UDC, who present increased incidence of complications related to severe portal hypertension, such as circulatory dysfunction at enrollment, increased incidence of gastrointestinal hemorrhage and TIPS placement during the six-month observational period and higher mortality due to hypovolemic shock. However, since the grade of systemic inflammation did not progress to the critical threshold level for inducing extrahepatic organ failure, only a minority of patients with USC developed ACLF. Consequently, they lived longer than patients with pre-ACLF. Finally, the third course of AD, which is by large the most frequent and corresponds to patients with SDC, is likely the consequence of a slow progression of these two pathophysiological mechanisms, leading to a relatively benign course and much longer survival.

This hypothesis is further supported by our findings showing that ascites, which is the complication associated with the most extensive organ dysfunction (liver, kidney, heart and systemic circulation) [16, 17], was associated with the most intense

systemic inflammation in comparison with hepatic encephalopathy and gastrointestinal hemorrhage.

Bacterial infections were frequently associated with AD. Roughly, one every 3-4 patients included in each group were infected at the time of AD development. Two mechanisms have been proposed for this association. The first is that bacterial infections, by increasing the intensity of systemic inflammation, precipitate the development of AD [2, 5, 6]. The second is that bacterial infections would be the consequence of a compensatory immunomodulatory reaction to systemic inflammation, which impairs the antibacterial activity of the immune cells (immunoparalysis) [18-20]. Our findings suggests that these two mechanisms are not mutually exclusive.

In summary, the PREDICT study suggests that AD in cirrhosis is a clinical condition with three different courses and two major pathophysiological mechanisms. The pre-ACLF is predominantly related to rapid progression of systemic inflammation, ACLF development and extremely high short-term mortality rate. The UDC, occurs in the context of rapid progression of portal hypertension and is associated with a less severe clinical course and lower short-term mortality. Finally, in SDC both mechanisms progress slowly, and patients follow a relatively benign course with longer survival. Predicting the outcome of patients who present with AD is a major challenge for future research.

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Figure legends

Figure 1. Scheme of visits and collection of data and samples during the 6-month observational period, which included the 3-month period prior to enrollment, the enrollment's visit and the 3-month follow-up period after enrollment. At enrollment patients were initially stratified into 2 groups: high-risk group (CLIF-C AD-score≥50) and low-risk group (CLIF-C AD-score<50) of ACLF development [12]. In the high-risk group, the scheduled visits were performed at enrollment (visit 1) and 1, 4, 8 and 12 weeks after enrollment (visits 2-5). In the low-risk group, scheduled visits were performed only at enrollment (visit 1) and week 1 (visit 2) and 12 (visit 5) after enrollment. Any patient of both groups developing ACLF within the follow-up period received unplanned ACLF visits at the time of diagnosis of ACLF and 7 days later. Additional study visits were performed whenever a patient had to be readmitted for any reason except ACLF (readmission visits) in the high-risk group only, but the number of readmissions were recorded in both groups. Finally, data on liver transplantation or death and causes of death were prospectively collected 3, 6 and 12 months after enrollment in all patients.

Figure 2. Density curves of events during the 3-month follow-up period after enrollment in patients with pre-ACLF, unstable decompensated cirrhosis (UDC) and stable decompensated cirrhosis (SDC). The zero time-point corresponds to enrollment into the study. Bacterial infections are represented in red, ACLF in green, and deaths in blue.

Figure 3. *Panel A*. Cumulative rate of ACLF per week during the 90-day follow-up period in patients with pre-ACLF. *Panel B*. Cumulative incidence of death between

the 90-day (landmark) and one year after enrollment in patients with pre-ACLF, UDC and SDC.

Figure 4. *Panel A*. Density curves of liver transplantation or death during the 1-year follow-up period after enrollment in patients with pre-ACLF (in red), UDC (in blue) and SDC (in green) taking the zero-point as the onset of acute decompensation. The median time (interquartile range [IQR]) from the onset of clinically decompensated cirrhosis (as defined by the date of first the episode of acute decompensation) to death or liver transplantation (duration of the decompensated phase of cirrhosis) was significantly shorter in patients with pre-ACLF than in those with UDC, and in patients with UDC s than in those with SDC. *P*-values were obtained using Mann Whitney-U test. *Panel B*. Individual length of the time period between the onset of decompensated cirrhosis and liver transplantation, death or the end of the 1-year follow-up period after enrollment in the three groups of patients. For clarity reasons, the figure does not include patients with values over the 75% IQR. Differences between groups were highly significant (p<0.001). *P*-values were obtained using Kruskal-Wallis test. *Panel C*. Percentage of patients developing at least one bacterial infection during the 6-month observational period in patients with pre-ACLF, UDC and SDC. *P* values were obtained using chi-square test. *Panel D*. Plasma levels of CRP (median and 75% confidence interval) in a control group of 34 patients with compensated cirrhosis (CC, no prior history of AD), SDC, UDC, pre-ACLF and ACLF. Patients with CC were studied previously [5, 6]. The ACLF group includes 176 patients from the pre-ACLF group who develop ACLF during the 3-month follow-up period. Samples for PCR measurements in these patients were obtained at the time of ACLF development, *P*-values were obtained using Kruskal-Wallis test. *Panel E*. Serum concentration of C reactive protein in 134 patients without prior history of AD

who were enrolled only for ascites, encephalopathy or gastrointestinal hemorrhage. *P*-values were obtained using Kruskal-Wallis test. *Panel F.* Percentage of patients presenting at least one surrogate of severe portal hypertension during the 6-month observational period in the Pre-ACLF, UDC and SDC groups. *P* values were obtained using chi-square test.

Figure 5. *Panel A*. Comparison between the predictive ability of the CLIF-C ACLF-D score with those of the CLIF-C AD, MELD, MELD-sodium and Child-Pugh scores using Harrel' C-indexes and 95% confident intervals (Cis) both in the derivation and validation sets. Panel B. Decision Tree plot for the prediction of ACLF development during the 90-day follow-up period after enrollment. Each node shows the percentage of patients classified and their probability of ACLF development within the 90-day follow-up period after enrollment (also represented by the colors and color intensity). The blue color represents a probability of ACLF development >0.5. The green color represents a probability of ACLF development ACLF <0.5. The intensity of the color represents the estimated probability value. The upper node (root node) represents the entire population of patients (980 patients, 100%) included in the analysis and its corresponding probability of ACLF development before entering the model (0.22). Each node includes the estimated probability of subsequent subsets of patients.

Table 1. Characteristics Prior to, at, and After Enrollment, in Patients With "Pre-ACLF", "Unstable Decompensated Cirrhosis (UDC)" and "Stable Decompensated Cirrhosis" (SDC)

Characteristic	Pre-ACLF (n = 218)	UDC (n = 233)	SDC (n = 620)	*p* value
Data prior to enrollment				
Age, y, mean ± SD	61.1 ± 10.0	60.9 ± 10.6	57.9 ± 11.0[a]	<0.001
Female sex, n (%)	70 (32.1)	74 (31.8)	200 (32.3)	0.990
Etiology of cirrhosis, n (%)				
Alcohol	107 (49.1)	143 (61.4)[b]	346 (55.9)	0.032
Hepatitis C virus	14 (6.4)	12 (5.2)	41 (6.6)	0.727
Alcohol and hepatitis C virus	10 (4.6)	8 (3.4)	33 (5.3)	0.506
Nonalcoholic steatohepatitis	16 (7.3)	17 (7.3)	48 (7.8)	0.965
Other etiologies	70 (32.1)	51 (21.9)[b]	150 (24.2)[b]	0.028
Events prior to enrollment, n (%)				
Ascites	130 (66.7)	122 (65.9)	229 (47.7)[a]	<0.001
Hepatic encephalopathy	46 (25.4)	54 (31.4)	75 (17.1)[a]	<0.001
Gastrointestinal hemorrhage	17 (9.6)	29 (17.1)[b]	62 (13.9)	0.125
Any hospitalization	106 (56.7)	119 (65.0)	210 (45.6)[a]	<0.001
Data at enrollment				
Clinical data, organ failures and organ dysfunctions, n (%)				
Ascites	173 (79.4)	170 (73.0)	415 (66.9)[b]	0.002
Hepatic Encephalopathy	65 (29.8)	73 (31.3)	168 (27.1)	0.428
Gastrointestinal hemorrhage	16 (7.3)	39 (16.7)[b]	97 (15.6)[b]	0.005
No organ failure or dysfunction	50 (22.9)	80 (36.5)[b]	291 (46.9)[a]	<0.001
Liver failure	29 (13.3)	11 (4.7)[b]	30 (4.8)[b]	<0.001
Liver dysfunction	51 (23.4)	36 (15.5)[b]	84(13.5)[b]	0.003
Circulatory dysfunction	20 (9.2)	43 (18.5)[b]	50 (8.1)[c]	<0.001
Renal dysfunction	51 (23.4)	17 (7.3)[b]	40 (6.5)[b]	<0.001
Coagulation failure	8 (3.7)	4 (1.7)	7 (1.1)[b]	0.050
Coagulation dysfunction	29 (13.3)	19 (8.2)	46 (7.4)[b]	0.029
Brain failure	4 (1.8)	4 (1.7)	16 (2.6)	0.676
Brain dysfunction	59 (27.1)	67 (28.8)	144 (23.2)	0.197
Respiratory dysfunction	10 (4.6)	8 (3.4)	29 (4.7)	0.722
Main reason of hospitalization				
Ascites	105 (48.4)	106 (45.5)	267 (43.1)	0.382
Hepatic Encephalopathy	29 (13.4)	34 (14.6)	82 (13.2)	0.870
Gastrointestinal hemorrhage	13 (6.0)	37 (15.9)[b]	110 (17.7)[b]	<0.001
Bacterial infection	32 (14.7)	27 (11.6)	84 (13.5)	0.603
Others	38(17.5)	29(12.4)	77(12.4)	0.147

Table 1. (Continued.)

Biomarkers of systemic inflammation, median (IQR)				
White-cell count, x10^9/L	7.2 (4.9-9.8)	6.1 (4.3-8.5)[b]	6.0 (4.2-8.7)[b]	0.002
Serum C-reactive protein, mg/L	23 (11-41)	16 (8-35)[b]	15 (6-36)[b]	<0.001
Measurements estimating organ function				
Serum bilirubin, mg/dL, median (IQR)	3.9 (1.9-9.0)	2.6 (1.3-5.4)[b]	2.3 (1.4-4.5)[b]	<0.001
Serum albumin, g/dL, mean ± SD	2.7 ± 0.7	2.8 ± 0.6	3.0 ± 0.6[a]	<0.001
International Normalized Ratio, median (IQR)	1.6 (1.4-1.9)	1.4 (1.3-1.7)[b]	1.4 (1.2-1.7)[b]	<0.001
Serum creatinine, mg/dL, median (IQR)	1.1 (0.8-1.5)	0.9 (0.7-1.2)[b]	0.8 (0.7-1.1)[a]	<0.001
Plasma sodium, mEq/L, mean ± SD	134 ± 6	135 ± 5	136 ± 5[a]	<0.001
Severity scores, mean ± SD				
Child-Pugh	9.8 ± 1.8	9.2 ± 1.7[b]	8.7 ± 1.8[a]	<0.001
MELD	19 ± 5	16 ± 5[b]	15 ± 5[a]	<0.001
MELD-sodium	23 ± 5	19 ± 5[b]	18 ± 5[a]	<0.001
CLIF-C AD	57 ± 8	53 ± 8[b]	50 ± 8[a]	<0.001
Data after enrollment				
Mortality rates, n (%)				
90-day mortality rate	117 (53.7)	49 (21.0)	---------	
1-year mortality rate	147 (67.4)	83 (35.6)	59 (9.5)	
Main causes of death, n (%)				
ACLF	130 (88.4)	25 (30.1)[b]	29 (49.2)[a]	<0.001
Hypovolemic shock	4 (2.7)	14 (16.9)[b]	3 (5.1)[c]	<0.001
Other causes of death	6 (4.1)	15 (18.1)[b]	15 (25.4)[b]	<0.001
Unknown	7 (4.8)	29 (34.9)[b]	12 (20.3)[b]	<0.001
Liver transplantation within 12 months after enrollment	33 (15.1)	39 (16.7)	73 (11.8)	0.125
Indicators of severe portal hypertension, n (%)				
Transjugular intrahepatic portosystemic shunting (TIPS)[d]	18 (8.3)	33 (14.2)[b]	63 (10.2)	0.107
TIPS for gastrointestinal hemorrhage	4 (1.8)	12 (5.4)	26 (4.2)	0.145
Any episode of gastrointestinal hemorrhage[d]	48 (22.0)	76 (32.6)[b]	155 (25.0)[c]	0.016

NOTE: MELD denotes Model of End Stage Liver Disease score; CLIF-C AD denotes Chronic Liver Failure-Consortium acute decompensation score. *P* values were obtained using chi-square test.

[a] Significantly different from the pre-ACLF group and UDC groups.

[b] Significantly different from the pre-ACLF group.

[c] Significantly different from the UDC group.

[d] At any time of the 6-month observational period, this being defined by the 3 months prior to, and the 3 months as of enrollment.

Table 2. Characteristics of Infections at Enrollment and During the 90-day Follow-up Period in Patients With Pre-ACLF, Unstable Decompensated Cirrhosis (UDC) and Stable Decompensated Cirrhosis (SDC)

Characteristic	Pre-ACLF (n = 218)	UDC (n = 233)	SDC (n = 620)	*p* value
Number of patients with infections n (%*)				
3 months prior to enrollment	58 (31.0)	45 (26.5)	75 (17.1)[a]	<0.001
At enrollment	74 (33.9)	61 (26.2)	178 (28.7)	0.176
3 months after enrollment	106 (48.6)	83 (35.6)[b]	68 (11.0)[a]	<0.001
Throughout the 6-month observational period	158 (72.5)	133 (57.1)[b]	251 (40.5)[a]	<0.001
Infections at enrollment				
Number of infections	83	67	189	
Site of infection, n/N (%)*				
Urinary tract	19/83 (22.9)	15/67 (22.4)	44/189 (23.2)	0.985
Spontaneous bacterial peritonitis	18/83 (21.7)	13/67 (19.4)	26/189 (13.8)	0.232
Pneumonia	10/83 (12.0)	14/67 (20.9)	24/189 (12.8)	0.213
Spontaneous bacteremia	9/83 (10.8)	5/67 (7.5)	9/189 (4.8)	0.184
Cellulitis	4/83 (4.8)	6/67 (9.0)	18/189 (9.6)	0.414
Suspected infections	6/83 (7.2)	8/67 (11.9)	35/189 (18.6)[b]	0.040
Other [c]	17/83 (20.5)	6/67 (9.0)	32/189 (17.0)	0.150
Severity of infection, n/N (%)*				
Community-acquired	52/83 (62.6)	35/67 (52.2)	149/189 (78.8)[a]	<0.001
Health-care- or hospital-acquired	31/83 (37.4)	32/67 (47.8)	40/189 (21.2)[a]	<0.001
Sepsis	26/83 (31.3)	11/67 (16.4)[b]	28/189 (15.1)[b]	0.005
Infection caused by MDR	6/83 (7.2)	3/67 (4.9)	18/189 (10.3)	0.379
Infections during the 3-month follow-up period				
Number of infections	140	117	76	
Site of infection, n/N (%)*				
Urinary tract	35/140 (25.0)	31/117 (26.5)	22/76 (28.9)	0.821
Spontaneous bacterial peritonitis	21/140 (15.0)	24/117 (20.5)	5/76 (6.6)[c]	0.030
Pneumonia	27/140 (19.3)	10/117 (8.5)[b]	10/76 (13.2)	0.047
Spontaneous bacteremia	10/140 (7.1)	9/117 (7.7)	2/76 (2.6)	0.319
Cellulitis	6/140 (4.3)	8/117 (6.8)	4/76 (5.3)	0.665
Suspected infections	16/140 (11.4)	13/117 (11.1)	16/76 (21.1)	0.091
Other [c]	25/140 (17.9)	22/117 (18.8)	17/76 (22.4)	0.717
Severity of infection, n/N (%)*				
Community-acquired	14/140 (10.0)	15/117 (12.8)[b]	15/76 (19.7)[b]	0.129
Health-care- or hospital-acquired	126/140 (90.0)	102/117 (87.2)	61/76 (80.3)[b]	0.129

Table 2. (Continued.)

Sepsis	70/140 (50.0)	21/117 (18.1)[b]	4/76 (5.3)[a]	<0.001
Infection caused by multidrug-resistant bacteria	44/140 (33.8)	29/117 (28.2)	11/76 (16.2)[b]	0.031

NOTE: *P* values were obtained using chi-square, %* is calculated over the available data, no imputation was included in the table.
[a] Significantly different from the pre-ACLF group and UDC groups. [b] Significantly different from the pre-ACLF group. [c] Other: Catheter related infection, Cholecystitis, Cholangitis, Secondary peritonitis, Pseudomembranous colitis, Other gastrointestinal infection. [d] Significantly different from the UDC group. [e]

Table 3. Inflammatory Markers and Severity Scores at Enrollment and During the 90-day Follow-up Period in Patients with Pre-ACLF, Unstable Decompensated Cirrhosis, and Stable Decompensated Cirrhosis

	Enrollment	Follow-up	*P* value
Pre-ACLF (n=218)			
Blood biomarkers of systemic inflammation, median (IQR)			
White-cell count, $x10^9$/L	7.2 (4.9-9.8)	8.3 (5.7-12.9)	<0.001
Serum C-reactive protein, mg/L	23 (11-41)	29 (14-52)	0.033
Severity scores, mean ± SD			
MELD-sodium	23 ± 5	28 ± 6	<0.001
CLIF-C AD	57 ± 7	64 ± 9	<0.001
Unstable Decompensated Cirrhosis (n=233)			
Blood biomarkers of systemic inflammation, median (IQR)			
White-cell count, $x10^9$/L	6.1 (4.3-8.5)	5.9 (4.0-8.0)	0.343
Serum C-reactive protein, mg/L	16 (8-35)	12 (5-26)	0.004
Severity scores, mean ± SD			
MELD-sodium	19 ± 5	18 ± 6	0.006
CLIF-C AD	53 ± 7	51 ± 8	0.031
Stable Decompensated Cirrhosis (n=620)			
Blood biomarkers of systemic inflammation, median (IQR)			
White-cell count, $x10^9$/L	6.0 (4.2-8.7)	5.4 (3.9-7.3)	<0.001
Serum C-reactive protein, mg/L	15 (6-36)	8 (4-17)	<0.001
Severity scores, mean ± SD			
MELD-sodium	18 ± 5	16 ± 5	<0.001
CLIF-C AD	50 ± 8	48 ± 7	<0.001

NOTE: *P* values were obtained using the Wilcoxon signed rank test or the Student t-test where appropriate. ACLF denotes acute-on-chronic liver failure, MELD Model for End-Stage Liver Disease score, and CLIF-C AD Chronic Liver Failure-Consortium acute decompensation score.

Figure 1

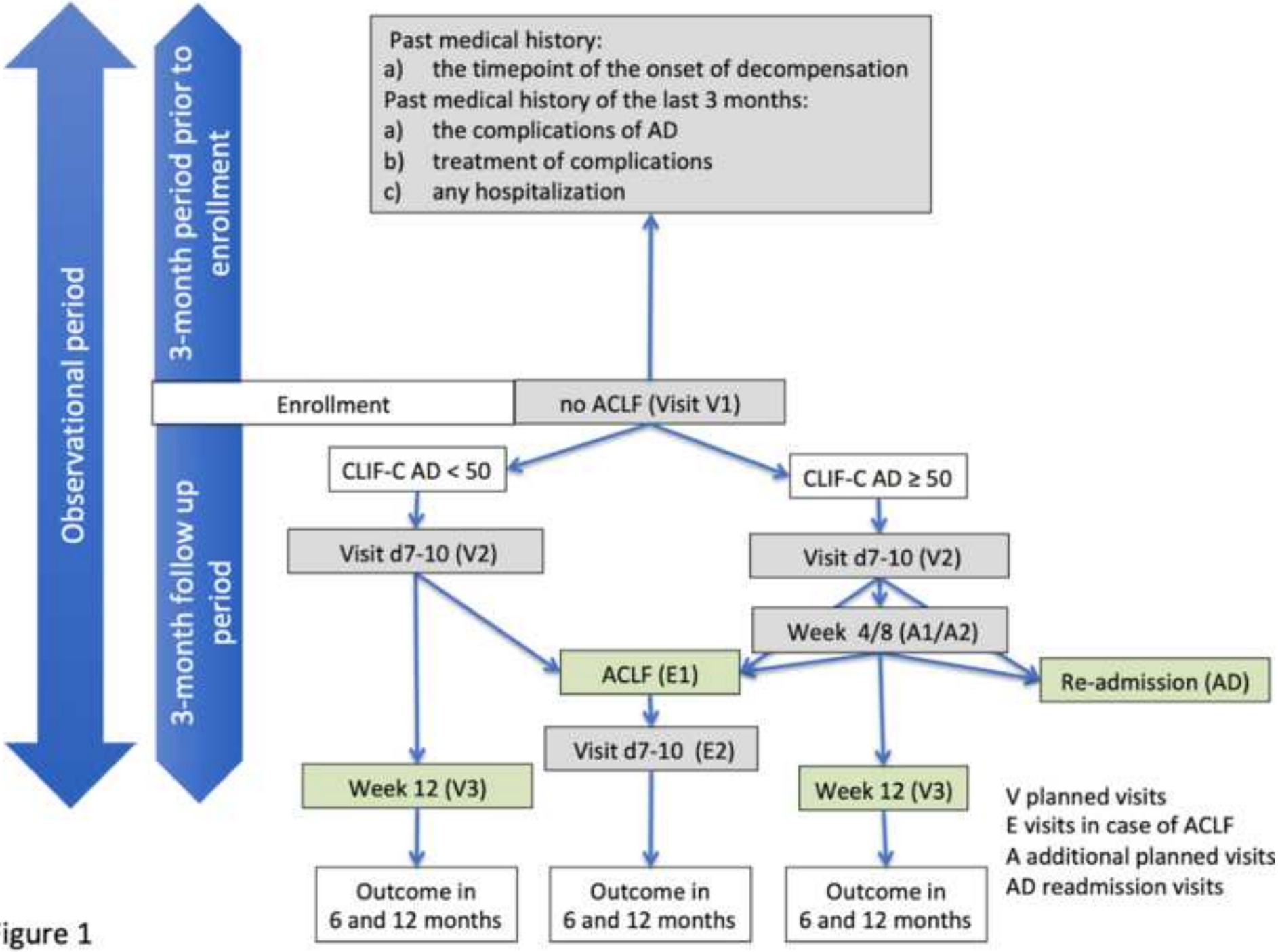


Figure 1

Figure 2

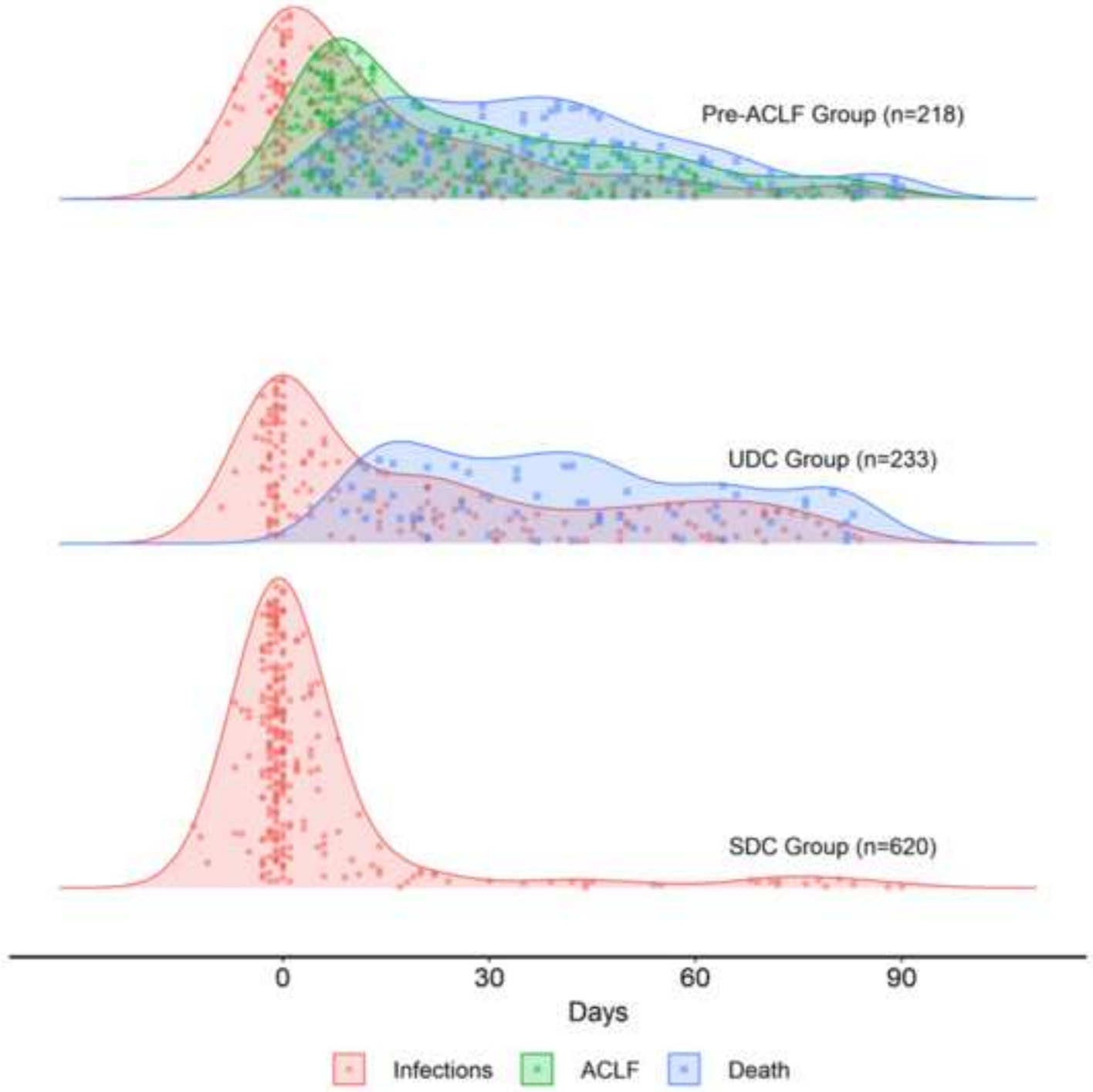
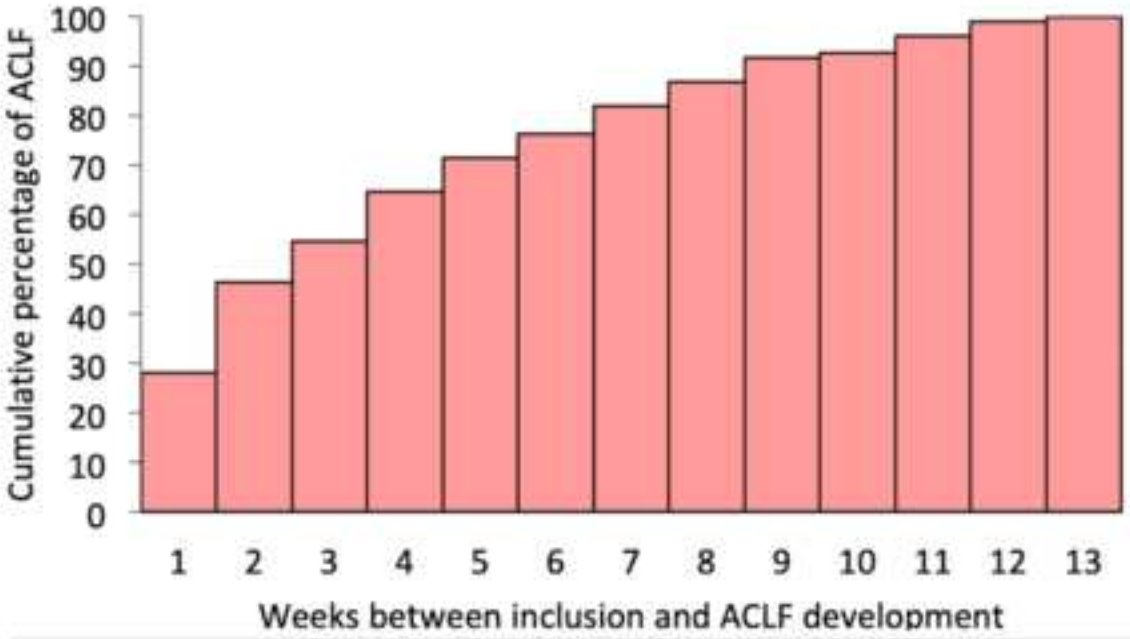


Figure 2

A



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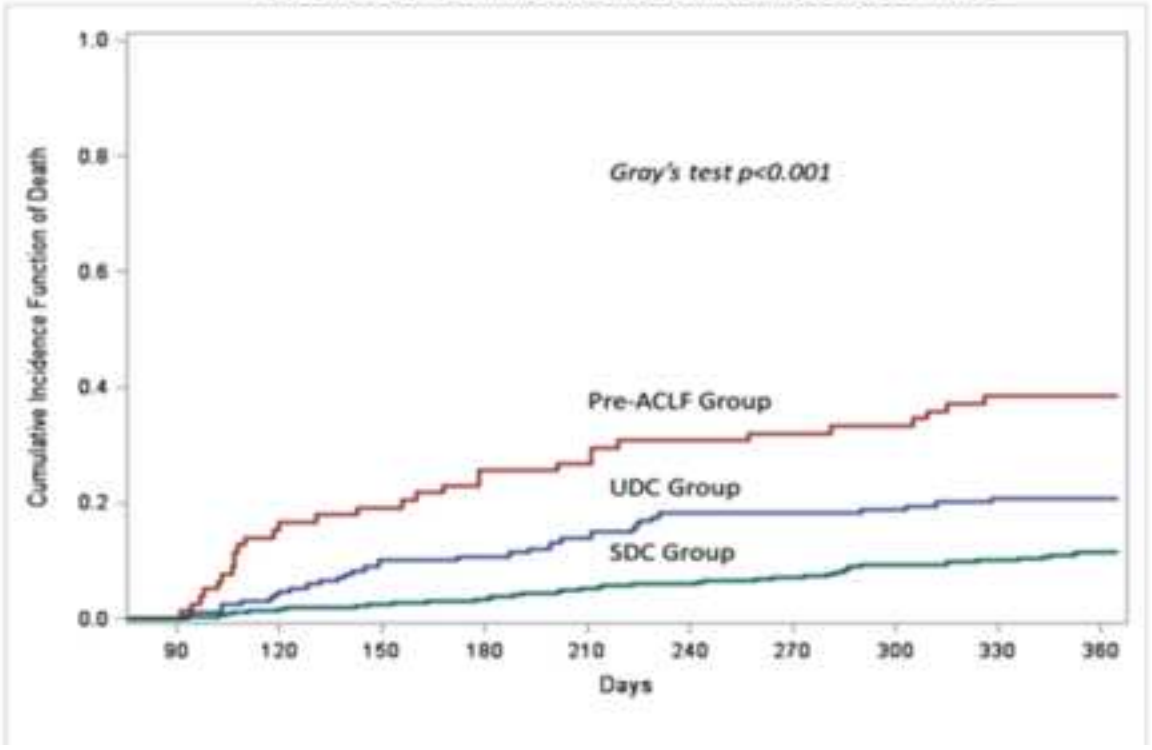

Figure 3

Figure 4

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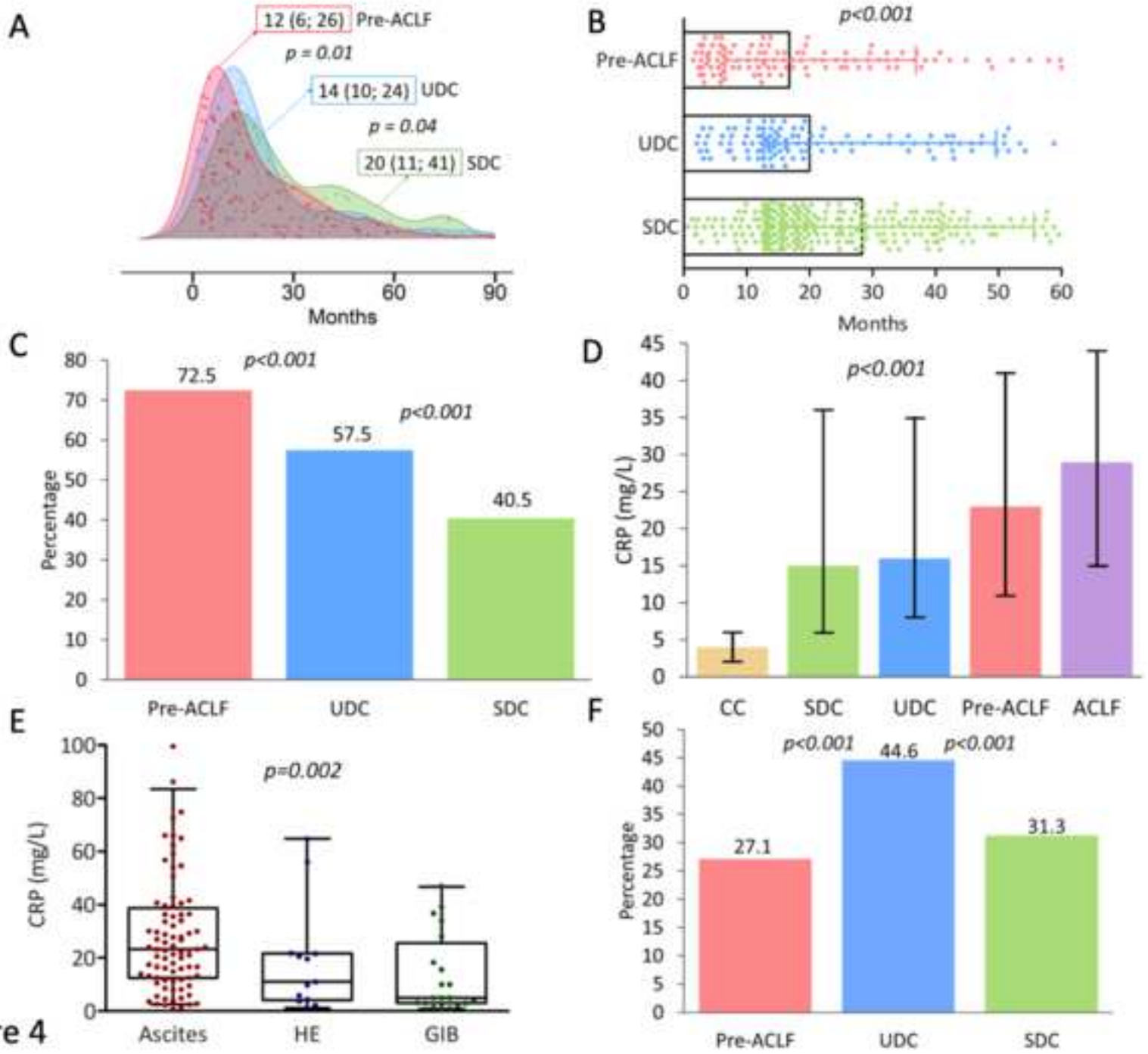


Figure 4

A

Severity Scores	Derivation set (n = 707)	Validation set (n = 364)
	Harrel' C-index (95% confidence interval)	
CLIF-C ACLF-D	0.76 (0.72-0.80)	0.77 (0.72-0.82)
CLIF-C AD	0.70 (0.66-0.74)	0.75 (0.70-0.80)
MELD-sodium	0.70 (0.66-0.74)	0.74 (0.69-0.80)
MELD	0.70 (0.66-0.74)	0.73 (0.67-0.79)
Child-Pugh	0.64 (0.59-0.68)	0.67 (0.60-0.73)

B

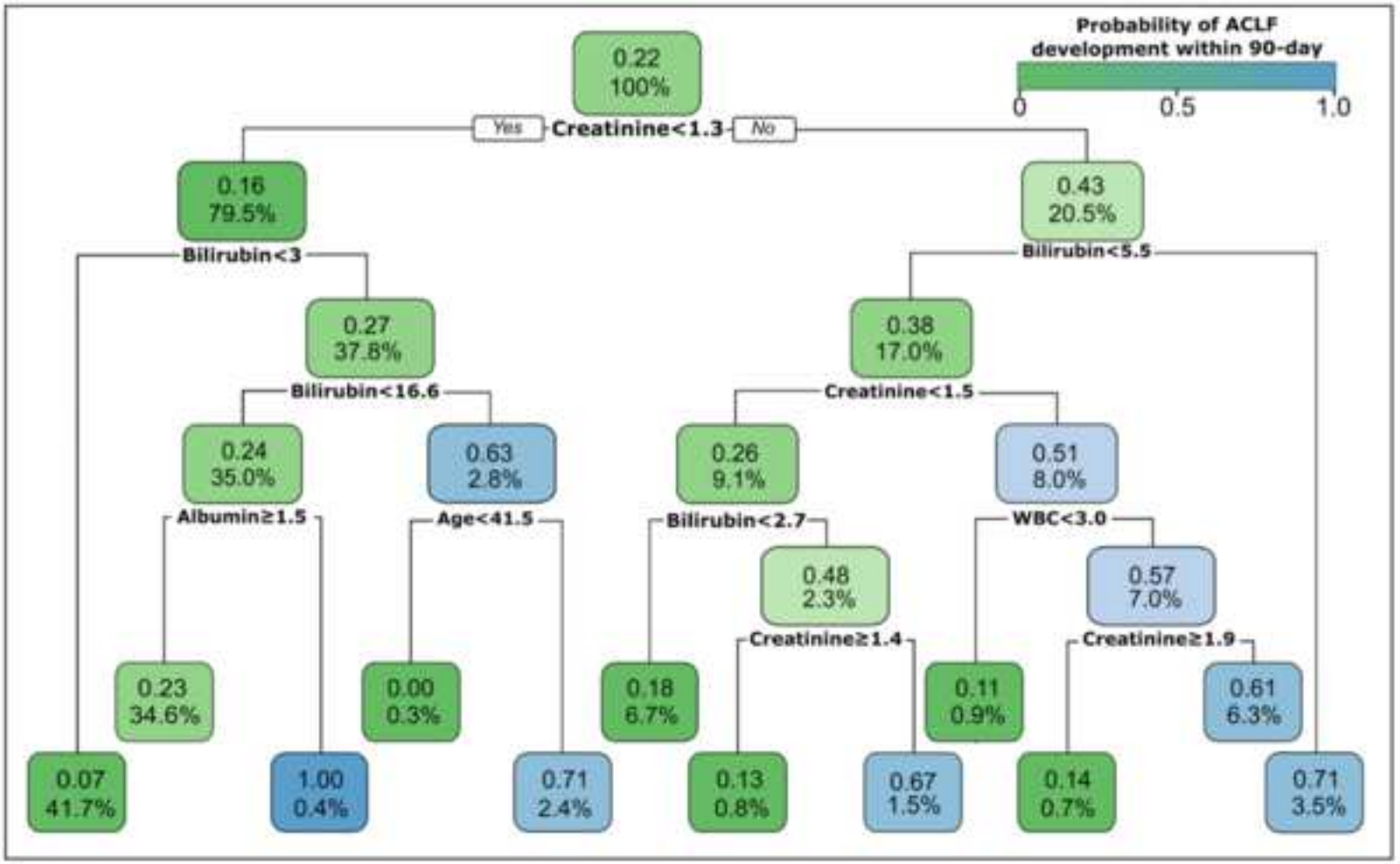
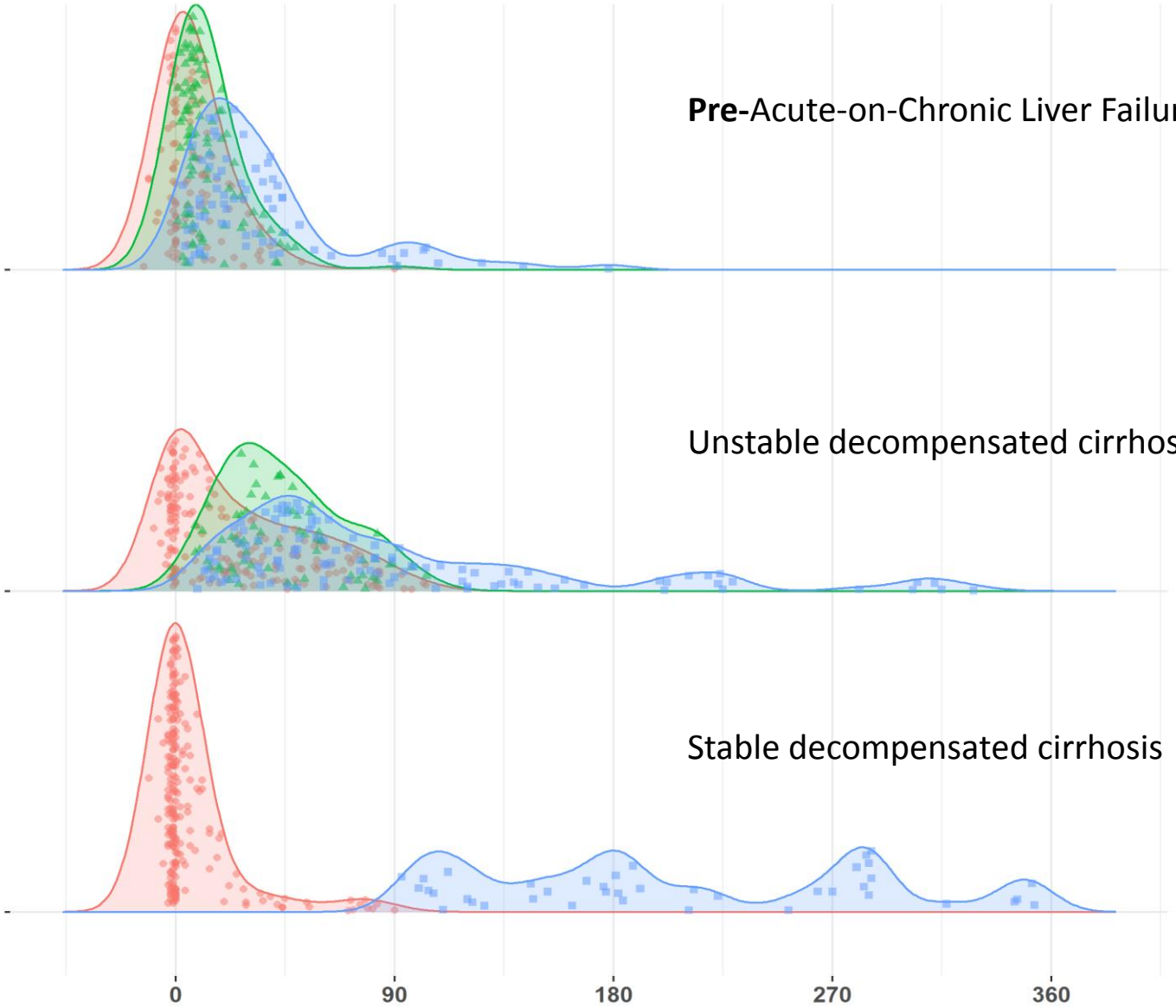


Figure 5

Graphical Abstract

PREDICT

Different clinical courses in acutely decompensated cirrhosis



ACLF

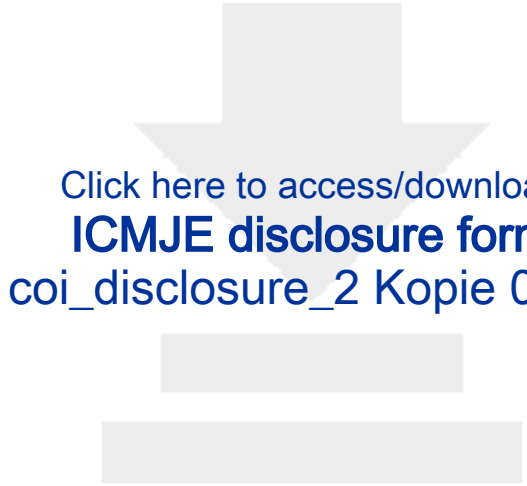
Death

Bacterial infection

Highlights

- Patients with acutely decompensated cirrhosis without ACLF develop three different clinical courses.
- Patients with pre-ACLF develop ACLF within 90 days and have high systemic inflammation and mortality.
- Patients with unstable decompensated cirrhosis show low-grade systemic inflammation but suffer characteristically from complications related to severe portal hypertension.
- Patients with stable decompensated cirrhosis do not present severe systemic inflammation or frequent complications related with portal hypertension, and show lower 1-year mortality risk.

protocol synopsis



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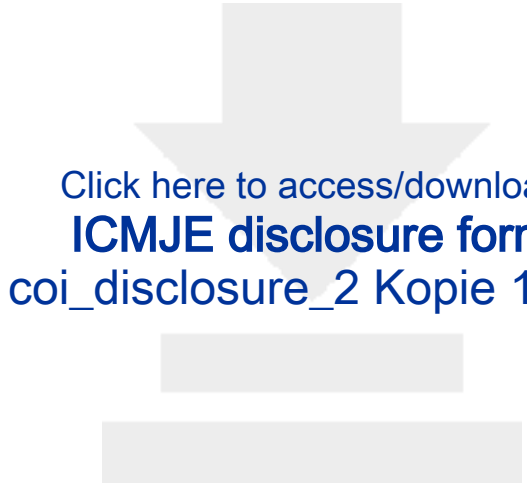

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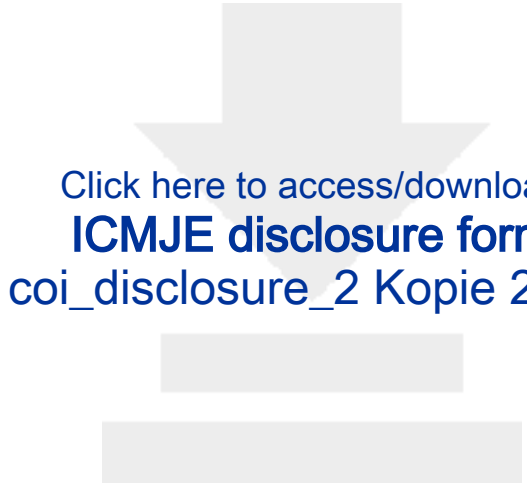

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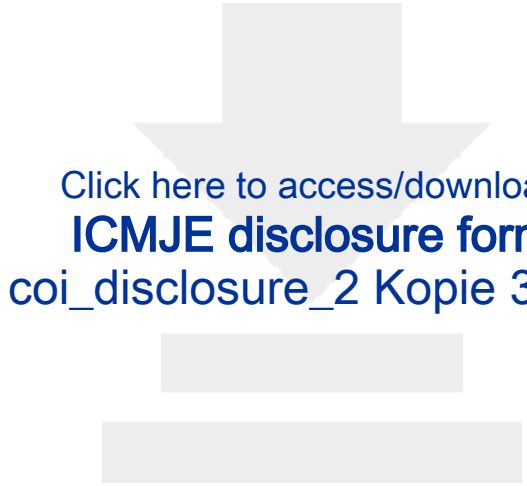

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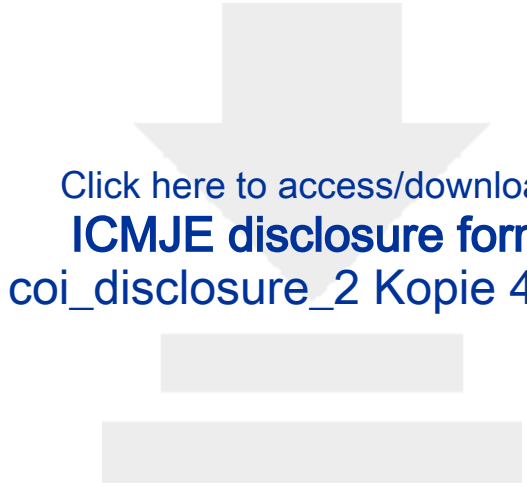

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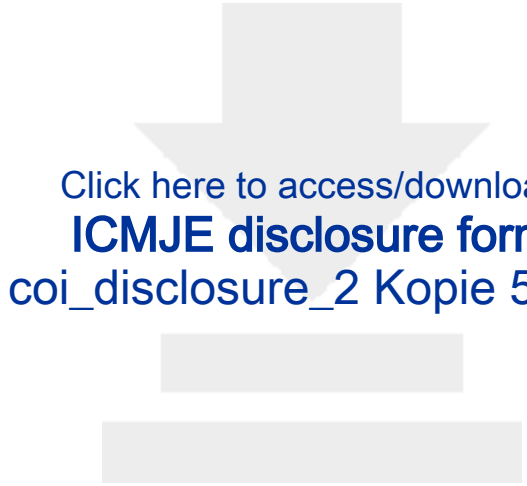

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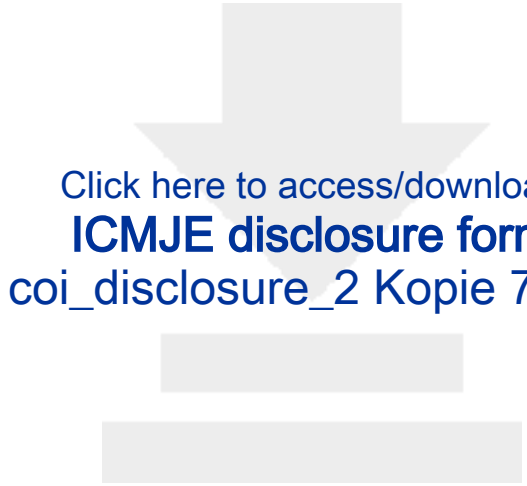

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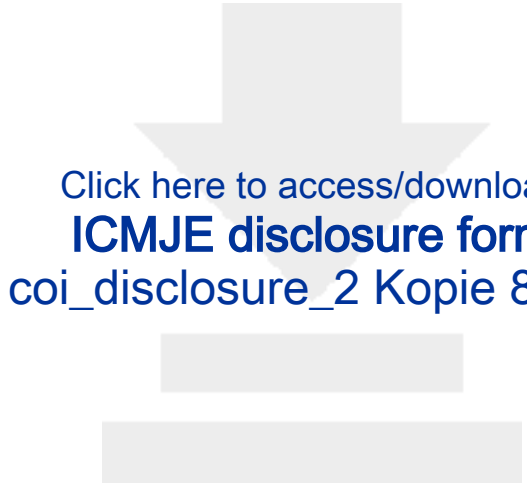

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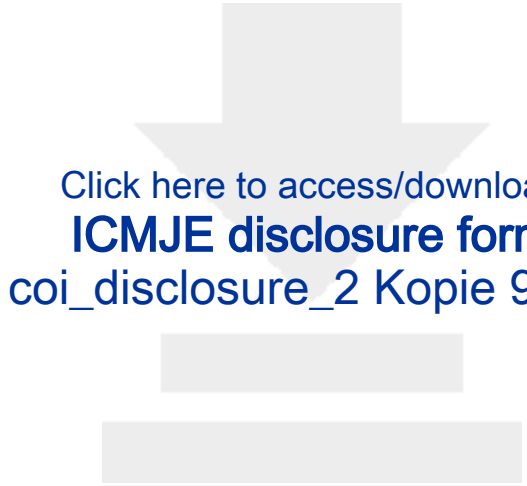

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