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PREDICT identifies Precipitating Events with Impact on Clinical Course and Outcome of Acutely Decompensated Cirrhosis.

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Abstract:	Introduction: Acute decompensation (AD) of cirrhosis may present without acute-on-chronic liver failure (ACLF) (AD-No ACLF), or with ACLF-phenotype (AD-ACLF) defined by organ failure(s). Precipitating events (PEs) may induce AD. This multicenter, prospective, observational PREDICT Study analyzes and characterizes the PEs leading to both AD-phenotypes. Patients and Methods: The PREDICT study included 1273 non-electively hospitalized patients with AD (No-ACLF=1071; ACLF=202). Medical history, clinical and laboratory data were carefully collected at enrolment and during 90-days follow up, focused on the characteristics of PEs, specifically induction of organ dysfunction/failure and/or systemic inflammation, chronology, intensity, and relationship to outcome in both AD phenotypes. Results: Among 16 events explored as potential PEs, four types of events were PEs consistently related to AD, including proven bacterial infections, severe alcoholic hepatitis, gastrointestinal (GI) bleeding with shock and toxic encephalopathy. Among patients in the AD-No ACLF cohort and the AD-ACLF cohort with PEs (38% and 71%, respectively), almost all (96% and 97%, respectively) showed proven bacterial infection and severe alcoholic hepatitis, either alone or in combination with other PEs. Interestingly, in both AD-phenotypes, proven bacterial infections and severe alcoholic hepatitis had a similar effect on survival, and the number of PEs was associated with significantly increased 90-day mortality, in parallel with surrogates of systemic inflammation proving the validity of the definition of PEs. Conclusions: This study identified PEs that significantly impact the clinical course and prognosis of patients with AD and specific preventive and therapeutic strategies to these events are required to improve outcome in decompensated cirrhosis.
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Dear Professor Paolo Angeli,

We are delighted to submit the second investigation of our European international multicenter observational prospective study PREDICT entitled: **“PREDICT identifies Precipitating Events with Impact on Clinical Course and Outcome of Acutely Decompensated Cirrhosis.”** for your kind consideration in the most prestigious **Journal of Hepatology**.

This study identified precipitating events that significantly impact the clinical course and prognosis of patients with acutely decompensated cirrhosis. This paper may pave the path for specific preventive and therapeutic strategies to these events in order to improve outcome in decompensated cirrhosis.

Since this paper addresses an important and practical issue, we hope that it will be found suitable for publication in the most prestigious **Journal of Hepatology**.

Sincerely yours

Jonel Trebicka on behalf of the authors

Title page

PREDICT identifies Precipitating Events with Impact on Clinical Course and Outcome of Acutely Decompensated Cirrhosis. (117/120)

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Short title: PRECIPITANTS OF AD AND ACLF

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

Data availability: The data of this paper will be partly available upon request, but the majority of the data are unsuitable to post and partly data confidential.

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Abbreviations: AD (acute decompensation), ACLF (acute-on-chronic liver failure), AST (aspartate aminotransferase), ALT (alanine aminotransferase), IQR (Interquartile Range), CLIF (chronic liver failure), CRP (C reactive protein), GI (gastrointestinal), OF (organ failure), CIF (cumulative incidence of function), MELD (model of end-stage liver disease), PE (precipitating event), SD (Standard Deviation), SDC (stable decompensated cirrhosis), UDC (unstable decompensated cirrhosis), WBC (white blood cell count)

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ABSTRACT

Introduction: Acute decompensation (AD) of cirrhosis may present without acute-on-chronic liver failure (ACLF) (AD-No ACLF), or with ACLF-phenotype (AD-ACLF) defined by organ failure(s). Precipitating events (PEs) may induce AD. This multicenter, prospective, observational PREDICT Study (NCT03056612) analyzes and characterizes the PEs leading to both AD-phenotypes.

Patients and Methods: The PREDICT study included 1273 non-electively hospitalized patients with AD (No-ACLF=1071; ACLF=202). Medical history, clinical and laboratory data were carefully collected at enrolment and during 90-days follow up, focused on the characteristics of PEs, specifically induction of organ dysfunction/failure and/or systemic inflammation, chronology, intensity, and relationship to outcome in both AD phenotypes.

Results: Among 16 events explored as potential PEs, four types of events were PEs consistently related to AD, including proven bacterial infections, severe alcoholic hepatitis, gastrointestinal (GI) bleeding with shock and toxic encephalopathy. Among patients in the AD-No ACLF cohort and the AD-ACLF cohort with PEs (38% and 71%, respectively), almost all (96% and 97%, respectively) showed proven bacterial infection and severe alcoholic hepatitis, either alone or in combination with other PEs. Interestingly, in both AD-phenotypes, proven bacterial infections and severe alcoholic hepatitis had a similar effect on survival, and the number of PEs was associated with significantly increased 90-day mortality, in parallel with surrogates of systemic inflammation proving the validity of the definition of PEs.

Conclusions: This study identified PEs that significantly impact the clinical course and prognosis of patients with AD and specific preventive and therapeutic strategies to these events are required to improve outcome in decompensated cirrhosis.

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INTRODUCTION

Acute decompensation of cirrhosis (hereafter called AD) defines the acute development of ascites, hepatic encephalopathy, gastrointestinal hemorrhage or bacterial infections, or any combination of these. In 2013, the CANONIC study identified the syndrome of acute-on-chronic liver failure (ACLF), the most severe phenotype of AD, in 20% of 1343 consecutive patients non-electively hospitalized for the treatment of an episode of AD [1]. ACLF was characterized by single or multiple organ failure and high 28-day mortality rate (30%).

In 2020, the PREDICT study, the second largest prospective observational investigation in 1273 hospitalized patients with AD, showed that patients without ACLF (AD-No ACLF phenotype) comprised 3 distinct sub-phenotypes defined according to ACLF development and readmission within 3 months after AD [2]. In brief, pre-ACLF patients developed ACLF and showed high short-term (90-day) mortality (67%); unstable decompensated cirrhosis (UDC) patients did not develop ACLF, but required readmission(s) and showed significant short-term mortality rate (35%); while stable decompensated cirrhosis (SDC) patients presented an uncomplicated course during the 3-month follow-up period and showed low 1-year mortality (9%).

In the traditional view, the development of AD is initiated by an acute worsening of stable cirrhosis through different pathophysiological mechanisms considered as precipitating events (PEs). The evidence from the CANONIC and the PREDICT studies challenges this view [1, 2], and suggests that AD manifests mainly as a result of systemic inflammation, inducing multiple organ dysfunction and presents with different clinical phenotypes [3, 4]. Indeed, systemic inflammation increases across the sub-phenotypes of AD-no ACLF (SDC, UDC and pre-ACLF), and reaches its maximum in patients with AD-ACLF [5, 6]. Moreover, in AD-ACLF

phenotype, the grade of systemic inflammation correlated with the number of organ failures, clinical course severity and prognosis [3, 4]. Therefore, for a PE to be of importance, it should have the ability to impair end-organ function.

Despite that AD-ACLF phenotype frequently develops in close chronological relationship with PEs, the critical time-period prior to AD-ACLF has not yet been explored in detail. Moreover, so far, there are no specific criteria for the diagnosis of PEs. Consequently, many clinical relevant aspects of PEs remain ill-defined.

The current study is the second investigation derived from the PREDICT study. It was aimed to provide the rationale for the diagnosis of PEs and to investigate the impact of the type and number of PEs on early clinical course and prognosis in patients hospitalized with AD-No ACLF and AD-ACLF phenotypes.

PATIENTS AND METHODS

Patients

The PREDICT study (ClinicalTrials.gov number, NCT03056612) is a European, investigator-initiated, multicenter, prospective, observational study performed in 48 university hospitals from 15 countries and promoted by the European Foundation for the Study of Chronic Liver Failure. The design of the study has been reported in detail elsewhere [2]. Briefly, 1071 patients with AD-No ACLF phenotype and 202 with AD-ACLF phenotype non-electively hospitalized for treatment were enrolled from March 2017 to July 2018. The diagnosis of cirrhosis was based on previous liver biopsy findings or a composite of clinical signs and laboratory test results and imaging. Diagnostic criteria of AD were based on the presence of ascites, encephalopathy, gastrointestinal hemorrhage or infections (the latter only in patients with prior decompensation) or any combination of these at non-elective hospital admission. Diagnosis of ACLF at enrolment or during follow-up was performed according to the EASL-CLIF criteria [1, 7]. Organ failure and organ dysfunction were defined according to the Chronic Liver Failure (CLIF)- Consortium organ failure (OF) score [8].

The stratification of patients who had the AD-No ACLF phenotype into the AD-pre-ACLF, AD-UDC and AD-SDC sub-phenotypes was performed using previously described criteria [2]. Therefore, patients included in the PREDICT study were stratified into four different groups (**Fig. 1**). 1. AD-ACLF: included 202 patients with ACLF at enrolment; 2. AD-Pre ACLF: included 218 patients without ACLF at enrolment that developed the ACLF during a 3-month follow-up period after enrolment; 3. AD-UDC: included 233 patients who did not develop ACLF during the 3-month follow-up period, but required at least one hospital readmission; 4. AD-SDC:

Included 620 patients who did not develop ACLF or required hospital readmissions during the 3-month follow-up period.

Study Design

The PREDICT study [2] was designed to explore in detail two important time-periods during the clinical course of AD. The first period covered the first 90-day prior to hospital admission, paying particular attention to the first two weeks prior to admission, which is the period in which most PEs can develop. The second period, the “follow-up period”, covered the first 3 months after admission, and was the period in which the early clinical course of patients with ACLF-phenotype and AD-No ACLF sub-phenotypes was assessed.

Pre-specified clinical and standard laboratory data were obtained at enrolment and during follow-up visits. The design of the PREDICT study is described in detail elsewhere [2].

Data obtained at enrolment.

Most patients were enrolled within the first or second day of hospital admission. Two categories of pre-specified information were obtained at enrolment. The first category included the general characteristics and demographic data, specific data related to the AD episode, physical examination, standard laboratory analysis at enrolment, and results from the bacteriological cultures routinely performed in patients with suspected bacterial infections.

The second category of pre-specified data obtained at enrolment were related to the past medical history. The electronic Case Report Form (eCRF) of the PREDICT study was specifically designed to capture the characteristics of any potential PE prior to enrolment, including severity and temporal relationship to the

onset of the AD.

Data obtained during follow-up

After enrolment, patients were closely followed-up for a period of 3 months with frequent pre-specified sequential visits and laboratory determinations. Data on liver transplantation or death and causes of death were prospectively collected 3, 6 and 12 months after enrolment in all patients.

Identification of PEs of AD-No ACLF and AD-ACLF

In order to identify the PE a Adjudication Committee of the PREDICT study, which included JT, JF, RM and VA was nominated to elaborate the list of potential PEs, and the general principles and specific criteria for diagnosis. This Committee identified relevant and "true" PEs (hereafter just called as PEs), highly probable of precipitating both phenotypes of AD according to the criteria defined below. The Adjudication Committee proposed the following events as potential precipitants according to prior experience by the CANONIC study and other investigation: bacterial infections, alcoholic hepatitis, GI bleeding, drug-induced organ injury, therapeutic interventions.

General principles for PE identification

To provide the PREDICT study with a reliable method to identify PEs, the following general principles were agreed: of AD-ACLF, specific diagnostic criteria were developed based on the following principles:

1. PEs should consist of events that have the potential to induce impairment in the function of the liver and/or other organs, either by direct organ injury (e.g., tissue

hypoperfusion) or, indirectly, through significant dysregulation of important pathophysiological mechanisms (e.g., immune responses to microbial or endogenous cause).

2. When assessing the potential of hepatotoxic, nephrotoxic or neurotoxic drugs as being PEs, the lack of liver, kidney or brain dysfunction or failure, respectively, as defined by the CLIF-C OF score [8] rule out drug-induced organ toxicity as a PE.

3. As suggested by the results of the CANONIC study [1, 7], clinically identifiable, relevant and true PEs should have a higher prevalence among patients with AD-ACLF than among those with AD-no ACLF.

4. PEs should precede or coincide with the onset of AD-ACLF. The time period between the PE and the onset of AD-ACLF, however, is heterogeneous, depending on the PE.

5. Any event developing after the onset of AD-ACLF is a complication or a coincidental event but not a PE.

Specific criteria for the identification of PEs from the list proposed by the adjudication comitee

Bacterial infections. Infections were considered potential PEs if they were diagnosed at the time of or solved within the 48-hour period that preceded the onset of AD. Infections occurring before AD but solved before this 48-hour time frame were considered as unrelated events. Previous data have shown that the cytokine response to bacterial infections, even efficiently treated, may last up to 48 hours and

may induce the onset of AD [9]. When infections were diagnosed between the first and the 10th day after the onset of AD, they were considered as complications of AD [10]. Proven bacterial infections were defined as previously described [10] and in accordance with the EASL guidelines [7].

Alcohol-related liver injury. Alcoholic hepatitis was diagnosed according to the clinical criteria of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) [11], which includes the presence of at least 3 of the following: 1. Active alcoholism, as defined by more than 3 consecutive months of an alcohol intake higher than 60 g/day for males and 40 g/day for females; 2. Serum bilirubin>3 mg/dl; 3. AST>50 IU/; 4. AST/ALT>1.5 (maximal value of AST or ALT not exceeding 400 U/l). These criteria are in line with the clinical diagnosis of alcoholic hepatitis according to the existing EASL guidelines [12]. Alcoholic hepatitis was considered severe if patients showed CLIF-Consortium AD score ≥ 50 points [13], ore presence of ACLF (**Table 1**).

Gastrointestinal bleeding. Gastrointestinal bleeding was considered a PE if occurring within 7 days prior to the onset of AD-ACLF. Moreover, because hemorrhagic shock, which is a potential cause of organ damage, had not been previously analyzed as a PE [1], hemorrhagic shock was included in our list of candidates for PEs (**Table.1**).

Drug-induced organ injury. 1. Drug-induced liver injury was considered a potential PE when the hepatotoxic drug was administered within 1 month prior to the onset of AD-ACLF and the patient presented with hepatocellular (serum AST or ALT exceeding 3-fold the upper limit of normal), cholestatic (serum alkaline phosphatase exceeding 2-fold the upper limit of normal) or mixed liver injury as defined by Hy´s

law and FDA guidance also described in the recent EASL guidelines [14]; as well as liver dysfunction (for patients with AD-No ACLF, bilirubin > 6 mg/dl) or liver failure (for patients with AD-ACLF, bilirubin > 12 mg/dl). Potential hepatotoxic drugs were classified as described elsewhere [15]. Only drugs from groups A and B of this classification were considered potential candidates for liver toxicity. 2. Drug-induced kidney injury was considered a potential PE when the nephrotoxic drug was administered within 7 days prior to the onset of AD-ACLF and patients presented with either renal dysfunction or renal failure according to the CLIF-C OF score. Diuretic-induced renal dysfunction or renal failure was not considered as a nephrotoxic condition. 3. Toxic encephalopathy was considered a potential PE when the neurotoxic drug was administered within 48 hours prior the onset of AD-ACLF and the patient presented with encephalopathy, with a severity similar to brain dysfunction or brain failure according to the CLIF-C OF score.

Therapeutic interventions. These including transjugular intrahepatic portosystemic shunting (TIPS), major surgical procedures and large volume paracentesis without albumin administration, were considered as potential PEs if performed within 7 days prior to the onset of AD-ACLF.

Other potential PEs identified by the investigators in the individual patients eCRF

The Adjudication Committee assessed nine additional, infrequent conditions (viral hepatitis and other viral infections, decompensated cardiopulmonary diseases, dehydration, large hematomas, acute pancreatitis, acute portomesenteric vein thrombosis, autoimmune diseases, cerebrovascular accident and intestinal occlusion) that were considered by the attending investigators as potential PEs.

Statistical analysis

Discrete variables are shown as counts (percentage) and continuous variables as mean ± standard deviation (SD). Non-normally distributed variables are summarized by the median (interquartile range [IQR]). In univariate statistical comparisons, the chi-square test or Fisher exact test, when at least 25% of expected counts were under 5, were used for categorical variables, whereas the Student t-test or analysis of variance were used for normally distributed continuous variables and the Wilcoxon rank-sum test or the Kruskal-Wallis test for continuous variables not normally distributed. For comparisons at different time-points in the same patients, paired tests were used: McNemar test was applied for dichotomic variables and a test of symmetry was performed for variables with 3 categories. In all statistical analyses, significance was set at $p<0.05$ and an Available-Data-Only approach was adopted.

The proportional-hazards model for the subdistributions of competing risks proposed by Fine and Gray was the base to estimate the cumulative incidence functions (CIF) of mortality [16]. This model was chosen in order to account for liver transplantation as an event ‘‘competing’’ with mortality, based on the consideration that transplantation clearly modifies the probability of mortality of a specific patient at each subsequent time-point. The equality of CIFs across groups was evaluated by means of the Gray's Test [17]. Statistical analysis was performed using SAS v9.4 and plots were performed with RStudio v1.2.5042 and GraphPad Prism v5 software.

RESULTS

Identification of PEs for AD at enrolment in the PREDICT Study Cohort.

The Predict Study Cohort includes 1273 patients, 202 patients with AD-ACLF and 1071 patients with AD-No ACLF (**Fig. 1**). There were four types of main PEs: bacterial infections, alcohol-related liver injury, gastrointestinal (GI) bleeding and toxic encephalopathy **(Table 1).**

Prevalence of patients with proven bacterial infections but not of suspected bacterial infections was significantly higher in AD-ACLF than in AD-No ACLF. Moreover, prevalence of suspected bacterial infections was very low and similar in both groups. Therefore, only proven bacterial infections were considered as PE of AD-ACLF. Proven bacterial infections were the most common PE, present in 44.0% of patients with AD-ACLF and in 22.3% of patients with AD-No ACLF (P<0.0001).

Prevalence of alcoholic hepatitis and particularly of severe alcoholic hepatitis (alcoholic hepatitis associated with CLIF-C AD score ≥ 50 or ACLF) was significantly higher in patients with AD-ACLF (43.6%) than in patients with AD-No ACLF (18.7%) (P<0.0001). Yet, the overall alcoholic hepatitis in AD-No ACLF patients was not always associated with organ dysfunction, therefore, only severe alcoholic hepatitis was identified as PE, and was the second most frequent.

Severe GI-bleeding associated with hypovolemic shock was the third most frequent PE, although its prevalence in the AD-ACLF group and AD-No ACLF group (5.9% and 1.2%, respectively, P<0.0001) was low.

Finally, among the three types of drug-induced organ injury examined, only the prevalence of toxic encephalopathy was significantly higher in the AD-ACLF group than in the AD-No ACLF group (5.9% and 1.2%, respectively, P<0.0001) and qualified as PE. All drugs associated with severe toxic encephalopathy were opioids

or benzodiazepines.

Therapeutic paracentesis without intravenous albumin and TIPS did not qualify as PEs, since their prevalence was not significantly higher in patients with AD-ACLF. Other extremely infrequent events proposed by the investigators showed similar frequency in patients with AD-No ACLF and AD-ACLF and, therefore, were also not considered PEs.

Overall 721 patients (56.6%) included in the PREDICT Study Cohort did not present identifiable PEs (indeterminate PE), 447 (35.1%) presented one PE, and 105 (8.2%) presented two PEs or more.

The clinical characteristics, laboratory data, prognostic scores, and 90-day mortality rate of patients with AD-No ACLF and AD-ACLF are presented in **Table S1**.

Prevalence and impact of PEs on the characteristics, clinical course and prognosis of patients included in the AD-No ACLF Cohort.

The AD-No ACLF cohort includes the 1071 patients with AD-No ACLF at enrolment (**Fig. 1**).

Prevalence of PE and their combinations

In 409 patients, AD-No ACLF was associated with one PE in 354 patients (33.0%) and with two or more PEs in 55 patients (5.1%), while in 662 patients (61.8%) from the AD-No ACLF Cohort no PE was identified (**Table 1**).

Fig. 2A illustrates the prevalence of combinations of PEs in the 409 patients of the AD-No ACLF cohort who had PEs. Most patients (354 [86.5%]) had one PE (proven bacterial infections in 188 patients; severe alcoholic hepatitis in 151; GI bleeding with shock in 9 and toxic encephalopathy in 6). In 54 patients (13.4%), there were five combinations of two PEs, including proven bacterial infections associated

with either severe alcoholic hepatitis, toxic encephalopathy or GI bleeding (in 44, 4 and 2 patients, respectively); and severe alcoholic hepatitis associated with toxic encephalopathy or with GI bleeding with shock in 2 patients each. Finally, there was only one patient with 3 PEs (bacterial infection, alcoholic hepatitis and toxic encephalopathy). Therefore, among the 409 patients with PEs, AD-No ACLF was related with proven bacterial infections or severe acute alcoholic hepatitis, either alone, in combination, or in association with other PEs, in 394 patients (96.3%) (either proven bacterial infections or severe alcoholic hepatitis in 339 patients, both precipitants in 44 and other combinations that included proven bacterial infections or severe alcoholic hepatitis in 11). In only 15 (3.7%) patients (9 patients with GI bleeding with shock and 6 with toxic encephalopathy alone), AD-No ACLF was unrelated with bacterial infections or alcoholic hepatitis.

PEs impact the clinical course and survival of patients with AD-No ACLF.

Prevalence of patients with proven bacterial infections and severe alcoholic hepatitis at enrolment was higher in AD-pre ACLF (29.4% and 26.6%, respectively) than in AD-UDC (21.0% and 19.3%) or AD-SDC (20.3%. and 15.6%) phenotypes. Moreover, the number of patients without PEs was significantly lower (50.9%) and the number of patients with one or two or more PEs higher (40.4% and 8.7%) in patients with AD-pre-ACLF than in those with AD-UDC (60.9%, 35.6% and 3.4%, respectively) and AD-SDC (66.0%, 29.5% and 4.5%). Moreover, these differences aggravate when compared at the time-point of ACLF development in AD-pre-ACLF group. These observations suggest that both the presence and number of PEs at enrolment are important determinants in the development of AD-pre-ACLF, the most severe sub-phenotype among patients with AD-No ACLF (**Table 2**). The difference in presence and number of PEs is even more pronounced at ACLF diagnosis (**Table 2**).

Interestingly, patients with single PE of the two major groups (proven bacterial infection and severe alcoholic hepatitis) showed comparable 90-days mortality (**Fig. 2B**). This is striking, especially since there were important differences between the two groups of patients (**Table S2**), showing higher levels of WBC, liver failures and CLIF-C AD scores in severe alcoholic hepatitis, but higher CRP in patients with proven bacterial infection, underlining that the type PE is not crucial for outcome if correctly defined.

Despite the event precipitating AD playing a similar role in mortality, the number of PEs observed simultaneously at AD played a role in the outcome of the patients, with the highest 90-day mortality in patients with two or more PEs and the lowest mortality in patients without identifiable PE (**Fig. 2C**). This finding is confirmed by the activation of systemic inflammation assessed by surrogates at enrolment, since the number of PEs increased with higher levels of leukocytes, neutrophils, monocytes and CRP (**Fig. 2D-G**), organ dysfunction and failures and scores overall (**Table S3**).

Results derived from the Integrated ACLF Cohort

This cohort included a total of 420 patients. Of those, 202 had AD-ACLF at the time of enrolment in the PREDICT study (AD-ACLF phenotype group), while the other 218 patients developed AD-ACLF from the AD-pre-ACLF (at enrolment) during the study, and were included in the integrated cohort at the time of the development of ACLF (**Fig. 1**). The Integrated AD-ACLF Cohort was developed with two objectives: 1. the comprehensive characterization of the AD-ACLF phenotype, including patients with community acquired and hospital acquired ACLF; 2. sufficiently sized AD-ACLF cohort to analyze the differences in PE.

Prevalence of PEs and of its combinations

Among the 420 patients included in the Integrated AD-ACLF Cohort, 273 patients, AD-ACLF was triggered by one (191 patients, 45.5%) or two or more (82 patients, 19.5%) PEs, while 147 patients (35.0%) did not show PEs at diagnosis (**Table 3**).

Fig. 3A shows the different combinations of PEs in the Integrated AD-ACLF Cohort. Among the 191 patients with one PE, proven bacterial infection, severe alcoholic hepatitis, and GI bleeding with shock were identified in 111, 73 and 6 patients, respectively. Among the 70 patients with two PEs, proven bacterial infection was associated with severe alcoholic hepatitis in 55, toxic encephalopathy in 4 or GI bleeding with shock in 4 patients, while severe alcoholic hepatitis was associated with GI bleeding with shock in 4 patients. Finally, among the 12 patients with three PEs, proven bacterial infection was associated with severe alcoholic hepatitis and toxic encephalopathy in 6, with severe alcoholic hepatitis and GI bleeding with shock in 4, and with toxic encephalopathy and GI bleeding with shock in 2 patients. Therefore, out of the 273 patients in the Integrated AD-ACLF Cohort with PEs, 266 (97.4%) had proven bacterial infections or severe acute alcoholic hepatitis as either alone or combined PEs. The relative prevalence of the different combinations of PEs was similar in the Integrated AD-ACLF Cohort and in AD-No ACLF cohort (**Fig. 2A and 3A**).

*The type of PE significantly impacts clinical characteristics, but not clinical course and mortality of patients with AD-ACLF in the integrated cohort***.**

There were significant differences between patients with AD-ACLF triggered by proven bacterial infections or severe alcoholic hepatitis as single PEs (**Table 4**). Patients with AD-ACLF and proven bacterial infections were significantly older,

presented significantly lower mean arterial pressure and heart rate, indicative of arterial vasodilation and of cardiac chronotropic dysfunction, higher prevalence of circulatory, renal and respiratory failure and of vasopressors requirements, and lower prevalence of liver and coagulation failure than patients with severe alcoholic hepatitis. Serum levels of CRP were also higher in patients with infections. These differences, however, did not impact the clinical course and prognosis, since there were no significant differences between groups in the rate of intensive care unit admission, liver transplantation or 90-day mortality rate. **Fig. 3B** shows that there were also no significant differences in 90-day cumulative incidence of mortality between these two groups.

Number of PEs significantly impacts the clinical course and mortality of patients with AD-ACLF.

The number of PEs in patients included in the Integrated AD-ACLF Cohort (no PE, one PE, and two or three PEs) correlated positively with the prevalence of liver, brain, coagulation and cardio-circulatory failure and inversely with the prevalence of renal failure. These findings were due to differences in the predominance of specific organ failures among patients with distinct number of PEs. While the predominant organ failure in patients with no PE or with only one PE was kidney, liver failure was the predominant organ failure in patients with two or three PEs. Moreover the prevalence of other organ failures was also higher in patients with two or three PEs. Consistent with these results, the number of PEs at diagnosis also correlated directly with the grade of severity of ACLF (I, II or III), the severity of prognostic scores, the need of intensive care, the frequency of treatment with mechanical ventilation or renal replacement therapy, and the 90-day cumulative incidence of mortality (**Table 5**, **Fig. 3C).** Systemic inflammation, as estimated by the

WBC and blood levels of neutrophils and monocytes, increased in parallel with the number of PEs (**Table 5**, **Fig. 3D-G**). The serum levels of CRP were also significantly higher in patients with one or two or more PEs than in patients with no PEs. Overall, these findings suggest that PEs at diagnosis impact the severity of systemic inflammation and of the ACLF grade at diagnosis, clinical course severity and mortality in patients with AD-ACLF.

DISCUSSION

The relationship between clinical events precipitating extra-hepatic organ failures in patients with cirrhosis (e.g. spontaneous bacterial peritonitis, hepatorenal syndrome) has been well established for decades, and traditional treatments currently used (e.g. norfloxacin or albumin), were developed more than 30 years ago to prevent organ failures with high mortality [18, 19]. Yet unified and comprehensive investigations to elaborate on characteristics and impact of PEs are missing. The second investigation of the PREDICT Study fills this gap and offers PEs based on prospective data.

The CANONIC and the PREDICT studies are complementary, large-scale, prospective, observational investigations consecutively performed to investigate AD in cirrhosis. The CANONIC study was the first investigation stratifying patients with AD based on the presence (AD-ACLF phenotype) or absence (AD-No ACLF phenotype) of organ failure(s). Moreover, it suggested that PEs play an important role in the pathogenesis and clinical course in patients with AD-ACLF. However, since this study was restricted to the time frame of hospitalization, PEs developing prior to admission and the clinical course after hospitalization were insufficiently assessed. In contrast, the PREDICT study was specifically designed to explore patients with AD enrolled at non-elective hospital admission, and assess the 90-day period prior to and the 90-day period following enrolment [2]. The first investigation derived from the PREDICT study identified three different clinical courses in patients admitted with the AD-No ACLF phenotype: the pre-ACLF, the AD-UDC and the AD-SDC sub-phenotypes. The current article reports the results of the second PREDICT study investigation, which was aimed to assess if the type and number of PEs

influence clinical course and prognosis in patients with AD-No ACLF and AD-ACLF.

The PREDICT study is an observational study in highly complex patients, which were prospectively enrolled and followed for 90 days. At enrolment, data related to PEs (e.g. time between PEs and AD) were also obtained, although retrospectively. More importantly, during this extensive prospective observation, the patients PEs leading to ACLF are assessed and characterized. Based on those data, this study offers for the first-time diagnostic criteria for PEs and constitutes the first attempt to rationalize the identification of PEs in patients with cirrhosis and AD.

The PREDICT design took into account inherited limitations of observational studies. First, the extremely detailed eCRF was able to capture all potentially important events prior to and at the time of enrolment. Second, patients were carefully controlled within a 90-day follow-up period after enrolment by frequent visits and laboratory assessments. Third, we enrolled a large series of 1273 non-elective patients with AD hospitalized for treatment; 202 with AD-ACLF and 1071 with AD-No ACLF. Fourth, in order to increase the power analyzing PEs in AD-ACLF, the visits of patients with AD-Pre ACLF at the time of development of AD-ACLF were included to form the Integrated ACLF Cohort (420 patients). Finally, the criteria used for the diagnosis of PEs considered the severity of the PE, the time interval between the onset/resolution of the PE and the onset of the AD episode, and the concept that any PE should be significantly more prevalent in patients with AD-ACLF than in patients with AD-No ACLF. These criteria are more objective than the traditional principles of chronology and vague possibility for inducing organ injury of a specific event at the discretion of attending physicians.

Despite their limitations, large-scale prospective observational studies may give rise to important pathophysiological insight. As such, the CANONIC study showed close association between surrogates of systemic inflammation (WBC, CRP) and the presence and severity of AD-ACLF at enrolment and on follow-up, suggesting that systemic inflammation is the most likely mechanism underpinning ACLF [1, 20], a concept confirmed with sophisticated cytokines, lipidomic and metabolomics studies [5, 6, 21, 22]. Therefore, PEs should occur more frequently in patients with AD-ACLF than in patients with AD-No ACLF, as shown in the CANONIC study with bacterial infections and active alcoholism (surrogate of alcoholic hepatitis) both correlating with the severity of systemic inflammation and number of organ failures.

Among the 16 events recorded and evaluated in the current study, only four fulfilled the properties of PEs (chronology, severe organ injury or higher prevalence in the AD-ACLF phenotype): proven bacterial infections, severe alcoholic hepatitis, GI bleeding with shock and toxic encephalopathy. While paracentesis without intravenous albumin administration and TIPS (even improves survival in GI bleeding and ACLF [23, 24]) did not induce organ impairment, the prevalence of drug-induced liver or renal injury and of other potential PEs proposed by the investigators was extremely low, frequently below 1%, suggesting that they could be coincidental events rather than PEs.

Proven bacterial infections and severe alcoholic hepatitis were by far the most prevalent PEs observed in the AD-No ACLF and the AD-ACLF cohorts within the 1273 patients included in the PREDICT study. Prevalence of GI bleeding associated with shock and toxic encephalopathy was very much lower in both groups. Among

patients with PEs, 409 in the AD-No ACLF Cohort and 273 in the Integrated AD-ACLF Cohort, almost all (96.3% and 97.4%, respectively) showed proven bacterial infection and severe alcoholic hepatitis, either alone or in combination with other PEs. This overwhelming prevalence of proven bacterial infections and/or severe alcoholic hepatitis as PEs of AD-No ACLF and AD-ACLF suggests that preventing these PEs, or if not possible diagnosing and treating them as early as possible after onset, is paramount to improving the prognosis in decompensated cirrhosis.

Importantly, the majority of the 1071 patients in the AD-No ACLF Cohort, 61.8% did not present PEs at enrolment. In contrast the rate of patients with no PEs at enrolment in the 420 patients with AD-ACLF from the Integrated ACLF Cohort was only 35%. The prevalence of patients with one or multiple PEs were 33.0% and 5.1%, respectively, in the AD-No ACLF Cohort, and 45.5% and 19.5% in the Integrated AD-ACLF Cohort. These data suggest that AD-No ACLF develops in the context of endogenous mechanisms (e.g. progressing liver disease, bacterial translocation) than AD-ACLF. These observations using the PEs support the CANONIC study, which underlines the solidity of the present investigation. Moreover, whilst multiple (two or more) PEs trigger AD-ACLF (1 in 5 patients), it is exceptional (1 in 20 patients) in AD-No ACLF.

In patients with AD-No ACLF, the prevalence of proven bacterial infections or severe alcoholic hepatitis and the number of PEs present at enrolment were higher in patients with AD-Pre ACLF than in patients with AD-UDC and AD-SDC. These findings suggest that PEs are determinants of the development of the AD-Pre ACLF sub-phenotype, which is associated with a worse clinical course and prognosis in patients with AD-No ACLF. In contrast, no differences were found in the prevalence

of these PEs between patients with UDC and SDC. It is known that AD-Pre ACLF develops in the setting of severe systemic inflammation, while the grade of systemic inflammation associated with the UDC and the SDC is moderate [2]. Therefore, it is likely that proven bacterial infections or severe acute liver injury impact clinical course and prognosis in patients with AD-No ACLF by acting as inducers of systemic inflammation.

This study describes for the first time that the type of PE differentially impacted the clinical characteristics of AD-ACLF patients. AD-ACLF triggered by severe alcoholic hepatitis was associated with less systemic inflammation, higher prevalence of liver and coagulation failure and lower prevalence of renal and circulatory failure than AD-ACLF triggered by proven bacterial infections. Importantly, the type of PE did not impact clinical course severity and the 90-day cumulative incidence of mortality. This finding is not surprising, since Shi et al [25] showed that other hepatic PEs (hepatitis B reactivation or superimposed hepatitis A and E) led to higher prevalence of liver and coagulation failure and lower prevalence of renal and circulatory failure than AD-ACLF triggered by extra-hepatic precipitants (bacterial infections or GI bleeding). Therefore, each of the major types of PEs likely promotes specific organs failures in AD-ACLF [6]. Bacterial infections would induce systemic inflammation as the primary mechanism, leading to predominantly circulatory and renal dysfunction or failure. In contrast, the direct insult of alcohol toxicity induces hepatic inflammation and cell death as primary mechanisms culminating in liver and coagulation dysfunction or failure. Yet in both cases systemic inflammation aggravates and leads to an identical syndrome through distinct pathophysiological pathways. For this reason the criterion of the severity (either systemic inflammation or organ injury) of PE is crucial to identify PE.

Our results finally showed that the number of PEs was an important determinant of the characteristics, clinical course severity and 90-day cumulative incidence of mortality of patients included in the Integrated AD-ACLF Cohort. The intensity of systemic inflammation, the prevalence of liver, brain, coagulation, cardio-circulatory and respiratory failures; the ACLF grade; and the prognostic scores increased progressively from patients with no PEs to patients with one and multiple PEs. Moreover, the need for intensive care, mechanical ventilation, renal replacement therapy or treatment with vasoconstrictors and the 90-day cumulative incidence of mortality rate also increased in parallel with the number of PEs in these patients. Therefore, when PEs are defined according to these criteria, they are synergistic and additive in the worsening of outcome, despite different clinical characteristics.

In summary, among the 16 events explored as potential PEs in the Predict study only four (proven bacterial infections, severe acute alcoholic hepatitis, GI bleeding associated with shock and toxic encephalopathy) fulfilled the diagnostic criteria of PEs. Proven bacterial infections and severe alcoholic hepatitis were present in more than 95% of patients. However, it is important to remark that no PE could be identified in 2/3 of AD-No ACLF patients and in 1/3 AD-ACLF patients. The prevalence and number of PEs increased with severity of the AD-sub-phenotype form SDC/UDC to Pre-ACLF and ACLF, which were also directly related with clinical course severity and short-term mortality in patients with AD. Our data, therefore, strongly suggest that PEs significantly influence the clinical course and prognosis of patients with AD and specific preventive and therapeutic strategies for these PEs are required to improve outcomes in decompensated cirrhosis.

REFERENCES

[1] Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. Gastroenterology 2013;144:1426-1437, 1437 e1421-1429.
[2] Trebicka J, Fernandez J, Papp M, Caraceni P, Laleman W, Gambino C, et al. The PREDICT study uncovers three clinical courses in acutely decompensated cirrhosis with distinct pathophysiology. Journal of hepatology 2020.
[3] Arroyo V, Moreau R, Kamath PS, Jalan R, Gines P, Nevens F, et al. Acute-on-chronic liver failure in cirrhosis. Nat Rev Dis Primers 2016;2:16041.
[4] Arroyo V, Moreau R, Jalan R. Acute-on-Chronic Liver Failure. The New England journal of medicine 2020;382:2137-2145.
[5] Claria J, Stauber RE, Coenraad MJ, Moreau R, Jalan R, Pavesi M, et al. Systemic inflammation in decompensated cirrhosis: Characterization and role in acute-on-chronic liver failure. Hepatology 2016;64:1249-1264.
[6] Trebicka J, Amoros A, Pitarch C, Titos E, Alcaraz-Quiles J, Schierwagen R, et al. Addressing Profiles of Systemic Inflammation Across the Different Clinical Phenotypes of Acutely Decompensated Cirrhosis. Front Immunol 2019;10:476.
[7] European Association for the Study of the Liver. , Collaborators:, Angeli P, Bernardi M, Villanueva C, Francoz C, et al. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. Journal of hepatology 2018;69:406-460.
[8] Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Gines P, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. Journal of hepatology 2014;61:1038-1047.
[9] Byl B, Roucloux I, Crusiaux A, Dupont E, Deviere J. Tumor necrosis factor alpha and interleukin 6 plasma levels in infected cirrhotic patients. Gastroenterology 1993;104:1492-1497.
[10] Fernandez J, Acevedo J, Wiest R, Gustot T, Amoros A, Deulofeu C, et al. Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis. Gut 2018;67:1870-1880.
[11] Crabb DW, Bataller R, Chalasani NP, Kamath PS, Lucey M, Mathurin P, et al. Standard Definitions and Common Data Elements for Clinical Trials in Patients With Alcoholic Hepatitis: Recommendation From the NIAAA Alcoholic Hepatitis Consortia. Gastroenterology 2016;150:785-790.
[12] European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL Clinical Practice Guidelines: Management of alcohol-related liver disease. Journal of hepatology 2018;69:154-181.
[13] Jalan R, Pavesi M, Saliba F, Amoros A, Fernandez J, Holland-Fischer P, et al. The CLIF Consortium Acute Decompensation score (CLIF-C ADs) for prognosis of hospitalised cirrhotic patients without acute-on-chronic liver failure. Journal of hepatology 2015;62:831-840.
[14] European Association for the Study of the Liver. Electronic address eee, Clinical Practice Guideline Panel C, Panel m, representative EGB. EASL Clinical Practice Guidelines: Drug-induced liver injury. Journal of hepatology 2019;70:1222-1261.
[15] Bjornsson ES, Hoofnagle JH. Categorization of drugs implicated in causing liver injury: Critical assessment based on published case reports. Hepatology 2016;63:590-603.

[16] Fine J, Gray R. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496-509.
[17] Gray R. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat 1988;16:1141-1154.
[18] Gines P, Rimola A, Planas R, Vargas V, Marco F, Almela M, et al. Norfloxacin prevents spontaneous bacterial peritonitis recurrence in cirrhosis: results of a double-blind, placebo-controlled trial. Hepatology 1990;12:716-724.
[19] Sort P, Navasa M, Arroyo V, Aldeguer X, Planas R, Ruiz-del-Arbol L, et al. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. The New England journal of medicine 1999;341:403-409.
[20] Gustot T, Fernandez J, Garcia E, Morando F, Caraceni P, Alessandria C, et al. Clinical Course of acute-on-chronic liver failure syndrome and effects on prognosis. Hepatology 2015;62:243-252.
[21] Moreau R, Claria J, Aguilar F, Fenaille F, Lozano JJ, Junot C, et al. Blood metabolomics uncovers inflammation-associated mitochondrial dysfunction as a potential mechanism underlying ACLF. Journal of hepatology 2020;72:688-701.
[22] Lopez-Vicario C, Checa A, Urdangarin A, Aguilar F, Alcaraz-Quiles J, Caraceni P, et al. Targeted lipidomics reveals extensive changes in circulating lipid mediators in patients with acutely decompensated cirrhosis. Journal of hepatology 2020.
[23] Trebicka J, Gu W, Ibanez-Samaniego L, Hernandez-Gea V, Pitarch C, Garcia E, et al. Rebleeding and mortality risk are increased by ACLF but reduced by pre-emptive TIPS. Journal of hepatology 2020.
[24] Kumar R, Kerbert AJC, Sheikh MF, Roth N, Calvao JAF, Mesquita MD, et al. Determinants of mortality in patients with cirrhosis and uncontrolled variceal bleeding. Journal of hepatology 2020.
[25] Shi Y, Yang Y, Hu Y, Wu W, Yang Q, Zheng M, et al. Acute-on-chronic liver failure precipitated by hepatic injury is distinct from that precipitated by extrahepatic insults. Hepatology 2015;62:232-242.

LEGEND OF FIGURES

Figure 1. AD phenotype groups and subgroups included in each of the AD cohorts used for the study analysis. For more explanation see the text.

Figure 2. Combinations of PEs in the AD-No ACLF Cohort shown in four-set circle Venn's diagram (Panel A). Cumulative incidence of mortality in patients with AD-No ACLF according to the type of PE (proven infections alone versus severe alcoholic hepatitis alone; panel B) and the number of PEs (no PE, one PE, and two or more PEs; Panel C); p-values were obtained from Gray's Test. Blood levels of leukocytes (panel D), neutrophils (panel E), monocytes (panel F) and the serum concentration of CRP (panel G) in patients with AD-No ACLF and indeterminate PE (no PEs), one PE and two or more PEs.Boxes show median and IQR and whiskers show 10-90 percentiles. Kruskal-Wallis test was performed with all values in each comparison. Differences were statistically significant (P<0.0001) for all biomarkers.

Figure 3. Combinations of PEs in the Integrated AD-ACLF Cohort shown in four-set circle Venn's diagram (Panel A). Cumulative incidence of mortality in patients with AD-ACLF according to the type of PE (proven infections alone versus severe alcoholic hepatitis alone; panel B) and the number of PEs (no PE, one PE, and two or more PEs; Panel C); p-values were obtained form Gray's Test. Blood levels of leukocytes (panel D), neutrophils (panel E), monocytes (panel F) and the serum concentration of CRP (panel G) in patients with AD-ACLF and indeterminate PE (no PEs), one PE and two or more PEs. Boxes show median and IQR and whiskers show 10-90 percentiles. Kruskal-Wallis test was performed with all values in each comparison. Differences were statistically significant (P<0.0001) for all biomarkers.

TABLES

Table 1. Candidates for Precipitating Events (PEs), PEs, and the Combination of PEs in patients with AD-No ACLF and with AD-ACLF.

	AD-No ACLF (n = 1071)	AD-ACLF (n = 202)	*p* value[a]
Candidates for PEs *n (%)*			
Bacterial infections			
Any infection	314 (29.32)	101 (50.00)	<.0001
Suspected Bacterial Infection	74 (6.91)	12 (5.94)	0.6148
Proven Bacterial Infections[b]	239 (22.32)	89 (44.06)	<.0001
Alcohol-related liver injury			
Alcoholic Hepatitis	275 (25.68)	88 (43.56)	<.0001
Severe Alcoholic Hepatitis[b]	200 (18.67)	88 (43.56)	<.0001
GI Bleeding			
Any GI Bleeding	176 (16.43)	40 (19.80)	0.2420
GI Bleeding with hypovolemic shock[b]	13 (1.21)	12 (5.94)	<.0001
Drug-induced brain injury			
Patients treated with neurotoxic drugs	84 (7.84)	17 (8.42)	0.7824
Toxic Encephalopathy[b]	13 (1.21)	12 (5.94)	<.0001
Other candidates *n (%)*			
Paracentesis without albumin	110 (10.28)	21 (10.40)	0.9604
TIPS	49 (4.58)	8 (3.96)	0.6965
Drug-induced liver injury	16 (1.49)	4 (1.98)	0.5431
Viral hepatitis or other viral Infections	13 (1.21)	3 (1.49)	0.7299
Drug-induced kidney injury	3 (0.28)	1 (0.50)	-
Surgery	3 (0.28)	0 (0.00)	-
Decompensated cardiopulmonary disease	4 (0.37)	3 (1.49)	-
Dehydration	3 (0.28)	1 (0.50)	-
Large hematomas	3 (0.28)	0 (0.00)	-
Acute pancreatitis	1 (0.09)	1 (0.50)	-
Portomesenteric vein thrombosis	2 (0.19)	1 (0.50)	-
Extra-hepatic autoimmune disease	2 (0.19)	0 (0.00)	-
Cerebrovascular accident	0 (0.00)	1 (0.50)	-
Bowel occlusion	1 (0.09)	0 (0.00)	-
Number of PEs			
Indeterminate PE (No PEs)	662 (61.81)	59 (29.21)	<.0001
One PEs	354 (33.05)	93 (46.04)	
Two or more PEs	55 (5.14)	50 (24.75)	

[a] Certain *p* value were not determined because of the low number of patients.

[b] Underlined precipitating events are those considered as precipitating events of AD-ACLF

Chi-square or Fisher tests performed in percentages comparisons.

Table 2. Type and Number of Precipitating Events (PEs) in Patients with Pre-ACLF, Unstable Decompensated Cirrhosis (UDC) and Stable Decompensated Cirrhosis (SDC)

	Pre-ACLF (n=218)		UDC (n=233)	SDC (n=620)
	At enrolment	At ACLF development		
Type of PEs, n (%)				
Proven Bacterial Infections	64 (29.4)	97 (44.5)**	49 (21.0) *,##	126 (20.3) **,##
Severe Alcoholic Hepatitis	58 (26.6)	57 (26.1)	45 (19.3) +	97 (15.6) **,#
GI Bleeding with Shock $	2 (0.9)	8 (3.7)	2 (0.9)	9 (1.5)
Toxic Encephalopathy $	3 (1.4)	4 (1.8)	3 (1.3)	7 (1.1)
Number of PEs, n (%)				
Indeterminate PE (No PEs)	111 (50.9)	88 (40.4)**	142 (60.9) *,##	409 (66.0) **,##
One PE	88 (40.4)	98 (45.0)**	83 (35.6) ##	183 (29.5) ##
Two or more PEs	19 (8.7)	32 (14.7)**	8 (3.4) ##	28 (4.5) ##

+ $p < 0.07$, * $p < 0.05$ and ** $p < 0.01$ versus the Pre-ACLF group at enrolment

# $p < 0.001$ and ## $p < 0.0001$ vs Pre-ACLF group at ACLF development

$ p value not determined due to the low number of patients

Chi-square or Fisher tests performed in percentages comparisons among groups.

McNemar test used in paired comparisons for the types of PEs between the 2 time-points in Pre-ACLF group

Symmetry test used in paired comparisons for the number of PEs between the 2 time-points in Pre-ACLF group

Table 3. Demographic Data and Etiology, Types and Number of Precipitating Events (PEs), Clinical and Laboratory Data at Diagnosis, Special Treatments during Follow-up and Mortality in the Integrated ACLF cohort (n = 420).

Demographic data and etiology	
Age, yr, mean ± SD	59.1 +/- 11.74
Male sex, n (%)	288 (68.6)
Alcoholic cirrhosis, n (%)	302 (71.9)
Precipitating events at diagnosis*	
Type of PEs, n (%)	
Proven Bacterial Infections	186 (44.3)
Severe Alcoholic Hepatitis	145 (34.5)
GI Bleeding with Shock	20 (4.8)
Toxic Encephalopathy	16 (3.8)
Number of PEs, n (%)	
Indeterminate PE (No PEs)	147 (35.0)
One PE	191 (45.5)
Two or more PEs	82 (19.5)
Clinical and laboratory data	
Systemic hemodynamics, mean ± SD	
Mean arterial pressure (mmHg)	79.0 +/- 13.08
Heart rate (bpm)	83.4 +/- 18.06
Complications, n (%)	
Ascites	295 (77.2)
Hepatic Encephalopathy	235 (61.5)
Gastrointestinal bleeding	51 (13.4)
Organ failures, n (%)	
Liver failure	138 (36.1)
Renal failure	215 (56.3)
Brain failure	71 (18.6)
Coagulation failure	94 (24.7)
Cardiovascular failure	58 (15.3)
Respiratory failure	37 (9.7)
Biomarkers of systemic inflammation, median (IQR)	
White-cell count, x109/L	8.69 (6.10 - 13.14)
Neutrophil count, x109/L	6.74 (4.12 - 10.45)
Lymphocyte count, x109/L	0.96 (0.59 - 1.50)
Monocyte count, x109/L	0.83 (0.51 - 1.24)
Serum C-reactive protein, mg/L	26.75 (12.40 - 52.00)
Measurements estimating organ function	
Serum bilirubin, mg/dL, median (IQR)	5.65 (2.00 - 15.95)
Serum albumin, g/dL, mean ± SD	2.9 +/- 0.72
Total cholesterol, mg/dL, median (IQR)	75.49 (50.19 - 107.34)
International normalized ratio, median (IQR)	1.78 (1.44 - 2.40)
Serum creatinine, mg/dL, median (IQR)	2.04 (1.05 - 2.61)
Serum sodium, mEq/L, mean ± SD	133.8 +/- 7.03
Scores at diagnosis	
Prognostic scores, mean ± SD	
Child-Pugh score	10.5 +/- 2.28
MELD score*	26.0 +/- 6.58
MELD-Na score*	28.2 +/- 6.13
CLIF-C Organ Failure score**	9.8 +/- 2.12
CLIF-C ACLF score**	49.5 +/- 9.05
ACLF Grade	
ACLF-Grade I	222 (58.7)
ACLF-Grade II	110 (29.1)
ACLF-Grade III	46 (12.2)
Special treatments and mortality	
Special treatments from ACLF, n (%)	

Intensive Care	88 (21.0)
Renal replacement therapy	35 (8.3)
Mechanical ventilation	47 (12.3)
Vasopressors	159 (37.9)
90-day liver transplantation	49 (11.67)
Mortality from diagnosis	
90-day mortality, n (%)	209 (49.76)

* MELD: Model for End-Stage Liver Disease score

** CLIF-C: Chronic Liver Failure Consortium

Table 4. Demographic Data and Etiology, Clinical and Laboratory Data at Diagnosis, Specific Treatments during Follow-up and Mortality in Patients included in the Integrated AD-ACLF cohort with Proven Bacterial Infection or Severe Alcoholic Hepatitis as Unique Precipitating Events

	Proven Bacterial Infections (n = 111)	Severe Alcoholic Hepatitis (n = 73)	*p* value
Demographic data and etiology			
Age, yr, mean ± SD	63.5 +/- 10.08	56.3 +/- 11.21	<.0001
Male sex, n (%)	81 (73.0)	50(68.5)	0.5115
Alcoholic cirrhosis, n (%)	68 (61.3)	73 (100.0)	<.0001
Clinical and laboratory data			
Systemic hemodynamics, mean ± SD			
Mean arterial pressure (mmHg)	77.2 +/- 12.89	82.6 +/- 12.56	0.0070
Heart rate (bpm)	79.7 +/- 16.72	84.8 +/- 17.17	0.0546
Complications, n (%)			
Ascites	78 (75.0)	54 (79.4)	0.5031
Hepatic Encephalopathy	63 (60.6)	44 (64.7)	0.5850
Gastrointestinal bleeding	8 (7.7)	2 (2.9)	0.3187
Organ failures, n (%)			
Liver failure	22 (21.2)	38 (55.9)	<.0001
Renal failure	68 (65.4)	26 (38.2)	0.0005
Brain failure	20 (19.2)	8 (11.8)	0.1947
Coagulation failure	18 (17.5)	23 (33.8)	0.0143
Cardiovascular failure	20 (19.4)	2 (2.9)	0.0016
Respiratory failure	17 (16.5)	3 (4.5)	0.0174
Biomarkers of systemic inflammation, median (IQR)			
White-cell count, $x10^9$/L	9.21 (6.33 - 13.35)	10.36 (7.61 - 13.60)	0.1606
Neutrophil count, $x10^9$/L	7.15 (4.67 - 10.74)	7.70 (5.08 - 9.35)	0.8042
Lymphocyte count, $x10^9$/L	0.70 (0.43 - 1.24)	1.21 (0.78 - 1.80)	0.0005
Monocyte count, $x10^9$/L	0.80 (0.60 - 1.20)	1.00 (0.67 - 1.32)	0.0860
Serum C-reactive protein, mg/L	40.50 (18.00 - 83.50)	24.46 (11.00 - 41.60)	0.0025
Measurements estimating organ function			
Serum bilirubin, mg/dL, median (IQR)	3.24 (1.89 - 9.87)	13.30 (4.71 - 20.82)	<.0001
Serum albumin, g/dL, mean ± SD	3.0 +/- 0.67	2.8 +/- 0.72	0.1874
Total cholesterol, mg/dL, median (IQR)	54.00 (39.77 - 92.66)	91.00 (67.00 - 120.00)	0.0033
International normalized ratio, median (IQR)	1.70 (1.40 - 2.18)	1.98 (1.52 - 2.67)	0.0231
Serum creatinine, mg/dL, median (IQR)	2.15 (1.29 - 2.68)	1.39 (0.79 - 2.13)	0.0002
Serum sodium, mEq/L, mean ± SD	134.0 +/- 6.41	132.6 +/- 6.03	0.1421
Prognostic scores, mean ± SD			
Child-Pugh score	10.2 +/- 2.23	11.1 +/- 1.96	0.0094
MELD score*	24.8 +/- 6.72	27.2 +/- 5.39	0.0142
MELD-Na score*	27.3 +/- 5.93	29.5 +/- 5.19	0.0150
CLIF-C Organ Failure score**	9.8 +/- 2.21	9.7 +/- 1.55	0.7228
CLIF-C ACLF score**	51.2 +/- 8.75	48.6 +/- 6.54	0.0316
ACLF grades, n (%)			
ACLF grade I	61 (59.8)	41 (61.2)	0.2391
ACLF grade II	27 (26.5)	22 (32.8)	
ACLF Grade III	14 (13.7)	4 (6.0)	
Special treatments and mortality			
Special treatments from ACLF, n (%)			
Intensive care	26 (23.4)	11 (15.1)	0.1666
Renal replacement	10 (9.0)	3 (4.1)	0.2045
Mechanical ventilation	17 (16.3)	4 (5.9)	0.0404
Vasopressors	47 (42.3)	19 (26.0)	0.0240
90-day Liver transplantation	15 (13.89)	9 (12.68)	0.8158
Mortality from ACLF diagnosis, n (%)			
90-day Mortality	58 (52.25)	36 (49.32)	0.6966

* MELD: Model for End-Stage Liver Disease score; ** CLIF-C: Chronic Liver Failure Consortium

Chi-square or Fisher tests performed in percentages comparisons. For continuous variables comparisons, Student T-test for normally distributed variables or Wilcoxon Rank-Sum Test for not-normally distributed variables were used.

Table 5. Demographic Data and Etiology, Clinical and Laboratory Data at Diagnosis, Special Treatments during Follow-up and Mortality in Patients included in the Integrated AD-ACLF cohort according to the Number of Precipitating Events.

	No PEs (n=147)	One PE (n=191)	Two or more PEs (n=82)	*p* value
Demographic data and etiology of cirrhosis				
Age, yr, mean ± SD	61.2 +/- 11.38	60.5 +/- 11.06	52.1 +/- 11.41[a]	<.0001
Male sex, n (%)	99 (67.3)	137 (71.7)	52 (63.4)	0.3684
Alcoholic cirrhosis, n (%)	81 (55.1)	144 (75.4)[b]	77 (93.9)[a]	<.0001
Data at ACLF diagnosis				
Systemic hemodynamics, mean ± SD				
Mean arterial pressure (mmHg)	80.8 +/- 12.51	79.0 +/- 13.05	76.1 +/- 13.65[b]	0.0419
Heart rate (bpm)	79.4 +/- 15.80	82.0 +/- 17.26	92.9 +/- 19.93[a]	<.0001
Complications, n (%)				
Ascites	90 (73.2)	134 (74.9)	71 (88.8)[a]	0.0206
Hepatic encephalopathy	61 (49.6)	112 (62.6)[b]	62 (77.5)[a]	0.0003
Gastrointestinal bleeding	16 (13.1)	16 (8.9)	19 (23.8)[c]	0.0053
Organ failures, n (%)				
Liver failure	29 (23.6)	60 (33.5)	49 (61.3)[a]	<.0001
Renal failure	84 (68.3)	98 (54.7)[b]	33 (41.3)[a]	0.0006
Brain failure	13 (10.6)	31 (17.3)	27 (33.8)[a]	0.0002
Coagulation failure	25 (20.3)	41 (23.0)	28 (35.0)[a]	0.0474
Cardiovascular failure	6 (4.9)	25 (14.0)[b]	27 (33.8)[a]	<.0001
Respiratory failure	3 (2.4)	21 (11.9)[b]	13 (16.3)[b]	0.0022
Biomarkers of systemic inflammation, median (IQR)				
White-cell count, x10^9/L	7.19 (5.03 - 9.40)	9.72 (6.39 - 13.50)[b]	12.14 (8.57 - 18.10)[a]	<.0001
Neutrophil count, x10^9/L	4.44 (2.72 - 6.71)	7.22 (4.70 - 10.52)[b]	9.15 (6.42 - 15.75)[a]	<.0001
Lymphocyte count, x10^9/L	0.85 (0.60 - 1.32)	0.90 (0.52 - 1.47)	1.10 (0.70 - 1.90)[b]	0.0794
Monocyte count, x10^9/L	0.58 (0.39 - 0.89)	0.87 (0.60 - 1.21)[b]	1.21 (0.90 - 1.72)[a]	<.0001
Serum C-reactive protein, mg/L	17.60 (8.80 - 32.00)	32.30 (15.00 - 58.90)[b]	36.15 (18.00 - 75.00)[b]	<.0001
Measurements estimating organ function				
Serum bilirubin, mg/dL, median (IQR)	2.29 (1.12 - 11.04)	5.70 (2.12 - 14.80)[b]	14.53 (6.55 - 23.08)[a]	<.0001
Serum albumin, g/dL, mean ± SD	3.0 +/- 0.82	2.9 +/- 0.68	2.9 +/- 0.65	0.4571
Total cholesterol, mg/dL, median (IQR)	86.50 (57.73 - 122.78)	70.25 (48.52 - 104.13)[b]	63.39 (42.00 - 83.01)[b]	0.0145
International normalized ratio, median (IQR)	1.53 (1.32 - 2.13)	1.75 (1.45 - 2.34)[b]	2.18 (1.80 - 2.78)[a]	<.0001
Serum creatinine, mg/dL, median (IQR)	2.15 (1.54 - 2.80)	2.00 (1.04 - 2.50)[b]	1.55 (0.82 - 2.81)[b]	0.0024
Serum sodium, mEq/L, mean ± SD	133.6 +/- 6.77	133.6 +/- 6.36	134.4 +/- 8.71	0.7078
Prognostic scores, mean ± SD				
Child-Pugh score	9.5 +/- 2.41	10.5 +/- 2.18[b]	11.8 +/- 1.50[a]	<.0001
MELD score*	24.3 +/- 6.21	25.6 +/- 6.41	29.8 +/- 6.13[a]	<.0001
MELD-Na score*	26.6 +/- 6.11	27.9 +/- 5.81	31.2 +/- 5.83[a]	<.0001
CLIF-C organ failure score**	8.9 +/- 1.70	9.7 +/- 1.97[b]	11.3 +/- 2.20[a]	<.0001
CLIF-C ACLF score**	45.7 +/- 7.45	50.1 +/- 8.05[b]	54.1 +/- 10.86[a]	<.0001
ACLF grades, n (%)				
ACLF grade I	93 (76.2)	105 (59.7)[b]	24 (30.0)[a]	<.0001
ACLF grade II	23 (18.9)	53 (30.1)[b]	34 (42.5)[a]	
ACLF grade III	6 (4.9)	18 (10.2)[b]	22 (27.5)[a]	
Specific treatments and mortality				
Specific treatments from ACLF, n (%)				
Intensive care	15 (10.2)	41 (21.5)[b]	32 (39.0)[a]	<.0001
Renal replacement	8 (5.4)	13 (6.8)	14 (17.1)[a]	0.0055
Mechanical ventilation	3 (2.4)	22 (12.3)[b]	22 (27.5)[a]	<.0001
Vasopressors	35 (23.8)	72 (37.7)[b]	52 (63.4)[a]	<.0001
90-day Liver Transplantation	19 (13.1)	25 (13.4)	5 (6.3)	0.2290
Mortality after ACLF diagnosis, n (%)				
90-day Mortality	62 (42.2)	95 (49.7)	52 (63.4)[a]	0.0087

* MELD: Model for End-Stage Liver Disease score; ** CLIF-C: Chronic Liver Failure Consortium; [a] p<0.05 versus No PE and 1 PE; [b] p<0.05 versus No PE; [c] p<0.05 versus 1 PE. Chi-square or Fisher tests performed in percentages comparisons. For continuous variables comparisons, Analysis of Variance for normally distributed variables or Kruskal-Wallis test for not-normally distributed variables were used.

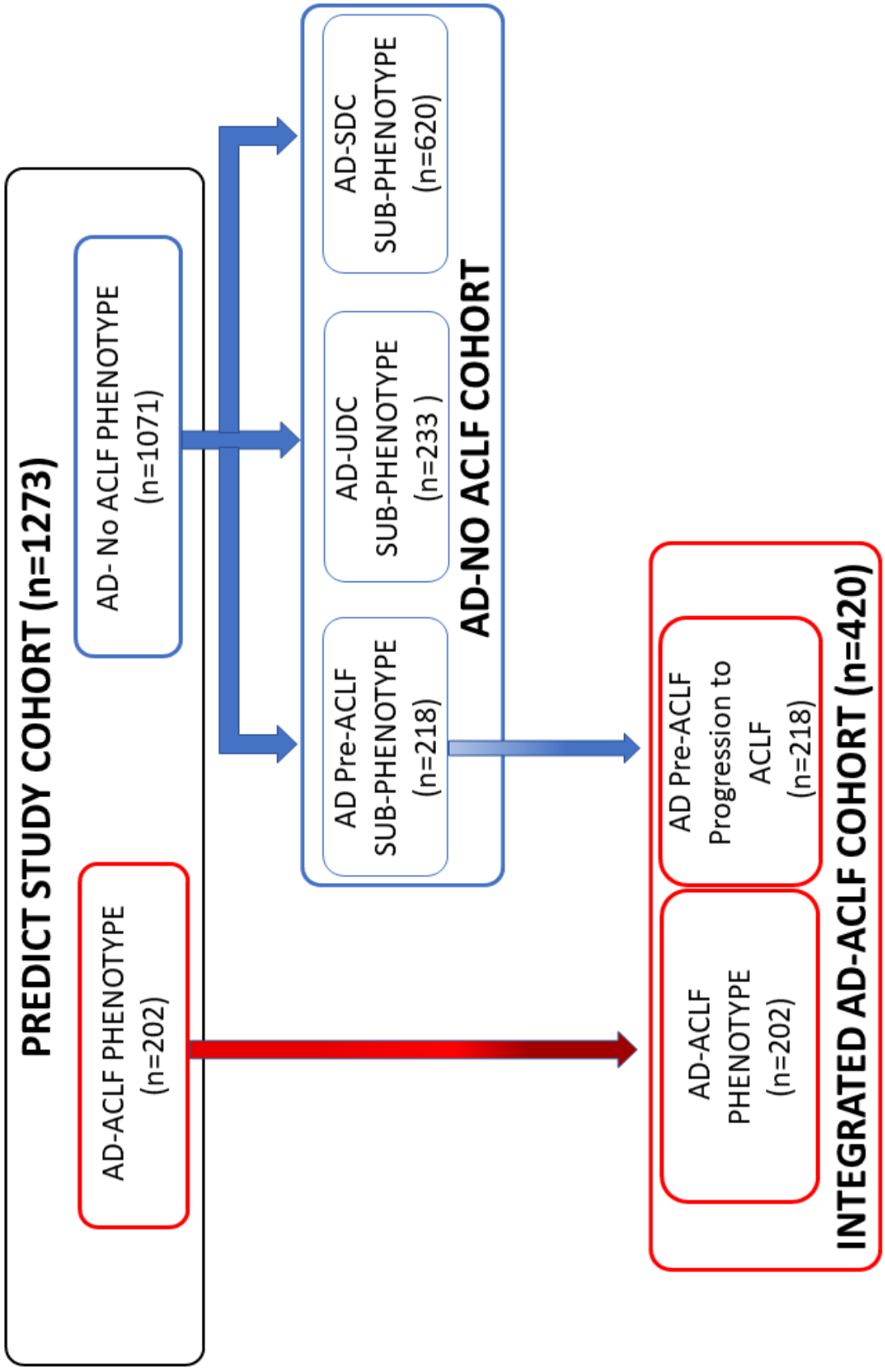

PREDICT STUDY COHORT (n=1273)
AD-ACLF PHENOTYPE (n=202)
AD- No ACLF PHENOTYPE (n=1071)
AD Pre-ACLF SUB-PHENOTYPE (n=218)
AD-UDC SUB-PHENOTYPE (n=233)
AD-SDC SUB-PHENOTYPE (n=620)
AD-NO ACLF COHORT
AD-ACLF PHENOTYPE (n=202)
AD Pre-ACLF Progression to ACLF (n=218)
INTEGRATED AD-ACLF COHORT (n=420)

Figure 1

Figure 2

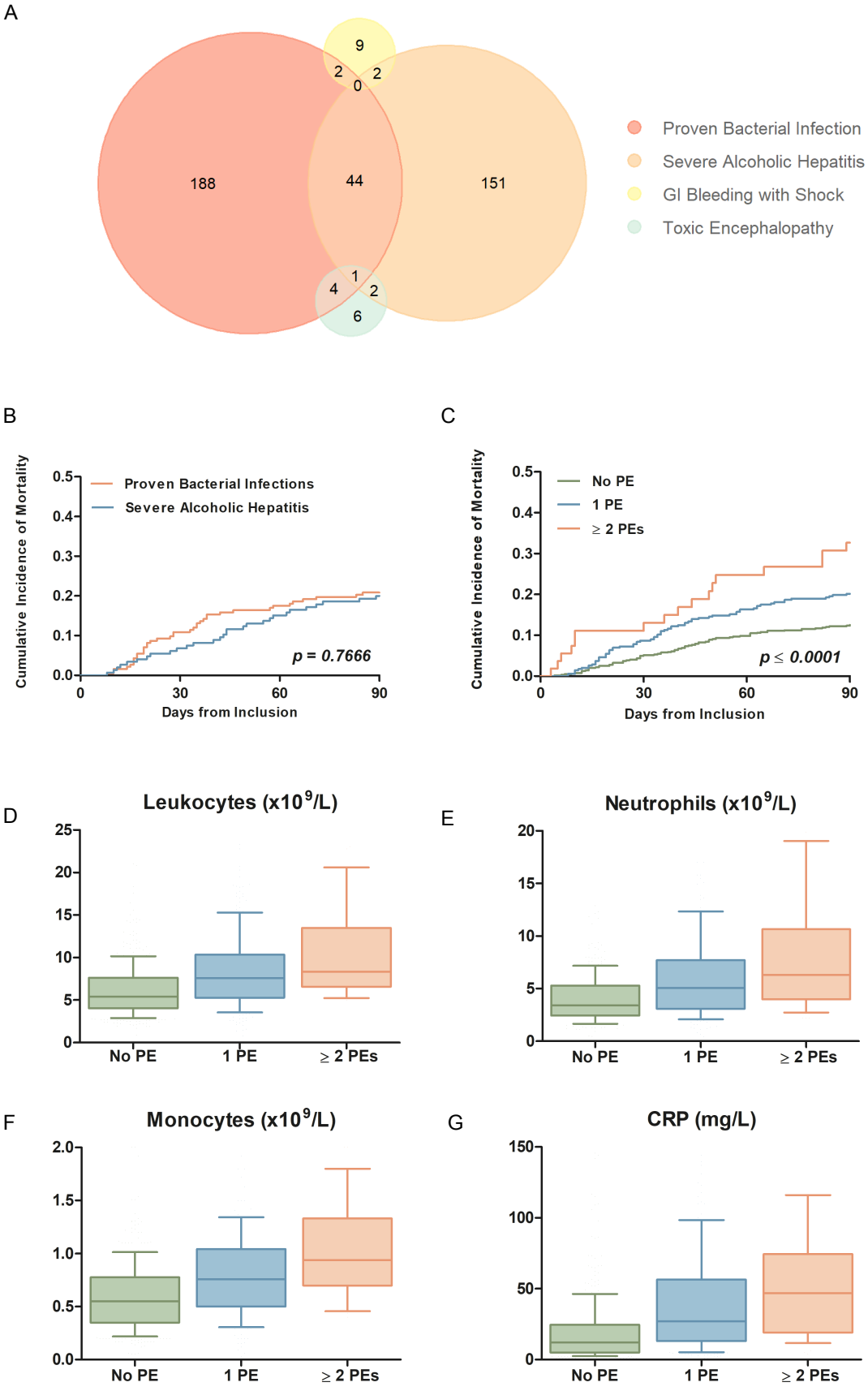
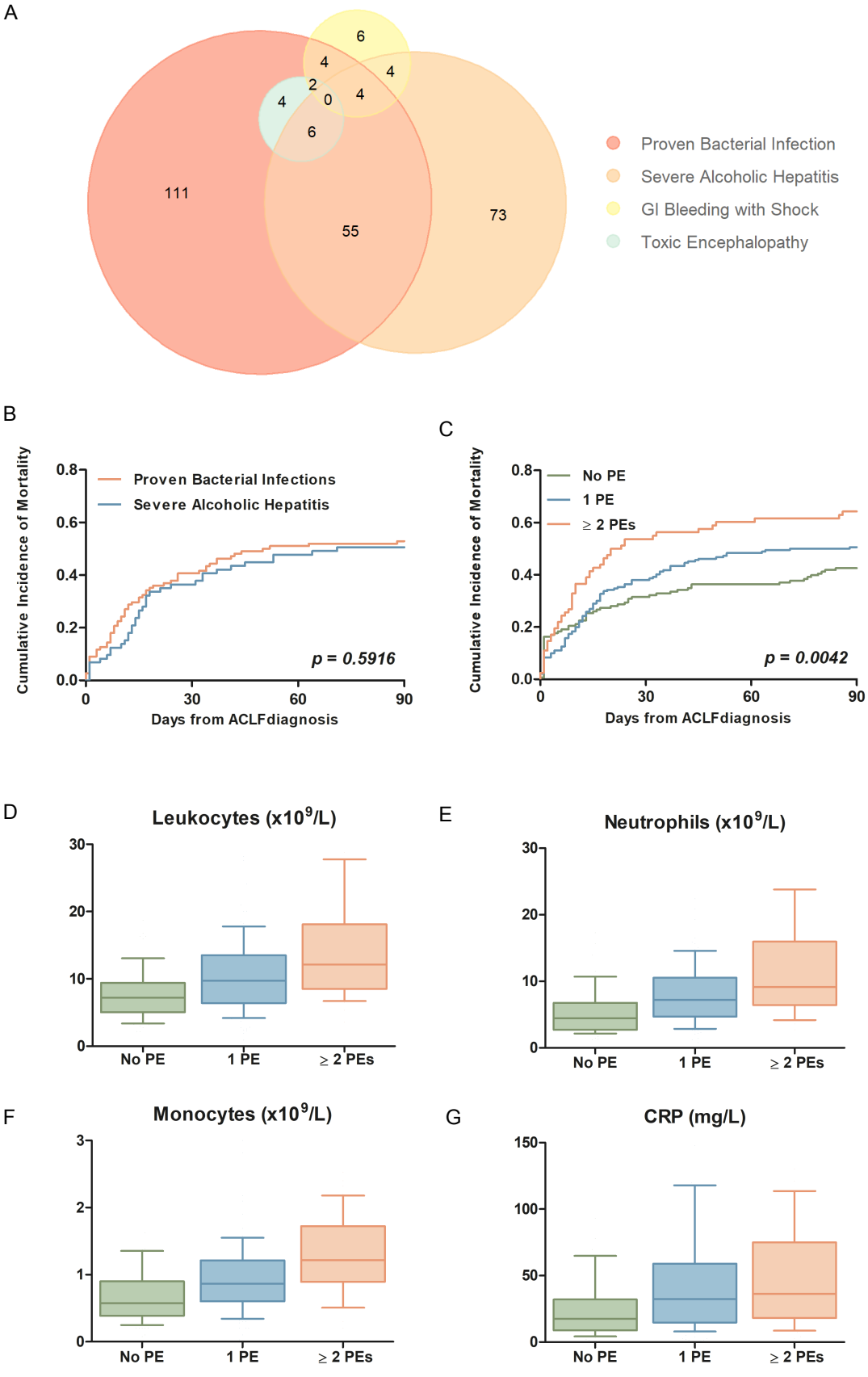

A
9
2
2
0
188
44
151
1
4
2
6
Proven Bacterial Infection
Severe Alcoholic Hepatitis
GI Bleeding with Shock
Toxic Encephalopathy
B
Cumulative Incidence of Mortality
Proven Bacterial Infections
Severe Alcoholic Hepatitis
p = 0.7666
Days from Inclusion
C
Cumulative Incidence of Mortality
No PE
1 PE
≥ 2 PEs
p ≤ 0.0001
Days from Inclusion
D
Leukocytes (x10⁹/L)
No PE
1 PE
≥ 2 PEs
E
Neutrophils (x10⁹/L)
No PE
1 PE
≥ 2 PEs
F
Monocytes (x10⁹/L)
No PE
1 PE
≥ 2 PEs
G
CRP (mg/L)
No PE
1 PE
≥ 2 PEs

Figure 3


A
6
4
4
2
0
4
4
6
111
55
73
Proven Bacterial Infection
Severe Alcoholic Hepatitis
GI Bleeding with Shock
Toxic Encephalopathy
B
Cumulative Incidence of Mortality
Proven Bacterial Infections
Severe Alcoholic Hepatitis
p = 0.5916
Days from ACLFdiagnosis
C
Cumulative Incidence of Mortality
No PE
1 PE
≥ 2 PEs
p = 0.0042
Days from ACLFdiagnosis
D
Leukocytes (x10⁹/L)
No PE
1 PE
≥ 2 PEs
E
Neutrophils (x10⁹/L)
No PE
1 PE
≥ 2 PEs
F
Monocytes (x10⁹/L)
No PE
1 PE
≥ 2 PEs
G
CRP (mg/L)
No PE
1 PE
≥ 2 PEs