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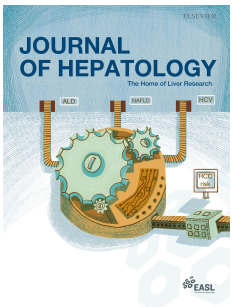

PREDICT identifies precipitating events associated with the clinical course of acutely decompensated cirrhosis

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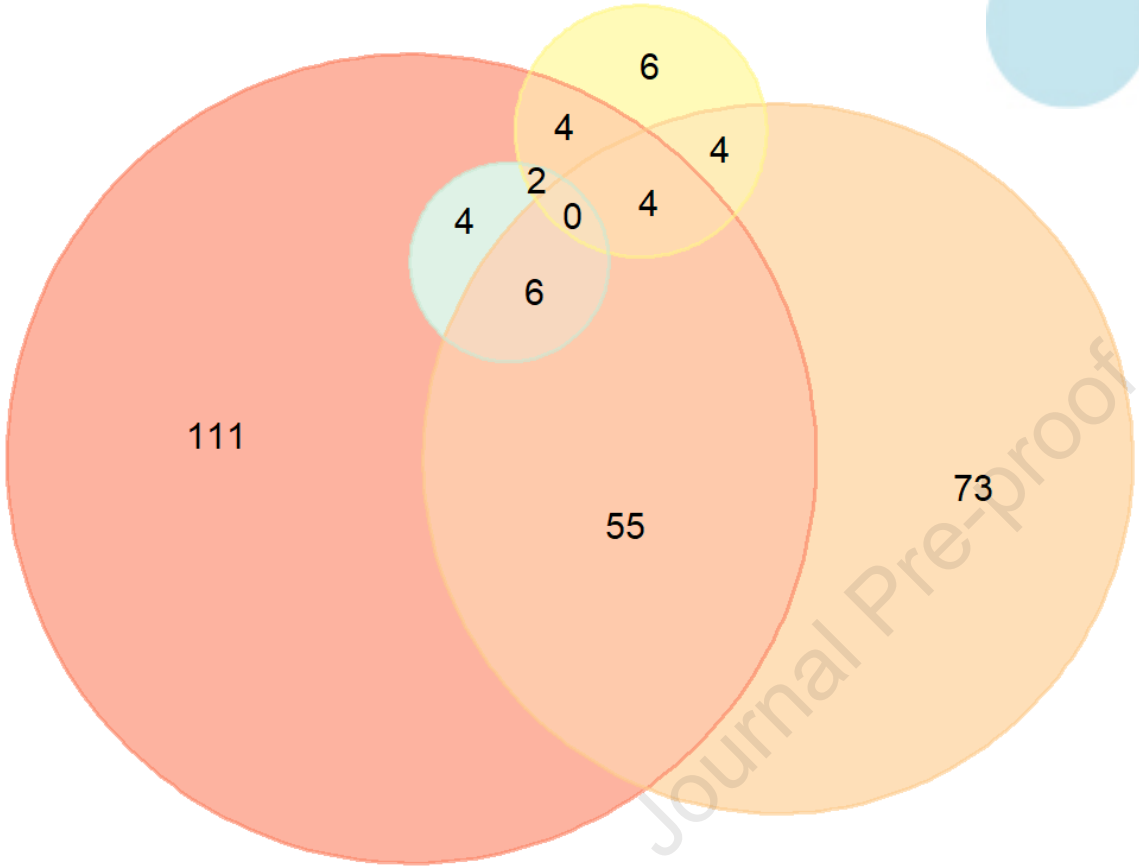
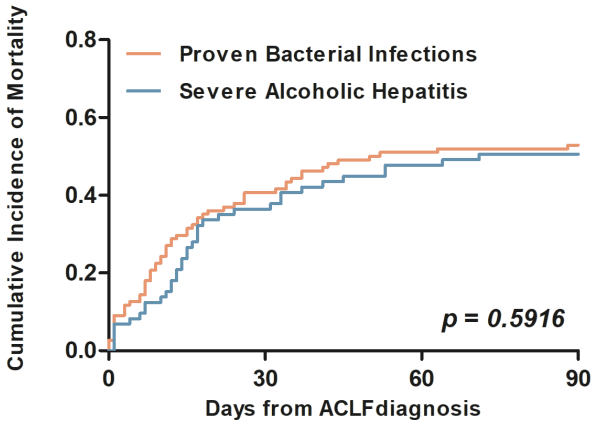


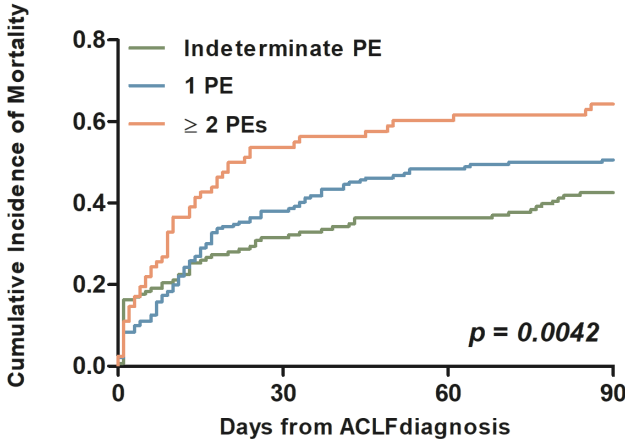
Proven Bacterial Infection

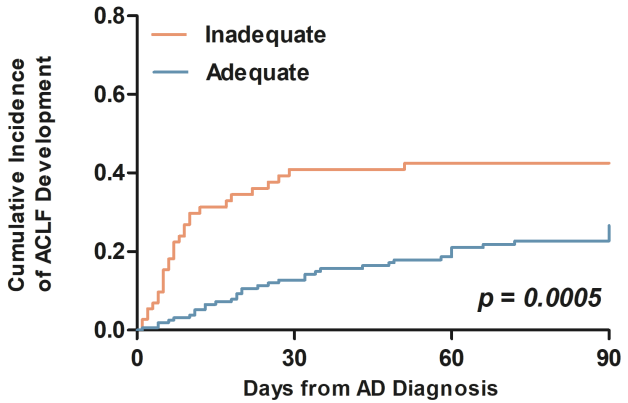
Severe Alcoholic Hepatitis

GI Bleeding with Shock

Toxic Encephalopathy







Title page

PREDICT identifies precipitating events associated with the clinical course of acutely decompensated cirrhosis

Authors:

Jonel Trebicka[1,2], Javier Fernandez[1,4], Maria Papp[5], Paolo Caraceni[6], Wim Laleman[13], Carmine Gambino[7], Ilaria Giovo[8], Frank Erhard Uschner[2], Christian Jansen[3], Cesar Jimenez[9], Rajeshwar Mookerjee[10], Thierry Gustot[11], Agustin Albillos[12], Rafael Bañares[14], Peter Jarcuska[15], Christian Steib[16], Thomas Reiberger[17], Juan Acevedo[18], Pietro Gatti[19], Debbie L. Shawcross[20], Stefan Zeuzem[2], Alexander Zipprich[21], Salvatore Piano[7], Thomas Berg[22], Tony Bruns[23,34], Karen Vagner Danielsen[24], Minneke Coenraad[25], Manuela Merli[26], Rudolf Stauber[27], Heinz Zoller[28], José Presa Ramos[29], Cristina Solé[4], Germán Soriano[30], Andrea de Gottardi[31], Henning Gronbaek[32], Faouzi Saliba[33], Christian Trautwein[34], Haluk Tarik Kani[35], Sven Francque[36], Stephen Ryder[37], Pierre Nahon[38], Manuel Romero-Gomez[39], Hans Van Vlierberghe[40], Claire Francoz[41,42], Michael Manns[43], Elisabet Garcia-Lopez[1], Manuel Tufoni[6], Alex Amoros[1], Marco Pavesi[1], Cristina Sanchez[1], Michael Praktiknjo[3], Anna Curto[1], Carla Pitarch[1], Antonella Putignano[11], Esau Moreno[1], William Bernal[20], Ferran Aguilar[1], Joan Clària[1,4], Paola Ponzo[8], Zsuzsanna Vitalis[5], Giacomo Zaccherini[6], Boglarka Balogh[5], Alexander Gerbes[16], Victor Vargas[9], Carlo Alessandria[8], Mauro Bernardi[6], Pere Ginès[4], Richard Moreau[1,41,42], Paolo Angeli[1,7], **Rajiv Jalan[1,10], and Vicente Arroyo**[1] for the PREDICT STUDY group of the EASL-CLIF CONSORTIUM

Affiliations:

[1]European Foundation for Study of Chronic Liver Failure, EF-Clif, Barcelona, Spain

[2]Department of Internal Medicine I, Goethe University Frankfurt, Frankfurt, Germany

[3] Department of Internal Medicine I, University Hospital Bonn, Bonn, Germany

[4]Hospital Clinic of Barcelona, University of Barcelona, CIBEReHD, IDIBAPS, Barcelona, Spain

[5]University of Debrecen, Faculty of Medicine, Institute of Medicine, Department of Gastroenterology, Debrecen, Hungary

[6]University of Bologna, Bologna, Italy

[7]University of Padova, Padova, Italy

[8]A.O.U. Città della Salute e della Scienza Torino, Torino, Italy

[9]Liver Unit, Hospital Vall d'Hebron, Universitat Autònoma de Barcelona, CIBEREHD, Barcelona, Spain

[10]UCL Medical School,Royal Free Hospital, London, UK

[11]C.U.B. Erasme, Bruxelles, Belgium

[12]Department of Gastroenterology, Hospital Universitario Ramón y Cajal, IRYCIS, University of Alcalá, CIBEREHD, Madrid, Spain

[13] Department of Gastroenterology and Hepatology, Section of Liver and Biliopancreatic disorders, University of Leuven, Leuven, Belgium

[14] Hospital General Universitario Gregorio Marañón. Facultad de Medicina (Universidad Complutense of Madrid), CIBERehd, Madrid, Spain

[15]Pavol Jozef Safarik University in Kosice, Kosice, Slovakia

[16]Department of Medicine II, Liver Centre Munich, University Hospital, LMU, Munich, Germany

[17]Medical University of Vienna, Vienna, Austria

[18]University Hospitals Plymouth NHS Trust, Plymouth, UK

[19]Internal Medicine PO Ostuni, ASL Brindisi, Italy

[20]King's College Hospital, London, UK

[21]University Hospital Halle-Wittenberg, Halle(Saale), Germany

[22]Division of Hepatology, Department of Medicine II, Leipzig University Medical Center, Leipzig, Germany

[23]Jena University Hospital, Jena, Germany

[24]Gastrounit, Medical Section, Hvidovre Hospital and Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

[25]Leiden University Medical Center, Leiden, The Netherlands

[26]Universitá Sapienza Roma, Roma, Italy

[27]Medical University of Graz, Graz, Austria

[28]Medical University of Innsbruck, Innsbruck, Austria

[29]CHTMAD Vila Real-Blueclinical, Vila Real, Portugal

[30]Hospital de la Santa Creu i Sant Pau and CIBERehd, Barcelona, Spain

[31]University Clinic of Visceral Surgery and Medicine-Inselspital, Bern and Ente Ospedaliero Cantonale, Universita della Svizzera Italiana, Lugano, Switzerland

[32]Aarhus University Hospital, Aarhus, Denmark

[33]AP-HP Hôpital Paul Brousse, Centre Hépato-Biliaire, Universite Paris Saclay, INSERM Unit 1193, Villejuif, France

[34]Aachen University Hospital, Aachen, Germany

[35]Marmara University, Kadiköy, Turkey

[36]University Hospital Antwerp, Antwerp, Belgium

[37]NIHR Biomedical Research Centre at Nottingham University Hospitals NHS Trust and the University of Nottingham, Nottingham, UK

[38]AP-HP, Hôpital Jean Verdier, Service d'Hépatologie, Bondy; Université Paris 13, Sorbonne Paris Cité, "Equipe labellisée Ligue Contre le Cancer", Saint-Denis; Inserm, UMR-1162, "Génomique fonctionnelle des tumeurs solides", Paris, France

[39]Virgen del Rocío University Hospital, Sevilla, Spain

[40]Ghent University Hospital, Ghent, Belgium

[41]APHP, Hôpital Beaujon, Service d'Hépatologie, Clichy, France

[42]Inserm, Université de Paris, Centre de Recherche sur L´Inflammation, Paris, France

[43]Hannover Medical School, Hannover, Germany

[44]Munster University Hospital, Münster, Germany

[45]University of Basel-St Gall Cantonal Hospital, Switzerland

[46]Hôpitaux Universitaires de Genève, Genève, Switzerland

[47]University of Birmingham, Birmingham, UK

Authors in bold share either first or last authorship.

Collaborators:

Miriam Maschmeier[1], David Semela[2], Laure Elkrief[3], Ahmed Elsharkawy[4], Tamas Tornai[5], Istvan Tornai[5], Istvan Altorjay[5], Agnese Antognoli[6], Maurizio Baldassarre[6], Martina Gagliardi[6], Eleonora Bertoli[7], Sara Mareso[7], Alessandra Brocca[7], Daniela Campion[8], Giorgio Maria Saracco[8], Martina Rizzo[8], Jennifer Lehmann[9], Alessandra Pohlmann[9], Maximilian J. Brol[9], Johannes Chang[9], Robert Schierwagen[18], Elsa Solà[10], Nesrine Amari[11], Miguel Rodriguez[12], Frederik Nevens[13], Ana Clemente[14], Martin Janicko[15], Daniel Markwardt[16], Mattias Mandorfer[17], Christoph Welsch[18], Tanja M. Welzel[18], Emanuela Ciraci[19], Vish Patel[20], Cristina Ripoll[21], Adam Herber[22], Paul Horn[23], Flemming Bendtsen[24], Lise Lotte Gluud[24], Jelte Schaapman[25], Oliviero Riggio[26], Florian Rainer[27], Jörg Tobiasch Moritz[28], Mónica Mesquita[29], Edilmar Alvarado-Tapias[30], Osagie Akpata[31], Luise Aamann[32], Didier Samuel[33], Sylvie Tresson[33], Pavel Strnad[34], Roland Amathieu[35], Macarena Simón-Talero[36], Francois Smits[11], Natalie van den Ende[13], Javier Martinez[12], Rita Garcia[14], Harald Rupprechter[17], Cornelius Engelmann[22], Osman Cavit Özdogan[37]

Affiliations:

[1]Munster University Hospital, Münster, Germany

[2]University of Basel-St Gall Cantonal Hospital, Switzerland

[3]Hôpitaux Universitaires de Genève, Genève, Switzerland

[4]University of Birmingham, Birmingham, UK

[5]University of Debrecen, Faculty of Medicine, Institute of Medicine, Department of Gastroenterology, Debrecen, Hungary

[6]University of Bologna, Bologna, Italy

[7]University of Padova, Padova, Italy

[8]A.O.U. Città della Salute e della Scienza Torino, Torino, Italy

[9]University Hospital Bonn, Bonn, Germany

[10]Hospital Clinic of Barcelona, Barcelona, Spain

[11]C.U.B. Erasme, Bruxelles, Belgium

[12]Department of Gastroenterology, Hospital Universitario Ramón y Cajal, IRYCIS, University of Alcalá, CIBEREHD, Madrid, Spain

[13]Department of Gastroenterology and Hepatology, Section of Liver and Biliopancreatic disorders, University of Leuven, Leuven, Belgium

[14] Hospital General Universitario Gregorio Marañón. Facultad de Medicina (Universidad Complutense of Madrid), CIBERehd, Madrid, Spain

[15]Pavol Jozef Safarik University in Kosice, Kosice, Slovakia

[16]Department of Medicine II, Liver Centre Munich, University Hospital, LMU, Munich, Germany

[17]Medical University of Vienna, Vienna, Austria

[18]Department of Internal Medicine I, Goethe University Frankfurt, Frankfurt Germany

[19]Internal Medicine PO Ostuni, ASL Brindisi, Italy

[20]King's College Hospital, London, UK

[21]University Hospital Halle-Wittenberg, Halle(Saale), Germany

[22]Division of Hepatology, Department of Medicine II, Leipzig University Medical Center, Leipzig, Germany

[23]Jena University Hospital, Jena, Germany

[24]Gastrounit, Medical Section, Hvidovre Hospital and Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

[25]Leiden University Medical Center, Leiden, The Netherlands

[26]Universitá Sapienza Roma, Roma, Italy

[27]Medical University of Graz, Graz, Austria

[28]Medical University of Innsbruck, Innsbruck, Austria

[29]CHTMAD Vila Real-Blueclinical, Vila Real, Portugal

[30]Hospital de la Santa Creu i Sant Pau and CIBERehd, Barcelona, Spain

[31]UCL Medical School,Royal Free Hospital, London, UK

[32]Aarhus University Hospital, Aarhus, Denmark

[33]AP-HP Hôpital Paul Brousse, Centre Hépato-Biliaire, Universite Paris Saclay, INSERM Unit 1193, Villejuif, France

[34]Aachen University Hospital, Aachen, Germany

[35]AP-HP, Hôpital Jean Verdier, Service d'Hépatologie, Bondy; Université Paris 13, Sorbonne Paris Cité, "Equipe labellisée Ligue Contre le Cancer", Saint-Denis; Inserm, UMR-1162, "Génomique fonctionnelle des tumeurs solides", Paris, France

[36] Liver Unit, Hospital Vall d'Hebron, Universitat Autònoma de Barcelona, CIBEREHD, Barcelona, Spain

[37]Marmara University, Kadiköy, Turkey

Short title: PRECIPITANTS OF ACUTE DECOMPENSATION AND ACLF

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Keywords: chronic liver disease, non-elective admission, acute complications, outcome, risk factors.

Data availability: While some of the data of this paper will be available upon request, the majority of the data are not suitable for posting as some of it is confidential.

Corresponding author: Professor Dr. med. Jonel Trebicka, MD, PhD, European Foundation for Study of Chronic Liver Failure, EF-Clif, Travesera de Gracia 11, 7th Floor, 08021 Barcelona, Spain

Abbreviations: 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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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, DLS, AA, WL, ES, RB, MJ, CS, TR, JA, PG, WB, SZ, CR, TB, AS, KVD, 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

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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). Precipitants may induce AD. This multicenter, prospective, observational PREDICT study (NCT03056612) analyzes and characterizes the precipitants leading to both of these 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 collected at enrolment and during 90-day follow up, with particular attention to the following characteristics of precipitants: induction of organ dysfunction or failure, systemic inflammation, chronology, intensity, and relationship to outcome.

Results: Among various clinical events, four distinct events were precipitants consistently related to AD, including proven bacterial infections, severe alcoholic hepatitis, gastrointestinal (GI) bleeding with shock and toxic encephalopathy. Among patients with precipitants in the AD-No ACLF cohort and the AD-ACLF cohort (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 events. In both AD phenotypes, patients with proven bacterial infections or severe alcoholic hepatitis had a similar survival. The number of precipitants was associated with significantly increased 90-day mortality, and was paralleled by increasing levels of surrogates for systemic inflammation. Importantly, adequate first-line antibiotic treatment of proven bacterial infections was associated with lower ACLF development rate and lower 90-day mortality.

Conclusions: This study identified precipitants that are significantly associated with a distinct clinical course and prognosis of patients with AD and specific preventive and therapeutic strategies targeting these events may improve outcome in decompensated cirrhosis.

word count (max. 275): 266

Layman summary

Acute decompensation (AD) of cirrhosis is a rapid deterioration of the health condition. The maximal form is acute-on-chronic liver failure (ACLF), organ failures and high short-term mortality. This multicenter, prospective, observational PREDICT study analyzes and characterizes the precipitants leading to AD. The PREDICT study included 1273 patients with AD and focused on the characteristics of precipitants, specifically induction of organ dysfunction/failure and/or systemic inflammation, chronology, intensity, and relationship to outcome in both AD phenotypes (No-ACLF and ACLF). In particular, proven bacterial infections and severe alcoholic hepatitis, either alone or in combination, accounted for almost all (96-97%) acute decompensation and ACLF. Whilst type of the precipitants was not associated with mortality, the number was. This study identified precipitants that are significantly associated with a distinct clinical course and prognosis of patients with AD. Specific preventative and therapeutic strategies targeting these events may improve outcome in decompensated cirrhosis.

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, a prospective observational investigation of 1273 hospitalized patients with AD, showed that patients without ACLF (AD-No ACLF phenotype) comprised three distinct sub-phenotypes defined according to ACLF development and readmission within three 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 (35%); 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 precipitants. 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 peak 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]. Hence, for a precipitant to be of importance, it must have the ability to impair end-organ function.

Despite the fact that AD-ACLF phenotypes frequently develop in close chronological relationship with precipitant, the critical time period prior to AD-ACLF has not yet been explored in detail. Moreover, no specific criteria for the diagnosis of precipitants have been identified to date. Consequently, many clinically relevant aspects of precipitants remain ill-defined.

The current study is the second investigation derived from the PREDICT study. Its aim was to provide the rationale for the diagnosis of precipitants and to investigate the association of type and number of precipitants with 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 (approved by the respective ethics committees) 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 cirrhosis 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 after providing their informed consent. AD was diagnosed as previously described [2] and ACLF according to the EASL-CLIF criteria [1, 7]. Stratification of patients with the AD-No ACLF phenotype into the AD-pre-ACLF, AD-UDC and AD-SDC sub-phenotypes was performed using previously described criteria [2] and outlined in **Fig. 1 (for detailed description please see supplementary appendix)**.

Study Design

The PREDICT study [2] was designed to explore the last 90 days prior to hospital admission (especially the last two weeks), and the first three months after admission (follow-up period), in which the early clinical course of patients 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 in the supplementary appendix and elsewhere [2].

Identification of precipitants of AD-No ACLF and AD-ACLF

PRECIPITANTS OF ACUTE DECOMPENSATION AND ACLF

In order to identify the precipitants an adjudication committee of the PREDICT study, which included JT, JF, RM and VA, was nominated to elaborate a list of clinical events with the potential to precipitate AD or ACLF, and also the general principles and specific criteria for diagnosis. This committee identified precipitants according to the criteria defined below.

General principles for precipitants identification

1. Precipitants 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 precipitants, 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 precipitant.
3. As suggested by the results of the CANONIC study [1, 7], clinically identifiable, relevant and true precipitants should have a higher prevalence in patients with AD-ACLF than in those with AD-no ACLF.
4. Precipitants should precede or coincide with the onset of AD-ACLF. The time period between the precipitants and the onset of AD-ACLF, however, is heterogeneous, depending on the precipitants.
5. Any event developing after the onset of AD-ACLF is a complication or a coincidental event but not a precipitant.

Specific criteria for the identification of precipitants from the list proposed by the adjudication committee **(for detailed description see supplementary appendix).**

PRECIPITANTS OF ACUTE DECOMPENSATION AND ACLF

The adjudication committee evaluated the following events as potential precipitants as proposed by the CANONIC study and other investigations: bacterial infections, alcoholic hepatitis, GI bleeding, drug-induced organ injury, therapeutic interventions.

Bacterial infections (details in supplementary appendix). Infections were considered to be potential precipitants if they were diagnosed at the time of or solved within the 48-hour period that preceded the onset of AD. Proven bacterial infections were defined as previously described [9] and in accordance with the EASL guidelines [7] **(detailed definition in supplementary appendix)**.

Alcohol-related liver injury (details in supplementary appendix). Alcoholic hepatitis was diagnosed according to the clinical criteria of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) [10]. These criteria are in line with the clinical diagnosis of alcoholic hepatitis according to the existing EASL guidelines [11]. Alcoholic hepatitis was considered severe if patients showed CLIF-Consortium AD score ≥ 50 points [12], or presence of ACLF (**Table 1**).

Gastrointestinal bleeding (details in supplementary appendix). Gastrointestinal bleeding was considered a precipitant if occurring within seven days prior to the onset of AD-ACLF. Moreover, hemorrhagic shock was indicative of severe bleeding (**Table.1**).

Drug-induced organ injury (details in supplementary appendix).

1. *Drug-induced liver injury* was considered a potential precipitant when the hepatotoxic drug was administered within one month prior to the onset of AD-ACLF and the patient presented with liver injury as defined by Hy´s law and FDA guidance as described in the recent EASL guidelines [13]; as well as liver dysfunction (in patients with AD-No ACLF, bilirubin > 6 mg/dl) or liver failure (in patients with AD-ACLF, bilirubin > 12 mg/dl). Only drugs from groups A and B of potential hepatotoxic drugs, described elsewhere [14], were considered potential candidates for liver toxicity.

2. *Drug-induced kidney injury* was considered a potential precipitant when the nephrotoxic drug was administered within seven 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 a nephrotoxic condition.

3. *Toxic encephalopathy* was considered a potential precipitant when the neurotoxic drug was administered within 48 hours prior to the onset of AD-ACLF and the patient presented with encephalopathy in severity similar to brain dysfunction or brain failure according to the CLIF-C OF score.

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

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

The adjudication committee assessed nine additional, infrequent clinical events **(details in supplementary appendix)**.

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's exact test, when at least 25% of expected counts were below 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 three categories. In all statistical analyses, significance was set at $p<0.05$.

Overall, the proportion of missing values in the main reported characteristics (demographics, clinical variables, laboratory values, precipitants and clinical outcomes) rounded 1% at most. Only complete clinical blood counts and total cholesterol showed higher proportions of missing data, which were mainly due to common problems with sample availability or with technical laboratory processes that occurred in several site laboratories and can be considered to be completely random. A simple imputation approach was used to impute the missing values for each of the four variables mentioned above (neutrophil, lymphocyte and monocyte counts and total cholesterol). SAS PROC MI was used assuming an arbitrary pattern for missing values and adopting a fully conditional specification (FCS) regression method. Model covariates included age, sex, CLIF-C OF score and number of PE's or presence of bacterial infections or alcoholism or ACLF at inclusion, depending on the analysis that was to be performed. For each variable, missing values were imputed by computing the median of the values obtained by fitting the model on 100 repetitions generated from the original dataset.

Cumulative Incidence Functions (CIF's) were used to estimate survival curves accounting for liver transplantation as an event 'competing' with mortality, as well as to estimate ACLF development accounting for both mortality and liver transplantation as events "competing" with ACLF development, using common non-parametric methods. The equality of CIFs across groups was evaluated by means of the Gray's test [15]. Statistical analysis was performed using SAS v9.4 and plots were performed with R 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, of whom 202 patients presented 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 was significantly higher in AD-ACLF than in AD-No ACLF cases, while prevalence of suspected bacterial infections was very low and similar in both groups. Therefore, only proven bacterial infections were considered as precipitants of AD-ACLF, and this was the most common precipitant (44% in AD-ACLF and in 22.3% in AD-No ACLF (P<0.0001)).

Prevalence 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% vs. 18.7% in AD-No ACLF). Overall, alcoholic hepatitis was not always associated with organ dysfunction. Therefore, only severe alcoholic hepatitis was identified as a precipitant, and was the second most frequent.

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

Finally, of the three examined types of drug-induced organ injury, 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 it thus qualified as precipitant. All drugs associated with severe toxic encephalopathy were opioids or benzodiazepines.

Neither therapeutic paracentesis without intravenous albumin nor TIPS qualified as

precipitants, since their prevalence was not significantly higher in patients with AD-ACLF than in patients with AC-no ACLF.

In total, 721 patients (56.6%) included in the PREDICT study cohort did not present any identifiable precipitant (indeterminate precipitant), 447 (35.1%) presented one precipitant, and 105 (8.2%) presented two or more precipitants.

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 association of precipitants with characteristics, clinical course and prognosis of patients included in the AD-No ACLF cohort (N=1071)

Prevalence of precipitants and their combinations

AD-No ACLF was associated with one precipitant in 354 patients (33.0%), and with two or more precipitants in 55 patients (5.1%), as illustrated in **Fig. 2A.** In the AD-No ACLF cohort, 662 patients (61.8%) presented with indeterminate precipitants (**Table 1**). Therefore, in 394 patients (96.3%), AD-No ACLF was related to proven bacterial infections or severe acute alcoholic hepatitis, either alone or in combination. In only 15 (3.7%) patients, AD-No ACLF was unrelated to bacterial infections or alcoholic hepatitis.

Precipitants are associated with 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 with indeterminate precipitants was significantly lower (50.9%) and the number of patients with one or two or more precipitants was 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 were even more pronounced, when UDC or SDC groups at baseline were compared with the AD-pre-ACLF group at the time point of ACLF development. These observations suggest that the presence and the number of precipitants at enrolment are important determinants in the development of AD-pre-ACLF, the most severe sub-phenotype in patients with AD-No ACLF (**Table 2**).

Interestingly, patients with a single precipitant of the two major groups of precipitants (proven bacterial infection and severe alcoholic hepatitis) showed a comparable 90-day mortality (**Fig. 2B**). This is the case, despite the significant differences in clinical and laboratory parameters between patients with either proven bacterial infection or severe alcoholic hepatitis as sole precipitant (**Table S2**), indicating that the type of precipitant is not crucial for outcome, if correctly defined.

As shown in Figure 2C, 90-day mortality was highest in patients with two or more precipitants and lowest in patients without any identifiable precipitant (**Fig. 2C**). In parallel, levels of leukocytes, neutrophils, monocytes and CRP (**Fig. 2D-G**), organ dysfunction and failures and overall scores increased with the number of precipitants (**Table S3**).

Results derived from the Integrated ACLF cohort (n=420)

This integrated cohort included 202 patients with AD-ACLF at the time of enrolment (AD-ACLF group) and 218 patients in AD-pre-ACLF group who developed AD-ACLF during the study and who were included at the time of development of ACLF (**Fig. 1**). The integrated AD-ACLF cohort was developed with two objectives: 1) a further characterization of the AD-ACLF phenotype in patients with community-acquired and hospital-acquired ACLF; and 2) an analysis of precipitants in sufficiently sized AD-ACLF

cohort.

Prevalence of precipitants and their combinations

Of the 420 patients included in the integrated AD-ACLF cohort, AD-ACLF was triggered by one precipitant in 191 patients (45.5%), and by or two or more precipitants in 82 patients (19.5%), while precipitant was indeterminate in 147 patients (35.0%) (**Table S4**). **Fig. 3A** shows the different combinations of PEs in the Integrated AD-ACLF cohort. Similarly to AD-No ACLF, 266 (97.4%) of patients with identifiable precipitants had proven bacterial infections or severe acute alcoholic hepatitis as either a single or as combined precipitants.

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

Similarly to AD-No ACLF patients (**Table S2)**, also in patients with AD-ACLF showed differences (among others higher bilirubin but lower CRP values in severe alcoholic hepatitis) between patients with proven bacterial infections or severe alcoholic hepatitis as single precipitant (**Table S5**). Similar to AD-No ACLF patients, these differences did not impact the clinical course and prognosis, as shown in **Fig. 3B**.

Number of precipitants is significantly associated with the clinical course and mortality of patients with AD-ACLF.

The number of precipitants in patients included in the integrated AD-ACLF cohort (indeterminate, one PE, and two or three precipitants) 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 a distinct number of precipitants. The predominant organ failure in patients with an indeterminate precipitant or with only one precipitant was renal failure. By contrast, liver failure was the predominant organ failure in patients with two or three precipitants. Moreover, the prevalence of other organ failures was also higher in patients with two or three precipitants. Consistent with these results, the number of precipitants at diagnosis also correlated directly with the grade of severity of ACLF (I, II or III), the severity of prognostic scores, the need for intensive care, the frequency of treatment with mechanical ventilation or renal replacement therapy, and the 90-day cumulative incidence of mortality (**Table 3**, **Fig. 3C).** Systemic inflammation, as estimated by the WBC and blood levels of neutrophils and monocytes, increased in parallel with the number of precipitants (**Table 3**, **Fig. 3D-G**). Serum levels of CRP were also significantly higher in patients with one or two or more precipitants than in patients with indeterminate precipitants.

Role of treatment of precipitant in prevention of AD-ACLF and improvement of survival

*Proven bacterial infections (***details in supplementary appendix***)*

A total of 376 patients (29.5%) developed 440 bacterial infections, of which 66.2% were culture-positive. Nosocomial episodes and severe sepsis or shock predominated in infections diagnosed as a precipitant of ACLF during follow-up, while multi-drug resistant (MDR) strains were involved in 18.9% of all infections and 29.4% of culture-positive episodes. Also, prevalence of infections caused by MDR strains was significantly higher in infections precipitating ACLF during follow-up and in those causing severe sepsis/shock (**Table S6**). Overall, resolution of infection was significantly lower in episodes caused by MDR bacteria (57.8% vs. 82.1%, P<0.0001). The lower resolution rate of MDR-infections

was associated with higher 28-day and 90-day mortality in patients with AD-ACLF, but not in infections precipitating AD (**Table S7**).

Classic antibiotic strategies were used frequently in community-acquired and health-care associated infections as first-line therapy (**Table 4**). In contrast, nosocomial episodes were more frequently treated with piperacillin-tazobactam (20.4%) or with broader MDR-covering strategies (38.8%). Remarkably, a significant percentage of patients with severe sepsis/shock still received classic schemes not covering MDR strains (40.5%). Empirical MDR-covering strategies were more effective in infection resolution (with regard to clinical response and microbiological susceptibility) than classic schemes (**Table 4, Table S8)**. Adequacy of empirical antibiotic therapy was defined as resolution of infection without further escalation or bacterial susceptibility to initial antibiotics in culture positive infections. Importantly, adequacy of first-line antibiotic strategies decreased the cumulative incidence of developing ACLF in patients with AD (21.3% vs 39.2%, **Fig. 4A**) and 90-day mortality in both AD (16.9% vs. 36.5%, **Fig. 4B**) and ACLF patients (44.2% vs. 66.2%, **Fig. 4C**).

Severe alcoholic hepatitis

Steroids were administered in 49 patients with severe alcoholic hepatitis (18.9%), 30 patients with AD and 19 patients with ACLF at inclusion. The 28-day and 90-day mortality rates were not significantly different between patients receiving or not receiving steroids, neither in the whole population nor in patients with AD or ACLF at inclusion (**Table S9**).

DISCUSSION

The PREDICT study offers a comprehensive investigation characterizing the precipitants of AD and demonstrating their impact on the development of AD-ACLF and prognosis of cirrhosis patients.

The CANONIC study characterized the AD-ACLF and attributed an important role to precipitants in its development. The PREDICT study, designed to assess the period prior to ACLF [2], identified three different clinical courses in AD-No ACLF: pre-ACLF, AD-UDC and AD-SDC. Moreover, the PREDICT study assessed how type and number of precipitants influence the clinical course and the prognosis in patients with both AD-No ACLF and AD-ACLF. This prospective and detailed characterization offers diagnostic criteria for precipitants and rationalizes the identification of precipitants in patients with cirrhosis and AD. The criteria used for the diagnosis of precipitants considered the severity of the precipitant, the time interval between onset/resolution of the precipitant and onset of the AD episode, and their higher prevalence in patients with AD-ACLF than in patients with AD-No ACLF, which are more objective than the traditional principles of chronology and potential of organ injury.

Among the events recorded and evaluated in the PREDICT study, only four fulfilled the properties of precipitants (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 did not induce organ impairment (TIPS even improves survival in GI bleeding and ACLF [21, 22]), the prevalence of drug-induced liver or renal injury and of other potential precipitants proposed by the investigators was extremely low, frequently below 1%, suggesting that they could be coincidental rather than precipitants.

Proven bacterial infections and severe alcoholic hepatitis were by far the most

prevalent precipitants observed in the PREDICT study. Prevalence of GI bleeding associated with shock and toxic encephalopathy was considerably lower in both groups. In patients with AD-No ACLF, the prevalence of proven bacterial infections or severe alcoholic hepatitis and the number of precipitants present at enrolment were higher in patients with AD-Pre ACLF than in patients with AD-UDC and AD-SDC. In contrast, no differences were found in the prevalence of these precipitants between patients with UDC or SDC. These findings suggest that precipitants 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. Importantly, in >60% of the patients in the AD-No ACLF cohort, precipitating events were indetermined at enrolment, while this was the case in only 35% of the patients with AD-ACLF. These data suggest that AD-No ACLF develops more frequently in the context of endogenous mechanisms (e.g. progressive liver disease, bacterial translocation), confirming the CANONIC study and underlining the solidity of the PREDICT study.

The type of precipitant was associated with different clinical characteristics, but a similar clinical course and mortality. This finding is not surprising, since reactivation or superimposed hepatitis, also showed different prevalences of specific organ failures in AD-ACLF, but similar out come as AD-ACLF triggered by extra-hepatic precipitants (e.g GI-bleeding) [23]. The explanation may be due to the sequence of mechanisms. 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. Importantly, systemic inflammation aggravates and leads to an identical syndrome through distinct pathophysiological pathways. For this reason, the criterion of severity (either systemic

inflammation or organ injury) of the event is crucial to identify the precipitant.

Finally, our results show that the number of precipitants was an important determinant for the characteristics, the clinical course severity and the 90-day cumulative incidence of mortality. Not only that multiple (two or more) precipitants trigger AD-ACLF (one in five patients) and is exceptional (one in 20 patients) in AD-No ACLF, but also the intensity of systemic inflammation, the prevalence of organ failures, the need of for organ support, and the prognostic scores increased progressively from patients with indetermined precipitants to patients with one and multiple precipitants. Therefore, when precipitants are defined according to these criteria, they are synergistic and additive in the worsening of outcome, despite different clinical characteristics.

Almost all (>96%) patients with precipitants showed proven bacterial infection and/or severe alcoholic hepatitis, either alone or in combination with other precipitants. This overwhelming prevalence of proven bacterial infections and/or severe alcoholic hepatitis as precipitants suggests that diagnosing, preventing and treating these precipitants is paramount to improve the prognosis in decompensated cirrhosis.

PREDICT demonstrates that proven bacterial infections requires specific and adequate treatment for prevention of AD-ACLF. This is of particular importance since MDR may challenge empirical treatments. The overall prevalence of MDR bacterial infections in PREDICT was in line with that reported in recently published multicenter investigations [24, 25] and MDR bacterial infections were more severe (higher rate of severe sepsis/shock and of ACLF), associated with a lower resolution rate and higher 28-day and 90-day mortality [24-27], underlining the importance of treatment of precipitants, thus confirming that definition and selection of precipitants has been chosen adequately. Importantly, classic antibiotics (1^{st}-3^{rd} generation cephalosporins, quinolones) have an unacceptable efficacy (<40%) in nosocomial infections, or in those with severe sepsis or

shock. These findings support the current recommendations on the use of empirical broad schemes, according to specific epidemiological pattern of antibiotic resistance [28-30], in the nosocomial setting and in severe sepsis/shock with rapid de-escalation strategies [13].

In summary, of the clinical events explored as potential precipitants 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 precipitants. Proven bacterial infections and severe alcoholic hepatitis were present in the absolute majority (>96%) of patients. However, no precipitating event could be identified in 2/3 of AD-No ACLF patients and in 1/3 AD-ACLF patients. The prevalence and number of precipitants 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 precipitants are significantly associated with the clinical course and prognosis of patients with AD and specific preventive and therapeutic strategies for these precipitants are required to improve outcomes in decompensated cirrhosis.

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LEGEND OF FIGURES

Figure 1. Schematic outline of the study. 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. Precipitants in AD-No ACLF. Combinations of precipitants (PE) in the AD-No ACLF cohort shown in a four-set circle Venn diagram (panel A). Cumulative incidence of mortality in patients with AD-No ACLF according to the type of precipitant (proven infections alone versus severe alcoholic hepatitis alone; panel B) and the number of precipitants (indeterminate 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, 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. Precipitants in AD-ACLF. Combinations of PEs in the integrated AD-ACLF cohort shown in a four-set circle Venn 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 (indeterminate 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-ACLF and indeterminate PE, 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 4. Treatment of bacterial infections. Prognostic impact of inappropriate empirical antibiotic therapy in patients with AD and ACLF. Probability of ACLF at day 90 in infected patients with AD receiving adequate or inadequate empirical antibiotic strategies (panel A). Probability of death at day 90 in patients with acute decompensation (AD; panel B) and in ACLF patients (panel C). Inadequacy of empirical strategies significantly increased the probability of ACLF and death in the different populations. P-values were obtained from Gray's test.

TABLES

Table 1. Clinical events, precipitants (PEs) and 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]
Clinical events, PEs *n (%)*			
Bacterial infections			
Any infection	314 (29.32)	101 (50.00)	<.0001
Suspected bacterial Infection	74 (6.91)	12 (5.94)	0.61
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)	0.24
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)	0.78
Toxic encephalopathy[b]	13 (1.21)	12 (5.94)	<.0001
Other candidates *n (%)*			
Paracentesis without albumin	110 (10.28)	21 (10.40)	0.96
TIPS	49 (4.58)	8 (3.96)	0.69
Drug-induced liver injury	16 (1.49)	4 (1.98)	0.54
Viral hepatitis or other viral Infections	13 (1.21)	3 (1.49)	0.72
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	662 (61.81)	59 (29.21)	<.0001
One PE	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 precipitants are those considered as precipitants of AD-ACLF
Chi-square or Fisher's tests performed in percentages comparisons.

Table 2. Type and number of precipitants (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	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) ##

Comparison between all groups to the pre-ACLF group at enrolment is displayed by the following symbols:
+ $p < 0.07$, * $p < 0.05$ and ** $p < 0.01$ versus the pre-ACLF group at enrolment.

Comparison between all groups to the pre-ACLF group at ACLF development is displayed by the following symbols:
# $p < 0.001$ and ## $p < 0.0001$ versus pre-ACLF group at ACLF development.

Chi-square or Fisher's tests performed in percentages comparisons among groups.
McNemar test used in paired comparisons for the types of PEs between the two time points in pre-ACLF group
Symmetry test used in paired comparisons for the number of PEs between the two time-points in pre-ACLF group
$ p value not determined due to the low number of patients

Table 3. 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 according to the number of precipitants

	Indeterminate PE (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)	0.36
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
GI 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 blood 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	4.23 (2.25 - 6.85)	7.32 (4.60 - 10.45)[b]	9.56 (6.44 - 15.50)[a]	<.0001
Lymphocyte count, $x10^9$/L	0.85 (0.65 - 1.40)	0.94 (0.56 - 1.56)	1.20 (0.73 - 1.97)[b]	0.0794
Monocyte count, $x10^9$/L	0.60 (0.40 - 0.92)	0.92 (0.65 - 1.22)[b]	1.32 (0.95 - 1.77)[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	0.45
Total cholesterol, mg/dL, median (IQR)	86.70 (57.75 - 123.80)	70.50 (48.50 - 104.00)[b]	64.50 (42.00 - 83.50)[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	0.70
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)	0.22
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 indeterminate PE; [c] p<0.05 versus 1 PE. Chi-square or Fisher's 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.

PRECIPITANTS OF ACUTE DECOMPENSATION AND ACLF

Table 4. Adequacy of initial antibiotic strategies according to clinical criteria (resolution of infection without further escalation or bacterial susceptibility to initial antibiotics in culture positive infections) and the microbiological criterion (bacterial susceptibility to initial antibiotics in culture positive infections) in the whole series of proven infections and in infections precipitating AD and ACLF, according to empirical antibiotic strategies.

Type of empirical antibiotic strategies		Total	Classic*	Piperacillin-tazobactam	MDR Coverage**	*p*-value
Number of all proven bacterial infections		**440**	**273**	**70**	**92**	
Adequacy	Resolution of infection without further escalation or bacterial susceptibility to initial antibiotics in culture positive infections **(%)**	62.5	54.2	68.6	82.6	<.0001
	Bacterial susceptibility to initial antibiotics in culture positive infections **(%)**	61.6	48.3	70.7	93.4	<.0001
Number of proven bacterial infections precipitating AD		**265**	**187**	**34**	**39**	
Adequacy	Resolution of infection without further escalation or bacterial susceptibility to initial antibiotics in culture positive infections **(%)**	68.1	62.0	73.5	92.3	0.0008
	Bacterial susceptibility to initial antibiotics in culture positive infections **(%)**	63.9	54.4	75.0	100.0	<.0001
Number of proven bacterial infections precipitating ACLF		**175**	**86**	**36**	**53**	
Adequacy	Resolution of infection without further escalation or bacterial susceptibility to initial antibiotics in culture positive infections **(%)**	54.3	37.2	63.9	75.5	<.0001
	Bacterial susceptibility to initial antibiotics in culture positive infections **(%)**	58.5	36.7	66.7	89.2	<.0001

*One to third generation cephalosporins, amoxicillin-clavulanic acid, quinolones; **carbapenem±glycopeptide/linezolid/daptomycin or tigecycline; Chi-square or Fisher's tests used to compare percentages.

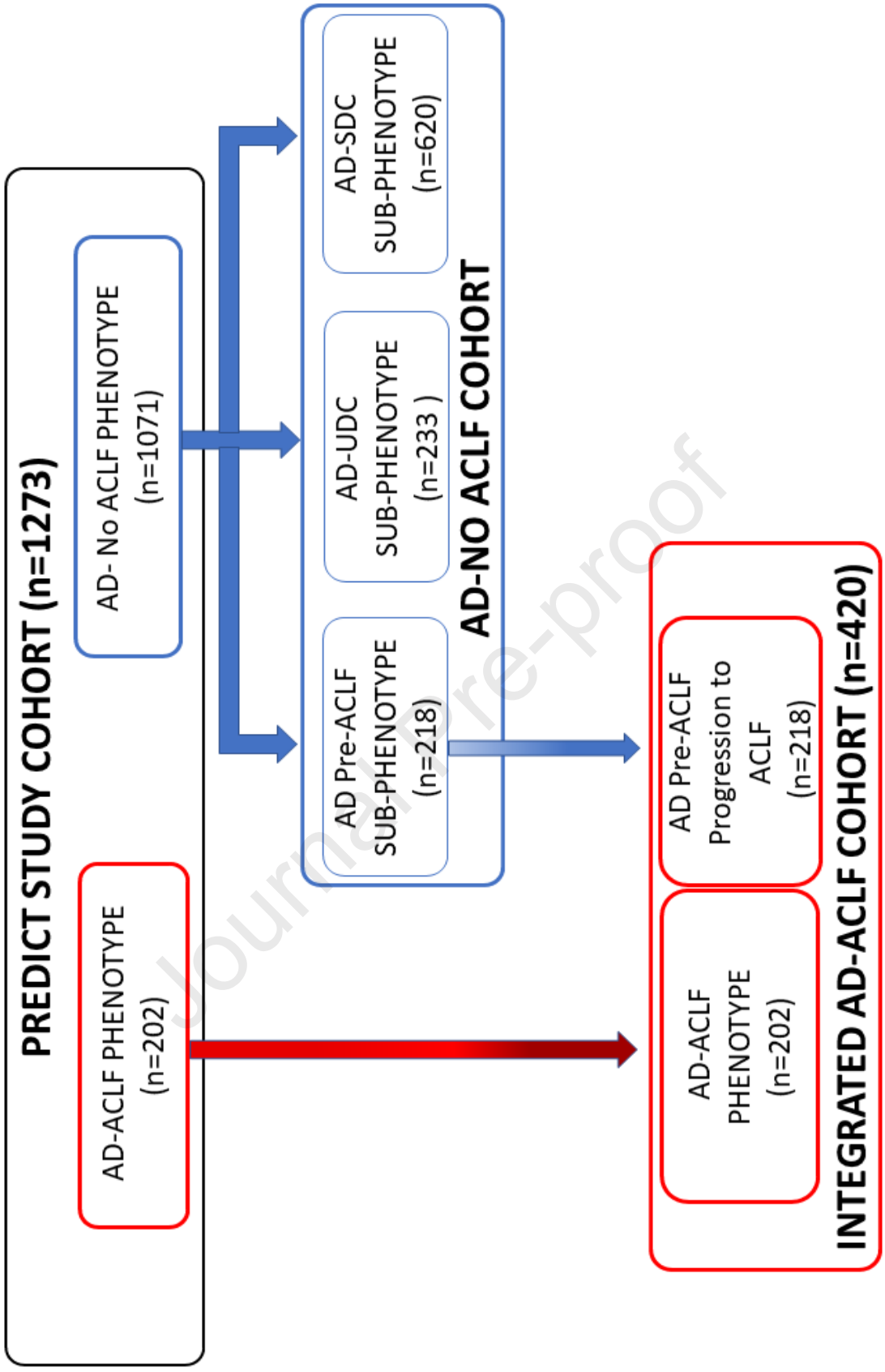


Figure 1

Figure 2

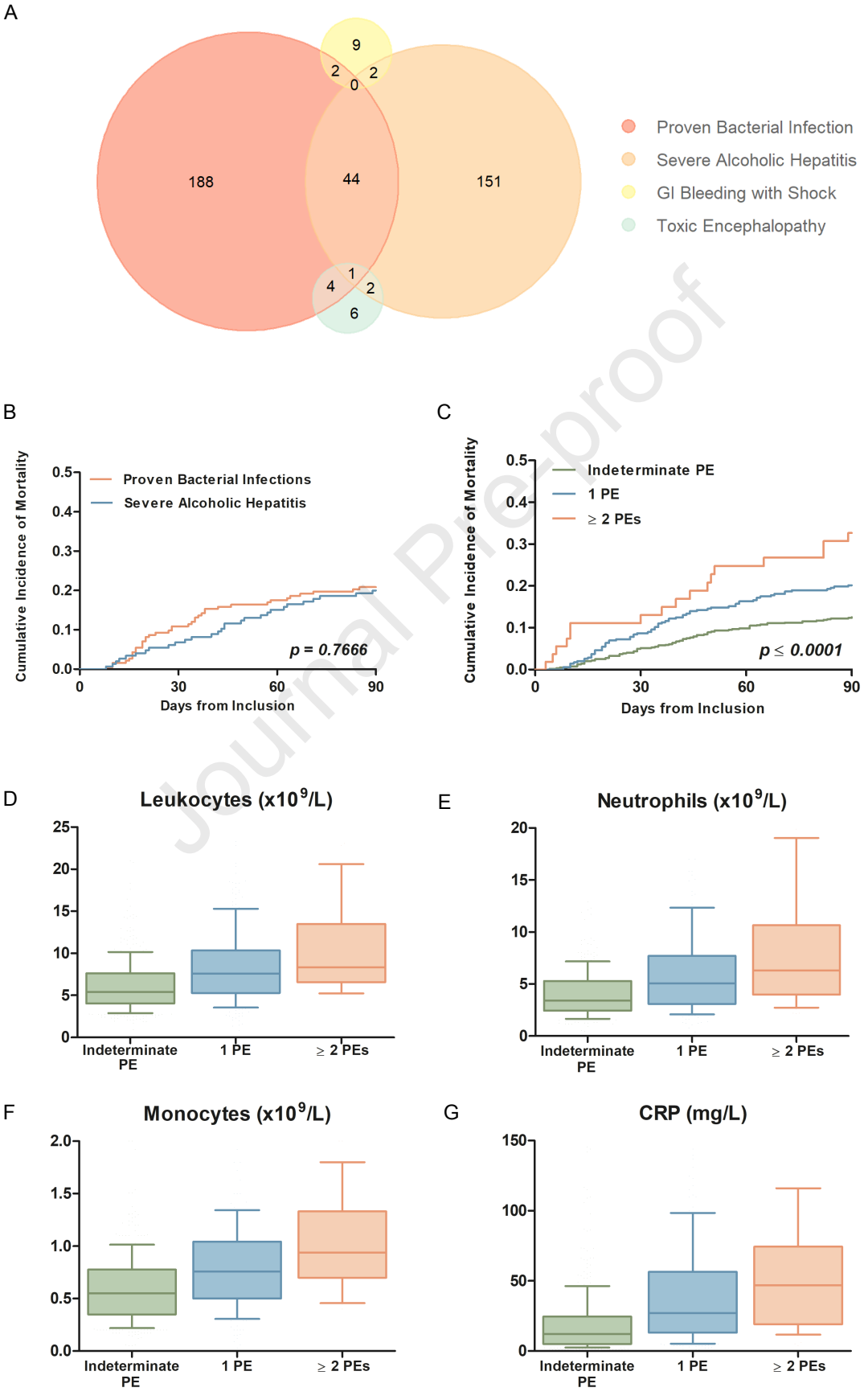


Figure 3

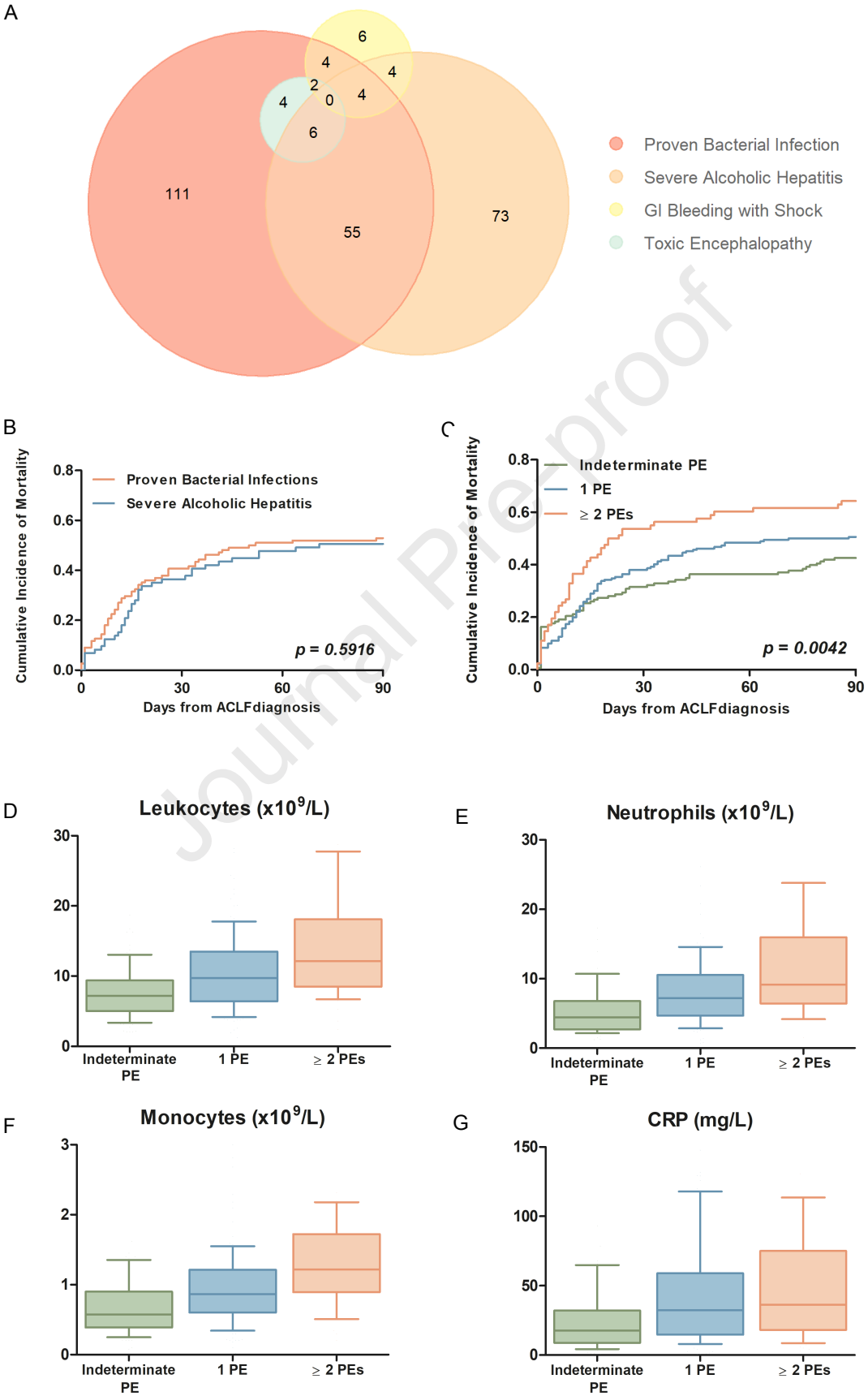
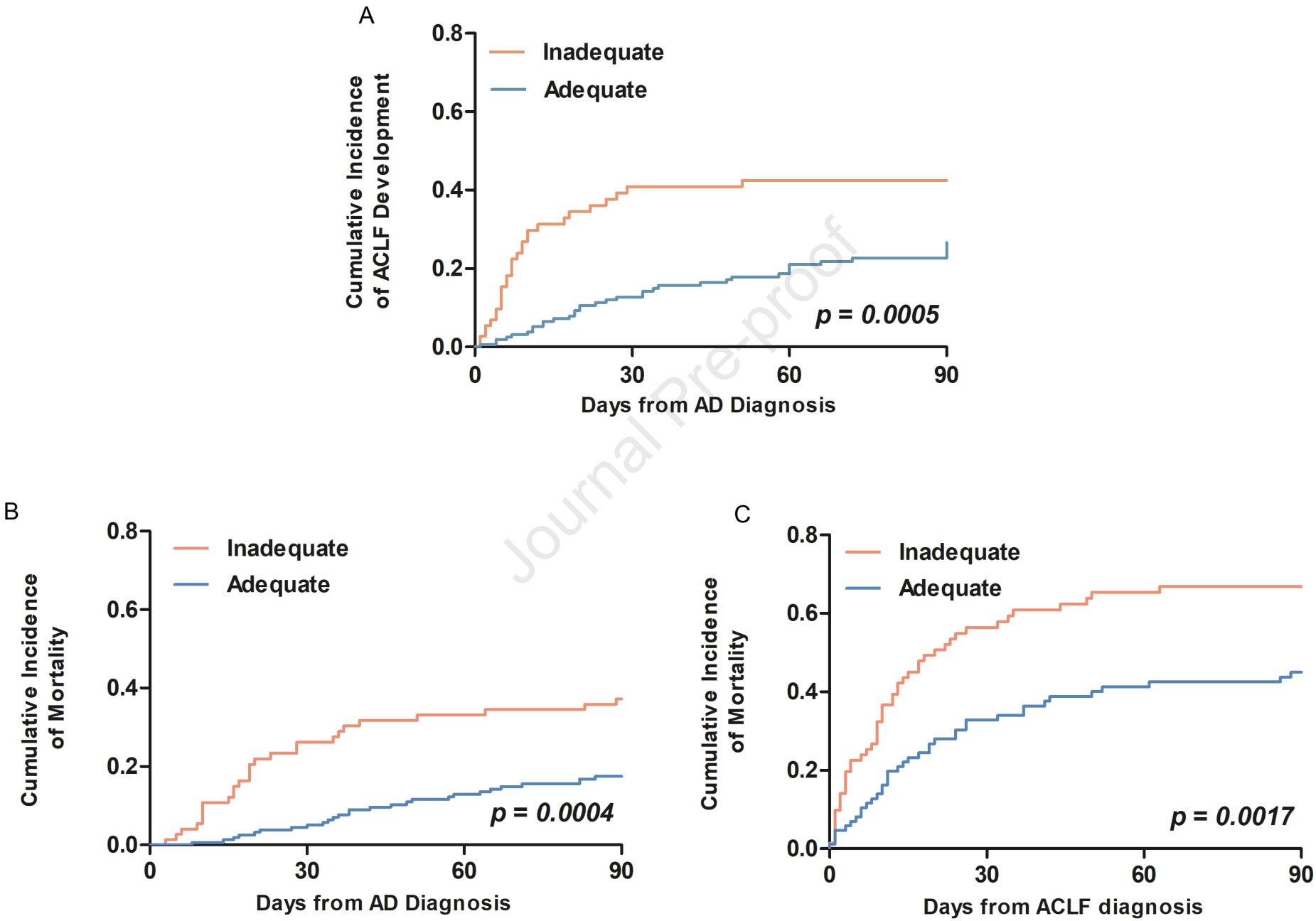


Figure 4



Highlights

- Proven bacterial infections and severe alcoholic hepatitis, alone or in combination, are the major precipitating events for acute decompensation and ACLF.
- While the type of precipitating event has no association with survival, the number of identifiable events was significantly associated with surrogates of systemic inflammation and increased 90-day mortality.
- Adequate first-line antibiotic treatment of proven bacterial infections was associated with lower ACLF development rate and higher 90-day survival.
- Specific preventive and therapeutic strategies to precipitating events may improve outcome in decompensated cirrhosis.