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EUROPEAN
FOUNDATION
FOR THE STUDY
OF CHRONIC
LIVER FAILURE

1ST INTERNATIONAL MEETING

Systemic Inflammation and Organ Failure in Cirrhosis. The Acute-on-Chronic Liver Failure Syndrome (ACLF)

ELSEVIER

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Vicente Arroyo

Mauro Bernardi

For the Canonic Study Investigators of the EASL-CLIF Consortium

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EASL-CLIF Consortium.
Grifols Chair for Research and Education.
European Foundation for the Study of
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IN MEMORIAM

This book is dedicated to the memory of Joan Córdoba Cardona, member of the EASL-CLIF Consortium Steering Committee, excellent clinician and outstanding clinical investigator.

Joan Córdoba Cardona

We would like to dedicate this book to the memory of the much loved and respected friend, Professor Joan Córdoba, who passed away prematurely at the age of 49 from cancer. Joan was a dedicated clinician, great scientist, mentor, friend, collaborator, critic and was extremely enthusiastic about trying to find solutions for patients with cirrhosis. Although his main interest was in studying hepatic encephalopathy, he was passionate about cirrhosis research. He was critical to the formation of the CLIF Consortium and provided leadership to organisation and contributed significantly to its ultimate success.

Joan's seminal scientific contributions are many. He was the first to show the importance of cerebral hyperemia in acute liver failure, the deleterious consequences of hyponatremia in the development of brain edema, evidence that the brain of patients with hepatic encephalopathy was irreversibly injured, the negative consequences of administering a low protein diet to patients with hepatic encephalopathy, providing evidence for white matter lesions in patients with hepatic encephalopathy, generating the first evidence of the safety and efficacy of a novel ammonia lowering compound, ornithine phenylacetate and showing for the first time that the brain in patients with acute on chronic liver failure was clinically and pathophysiologically distinct. However, his greatest contribution was his mentorship of many, many colleagues in the field of hepatic encephalopathy without asking for anything in return.

Joan is desperately missed by the whole of the Hepatology community and remembered very fondly by all who knew him.

Rajiv Jalan

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CONTRIBUTORS

Agustín Albillos
Hospital Universitario Ramón y Cajal, Universidad de Alcalá, Madrid, Spain

Paolo Angeli
Unit of Hepatic Emergencies and Liver Transplantation, University of Padova, Padova, Italy

Vicente Arroyo
Hospital Clinic, University of Barcelona, Barcelona, Spain

Jasmohan Bajaj
Virginia Commonwealth University; McGuire VA Medical Center, Richmond, VA, USA

Ramón Bataller
University of North Carolina at Chapel Hill, NC.

Mauro Bernardi
University of Bologna, Bologna, Italy

Roger F. Butterworth
Department of Medicine, University of Montreal, Montreal, Qc., Canada

Paolo Caraceni
University of Bologna, Bologna, Italy

Joan Clària
Hospital Clínic, University of Barcelona, Barcelona, Spain

Javier Fernández
Hospital Clinic, University of Barcelona, Barcelona, Spain

Guadalupe García-Tsao
Yale University, New Haven, CT, USA

Pere Ginès
Hospital Clinic, University of Barcelona, Barcelona, Spain

Thierry Gustot
Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium.

Rajiv Jalan
Institute of Liver and Digestive Health, Royal Free Campus, London, UK

Patrick Kamath
Division of Gastroenterology and Hepatology, Mayo Clinic Rochester MN, USA

Richard Moreau
Hôpital Beaujon, Clichy; Université Paris Diderot-Paris, Paris, France

Frederik Nevens
University Hospital Gasthuisberg, KU Leuven, Leuven, Belgium

Faouzi Saliba
Centre Hépato-Biliaire, Hôpital Paul Brousse, Villejuif, France.

Shiv K Sarin
Institute of Liver and Biliary Sciences, New Delhi, India

Bern Schnabl
University of California San Diego, CA, USA

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LIST OF THE CANONIC STUDY INVESTIGATORS

Patricia Aguilar Melero, Hospital Universitario Reina Sofía, Córdoba, Spain.

Agustín Albillos, Hospital Universitario Ramón y Cajal, Universidad de Alcalá, Madrid, Spain.

Carlo Alessandria, San Giovanni Battista Hospital, University of Turin, Turin, Italy.

Paolo Angeli, Unit of Hepatic Emergencies and Liver Transplantation, University of Padova, Padova, Italy.

Vicente Arroyo, Hospital Clinic, University of Barcelona, Barcelona, Spain.

Rafael Bañares, Hospital General Universitario Gregorio Marañón, Universidad Complutense, Madrid, Spain.

Daniel Benten, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Mauro Bernardi, University of Bologna, Bologna, Italy.

Massimo Bocci, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium.

Paolo Caraceni, University of Bologna, Bologna, Italy.

María-Vega Catalina, Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Jun Liong Chin, St Vincent's University Hospital, Dublin, Ireland.

Minneke J. Coenraad, Leiden University Medical Centre, Leiden, The Netherlands.

Audrey Coilly, Hôpital Paul Brousse, Villejuif, France.

Mar Concepción, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain.

Joan Cordoba, Hospital Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain.

Andrea de Gottardi, University Clinic of Visceral Surgery and Medicine of Bern, Bern, Switzerland.

Manuel de la Mata García, Hospital Universitario Reina Sofía, Córdoba, Spain.

Carme Deulofeu, Hospital Clinic, Barcelona, Spain.

Marco Domenicali, University of Bologna, Bologna, Italy.

Livia Dorn, Innsbruck Medical University, Innsbruck, Austria.

François Durand, Hôpital Beaujon, Clichy; Université Paris Diderot, Paris, France.

Laure Elkrief, Hôpital Beaujon, Clichy; Université Paris Diderot, Paris, France.

Javier Fernandez, Hospital Clinic, University of Barcelona, Barcelona, Spain.

Elisabet Garcia, Hospital Clinic, Barcelona, Spain.

Angelo Gatta, University of Padova, Padova, Italy.

Ludmila Gerber, JW Goethe University Hospital, Frankfurt, Germany.

Alexander Gerbes, Klinikum der LMU München-Grosshadern, Munich, Germany.

Pere Ginès, Hospital Clinic, University of Barcelona, Barcelona, Spain.

Henning Grønbæk, Aarhus University Hospital, Aarhus, Denmark.

Monica Guevara, Hospital Clinic, University of Barcelona, Barcelona, Spain.

Thierry Gustot, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium.

Anne Kristin Hausen, University Hospital of Bonn, Bonn, Germany.

Corinna Hopf, Klinikum der LMU München-Grosshadern, Munich, Germany.

Rajiv Jalan, Institute of Liver and Digestive Health, Royal Free Campus, London, UK.

Stine Karlsen, Aarhus University Hospital, Aarhus, Denmark.

Wim Laleman, University Hospital Gasthuisberg, KU Leuven, Leuven, Belgium.

Ansgar W. Lohse, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Caterina Maggioli, University of Bologna, Bologna, Italy.

Daniel Markwardt, Klinikum der LMU München-Grosshadern, Munich, Germany.

Javier Martinez, Hospital Universitario Ramón y Cajal, Madrid, Spain.

Alfredo Marzano, San Giovanni Battista Hospital, University of Turin, Turin, Italy.

P. Aiden McCormick, St Vincent's University Hospital, Dublin, Ireland.

Francisco Mesonero, Hospital Universitario Ramón y Cajal, Madrid, Spain.

José Luis Montero Álvarez, Hospital Universitario Reina Sofía, Córdoba, Spain.

Rajeshwar P. Mookerjee, Institute of Liver and Digestive Health, Royal Free Campus, London, UK.

Filippo Morando, University of Padova, Padova, Italy.

Richard Moreau, Hôpital Beaujon, Clichy; Université Paris Diderot-Paris, Paris, France.

Christophe Moreno, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium.

Bernhard Morrell, University Clinic of Visceral Surgery and Medicine of Bern, Bern, Switzerland.

Christian Mortensen, Hvidovre Hospital, University of Copenhagen, Copenhagen, Denmark.

Frederik Nevens, University Hospital Gasthuisberg, KU Leuven, Leuven, Belgium.

Marco Pavesi, Hospital Clinic, Barcelona, Spain.

Markus Peck-Radosavljevic, Medical University of Vienna, Vienna, Austria.

Gustavo Pereira, Hospital Clinic, University of Barcelona, Barcelona, Spain.

Alessandro Risso, San Giovanni Battista Hospital, University of Turin, Turin, Italy.

Mario Rizzetto, San Giovanni Battista Hospital, University of Turin, Turin, Italy.

Ezequiel Rodriguez, Hospital Clinic, University of Barcelona, Barcelona, Spain.

Antonietta Romano, University of Padova, Padova, Italy.

Faouzi Saliba, Centre Hépato-Biliaire, Hôpital Paul Brousse, Villejuif, France.

Didier Samuel, Hôpital Paul Brousse, Hôpitaux de Paris, Villejuif, France.

Tilman Sauerbruch, University Hospital of Bonn, Bonn, Germany.

Macarena Simon-Talero, Hospital Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain.

Pablo Solis-Muñoz, Institute of Liver Studies and the Cellular Biology of Inflammation, King's College London, London, UK.

German Soriano, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain.

Jan Sperl, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Walter Spindelboeck, Medical University of Graz, Graz, Austria.

Rudolf Stauber, Medical University of Graz, Graz, Austria.

Christian Steib, Klinikum der LMU München-Grosshadern, Munich,Germany.

Jonel Trebicka, University Hospital of Bonn, Bonn, Germany.

Dominique Valla, Hôpital Beaujon, Clichy; Université Paris Diderot, Paris, France.

Hans Van Vlierberghe, Ghent University Hospital, Ghent, Belgium.

Len Verbeke, University Hospital Gasthuisberg, KU Leuven, Leuven, Belgium.

Wolfgang Vogel, Innsbruck Medical University, Innsbruck, Austria.

Henninge Wege, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Tania Welzel, JW Goethe University Hospital, Frankfurt, Germany.

Julia Wendon, King's College London, London, UK.

Chris Willars, King's College Hospital, London, UK.

Maria Yago Baenas, Institute of Liver and Digestive Health, Royal Free Campus, London, UK.

Giacomo Zaccherini, University of Bologna, Bologna, Italy.

Stefan Zeuzem, JW Goethe University Hospital, Frankfurt, Germany.

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EUROPEAN FOUNDATION FOR THE STUDY OF CHRONIC LIVER FAILURE (EF-CLIF)

MILESTONES

1. *Boston, November 2006* The decision to create an International Chronic Liver Failure (CLIF) Consortium was taken by several European and American hepatologists in an informal dinner during the annual meeting of the American Association for the Study of Liver Diseases in Boston. Soon it became clear that there should be two Consortiums, one American and one European.

2. *Paris, February 2008* First informal contact with the European Association for the Study of the Liver (JM Pawlotsky, EASL Secretary General) looking for an academic support of the European CLIF Consortium (fig. 1).

3. *Milan, April 2008* First presentation of the CLIF Consortium to EASL members.

4. *Paris, July 2008* First presentation of the CLIF Consortium to the EASL-Governing Board.

5. *January 2009* Formal proposal signed by the 19 EASL members asking for EASL official support to the CLIF-Consortium.

6. *February 2009* The EASL-Governing Board decided to endorse the CLIF Consortium under the official name of "EASL-CLIF Consortium".

7. *March 2009* Invitation call for European Centers to join the EASL-CLIF Consortium. Sixty-two university hospitals with liver units and facilities to perform clinical research were recruited.

8. *Barcelona, July 2009* Agreement signed by the Fundació Clinic, Grifols and the Chairman of the EASL-CLIF Consortium by which Fundació Clinic gave legal support to the Consortium and Grifols provided an unrestricted grant of 3.5 millions euros for a period of 5 years (fig. 2).

Figure 1. Jean Michel Pawlotsky, Secretary General of the EASL at the time of the development of the European CLIF Consortium.

Figure 2. Agreement signed by Mr Victor Grifols, President and CEO of Grifols, and Prof. Vicente Arroyo, Chairman of the EASL-CLIF Consortium, on the unrestricted grant given by Grifols to the EASL-CLIF Consortium (July, 2009).

9. *Barcelona, July 2009* First EASL-CLIF Consortium Steering Committee Meeting (fig. 3). It was decided that the first study of the Consortium should be devoted to Acute-on-Chronic Liver Failure (ACLF).

10. *January 2011* First patient included in the CANONIC study, a prospective observational investigation in 1342 patients with decompensated cirrhosis hospitalized in 21 European Hospitals. The aim of the study was to characterize ACLF. Data collection was completed in less than one year.

11. *June 2013* Publication of the first CANONIC study describing the clinical characteristics of ACLF (Gastroenterology. 2013;144:1426-37). At present, 21 studies or review articles derived from the CANONIC have been published or are in the process of publication. It is estimated that 10-15 additional studies will appear in the next 2 years before closing definitely the CANONIC investigation. The CANONIC study has allowed a comprehensive assessment of the epidemiology, clinical characteristics, diagnostic criteria, clinical course, prognosis and mechanism of ACLF and represents a solid base for future investigations of the pathophysiology and treatment of this extremely relevant syndrome. It is a clear example of the efficacy of networking research in the approach of a complex clinical problem.

12. *Barcelona, June 2015* Creation of the European Foundation for the Study of Chronic Liver Failure to give legal support to the EASL-CLIF Consortium. Grifols increased the unrestricted grant to 1.5 million per year to support the EASL-CLIF Consortium and the Grifols-Chair, a new activity dealing with Translational Research and Education in Chronic Liver Failure.

13. *Barcelona, September 2015* The EF-CLIF approved to provide economical resources to the CLIF-Consortium for a new large observational study, the PREDICT study, in 1200 patients hospitalized with acute decompensation of cirrhosis. The CANONIC study assessed ACLF from the onset of the syndrome. The PREDICT study will be focused on patients with decompensated cirrhosis during the critical period prior to ACLF development. The study is aimed to identify predictors of ACLF development and to better assess the mechanisms leading to the development of the syndrome. The PREDICT study will be lead by young European investigators.

Figure 3. First Steering Committee of the EASL-CLIF Consortium (July 2009): From left to right: Francesco Salerno (Milan), Joan Cordoba (Barcelona), Richard Moreau (Paris), Pere Ginès (Barcelona), James O'Beirne (London), Paolo Angeli (Padova), Fin Stolze Larsen (Copenhagen), Vicente Arroyo (Barcelona), Frederik Nevens (Leuven), Dieter Häussinger (Düsseldorf), Mauro Bernardi (Bolonia), Alexander Gerbes (Munich), Faouzi Saliba (Paris), Rajiv Jalan (London). Kinan Rifai (Hannover) and Jules Wendon (London) could not attend the meeting.

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INTRODUCTION

Mauro Bernardi

Member of the EASL Executive Committee and Vice-Chairman of the CLIF Consortium

Vicente Arroyo

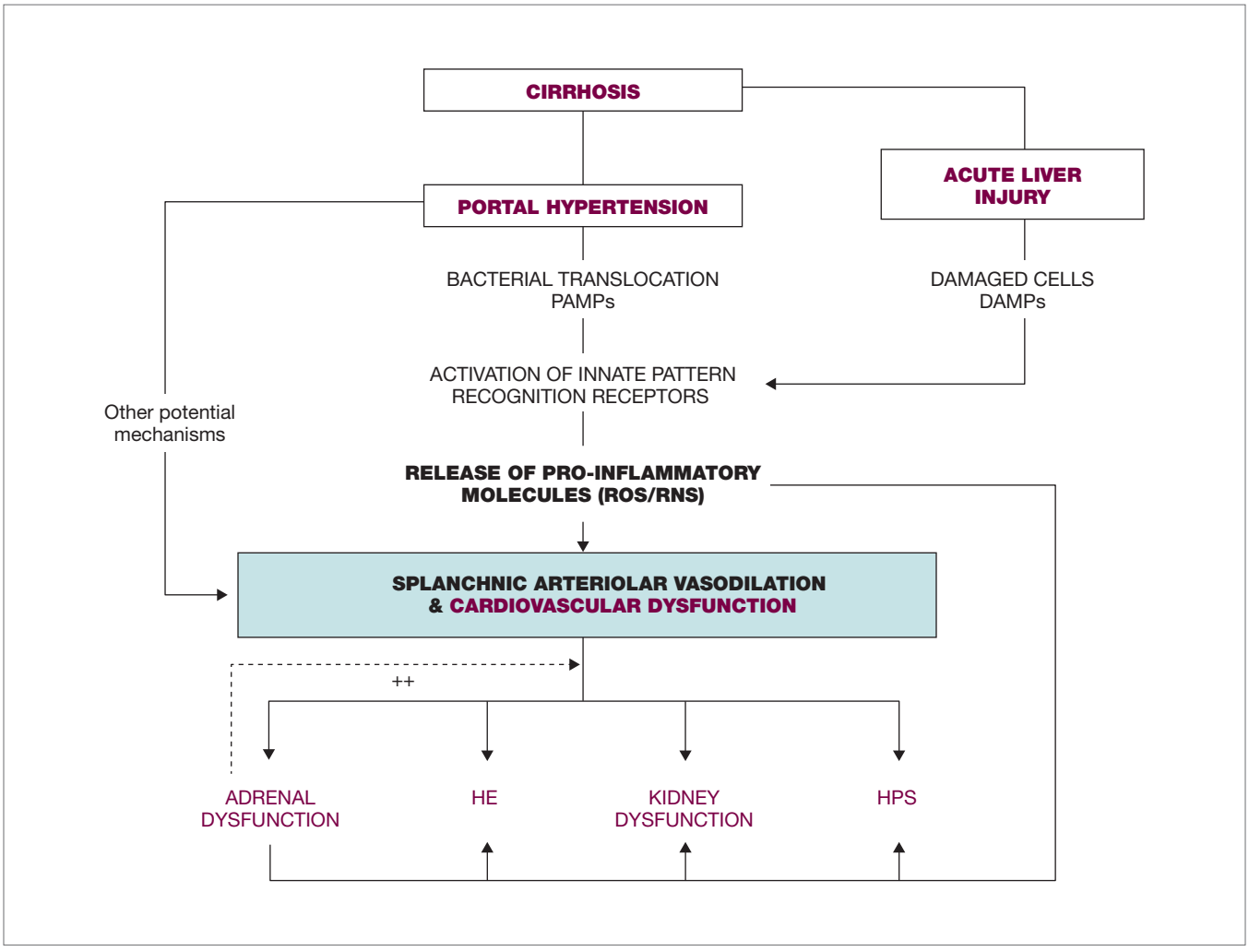
Chairman of the EF-CLIF and EASL-CLIF Consortium

Cirrhosis, which represents the background for chronic liver failure, evolves through two main stages. The compensated stage is usually asymptomatic and has no major impact on patient quality of life, although histological liver lesions and portal pressure steadily progress. The occurrence of complications, that is gastrointestinal bleeding, ascites, hepatic encephalopathy and clinically evident signs of liver dysfunction such as jaundice, marks the following decompensated stage where the patient becomes at high risk of medium- or short-term mortality. The course of cirrhosis seldom follows a steady downward path. Indeed, acute deteriorations of clinical conditions, usually triggered by a precipitating event and requiring hospital admission, can occur. In these circumstances, patient short-term survival is abruptly endangered. However, contrary to cirrhosis that has reached an irreversible end-stage, recovery to the pre-existing condition is possible with prompt recognition and aggressive treatment. We are accustomed to define this condition as acute-on-chronic liver failure (ACLF), and its potential reversibility is a first distinctive feature with respect to end-stage liver disease. In both conditions, the high risk of mortality is heralded by the occurrence of hepatic and/or extrahepatic organ failure, which makes the clinical picture of patients with advanced cirrhosis very complex and multifaceted.

ACLF is highly challenging for physicians and poses difficult questions related to the recognition of precipitating factors, prognosis, evolution, choice of the most appropriate treatment, early recognition of patients requiring admission to intensive care and/or urgent liver transplantation and, finally, the identification of those situations that may render futile intensive and expensive care.

Until recently, there was no evidence-based definition of ACLF. After the first definition proposed by Jalan and Williams, others followed as result of personal opinions or consensus agreement among experts. Not surprisingly, even major discrepancies among these definitions emerged, also conveying the influence of the most prevalent conditions leading to ACLF in different geographical settings. A major accomplishment by the Chronic Liver Failure Consortium of the European Association for the Study of the Liver (EASL-CLIF) is to have provided the first and, up to now, unique large prospective study designed to define ACLF and its phenotype: the CANONIC study. Briefly, 1,343 patients with cirrhosis admitted to 29 European hospitals for an acute deterioration of their conditions, such as acute development of large ascites, hepatic encephalopathy, gastrointestinal bleeding, bacterial infection, or any combination of these were enrolled. The pre-defined requirements to diagnose ACLF were the presence of organ failure, identified by a SOFA score modified to take into account specific features of cirrhosis, and a short-term (28-day) mortality equal to or greater than 15%. According to the number of organ failures, patients were stratified into four grades, grade 0 being the absence of organ failure and grades 1 to 3 referring to one, two and three or more organ failures. The severity of prognosis increased in parallel with ACLF grade, as 28-day mortality was 22% in grade 1 and 79% in grade 3

The first lesson that emerged from the CANONIC study was that patients with a single organ failure could not automatically be assigned to grade 1 ACLF, as only single kidney failure was associated with a 28-d mortality greater than the pre-defined limit warranting the diagnosis of ACLF. In fact, any other single failure was followed by mortality exceeding 15% only when it was associated with kidney dysfunction, defined as serum creatinine ranging from 1.5 to 1.9 mg/dL. Moreover, non-kidney



Mechanisms leading to multiorgan dysfunction / failure in advanced cirrhosis. The activation of innate pattern recognition receptors by pathogen and danger associated molecular patterns (PAMPs; DAMPs) leads to the release of pro-inflammatory cytokines / chemokines and oxidative stress. These molecular events are responsible for cardiovascular dysfunction and, in concert with it, to multiorgan dysfunction / failure.

HE: hepatic encephalopathy; HPS: hepatopulmonary syndrome.

Modified from: Bernardi M, Moreau R, Angeli P, Schnabl B, Arroyo V. Mechanisms of decompensation and organ failure in cirrhosis: From peripheral arterial vasodilation to systemic inflammation hypothesis. J Hepatol. 2015;63:1272-84.

and non-brain single failure associated with hepatic encephalopathy grade I or II was also associated with a mortality rate justifying the diagnosis of ACLF grade 1.

The CANONIC study demonstrated that development of ACLF in patients hospitalized because of an acute decompensation of cirrhosis is far from being a rare event, as about one-third of them underwent this complication, a prevalence that has been confirmed by subsequent studies. Another important finding was that patients with ACLF, with respect to patients with acute deterioration of cirrhosis but without ACLF, are younger, more frequently are affected by alcoholic liver disease, more often present ascites and have higher white blood cell count and serum C-reactive protein concentration. A further interesting and somehow unexpected finding was that ACLF developed in patients without a history of decompensation or with a time to the first episode of decompensation shorter than three months in more than 40% of cases. Thus, ACLF cannot be seen as a terminal event in long-lasting decompensated cirrhosis.

The CANONIC study also provided an insight of the most common precipitating events leading to ACLF: in about one-third of cases the syndrome followed sepsis and active alcoholism in about one-fourth. However, a most interesting finding was that a precipitating event was not identified in more than 40% of cases. Even though this may be due in part to undiagnosed drug-

induced liver injury or bacterial infection, clearly suggests that the definition of the mechanisms leading to ACLF still needs further insight.

The original CANONIC study has constituted the ground from which several ancillary studies germinated shedding light to further aspects of ACLF, such as its clinical course, thus helping the decision-making on subsequent management, i.e. continuation of intensive care and/or urgent liver transplantation, as well as discontinuation due to futility. Furthermore, specific prognostic indicators for acute decompensation and ACLF were developed that proved to be more reliable than widely used prognostic scores such as MELD and Child-Pugh. Finally, the clinical features of hepatic encephalopathy and acute kidney injury in the setting of ACLF were defined.

White blood cell count and serum C-reactive protein concentration are significantly higher in patients with ACLF than in those with acute decompensation, irrespective of the presence of bacterial infections. Moreover, there is a clear trend for a parallel increases in these abnormalities, the ACLF grade and 28-day transplant-free mortality. As a whole, these findings strongly suggest that inflammation plays an important role in the pathogenesis of the syndrome. Indeed, further studies in patients enrolled in the CANONIC study have shown that the plasma levels of pro-inflammatory cytokines/chemokines are higher in patients with ACLF than in those with acute decompensation without the syndrome. Moreover, the higher was the ACLF grade, the higher were plasma pro-inflammatory cytokine/chemokine levels. Thus, immunopathology likely represents a prominent contributor to the development of organ failures in this setting. This finding is in line with the recently proposed systemic inflammation hypothesis, which identifies in a systemic inflammatory process and the consequent systemic oxidative stress the pathogenetic background ultimately leading to hemodynamic disturbances and multi-organ dysfunction / failure in advanced cirrhosis (Figure). This process likely originates in the intestine following translocation of viable bacteria and/or bacterial products (PAMPs) from the lumen to the gut wall, ultimately spreading to the systemic circulation. In particular settings, such acute liver injury induced by acute alcoholic hepatitis, HBV-related hepatitis flares or drug-induced liver injury, the origin of the inflammatory responses could be identified in the liver itself, spreading danger-associated molecular patterns (DAMPs) from damaged liver cells and activated immune cells. Thus, ACLF can be seen as the result of an acute and severe systemic inflammatory response to bacterial infections, liver injury or, hypothetically, to a burst of PAMPs translocation that could be involved in those cases where a potential precipitating factor cannot be identified.

The knowledge originated by the CANONIC study, although impressive, is far from having shed light on all pathophysiological and clinical aspects related to ACLF and further investigations are warranted. Namely, prospective studies, both pathophysiological and clinical, focusing in the time frame preceding the development of the syndrome would provide essential information for developing management strategies aimed at reducing its incidence. The identification of biomarkers more sophisticated and sensitive than white blood cell count or C-reactive protein would help in establishing an early diagnosis and assessing response to treatments. Recent studies have shown that the pathological background of ACLF is characterized by bilirubinostasis and cholestasis. Interestingly, these abnormalities have been described in septic patients without cirrhosis and could be attributed to liver injury induced by PAMPs, DAMPs or oxidative stress. The study of liver histology in patients with ACLF, although difficult to be performed, would therefore be warranted. Other aspects needing a further insight are those related to the fragile and unstable balance between pro- and anti-inflammation. The putative intense inflammatory storm leading to ACLF could be followed by immune paralysis, a recognized cause of delayed mortality in septic patients with and without cirrhosis. Indeed, evidence of this process has already been provided in patients with ACLF and need to be better defined. Finally, the observation that ACLF is more severe in cirrhotic patients without previous decompensation seems to be consistent with the concept that inflammation-induced tissue damage depends not only on the intensity of the inflammatory response, but also on the intrinsic capacity of host organs to tolerate its effects. Thus, the chronic organ exposure to low-grade inflammation in decompensated cirrhosis may render vital organs more tolerant the acute bursts of inflammation. Studies characterizing tolerance to inflammatory insults in patients with cirrhosis with and without ACLF would be of great importance and may assume clinical relevance.

SECTION I

ACLF: A new syndrome associated to systemic inflammation

Chairpersons:

Frederik Nevens

University Hospital Gasthuisberg, KU Leuven, Leuven, Belgium

Guadalupe García-Tsao

Yale University, New Haven, CT, USA

Acute systemic inflammation, a condition frequently associated with severe sepsis or trauma, is characterized by an acute and generalized activation of the immune system, intense cytokine release by the immune cells, high circulating levels of inflammatory cytokines and increased release of reactive oxygen species by the inflammatory process. If severe, it may cause acute organ failure(s) due to direct deleterious effects of cytokines and reactive oxygen species in cardiovascular function, organ microcirculation and cell function. Chronic Systemic Inflammation is also characterized by increased circulating levels of inflammatory cytokines and oxidative damage but at a considerably lower level of severity. Moderate chronic systemic inflammation is currently considered an important driver of peripheral organ dysfunction or lesions in a large number of non-autoimmune chronic diseases including chronic obstructive pulmonary disease, congestive heart failure, chronic renal failure, alcoholism and metabolic syndrome. The effects of this chronic increase in cytokine secretion and reactive oxygen generation may cause damage in remote organs such as the brain, bone, muscle, heart, circulatory system, lung, gonads, adrenal glands, peripheral nerves, liver and pancreas. Translocation of bacterial products from the intestinal lumen to the systemic circulation has been identified as a potential mechanism of chronic systemic inflammation in these diseases. Chronic systemic inflammation and oxidative stress are also characteristic features in decompensated cirrhosis. The degree of systemic inflammation in patients with decompensated cirrhosis is much more intense than that reported in other chronic diseases. Not surprisingly it has been proposed as a potential mechanism of complications associated with decompensated cirrhosis, including cardiovascular dysfunction, ascites, hepatic encephalopathy, sarcopenia, and relative adrenal insufficiency. Acute-on-Chronic Liver Failure in cirrhosis is a recently characterized syndrome defined by acute decompensation, organ failure(s) and high short term mortality. Evidences have been presented that this syndrome is associated with an acute exacerbation of the chronic systemic inflammation of cirrhosis promoted by precipitating events such as infections, acute alcoholism and presumably, in cases undetectable precipitating events, a severe burst of bacterial translocation. The prevalence of Acute-on-Chronic Liver Failure in patients hospitalized by acute decompensation of cirrhosis is high and one third of these patients die shortly after diagnosis.

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SECTION I

SYSTEMIC INFLAMMATION AS A DRIVER IN HUMAN DISEASE

Agustín Albillos[1,2,3], María Úbeda[2,3], Leticia Muñoz[2,3] and Melchor Álvarez-Mon[2,3,4]

[1]Servicio de Gastroenterología y Hepatología, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

[2]Laboratorio de Enfermedades del Sistema Inmune, Departamento de Medicina, Universidad de Alcalá, Alcalá de Henares, Spain

[3]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain

[4]Servicio de Enfermedades del Sistema Inmune y Oncología, Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Spain

A. Albillos

Inflammation is both a protection mechanism responsible for confining and clearing damaging factors, and a healing mechanism aimed at repairing injured tissue. Various factors, such as microbial infection or tissue damage, will activate an inflammatory response. The triggering agent for such a response is initially sensed by innate patterns recognition receptors (PRR), which set off intracellular signalling cascades leading to the expression of inflammatory mediators. The inflammatory response then makes sure pathogens are eliminated and damaged tissue is repaired. An abnormal inflammatory response can arise due to factors related to the host or noxious agent, such as genetic susceptibility or virulence/load, respectively, and this response may systemically spread. Systemic spillover of inflammation involves a complex cascade of pathways that lead to massive production of pro- and anti-inflammatory cytokines, circulatory abnormalities, coagulopathy, organ dysfunction and, paradoxically, also to an increased susceptibility to infection. Contrary to acute inflammation, chronic low-grade systemic inflammation accompanies chronic diseases and physiological processes, such as aging, due to persistence of pro-inflammatory factors. Low-grade inflammation as present in chronic diseases contributes to comorbidities and accelerates the course of the underlying disease. This chapter reviews the concepts and mechanisms of transit from a local inflammation response to systemic spread of inflammation, highlighting differences between acute and chronic low-grade inflammation.

Defining inflammation

Inflammation is a host response to an infectious agent, tissue damage, or to irritants such as toxins. When immune cells encounter any of these noxious factors, they are activated to release an array of pro- and anti-inflammatory mediators. These, in turn, act on local vessels leading to the well-known clinical signs of inflammation.

The inflammatory cascade in local tissue can be broadly categorized into two stages: one of damaged cell/tissue removal and one of new tissue growth. In the first stage, injured cells are eliminated through apoptosis and/or their attachment to microvessels, along with the infiltration at the site of injury of a variety of circulating immune cells (firstly neutrophils and macrophages, and secondly lymphocytes). These immune cells act in concert with local tissue cells to remove harmful agents and clear damaged tissue components. This stage of inflammation depends strictly on a permissive microvasculature, which normally has the opposite function of preventing the indiscriminate influx of immune cells into a tissue[1]. In the second stage, the tissue repair process is initiated by mitosis and angiogenesis, the release of growth factors, and by a new extracellular matrix[2]. This local inflammatory response and accompanying immune response is directed at restoring homeostasis and may be defined as the early physiological inflammatory reaction. The host inflammatory response is the result of the actions of a plethora of cell-derived mediators (e.g., chemokines, cytokines, antimicrobial peptides, and reactive oxygen and nitrogen species) and activated biochemical cascades that originate in the blood system (e.g., complement, coagulation, and fibrinolytic systems). The described response, known as acute inflammation (fig. 1), ceases within hours or days, once the noxious agents have been removed, and damaged tissue heals.

Chronic inflammation, by contrast, is the continued presence, sometimes over many years, of proinflammatory factors at levels higher than baseline, but many-fold lower than those found in acute inflammation. Chronically inflamed tissues are characterized by the presence of infiltrating lymphocytes and macrophages, abundant blood vessels,

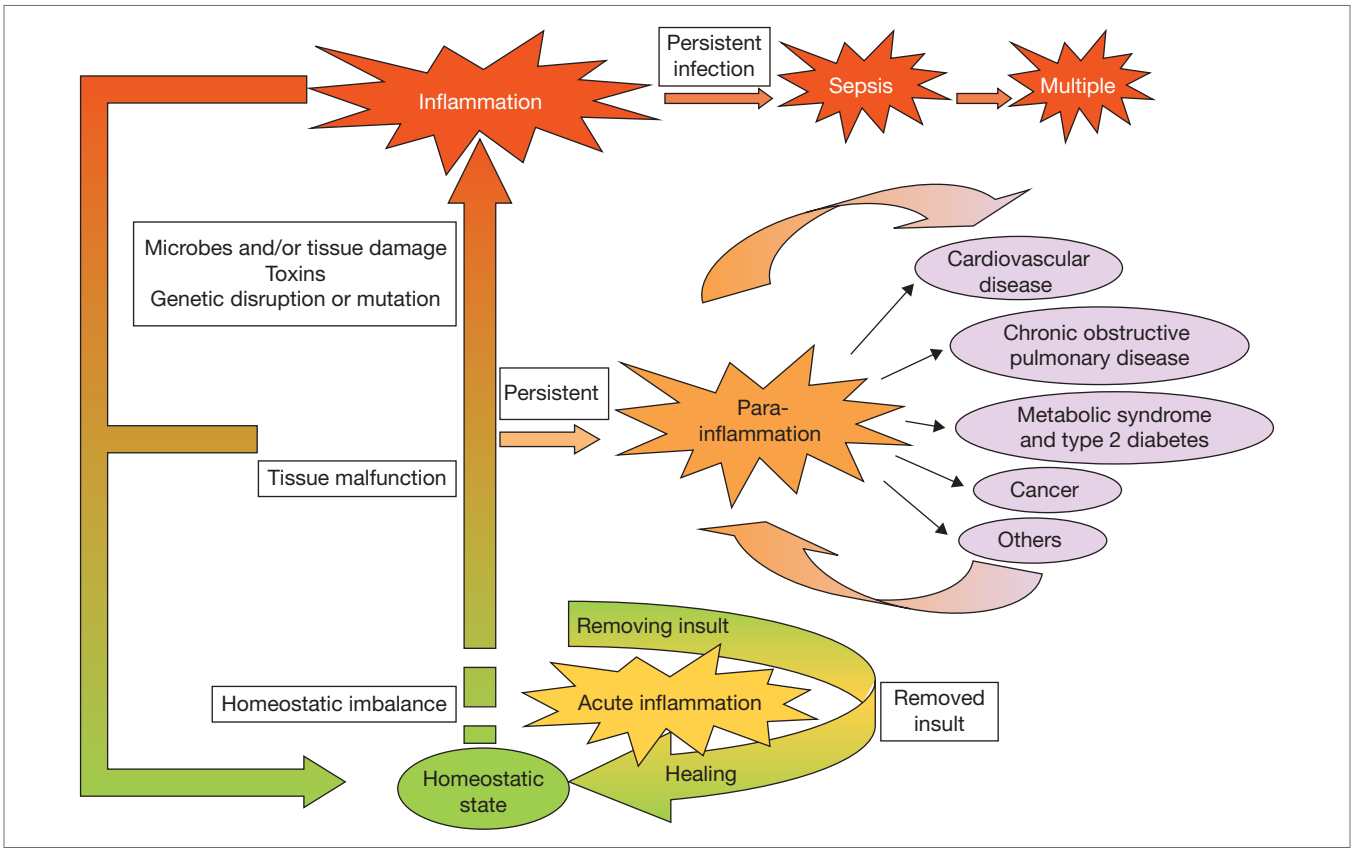

Figure 1. The spectrum of inflammation. An inflammatory response is at the extreme end of a spectrum that ranges from homeostatic state to inflammation, depending on the kind and persistence of the insult.

fibrosis and, often, tissue necrosis causing tissue damage and loss of function (fig. 1). When insults giving rise to a local immune response are released in sufficient amount or persist, activate a systemic inflammation response. In brief, this systemic response is propagated by peripheral as well as local immune cells, which become activated to step up their production and release of inflammatory cytokines, chemokines, and other immunologically active peptides into the bloodstream. Such mediators can induce organ dysfunction directly or indirectly by interfering with homeostasis or disrupting the milieu of an organ. The end-result is a continuum of clinical manifestations from local and transient to diffuse and persistent increasing the rate of comorbidities in chronic diseases[3] (fig. 1).

Para-inflammation, also known as low-grade chronic systemic inflammation, defined as a two- to fourfold elevation in serum levels of proinflammatory and anti-inflammatory mediators, is associated with many chronic diseases, including rheumatoid arthritis, metabolic syndrome and type 2 diabetes, atherosclerosis, inflammatory bowel diseases, osteoarthritis, chronic obstructive pulmonary disease and cancer, among others[3]. Chronic inflammation is also associated with normal aging. Large loads of pathogens, or infection by highly virulent pathogens, can trigger a massive systemic response that leads to sepsis and multiple organ failure[4] (fig. 1).

Many factors may trigger inflammation

Inflammation can be viewed as the end stage of a wide spectrum of mechanisms that maintain and defend homeostasis[5,6]. This spectrum consists of: (i) homeostatic mechanisms that operate under normal conditions; (ii) stress and defence responses that come into play when homeostatic capacity is outstripped; (iii) para-inflammation, a tissue-level stress response intermediate between a basal homeostatic state and the classic inflammatory response that relies mainly on tissue-resident macrophages. This adaptive response is thought to be responsible for the chronic inflammation associated with modern human diseases[6]; and, finally (iv) inflammation proper, which occurs when other mechanisms fail to rescue homeostasis[5]. The sensory cells that initiate the inflammatory response are also the cells responsible for tissue level stress and defence responses[7]. Both stress and inflammation responses strive to eliminate the stressor, promote host adaptation to the stressor, and ultimately to return to a homeostastic state (fig. 1).

This means that inflammation is induced by extreme deviations from cell or tissue homeostasis (e.g., by hypoxia, heat shock, or oxidative stress) or by the factors that provoke such deviations (i.e. pathogens, toxins, and tissue damage). The former are detected by sensors (such as HIF-1a and

HSF-1) of variables that are regulated in cell and tissue homeostasis (oxygen and protein folding state, respectively), and induce stress responses. The latter are detected directly (some pathogens) by receptors (i.e. pattern recognition receptors (PRRs) specialized at identifying insults that can disrupt homeostasis, or indirectly (i.e. some pathogens, most toxins and poisons, and the majority of allergens) through their functional features (i.e. enzyme activities and disruption of membrane integrity), and induce defence responses.

Inflammation has been traditionally considered a defence response to infection or injury. However, the presence of microbial products in the absence of tissue injury does not trigger an inflammatory response. Sterile tissue injury, as occurs in well-performed surgery, provokes little or no clinically apparent inflammation. Transient, functionally consequential inflammation can arise when large numbers of host cells undergo necrotic death without involvement of microbial products, most often due to an ischaemic event such as heart attack or stroke. Indeed, the products of dying cells activate many of the receptors that detect microbes. However, it is when signals arising from tissue injury coincide with signals arising from microbes that inflammation usually ensues[8]. This occurs in cirrhosis, whereby increased intestinal permeability enhances contact between intestinal flora and local immune system cells, inducing intestinal inflammation and gut bacteria translocation. This, in turn, leads to bacterial infection in these patients[9].

Notwithstanding, it is increasingly being accepted that chronic inflammation can accompany a wide variety of pathological states (e.g. metabolic syndrome, type 2 diabetes, cardiovascular and neurodegenerative diseases, inflammatory bowel disease, obesity, cancer, asthma, and ageing) in the absence of infection or injury. In these cases, the inflammatory response appears to be supported by tissue malfunction or homeostatic imbalance. In this type of response, inflammation is of a lesser magnitude than in classic reactions[3,6] (fig. 1).

In addition, numerous genes exist whose disruption predisposes a host to inflammation in the absence of any evident factor known to elicit inflammation[8]. This determines that the classic instigators of inflammation –infection and tissue injury– are at one end of a large spectrum of conditions that induce inflammation (fig. 1).

Inflammation may be beneficial or detrimental depending on multiple factors

Regardless of the origin of inflammation, the inflammatory response has the effect of protecting the host from further tissue damage and restoring tissue function, reestablishing homeostasis. It is also true, however, that, depending on the circumstances, anti-inflammatory mechanisms avoiding spontaneous inflammation will sometimes fail, and then the innocuous immune response becomes a problem[10].

Spontaneous inflammation may firstly occur whenever we lose a non-redundant component of the mechanism that regulates proliferation or signalling in lymphoid, myeloid or epithelial cells responding to antigens, microbes or injury. Secondly, inflammation may not be resolved if the secretion or extracellular formation of the soluble products that drive this process is delayed or reduced. Thirdly, if insult persists, the elicited immune responses themselves can damage tissue or result in chronic inflammatory processes[8,11-13]. In these cases, inflammation progresses from acute to chronic and then stalls for a prolonged period as local inflammatory mediators spread into the systemic circulation to propagate a systemic inflammatory response. However, signs of acute inflammation, such as accumulation of neutrophils, may reappear later accelerating the course of the disease. Classic examples are persistent infections. The chronic inflammatory response in this case is typically restricted to the site where the inflammatory inducer is present, and often gives rise to different types of local tissue remodelling. For example, persistent infection can lead to granulomas and tertiary lymphoid organs at the site of infection, or to fibrosis in a tissue with collagen bundles. Similarly, persistent airway inflammation induced by allergens can lead to respiratory epithelial tissue remodelling resulting in asthma. Moreover, persistent inflammation may also oxidize DNA badly enough to promote neoplastic transformation. Interestingly, it has been recently shown that resolved infections leave scars of another sort: long lasting immune dysfunction consisting of chronic inflammation and an inability to initiate subsequent immune responses, which may account for a wide range of chronic diseases[12,13].

Fourthly, non-resolving inflammation may also be the outcome of a prolonged or excessive response. This can take place when tissue injury is associated to infection. The persistent infection in the wound or the existence of any additional source of infection increases the local and systemic immune disorders, which can lead to sepsis and multiorgan failure (figs. 1 and 2). Sepsis is defined as severe systemic inflammation produced in response to invading pathogens, or an uncontrolled hyperinflammatory response, as it is mediated by the release of various proinflammatory mediators. Although some patients may die rapidly from septic shock accompanied by an overwhelming systemic inflammatory response syndrome (SIRS) triggered by a highly virulent pathogen, most patients survive the initial phase of sepsis, only to show multiple organ damage days or weeks later. These patients often demonstrate signs of immune suppression accompanied by enhanced inflammation. An unbalanced systemic compensatory anti-inflammatory response (CARS) can result in anergy and immunosuppression. Thus, pro- and anti-inflammatory forces may ultimately reinforce each other, creating a state of increasingly destructive immunologic dissonance. Sepsis is therefore the result of a complex process involving interactions among several pathways such as inflammation, immunity, coagulation, as well as neuroendocrine mechanisms, which potentially drive an organism to death[4].

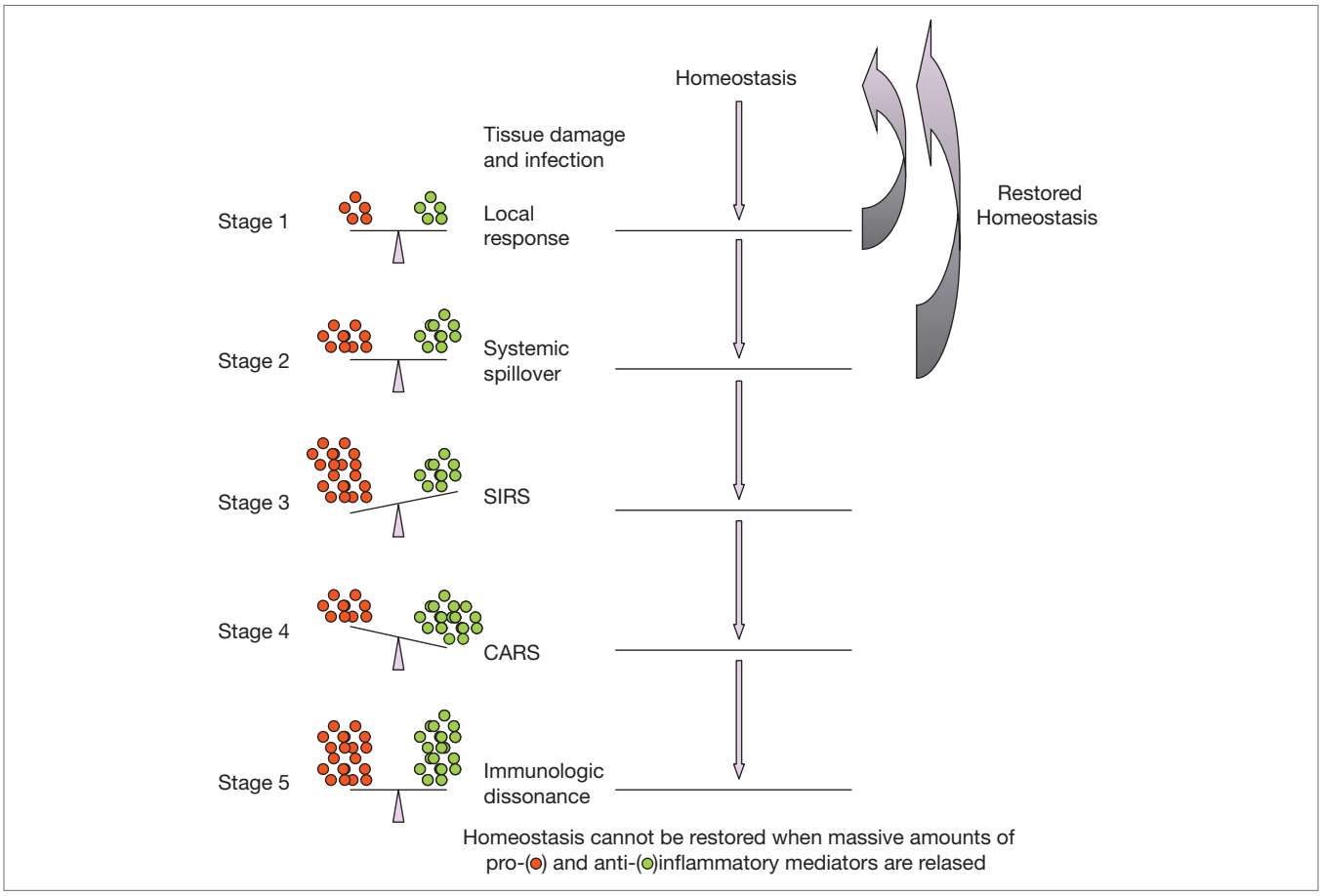

Figure. 2. Transit from a local to a systemic inflammatory response. Prolonged or excessive local immune response might lead to tissue injury, systemic spillover of inflammation and multiple organ failure.

Lastly, a subnormal immune response may, as well, be linked to non-resolving inflammation. An example of this has recently been illustrated[14]. In cirrhotic rats with ascites, the continuous pressure of gut bacteria shapes the phenotypic and functional profile of intestinal dendritic cells and produces effects that range from their activation and enhanced function to their exhaustion and tolerance, the latter contributing to gut bacteria translocation and systemic inflammation.

The role of inflammation in pathogenesis warrants special attention when it is the host inflammatory response and not inflammation triggered by some sort of insult that is responsible for tissue damage. Indeed, rather than being a primary cause of several heterogenic multifactorial diseases, non-resolving inflammation has been described as the unifying force driving the progression of diseases such as cirrhosis, atherosclerosis, obesity, cancer, chronic obstructive pulmonary disease, asthma, inflammatory bowel disease, neurodegenerative disease, multiple sclerosis, or rheumatoid arthritis[3,8-10]. In these different settings, we can find three situations:

(i) If targeted destruction and assisted repair occurring after an inflammatory response are not properly phased, this gives rise to scar tissue with reduced cell functions. However, in the presence of stem cells, the wound repair process may rescue original tissue functions present before injury[11,15]. Successful post-inflammatory tissue repair requires the coordinated replacement of different cell types and structures, including extracellular matrix and blood vessels besides epithelial and mesenchymal cells. Chemokines are critical for vascular remodelling after inflammation. Without appropriate neovascularization, impaired tissue oxygenation may preclude normal repair, resulting in atrophy or fibrosis. Atrophy (i.e., loss of parenchymal cells) is often accompanied by expansion of extracellular tissue elements, particularly collagen, resulting in fibrosis via the deposition of excess connective tissue. It is likely that atrophy may promote fibrosis, that fibrosis may promote atrophy, and that each can occur independently. Fibrosis sufficient to interfere with organ function is a principal cause of disease following inflammation[10].

(ii) A growing number of chronic inflammatory conditions are being identified in which the initiating trigger is not well defined but does not seem to involve infection or tissue damage. These inflammatory states are of particular

interest because they accompany diseases typical of industrialized countries including obesity and type 2 diabetes, atherosclerosis, neurodegenerative diseases, and cancer. Interestingly, in these cases of chronic inflammation there appear to be vicious cycles connecting inflammation to the disease process it accompanies. For example, obesity can lead to inflammation, whereas chronic inflammation can contribute to obesity-associated diabetes by inducing insulin resistance. Similar positive feedback loops are present in atherosclerosis, cancer, and other chronic inflammatory diseases. Indeed, this type of reciprocal relationship may be responsible, at least in part, for the chronic nature of these inflammatory conditions.

(iii) Finally, there are diseases of infectious origin in which inflammation may contribute as much to the disease as does microbial toxicity. This feature distinguishes them from the chronic inflammation mentioned above, which is caused by persistence of the inflammation inducer.

Innate immune system defence mechanisms: PAMPs and DAMPs

The innate immune system is the first line of defence against pathogens, and is mediated by phagocytes, mainly neutrophils, macrophages and dendritic cells. Immune cells express a set of receptors known as pattern-recognition receptors (PRRs) that rapidly initiate host defence responses when they detect tissue damage or microbial infection. PRRs recognize structures conserved among microbial species called pathogen-associated molecular patterns (PAMPs) e.g., wall lipopolysaccharides (LPS) of Gram-negative bacteria. PRRs also recognize endogenous molecules released from damaged cells called damage-associated molecular patterns (DAMPs) or alarmins (e.g., heat shock proteins and high mobility group box1 (HMGB1), a nuclear protein with DNA-binding capacity derived from necrotic cells). These molecules also spark an inflammatory response in the absence of infection that has been termed “sterile inflammation”.

Two types of PRRs have been identified according to their subcellular localization: i) transmembrane, as toll-like receptors (TLRs) and C-type lectin receptors (CLRs), and ii) intracytoplasmic, as retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) and NOD-like receptors (NLRs)[16]. TLRs are perhaps the best-characterized PRR family and are responsible for sensing invading pathogens outside the cell and within intracellular endosomes and lysosomes. TLRs recognise bacterial LPS, peptidoglycans, unmethylated CpG motifs of bacterial DNA or double-stranded RNA of viruses. The most investigated TLR is TLR4, which recognizes LPS. LPS, also known to be an endotoxin, is generally the most potent immunostimulant among the cell wall components of Gram-negative bacteria. A lipid portion of LPS termed “lipid A” is responsible for most of the pathogenic events associated with Gram-negative bacterial infection. LPS released from Gram-negative bacteria associates with LPS binding protein, an acute-phase protein present in the bloodstream, and then binds to CD14, a protein expressed on the cell surface of phagocytes.

CLRs act as an adhesion receptor in leukocyte-leukocyte or leukocyte-endothelium interactions, and also function as PRRs on macrophages and dendritic cells, recognizing carbohydrate groups on microorganisms such as viruses, bacteria, and fungi[16,17]. Some of these receptors can induce signalling pathways that directly activate nuclear factor-kappa B, whereas others regulate TLR signalling by synergising or antagonising TLR signals.

In contrast, RLRs are located in the host cell cytoplasm and recognize the genomic RNA of double-stranded RNA viruses and double-stranded DNA generated as the replication intermediate of single-stranded RNA viruses. The expression of RLRs is greatly enhanced in response to type I interferon (IFN) stimulation or virus infection. Finally, the members of the NLR family, NODs and NALPs, are cytoplasmic pathogen sensors[17]. The NOD class of proteins includes NOD1 and NOD2, which like TLRs recognize the structures of bacterial peptidoglycans and induce the transcriptional up-regulation of pro-inflammatory cytokine genes. NALPs are components of inflammasomes, responsible for regulating caspase-1 activation. Inflammasomes are multimeric protein complexes that assemble in the cytosol after sensing PAMPs or DAMPs[18]. Caspase-1 is constituently expressed in monocytes, macrophages, and neutrophils. This enzyme is a prerequisite for initial attempts of bacterial clearance by the innate immune system because it cleaves the cytokine precursors pro-IL-1β and pro-IL-18 to generate the biologically active cytokines IL-1β and IL-18, respectively.

Early innate immune responses are regulated mostly by TLRs and NLRs. TLRs initiate the inflammatory response via adapter molecules, MyD88 or TRIF, and NODs activate the inflammatory cascade using receptor-interacting protein 2 as an adapter protein[15,16]. Activation of these PRRs triggers an intracellular signalling pathway that leads to the nuclear translocation of a set of transcription factors, NF-kB, AP-1, IRFs, and C/EBPb, which, in turn, control/drive the induction of pro-inflammatory cytokines (IL-1, IL-6, TNFα and IL-12), chemokines, type I interferon (IFN-α or -β), adhesion and co-stimulatory molecules, growth factors, metalloproteinases, cyclo-oxygenase and inducible nitric oxide synthase. PRR activation also causes dendritic cell maturation triggering adaptive immunity. Different external agents set off multiple pathways in different cell types and induce the expression of certain gene subsets. For instance, it is known that HMGB1 and LPS induce distinct patterns of gene expression in neutrophils such that the expression of monoamine oxidase B and the anti-apoptosis protein Bclxl is enhanced by HMGB1 but not by LPS. Further, whilst the cytokine expression profile induced by HMGB1 versus LPS seems similar, the slower induction of TNFα mRNA upon LPS stimulation compared to HMGB1 has been reported[11].

Transit from a "local" to a "systemic" inflammatory response

Although infectious (sepsis) and noninfectious (trauma) inflammation differ in their pathophysiology, they share many common mechanisms. Neutrophils and macrophages are the first cell types to migrate across damaged endothelium and remove dead cells and cellular debris. These immune cells release pro-inflammatory cytokines, which activate endothelial cells to up-regulate the expression of adhesion molecules in an effort to increase immune cell recruitment (selectins, intercellular and vascular cell adhesion molecules). More leukocytes become trapped generating reactive oxygen species, inducible nitric oxide synthase, additional growth factors, proteases and more cytokines[16]. In addition, endothelial cells express tissue factor triggering the extrinsic coagulation cascade and leading to further endothelial damage. The expression of co-stimulatory molecules induces efficient T cell activation and the adaptive immune response. Cytokines regulate the cell death of inflammatory tissues, modify vascular endothelial permeability, recruit blood cells to inflamed tissues, and induce the production of acute-phase proteins[19].

Cell damage is initially restricted to the area of infection or trauma. However, delocalization of the inflammatory response can occur causing wide-spread cell and tissue damage remote from the site of origin. The release of large amounts of PAMPs and/or DAMPs results in the overstimulation of PRRs on immune cells, leading to a vicious cycle and eventually to a systemic inflammatory response syndrome (SIRS). Of note, SIRS could be the consequence of different underlying conditions, including infection and trauma or other types of sterile injury[20]. Sepsis is defined as infection with evidence of systemic inflammation. During SIRS, the innate immune system is activated and pro-inflammatory cytokines, as well as vasoactive substances are released. This in turn causes activation of the complement system, coagulation, capillary leakage and vasodilatation, along with organ damage.

The inflammatory response is effectively mediated by a number of overlapping and interrelated cascades. These include pathways of the complement system, coagulation and fibrinolysis (fig. 3). Activation of coagulation with concurrent down-regulation of anticoagulant systems and fibrinolysis are almost universally present in septic patients with SIRS, leading sometimes to its more severe clinical form of disseminated

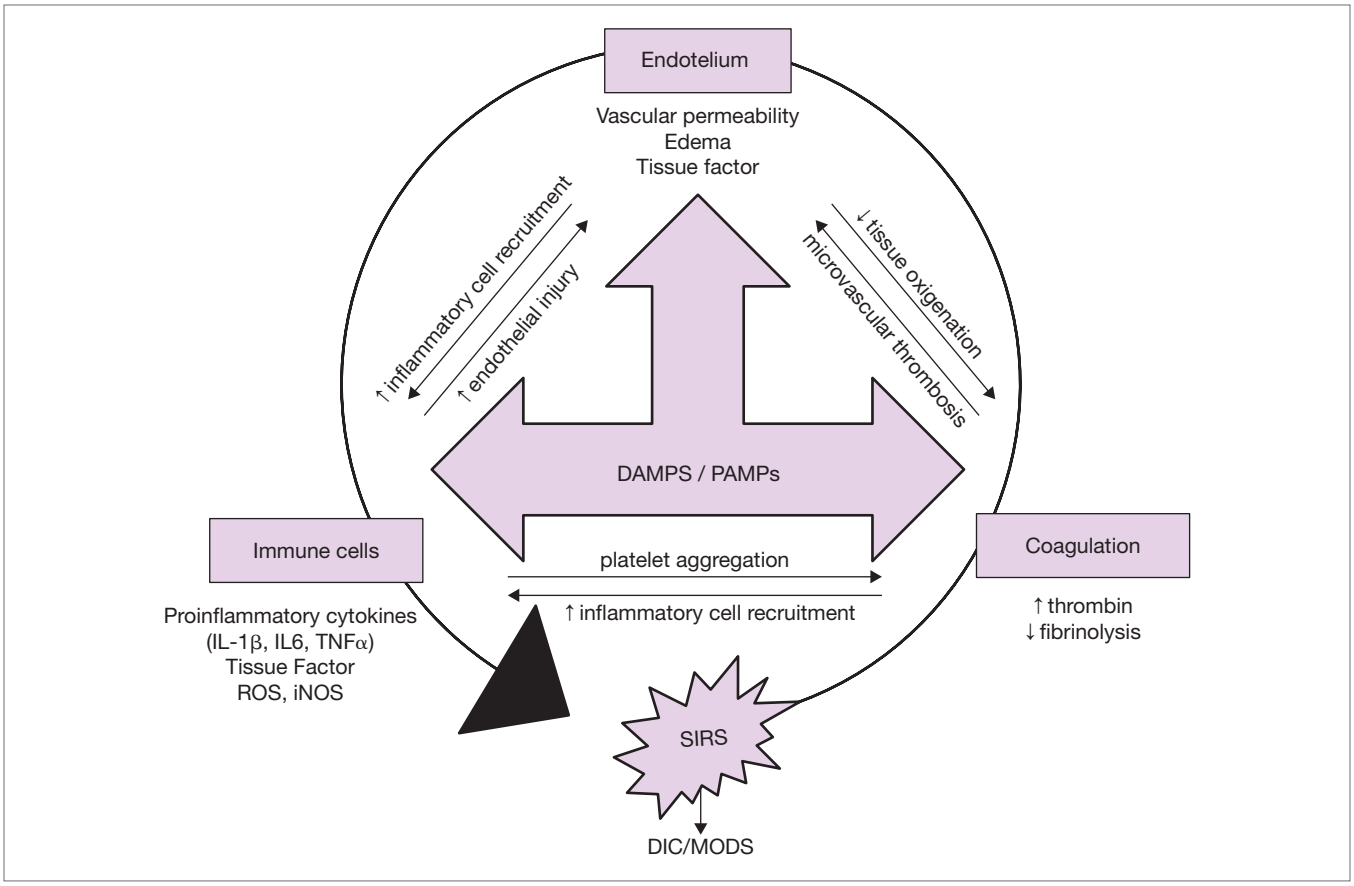

Figure 3. Inflammatory response to tissue damage and/or infection. Neutrophils and monocytes release pro-inflammatory cytokines that activate endothelial cells to up-regulate adhesion molecules and tissue factor, and trigger coagulation cascade. These events are interrelated, and conform a vicious cycle that leads to the systemic inflammatory response syndrome (SIRS) and eventually to disseminated intravascular coagulation (DIC) and multiple organ dysfunction syndrome (MODS).

intravascular coagulation (DIC). DIC consists of a deranged coagulation system involving enhanced coagulation activation, suppressed coagulation inhibition mechanisms and an inhibited fibrinolytic system. The depletion of platelets and coagulation proteins due to extensive ongoing activation of the system gives rise to a paradoxical situation in which there is a high risk of simultaneous fatal thrombosis and large-scale haemorrhage, which clinically define DIC. In sepsis, the pro-inflammatory environment, mainly created by TNFα, determines that endothelial and mononuclear cells upregulate the expression of tissue factor on their surface. This initiates the clotting and proteolytic cascade, leading to thrombin formation and fibrin deposits simultaneous with fibrinolysis blockade due to increased levels of plasminogen-activator inhibitor 1 and thrombin-activatable fibrinolysis inhibitor. Also, the consumption of various factors that normally regulate the generation of thrombin, such as antithrombin III, protein C and tissue-factor pathway inhibitor, contributes to the development of DIC[20,21]. The complement system amplifies coagulation through phospholipid membrane modification by activating platelets and by inducing the leukocyte expression of tissue factor and plasminogen-activator inhibitor 1.

Simultaneous coagulation system activation and fibrinolysis inhibition along with microvessel occlusion and direct tissue-toxicity caused by the inflammatory immune reaction impair tissue oxygenation leading to tissue necrosis/apoptosis[20]. Tissue oxygen demands increase significantly and, if oxygen delivery is inadequate, a diffuse cellular ischaemia is produced, which exacerbates cell damage and inflammation. In this manner, a vicious circle sets in, which, if uncorrected, eventually leads to widespread organ failure (multiple organ dysfunction syndrome MODS), severe functional deterioration, and eventually death[21] (fig. 3).

Organ damage related to the extreme systemic inflammatory response

During the extreme inflammatory response that is part of SIRS and/or sepsis, the organs that mainly show dysfunction are lung, liver, kidney, gastrointestinal tract and heart, though other systems like the brain may be also affected. The lung is usually the first organ to show signs of damage. Hence, coagulation activation, insufficient anticoagulation and impaired fibrinolysis lead to fibrin deposition in the alveolar space, and the sequestering and activation of neutrophils causes neutrophil-mediated epithelial lung injury[19,21]. The latter is manifested by increased capillary permeability, loss of alveolar epithelial cell surfactant, oedema, alveolar flooding and finally, collapse and hypoxaemia. The myocardium is the second most common organ to be affected in MODS due to elevated nitric oxide and pro-inflammatory cytokine production. The brain is often affected by multifactorial mechanisms including blood–brain barrier disturbances involving increased permeability to circulating cytokines, brain inflammation, neuron degeneration and apoptosis leading to brain dysfunction or encephalopathy. In addition, there is initial acute hepatic dysfunction related to decreased perfusion, hepatocellular necrosis and fibrin thrombi forming in the sinusoids around the necrotic area. After a latent period, however, more prolonged hepatic dysfunction can follow. The gut is an immunologically active organ and gut dysfunction usually occurs early in MODS. The high-density immune cells in the intestinal mucosa and mesenteric lymph nodes have an enormous potential to synthesize various inflammatory mediators that induce epithelial cell damage, tight junction disruption and mucosal cell death. This, in turn, increases intestinal permeability and barrier dysfunction leading to translocation of viable bacteria and/or endotoxins and to secondary infection. Contrary to previous belief, the kidneys maintain their perfusion capacity during sepsis, and the mechanism of renal failure during MODS seems to be cytokine-induced apoptosis and a glomerular filtration rate that is reduced through a dilation gradient between efferent and afferent arterioles[22].

The immune system is highly dynamic and the immune response progresses during the course of SIRS and sepsis. Cytokine composition changes from an early, hyperinflammation stage to a compensatory anti-inflammatory response syndrome involving markedly elevated levels of anti-inflammatory cytokines such as IL-4, IL-10, IL-13 IL-1ra and TGFβ[20]. This state, called immunoparalysis, predisposes the patient to secondary infections and is characterized by both innate and adaptive immunodysfunction. Several mechanisms have been implicated in immunoparalysis: i) functional defects in antigen-presenting ability, i.e. the reduced expression of human leukocyte antigen (HLA)-DR in monocytes, which is the most widely accepted biomarker of immunoparalysis; ii) a shift in leukocyte composition towards increased numbers of regulatory immune cells with potent anti-inflammatory activity, and enhanced apoptosis of other immune cells, like B and dendritic cells; and iii) lymphocyte anergy, the impaired response to antigen involving the reduced release of cytokines by T-cells. The shift towards anti-inflammatory cytokine production that occurs in later stages of sepsis, manifesting as an increased IL-10 to TNF-γ ratio, correlates with mortality.

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SECTION I

ACUTE ON CHRONIC LIVER FAILURE: THE SYNDROME

Cristina Solé, Elsa Solà and Pere Ginès

Liver Unit, Hospital Clínic, University of Barcelona, Barcelona, Spain. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD)


P. Ginès

Acute decompensation of cirrhosis and acute-on-chronic liver failure (ACLF) have been recognized as separate entities for many years. Physicians taking care of patients with cirrhosis identified patients with acute decompensation of cirrhosis (AD) without any other associated complication, while in other patients AD was associated with organ failure(s) (OF) which markedly increased short-term mortality. The latter situation was identified as ACLF, however, there were no universally accepted diagnostic criteria available[1,2]. The concept of ACLF was described by the Asia-Pacific Association for the Study of the Liver (APASL) and also by Western studies, but these definitions were based on single-centre studies and differed from each other not only in the proposed diagnostic criteria but also in their accuracy to predict prognosis[1-3].

The CANONIC study was the first prospective study designed to describe the concept, diagnostic criteria, precipitating events, natural history and prognosis of ACLF in a large series of patients admitted to hospital with acute decompensation of cirrhosis. The study was developed in the setting of the EASL-Chronic Liver Failure (EASL-CLIF) Consortium and included 1343 consecutive patients from 21 European hospitals[4]. According to this study, ACLF is defined as a specific syndrome characterized by acute decompensation of cirrhosis associated with OF and high short-term mortality (28-day mortality ≥ 15%)[4]. The existence of OF was assessed by a modified version of the Sequential Organ Failure Assessment score (SOFA), called CLIF-SOFA score or its simplified version, CLIF Organ Failure score (CLIF-OFs) (table 1). According to the number and type of OF, ACLF is classified into three grades with different prognosis[4]:

- ACLF-1:
  - patients with single kidney failure;
  - single non-renal OF plus renal dysfunction (creatinine ranging from 1.5-1.9 mg/dL) and/or grade 1-2 hepatic encephalopathy.
- ACLF-2: two OF.
- ACLF-3: ≥ three OF.

Epidemiology

ACLF is a frequent complication in patients with cirrhosis and is a common cause of hospital admission[4,5]. Prevalence of ACLF in the CANONIC study was 30%; 20% of patients presented with ACLF at hospital admission and 10% developed it during hospitalization[4]. ACLF grade 1 and grade 2 were the most frequent (16% and 11%, respectively) while ACLF grade 3 represented only 4% of cases. Overall 28-day and 90-day mortality rates were 33% and 51%, respectively. However, mortality rate depended on ACLF grade, and increased progressively from grade 1 to grade 3[4] (fig. 1).

Table 1. The CLIF-organ failure score system

Organ/system	Subscore = 1	Subscore = 2	Subscore = 3
Liver, bilirubin (mg/dL)	< 6	≥ 6-< 12	≥ 12
Kidney, creatinine (mg/dL)	< 2	≥ 2-< 3.5	≥ 3.5 or RRT
Brain, (West-Haven grade HE)	0	1-2	3-4
Coagulation (INR)	< 2.0	≥ 2.0-< 2.5	≥ 2.5
Circulation; MAP (mmHg)	≥ 70	< 70	Vasopressors
Respiratory			
PaO_2/FiO_2 or	> 300 or	≤ 300 and > 200 or	≤ 200 or
SpO_2/FiO_2	> 357	> 214 and ≤ 357	≤ 214

The shade area describes criteria for diagnosing organ failures.
HE; hepatic encephalopathy; RRT, renal replacement therapy; INR; international normalized ratio; MAP, mean arterial pressure; FiO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; SpO_2 pulse oximetric saturation.

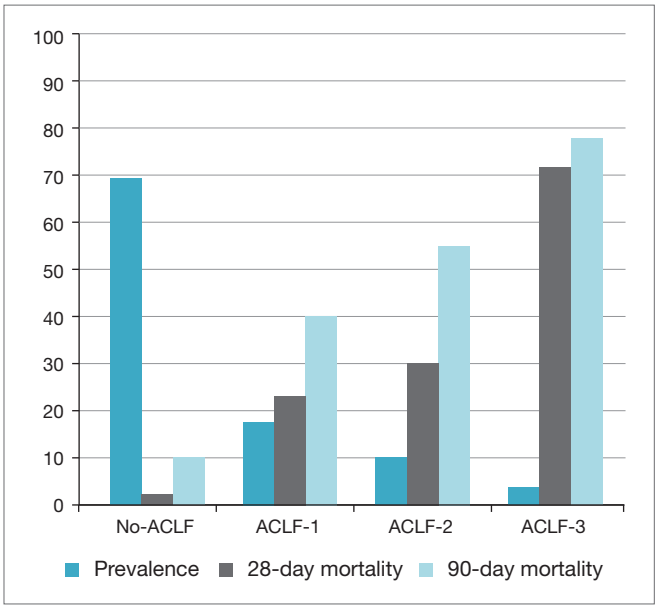

Figure 1. Prevalence and mortality of patients admitted to hospital with acute decompensation of cirrhosis, categorized according to the presence or absence of ACLF and ACLF grade.

A recent study from Asia that investigated characteristics of ACLF in a series of 890 patients with HBV-related cirrhosis using the diagnostic criteria from the CANONIC study described a prevalence of ACLF that was higher than that reported in the CANONIC study (40%)[5]. In this series, ACLF grade 2 was the most frequent one, followed by grade 1 and grade 3. However, mortality rate was very similar to that reported in the Canonic Study.

Clinical features and precipitating factors

Data from the CANONIC study showed that patients with ACLF were younger than those patients with AD without ACLF[4]. The main aetiologies of underlying cirrhosis were alcoholism (60%), hepatitis C infection (13%) and alcoholism plus hepatitis C infection (9%). Interestingly, the prevalence of alcoholic cirrhosis was higher than that of active alcoholism, suggesting that active alcoholism represents only part of the episodes of ACLF associated with alcoholic cirrhosis and that patients with alcoholic cirrhosis without active alcoholism may still develop ACLF associated with other precipitating factors. The characteristics of patients with alcoholic cirrhosis and active alcoholism were significantly different from those of patients with alcoholic cirrhosis and no active alcoholism and patients with non-alcoholic cirrhosis. Patients with active alcoholism were younger and had more marked liver function impairment; however, mortality rate was not significantly different between patients with no active alcoholism and non-alcoholic cirrhosis[4].

Traditionally, ACLF has been understood as a terminal event in patients with already decompensated cirrhosis. Strikingly, data from recent studies show that previous episodes of AD were absent in 23% of patients with ACLF[4]. Moreover, those patients without previous decompensations of the disease developed more severe forms of ALCF, showed higher levels of inflammatory mediators (C-reactive protein and leukocyte count) and had higher mortality rates compared to those of patients with ACLF with previous decompensated cirrhosis (28-day mortality of 42% vs 29%, respectively)[4]. Although there are no data to explain these differences, it has been suggested that tolerance to the excessive inflammatory response associated with ACLF could be significantly decreased in patients with previous compensated cirrhosis compared to patients with decompensated cirrhosis.

In most cases, ACLF is associated with a precipitating factor. The most common precipitating events are bacterial infections, followed by active alcoholism and reactivation of hepatitis B virus (HBV). Other factors that may trigger ACLF are gastrointestinal (GI) bleeding, TIPS, major surgery or therapeutic paracentesis without the administration of albumin[4,6-8]. Available data show that among patients with ACLF, the presence and the type of precipitating event is not associated with mortality, suggesting that mortality depends on other factors more than triggers, such as clinical course and number of OFs[4,6,7].

As it occurs with the definition of ACLF, the type and frequency of precipitating factors differ between the East and the West. While in Western countries the most common precipitating factors are bacterial infections followed by active alcoholism, in the East, the most common precipitating factor is flare-up of HBV superimposed to patients with chronic liver disease, followed by bacterial infections[4,6-8]. Interestingly, a recent study suggests that patients with ACLF could be classified according to the type of precipitating factor categorized into hepatic and extrahepatic insults. "Hepatic-ACLF" was considered when precipitating factors were exacerbation of HBV, superimposed hepatitis A or hepatitis E virus infection, hepatotoxic drugs or active alcoholism. In contrast, "extrahepatic-ACLF" was considered when precipitating factors were bacterial infections, gastrointestinal (GI) bleeding or surgery among others[5]. The study shows that patients with "hepatic-ACLF" and those with "extrahepatic-ACLF" have different clinical characteristics and prognosis. Patients with "hepatic-ACLF" were characterized by relatively well-compensated underlying cirrhosis, higher transaminases and bilirubin levels and more frequent liver and coagulation organ failures. In contrast, patients with "extrahepatic-ACLF" had more severely decompensated underlying cirrhosis, higher leukocyte count levels and higher incidence of extrahepatic organ failures[8].

The identification of a precipitating factor is not a *sine qua non* condition for the diagnosis of ACLF. Interestingly, in up to 40% of patients with ACLF no precipitating event can be identified[4,6-8]. As with other patients with ACLF, this group of patients also showed data suggesting the existence of an excessive inflammatory reaction. It is evident that in some

cases, the existence of an infection as precipitating event could have been missed; however, this could have happened in a small percentage of patients, while in the remaining patients mechanisms leading to ACLF are still not clear. It is suggested that bacterial products resulting from bacterial translocation or damage-associated molecular patterns (DAMPs) resulting from injured liver tissue may act as triggering factors leading to the development of ACLF in these cases.

Pathophysiology of the syndrome

The pathophysiology of ACLF is still not well understood. The CANONIC study provided indirect data suggesting the existence of an excessive inflammatory response in patients with ACLF. These patients showed significantly higher levels of leukocyte count and C-reactive protein (CRP) compared to those of patients without ACLF. The intensity of this inflammatory response correlated with mortality[4,6,7]. A few recent studies showed results in keeping with this hypothesis and also some data suggest that patients with ACLF may also present features of immunodeficiency that could play a role in its pathophysiology[9,10]. Overall, although information is limited it seems that the pathogenesis of ACLF involves a complex alteration of immune response.

Inflammation vs immunodeficiency

Systemic inflammation in ACLF

There is growing evidence suggesting that ACLF is associated to an excessive systemic inflammatory reaction, independently of the existence of associated bacterial infections. Besides data from the CANONIC study showing higher levels of leukocytes and CRP in patients with ACLF, a few recent studies show that patients with ACLF have increased circulating levels of cytokines compared to patients with cirrhosis without ACLF[4,6,10-11].

As described above, the most common precipitating factor of ACLF are bacterial infections[4]. In these patients, whole bacteria express different pathogen-associated molecular pattern (PAMPS) that are specifically recognized by pattern-recognition receptors (PRRs), such as toll-like receptors (TLR) or NOD-like receptors (NOD). PRRs are expressed in innate immune cells and the engagement with PAMPs induces genes coding for proinflammatory cytokines. In patients with ACLF there is a "cytokine storm" leading to an excessive proinflamatory state[6,11]. Although inflammatory response is beneficial for host resistance to infection, an excessive and chronic response may cause tissue damage (immunopathology) and subsequent multiorgan failure[6,11], such as in patients with spontaneous bacterial peritonitis (SBP) in whom higher levels of proinflammatory cytokines were associated with an increased risk of renal failure[12]. Immunopathology may cause OF as a consequence of organ hypoperfusion and/or by direct effect of inflammatory mediators on cell function and death. On the other hand, OF may be developed due to an impairment of mechanisms involved in tissue homeostasis or tolerance, which means a decrease in the capacity of the host organs to tolerate this excessive inflammatory response[6,11].

Patients with ACLF without bacterial infections also show signs of excessive inflammatory response. In these patients, systemic inflammation may be initiated by PAMPs released by products resulting from bacterial translocation or by damaged-associated molecular pattern (DAMPs) released from tissue injury[13]. Both PAMPs and DAMPs may be recognized by PRR and trigger inflammation.

Immunodeficiency

Recent studies suggest that in patients with ACLF there is also a state of immunoparalysis similar to that occurring in patients with severe sepsis or septic shock. Data from these studies show that there is impaired peripheral immune response to microbial challenges, characterized by monocyte dysfunction with reduced expression of HLA-DR, increased number of MERTK receptors and decreased cytokine production[9,10]. The existence of immunodeficiency could explain, at least in part, the high susceptibility of these patients to develop secondary infections that is an independent predictor of mortality in patients with ACLF.

Histopathology

There is paucity of data on the liver histological spectrum of ACLF. A prospective study that compared 54 biopsies from patients with alcoholic-ACLF with 48 biopsies from patients with AD without ACLF showed that in patients with ACLF ductular bilirubinostasis, cholangiolitis, Mallory bodies, hepatocellular ballooning and steatosis were more frequent. Interestingly, ductular bilirubinostasis and Mallory bodies were independently related to mortality and ductular bilirubinostasis had a positive association with in-hospital infections[14]. Data from a retrospective study evaluating liver biopsies of patients with HBV or alcohol-related ACLF showed that fibrosis, ductular proliferation, and apoptosis were independently associated with poor outcome[15]. However, further studies are needed to investigate whether histology of ACLF shows any specific features different from those of patients with AD without ACLF and independent of the etiology of ACLF.

Clinical course and prognosis

As described above, ACLF is associated with high short-term mortality[2,4-6] (fig. 1). Nevertheless, ACLF is a very

dynamic syndrome with a resolution rate of approximately 40%. In fact, in the CANONIC study ACLF resolved or improved in 50%, had a steady or fluctuating course in 30% and worsened in 20%. However, the frequency of resolution depends on the initial ACLF grade. While ACLF resolved in 55% of patients with ACLF grade 1, it only resolved in 15% of patients with ACLF grade 3. Although the ACLF grade at diagnosis correlated with prognosis, the clinical course of the syndrome during hospitalization was the most important determinant of short-term mortality[4,16]. Since the majority of patients achieved their final grade of ACLF within a week, ACLF grade at days 3-7 after diagnosis predicted 28 and 90-day mortality more accurately than ACLF grades at diagnosis[4,16].

On this background, it is very important to stratify patients according to prognosis, in order to monitor treatment, determine emergency for transplantation, decide allocation in the intensive care unit (ICU) and also have a rational basis to decide futility. Different prognostic scores have been described so far derived from data of the CANONIC Study. In summary, when a patient is admitted to hospital with acute decompensation of cirrhosis, CLIF Consortium Organ Failure score (CLIF-C OFs) should be applied. This score divides patients according to the presence or absence of ACLF[4]. If patient presents ACLF, the prognosis should be assessed by the CLIF Consortium ACLF score (CLIF-C- ACLFs), which includes CLIF-C OFs, age and white cell count[17]. If patient does not have ACLF, prognosis should be assessed by the CLIF Consortium Acute Decompensation score (CLIF-C ADs), which includes age, white cell count, serum sodium, serum creatinine and INR[18]. All these scores can be calculated in the online application: http://www.clifconsortium.com.

With all this information, proposed stepwise algorithms have been built to assess prognosis and to help decision making in patients with cirrhosis and AD with or without ACLF[16-18].

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SECTION I

THE BURDEN OF ACUTE ON CHRONIC LIVER FAILURE IN USA

Alina A. Allen and Patrick S. Kamath

Division of Gastroenterology and Hepatology, Mayo Clinic

Rochester MN, USA


P.S. Kamath

Acute on chronic liver failure (ACLF) is a syndrome in patients with chronic liver disease and compensated or decompensated cirrhosis, associated with acute deterioration in liver function and one or more extra-hepatic organ failures associated with high short-term mortality. In this chapter, only features of ACLF unique to the United States will be discussed.

Burden of ACLF in the United States

The burden of ACLF in the United States has been studied from the National Inpatient Sample (NIS). This database is the largest US inpatient care database and is maintained by the US Agency for Healthcare Research and Quality as part of the Healthcare Cost and Utilization Project. The database represents a 20% stratified sample of hospitals across 46 states which comprises more than 97% of the US population. Approximately 8 million individual discharge records per year from more than 1,000 community and academic hospitals are included in the database. The primary and up to 24 secondary discharge diagnoses, procedure codes, demographic data, hospitalized inpatient mortality indicators, payer status and various other indicators are included. In 2013 chronic liver disease and cirrhosis represented the 12th leading cause of death in the United States[1].

The mortality related to cirrhosis has increased from 9.4/100,000 in 1999 to 11.5/100,000 in 2013. Mortality has increased, especially in the patients in the 55-64 years age group, which represents the aging of the hepatitis C cohort. The specific burden of alcoholic liver disease and alcoholic hepatitis, which is a common cause of ACLF, has not been reported. However, the burden of alcohol use in the United States has increased. In 2013 almost 90% of people aged 18 or older reported alcohol use at some point in their lifetime, 70% reported drinking within the past year, and 56% reported drinking within the previous month. Up to 25% of people reported binge drinking in the previous month, and 6.8% confirmed regular heavy drinking. The cost of problems related to alcohol misuse in the United States is $ 223.5 billion, with three-quarters of the cost related to binge drinking[2]. The cost related to managing patients with alcoholic hepatitis is, therefore, likely to have increased.

The prevalence of ACLF in the United States has been determined from the NIS database using the definition of the North American Consortium for the Study of End-Stage Liver Disease (NACSELD) which focused on infection as a precipitating cause for ACLF[3]. ACLF was defined as two or more extra-hepatic organ failures (cardiovascular, respiratory, renal or cerebral) in patients with cirrhosis. According to the NIS database, hospitalization rates for ACLF have increased six-fold from 5407 in 2001 to 32,335 in 2011 (fig. 1). The proportion of cirrhotic patients admitted with ACLF now accounts for 4.9% of hospitalizations, an increase from 1.46% in 2001. Of note, this higher proportion of patients with ACLF is in spite of the total number of hospitalizations for cirrhosis increasing from 371,104 to 658,884 within the same time period. Throughout this entire period the mean length of hospital stay for patients with ACLF has remained unchanged at 16 days as compared to only 7 days in patients with cirrhosis without ACLF. Hospital mortality rates have decreased but remain above 50% in patients with ACLF. On the other hand, hospitalizations for cirrhosis without ACLF are associated with only a 7% death rate.

The mean cost per ACLF hospitalization in 2011 was $53,570, as opposed to the median cost of $15,193 for patients with cirrhosis without ACLF. To put these figures

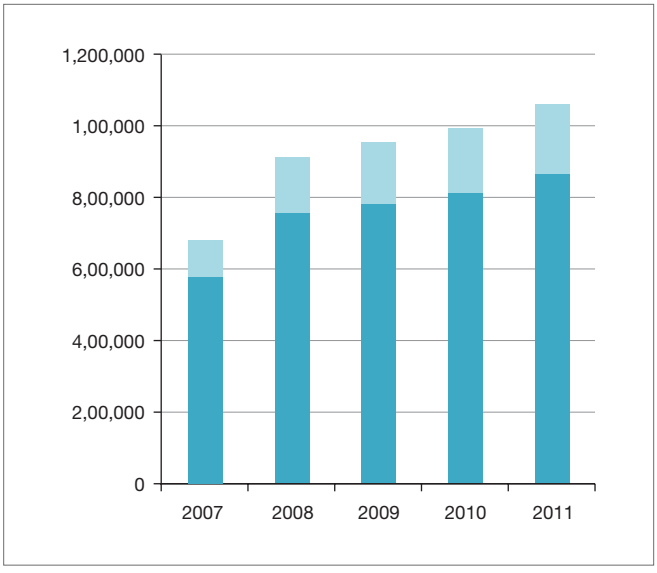

Figure 1. Cirrhosis and ACLF in the United States: hospitalization rates.

in perspective, the mean cost of a liver transplant in the United States is $201,110 ($178,760-223,460); the cost of managing variceal hemorrhage is $25,595; refractory ascites is $24,755; and hepatic encephalopathy is $16,430. The total annual hospitalization cost for ACLF in the US is currently $1.6 billion; in 2001 the annual cost was $320 million. Of note, the higher cost is related to the increase in the number of hospitalizations, and not to the cost per hospitalization which has been stable.

The costs for managing ACLF are higher when compared with chronic medical conditions such as pneumonia, congestive heart disease, and cerebrovascular disease. These conditions represent the three highest indications for hospitalizations in the US, each approximately one million admissions per year. The cost for each of these admissions is approximately $8 billion per year. Of note, the cost for looking after all patients with cirrhosis is $9.8 billion, even though the volume of hospitalizations for cirrhosis is far lower. As noted in table 1, ACLF is 7 times more expensive to manage in hospital than pneumonia, and 3.5 times more expensive than septicemia.

Etiology of ACLF

Infection

Infection is the leading cause of hospitalization, hepatic decompensation, and death in patients with cirrhosis. NACSELD analyzed 453 patients with cirrhosis from 18 centers in the United States and Canada with infection. Approximately 25% of patients developed ACLF as defined by the presence of 2 or more extrahepatic organ failures[3]. The criteria for failure were as follows: cardiovascular (shock), cerebral (grade3-4 hepatic encephalopathy), renal (need for hemodialysis), and pulmonary (need for mechanical ventilation). Among patients who developed infection-related ACLF the mortality was 51%. Urinary tract infections, spontaneous bacterial peritonitis, and spontaneous bacteremia were the most prevalent infections. The predictors for developing ACLF were nosocomial infection and admission MELD scores. Of note, 22% of patients developed second infections. Among those patients with second infections, the prevalence of ACLF was 57%[3]. In patients without a second infection the prevalence of ACLF was 26%. Among patients with cirrhosis dismissed after hospitalization for infections, the 6-month re-infection rate was 45%[4]. Twenty-six percent of patients with reinfection had an infection in the same location as a previous hospitalization, whereas 74% developed a re- infection at a different site. *C. difficile* was likely to be the most common recurrent infection at 40%. The factors associated with recurrent infections were SBP prophylaxis, age, proton pump inhibitor use, and MELD score. The presence of SBP as the initial infection was associated with a lower risk of subsequent SBP. Among with patients with infection-associated ACLF, 42% were de-listed for liver transplantation within 6 months[5]. In patients with more than two extra-hepatic organ failures, 38% had either died or were de-listed within that time frame. Respiratory failure and circulatory failure were associated with the highest risk of de-listing.

Surgery-associated ACLF

Surgery is the ideal model to study ACLF since the timing of the onset of the insult (surgery) is definable, unlike with all other precipitating factors (example: viral or drug-induced hepatitis). Moreover, patients who undergo surgery are monitored carefully and risk factors can be determined. Retrospective studies done in the United States demonstrate that mortality risk within 7 days of surgery is best determined by the American Society for Anesthesia Class; that is, number of organ failures, specifically cardiopulmonary co-morbidity[6]. Survival at 90 days is best determined by MELD score, ASA class (for patients with liver disease, compensated cirrhosis vs. decompensated cirrhosis), and age over 70 years. In patients with MELD score >20 undergoing surgery, the 30-day survival was only 50%. There was no change in mortality based on the type of surgery carried out; that is, cardiac, orthopedic, or abdominal surgery.

Table 1. The economic burden of ACLF and cirrhosis compared to the most common medical conditions requiring hospitalization in the United States in 2010

Chronic disease	Number of hospitalizations in 2010	Length of stay	Inpatient mortality	Mean cost per hospitalization
Pneumonia	$1.1 million	5.2	3.3%	$7,581
Congestive heart disease	$1 million	5.0	3.0%	$8,315
Cerebrovascular disease	$1 million	6.1	4.7%	$8,117
Septicemia	$808,000	8.8	16.3%	$15,467
Cirrhosis	$606,288	6.7	7.5%	$15,732
ACLF	$28,637	16.1	53.3%	$54,727

Future directions and conclusions

Future directions in the United States are geared towards determining the varying etiologies of ACLF. Early diagnosis of organ failure is necessary and the individual organ failures need to be redefined[7]. Prospective studies of patients undergoing surgery would be required to determine risk factors for ACLF; the inflammatory response; the specific chemokines associated with an inflammatory response; and early diagnosis of organ failure. The high rate of hospital readmissions also needs to be reduced[8]. The role of bio-artificial liver support, early liver transplantation, and when therapy is futile need to be recognized.

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SECTION II

Extrahepatic organ failure and mechanism of ACLF in cirrhosis

Chairpersons:

Agustín Albillos

Hospital Universitario Ramón y Cajal, Universidad de Alcalá, Madrid, Spain

Richard Moreau

Hôpital Beaujon, Clichy; Université Paris Diderot-Paris, Paris, France

Translocation of viable gram negative bacteria across the intestinal barrier is a frequent feature in advanced cirrhosis and may lead to infection. Most often, however, the bacterial are killed by the intestinal immune system, but bacterial bio-products known as pathogen associated molecular patterns (PAMPS) are released and reach the systemic circulation. PAMPS released in the intestinal lumen by the microbiota could also translocate directly through the intercellular space between enterocytes and reach the systemic circulation. Both processes are thought to play a critical role in the pathogenesis of systemic inflammation in cirrhosis. The mechanism by which the intestinal mucosa is so permeable to intestinal bacteria and its bio-products is complex. Intestinal hypo-motility, reduced intestinal concentration of bile salts and impaired gastric secretion lead to quantitative and qualitative changes in the microbiota. On the other hand the intestinal barrier is seriously disturbed as a consequence of portal hypertension. Systemic inflammation in cirrhosis could also be related to the release of damage associated molecular patterns (DAMPS) by the cells of the diseased liver (for example in patients with acute alcoholic hepatitis). Both, PAMPS and DAMPS interact with specific receptors in the immune cells and lead to the inflammatory response. Organ failure is the differential characteristic of ACLF. Traditionally, organ failure(s) in cirrhosis have been attributed to specific mechanisms (I.e. cardio-circulatory dysfunction for hepatorenal syndrome, impaired ammonia metabolism for hepatic encephalopathy). Recent data, however, suggest that systemic inflammation could be an important common mechanism of organ dysfunction/failure in cirrhosis. Systemic inflammation may lead to organ failure(s) by directly interfering the essential processes of organ and cell homeostasis. It causes a heterogeneous (“patchy”) disruption of the microcirculation with marked reduction in functional capillary density. Moreover cytokine and reactive oxygen radicals cause cell injury, mitochondrial dysfunction, cellular bio-energetic failure, cell/cycle arrest and cell death.

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SECTION II

CHARACTERIZATION OF SYSTEMIC INFLAMMATION IN ACLF. ROLE IN ORGAN FAILURE

Joan Clària, Cristina López-Vicario, Esther Titos and José Alcaraz-Quiles

Department of Biochemistry and Molecular Genetics, Hospital Clínic, IDIBAPS, CIBERehd, University of Barcelona, Barcelona, Spain


J. Clària

Acute-on-chronic liver failure (ACLF) is defined as the acute deterioration of pre-existing chronic liver disease, usually related to a precipitating event and associated with increased mortality due to multi-system organ failure(s). ACLF typically progresses in patients with cirrhosis undergoing acute decompensation with ascites, jaundice, variceal haemorrhage, encephalopathy and bacterial infections. The epidemiology, diagnostic criteria, characteristics, clinical course and prognosis of ACLF have recently been detailed by the CANONIC Study, a large multicenter European prospective observational investigation in 1383 patients consecutively admitted to 29 European university hospitals for the treatment of acute decompensation of cirrhosis[1]. According to the CANONIC study, approximately 31% of patients admitted to the hospital for acute decompensation of cirrhosis present at admission (20%) or develop during hospitalization (11%) the ACLF syndrome. ACLF encompasses multiorgan failure(s) (liver, kidney, brain, coagulation, circulation and/or lung) and high short-term mortality[2]. Mortality rate depends on the number of failing organs as defined by the CLIF-C OFs (a simplified version of the CLIF-SOFA score). ACLF grade 1, defined as single kidney failure or single "non-kidney" organ failure with serum creatinine of 1.5-1.9 mg/dl and/or hepatic encephalopathy grade 1-2, is the most prevalent form of ACLF (15.8% of patients admitted to hospital with acute decompensation) and has a 28-day mortality rate of 23%. Patients with ACLF grade 2 (2 failing organs; prevalence 10.9%) have an intermediate prognosis (28-day mortality rate of 31%)[3]. Finally, patients with 3 or more organ failures (ACLF grade 3), the less frequent form of ACLF (4.4%), show extremely high mortality rates reaching 75% at 28 days.

Systemic inflammation and organ failure in ACLF

Although the mechanisms underlying ACLF were not specifically addressed by the CANONIC study, this investigation described several features suggesting that ACLF occurs in the setting of exacerbated systemic inflammation. Indeed, the white blood cell (WBC) count and the concentration of C-reactive protein (CRP) in plasma were significantly elevated in patients with ACLF as compared to patients without ACLF[1]. In addition, in patients with ACLF, the WBC count and CRP levels increased in parallel with the severity of the syndrome, as estimated by the number of organ failures. Finally, ACLF was frequently associated with precipitating events that promote systemic inflammation such as bacterial infections or acute alcoholic hepatitis. All these observations have led to the CANONIC study investigators to propose the Systemic Inflammation Hypothesis to explain the pathogenesis of ACLF in decompensated cirrhotic patients[4]. According to this hypothesis, acute decompensation in patients with pre-existing cirrhosis would occur in the setting of an exacerbated systemic inflammation evoked by the presence of circulating pro-inflammatory pathogen-associated molecular patterns (PAMPs). These pathogen-derived molecules interact with specific receptors (i.e. toll like receptors (TLRs) and NOD-like receptors (NLRs) in immune cells (monocytes and polymorphonuclear leukocytes (PMNs) promoting systemic inflammation. In contrast to other diseases in which the presence of circulating PAMPs is normally related to bacterial infections, in cirrhosis circulating PAMPs may occur by translocation of bacterial products from the intestinal lumen to the systemic circulation. This is a frequent feature in patients with decompensated cirrhosis due to increased intestinal production related to intestinal bacterial overgrowth, increase permeability of the intestinal mucosa, and impaired function of the intestinal innate immune system[5]. This fact may explain why some decompensated cirrhotic patients develop ACLF without an active bacterial infection. Alternatively, systemic inflammation in the absence of bacterial infections can be the consequence of the release of damage-associated molecular patterns (DAMPs) from the injured liver. In this case, necrotic or apoptotic cells release DAMPs such as HMGB1, fragments of mitochondrial DNA, histones, ATP, cholesterol and urate crystals, that also interact with TLRs and other specific receptors and activate the innate immune cells (fig. 1)[6].

The Systemic Inflammation Hypothesis also postulates systemic inflammation as the driver of organ failure in decompensated cirrhosis. According to this hypothesis,

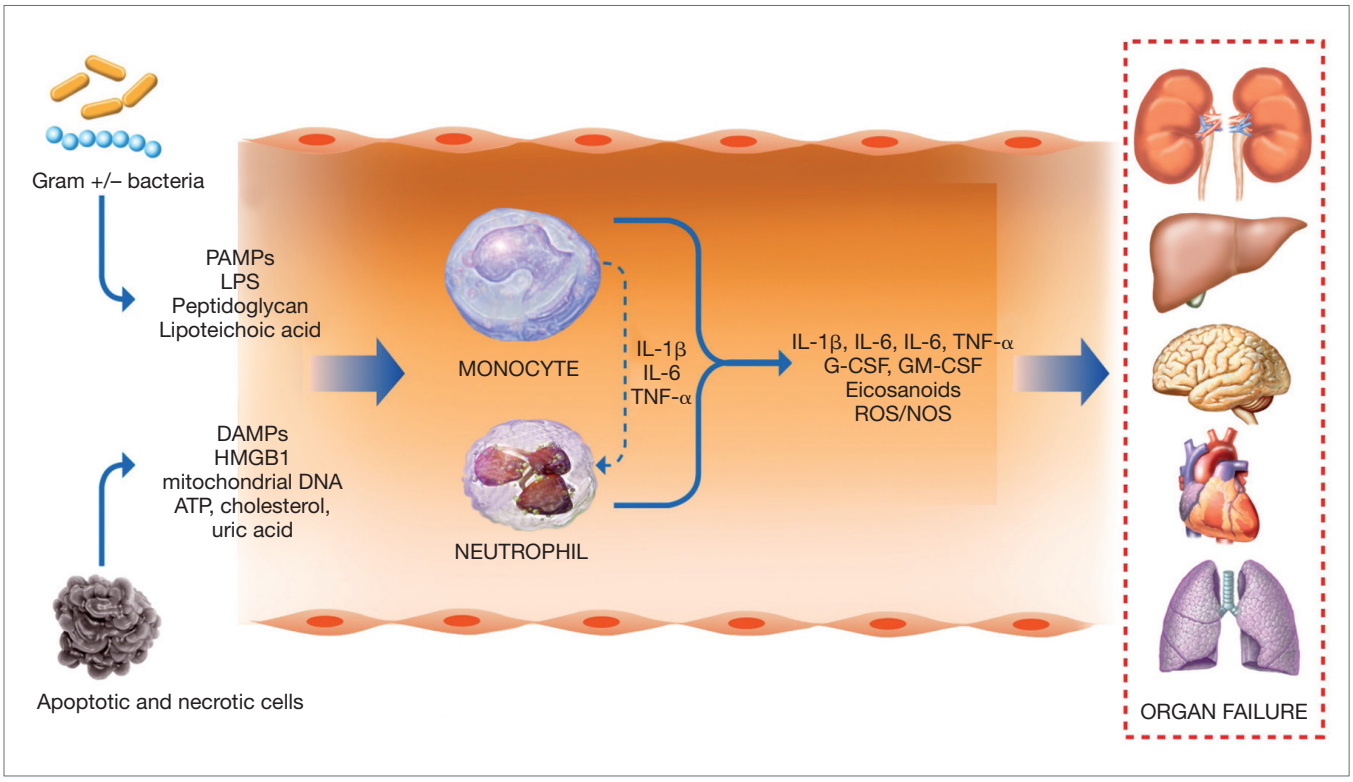

Figure 1. Schematic diagram summarizing the role of systemic inflammation in the progression of organ failure in patients with acutely decompensated cirrhosis. In this condition, systemic inflammation is likely due to the presence in the systemic circulation of pathogen associated molecular pattern (PAMP) molecules released by gram+/– bacteria (lipopolysaccharide (LPS), peptidoglycan and lipoteichoic acid) or damage associated molecular pattern (DAMP) molecules from necrotic or apoptotic dying cells (High mobility group box 1 protein (HMGB1), fragments of mitochondrial DNA, histones, ATP, cholesterol and urate crystals). These molecules interact with specific receptors present in cells of the innate immune system, especially monocytes which induce neutrophilic activation by mechanisms related to the cytokines interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α. The activation of both monocytes and neutrophils results in the bulk release of inflammatory (IL-1α, IL-6, IL-8 and TNF-α) and hematopoietic (granulocyte-macrophage colony stimulating factor (GM-CSF) and G-CSF) cytokines, accompanied by the production of eicosanoids (small lipid mediators with inflammatory properties) and reactive oxygen and nitric oxide species (ROS and NOS). The concerted action of these inflammatory mediators may cause organ failure through mechanisms related to organ hypoperfusion, tissue ischemia, tissue cell dysfunction/necrosis and hypercoagulopathy.

organ failure in these patients is not only due to impairment in systemic circulatory function and organ perfusion but also to the direct deleterious effects of the over-activated innate immune system on the microcirculation and tissue cell homeostasis (fig. 1)[4]. Renal failure, the paradigm of organ failure in cirrhosis, has been considered for many years the result of intense renal vasoconstriction promoted by the homeostatic stimulation of the renin-angiotensin system, the sympathetic nervous system and antidiuretic hormone[7]. Since these endogenous vasoconstrictors also increase intrahepatic and cerebral vascular resistance, impaired blood perfusion was proposed as the leading mechanism contributing to liver and cerebral failure in cirrhosis[8,9]. However, studies in patients with severe sepsis clearly indicated that the pathogenesis of organ failure was also associated with systemic inflammation. In fact, systemic inflammation in sepsis leads to organ failure by a direct effect of the inflammatory mediators on microvascular function, tissue cell function and cell death mechanisms[10-12]. This also appears to be the case for ACLF in patients with decompensated cirrhosis. For example, TNFα-induced activation of the NF-B-iNOS pathway was reported to account for impaired left ventricular contractility and cardiac dysfunction in cirrhosis[13]. Similarly, systemic inflammation could also be implicated in the pathogenesis of pulmonary dysfunction characterized by increased nitric oxide release in pulmonary circulation in parallel with overactivation of chemokines and macrophage accumulation in lung microvasculature[14]. Therefore, systemic inflammation may cause organ failure in patients with decompensated cirrhosis through mechanisms not only related to arterial vasodilation and impairment in left ventricular function, organ hypoperfusion and tissue ischemia but also to mechanisms leading to cell dysfunction/necrosis. In addition, it is also plausible that inflammation triggers organ failure by increasing the release of local pro-coagulant factors (including tissue factor and membrane microparticles) from the endothelial cells, inducing microthrombosis in the microcirculation.

The Systemic Inflammation Hypothesis has been widely accepted and has opened new avenues for the study of ACLF pathophysiology. Unfortunately, information on this subject is scarce and the full characterization of systemic inflammation in patients with decompensated cirrhosis is still a matter of investigation. The following pages describe most of the characteristics of systemic inflammation described up-to-date in patients with acutely decompensated cirrhosis and ACLF.

Characterization of systemic inflammation in ACLF

Several studies have attempted to properly address the distinctive profile of the innate immune system in patients with decompensated cirrhosis. The innate immune system, which protects the body against potentially disease–causing micro–organisms (pathogens) and other foreign or damaging molecules, is composed of different circulating blood immune cell types, of most importance PMNs and monocytes[15]. These cells have the ability to ingest invading bacteria and other microorganisms, foreign molecules and dying cell corpses. Monocytes also participate in the cross-talk with cells of the adaptive immune system (i.e. lymphocytes) and therefore further enhancing the body's response to the pathogen or injury by displaying proteins called antigens (derived from ingested pathogens and other molecules) on their surface. Monocytes have the ability to stop circulating in the blood and enter the tissues to become macrophages, which have similar functions to circulating monocytes. Monocytes, as well as PMN, also have the ability to orchestrate immune responses by releasing cytokines and other soluble factors such as inflammatory lipid mediators. Since these mediators are the primary determinants of the innate immune response, the term "cytokine and eicosanoid storms" has been coined for their inappropriate production under inflammatory conditions. In "sepsis-like" pathologies, such is the case of cirrhosis with ACLF, the bulk release of inflammatory mediators is closely related to the appearance of immune cell dysfunction and altered viability, also known as immune paralysis[16].

The cytokine storm

The term "cytokine storm" was originally coined to describe the excessive release of pro-inflammatory cytokines in graft-versus-host diseases[17]. The term consolidated in disease and bacterial sepsis along with other examples of tissue injury (trauma, burns), pathologies that all have in common an excessively activated innate immune system along with systemic inflammation[17]. Cytokines are low-molecular-weight proteins produced and released by immune cells in response to damage and stress stimuli[18]. The production of cytokines by immune cells is one of the initial steps of the inflammatory cascade. Once released, cytokines interact with specific receptors in their target cells (mainly neutrophils and monocytes/macrophages), where they induce multiple responses in both autocrine and paracrine fashion (i.e. interacting with the same cell or with the neighboring cells). Many cytokines act synergistically either by binding to the same cell-surface receptor or by exerting multiple overlapping effects[18]. Moreover, cytokines tend to have pleiotropic functions that may alter different cell functions such as proliferation, migration, adhesion and apoptosis, although they are best known by their immunomodulating actions. A general agreement exists that cytokines are the major determinants of systemic inflammation, since they not only favor a pro-inflammatory environment but also amplify the inflammatory process in a positive feedback loop[18].

Cytokines can be classified according to their specific activities in different subgroups, which include TNF-α, interleukins (ILs) (there are currently 18 different interleukins), growth factors (i.e. transforming growth factor (TGF) family), interferons and chemokines. Cytokines can also be classified according to their primary role in inflammation. For example, TNF-α, IL-1 and IL-6 are cytokines with primarily pro-inflammatory actions determining the classical signs of inflammation (table 1). Similar role is played by the chemokines such as IL-8, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1β (MIP-1β). In contrast, IL-4, IL-10 and IL-1 receptor antagonist (IL-1ra) are cytokines with primary anti-inflammatory properties. Other cytokines including interferon (IFN) γ, IL-17a and IL-7 are involved in the activation and shaping of the adaptive immune system. Finally, cytokines such as granulocyte-macrophage colony stimulating factor (GM-CSF) and G-CSF are implicated in hematopoiesis targeting committed progenitors to promote differentiation and activation of neutrophils and monocytes.

TNF family

TNF-α is the most relevant member of the TNF family and plays a prominent role in inflammation[19]. TNF-α secretion is induced by conserved structural elements common to microbial pathogens, which are recognized by Toll-like receptors present on the surface of immune cells[19]. Once secreted, TNF-α exerts its biological effects through binding to structurally-related receptor proteins known as the TNF receptor superfamily that embraces at least 12 different receptors. The biological activities of TNF-α are mediated through the convergence of NF-κB and NF-AT signalling pathways, which trigger an inflammatory response and promote tissue injury. Regarding liver diseases, circulating levels of TNF-α are markedly increased in patients with fulminant hepatic failure, whose levels directly correlate with disease severity[20,21]. TNF-α is also implicated in the pathogenesis of liver allograft rejection[22], chronic hepatitis B virus infection[23] and alcoholic hepatitis[24]. In cirrhotic patients, increased serum and plasma levels of

Table 1. List of the most relevant mediators of inflammation released during the "Cytokine/Eicosanoid Storm"

Mediators	Function
Cytokine*	
Tumor necrosis factor (TNFα)	Innate immune response and inducer of local and systemic inflammation; promotes activation and production of acute-phase proteins.
Interleukin-6 (IL-6)	Inflammatory and co-stimulatory action; induces proliferation and differentiation; synergizes with TGF-β to drive Th17; promotes activation and production of acute-phase proteins.
Interleukin-8 (IL-8)	Pro-inflammatory mediator, chemotactic factor for neutrophils, induces respiratory burst and phagocytosis.
C-C motif chemokine 2 (MCP-1)	Pro-inflammatory; chemotactic activity for monocytes and basophils.
Granulocyte-macrophage colony-stimulating factor (GM-CSF)	Differentiation and activation of granulocytes and monocytes/macrophages.
Granulocyte colony-stimulating factor (G-CSF)	Differentiation and activation of granulocytes.
Interleukin-10 (IL-10)	Immune suppression; decreases antigen presentation and MHC class II expression of dendritic cells; down-regulates pathogenic Th1, Th2, and Th17 responses.
Interleukin-1 receptor antagonist (IL-1ra)	IL-1ra and the soluble decoy receptor complex (composed of IL1R2 and IL1RAP) inhibit IL-1-mediated inflammatory responses.
ROS and NOS	
Superoxide anion ($O2^{-}$)	Endogenous oxygen-dependent killing mechanism of invading pathogens. Cell damage of proteins, lipids and DNA. Inflammation.
Peroxynitrite ($ONOO^{-}$)	Nitration/oxidation of proteins, lipids and DNA. Cellular damage, induces cell death by apoptosis and/or necrosis. Inflammation.
Eicosanoids	
F_2-Isoprostanes (8-epi-$PGF_{2\alpha}$)	Biomarkers of oxidative stress and lipid peroxidation. Vasoconstriction.
Prostaglandin $F_{2\alpha}$	Promotes vasoconstriction and bronchoconstriction.
Prostaglandin E_2	Pro-inflammatory actions, immunesuppression. Vasodilation
Thromboxane A_2	Platelet aggregation (hipercoagulopathy), vasoconstriction, tissue damage and fibrosis.
Leukotriene (LT) B_4	Inflammatory cell recruitment, inflammation.
$LTC_4/LTD_4/LTE_4$	Vasoconstriction, bronchospasm. Increase permeability. Inflammation. Anaphylaxis.
Other	
Platelet activating factor (PAF)	Permeability of blood vessels. PMN and platelet activation. Acute inflammation, tissue injury.
Plasminogen activator inhibitor-1 (PAI-1)	Inhibitor of fibrinolysis, coagulopathy. Cell migration. Tissue injury.

*Protein name and protein short name.

TNF-α have been reported in infected patients as compared to those non-infected[25,26]. Increased plasma TNF-α levels have also been observed in decompensated cirrhotic patients with ACLF as compared to those without, although the levels of this cytokine did not show a linear relationship with the severity of ACLF (Clària et al, unpublished observation). However, *ex vivo* TNF-α production by monocytes is remarkably decreased in patients with decompensated cirrhosis and ACLF, suggesting that these patients present a reduced cellular immune function or "sepsis-like" immune paralysis[27,28].

IL-6 family

IL-6 is the most prominent inflammatory cytokine of the IL-6 family[29]. The presence of increased serum and intrahepatic IL-6 levels has been reported in patients with acute and chronic liver diseases[24]. Similar to TNF-α, increased serum and plasma levels of IL-6 have been reported in cirrhotic patients, in particular in patients with an undergoing bacterial infection[25,26]. In a series of 522 patients with decompensated cirrhosis from the CANONIC study, plasma IL-6 levels were found to be consistently increased and to follow a parallel trend with the severity of ACLF (Clària et al, unpublished observation). In this study, IL-6 and IL-8 were the systemic inflammatory markers more closely associated with ACLF. Moreover, IL-6 showed a clear relationship with the clinical course of ACLF, in such a way that lower levels were associated with improvement whereas high levels were associated with worsening of ACLF. In these patients, plasma IL-6 levels were particularly higher in patients with bacterial infection-associated ACLF. Finally, plasma IL-6 levels were strongly associated with 28- and 90- day mortality. It is important to mention that IL-6 is one of the most important mediators of the hepatic acute phase response and potently increases the synthesis of positive acute phase proteins such as CRP[30].

Therefore, high IL-6 concentrations probably contribute to increased CRP levels in patients with ACLF, despite these patients having liver dysfunction or failure. In any event, and although IL-6 is an established pro-inflammatory cytokine, its overall role in liver disease is still intriguing because it appears to have essential functions in protecting the integrity of liver tissue organ during acute or chronic injury[31].

IL-8 and the chemokine family

The chemokine family plays an important role in attracting granulocytes into sites of inflammation. Up to date, four different subfamilies of chemokines have been described according to highly conserved cysteine motifs in their aminoterminal domain[32]. CXC and CC chemokines are the two major subfamilies of chemokines, which differ in their cell targets. Whereas CXC chemokines such as IL-8 and GRO activate PMN predominantly, CC chemokines such as RANTES and MCP-1 mainly activate monocytes[32]. Among these, IL-8 plays a relevant role in acute inflammation not only by recruiting PMN into tissues and sites of inflammation but also by inducing degranulation and release of lysosomal enzymes by activated PMN. In these granulocytes, IL-8 binds with high affinity to two different receptors: CXCR1 (IL-8RA) and CXCR2 (IL-8RB). In the context of liver diseases, increased levels of IL-8 and its receptors have been reported in patients with severe alcoholic hepatitis and hepatic protein expression of IL-8 is an independent predictor of short-term mortality[33,34]. In a recent study from our laboratory, IL-8 levels were observed to be also significantly increased in patients with decompensated cirrhosis and ACLF, in whom this chemokine was a discriminate marker of active alcoholism as the ACLF precipitating event (Clària et al, unpublished observation). Moreover, in these patients, IL-8, similar to IL-6, followed a parallel trend with the severity of ACLF.

IL-1 family

The IL-1 family forms a gene cluster located on chromosome 2q and contains within a 430-kb region three related genes (IL1A, IL1B and IL1RN) that encode for three of the most important cytokines of the inflammatory process, namely IL-1α, IL-1β and IL-1ra[35,36]. Among these, IL-1α, which is produced via non-classical pathways of cytokine secretion is the most relevant. Indeed, PAMPs and DAMPs launch a unique signaling pathway called the inflammasome, a multiprotein complex that turns on the protease caspase-1, which in turn cleaves pro-IL-1α into its active mature form, IL-1α, before it is transported outside the cell[37]. It is important to mention that both pro-IL1α and IL-1α are very vulnerable to degradation in inflammatory fluids, which may explain why this cytokine is virtually undetectable in human plasma[38]. In our hands, plasma IL-1β levels range from 0.8 to 82.6 pg/ml in patients with decompensated cirrhosis, although this cytokine was only detectable in 16% of these patients[39]. In contrast, IL-1α, the synthesis of which parallels the formation of IL-1α, and IL-1ra, which has a relatively longer half-life compared to IL-1, were readily detectable in plasma in 65% and 94% of patients with decompensated cirrhosis, respectively, suggesting that circulating IL-1 in plasma does not properly reflect the real activity of the IL-1 system.

In a recent study we have identified two single nucleotide polymorphisms (SNPs) within the IL-1 gene cluster that were found to influence the degree of systemic inflammation in patients with decompensated cirrhosis and therefore the susceptibility of these patients to develop ACLF[39]. Specifically, we identified a SNP in the promoter of the gene coding for IL-1β, which was associated with a lower risk of developing ACLF. This SNP had functional significance, and carriers of this SNP presented reduced circulating levels of IL-1α accompanied by an attenuated degree of systemic inflammation as estimated by lower IL-1α, IL-6, G-CSF and GM-CSF levels and reduced CRP and WBC count. In addition to the IL-1α SNP, we identified another SNP in the promoter of the IL-1ra gene, a cytokine that inhibits inflammation by antagonizing the binding of IL-1 to its receptor. This observation open new avenues for exploring anti-inflammatory therapies based on recombinant human IL-1ra, which has been shown to be effective in critically ill pediatric patients and in patients with sepsis and organ dysfunction and/or a predicted risk of mortality of 24% or greater[38,40].

The eicosanoid storm

Eicosanoids, which are small bioactive lipids originating from the cleavage of structural lipid components of cellular membranes, constitute one of the most well-established classes of endogenous mediators of inflammation[41]. The eicosanoid storm, similar to the cytokine storm, plays a primordial role in infection and inflammation and inhibiting the formation or blocking the receptor-mediated actions of classical eicosanoids (that is, prostaglandins and leukotrienes) by aspirin and other anti-inflammatory drugs (i.e. NSAIDs) remains a prevailing strategy to alleviate inflammation. Contrary to cytokines, eicosanoids are immediately produced on demand by immune cells and act locally on nearby cells and tissues.

Eicosanoids are generated from the essential omega-6 polyunsaturated fatty (PUFA) acid arachidonic acid[42]. Their biosynthesis is initiated by the activation of phospholipase A_2 and the release of arachidonic acid from membrane phospholipids in response to the interaction of a danger stimulus with a receptor on the cell surface. Free arachidonic acid is then available as a substrate for the intracellular biosynthesis of eicosanoids through two major enzymatic routes, namely the cyclooxygenase (COX) pathway and the lipoxygenase (LO) pathway. The COX pathway results in the formation of prostaglandins (PGs) which are known for their

powerful physiological properties and their critical role in the inflammatory response (table 1). On the other hand, the LO pathway comprises three major LOs, designated 5-LO, 12-LO and 15-LO, being 5-LO, which converts arachidonic acid into leukotrienes (LTs), also a consolidated pharmacological target in inflammation. Alternatively, arachidonic acid can be converted through free radical-catalyzed peroxidation to a unique series of PG-like compounds, known as isoprostanes (i.e. 8-epi-$PGF_{2\alpha}$, which work as accurate markers of oxidative stress.

The formation and actions of eicosanoids in patients with liver cirrhosis has extensively been studied in the context of renal dysfunction[43]. However, scarce information is available on these mediators in the context of systemic inflammation in patients with decompensated cirrhosis. Early studies have demonstrated an altered biosynthesis of eicosanoids and a defective response to these mediators of PMN and monocytes from cirrhotic patients[44]. These findings were interpreted as the regulation of leukocyte function by eicosanoids is impaired in cirrhosis, contributing to the development of "immune paralysis" in these patients. This idea has been reinforced by a recent publication by O'Brien et al demonstrating that the eicosanoid PGE_2 drives immunosupresion and increases the risk of infection in acutely decompensated cirrhosis[28]. In any event, since hundreds of structurally and stereochemically distinct eicosanoid species can be generated from arachidonic acid and other omega-6-derived PUFA such as dihomo-γ-linolenic acid — the origin of which is the 18-carbon essential fatty acid linoleic acid — as well as from omega-3-derived PUFAs such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), the cytokine storm that accompanies inflammation needs to be exhaustively profiled in biological samples from patients with decompensated cirrhosis and ACLF. This task can be facilitated by the use of mass spectrometry-based lipidomic technologies able to profiling and quantify hundreds of distinct eicosanoids and related lipid mediators involved in inflammation.

Conclusions

In this chapter, we have summarized and discussed our current understanding of the salient characteristics of systemic inflammation in patients with decompensated cirrhosis and ACLF. We have described the most important hallmarks of systemic inflammation in this disease and highlight the role of cytokine and eicosanoid storms as drivers for the development of organ failure(s) in acutely decompensated cirrhosis.

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SECTION II

MECHANISM OF SYSTEMIC INFLAMMATION IN ACLF IN CIRRHOSIS

Richard Moreau

Inserm, U1149, Centre de Recherche sur l'Inflammation (CRI), Clichy and Paris, France

UMRS1149, Université Paris Diderot-Paris 7, Paris, France

Département Hospitalo-Universitaire (DHU) UNITY, Service d'Hépatologie, Hôpital Beaujon, Assistance Publique-Hôpitaux de Paris, Clichy, France

Laboratoire d'Excellence Inflamex, ComUE Sorbonne Paris Cité, Paris, France


R. Moreau

Systemic inflammation is a hallmark of acute-on-chronic liver failure (ACLF) which complicates cirrhosis[1]. Indeed, among patients with acutely decompensated cirrhosis, white-cell count as well as plasma levels of C-reactive protein and major cytokines (i.e., interleukin (IL-6, tumor necrosis factor (TNF-α) or chemokines (e.g., IL-8) are higher in patients with ACLF than in those without[1,2]. Before commenting on inflammation in ACLF, one should have in mind general principles of inflammation.

General principles of inflammation

The inflammatory response involves stimuli (inducers; that can be exogenous or endogenous), sensors that are molecules of the host which detect stimuli, and the effectors engaged by sensors.

Inducers and sensors of inflammation

Exogenous inducers of inflammation

Exogenous inducers can be microbial or non-microbial[3]. Here we will concentrate on bacterial stimuli because bacterial infection is a trigger of 30% of cases of ACLF.

Bacterial inducers include two classes of molecules: pathogen-associated molecular patterns (PAMPs) and virulence factors[3]. PAMPs are sensed directly via structural feature recognition[3-5]. Indeed, PAMPs are unique microbial (here bacterial) structures that are detected by pattern-recognition receptors (PRRs) of the host[3-5] (table 1). Among PRRs, there are Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD-like receptors (NLRs), retinoic acid-inducible gene (RIG-I (a member of the RIG-I-like receptor family), cytosolic DNA sensors. PRRs have different localization (table 1): PAMP recognition by PRRs stimulates signaling pathways that activate transcription factors (TFs) including nuclear factor (NF-κB, activator protein 1, interferon regulatory factors, among others[4]. Activated TFs induce a battery of genes encoding antimicrobial effectors; cytokines and chemokines that orchestrate the inflammatory and innate immune responses as well as molecules inducing the adaptive immunity (e.g., molecules involved in antigen presentation)[3,4]. A given PAMP can be recognized by different PRRs. For example, extracellular flagellin is detected by TLR5 and cytosolic flagellin by the protein NAIP5 (for NLR family, apoptosis inhibitory protein 5) (table 1). Fagellin detection by NAIP5 leads to a physical interaction of NAIP5 with the canonical inflammasome protein NLRC4 (NLR family, CARD domain containing 4)[6]. This engages the adaptor called apoptosis-associated speck-like protein containing a CARD (also known as PYCARD or ASC) and activates caspase-1 (a pro-inflammatory caspase) to promote cleavage and secretion of IL-1β and IL-18. Interestingly, intracellular LPS (a PAMP expressed by Gram-negative bacteria) can activate the noncanonical inflammasome; it is non-canonical in that another inflammatory caspase (caspase-11 in mice; caspase-4 or -5 in humans) is required in addition to ASC and NLRC4[7]. This non-canonical inflammasome activation is independent of TLR4[7]. Caspase-11 (or its orthologs in humans) are immune receptors for intracellular LPS[8]. Interestingly, engagement of inflammatory caspases can drive a form of programmed cell death called pyroptosis whose purpose is to suppress the infected cell[5].

The presence of virulence factors (and hence that of the invading bacteria that produce these factors) is sensed by a process called functional feature recognition[5]. In other words, virulence factors are sensed not directly (through their molecular structure) but indirectly via their functional effects that disrupting host tissue homeostasis[3,5]. For example, some bacterial exotoxins form pores in host cell membranes that result in K+ transmembrane movements K+ movement that are detected by NLRP3 inflammasome and leads to IL-

Table 1. Examples of bacterial, fungal and endogenous inducers of inflammation with their receptors and localization of receptors

Inducers	Receptors*	Receptor localization
Bacteria		
PAMPs		
Triacylated lipopeptides	TLR1	Cell surface
	TLR2	Cell surface
Diacylated lipopeptides	TLR2	Cell surface
	TLR6	Cell surface
Peptidoglycan	TLR2	Cell surface
Lipopolysaccharides	TLR4	Cell surface
	inflammatory caspases**	Cytosol
Flagellin	TLR5	Cell surface
	NAIP5 and NLRC4	Cytosol
Lipoteichoid acid	TLR6	Cell surface
Single-stranded RNA	TLR7	Endolysosome
CpG-DNA	TLR9	Endolysosome
γ-D-glutamyl-mesodiaminopimelic acid	NOD1	Cytosol
Muramyl dipeptide	NOD2	Cytosol
	NLRP1	Cytosol
mRNA	NLRP3	Cytosol
	DDX58 (alias: RIG-I)	Cytosol
Double-stranded DNA	AIM2	Cytosol
	IFI16	Cytosol
	ZBP1	Cytosol
	MB21D1 (alias: cGAS)	Cytosol
Double-stranded DNA, single-stranded DNA	LRRFIP1	Cytosol
Cyclic-di-GMP, cyclic-di-AMP	TMEM173 (alias: STING)	Endoplasmic reticulum surface
Double-stranded DNA, single-stranded DNA	HMGB proteins	Cytosol
Bacterial pigmented virulence factors		
Phenazines, naphtoquinone phtiocol	AHR	Cytosol (unbound form)
Fungus		
high mannose, fucose	Mannose receptor, CD206, CLEC13D	Cell surface
mannose, fucose, N-acetyl-glucosamine, β-glucan	Langerin, CD207, CLEC4K	Cell surface
high mannose and fucose	DC-SIGN, CLEC4L	Cell surface
β-1,3 glucans	Dectin-1, CLEC7A	Cell surface
high mannose, α-mannans	Dectin-2, CLEC6A, CLEC4N	Cell surface
α-mannose, glycolipids	Mincle, CLEC4E	Cell surface
Mannose, fucose	BDCA-2, CD303, CLEC4C	Cell surface
Mannose, fucose	DCIR, CLEC4A	Cell surface
O-linked mannan	TLR4	Cell surface
Phospho-lipomannan	TLR2	Cell surface
Endogenous molecules (DAMPs)		
Released by necrotic cells		
ATP	Purinoceptors	Cell surface
HMGB1	AGER (alias: RAGE)	Cell surface
	TLRs	Cell surface
Histones	TLR2, TLR4	Cell surface
	TLR9	Endolysosome
IL-1 family		
IL-1α	IL1R1 and IL1RAP	Cell surface
IL-33	IL1RL1 and IL1RAP	Cell surface
S100 calcium-binding protein family		
S100A8, S100A9	TLR4	Cell surface
S100A12	AGER	Cell surface
Mitochondrial DAMPs		
mtDNA	TLR9	Endolysosome
N-Formylated peptides	FPR	Cell surface
Peroxideroxins	TLR2, TLR4	Cell surface
Heat shock proteins	TLRs?	?

Table 1. *(Cont.)*

Inducers	Receptors*	Receptor localization
Products of extracellular matrix breakdown		
Low-molecular weight fragments of hyaluronic acid	TLR4	Cell surface

*Unless specified, symbols used for receptors are official gene symbols provided by Hugo Gene Nomenclature Committee (HGNC; http://www.genenames.org)
**Inflammatory caspases are caspase-4, -5 in humans and caspase-11 in mice.
***NAIP in humans
Abbreviations: PAMPs, pathogen-associated molecular patterns; TLR, Toll-like receptor; Ly-96, lymphocyte antigen 96; LBP, lipolysaccharide-binding protein; NAIP, nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family, apoptosis inhibitory protein; NLRC4, NLR family, CARD domain containing 4; NLRP1, NLR family, pyrin domain containing 1; NLRP3, NLR family, pyrin domain containing 3; DDX58, DEAD (Asp-Glu-Ala-Asp) box polypeptide 58; RIG-I, retinoic acid-inducible gene I; CpG-DNA, DNA containing the unmethylated phosphate-guanine(CpG) dideoxynucleotide motif; AIM2, absent in melanoma 2; IFI16, interferon, gamma-inducible protein 16; ZBP1, Z-DNA binding protein 1, MB21D1, Mab-21 domain containing 1; cGAS, cyclic GMP-AMP synthase; LRRFIP1, leucine rich repeat (in FLII) interacting protein 1; cyclic-diGMP, cyclic diguanylate monophosphate; cyclic-di-AMP, cyclic diadenosine monophosphate; TMEM173, transmembrane protein 173; STING, stimulator of interferon genes protein; HMBG, high-mobility group box; AHR, aryl hydrocarbon receptor; DAMPs, damage-associated molecular patterns; AGER, advanced glycation end-product-specific receptor; IL, interleukin; mtDNA, mitochondrial DNA; FPR, formyl peptide receptor.

1β production[9]. Some virulence factors such as cytotoxin TcdB6-8, a major virulence factor of Clostridium difficile cause modifications and inactivation of host Rho GTPases and these alterations are sensed by the Pyrin inflammasome[10]. Interestingly there are virulence factors which are sensed directly via structural feature recognition. This is the case of bacterial pigmented virulence factors such as phenazines from Pseudomonas aeruginosa that are ligands of the arylhydrocarbon receptor (AhR). Phenazine-induced AhR activation leads to degradation of this virulence factor and induction of inflammation[11]. Therefore phenazines may be PAMPs and AhR as a PRR (table 1).

Endogenous inducers of inflammation

Endogenous inducers are produced as a result of tissue damage or injury. They include molecules released by necrotic cells and products of breakdown of the extracellular matrix (ECM). They may be recognized by sensors expressed at surface of resident macrophages and trigger inflammation. These endogenous inducers of inflammation are called danger-associated molecular patterns (DAMPs)[12].

Necrosis can be accidental (due to decreased oxygen delivery) or a result of the induction of programmed cell death. Programmed cell necrosis includes pyroptosis and necroptosis. As mentioned earlier, pyroptosis results from inflammasome activation and necroptosis from engagement of the receptor-interacting protein kinase (RIPK)-3/mixed lineage kinase domain-like (MLKL) pathway[13]. Necrotic cells can release ATP[3,12], high-mobility group box 1 protein (HMGB1)[3,12], histones[14,15], members of the IL-1 family (IL-1α, IL-33)[13], members of the S100 calcium-binding protein family (S100A8, S100A9 and S100A12)[3,12], mitochondrial DAMPs (mitochondrial DNA (mtDNA), formyl peptides)[16], peroxideroxins[17], and heat shock proteins[3,12] (table 1). ATP binding to purinoceptors may contribute to NLRP3 inflammasome activation in macrophages. HMGB1 engages the receptor called advanced glycation end-product-specific receptor (RAGE), and this receptor may cooperate with TLRs to evoke an inflammatory response. Histones may engage TLR2, TLR4, or TLR9. IL-1α and IL-33 engage specific receptors mediating inflammatory signals. S100A8 and S100A9 signal through TLR4. S100A12 engage the receptor RAGE. Mitochondrial DAMPs activate neutrophils; this activation is mediated by both TLR9 (that recognizes mtDNA) and the G-protein-coupled receptors formyl peptide receptor 1 (that are engaged by mitochondrial formyl peptides). Peroxiredoxins are antioxidant molecules that can engage TLR2 and TLR4. Some of the heat shock proteins released by necrotic cells may induce inflammation through cell surface TLRs; however, these findings are controversial.

Breakdown products of the ECM are generated during tissue damage. For example, tissue injury promotes hyaluronate breakdown into low-molecular-weight fragments, which are inflammatory, activating TLR4[3,4].

Areas of uncertainty

Nature of the stimulus

The presence a pathogen triggers inflammation because it represents a threat for host tissue homeostasis. It has been suggested that disruption of cell homeostasis by some stressors could trigger inflammation[3]. For example, epithelial cells can be affected by oxidative stress, hypoxia, glucose deprivation, endoplasmic reticulum (ER) stress, among others.

Stressed cells, via elusive messengers, may stimulate resident macrophages to produce chemokines that recruit circulating myeloid cells[3]. This would cause tissue inflammation. This interesting hypothesis deserves future studies to be confirmed.

Outcomes of the inflammatory response

The aim of the inflammatory response to bacterial infection differs from the aim of the DAMP-mediated response to tissue injury: the purpose of the former is to promote host resistance by reducing bacterial burden while the aim of the latter primarily is to promote tissue repair[3,5]. This question should be addressed because of important therapeutic implications.

It is generally considered that infection-induced organ failure is the result of collateral tissue damage caused by an excessive immune response of the host. This process is called immunopathology. However, infection can also cause direct tissue damage via toxins and virulence factors[3,5]. There are other cases in which severe outcome is related to bacterial overgrowth due to failed host resistance (immune suppression)[18]. On the other hand, recent studies have shown that severe outcome of bacterial infection can be caused by failure of protective tissue-intrinsic mechanisms. In other words, severity of the disease may be related to failed disease tolerance (i.e., endurance)[18-20]. All these mechanisms should be investigated in the future.

Acute on chronic liver failure

A limitation of research in the field of ACLF is the lack of available murine models of ACLF. Therefore, information on pathophysiology of this syndrome come from observational and translational studies performed in patients. Mechanisms of the systemic inflammatory response differ according to the context in which it develops: bacterial sepsis, severe alcoholic hepatitis, or no identifiable trigger for ACLF.

Sepsis-induced ACLF

Spontaneous bacterial peritonitis (SBP) caused by Gram-negative bacteria of intestinal origin is the most common infection in patients with cirrhosis[20]. The mechanisms explaining how viable bacteria migrate across the intestinal barrier are unclear[21,22]. SBP is more common in patients with ACLF than in those without[1]. However not all patients with SBP will develop ACLF[1] and the reasons for this are unclear. In patients with SBP, the greater the intensity of the immune response, the higher the risk of developing type 1 hepatorenal syndrome (HRS, a form of ACLF)[22]. Therefore, immunopathology may be a major mechanism explaining the development of ACLF in patients with SBP. Patients with ACLF are younger than those without ACLF and this difference in age may contribute to differences in immune responses between the two groups[1]. Indeed, younger age is known to be a factor of more vigorous immune response to infection[18]. In addition, genetic factors may play a role in the excessive immune response measured in some patients with SBP. Single-nucleotide polymorphisms (SNPs) in gene encoding PRRs (NOD2, *TLR4*, *TLR2*) or nuclear receptor (*NR1H4* encoding FXR) are associated with increased risk of severe bacterial infection in patients with cirrhosis[21]. The impact of genetic variants on the host immune response to PAMPs should be extensively studied in cirrhosis.

Severe alcoholic hepatitis

Severe alcoholic hepatitis is a form of ACLF[1]; it is present in 20% of the cases of ACLF. Pathological features of alcoholic hepatitis include hepatocyte death and liver inflammation, with prominent neutrophil infiltration[23]. Several mechanisms may contribute to these features. Excessive alcohol consumption induces gut dysbiosis and increases permeability of the intestinal barrier[23]. As a result, there may be translocation of bacterial PAMPs which are TLR ligands (e.g., LPS) (table 1). These PAMPs could then reach the liver where they are recognized by TLRs expressed in Kupffer cells (i.e., resident macrophages). This recognition stimulates the production of pro-inflammatory CXCL chemokines (e.g., IL-8) and TNF-α[23,24]. CXCL chemokines are potent neutrophil-attracting and –activating cues. Activated neutrophils produce large amounts of reactive oxygen species (ROS) that may cause deleterious oxidative stress in environing hepatocytes[23]. Moreover, in hepatocyte alcohol metabolism by cytochrome P450 2E1 generates substantial amount of ROS causing cell stress (including ER stress). Stressed hepatocyte stress may produce as-yet unidentified signals that activate Kupffer cells to produce inflammatory molecules (see above). In addition, LPS-induced TNF-α can cause hepatocyte necrosis[23] that would result in the release of DAMPs and subsequent accentuation of inflammation (table 1).

Patients with severe alcoholic hepatitis have a systemic inflammatory response syndrome (SIRS)[25] whose trigger is unclear: it may originate from liver inflammation and/or high systemic levels of LPS. Studies are needed to determine the respective role of these mechanisms in the development of SIRS in the context of severe alcoholic hepatitis.

Areas of uncertainty

ACLF of unknown origin

ACLF is of unknown origin in ~50% of cases[1]. These cases are also associated with systemic inflammation[1]. There is no clear explanation for the induction of systemic inflammation in patients with ACLF of unknown origin. Three hypotheses

have been proposed to explain inflammation in these patients: the first one involves gut dysbiosis; the second suggests a role for translocation of bacterial PAMPs and the third suggests an action of endogenous inducers of inflammation (see earlier and table 1).

Patients with cirrhosis exhibit gut dysbiosis whose pattern changes according to the stage of cirrhosis (compensated vs. decompensated) and its severity (survivors vs. non-survivors)[22]. Moreover some changes of gut microbiome were found to be correlated with a more intense systemic inflammation[22], suggesting that metabolites produced by the intestinal microbiome may contribute to inflammation. Clearly, metagenomics and metabolomics are required for further investigations in patients with cirrhosis, at different stages of the liver disease.

The second hypothesis is based on the finding that patients can have bacterial translocation but do not develop bacterial infection[22], suggesting that bacteria are killed soon after they crossed the intestinal barrier. Nevertheless, bacteria could release PAMPs such as LPS or CpG DNA that may reach the systemic circulation, be recognized by TLRs at different sites and trigger inflammation[22]. This hypothesis is plausible because TLR recognition is generally not dependent on microbial viability or invasiveness[5]. The third hypothesis for inflammation in ACLF of unknown origin involves DAMPs (table 1). Together these findings indicate that future studies should investigate the profile of circulating PAMPs and DAMPs in patients with cirrhosis, at different stages of the liver disease.

Role of immune suppression

Immune suppression may develop in patients with ACLF. For example, circulating CD14+ immune cells from patients with ACLF are enriched with a MER-expressing subset of cells[2]. These cells have reduced inflammatory responses to ex vivo LPS stimulation. These findings are consistent with the well-known effects of MER receptors which are receptor-tyrosine kinases of the TAM family that inhibit TLR4-mediated pro-inflammatory signaling. It is important to note that, like other TAM receptors such as AXL, MER plays a major role in the engulfment and clearance of apoptotic cells by phagocytes. However, unlike the induction of *AXL*, the induction of *MERTK* (the gene coding for MER) does not occur in response to inflammatory signals but occurs in response to stimuli homeostatic-restoring signals[26]. Therefore MER-expressing CD14+ cells may be engaged in the context of ACLF to protect damaged tissues. This question should be addressed in future studies.

Another study found that plasma from acutely decompensated patients with cirrhosis and ACLF had increased levels of prostaglandin E2 (PGE2) which may inhibit the macrophage TNF-α production in response to LPS[27]. In addition, PGE2 was found to reduce the macrophage ability to kill bacteria[27]. These results suggest that increased plasma PGE2 levels in cirrhotic patients may play a role in immune suppression and increased susceptibility to bacterial infection. Future studies are required to confirm these hypotheses.

Conclusions

Future studies should develop mouse models of ACLF. In addition, translational research using prospectively collected biological samples should be performed in patients with cirrhosis at different stages of the liver disease: compensated, decompensated stable, acute decompensation with or without ACLF. This research should also be longitudinal with information obtained in the same patients at different stages of the disease. Translational research should describe the profiles of plasma components such as cytokine/chemokines, bacterial PAMPs, DAMPs, among others at different stages of the disease and changes in these profiles during disease progression. Translational research should include ‘omics’ approaches to better understand the gene-by-environment interactions that may determine the outcome of bacterial infections as well as the impact of alterations in gut microbiome on progression and severity of cirrhosis.

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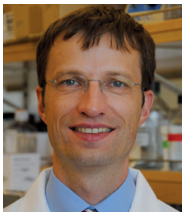
TRANSLOCATION OF BACTERIA AND BACTERIAL PRODUCTS FROM THE INTESTINAL LUMEN IN CIRRHOSIS. ROLE OF MICROBIOTA, INTESTINAL MUCOSAL INTEGRITY AND LOCAL IMMUNE FUNCTION

Phillipp Hartmann[1] and Bernd Schnabl[1,2]

[1]*Department of Medicine, University of California San Diego, USA*

[2]*Department of Medicine, VA San Diego Healthcare System, San Diego, USA*


B. Schnabl

Cirrhosis is amongst the 14 most common causes of death worldwide with a 1-year mortality from 1 to 57%, largely depending on the occurrence of decompensating events[1]. In contrast, the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) define Acute-on-Chronic Liver Failure (ACLF) as an acute deterioration of pre-existing decompensated liver cirrhosis, typically related to a precipitating event (including bacterial infections) and associated with increased mortality at 3 months due to multisystem organ failure[2,3]. Organ failure in this context can be diagnosed with a bilirubin > 12 mg/dL (liver), creatinine ≥ 2 mg/dL (kidney), West-Haven grade ≥ 3 (brain), international normalized ratio (INR) ≥ 2.5 (coagulation), low mean arterial pressure (MAP) necessitating vasopressors (circulation), $PaO_2/FiO_2 \leq 200$ or $SpO_2/FiO_2 \leq 214$ (respiration), according to the EASL-Chronic Liver Failure (CLIF) Consortium Organ Failure score (CLIF-C OFs)[4]. Liver cirrhosis is associated with pathological processes in the gut which contribute to further deterioration of liver cirrhosis. These are related to pathological bacterial translocation. Bacterial translocation describes the passage of live bacteria or bacterial products from the gastrointestinal tract to mesenteric lymph nodes or other extra-intestinal organs[5]. Liver cirrhosis is further accompanied by several changes of the intestinal microbiome leading to intestinal dysbiosis with reduced microbial diversity, decreased abundances of beneficial bacteria and increased quantities of potentially pathogenic bacteria[6]. Several studies have conclusively shown that subjects with liver cirrhosis display significantly smaller amounts of the probiotic *Bifidobacterium* spp. and *Lactobacillus* spp. but more *Enterococcus faecalis* at the genus level, and more abundant Enterobacteriaceae and fewer Bacteroidaceae at the family level relative to healthy controls[6-12]. Interestingly, Lachnospiraceae (positively) and Streptococcaceae (negatively) also seem to correlate with the severity of liver cirrhosis[10]. Changes in the bacterial composition associated with cirrhosis have been summarized in recent reviews[6,7].

Because compensated and in particular decompensated liver cirrhosis is associated with dysbiosis, a disrupted gut barrier and bacterial translocation, we will highlight additional changes that occur during ACLF and possibly contribute to the onset of disease. How does the gut microbiota change in ACLF? In what way is the gut further affected in ACLF, in particular its mucosal integrity and its local immune function? And how does the gut in return communicate with the liver during ACLF?

Role of the intestinal microbiome

As mentioned above, significant changes in the composition of the intestinal microbiota occur in cirrhotics[6,7]. Furthermore, in one out of three cirrhotic patients ACLF has been induced by a bacterial infection; bacterial infections are hence identified as the most frequent precipitating event of ACLF[3,4,13]. The intestinal microbiota plays therefore a central part in the pathogenesis of ACLF. 16S ribosomal DNA pyrosequencing of fecal microbiota evidenced that, similar to cirrhotics, subjects with ACLF exhibit a significantly decreased diversity and richness of their microbial communities compared with healthy controls (according to observed operational taxonomic units (OTUs), Chao1, Shannon, and Simpson indices)[14]. Patients with ACLF (relative to controls) show less Bacteroidetes, more Firmicutes and Proteobacteria at the phylum level; similar changes at the respective class level: less Bacteroidia, more Bacilli and Gammaproteobacteria; and also analogues changes at the respective family level: less Bacteroidaceae and Porphyromonadaceae; more Streptococcaceae and

Enterococcaceae; and more Pasteurellaceae[14] (table 1). The Firmicutes family Veillonellaceae was enriched and proportions of the Firmicutes families Ruminococcaceae and Lachnospiraceae were found lower in ACLF subjects compared with healthy controls[14]. Ruminococcaceae and Lachnospiraceae produce the short-chain fatty acid (SCFA) butyrate which has been linked to promoting gut health by increasing the intestinal barrier function[6,14,15]. Their role could hence be viewed as protective. In addition, the level of Ruminococcaceae was inversely proportional to the blood level of the membrane component of Gram-negative bacteria lipopolysaccharides (LPS, or endotoxin)[9]. It was also negatively correlated to the model for end-stage liver disease (MELD) scores in cirrhotic patients[9]. Moreover, fecal analysis of ACLF patients with hepatic encephalopathy (HE) found depressed levels of Lachnospiraceae relative to ACLF subjects without HE[14]. Non-survivors of an ACLF also showed reduced proportions of the family Lachnospiraceae compared with ACLF survivors. Patients who did not survive ACLF had an augmented relative abundance of the phylum Proteobacteria, its class Gammaproteobacteria and its family Pasteurellaceae. Interestingly, the relative abundance of Pasteurellaceae was found to be an independent predictor of mortality in patients suffering ACLF besides the MELD score itself. The overall microbial structure appears stable in the first 28 days post-ACLF[14]. Antibiotic treatment of ACLF patients resulted in a significant enrichment of Ruminococcaceae. Ruminococcaceae and Lachnospiraceae displayed a negative correlation with the cytokines Tumor necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6)[14]. How far this is causatively related requires further studies, as the blood levels of several pro- and anti-inflammatory cytokines such as TNF-α and IL-6 are augmented in ACLF[16]. Subjects with ACLF show less abundant Clostridiales Family

Table 1. Changes in intestinal microbiota associated with Acute-on-Chronic Liver Failure (ACLF)

		Implicated microbiota				
Disease	Comparison	Phylum	Class	Family	Methodology	Reference
Healthy (n=50) ACLF patients (n=79; HBV=61, alcohol=9, HCV=5, autoimmune=2, PBC=2)	Healthy vs. ACLF	Bacteroidetes ↓	Bacteroidia ↓	Bacteroidaceae ↓ Porphyromonadaceae ↓	16S rRNA gene pyrosequencing, stool sample	(14)
		Firmicutes ↑	Bacilli ↑	Enterococcaceae ↑ Streptococcaceae ↑		
			Clostridia	Lachnospiraceae ↓ Ruminococcaceae ↓		
			Negativicutes	Veillonellaceae ↑		
		Proteobacteria ↑	Gammaproteobacteria ↑	Pasteurellaceae ↑		
	ACLF without vs. ACLF with HE	Firmicutes	Clostridia	Lachnospiraceae ↓		
	ACLF survivor vs. ACLF non-survivor	Firmicutes	Clostridia	Lachnospiraceae ↓		
		Proteobacteria ↑	Gammaproteobacteria ↑	Pasteurellaceae ↑		
	ACLF Pre-Abx vs. Post-Abx	Firmicutes	Clostridia	Ruminococcaceae ↑		
Healthy (n=25) Cirrhotic patients (n=219; HCV=87, alcohol=43, HCV+ alcohol=32, NASH=32, other=25; 9 ACLF in 38 with infections vs. 38 matched without infections)	Cirrhotic patients without ACLF vs. cirrhotic patients with ACLF	Firmicutes	Bacilli Clostridia	Leuconostocaceae ↑ Family XIV *Incertae Sedis* ↓	16S rRNA gene pyrosequencing Stool sample	(8)

Abx, antibiotics; ACLF, Acute-on-Chronic Liver Failure; HBV, hepatitis B-virus; HCV, hepatitis C-virus; NASH=non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis.
A comparison of condition A vs. condition B: F, increase in condition B relative to condition A; f, decrease in condition B relative to condition A. Taxonomy was updated using the National Center for Biotechnology Information (NCBI) Taxonomy Browser.

XIV *Incertae Sedis* and more numerous Leuconostocaceae than cirrhotics without ACLF[8].

As stated above, bacterial infection is the most common cause for ACLF in cirrhotic patients[3,4,13]. Cirrhotics with evidence of bacterial infection displayed higher endotoxemia, and also significant changes in the microbial composition such as higher Enterobacteriaceae and lower Clostridiales Family XIV *Incertae Sedis*, Lachnospiraceae, Ruminococcaceae, and Veillonellaceae, as well as a trend toward lower Bacteroidaceae[8] (table 2).

Furthermore, when comparing decompensated with compensated cirrhotics, decompensated patients showed higher levels of Enterobacteriaceae, the Pasteurellaceae genera *Haemophilus parainfluenzae* and *Aggregatibacter segnis*, several *Veillonella* spp., *Fusobacterium nucleatum*, *Campylobacter* sp., *Streptococcus oralis* and *vestibularis*; in contrast, *Oscilibacter*, *Bilophila wadsworthia*, *Coprococcus comes*, *Clostridium symbiosum* and *Alistipes* spp. were found at lower levels[15,17]. These findings are not surprising, as Enterobacteriaceae are responsible for many life-threating infections occurring in advanced cirrhosis[9], and Enterobacteriaceae, Veillonellaceae, and Fusobacteriaceae were associated with inflammation in cirrhotic patients with HE[9]. Similarly, cirrhotics with minimal hepatic encephalopathy (MHE)/HE exhibited significantly more abundant Enterobacteriaceae, Veillonellaceae, Prevotellaceae, and less Sutterellaceae, Eubacteriaceae, unclassified taxonomic units of Clostridiales at the family level; and more *Prevotella*, *Coprobacillus*, *Flavonifractor*, *Odoribacter*, and less *Parasutterella*, *Lachnospira*, *Eubacterium*, unclassified taxonomic units of Erysipelotrichaceae at the genus level than cirrhotic subjects without MHE/HE[9,18]. Interestingly, patients with liver cirrhosis and MHE harbored more copious quantities of the urease-containing bacterium *Streptococcus salivarius* than cirrhotics without MHE[18]. *Streptococcus salivarius* positively correlated with ammonia accumulation in these patients which has been linked to HE. This gives another indication of the role of the dysbiotic microbiota in the pathogenesis and disease progression in liver cirrhosis and its complications. Additional metagenomic and metabolomic analyses are required to elucidate functional contributions of a dysbiotic intestinal microbiota to ACLF. Moreover, as factors related to each cirrhosis etiology might affect the microbial structure at baseline, more etiology-specific studies are required to better understand and classify the respective changes of the microbiome. For example, alcoholic hepatitis is a trigger for the onset of ACLF in a subset of patients[3]. One could imagine that changes in the microbiota are different in these than in cirrhotic patients with viral hepatitis.

Intestinal mucosal integrity

Changes of the intestinal microbiome also have repercussions on the intestinal barrier function and thus intestinal permeability. Experimentally induced small intestinal bacterial overgrowth (SIBO) in rodents can result in liver inflammation and injury[19]. However, there is evidence in cirrhotic rats that for bacterial translocation to occur intestinal bacterial overgrowth (IBO) has to be coupled with increased intestinal permeability; IBO with normal intestinal permeability does not lead to bacterial translocation[20].

Nevertheless, the development of an elevated intestinal permeability might necessitate more time in the latter group, as IBO and other dysbiotic changes are usually associated with a disrupted gut barrier in liver disease[7]. Intestinal epithelial and mucosal integrity has hence to be disturbed for bacterial translocation to occur. Cirrhotic rats with bacterial translocation displayed lower expression of tight junction (TJ) proteins (zonula occludens-1, occludin) and greater intestinal permeability than cirrhotic rats without bacterial translocation[21]. Subjects with liver cirrhosis showed enlarged intercellular spaces below the TJs in duodenal biopsies relative to healthy controls[22,23]. The intestine of cirrhotics also showed shortened and wider microvilli, a reduced villus to crypt ratio, edema of the lamina propria, and an increased rate of apoptosis[23]. Moreover, the expression of the TJ proteins occludin and claudin-1 was decreased in duodenal biopsies of cirrhotic patients vs. controls, and it was significantly lower in decompensated than in compensated cirrhotics[24]. This dysfunction of TJs was linked to a more pronounced endotoxemia in decompensated in relation to compensated subjects, and correlated with a higher Child-Pugh score. The expression of occludin also seems to wane from the crypt to the tip of the villi. An increased duodenal permeability in decompensated cirrhotic patients appears to be at least partially mediated via intestinal macrophages releasing IL-6 and nitric oxide[25]. Several factors have been found to be involved in disturbing the intestinal integrity in cirrhotic patients: the causative element of cirrhosis itself (e.g. alcohol), reactive oxygen species (damaging cells and TJs), genetic disposition (e.g. NOD2 and TLR2 variants), intestinal inflammation, and portal hypertension (via formation of intestinal wall edema and dilatation of epithelial intercellular spaces)[6,7,23,26]. Portal hypertension appears to correlate with intestinal permeability, blood levels of LPS-binding protein and IL-6 in cirrhotic patients, and interestingly, all three were improved following blood pressure lowering treatment with non-selective beta-blockers in cirrhotic patients[26].

Local immune function

As liver cirrhosis progresses, global immune function deteriorates. In the very late stage ACLF a sepsis-like state of immune paralysis occurs[27]. Similarly, the immune function in the gut worsens as well as liver disease aggravates. The local intestinal immune function has a considerable impact on bacterial translocation. In cirrhotic rats with ascites, bacterial translocation was linked to compromised Paneth

Table 2. Changes in intestinal microbiota associated with decompensated liver cirrhosis

		Implicated microbiota					
Disease	Comparison	Phylum	Class	Family	Genus/Species	Methodology	Reference
Healthy (n=25) Cirrhotics (n=219; HCV=87, alcohol=43, HCV+ alcohol=32, NASH=32, other=25; 38 with infections vs. 38 matched without infections)	Healthy vs. cirrhotics	Bacteroidetes	Bacteroidia	Porphyromonadaceae ↓		16S rRNA gene pyrosequencing, Stool sample	(8)
		Firmicutes	Bacilli	Enterococcaceae ↑			
				Staphylococcaceae ↑			
			Clostridia	Family XIV *Incertae Sedis* ↓			
				Lachnospiraceae ↓			
				Ruminococcaceae ↓			
			Negativicutes	Veillonellaceae ↓			
		Proteobacteria	Gammaproteobacteria	Enterobacteriaceae ↑			
	Cirrhotics with-out infection vs. cirrhotics with infection	Actinobacteria	Actinobacteria	Coriobacteriaceae ↓			
		Firmicutes	Clostridia	Family XIV *Incertae Sedis* ↓			
				Lachnospiraceae ↓			
				Ruminococcaceae ↓			
			Negativicutes	Veillonellaceae ↓			
		Proteobacteria	Gammaproteobacteria	Enterobacteriaceae ↑			
	Surviving, infected cirrhotics vs. non-surviving infected cirrhotics at 30 days	Actinobacteria	Actinobacteria	Propionibacteriaceae ↑			
		Proteobacteria	Gammaproteobacteria	Halomonadaceae ↑			
	Cirrhotics with-out organ failure vs. cirrhotics with at least one organ failure at 30 days	Firmicutes	Clostridia	Lachnospiraceae ↓			
			Negativicutes	Veillonellaceae ↓			
Several cohorts: healthy (n=114), cirrhotic patients (n=123); healthy (n=45) vs. compensated (n=171) vs. decompensated cirrhotic patients (n=141)	Compensated cirrhotic patients vs. decompensated cirrhotic patients	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae ↑		Metagenomic sequences(MGS), 16S rRNA gene pyrosequencing, Stool sample	(17) based on (15)
				Pasteurellaceae	*Aggregatibacter segnis* ↑		
					H. parainfluenzae ↑		
			Deltaproteobacteria	Desulfovibrionaceae	*Bilophila wadsworthia* ↓		
			Epsilonproteobacteria	Campylobacteriaeae	*Campylobacter* sp. ↑		
		Bacteroidetes	Bacteroidia	Rikenellaceae	*Alistipes* spp. ↓		
		Firmicutes	Bacilli	Streptococcaceae	*Streptococcus vestibularis* ↑		
					Streptococcus oralis ↑		
			Clostridia	Clostridiaceae	*Clostridium symbiosum* ↓		
				Lachnospiraceae	*Coprococcus comes* ↓		
				Oscillospiraceae	*Oscillibacter* ↓		
			Negativicutes	Veillonellaceae	*Veillonella* spp. ↑		
		Fusobacteria	Fusobacteriia	Fusobacteriaceae	*Fusobacterium nucleatum* ↑		

HCV, hepatitis C-virus; NASH=non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis.
A comparison of condition A vs. condition B: ↑, increase in condition B relative to condition A; ↓, decrease in condition B relative to condition A. Taxonomy was updated using the National Center for Biotechnology Information (NCBI) Taxonomy Browser.

cell function with depressed levels of α-cryptdin 5 and 7 in particular in ileum and cecum[28]. Rats with experimental liver cirrhosis showed diminished amounts of antimicrobials in the ileum such as angiogenin-1 and α-5-defensin (worse when bacterial translocation was present)[21]. They also exhibited increased intestinal inflammation such as augmented levels of TNF-α and interferon-γ in the ileum, and higher numbers of inflammatory monocytes and activated T-helper lymphocytes in the ileal lamina propria and in the systemic circulation compared with controls. More ileal and systemic inflammation was detected in cirrhotic rats with bacterial translocation vs. cirrhotic rats without bacterial translocation. In a similar experimental setup, cirrhotic rats with detectable bacterial DNA but no bacteria in their mesenteric lymph nodes (MLNs) harbored $CD103^+$-dendritic cells (DCs) in the gut and MLNs that showed features of activation, expansion of the pro-inflammatory $CD4^+$-DC subpopulation, elevated TNF-α synthesis, and heightened phagocytosis and migration capacities[29]. In contrast, rats with cultivatable bacteria in their MLNs displayed non-activated $CD103^+$-DCs with suppressed TNF-α production and deficient phagocytosis and migration capacities[29]. This scenario could represent what is occurring in human subjects with liver cirrhosis experiencing ACLF, as these patients exhibit an immune exhaustion with lower human leukocyte antigen-DR expression and LPS-stimulated TNF-α production by circulating monocytes[4,27,29]. Intriguingly, intestinal decontamination with antibiotics normalized the activation state, function and TNF-α production of $CD103^+$-DCs in cirrhotic rats[29]. This indicates that the local immune response depends on dysbiotic changes of the microbiome. A decreased bile flow is observed in patients with liver cirrhosis[6,8]. Interestingly, experimental administration of the bile acid obeticholic acid to cirrhotic rats heightens the intestinal antimicrobial defense, lessens intestinal inflammation, fights ileal mucosa-associated bacteria and bacterial translocation, and improves the liver phenotype[21]; this is at least partly mediated via farnesoid X receptor (FXR)[21]. Obeticholic acid treatment also led to an enhanced expression of the TJ proteins zonula occludens-1 and occludin. Taken together, a local inflammatory response in the lamina propria contributes to tight junction disruption and increased paracellular permeability. At the same time, an impaired immune surveillance function facilitates bacterial translocation in advanced stages of liver cirrhosis.

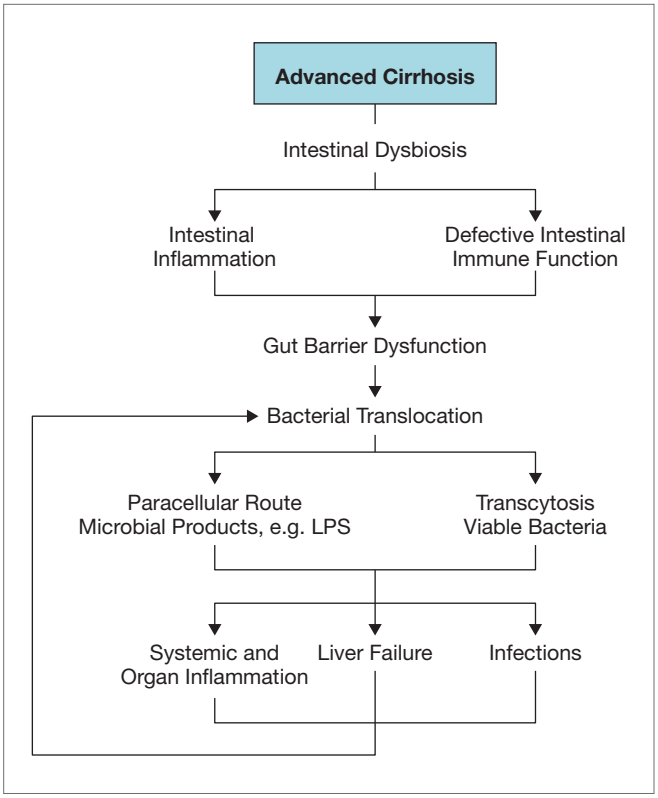


Figure 1. Intestinal dysbiosis, gut barrier dysfunction and defective immune function facilitate bacterial translocation during advanced stages of liver cirrhosis.

Advanced liver cirrhosis is associated with quantitative and qualitative changes of the intestinal bacteria (dysbiosis) which result in inflammation of the intestinal wall and dampened intestinal immune function. Intestinal inflammation leads to elevated intestinal permeability (gut barrier dysfunction) with increased bacterial translocation. Suppressed local immune function might contribute to intestinal barrier dysfunction and bacterial translocation through an impaired removal of translocated bacteria and possibly microbial products. Translocation of microbial ligands such as lipopolysaccharide (LPS, or endotoxin) occurs via the paracellular route in all stages of liver disease. Viable bacteria translocate via the transcellular route (transcytosis) through intestinal epithelial cells during decompensated stages of liver cirrhosis. Bacterial translocation can result in systemic and organ inflammation, liver failure and infections that can further exacerbate gut barrier dysfunction.

Bacterial translocation

Compositional changes in the intestinal microbiota, increased intestinal permeability and changes in the local immune function, commonly observed in chronic liver disease, all promote bacterial translocation (fig. 1). So do patients with liver cirrhosis more frequently display SIBO and bacterial translocation than healthy controls[6,7]. The severity of liver injury correlates with blood levels of translocated bacterial products, such as lipopolysaccharides (LPS, or endotoxin), a membrane component of Gram-negative bacteria[6]. Endotoxemia is more pronounced in the presence of ACLF vs. liver cirrhosis without ACLF[8]. It was found highest in the peak phase compared with the progression or remission phase of ACLF[30]. Blood LPS is also more elevated in cirrhotics with MHE relative to cirrhotics showing no disturbance in brain function, as are cytokines IL-10, IL-6, IL-2, TNF-α, and IL-13[9]. Subjects with decompensated cirrhosis without identified bacterial infection who tested positive for bacterial DNA (bactDNA+) in serum and ascitic fluid had a higher mortality than those who did test negative (bactDNA-)[31]. The most common cause of death in these patients was ACLF;

the predictive value for bactDNA herein was particularly high when short-term mortality was considered (7/48 deaths in bactDNA+ vs. 0/108 in bactDNA- at 30 days), and in patients with lower MELD scores (< 15). The most frequently identified bacterium in bactDNA+ was *Escherichia coli* in two thirds of the cases. Moreover, the rate of bacterial infections appears to correlate with the severity of ACLF[13]. Patients suffering from ACLF displayed higher levels of leukocytes and C-reactive protein (CRP); and leukocyte counts were also an independent predicting factor of mortality in these subjects. In light of potential bacterial infections undetected by traditional means due to limited sensitivity[13], these inflammatory markers might serve as substitute indicators for bacterial infections and as a prognostic guide for the clinician in this scenario. Translocation of bacteria and bacterial products leads to additional liver damage by promoting liver inflammation by Kupffer cells, liver fibrosis by hepatic stellate cells, and hepatocyte death[6,7]. It also causes other complications of end-stage liver disease such as systemic inflammation, hemodynamic derangement or spontaneous bacterial peritonitis (SBP)[6,7]. This is mediated via interaction of these pathogen associated molecular patterns (PAMPs) with pattern recognition receptors such as Toll-like receptors (TLRs) on immune cells and liver cells[32]. Systemic inflammation might contribute to TJ disruption and might promote further damage to the gut barrier. Already at pre-cirrhotic and at cirrhotic stages of liver disease dysbiosis occurs, leading to intestinal inflammation and subsequent TJ disruption and paracellular translocation of bacterial products such as LPS[33]. Decompensation of cirrhosis coincides with transcellular translocation (transcytosis) of bacteria[34]. In ACLF, even higher LPS levels and rates of bacterial infections are noted than in cirrhosis[4,8,13]. We can only speculate what additional mechanisms might contribute to further increases in intestinal permeability and bacterial translocation in ACLF. The exacerbated systemic inflammation in ACLF might damage the intestinal barrier further thereby facilitating bacterial translocation. Additionally, the described dysbiotic changes in the intestinal microbiome and the associated shifted levels of its metabolites might impair the barrier as well.

Conclusion

ACLF has been identified as a distinct entity from other liver diseases fairly recently[4]. As the current knowledge of this pathology is limited, ACLF is still difficult to manage clinically with a resulting high short-term mortality. Bacterial infections account for a considerable number of the deaths in ACLF[3,4,13]. As these might originate from the intestine, it is important to investigate central events in the intestine in ACLF. ACLF is associated with intestinal dysbiosis. How exactly dysbiosis contributes to the onset of ACLF is largely unknown. Second, if the mechanisms are established how certain bacteria can alleviate or possibly protect from ACLF, the next question would be how to modulate the intestinal microbiome to induce these protective properties. The potentially positive effects of probiotics or antibiotics have been evidenced in several stages of liver disease and also in liver disease with different etiologies[6,7]. Furthermore, the more advanced the liver disease the more pronounced is the intestinal permeability[20,21,24]. A number of factors have been identified to negatively impact the intestinal integrity such as oxidative stress, the genetic disposition, intestinal inflammation, and portal hypertension. While some factors cannot be easily modified in an everyday clinical setting (i.e. genes), others might be promising to improve the intestinal barrier function and hence the outcome (e.g. antioxidants). The local immune function in the gut might be another target to modulate liver disease and possibly ACLF. As the microbiota affects the local immune function and vice-versa, enhancing immune surveillance mechanisms without increasing local inflammation in the gut might be beneficial. Administration of certain bile acids and/or FXR-agonists (such as obeticholic acid) might be an option, as it has been shown that they can induce the expression of antimicrobial proteins and bactericidal activity, and reduce bacterial translocation[21]. The aforementioned changes in the intestinal microbiome, intestinal integrity and local immune function facilitate bacterial translocation in all stages of liver disease, also in advanced stages such as ACLF. If paracellular translocation of bacterial ligands (e.g. LPS) and transcellular translocation of bacteria can be suppressed, the liver phenotype could be rescued in all stages of liver disease, from pre-cirrhotic stages to decompensated cirrhosis, and possibly as well in ACLF[6,7,33,34]. However, further studies are required to identify known or novel agents that positively impact the intestinal microbiome to help to manage ACLF.

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EUROPEAN FOUNDATION FOR THE STUDY OF CHRONIC LIVER FAILURE
SECTION II

BACTERIAL INFECTION IN ACLF: AN ETIOLOGIC FACTOR AND A COMPLICATION

Javier Fernández

Liver Unit, Hospital Clínic, University of Barcelona, Barcelona, Spain. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD)


J. Fernández

Bacterial infections constitute a major complication of cirrhosis and are associated with high mortality rate. Infections can occur in compensated patients and can precipitate clinical decompensations (variceal hemorrhage, hepatic encephalopathy) or may further deteriorate an already decompensated patient (variceal rebleeding and hepatorenal syndrome: HRS)[1-4]. Bacterial infections are therefore associated with an increased in-hospital mortality (4-5 fold), and higher risk of death from sepsis (2-fold)[3].

Acute-on-chronic liver failure (ACLF) is a recently redefined syndrome that develops in cirrhotic patients with acute decompensation and is characterized by the development of extrahepatic organ failures[5-8]. In most cases, the development of ACLF is associated to a precipitating factor (table 1), although frequency and characteristics differ between Eastern and Western countries[9-11]. While in the West, bacterial infections are the most common precipitating factors, in the East flares of hepatitis B virus are the most common insult identified[5,10-12]. Interestingly, precipitating events cannot be detected in a relatively high percentage of patients (from 20% to 40%)[5,9]. The pathophysiology of the syndrome is incompletely understood but exaggerated inflammatory response and failed tolerance to organ damage seem to be involved[5,6,13]. Although triggers may be important in the development of ACLF, data from the CANONIC study show that mortality is independent of the type of precipitating factor and that it is mainly related to other factors such as the type and number of organ failures, the intensity of inflammatory response and the early clinical course of the syndrome[5,6,14].

Table 1. Precipitating events of ACLF

Precipitating Factors	Prevalence
Type of insult	
Bacterial infections	30-57%
Flare of HBV infection	8-36%
Active alcoholism	~25%
Gastrointestinal bleeding	~10%
Superimposed HAV or HEV infection	<1-6%
Hepatotoxic drugs	<1-3%
Others (surgery, TIPS, large volume paracentesis without albumin)	<10%

Pathogenesis of organ failure in infection-related ACLF

In cases of ACLF triggered by infection, the systemic spread of bacteria and/or bacterial products represents the primary pathogenic event that activates the host innate immune response[6,13,15]. It is well-known that during the first hours of bacterial infection, recognition of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) in patients with cirrhosis results in higher plasma pro-inflammatory cytokine levels compared to those in patients without cirrhosis[6,13,16]. This 'cytokine storm' may cause collateral tissue damage[15]. Moreover, bacteria can also directly cause tissue damage. "Failed tolerance" or the failure in the mechanisms of resistance to the damage induced by immunopathology and bacterial strains could also be involved in the pathogenesis of ACLF[17]. Among patients with ACLF, the higher the ACLF grade, as estimated by the number of organ failures, the higher plasma pro-inflammatory cytokine/chemokine levels.

Patients with ACLF without precipitating factors also show features of excessive inflammatory reaction. In some cases, the existence of a bacterial infection could have been missed; however, in others, subclinical bacterial translocation could be the real insult of the syndrome[18]. PAMPs resulting from intestinal bacterial translocation reach the liver and systemic circulation in advanced cirrhosis[18,19]. These PAMPs may be then recognized by PRRs thus triggering inflammation and the development of ACLF. On the other hand, the release of danger-associated molecular patterns (DAMPs) resulting from dying hepatocytes after acute liver

tissue injury could also act as precipitating event leading to the development of ACLF[20].

Prevalence of bacterial infection as cause of ACLF

Studies performed in patients with spontaneous bacterial peritonitis (SBP) some decades ago demonstrated that despite resolution of infection with antibiotics, patients with SBP frequently develop an exaggerated inflammatory response, severe impairment in cardiovascular function, renal failure and failure in the function of other organs, a condition associated with high hospital mortality[21-23]. The prevalence of type-1 HRS, a type of ACLF, and hospital mortality following SBP is markedly reduced (from 30% to 10%) if plasma volume is expanded by intravenous albumin at infection diagnosis[23]. HRS may be also precipitated by other bacterial infections, although the risk is substantially lower (around 10%)[24].

Recent studies confirm these data and show that bacterial infections constitute major precipitants of ACLF[5,9]. In the CANONIC study nearly 40% of patients with ACLF had a bacterial infection as precipitating event compared to 25% of patients with decompensated cirrhosis without ACLF. This rate of infection-related ACLF is almost 2 times than that reported for active alcoholism-related ACLF (23% of the cases) (table 1)[5]. Bacterial infection caused 33% of ACLF episodes diagnosed at enrollment and 57% of those developed during hospitalization. Nosocomial infections, therefore, could be associated to a higher risk of ACLF[5]. In fact, nosocomial first infections and high MELD score have been identified as risk factors for infection-related ACLF in a multicenter North American study[25]. Data derived from the CANONIC study also show that bacterial infections tend to cause ACLF more frequently in patients without previous history of decompensation compared to patients with previous decompensation (46% vs. 38%, respectively), finding that suggests that factors other than infection such as genetic determinants of the intensity of inflammatory response and host tolerance to organ damage could also play a role in the pathogenesis of ACLF[5].

Bacterial infections are also an important insult of ACLF in Eastern countries. In an Asian study, bacterial infections were recognized as the main trigger of ACLF in 35% of cases in the whole series (44% if only patients with definitive precipitating event were considered), being therefore the second most common cause of ACLF just behind flare-up or reactivation of HBV[9]. This figure is similar to that reported in the CANONIC study[5]. Bacterial infections were the main insult identified in patients with extrahepatic ACLF (80%), and represented a clinically relevant event in patients with hepatic ACLF (16%)[9]. A recent Chinese study confirms the relevance of bacterial infections in the development of ACLF in Eastern countries. Both, bacterial infections and HBV reactivation were triggering factors of 30% of cases of ACLF each[12].

Type and characteristics of bacterial infections causing ACLF

Initial analyses derived from the CANONIC series show that although any bacterial infection can trigger an ACLF, the risk is greater in SBP, secondary peritonitis and pneumonia, the only three infections that were more prevalent in patients with ACLF compared to those with acute decompensation without ACLF (11% vs. 6% in SBP, 3% vs. 0% in secondary peritonitis and 6% vs. 2% in pneumonia) (Table 2)[5]. Severity of infection also increases the risk of ACLF. Infections associated with sepsis or severe sepsis triggered more frequently the syndrome than those without systemic inflammatory response syndrome (15% vs. 4%). In this study, the intensity of the inflammatory response (high blood leukocyte count and plasma C-reactive protein levels) also paralleled the severity of ACLF, finding that was independent of the presence or absence of infection[5]. All these data indicate that exaggerated systemic inflammation contributes to the development of ACLF, determines its severity and also impacts its outcome.

The risk of ACLF related to the development of multidrug-resistant bacterial infections is currently unknown, although different studies have demonstrated that these infections are associated with low resolution rates, high incidence of septic shock and increased mortality[4,26].

Prognosis of infection-related ACLF

Preliminary data suggest that bacterial infections could be associated with more severe forms of ACLF. In the CANONIC study, they were identified as the potential precipitating event of the syndrome in 45% of patients with ACLF grade 3 compared to 30% and 31% in those patients with less severe forms of the syndrome (grade 1 and 2, respectively)[5]. In the Asian study, patients with extra-hepatic ACLF, mainly caused

Table 2. Characteristics of bacterial infections causing ACLF

Precipitating Factors	Prevalence	
Type of infection	**ACLF**	**AD**
Spontaneous bacterial peritonitis	11%	6%
Pneumonia	6%	2%
Secondary peritonitis	2%	—
Urinary tract infection	6%	4.5%
Cellulitis	2%	2%
Unproved suspected infection	6%	6%
Severity of infection		
Sepsis	12%	3.5%
Septic shock	3%	0.1%

AD: acute decompensation. Modified from Moreau et al[5]

by bacterial infections, developed higher frequency of extra-hepatic organ failures (kidney, circulation, respiratory and brain systems)[9]. One might expect that this different clinical profile and greater initial severity of infection-related ACLF would translate into a worse short-term prognosis in these patients in comparison to ACLF caused by other insults. However, the 2 largest studies on ACLF published so far show no differences in 28-day mortality among infection-related and infection-unrelated ACLF and failed to identify bacterial infection as an independent predictor of mortality[5,9]. Development of "de novo" bacterial and/or fungal infections in patients with infection-unrelated ACLF could explain at least partially this finding. Short-term prognosis in ACLF, therefore, seems to be related to the severity of the syndrome, to the intensity of the inflammatory response and to the initial evolution of ACLF (3-7 days) and is probably independent of the type of precipitating event.

According to the results of the North American multicenter study, 30-day probability survival in infection-related ACLF is of only 23% in patients with four organ failures (shock, mechanical ventilation, severe hepatic encephalopathy and dialysis) and ranges from 32 to 55% in patients with different combinations of 3 organ failures. For those with 2 organ failures, 30-d survival ranges from 57% in patients requiring vasopressors and mechanical ventilation to 82% in those with severe hepatic encephalopathy and renal support[25].

Infections that complicate the evolution of ACLF

It is well known that infections frequently complicate the evolution of ACLF and in fact constitute the main cause of death in these patients. ACLF patients are highly susceptible to the development of new infections that can act as second hit of the syndrome. This higher susceptibility to bacterial and fungal infections in ACLF might be associated with immunosuppression status and with the high degree of instrumentation of the patients[19]. However, the precise risk of bacterial and fungal superinfections in ACLF patients is unknown. Bañares et al reported a high rate of new bacterial infections in patients with ACLF receiving standard medical therapy and albumin dialysis (49% and 59%, respectively)[27]. Similar figures were reported in a multicenter study evaluating the efficacy of fractionated plasma separation and adsorption (Prometheus) in patients with ACLF: from 54% to 62%[28]. Pneumonia, bacteremia and SBP were the most frequent second infections reported in these series. However, definition of ACLF used in these studies differs markedly from that currently accepted.

Since immunoparalysis can have a relevant impact on the prognosis of patients with ACLF, some groups have suggested that these patients could benefit from treatments aimed at restoring the patients' immune function, and that therefore this may prevent death from secondary infections[29]. Drugs with immunomodulatory potential are albumin, N-acetylcysteine and granulocyte-colony stimulating factor (G-CSF). A recent Indian RCT suggest that the administration of G-CSF could prevent the development of sepsis and improve short-term survival in patients with less severe forms of ACLF[30]. As patients with more severe forms of the syndrome were excluded (those with sepsis, cerebral failure and multi-organ failure), the real applicability of this treatment in ACLF deserves further evaluation.

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SEVERE ALCOHOLIC HEPATITIS, SYSTEMIC INFLAMMATION AND MULTIORGAN FAILURE

Zahid M. Vashora, Joaquín Cabezas and Ramón Bataller

Division of Gastroenterology and Hepatology, Department of Medicine and Nutrition, University of North Carolina at Chapel Hill, NC


R. Bataller

Alcoholic liver disease (ALD) is a major cause of preventable liver-related morbidity and mortality worldwide. The WHO reported 3.3 million deaths occur yearly worldwide secondary to the harmful use of alcohol, which equates to 5.9% of all death[1]. ALD comprises of a wide spectrum of stages related to the susceptibility factors and duration of alcohol consumption; listed from least to most severe, are steatosis, alcoholic steatohepatitis (ASH), progressive fibrosis to cirrhosis, decompensated cirrhosis and superimposed hepatocellular carcinoma (HCC). In addition to this, patients with heavy alcohol intake but also with underlying ALD can develop a form of acute-on-chronic liver injury known as alcoholic hepatitis (AH). AH is characterized by an abrupt development of jaundice and clinical decompensations including ascites, encephalopathy and variceal bleeding. AH is one of the deadliest diseases in clinical hepatology and bears a very high short-term mortality (20-40% at 3 months)[2]. While there continues to remain a large economic and health burden from ALD, this has unfortunately garnered very little attention from health policy makers, pharmaceutical companies, and private funding agencies unlike advancements in viral hepatitis and non-alcoholic fatty liver disease[3]. Within ALD, AH is a field that requires an urgent need for novel targeted therapies. This urgent need was further compounded by the recent STOPAH trial that showed limited efficacy of prednisolone[4].

There are several important unmet needs in diagnosis, management and therapy of AH. First, patients often present in the emergency room with very advanced disease, severe sepsis and poor physical status, having high mortality within few days. Informative campaigns in primary care centers, addiction centers and in the general population about the clinical relevance of jaundice in patients with alcohol abuse should be emphasized. Second, the specific mechanisms, outcome, and responses to therapy of the most common secondary complications (encephalopathy, infections, renal failure, etc.) are largely unknown. There are only few studies characterizing these complications in this particular population and additional research to understand their underlying mechanisms are warranted. Third, the extra-hepatic consequences of AH that lead to multi-organ failure (MOF) and death are not well defined. In patients with acute-on-chronic liver failure, mortality is not only due to end-stage liver failure, but is often related to MOF[5]. The existence of systemic inflammatory response syndrome (SIRS) is a major predictor of acute organ failure in these patients, suggesting that the extra-hepatic consequences of AH play a major role on disease severity[6]. Finally, a meaningful molecular classification of these patients could guide the use of targeted therapies. Not all patients are seen at the same stage of the disease and it is likely that different types and patterns of hepatic inflammation play defining roles for silent ASH, moderate and severe AH; whereas impaired liver regeneration could be the major event in patients with severe AH[7,8]. Besides intrahepatic inflammation, identifying the molecular mechanisms leading to hepatocellular failure as well as SIRS will facilitate the development of targeted therapies.

In this chapter, we discuss a general review of AH including the molecular pathogenesis of AH in terms of intrahepatic and systemic inflammation as well as the impact of SIRS on the development of multiorgan dysfunction and death. Moreover, the usefulness of biomarkers of sterile and bacterial infections in the setting of SIRS in AH is discussed.

Alcoholic hepatitis: clinical and pathogenic basis

AH is a form of acute-on-chronic liver injury in patients with underlying ALD (in most cases with severe fibrosis or cirrhosis) in the setting of heavy alcohol intake[2]. The incidence of AH is not truly known, with many cases likely undiagnosed, especially when patients present with sepsis or variceal bleeding. Population-based studies in Denmark estimate approximately 4.5 hospitalizations for AH per 100,000 persons each year, with a slight male predominance[9]. Clinically, AH is characterized by an abrupt rise in serum bilirubin levels, jaundice, coagulopathy, and liver-related complications. AH is a life-threatening disorder with a high morbidity and mortality that often is the first manifestation of previously silent or undiagnosed ALD or cirrhosis. In patients with ALD, it is also important to differentiate between AH with ALD versus other conditions which can also present with jaundice and liver decompensation, such as severe sepsis, biliary obstruction, diffuse HCC, drug-induced liver injury, ischemic

hepatitis (i.e., due to massive bleeding or cocaine use), and other misdiagnosed entities: alcoholic foamy degeneration and alcoholic fatty liver with jaundice[10]. Given that not all acute jaundice with ALD is AH, in patients with other potential causes of jaundice, with severe forms of ALD, or involved in clinical trials, a transjugular liver biopsy is recommended to confirm the existence of AH[11,12].

Short-term mortality in patients with severe AH is due to sepsis, liver failure and MOF[13]. Of the several clinical scoring systems to assess the severity of AH, including Maddrey's discriminant function -DF-, MELD, ABIC and Glasgow, Maddrey's DF is the most widely used, however none of these include liver histology[14]. Alcoholic Hepatitis Histological Score (AHHS) is a histological scoring system to predict short term survival in AH that was recently developed through a multi-center study[9]. AHHS comprises 4 parameters that are independently associated with patients' survival: fibrosis stage, neutrophil infiltration, type of bilirubinostasis, and presence of megamitochondria. By combining these parameters in a semiquantitative manner, patients can be stratified into low, intermediate, or high risk for death within 90 days (table 1)[11].

The specific management of patients with AH has not seen substantial advancement over the last decades. Admission to an intensive care unit is often warranted in severe AH which may present with poor mental status or hypotension. Close observation and management to prevent alcohol withdrawal symptoms and Wernicke's encephalopathy is also recommended. In terms of diet, malnutrition is a common finding in alcoholic patients and may play a role in bacterial infections. Therefore, daily protein intake of 1.5 g/kg body weight is also advised with oral or enteral feeds favored over total parenteral nutrition so as to avoid gram-positive infections[15]. Importantly, given the predisposition of severe infections in patients with AH, early diagnosis and empiric antibiotic treatment are advised. A major determinant of long-term survival is sustained abstinence, therefore on discharge; patients should follow alcohol counseling to reach sustained abstinence[16]. Current pharmacological therapy has limited results as prednisolone for 4 weeks has shown to improve only short-term survival in some patients with severe AH and many patients do not respond to prednisolone, as seen in the STOPAH trial, thus novel targeted therapies are urgently needed[2]. The efficacy of pentoxifylline remains questionable[2,4,17]. N-acetylcysteine, a potent antioxidant has shown some beneficial effect in a recent study[18]. Liver transplantation, albeit in highly selected AH patients, is shown to improve survival significantly[19]. However, the eligibility of liver transplantation in AH patients raises clinical and ethical concerns particularly recidivism and the relative shortage of donor organs.

Table 1. Alcoholic Hepatitis Histological Score (AHHS) for Prognostic Stratification of Alcoholic Hepatitis (from Altamirano et al11)

	Points	
Fibrosis stage		**AHHS categories (0-9 points)**
None Fibrosis or Portal fibrosis	0	Mild: 0-3
Expansive fibrosis	0	Intermediate: 4-5
Bridging fibrosis or Cirrhosis	+3	Severe: 6-9
Bilirubinostasis		
None	0	
Hepatocellular only	0	
Canalicular or ductular	+1	
Canalicular or ductular plus Hepatocellular	+2	
PMN infiltration		
Mild PMN Infiltration	+2	
Severe PMN Infiltration	0	
Megamitochondria		
No Megamitochondria	+2	
Megamitochondria	0	

Recent studies have identified some key pathogenic mechanisms in AH (fig. 1). Excessive drinking in patients with ALD causes hepatocellular injury and impaired regeneration, leading to liver failure[20]. Alcohol abuse induces gut bacterial overgrowth, increases gut permeability and the subsequent translocation of bacterial products including LPS to the portal circulation[21]. Importantly, lipopolysaccharide (LPS) induces impaired phagocytic function and immune paralysis, favoring bacterial infections[22]. Damaged hepatocytes subsequently release a variety of damage-associated molecular pattern molecules (DAMPs) such as HMGB1 that might contribute to hepatic and systemic inflammatory response[23]. In addition to DAMPs, bacterial products named pathogen associated molecular patterns (PAMPs) might also favor SIRS in AH patients[24]. These elevated PAMPs then activate Kupffer cells and other inflammatory cells to produce a variety of pro-inflammatory cytokines such as TNFα, MCP-1 and IL-1β, which contribute to the pathogenesis of AH. In addition to inflammation, poor hepatic regenerative response is probably another important mechanism contributing to the liver failure in some AH patients. A detailed analysis of liver explants from AH patients that underwent liver transplantation revealed that patients who failed to respond to medical therapy had reduced hepatic expression of liver regeneration-related cytokines and lack of proliferative hepatocytes[8]. Moreover, a massive expansion of liver progenitor cells called "ductular reaction" is often observed in AH patients, but these LPCs fail to differentiate into mature hepatocytes and correlate positively with severity of liver disease and short-term mortality in these patients[7]. At present, the mechanisms leading to inefficient liver regeneration in AH are unknown and deserve further investigation. There are two more important cellular events in AH that need pathogenic studies: "chicken-wire" fibrosis and cellular and ductular bilirubinostasis. Because the animal models of ALD do not reproduce these two features of AH,

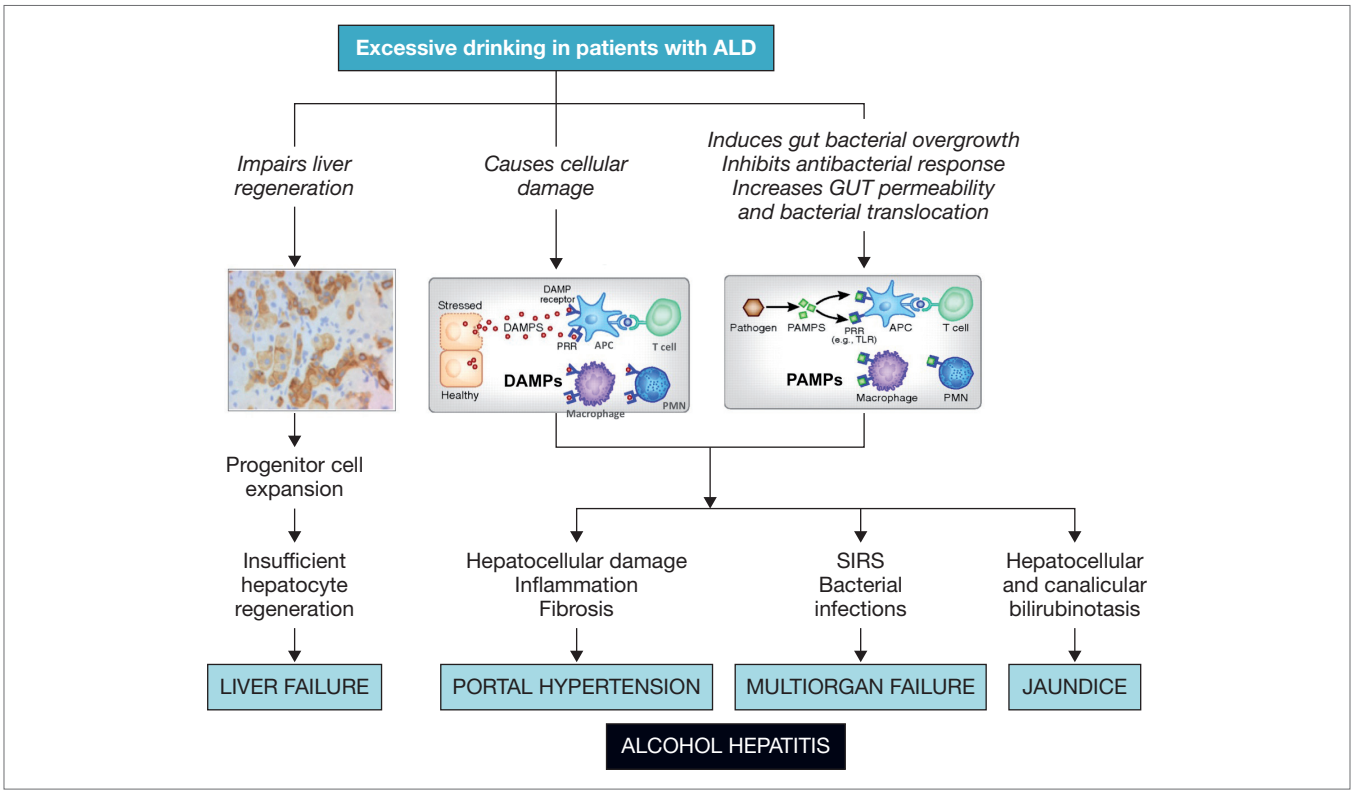

Figure 1. Main pathogenic events in AH. Excessive alcohol intake in patients with underlying ALD induces hepatocellular damage, bacterial translocation from the gut and impaired liver regeneration. Products from bacteria (Pathogen-Associated Molecular Patterns -PAMP-) and from dying cells (Damage-Associated Molecular Patterns -DAMP-) induce systemic inflammation response syndrome (SIRS) and impair liver regeneration, resulting in liver failure, jaundice, portal hypertension and their related complications, leading to multiorgan dysfunction and death.

there is a clear need of translational studies in human samples The combination of massive fibrosis, hepatocellular failure, jaundice, systemic inflammation and immune paralysis lead to main of the complications of AH. Targeted therapies other than anti-inflammatory drugs should target these important pathogenic events in order to improve the prognosis of these patients.

Multiorgan failure in AH: role of SIRS

The mechanisms leading to early death in patients with AH are largely unknown. The degree of liver failure and the development of renal failure are associated with a poor outcome[25]. In patients with acute-on-chronic liver failure, mortality is not only due to end-stage liver failure, but is often related to MOF[5]. We recently found that the development of acute kidney injury (AKI) is an early prognostic factor of mortality in AH[6]. In that study, we observed that the presence of SIRS at admission may have an important role in the development of AKI. The role of SIRS on AKI development was confirmed in a recent study from India[26]. Moreover, SIRS has recently been associated with a poor prognosis in patients with acute-on-chronic liver failure[27].

We recently studied a large cohort of patients with biopsy-proven AH[13]. More than one third of the patients developed MOF during the hospitalization. The organs most frequently involved were liver and kidney (70.8% and 33.3% of patients, respectively), while circulatory failure was present in 9.8%, coagulopathy in 9.2%, respiratory failure in 5.9% and neurological failure in 5.2%. 37 patients (22.8%) presented no organ failure, while 67 patients (41.4%) presented 1 organ failure, 34 patients (21%) presented 2 organ failure and 24 (14.8%) 3 or more organ failure. As expected, 90-day mortality was significantly higher in patients that developed MOF (62.1% vs. 3.8%, in patients with and without MOF, respectively; $p < 0.001$) (fig. 2). Importantly, the association of MOF with mortality was independent of the degree of liver dysfunction, as assessed by the ABIC or MELD and Maddrey's DF, and the Lille score. This study clearly indicates that the development of multiple organ failure carries an ominous prognosis in these patients and that strategies aimed at preventing extrahepatic complications are required to improve patient outcome.

The prevalence of SIRS in patients with AH and its impact on the development of MOF and mortality are not well known. As stated above, in our early study we found that the presence of SIRS is an independent predicting

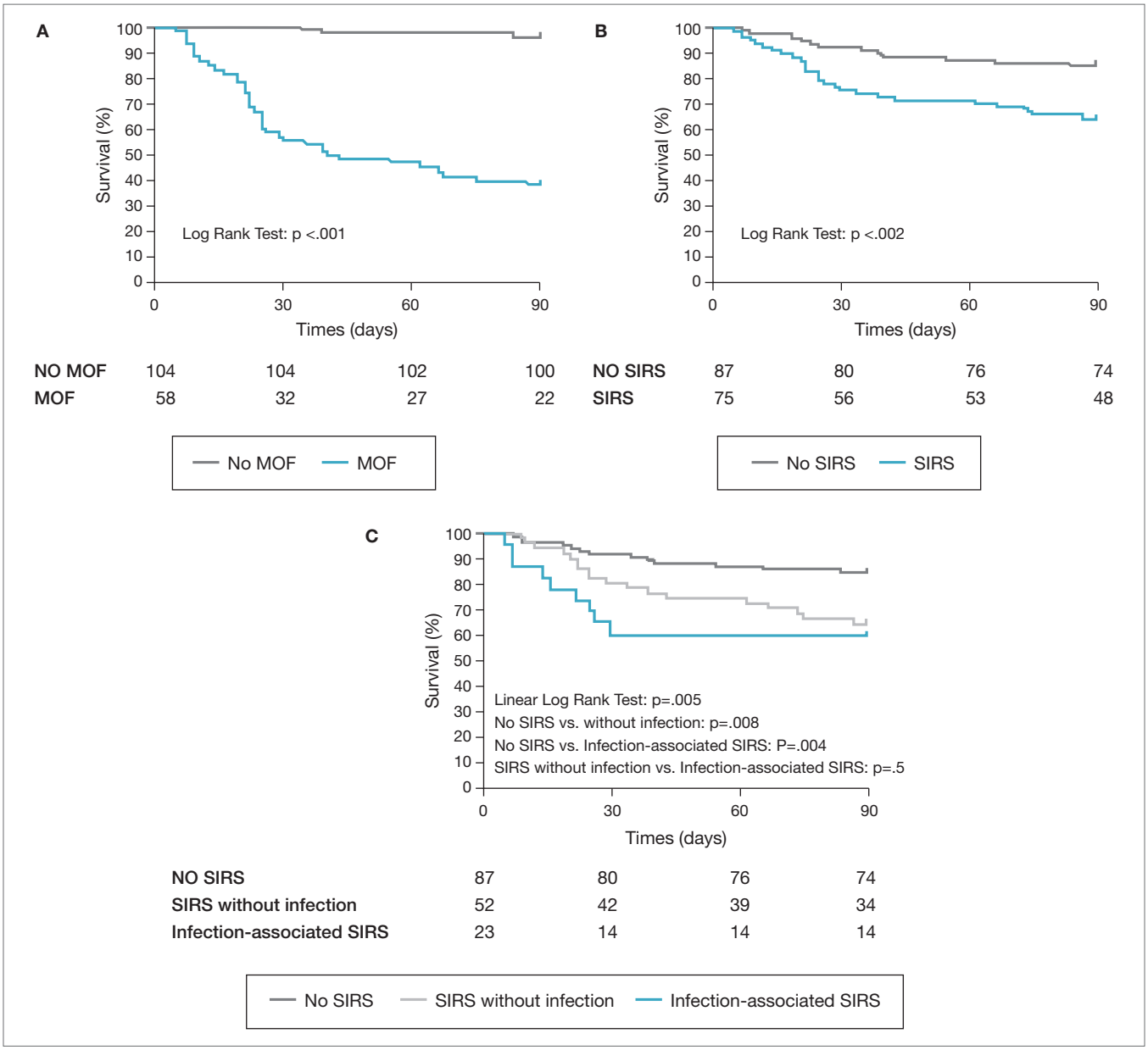

Figure 2. Impact of MOF and SIRS in mortality in patients with AH. Ninety-day mortality according to (A) the presence of MOF, (B) the presence of SIRS, and (C) the SIRS-associated conditions. Reproduced with permission, Michelena et al[13].

factor for the development of AKI[6]. SIRS in the setting of AH may be related to a bacterial infection (sepsis) given that around 25% of patients with AH present with a bacterial infection at admission[27]. However, many patients with AH show features of SIRS (e.g. leukocytosis, fever) without any identifiable bacterial infection. SIRS in these patients may be secondary to sterile inflammation of the liver, namely, an inflammatory response in the absence of a documented bacteria infection[28]. In the liver of patients with AH, there is a marked overexpression of pro-inflammatory cytokines such as interleukin 8 and CCL20[29,30]. The serum levels of both cytokines correlate with short-term mortality, suggesting that inflammatory mediators produced by the injured liver could play a role in the development of SIRS in patients with AH.

In our study assessing the presence of MOF in patients with AH[13], we extensively studied the potential role of SIRS. We found that nearly half of the patients with AH (46.3%) fulfilled SIRS criteria at admission. Interestingly, there were no differences in the prevalence of SIRS according to the severity of the AH. Patients with SIRS criteria showed a higher prevalence of infection at admission (30.7% vs. 10.3% with and without SIRS, respectively, while the incidence of in-hospital infections was comparable between patients with and without SIRS (46.7% vs. 41.4%). Importantly, the presence

of SIRS at admission negatively impacted patients' outcome. Overall 90-day mortality was significantly higher among patients with SIRS at admission (36% vs. 14.9% in patients with and without SIRS, respectively) (fig. 2), independently of the degree of liver dysfunction. Moreover, patients with SIRS criteria showed a higher incidence of MOF during hospitalization than patients without SIRS (46.7% vs. 26.4%), also independently of the degree of liver. An additional relevant result from our study is that only one third of patients with SIRS at admission were diagnosed with an infection. Importantly, short-term mortality was similar in patients with infection-associated SIRS and SIRS without infection (39.1% and 34.6%, respectively) and was much higher than in patients without SIRS (14.91%). Similarly, both causes of SIRS equally predicted MOF (42.3% in patients with SIRS without infection and 56.5% among patients with infection-associated SIRS). Overall, the results from our study clearly showed that, regardless of the origin, the presence of SIRS at admission predisposes to the development of MOF and death.

SIRS in patients with AH: role of biomarkers and clinical implications

The finding that SIRS at admission predicts MOF and is not always associated with bacterial infections has obvious therapeutic implications. When presenting with SIRS, all patients should undergo a full infectious work-up including chest X-ray, urine and ascitic fluid analysis and culture as well as blood cultures. The lack of evidence of infection may be related to an occult infection, but the role of sterile inflammation within the liver from AH causing systemic illness and multiple organ failure appears strong. As SIRS is related to MOF and thus a poor prognosis in AH, and not all SIRS in AH patients is infectious, it is unclear if prophylactic antibiotics will be beneficial. A clinical trial testing this hypothesis is underway. The concern for prophylactic antibiotics is further compounded by the concern that such an approach may predispose these patients to fungal infections. This raises the importance of the use of biomarkers in to discern the difference between sterile and infectious SIRS. In fact, while in some patients infections can be diagnosed early based on clinical, radiological and biochemical criteria, (e.g. spontaneous bacterial peritonitis or pneumonia) other patients have a positive culture identified after 2-3 days. Administering broad-spectrum antibiotics in patients under prednisolone therapy and with SIRS without infection may be unnecessary and recent reports have shown that broad-spectrum antibiotics can favor severe fungal infections[31]. Therefore, biomarkers capable of distinguishing between SIRS with and without infection would be of clinically useful. In our recent study, we selected a panel of biomarkers associated with systemic inflammation and infections in patients with cirrhosis including high-sensitivity C-reactive protein (hsCRP), procalcitonin and LPS[13] (fig. 3). Levels of procalcitonin, a well-known biomarker of infection, were higher among patients with infection associated SIRS than those in whom an infectious source was not isolated. Using levels of 0.45ng/mL, 83.3% of patients with procalcitonin levels > 0.45 ng/mL were infected at the time of admission while 29% of patients with procalcitonin levels < 0.45ng/mL were infected. Procalcitonin levels were also higher among patients who developed multiple organ failure during hospitalization. The role of procalcitonin in identifying sepsis

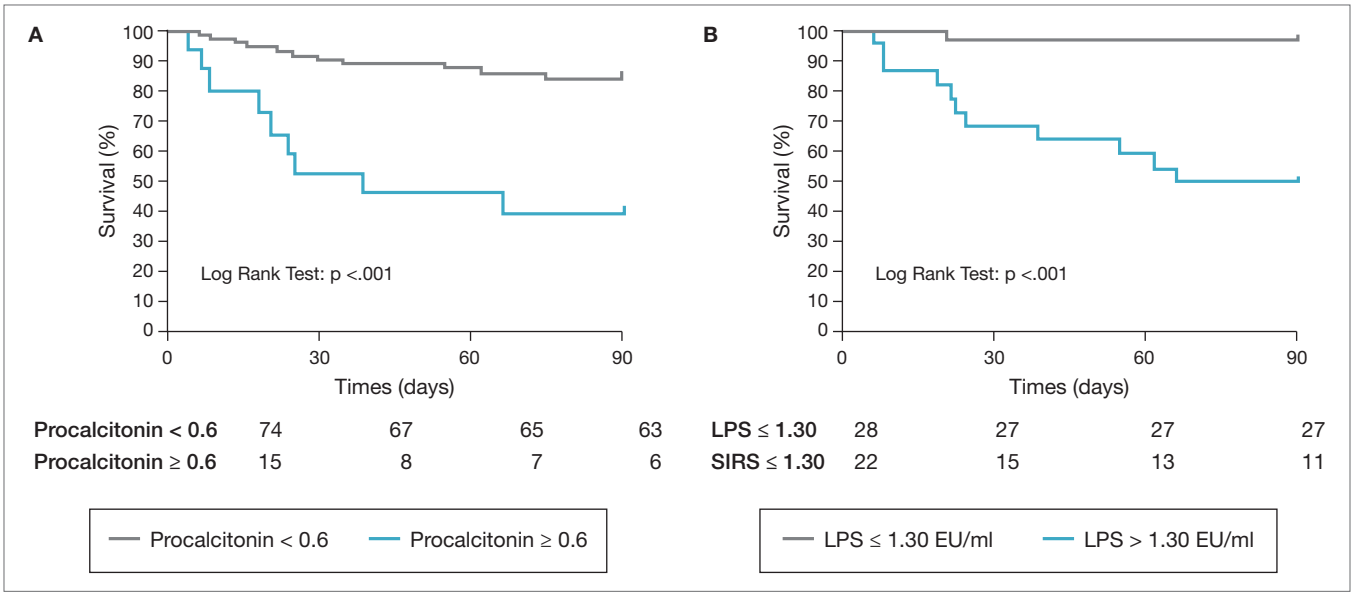

Figure 3. Usefulness of biomarkers in patients with AH. (A) Serum levels of procalcitonin according to the presence and severity of alcoholic liver disease, (B) serum levels of procalcitonin in patients with SIRS without infection versus infection-associated SIRS. Reproduced with permission, Michelena et al[13].

in AH was confirmed in an independent study[32]. In contrast, high reactive C-reactive protein was unable to discriminate between infectious and non-infectious SIRS. Finally, LPS is another potential biomarker in patients with AH. In our SIRS study, LPS levels did not differentiate between patients with infectious and non-infectious SIRS. In contrast, LPS levels did correlate with the severity of AH and also predicted the development of in-hospital infections. Interestingly, patients with low LPS levels were found to responders to corticosteroid therapy, while those with high levels were non-responders to prednisolone, as per the Lille score. These interesting results indicate that targeting LPS could be beneficial in patients with AH.

Finally, maneuvers aimed at preventing extrahepatic organ failure (i.e. AKI) in patients with SIRS should be considered. Although there are no studies assessing hepatic and systemic hemodynamics in this patient population, it is likely that the presence of SIRS, regardless of the cause, causes systemic vasodilatation and effective hypovolemia. Expanding the intravascular volume with albumin could prevent AKI in this subset of patients, yet this hypothesis has to be proven in prospective studies. An alternative approach to expand the intravascular volume is to target the mediators causing SIRS in these patients. Current research efforts are focus on identifying the molecular drivers of infection-associated and sterile SIRS.

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EUROPEAN FOUNDATION FOR THE STUDY OF CHRONIC LIVER FAILURE
SECTION II

CARDIOCIRCULATORY FAILURE AND HEPATORENAL SYNDROME IN CIRRHOSIS: ROLE OF SYSTEMIC INFLAMMATION

Salvatore Piano and Paolo Angeli

Unit of Internal Medicine and Hepatology, Department of Medicine (DIMED), University of Padova, Italy


P. Angeli

Liver cirrhosis is characterized by several cardiocirculatory abnormalities. These alterations were first described in 1953 and were characterized by a decrease in peripheral vascular resistance and an increase in cardiac output[1]. Further studies showed a notable splanchnic arterial vasodilation in these patients that was responsible for most of the circulatory changes observed in patients with cirrhosis. According to the "peripheral arterial vasodilation hypothesis" (PAVH), portal hypertension (PH) has a crucial role in the development of the splanchnic vasodilation since PH causes an overproduction of vasodilators, such as nitric oxide, carbon monoxide, adrenomedullin, endocannabinoids etc. in the splanchnic arterial system leading to a severe vasodilation. The consequent reduction of effective circulating volume causes the activation of baroreceptors and volume receptors leading to an overactivation of the endogenous vasoconstrictors systems such as the rennin angiotensin aldosterone system (RAAS), the sympathetic nervous system and the non osmotic release of vasopressin[2]. The compensatory mechanisms for these changes, namely an increase in heart rate and cardiac output, constitute the hyperdynamic circulation[2]. For several years, PAVH has been considered the main responsible of development of ascites, hyponatremia and hepatorenal syndrome in patients with cirrhosis. However, in the last 15 years it has been shown that in the most advanced stages of the disease the cardiac output fells, resulting in a further reduction of effective circulating volume, reduction in blood pressure and renal hypoperfusion[3,4]. These findings led to a partial revision of PAVH including the role of the cardiac dysfunction among factors involved in the developments of complications of cirrhosis and hepatorenal syndrome (HRS)[5]. The concept of acute-on-chronic liver failure (ACLF), a syndrome characterized by organ failures and a high short term mortality in patients with chronic liver disease, that may occur in any stage of the liver cirrhosis, has challenged the dogmas of PAVH. Indeed, the data from the CANONIC study suggested a crucial role of systemic inflammation in the development of organ failures in patients with an acute decompensation of cirrhosis[6]. In the light of these and other findings, the role systemic inflammation, triggered by pathological translocation of bacteria and/or bacterial products from the gut to systemic circulation, in the development of cardio-circulatory failure and renal failure leads experts to a further revision of PAVH[7]. In this chapter the pathogenesis and treatment of cardiocirculatory failure and hepatorenal syndrome have been reviewed.

Cardiocirculatory failure and cirrhotic cardiomyopathy

Several functional and morphological abnormalities have been found in the heart of patients with cirrhosis, leading to the definition of "cirrhotic cardiomyopathy"[8]. Diastolic, systolic and electromechanical dysfunctions are the main features of cirrhotic cardiomyopathy. For the purpose of this chapter only diastolic and systolic dysfunctions will be discussed.

Diastolic dysfunction

Diastolic dysfunction (DD) is characterized by a decreased left ventricular compliance and relaxation causing an abnormal filling pattern of the ventricles. Both clinical and experimental studies showed a significant increase in left ventricular mass and in left ventricular hypertrophy in cirrhosis vs controls[8-10]. The activation of RAAS and the consequent sodium retention has been claimed as responsible for these morphological alterations[8]. DD can be diagnosed by means of Doppler echocardiography. Traditionally DD has been diagnosed by a decreased E/A ratio which is measured from estimated velocities of the left ventricular inflow during early ventricular filling (E wave) and during atrial contraction (A wave). However, E/A ratio is strongly influenced by pre- and afterload and the tissue Doppler imaging (TDI) provides more accurate data in the diagnosis of DD. TDI allows to measure the tissue velocity at the basal part of the lateral and septal left ventricular wall during early filling (E' wave) which is primarily determined by the relaxation of the left ventricle and the E/E' ratio is considered the best index

of DD. The prevalence of DD varies across the studies according to the technique used for the definition of DD ranging from 46 to 57%[9-10]. The clinical impact of DD in patients with cirrhosis is controversial. Ruiz del Arbol et al. found DD being an independent predictor of the development of HRS and poor survival. Conversely, Nazar et al. found no association between DD and clinical outcomes. Further larger studies should be performed to clarify the clinical relevance of DD.

Systolic dysfunction

The systolic dysfunction (SD) closely relates to the size of the stroke volume, heart rate, and cardiac output. As reported above, in cirrhosis, the circulatory dysfunction is characterized by a hyperdynamic circulation and an increased cardiac output, at least in early stages of the disease. However, experimental studies supply a considerable amount of evidence for SD as a component of cirrhotic cardiomiopathy. SD seems to be the result of a) an impaired beta-adrenergic signaling, and b) systemic and cardiac inflammation.

The impaired beta adrenergic signaling is related to a reduction of the density of beta receptors at the cell surfaces of cardiomyocytes and by an overexpression of genes and protein that inhibit adenylate-cyclase such as G protein alpha-inhibiting subunit 2 and regulator of G-protein signaling 2 (RGS2) that inactivate cAMP[11].

Experimental studies showed that inflammation, probably due to bacterial translocation, plays a key role in the impairment of cardiac contractility in cirrhosis. Yang et al. showed that the impairment in cardiac contractility is strictly dependent by an hyperproduction of TNF alpha acting via Nuclear Factor kappa B and inducible nitric oxide sintase (iNOS) pathways. The consequent production of nitric oxide blunts cardiac contractility both increasing nitration of cardiac proteins and increasing cGMP production[12,13]. Furthermore, an increased infiltration of macrophages in cirrhotic heart with respect to sham controls has been recently shown[14].

In spite of these experimental evidences, when the left ventricular ejection fraction (LVEF) was evaluated at rest in patients with cirrhosis (not assuming beta blockers), it was found normal in most of the patients. This is probably related to a reduced afterload due to low systemic vascular resistance. As a consequence, systolic dysfunction can be unmasked only when a physical or pharmacological stress is induced. A subnormal increase of LVEF has been observed during a physiological exercise while a decrease of LVEF has been observed after the administration of a vasoconstrictor such as terlipressin[15,16]. The clinical relevance of the impairment of cardiac output has been clearly shown in patients with cirrhosis. The reduction of cardiac output has been found to predict the development of HRS and survival both in patients with spontaneous bacterial peritonitis and in stable patients with cirrhosis[17,18].

Hepatorenal syndrome

Patients with advanced liver disease and an acute decompensation of cirrhosis have a high prevalence of acute kidney injury (AKI). HRS represents the most life threatening type of AKI in patients with cirrhosis. According to the diagnostic criteria of HRS proposed by the International Club of Ascites (ICA) in 1996, HRS is a functional renal failure occurring in patients with cirrhosis and ascites. HRS occurs as a consequence of a severe reduction of effective hypovolemia not responsive to diuretic withdrawal and plasma volume expansion, in the setting of a severe activation of systemic vasoconstrictor systems with renal hypoperfusion. Two different types of HRS have to be considered[19]. Type 1 HRS was classified as a rapid progressive renal failure defined by a doubling of the initial serum creatinine (sCr) concentrations to a level greater than 226 mmol/l (2.5 mg/dl) in less than 2 weeks. Type 2 HRS is characterized by a moderate and steady or slowly progressive renal failure (sCr > 1.5 mg/dl). The dominant clinical features are different between the 2 types, being acute renal failure for type 1 HRS and refractory ascites for type 2. Precipitating events such as infections, bleeding and large-volume paracentesis without albumin administration can trigger type 1 HRS, bacterial infections being the most important trigger. Conversely, type 2 HRS usually occurs without any precipitating events.

Recently, new ICA criteria for the diagnosis of acute kidney injury (AKI) in patients with cirrhosis have been proposed[20]. Any previous final cut-off value for sCr has been removed from the definition of AKI and, consequently, from that of HRS in the context of AKI in these patients (Table 1). According to this definition type 2 HRS is now considered a type of chronic kidney disease.

Table 1. Diagnostic criteria of hepatorenal syndrome (HRS) according to International Club of Ascites (ICA) criteria*

Hepatorenal syndrome
Diagnosis of cirrhosis and ascites
Diagnosis of acute kidney injury according to ICA criteria
No response after 2 consecutive days of diuretic withdrawal and plasma volume expansion with albumin 1 g per kg of body weight
Absence of shock
No current or recent use of nephrotoxic drugs (NSAIDs, aminoglycosides, iodinated contrast media etc.)
No macroscopic signs of structural kidney injury, defined as: – absence of proteinuria (>500 mg/day) – absence of microhaematuria (> 50 RBCs per high power field), – normal findings on renal ultrasonography

ICA, International Club of Ascites; NSAIDs, nonsteroidal anti-inflammatory drugs; RBC, red blood cells.
*Modified from Angeli P et al. J Hepatol 2015[20].

Pathogenesis of HRS

As reported above, according to the revised PAVH, the pathogenesis of HRS-AKI in patients with cirrhosis involves two main factors: i) arterial vasodilation; ii) reduction in cardiac output. Furthermore, there is increasing evidence from experimental and clinical data that the degree of inflammation and/or the tolerance to inflammation are crucial in the pathogenesis of organ failures, including renal failure, in cirrhosis[6-7].

Nitric oxide (NO) and carbon monoxide (CO) are the main vasodilators involved in arterial splanchnic vasodilation. In the context of bacterial infections and particularly in that of sepsis, circulating endotoxins and proinflammatory cytokines increase portal hypertension further increasing its negative effect on the cardiovascular dysfunction in patients with cirrhosis. In addition, they stimulate per se iNOS and heme oxygenase type 1, leading to an increased production of NO[21] and CO[22], respectively. Therefore, the increased production of CO and NO results in a further enhancement of splanchnic vasodilation in patients with cirrhosis. Furthermore, sterile or non sterile systemic inflammation leads to a further impairment of cardiac output in patients with cirrhosis. More in detail, bacteria and/or bacterial products are recognized by monocytes through the bond with pattern recognition receptors such as toll-like receptors 4 (TLR4) and 2 (TLR2). This bond activates monocytes to release proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and interleukin 1 beta (IL-1β). TNF-α and IL-6 concentrations turn out to be strong predictors of AKI development in patients with SBP[17]. TNF-α stimulates iNOS synthesis via nuclear factor kappa B, resulting in a reduced cardiac contractility and enhanced peripheral arterial vasodilation.

Nevertheless, the effects of inflammation on cardiovascular function may not fully explain the pathogenesis of AKI and HRS in patients with cirrhosis. In fact, it has been observed that ACLF and AKI may occur in patients with previously compensated cirrhosis or even in patients with a previous diagnosis of a chronic liver disease without clinical, laboratory and/or instrumental signs of cirrhosis. This is important because, as mentioned before, inflammation induced by bacterial infections/translocation could precipitate AKI in decompensated cirrhotic patients by exacerbating the pre-existing degree of cardio-circulatory dysfunction. However, following the steps provided by the PAVH theory, this would be more difficult to achieve in cirrhotic patients without a history of previous episodes of decompensation or in patients with non-cirrhotic chronic liver disease. In the latter two types of patients, inflammation *per se* may lead to renal and/or other organ failures regardless its effect on cardiovascular function.

Indeed, it has been observed in both clinical[23] and experimental studies[24] that an up-regulation of renal tubular Toll like receptor 4 (TLR4) may occur in the setting of cirrhosis and inflammation and it is associated with the development of renal dysfunction, tubular damage and apoptosis suggesting a potential role of TLR4 as mediator of renal injury[23]. Although the mechanisms of up regulation of tubular TLR4 are not entirely clear, it seems likely to be the consequence of the continuous exposure to gut bacterial translocation[23] and may be prevented by gut decontamination with norfloxacin[24]. These data suggest that increased bacterial translocation may exert a detrimental effect in the kidney through the up-regulation of tubular TLR4.

Stretching this concept, a new hypothesis on the pathogenesis of AKI in patients with sepsis has been recently proposed, where AKI is the results of an adaptive response of the tubular cells to an injurious, inflammatory danger signal[25] (fig. 1). The interplay of inflammation and microvascular dysfunction amplify this signal leading tubular cells to a metabolic down-regulation and reprioritization which favors individual cell survival processes (such as the maintenance of membrane potential and cell cycle arrest), at the expense of "kidney function". All these features develop in the context of normal or even increased renal blood flow and provide the framework of the dramatic decrease in glomerular filtration rate and the development of uremia observed in these patients[25].

Prevention of HRS

Two strategies showed to be effective in preventing HRS and improving survival in patients with cirrhosis: a) plasma volume expansion with albumin in patients with SBP and b) primary prophylaxis of SBP with norfloxacin. In a randomized controlled trial, the i.v. administration of albumin (1.5 g per kg of body weight at the diagnosis of SBP and 1 g per kg of body weight at the third day) was able to reduce the incidence of HRS and to improve survival in patients with SBP[26]. However, in the context of bacterial infections other than SBP, results are discordant. Guevara et al. found a significant improvement in renal and circulatory function with albumin administration and a trend towards an improvement in survival[27]. Conversely, Thevenot et al. found albumin able to delay the onset of renal failure, although the three-month renal failure rate and survival rate were not different between the two groups[28]. In order to address this issue, a large multicenter randomized controlled trial is ongoing in Europe. The mechanisms underlying the therapeutic effects exerted by albumin are not fully understood, but they are more complex than the plasma volume expansion. Beyond its role as plasma volume expander, albumin is endowed with an array of non-oncotic properties. Albumin prevents HRS in patients with SBP through an improvement in peripheral vascular resistance and in cardiac function. The mechanism by which albumin ameliorates the peripheral arterial circulation is probably related to its scavenger effect on proinflammatory cytokines and vasodilator molecules released during infection[29]. The most likely mechanism for the improvement in left ventricular function may still be the increased venous return and cardiac

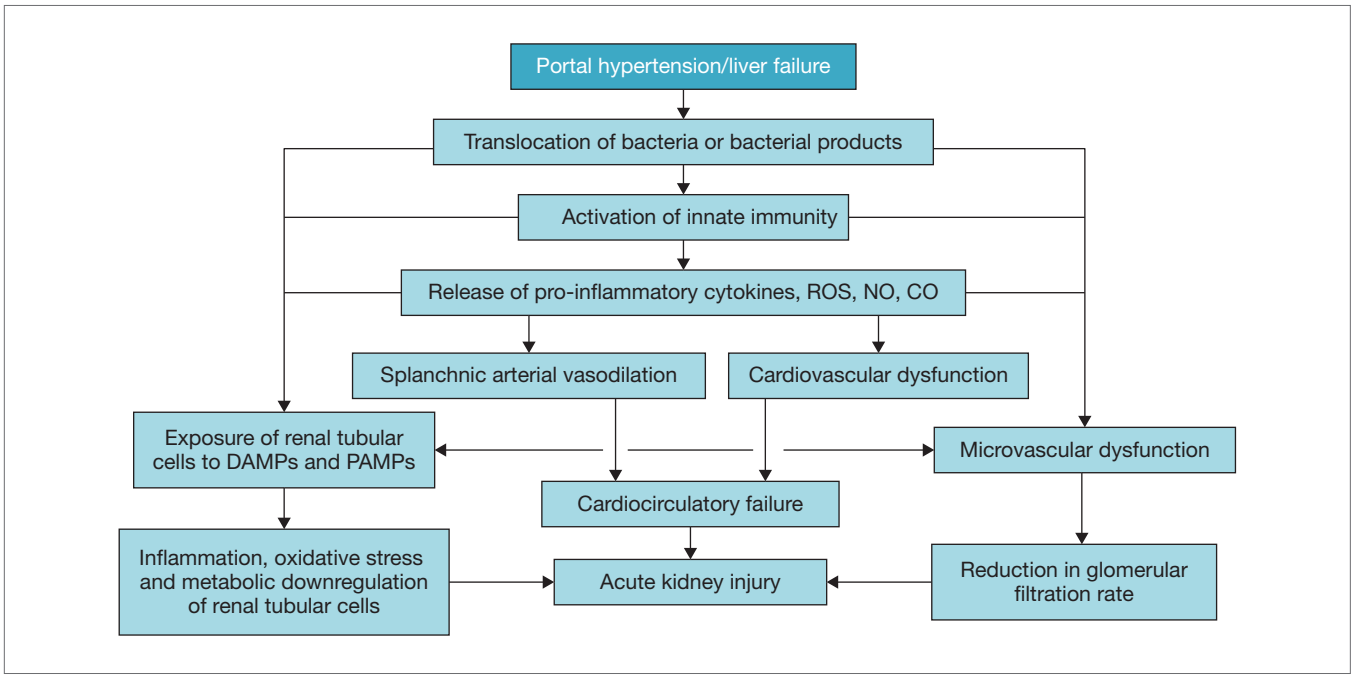

Figure 1. Pathogenesis of cardiocirculatory failure and acute kidney injury in patients with cirrhosis.

ROS, reactive oxygen species; NO, nitric oxide; CO, carbon monoxide; DAMPs, danger associated molecular patterns; PAMPs, pathogen associated molecular patterns.

preload. However, in an experimental study, albumin exerted an anti-oxidative and anti-inflammatory action capable of restoring cardiac contractility[30]. As far as primary prophylaxis of SBP is concerned, in a randomized placebo-controlled trial, Fernandez et al. found that the administration of norfloxacin (400 mg/day) in patients with advanced cirrhosis [Child-Pugh score ≥ 9 points with serum bilirubin level ≥ 3 mg/dl or impaired renal function (serum creatinine level ≥ 1.2 mg/dl, blood urea nitrogen level ≥ 25 mg/dl, or serumvsodium level≤130 mEq/L)] and low ascites protein levels (<15 g/l) was able to reduce the incidence of HRS and to improve survival[31].

Treatment of HRS

Liver transplantation is the treatment of choice in patients with HRS, however not all patients are eligible for liver transplantation, and the time needed to get a graft is unpredictable. Thus, in the past 15 years several medical treatments have been investigated in this field.

The administration of vasoconstrictors and albumin showed to be effective in improving renal function and in resolving HRS in patients with cirrhosis[32-35] (table 2). Vasoconstrictors were used in order to counteract the splanchnic arterial vasodilation, to reduce the portal pressure, and to improve effective circulating volume. Albumin was introduced to further improve the effective circulating volume. The complex mechanism of the action of albumin in improving the effective circulating volume in patients with cirrhosis has been already discussed. The molecular mechanisms of terlipressin, the most widely investigated vasoconstrictors in the treatment of HRS-AKI, are only partially known. Nevertheless, it should be highlighted that the activation of smooth muscle V1a-vasopressin receptors by terlipressin induces arterial vasoconstriction, not only through an increase in intracellular calcium via the phosphatidyl–inositol-bisphosphonate cascade, but also by inhibiting the expression of the inducible nitric oxide synthase in the arterial wall[36]. In two randomized controlled trials, terlipressin given as intravenous boluses (starting from 1 mg/4-6 h to 2 mg/4-

Table 2. Effective vasoconstrictors used in the treatment of hepatorenal syndrome

Treatment	Dose	Rate of full response (%)
Terlipressin	i.v. boluses starting from 1 mg/4-6 h to 2 mg/4-6 h or continuous i.v. infusion starting from 1 mg/4-6 h to 2 mg/4-6 h	34-56 §
Noradrenalin	continuous i.v. infusion starting from 0.5 mg/h to 3 mg/h	43 #

* Albumin (20-40 g/day) should be used in association with vasoconstrictors; §[32,33,35,37,38]; #[35].

6 h) plus albumin (20-40 g/day) was more effective than albumin alone in the treatment of HRS-AKI[32,33]. Midodrine (starting from 7.5 mg/8 h to 12.5 mg/8 h) plus octreotide (starting from 100 μg/8 h to 200 μg/8 h) and albumin were found to be effective in the treatment of HRS-AKI[34]. However, in a recent multicenter randomized controlled trial, terlipressin (given as continuous intravenous infusion starting from 3 mg/24 h to 12 mg/24 h) and albumin were more effective than midodrine plus octreotide and albumin in the treatment of HRS[37]. Noradrenalin was shown to have a similar efficacy than terlipressin in a randomized controlled trial[35]. Nonetheless, in the latter, the sample size was too small to detect a difference between the 2 groups and further studies are warranted. Finally, the way of administration of terlipressin deserves some comments. In fact, in a randomized controlled trial the continuous intravenous infusion showed to be better tolerated and effective at lower dosed than intravenous boluses in the treatment of type 1 HRS[38].

Implication of ACLF grade in HRS

Patients with HRS frequently show failure of organs other than the kidney. High baseline values of serum bilirubin, as well as a lower rise in mean arterial pressure, were identified as negative predictors of response to terlipressin plus albumin[39]. It seems, therefore, that the coexistence of a severe degree of other organ failures such as liver failure or cardiovascular failure can influence the response of HRS-AKI to medical treatment. These observations have been strongly supported by the data on acute-on-chronic liver failure (ACLF). In spite of a variety of ACLF definitions, ACLF is a syndrome that may occur in patients with chronic liver disease as a result of a precipitating event characterized by the development of organ failures, which is associated with a high three-month mortality rate[6]. Preliminary results in a small series of patients with type 1 HRS showed that the rate of response to terlipressin plus albumin is negatively affected by the severity of ACLF[40]. Non-responders had significantly higher values of CLIF-SOFA score compared to responders, thereby indicating a greater severity of ACLF. A CLIF-SOFA score ≥ 11 had 92% sensitivity and 100% specificity in predicting no response to therapy[40]. There are two possible explanations for this finding. One may argue that multiple organ failures are the expression of a more severe disease, however, the presence of multiple organ failures may be the expression of a more complex pathophysiological background, driven by systemic inflammation rather than severe renal arterial vasoconstriction and able to impair the response to treatment. Further studies should be performed to address these issues.

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SECTION II

ADRENAL INSUFFICIENCY IN CIRRHOSIS

Thierry Gustot[1,2], Verónica Prado[3,4,5] and Javier Fernández[3,4,5]

[1]*Erasme Hospital, Brussels,*

[2]*Université Libre de Bruxelles, Belgium,*

[3]*Liver Unit, Hospital Clinic;*

[4]*IDIBAPS, University of Barcelona;*

[5]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHED), Spain*


T. Gustot

Relative adrenal insufficiency (RAI) refers to a clinical entity in which normal or even high levels of cortisol are present, but not in the amount required to meet the increased demands of the organism, which is under stressing conditions (shock, burn, trauma)[1,2]. Cortisol is a pluripotent hormone essential to survive critical illness. It modulates the immune response leading to immunosuppression, and has also important anti-inflammatory properties, through the reduction of plasma levels of cytokines and nitric oxide and the regulation of NF-κβ. Cortisol has also metabolic effects through favoring catabolism of glycogen, fat and proteins, and delaying anabolic pathways. It has also key effects on the cardiovascular system, retaining intravascular fluid and maintaining vascular tone by increasing the vascular and cardiac response to catecholamines and angiotensin[1]. Several studies show that inadequate hypothalamic-pituitary-adrenal axis responses during critical illness are associated to refractory shock and higher mortality[3].

Physiological activation of the hypothalamic-pituitary-adrenal axis

The hypothalamic-pituitary-adrenal axis is activated during stress by cytokines and other factors (hypoxia, hypoglycemia, adipokines, catecholamines, angiotensin II among others), which stimulate the hypothalamus to release corticotrophin releasing hormone (CRH) and vasopressin[1,2]. CRH and vasopressin stimulate pituitary secretion of corticotrophin (ACTH), which finally induces adrenal production of cortisol. During critical illness, the levels of cortisol binding protein decrease markedly, leading to higher levels of free cortisol, the really active fraction of cortisol[4]. Furthermore, negative feedback of cortisol upon CRH and ACTH is inactivated, thus ensuring a sustained activation on the hypothalamic-pituitary-adrenal axis[1,2]. Finally, there is an increase in number and sensibility of cortisol receptors and a marked decrease in the catabolism of cortisol (reduced activity of 5-alpha and 5-β -reductase in the liver and of 11-β-HSD2 in the kidneys)[1,2]. All these multilevel adaptations in the axis optimize and increase the effects of cortisol in peripheral tissues during critical illness.

Diagnosis of relative adrenal insufficiency

Diagnosis of adrenal dysfunction is not possible on clinical grounds. Many different dynamic tests have been used to diagnose RAI. The most frequently employed consists of a baseline measurement of total cortisol levels followed by a single administration of adrenocorticotropic hormone (ACTH) with a second measurement of total cortisol levels taken 30 to 60 minutes later (peak level). The dose of ACTH employed can be of 250 µg (short synacthen test: SST) or more physiological (1 µg: low dose short synacthen test; LDSST)[5-7]. Definition of RAI depends on the test employed and can be based on baseline or peak cortisol levels criteria and/or on the difference between both values (delta cortisol).

The gold standard diagnostic test used in routine clinical practice is the SST. RAI is defined as an increase of total cortisol levels (delta value) in less than 9 µg/dL after the administration of 250 µg of ACTH intravenously. The advantage of using the delta cortisol value as the only diagnostic criterion of RAI (avoiding the baseline or peak cortisol criteria), is that as dynamic criterion it is not affected by changes in transcortin or albumin levels, frequently reduced in advanced cirrhosis[8]. Low baseline total serum cortisol levels (random cortisol < 10 µg/dL or baseline cortisol < 15 µg/dL in critical care and < 9 µg/dL in other settings) and/or peak total cortisol levels (< 18-20 µg/dL) have been used to diagnose RAI in several studies in patients with liver failure. However, these criteria tend to overestimate the prevalence of RAI when compared to the delta criterion or to free cortisol levels[5,6].

Circulating cortisol is mainly bound to transcortin and albumin, and only less than 10% is in the free biologically active form. Therefore, the ideal test to evaluate adrenal function and to diagnose RAI would be the measurement of free cortisol levels, especially in patients with important alterations in transcortin and albumin levels, such as cirrhotic patients. However, methodology to measure serum free cortisol is not easily accessible and the technique is complex, and therefore it cannot be applied in routine clinical practice[4-6]. Coolen´s equation, free cortisol index and salivary cortisol have been employed as surrogates of free cortisol.

Finally, other tests have been employed to diagnose RAI in the general population such as the intravenous administration of corticotrophin hormone (CRH), a complex and expensive technique, and the insulin-induced hypoglycemia test, which is considered the gold standard to assess the hypothalamic-pituitary-adrenal axis in endocrinology[5].

Pathogenesis of adrenal dysfunction in cirrhosis

Adrenal dysfunction has been reported in critically ill cirrhotic patients, in patients with acute decompensation admitted to the regular ward and even in stable cirrhosis. This finding suggests that adrenal insufficiency could be a pre-existing condition in cirrhosis that is further accentuated in critical illness or in acute decompensation. Prevalence of RAI varies from 7% to 75% in decompensated cirrhosis and from 22% to 77% in critical care, depending on the methodology used in its definition[5,6].

Mechanisms leading to adrenal dysfunction in cirrhosis remain largely unknown. Endotoxemia, decreased levels of apolipoprotein A-1, HDL cholesterol and LDL cholesterol, increased levels of proinflammatory cytokines, structural damage of the adrenal and pituitary glands due to infarction or hemorrhage, “exhaustion” of the adrenal cortex, and glucocorticoid resistance have been suggested as potential pathophysiologic mechanisms. Cholesterol is the main substrate in the synthesis of cortisol in the adrenal glands. Several studies reported a significant decrease in the levels of serum total cholesterol, HDL-cholesterol and LDL-cholesterol in cirrhotic patients with RAI that related to the severity of the disease. Furthermore, increased levels of circulating endotoxin (lipopolysaccharide) and TNF-α inhibit the synthesis of cortisol limiting the delivery of HDL cholesterol to the adrenal gland. In addition, TNF-α, IL-1 and IL-6 decrease the hepatocyte synthesis of apolipoprotein A-1, the major component of HDL cholesterol. The lack of substrate for steroidogenesis could contribute to the “adrenal exhaustion syndrome” and to the development of adrenal dysfunction. Reduction in adrenal blood flow due to circulatory dysfunction could also be involved in the pathogenesis of RAI in cirrhosis[5-10].

Changes in the adrenal microenvironment could also participate in the pathogenesis of adrenal insufficiency. Adrenal gland overt inflammation (increased expression of TLRs, proinflammatory cytokines and robust neutrophil infiltration) and adrenal vascular endothelium dysfunction have been demonstrated in experimental models of sepsis. These alterations are associated with increased adrenal cell death and hemorrhage[11].

Prevalence and prognostic impact

Severe sepsis or shock

RAI is extremely frequent in patients with cirrhosis and septic shock, prevalence ranging from 51% to 77% (table 1). RAI in this setting is associated with higher rate of renal failure (79% vs. 35%), lower blood pressure, worse liver function and higher severity of organ failure[12]. Patients with RAI present a poor outcome with higher ICU and hospital mortality (81% vs. 37%)[9,12-15]. Two single center studies have evaluated the impact of stress dose steroids on the prognosis of cirrhotic patients with septic shock, showing opposite results[13,14]. The first study compared a prospective cohort of 25 patients with evaluation of adrenal function and treatment of RAI (hydrocortisone 200

Table 1. Prevalence of relative adrenal insufficiency in critically ill patients with acute or chronic liver failure

Reference	Patients (n)	Critical illness	Prevalence
Harry et al (2003)	20	Acute or chronic liver failure and septic shock	69%
Marik et al (2005)	147	Cirrhosis, fulminant liver failure, liver transplantation	66%
Tsai et al (2006)	101	Cirrhosis and severe sepsis or shock	51%
Fernandez et al (2006)	25	Cirrhosis and septic shock	68%
Thierry et al (2007)	14	Cirrhosis and septic shock	77%
Cheyron et al (2008)	50	Cirrhosis	62%
Arabi et al (2010)	75	Cirrhosis and septic shock	76%
Tsai et al (2014)	143	Cirrhosis and variceal bleeding	30%
Graupera et al (2015)	36	Cirrhosis and severe variceal bleeding	22%

mg/d) with 50 patients from a historic cohort. Outcome was better in those patients included in the prospective series, with higher rate of shock resolution and higher ICU (68% vs. 38%) and hospital survival (64% vs. 32%)[13]. The second study was a RCT comparing 39 patients who received hydrocortisone (50 mg qds iv) with 36 patients who received placebo. The study showed that low-dose steroid treatment was associated to hemodynamic improvement (shock reversal: 62% vs. 39%), but failed to show an improvement in ICU or hospital survival[14]. Other studies performed in critically-ill patients with acute or chronic liver failure receiving low dose steroids also suggest that steroid supplementation is associated to shock reversal (table 2). A European RCT is currently ongoing to evaluate the impact of steroid therapy in cirrhotic patients with septic shock (The SCOTCH trial).

Variceal bleeding

According to the results of two recently published studies, RAI is also relatively frequent in patients with cirrhosis and variceal bleeding (22% to 30%)[16,17]. Its impact on prognosis is also noticeable. Patients with RAI seem to have higher rates of bacterial infection (37% vs. 14%) and treatment failure (62-64% vs. 11-25%) and worse short-term survival than patients with normal adrenal function. However, in these studies patients with RAI had more severe bleedings with higher prevalence of shock, active bleeding at endoscopy or transfusion requirements, feature that makes difficult to draw firm conclusions about the real impact of adrenal dysfunction on the prognosis of patients with variceal bleeding. In fact, RAI was not identified as an independent predictor of short-term mortality in none of these studies.

Acute decompensation

Recent studies show that RAI is also prevalent in patients with decompensated cirrhosis. RAI prevalence ranges from 7% to 75% in this setting depending on the criteria used in its diagnosis[5-8,18]. RAI is associated with a higher degree of circulatory and renal dysfunction, lower mean arterial pressure, higher activation of the vasoconstrictor systems (renin-angiotensin and sympathetic nervous systems) and lower serum sodium levels[8,18]. Moreover, patients with adrenal dysfunction present a more pronounced inflammatory response with higher plasma levels of pro-inflammatory cytokines. More importantly, patients with RAI show a higher risk of developing infections, severe sepsis, type-1 hepatorenal syndrome and death at short-term (22% vs. 7%) (figs. 1 and 2)[8]. In this study, delta cortisol and MELD score were found to be independent predictors of severe sepsis, type-1 HRS and mortality. The higher adrenergic tone observed in patients with RAI (as consequence of the higher degree of circulatory dysfunction), could have contributed to increase the risk of bacterial infection in these patients, since catecholamines promote bacterial translocation. This higher degree of circulatory dysfunction in patients with RAI also explains the higher risk of developing hepatorenal syndrome, severe sepsis and death[8]. Other 2 studies confirm that RAI has a negative impact on the probability of survival in patients with decompensated cirrhosis. Patients with adrenal dysfunction showed a higher mortality rate at medium (33% vs. 10%) and long-term (69% vs. 5%) than those with normal adrenal function[18,19].

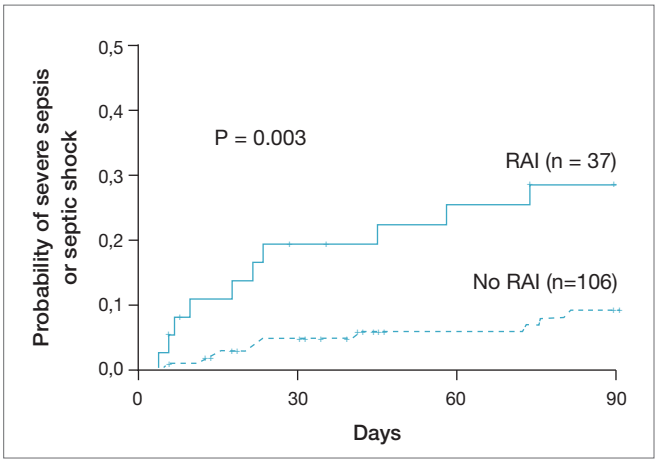

Figure 1. Probability of developing new episodes of severe sepsis or septic shock in patients with RAI (continuous line) and with normal adrenal function (doted line) at 3 months. Probability was significantly higher in patients with RAI.

Table 2. Clinical impact of stress dose steroids in critically ill patients with liver disease

Reference	Patients (n)	Critical illness	Outcome
Harry et al (2003)	20	Acute or chronic liver failure and septic shock	Reduction in vasopressors but not survival benefit Higher incidence of MDR bacterial infections
Marik et al (2005)	140	Acute or chronic liver failure and shock	Reduction in vasopressors and survival benefit
Fernandez et al (2006)	17	Cirrhosis and septic shock	Higher rate of shock reversal and survival benefit
Arabi et al (2010)	39	Cirrhosis and septic shock	Higher rate of shock reversal but not survival benefit at 28-day

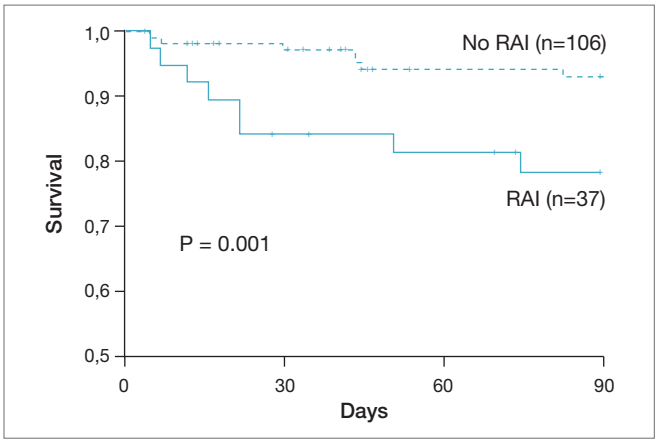

Figure 2. Probability of survival at 3 months in patients with RAI (continuous line) and with normal adrenal function (doted line). Probability was significantly lower in patients with RAI.

Conclusions

In summary, RAI frequently occurs during critical illness and decompensated disease in cirrhosis. Studies, however, do not agree on the prevalence of adrenal dysfunction in cirrhotic patients, mostly because of the different criteria and the methodology used in the evaluation of adrenal function. Diagnosis of RAI remains controversial as all diagnostic tests have some limitations. Probably the most appropriate test to diagnose RAI in cirrhosis is the SST (delta value criterion). Pathogenesis of adrenal dysfunction in liver cirrhosis is still unknown, although decreased levels of cholesterol (mainly HDL cholesterol) and increased levels of proinflammatory cytokines and circulating endotoxin play a role. Some data suggest that adrenal insufficiency may be a feature of cirrhosis per se. RAI has a negative impact on prognosis in both, critically ill and non-critically ill cirrhotic patients. There is still controversy regarding the administration of stress dose steroids in cirrhotic patients with septic shock, although patients with vasopressor resistant shock may benefit. Further prospective, randomized clinical trials are necessary to assess the effect of corticosteroid therapy in critically ill patients with cirrhosis.

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SECTION II

INFLAMMATION AND ENCEPHALOPATHY IN ACUTE AND ACUTE-ON-CHRONIC LIVER FAILURE

Roger F Butterworth

Department of Medicine, University of Montreal, Montreal, Qc., Canada


R.F Butterworth

Hepatic encephalopathy (HE) is a serious neuropsychiatric complication of acute, chronic and acute-on-chronic liver failure that is characterised by cognitive, psychiatric and motor disturbances progressing to stupor and coma. The appearance of HE heralds a poor prognosis with a significant impact on clinical outcome, health-related quality of life, priority for liver transplantation and survival.

A growing body of evidence supports the notion that systemic and central pro-inflammatory mechanisms (acting either alone or in concert with blood-borne toxins known to accumulate in the brain in liver failure) are of key importance in the pathogenesis of the neurological complications (HE, brain edema) in both acute and acute-on-chronic liver failure[1].

Inflammation and encephalopathy in acute liver failure (ALF)

The development of a systemic inflammatory response is a major predictor of the central nervous system (CNS) complications of ALF and polymorphisms of the gene coding for the pro-inflammatory cytokine tumour necrosis factor alpha (TNF-α) have been shown to influence the clinical outcome in ALF[2].

Neuropathological studies in brain from both ALF patients and from animal models reveal alterations of two types of neuroglial cells, namely the astrocyte and the microglial cell. While swelling of astrocytes resulting in cytotoxic brain edema and intracranial hypertension are the principal characteristic findings in ALF, more recent investigations reveal that activation of microglia, indicative of a neuroinflammatory response, has led to the widely-accepted concept that neuroinflammation (inflammation of the brain *per se*) also plays an important role in the pathogenesis of the CNS complications of ALF[3]. Microglial activation has been demonstrated in brain preparations from animals with ALF resulting from liver ischemia or toxic liver injury and has been confirmed in a patient with ALF resulting from viral hepatitis[4]. In the studies in experimental animals, microglial activation was accompanied by increased expression of genes coding for pro-inflammatory cytokines[5] (fig. 1) and the deletion of genes coding for receptors for these cytokines has been shown to delay the onset of severe HE and brain edema in mice with ALF resulting from toxic liver injury[6], consistent with a key role of inflammation in the pathogenesis of these complications.

Inflammation and encephalopathy in acute-on-chronic liver failure (ACLF)

ACLF is considered to be an acute deterioration of liver function in patients with established cirrhosis secondary either to super-imposed liver injury resulting from alcoholic hepatitis, drug-induced liver injury, portal vein thrombosis or ischemia[7] or to extra-hepatic precipitating factors culminating in end-organ dysfunction. Common extra-hepatic precipitating factors implicated in ACLF include infection, surgery and gastrointestinal bleeding and hyperammonemia, all of which are well-established activators of TNF-α production and/or release[8]. Moreover, an impressive positive correlation has been reported between HE severity precipitated by these extra-hepatic factors and circulating levels of TNF-α in patients with ACLF[8] (fig. 2). This latter report went on to demonstrate that improvements in severity of overt HE in these patients were accompanied by significant reductions of circulating TNF-α. Microglial activation, indicative of a neuroinflammatory response, has been described in autopsied brain tissue from patients with cirrhosis with HE[9]. Taken together, these observations support the notion that unregulated inflammation is a key contributing factor in the pathogenesis of HE in ACLF[7].

Inflammation and encephalopathy in acute and ACLF: role of blood-borne toxins

Evidence supports a role of inflammatory mechanisms, both systemic and central, in the pathogenesis of encephalopathy and brain edema in ALF and ACLF. There is a growing body of support for the notion that blood-borne toxins such as ammonia and manganese may act in concert with these

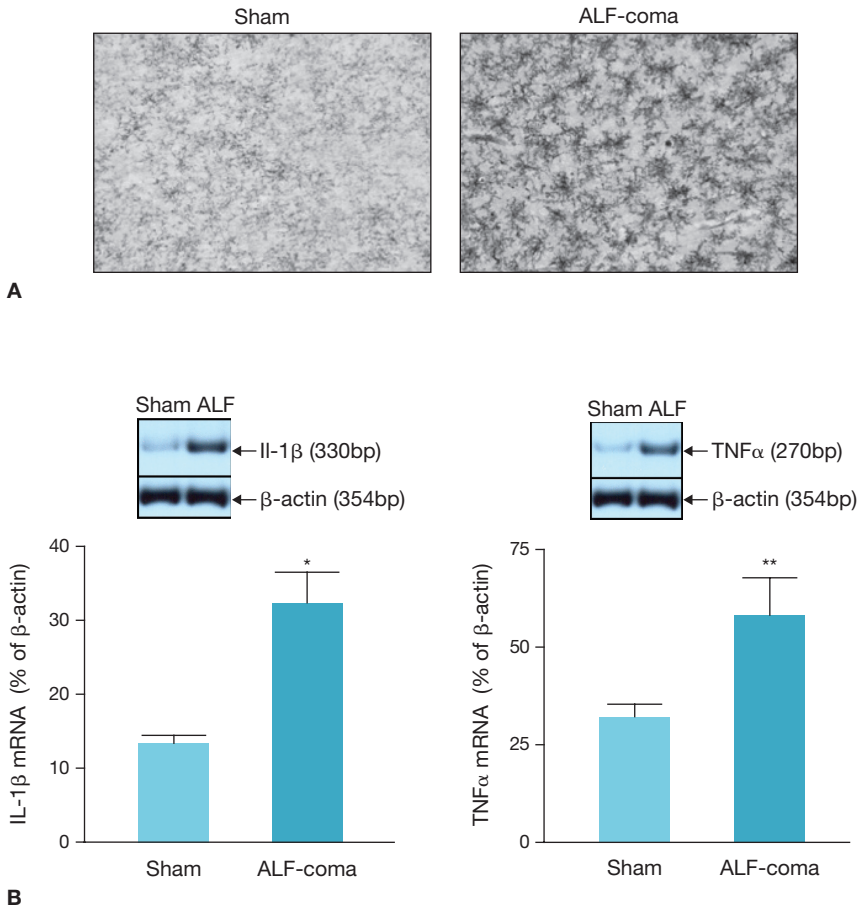

Figure 1. Neuroinflammation in ALF resulting from ischemic liver injury. Panel A: Activation of microglia indicated by increased OX-42 immunostaining in frontal cortex of a rat with ALF resulting from hepatic devascularisation at stage 4 HE (coma/edema) compared to sham-operated control (Sham). Original magnification: X200. Panel B: Increased expression of genes coding for the pro-inflammatory cytokines IL-1β and TNF-α in comparable material from similar groups of animals. Histograms indicate mean +/- SE from groups of n = 6 animals/group. Significant difference from control indicated by * $p < 0.02$, ** $p < 0.01$ by Student t test.

inflammatory mechanisms (and *vice versa*) resulting in HE and brain edema. Such interactions are evident at multiple cellular and sub-cellular levels. Examples include the following:

1. The presence of a significant systemic inflammatory response causes worsening of the central effects of hyperammonemia induced by feeding of amino acid solutions to patients with cirrhosis[10] where the ammonia-induced deterioration of neuropsychological function was prevented by antibiotics in support of the notion of ammonia-proinflammatory synergism.
2. Similar findings were reported following the finding of the synergistic enhancement by ammonia of the expression of genes coding for neuroglial proteins implicated in the pathogenesis of brain edema in cultured cerebral cortical astrocytes[11].
3. A significant positive correlation exists between circulating ammonia concentrations and circulating TNF-α in patients with ACLF[7].
4. TNF-α has the capacity to actively stimulate ammonia diffusion into cerebro-vascular endothelial cells[9] consistent with the existence of a molecular mechanism whereby inflammation has the potential to modulate blood-brain barrier transfer of ammonia leading to brain ammonia accumulation and enhanced ammonia neurotoxicity in liver failure in general. Modest systemic inflammation precipitates HE and increases the permeability of the blood-brain barrier in experimental ALF[12].
5. Manganese, a toxic metal known to accumulate in basal ganglia structures of the brain in cirrhosis[13], has been shown to potentiate the release of TNF-α and IL-1β from microglia[14].

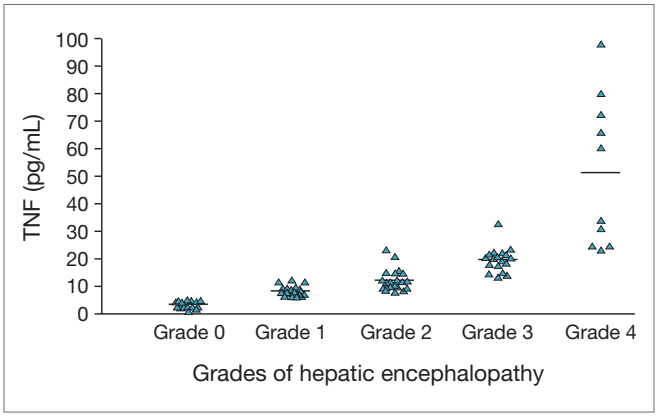

Figure 2. Circulating levels of TNF-α in patients with ACLF associated with a range of precipitating events at various stages of severity of HE. All patients with HE manifested increased circulating TNF-α and ANOVA revealed significant differences between grades of HE with every two grades showing significantly increases of TNF-α with $p < 0.001$. From reference 8 with permission.

Inflammation and encephalopathy in liver failure: role of impaired brain energy metabolism

Decreases of the cerebral metabolic rate for glucose (CMR_{glc}) have been consistently reported in patients with cirrhosis following an acute episode of mild HE[15]. Decreased glucose utilization occurred earliest in the anterior cingulate cortex, a brain structure implicated in the response to visual stimuli and was significantly positively correlated with impaired psychometric test scores in these patients.

Ammonia has potent actions on key enzymes involved in cellular energy metabolism at two distinct levels. Low millimolar concentrations of ammonia inhibit the tricarboxylic acid cycle enzyme α-ketoglutarate dehydrogenase and concomitantly activate the glycolytic enzyme phosphofructokinase. Together these actions of ammonia result in decreased energy production and increased brain lactate production. Brain and cerebrospinal fluid lactate accumulation has been consistently reported in HE patients irrespective of the etiology or acuteness of liver failure[16]. For example, nuclear magnetic resonance spectroscopic studies in rats with ALF resulting from liver ischemia reveal progressive increases of *de novo* lactate synthesis in brain that were closely correlated with brain edema and with the severity of HE[17]. Moreover, in a landmark study using in *vivo* brain microdialysis in patients with ALF, surges of in intracranial pressure were preceded by significantly increased extracellular brain lactate concentrations[18]. ACLF results in brain lactate accumulation and diminished levels of high energy phosphates[1]. Increased brain lactate has also been reported in an animal model of ACLF resulting from portacaval shunting and precipitation of HE by hyperammonemia where increases of brain lactate in excess of 10mM were recorded and found to be positively correlated with the severity of HE[19] (fig. 3). The precise mechanism(s) whereby exposure of brain to increased concentrations of lactate results in HE and brain edema are not fully understood. However, a role of neuroinflammatory factors has been suggested based upon studies demonstrating that exposure of microglia to concentrations of lactate in excess of 10mM (a concentration that is comparable to that reported in brain in ALF and ACLF) leads to increased release of pro-inflammatory cytokines including TNF-α and the interleukins IL-1β and IL-6 from these cells[20] and a neuro-inflammatory response.

Inflammation and encephalopathy in acute and ACLF: therapeutic Implications

Existing therapies for HE remain focussed upon the principle of the lowering of circulating ammonia using agents such as non-absorbable disaccharides, antibiotics, L-ornithine L-aspartate, probiotics and benzoate whose actions are focussed either on the reduction of gut-derived ammonia or on increased ammonia removal by various organs including muscle, kidney and residual hepatocytes. Many of the above agents have well-established anti-inflammatory actions. For

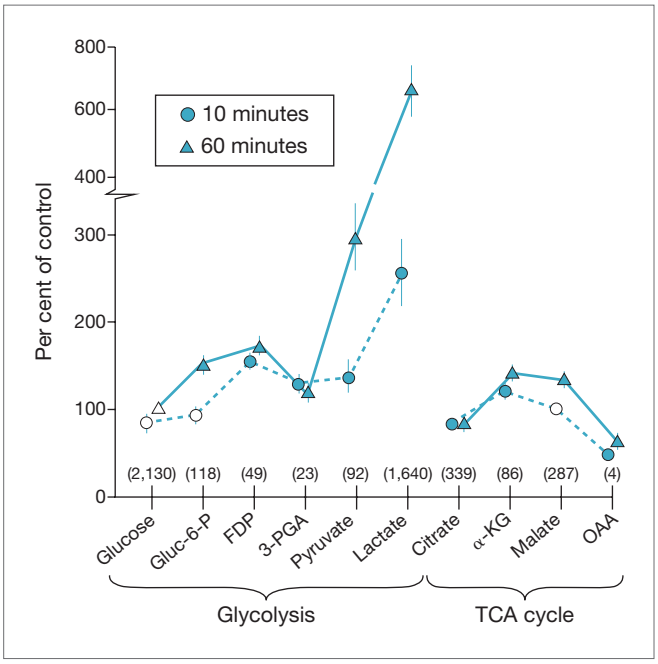

Figure 3. Brain lactate accumulation in an experimental animal model of ACLF. Brain metabolite profiles are indicated in 8 week-portacaval-shunted rats administered ammonium acetate (5.2 mmol/kg. Ip) to precipitate encephalopathy. Note increase in brain lactate to concentrations in excess of 10 mM. Controls received equimolar amounts of sodium acetate. Numbers in parentheses indicate mean metabolite concentrations expressed as umol/kg ww for n = 8 controls. Solid symbols indicate significant differences from control with $p < 0.05$. From reference 15 with permission.

example, lactulose has the potential to decrease circulating levels of pro-inflammatory cytokines such as TNF-α by decreasing intestinal bacterial overgrowth and translocation and/or by decreasing absorption of endotoxin from the intestine[8].

Sterilization of the gut by antibiotics such as neomycin results in decreased circulating levels of TNF-α as well as decreases in its production by Kupffer cells[8] and rifaximin inhibits bacterial overgrowth as well as the translocation of intestinal bacteria and endotoxin[8]. The antibiotic minocycline prevents brain edema in experimental ALF by mechanisms involving lowering of circulating ammonia and also via a direct central effect on the reduction of neuroinflammation via a direct inhibitory effect on microglial activation[21]. The molecular adsorbents re-circulating system (MARS) causes reductions of TNF-α production[8]. MARS has been shown to be effective in both ALF and ACLF where it effectively removes TNF-α leading to improved clinical outcome[3].

It is tempting to propose that one common mechanism whereby these ammonia-lowering agents exert their beneficial actions on the neurological consequences of ALF and ACLF could involve (via the reduction of brain ammonia) the prevention of the rise in brain lactate and the consequent reduction in release of pro-inflammatory cytokines from activated microglia as discussed in detail above[20].

N-acetyl cysteine (NAC) has well-established dual hepato-protective and neuro-protective properties and studies in experimental ALF demonstrate that NAC attenuates the neurological complications of non-acetaminophen-induced ALF via anti-inflammatory mechanisms[22].

Mild hypothermia is increasingly being employed as a bridge to liver transplantation. Experimental studies demonstrate effective reduction in severity of encephalopathy and prevention of brain edema that results, at least in part, from its central anti-inflammatory action[5].

Finally, research using experimental animals is currently underway to evaluate the potential benefits of anti-inflammatory agents such as infliximab and etanercept in the management of the neurological complications of liver failure. In the case of etanercept, the systemic sequestration of TNF-α using this agent was found to attenuate both systemic and neuro-inflammation in mice with ALF resulting from toxic liver injury resulting in delayed progression of liver disease and encephalopathy[23].

Conclusion

In summary, the presence of inflammation is a predictor of encephalopathy and brain edema in ALF and ACLF. Pro-inflammatory mechanisms may act synergistically with ammonia and manganese leading to these neurological complications and to increased permeability of the blood-brain barrier. Both ALF and ACLF result in increased brain lactate sufficient to cause release TNF-α and IL-1β from activated microglia providing a cogent signalling mechanism linking hyperammonemia and neuro-inflammation. Current therapies such as lactulose, antibiotics, probiotics, MARS, N-acetyl-cysteine and mild hypothermia have the potential to exert their beneficial effects, in part, as a consequence of their anti-inflammatory properties. Anti-inflammatory agents such as etanercept may hold promise for the future management of the neurological complications of ALF and ACLF.

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SECTION III

Prognosis, prevention and treatment of ACLF

Chairpersons:

Shiv K Sarin

Institute of Liver and Biliary Sciences, New Delhi, India

Jasmohan Bajaj

Virginia Commonwealth University; McGuire VA Medical Center, Richmond, VA, USA

One of the major advances following the characterization of ACLF has been the development of new prognostic scores specific for patients with decompensated cirrhosis with and without ACLF. Interestingly, the WBC count (a sensitive marker of systemic inflammation that correlates closely to the plasma levels of inflammatory cytokines in cirrhosis) has been found to be as important as bilirubin, creatinine and INR in the prediction of survival in both types of patients and therefore were included in these new scores. The prognostic accuracy of the Chronic Liver Failure Consortium Acute Decompensation Score (CLIF-C ADs) and the CLIF-C ACLFs, are significantly superior than that of other widely used scores such as the MELD, MELD-sodium and Child-Pugh scores in patients with and without ACLF. A score specifically designed to identify the risk of ACLF development is needed for prophylactic therapeutic studies. ACLF is a very dynamic syndrome that may resolve, improve, follow a steady course or worsen following standard medical treatment. These changes, which frequently occur within few days after diagnosis, correlate closely with survival. Therefore, the best prognostic approach in patients with ACLF consists in the assessment of the early clinical course. As in acute liver failure, the best treatment for patients with severe ACLF not improving or in whom the number of organ failures increases within few days following diagnosis is liver transplantation. Intensive care treatment and artificial organ support could serve as a bridge to liver transplantation. There is also data that long-term selective intestinal decontamination with poorly absorbable antibiotics or long term weekly administration of i.v. albumin could reduce the incidence of the syndrome. The recent advances in the mechanism, diagnostic criteria and prognosis of ACLF will be a powerful stimuli for the design of new prophylactic and therapeutic approaches to reduce the high mortality rate associated with this syndrome.

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SECTION III

PROGNOSTIC ASSESSMENT OF PATIENTS WITH DECOMPENSATED CIRRHOSIS AND ACLF AND ROLE OF EARLY LIVER TRANSPLANTATION

Jane Macnaughtan and Rajiv Jalan

Institute of Liver and Digestive Health, Royal Free Campus
London, UK


R. Jalan

Acute-on-chronic liver failure (ACLF) is a highly dynamic and heterogeneous clinical entity associated with a high 28-day mortality. Until recently, conventional scoring systems developed to define the prognosis of patients with cirrhosis such as the Child Pugh and Model for End-Stage Liver Disease (MELD) scores or their variations were the only tools available to prognosticate in this patient cohort. They were limited in their prognostic accuracy in ACLF due to a failure to incorporate two central prognostic determinants; (a) extra-hepatic organ failures and (b) measures of systemic inflammation, which fundamentally underlie the pathophysiological basis of the syndrome. Even the first descriptions of the syndrome in 2002 hypothesized that these factors were important and suggested their incorporation in the prognostic scoring systems[1].

To address this issue, the CANONIC study[2], a large scale multi-centre prospective clinical study evaluating over 1300 patients hospitalized with a complication of cirrhosis was conducted to describe the clinical phenotypes of patients with acute on chronic liver failure (ACLF). A further specific aim of the study was to assess the currently available prognostic scoring systems and develop a new score if required. Indeed, the aims of the study were met and led to the description of the ACLF phenotype and the development and validation of novel scoring systems for the prognosis of patients with ACLF[3] and acute decompensation (AD)[4]. The resultant CLIF Consortium ACLF score (CLIF-C ACLFs) has since been independently validated with proven superior prognostic accuracy for ACLF compared to conventional measures such as MELD, and Child-Pugh scores. The temporal clinical course of these patients was identified as an important prognostic indicator and dynamic assessment of the patient's clinical course using these scoring systems has also been validated as an important prognostic tool[5].

This chapter will consider the phenotype of ACLF and acute decompensation (AD) and how the nature of this influences outcome. Secondly, a description of the scoring systems developed from the CANONIC study data, how they compare to other scoring systems and a proposed algorithm of how they may be applied in clinical practice. Finally, we will discuss the role of liver transplantation in ACLF and how the available ACLF scoring systems may be applied to patient selection.

ACLF phenotype

The CANONIC study described several grades of ACLF classified using a modified sequential organ failure assessment (SOFA) score, evaluating liver, kidney, brain, coagulation, circulation and respiratory function (table 1). ACLF grades[1-3] were found to be highly predictive of mortality with strikingly different outcomes (fig. 1). In addition to ACLF score at presentation, the temporal course of ACLF (particularly over the first 7 days) was found to be strongly predictive of outcome. Heterogeneity was observed in the ACLF clinical course,

Table 1. Diagnostic criteria of ACLF (Data reproduced from Jalan et al. J Hepatology 2014)

Diagnosis	Criteria
No ACLF	• Patients with no organ failure • Patients with single hepatic, coagulation, circulation or respiratory failure, serum creatinine < 1.5 mg/dl and no HE • Patient with cerebral failure and serum creatinine < 1.5 mg/dl
ACLF-1	• Patients with renal failure • Patients with other single organ failure with serum creatinine ≥ 1.5 and < 2 mg/dl and/or HE grade 1-2
ACLF-2	• Patients with 2 organ failures
ACLF-3	• Patients with 3 or more organ failures

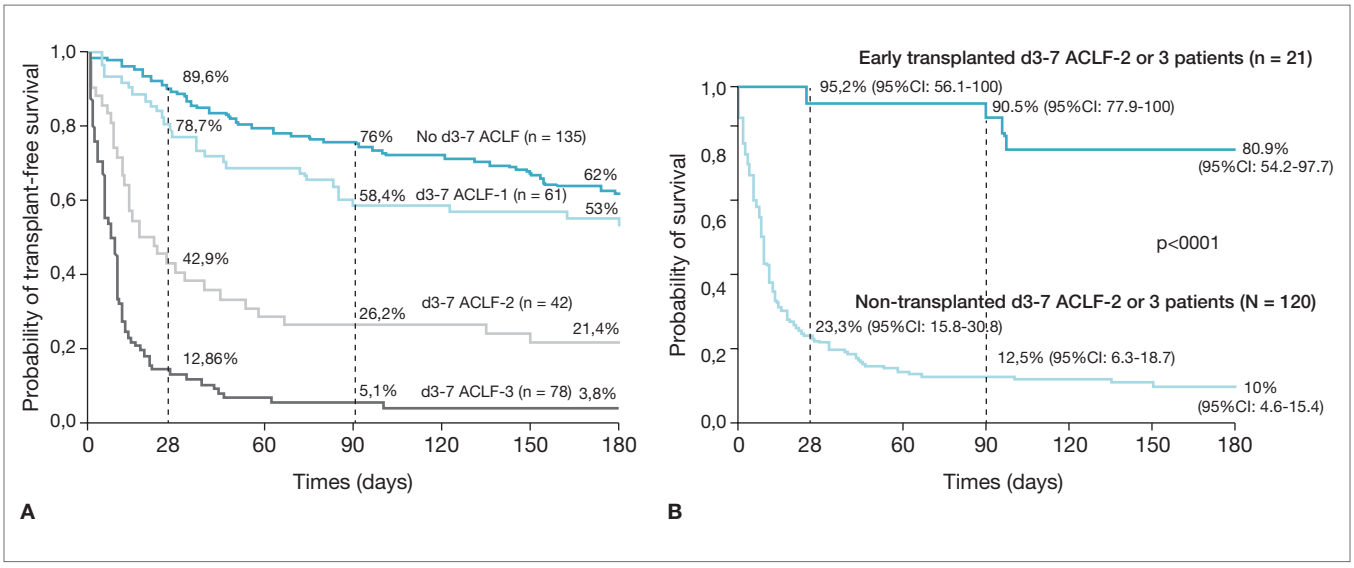

Figure 1. Transplant-free survival rates with differing grades of ACLF. (Gustot T et al, Hepatology. 2015;62: 243-52.)

in which improvement was observed in 50% of patients, a steady course in 30% with deterioration in 20% of cases. The frequency of the clinical course was dependent upon the initial ACLF grade. Patients who resolved to ACLF-1 were found to have outcomes similar to those with acute decompensation and no ACLF. Conversely, patients with ACLF-3 had a very high mortality. Once ACLF was established, the prognosis relied on other factors independent of the precipitating events such as the presence of systemic inflammation at the outset.

The presence of ACLF alters the natural history of acute decompensation events

Hepatic encephalopathy

Analysis of the CANONIC cohort identified that the presence of ACLF altered the clinical phenotype of hepatic encephalopathy and was associated with a worse outcome compared to patients with HE without ACLF[6]. Indeed the patient population developing hepatic encephalopathy with ACLF was markedly distinct from those with hepatic encephalopathy alone. The former populations were characterized by younger age, alcoholic aetiology, and a marked systemic inflammatory response syndrome (SIRS) with severe liver failure. Independent risk factors of mortality in patients with HE included age, HE grade, bilirubin, INR, creatinine and sodium.

Variceal haemorrhage

Clinical data describing the influence of ACLF on the natural history of variceal bleeding is limited. Mehta et al. observed that the occurrence of ACLF markedly changed the hepatic hemodynamics resulting in a marked increase in hepatic venous pressure gradient (HVPG) whereas the hepatic blood flow was reduced[7]. Garg et al.[8] confirmed this observation showing an increased HVPG in 57 ACLF patients with variceal bleeding compared to cirrhotic patients without ACLF. HVPG was an independent predictor of mortality in ACLF patients and significant improvements in HVPG were observed with resolution of ACLF as was previously observed in patients treated with anti-TNF and albumin dialysis[9,10]. A retrospective study of 132 patients hospitalized with acute gastric variceal bleeding demonstrated that the presence of ACLF was an independent predictor of mortality[11].

Renal failure

Renal failure is an important component of the CLIF scores and has been to be a key determinant of outcome in acute decompensation and ACLF. Indeed it has been shown that the ACLF classification has a greater prognostic accuracy in this patient cohort compared to the AKI classification in prediction of 28 and 90-day mortality[12]. It is recognized that prognosis of kidney failure is worse in patients with systemic inflammatory response than in those without. Indeed, in patients with hepato-renal syndrome, the CLIF–SOFA score at enrollment was the only predictor for renal response to standard medical therapy. Survival is significantly shorter in non-responders than in responders to terlipressin.

The NASCELD (North American Consortium for the Study of End-stage Liver Disease) study of 507 cirrhotic patients hospitalized with infection demonstrated that infection-related

ACLF was associated with poor 30 day survival and increased risk of secondary infection[13]. MELD score was found to be predictive of mortality, principally due to the contribution of creatinine.

Scoring systems to assess ACLF

A window of opportunity exists in ACLF to reverse organ failure and improve outcome but accurate prognostic tools are required to inform the clinical decision-making process. This allows for better stratification of patients to determine suitability for intensive care, urgent listing for liver transplantation, or determination of futility of further supportive care. Modified scoring systems validated in large prospective clinical studies such as the CANONIC study have facilitated a more accurate prognostication in patients with ACLF. The recently described CLIF scoring systems CLIF-C OF score, the CLIF-C ACLF score and the CLIF-C AD score discriminate between ACLF and acute decompensation and prognosticate allowing a step-wise algorithm for a rational management of patients with decompensated cirrhosis.

CLIF-OF score

The CLIF-OF (Organ Failure) score (table 2) may be used on admission to determine the presence or absence of ACLF. The scores are freely available on the CLIF Consortium website and also as an app that is downloadable on any mobile platform (ACLF Calculator, Cyberliver, UK). Response to treatment of ACLF patients may be monitored by daily calculation of the CLIF-C ACLF score, incorporating the CLIF-OF score, age and white cell count. Ultimately, resolution of ACLF is the most important determinant of short and medium term mortality and the CLIF-C scores provide an objective measure of this. Patients with high ACLF-C scores may be considered for liver transplantation. If ineligible for transplantation without a demonstrable treatment response in ACLF score by days 3-7, consideration should be made as to the appropriate ceilings of management.

CLIF-C acute decompensation score (CLIF-AD)

The CLIF-AD score may be used to stratify patients with acute decompensation but not ACLF into high, medium and low risk categories of mortality. The CLIF-C AD score includes age, white cell count, serum sodium, serum creatinine and INR. Variables in each score were combined to generate a score system ranging from 0 to 100. High risk acute decompensation has been shown to have similar outcomes to ACLF-1 and patients with this diagnosis should be managed in a level 2/3 care environment. Patients in medium risk (score 46-59) have a 3-month mortality of 31% and warrant further management within level 1 care. Conversely, low risk patients (score < 45) have a 3-month mortality of 1.8% and thus may be considered for early discharge. A proposed algorithm for the assessment of patients with ACLF and acute decompensation is highlighted in fig. 2.

Other scoring systems

The CLIF-OFs and CLIF-SOFA scores have been shown to have superior prognostic accuracy compared to conventional measures such as MELD, MELD-Na and Child-Pugh score[14]. Other scoring system that is relevant in the patients with infection was suggested by NACSELD and is a variant of the organ failure scoring system. Many aetiology specific scoring systems exist such as the Maddrey score, Lille score, Glasgow score that are specific for patients with acute alcoholic hepatitis. How the CLIF scores compare with these scores will have to be assessed in prospective studies in the future. Whilst capturing parameters relevant to hepatic failure, none of these commonly used scoring systems capture number of organ failures or incorporate markers of inflammation,

Table 2. The CLIF Organ Failure scoring system (Jalan et al., J Hepatol. 2015 62:831-40)

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥ 2 < 3.5	Creatinine ≥ 3.5 or renal replacement
Brain (West-Haven)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP < 70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤ 300 - > 200 > 214- ≤ 357	≤ 200 ≤ 214

The colored areas represent organ failure.
INR: International Normalised Ratio; MAP: Mean arterial pressure; PaO_2: Partial pressure of oxygen; SpO_2: Oxygen saturation; FiO_2: Fractional inspired oxygen.

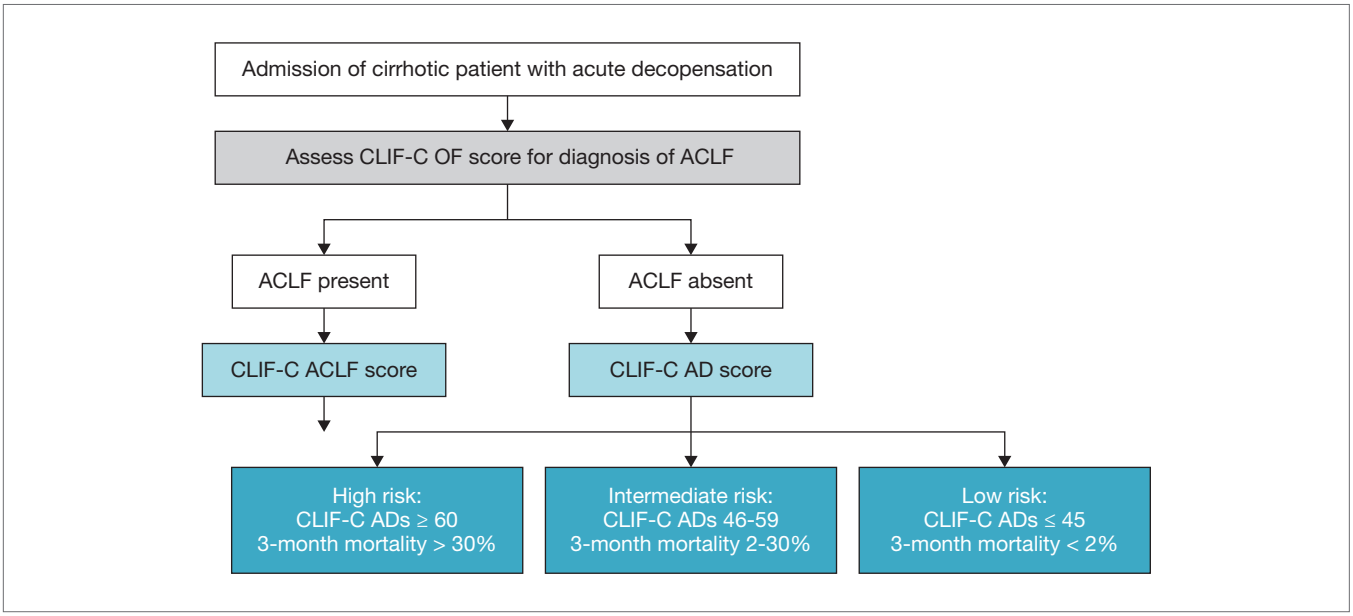

Figure 2. Proposed algorithm for diagnosis and risk stratification of ACLF and acute decompensation. (Arroyo V et al. J Hepatol. 2015;62:S131-43).

key prognostic determinants in ACLF. In context of acute decompensation, the predictive value of the CLIF-C AD score improves prediction of 3 month and 12 month mortality by 10-20% compared to MELD, MELD-Na, and Child-Pugh scores.

Patient selection for liver transplant in ACLF

At present, there is no priority given to patients being allocated organs for liver transplantation. 5-year survival outcomes following liver transplant for ACLF are good, ranging between 74 and 90%. Eligibility may be precluded by number of organ failures, sepsis, co-morbidity, age or active alcoholism. The pre-transplant condition of patients with ACLF plays a key role in determining outcome and therefore careful patient selection is crucial.

Given the labile and rapidly progressive nature of the disease, a narrow window of opportunity exists when patients are sufficiently stable to consider this option. Medical response to supportive therapy has been conventionally measured by scoring systems such as MELD. The limitations inherent in these are highlighted by Duan et al.[15] who observed that MELD score did not predict outcomes of patients with hepatitis B ACLF following orthotopic liver transplantation. The data from the CANONIC study also very clearly demonstrate that the MELD score, which is what is currently used for organ allocation, underestimates the risk of death of ACLF patients by 20-30% seriously disadvantaging ACLF patients. Dynamic assessment of ACLF-C scores may facilitate this decision-making process although further validation studies are required to determine a more precise algorithm for optimal timing of transplantation. Furthermore, transplantation is usually only considered in patients assessed and listed for transplantation before an episode of ACLF.

Data regarding liver transplantation outcomes for ACLF patients are limited and interpretation complicated by variable definitions of ACLF, small patient cohorts, retrospective analysis and lack of availability of long term follow up data. 4.9% and 15% of patients from the CANONIC patient cohort with ACLF underwent transplantation within 28 and 90 days of admission respectively. Survival of patients with ACLF-2 or -3 without transplantation was less than 20% but 80% with transplantation, comparable to patients transplanted without ACLF.

Only one study (n = 238)[16] used intention-to-treat analysis and showed a 5-year post-transplant survival of greater than 80% for patients eligible for transplantation (< 25% of patient cohort). The median transplant-free survival time was 48-days with deaths most commonly secondary to multi-organ failure. Successful transplant outcomes in carefully selected patients with corticosteroid-resistant acute alcoholic hepatitis further reinforce the importance of good patient selection using accurate prognostic criteria. Patients with ACLF appear to tolerate marginal grafts particularly well. Survival and post-transplant length of stay is known to be worse for patients hospitalized at the time of surgery than in those at home and markedly worse still for those in level 3 care. Increasing recipient age (> 60 years) is consistently associated with increased mortality.

Inclusion of high-risk ACLF subgroups as an indication for high urgency allocation is not currently practiced in most countries but should be the subject of further studies particularly given the good outcomes described. The US experience of this strategy is highly favorable with an

improvement in waiting list mortality of 30% with no significant increase in post-transplant mortality. Expedited transplantation assessment should also be considered for survivors of ACLF after discharge from the intensive care unit due to a substantial increase in medium-term mortality.

An algorithm detailing how patients with ACLF should be considered for liver transplantation is proposed for further evaluation (fig. 3).

ACLF biomarkers

ACLF biomarkers currently identified reflect the pathology of an exaggerated inflammatory response, organ injury and role of the microbiome driving this process. Evidence of immunological dysregulation has been shown in patients with ACLF in which monocyte MERTK$^+$ expression is increased and HLA-DR expression pathologically downregulated[17,18]. Concentrations of soluble CD163, a circulating marker of macrophage activation, were found to be associated with ACLF grade[19]. Levels were predictive of survival and improved the prognostic efficiency of clinical scoring systems. Plasma markers of cell death (M30-antigen, M65-antigen, AOPP, S100A12 and sRAGE) have been found to be independent risk factors for poor prognosis in ACLF[20-22]. Recently, copeptin, reflecting vasopressin levels in the plasma, was shown to improve the performance of the CLIF ACLF scores (unpublished). Urinary biomarkers with high accuracy for ACLF include NGAL, creatinine, osteopontin, albumin, and TFF-3[23].

The presence of hyponatremia (at inclusion or during hospitalization) was a predictive factor of survival both in patients with and without ACLF[24]. The presence of hyponatremia and ACLF was found to have an independent effect on 90-day survival after adjusting for the potential confounders. Patients with hyponatremia and ACLF had a three-month transplant-free survival of only 35.8% compared to 58.7% in those with ACLF without hyponatremia ($P < 0.001$). Microbiome composition has been identified an independent predictor of mortality in patients with ACLF with significant reductions in microbial diversity and richness observed in ACLF patients[25].

Further work is required to determine how ACLF biomarkers will be utilized in conjunction with clinical scoring systems to predict response to therapy and outcome measures.

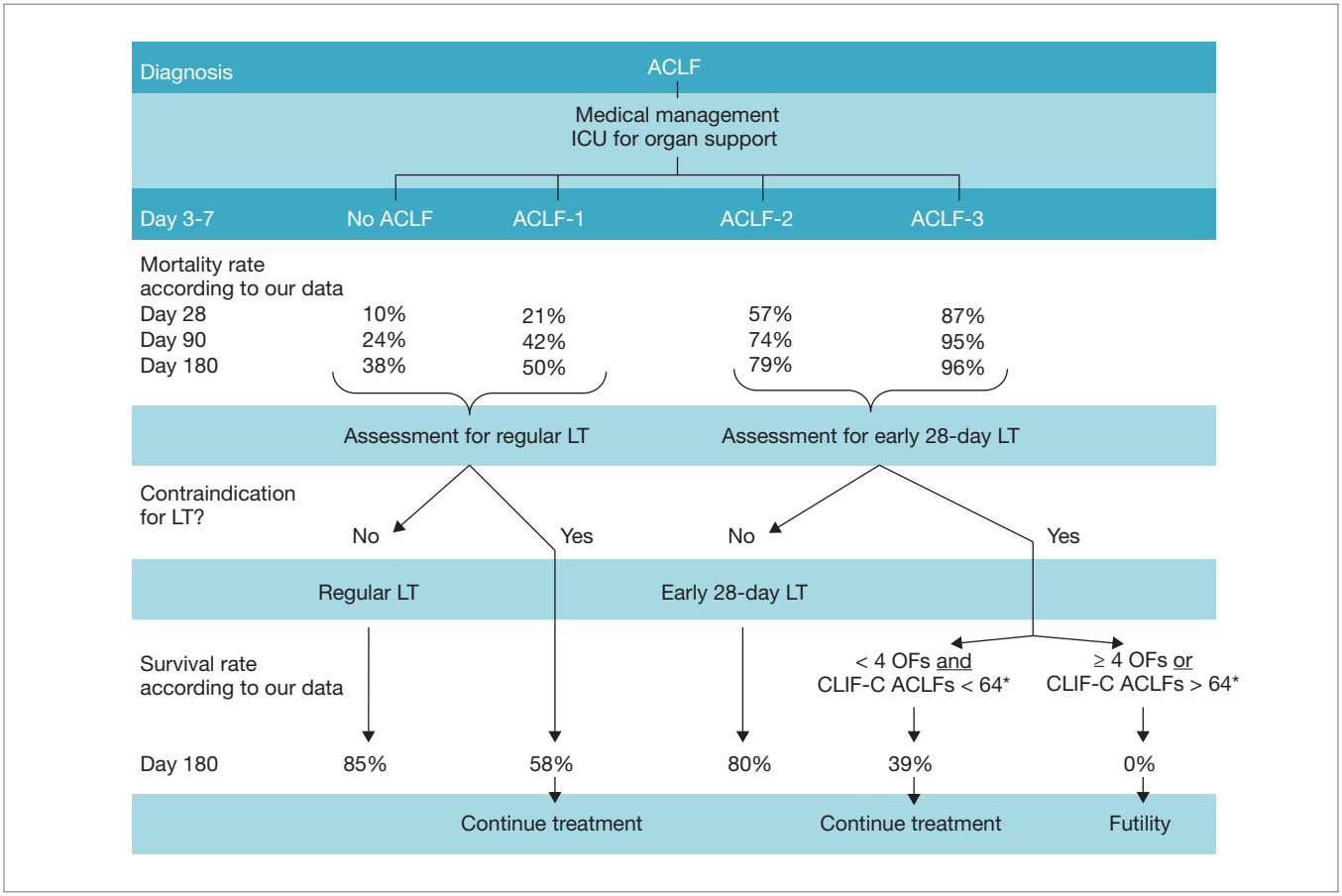

Figure 3. Proposed algorithm for management and further research studies of ACLF patients. (Gustot T et al. Hepatology. 2015;62: 243-52.)

Conclusions

ACLF is associated with a high mortality but the temporal course and prognosis may be highly variable requiring an accurate prognostic scoring system for clinical management. This must reflect the dynamic nature of the disease entity to be able to establish responsiveness to medical therapy. The CLIF ACLF and AD scoring systems are validated for ACLF and acute decompensation patient cohorts. They have been shown to be superior to conventional scoring systems and may be used to determine futility or need for transplantation. Further work is required to more clearly define patient selection, timing and priority for transplantation to appropriately manage this patient group with a narrow window of opportunity for therapeutic success. There is however, a huge opportunity to improve the performance of these scoring systems by the addition of novel biomarkers.

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PREVENTION OF ORGAN FAILURE AND MORTALITY IN DECOMPENSATED CIRRHOSIS

Paolo Caraceni, Maurizio Baldassarre and Mauro Bernardi

Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Italy


P. Caraceni

Each year, about 170,000 European citizens die from cirrhosis, which represents the 5th most common cause of death across Europe in individuals aged 45-65 years. Moreover, patients with cirrhosis cost for more than € 15.8 billions per annum in total health costs and loss in economic productivity[1].

Decompensation of cirrhosis, which is heralded by ascites, hepatic encephalopathy, gastrointestinal bleeding, and bacterial infections, develops in more than half of patients within 10 years from the diagnosis, and represents a hallmark in the natural history of cirrhosis. With decompensation, cirrhosis becomes a systemic disease with a dramatic worsening of the prognosis related more to the involvement of other organs rather than to the progressive exhaustion of liver function. The progressive clinical deterioration of these patients is often dramatically accelerated by the onset of acute-on-chronic liver failure (ACLF), a clinical syndrome characterized by acute decompensation (AD) of cirrhosis associated to organ failure(s) (liver, kidneys, brain, lungs, coagulation, circulation) and high short-term mortality[2].

As convincingly proposed by the Systemic Inflammation Hypothesis (SIH), decompensated cirrhosis is characterized by two major pathophysiologic features: circulatory dysfunction and chronic systemic inflammation. These events are closely inter-related and cooperate to cause multi-organ dysfunction and failure. If circulatory dysfunction and systemic inflammation are steadily moderate in patients with decompensated cirrhosis, they become abruptly severe in patients with ACLF[3].

Current strategies for prophylaxis and treatment of decompensation and organ failure in cirrhosis rely on measures aimed to prevent or improve the outcome of each complication (i.e. ammonia production or excretion in hepatic encephalopathy [HE], effective hypovolemia after large-volume paracentesis or during hepatorenal syndrome [HRS], renal failure after spontaneous bacterial peritonitis [SBP], and intestinal bacterial overgrowth in patients predisposed to develop infections).

Besides the improvement of the already established treatments for specific acute clinical complications, the scientific evidence emerged in the last decade clearly indicates that the future global management of patients with decompensated cirrhosis should be based on two major mainstays: 1) treatment of the underlying etiologic factor(s), whenever possible; and 2) long-term chronic approaches targeted to antagonize key pathophysiologic mechanisms aiming to reduce the incidence of AD and ACLF and improve patient survival and quality of life, as well as reducing the economic burden of the disease.

Etiologic treatments

If it has been clearly ascertained that the removal of the underlying etiologic cause in patients with compensated cirrhosis, namely persistent viral suppression for hepatitis B (HBV), viral eradication for hepatitis C (HCV), and sustained abstinence from alcohol, is associated with slower progression of portal hypertension and lower incidence rate of clinical complications, hepatocellular carcinoma and mortality, data in patients with decompensated cirrhosis are not so unequivocal. From the few studies available, it appears that HBV suppression, which is obtained in almost all cases, improves the all-cause mortality in patients with decompensated cirrhosis but not in those with ACLF[4]. The recent introduction of the interferon-free regimens with the new Direct Antiviral Agents (DAA), which couple high potency with a good safety profile, has extended the treatment for hepatitis C to patients in Child-Pugh class B or C who were previously excluded from antiviral therapy. Although lower as compared to that obtained in patients with a compensated disease, the achievement of sustained virological response (SVR) in decompensated cirrhosis appears to be consistently higher than 80-90%. Moreover, once viral eradication is achieved, about 30-50% of patients present a significant amelioration of MELD and Child-Pugh scores within few months, thus suggesting that SVR may lead to a significant reduction in the incidence of AD, ACLF, and mortality at least in a portion of patients with decompensated cirrhosis[5]. Although a "point of no-return" beyond which viral eradication does not produce any effect on the progression of the disease likely exists, a clear cut-off has not been established. At this regard, a recent trial indicates that

a higher benefit is observed in patients with MELD greater than 15[6].

Thus, all patients with HBV and HCV-related decompensated cirrhosis should be considered for antiviral therapy and the decision to deny treatment should be taken only after a careful assessment of several factors, such as concomitant contraindications to therapy, very short-term expectancy of survival, comorbidities affecting prognosis, indication of liver transplantation and expected time to transplant if already in the waiting list, given the possibility to start therapy in case of recurrent post-transplant hepatitis.

Finally, abstinence remains the cornerstone of therapy in patients with decompensated cirrhosis and active alcoholism and should include rehabilitation with a multidisciplinary approach, as its achievement lowers disease progression and increase candidacy to effective treatments, including transplantation.

Pathophysiologic treatments

Treatment approaches designed on the basis of the pathophysiology of the disease turn to be effective only if the proposed intervention is able to act on a "core" mechanism and/or on multiple steps of the pathogenetic process.

Based on the new SIH[3], the pathophysiology which drives cirrhosis and its complications is strongly associated with changes in the gut barrier function, which allow viable bacteria and/or bacterial products, known as pathogen-associated molecular patterns (PAMPs), to move into the gut wall mesenteric lymph nodes and then to other sites[7]. Bacterial translocation (BT) initiates both circulatory dysfunction, characterized by arterial vasodilation and, in a more advanced stage, by impaired cardiac contractility, leading to a progressive reduction of effective volemia, and chronic systemic inflammation, which is associated to oxidative stress and impairment of the immunological response. Although the exact contribution by each pathway and the chronological order of these events are far from be fully defined, the sum of these factors produce invariably cell damage/death and organ dysfunction/failure.

Thus, it appears that treatment strategies aimed at preventing AD, ACLF, and death in patients with decompensated cirrhosis should persistently antagonize BT, modulate systemic inflammation, restore the immunological response, and/or improve circulatory dysfunction. A list of potential interventions is reported in table 1.

Interventions acting on bacterial translocation

Due to its role of initiating "core" factor in the pathogenesis of decompensated cirrhosis, BT is a major target of therapy.

Antibiotic-based interventions

The most straightforward approach to abrogate translocation of microbes and their products is to decrease the burden of enteric bacteria with non- or poorly absorbed antibiotics. Norfloxacin is the most frequently used antibiotic for long-term selective intestinal decontamination being specifically active against Gram-negative bacteria. Norfloxacin is currently used in three specific sub-populations at high risk of infections, as primary prophylaxis for patients with active gastrointestinal bleeding or for those with low-protein ascites associated to poor liver function and as secondary prophylaxis with a prior SBP episodes[8]. Interestingly, the survival benefit observed in patients with low-protein ascites and poor liver function resulted not only from the lower number of SBP episodes, but also from the reduction in the incidence of non-infectious clinical complications, such as HRS type I[9]. This latter finding may be somewhat expected if we consider that norfloxacin, beside reducing translocation of viable bacterial and their products, has been shown to modulate the inflammatory response, as witnessed by a lower level of pro-inflammatory cytokines (i.e. TNF-α, IL-12, IFN-gamma), and to improve circulatory dysfunction by increasing systemic

Table 1. Therapeutic approaches potentially effective in preventing acute decompensation, acute-on-chronic liver failure, and mortality in patients with decompensated cirrhosis by acting with direct or indirect putative mechanisms on bacterial translocation, systemic inflammation, immune response and/or or circulatory dysfunction

	Bacterial translocation	Systemic inflammation	Immunologic response	Circulatory dysfunction
Norfloxacin	Direct	Indirect/direct	Indirect/direct	Indirect
Rifaximin	Direct	Indirect	Indirect	Indirect
Pre/probiotics	Direct	Indirect	Indirect	Indirect
Beta-blockers	Direct	Indirect	Indirect	Direct/indirect
Obeticholic acid	Direct	Indirect	Indirect	Indirect
Human albumin	—	Direct	Direct	Direct
Statins	—	Direct	Direct	Direct
Low-molecular weight heparin	Direct	—	—	—

vascular resistances and blood arterial pressure. Furthermore, a direct effect on the immune system has been also proposed[9]. Unfortunately, this approach carries the problem of the emergence of infections caused by multi-resistant bacteria, which are associated with high-mortality rates, rising doubts on the use of this preventive strategy even when strictly limited to patients with very advanced disease.

Recently, rifaximin has been proposed as an alternative antibiotic for intestinal decontamination in advanced cirrhosis. It is minimally adsorbed and carries a broad-spectrum activity against Gram-negative and Gram-positive aerobic and anaerobic bacteria, with a low risk of inducing bacterial resistance[10]. It has been also shown that rifaximin improves systemic hemodynamics and renal function in patients with decompensated alcohol-related cirrhosis as well as the circulating levels of endotoxin and pro-inflammatory cytokines (i.e., TNF-α and IL-6)[10]. These characteristics make rifaximin a very good candidate for long-term prophylactic treatment in advanced cirrhosis. Unfortunately, if the benefit of rifaximin on recurrent HE and HE-related hospitalizations is demonstrated by well-conducted randomized clinical trials[11], its efficacy in reducing the other clinical complications of cirrhosis and prolonging survival is still undetermined. Although non-randomized small-sized studies performed in retrospective and prospective series have shown that rifaximin treatment is associated to a lower incidence of HE, SBP, HRS, and variceal bleeding and to a longer survival[10], a more recent prospective study found a similar incidence of SBP in patients receiving rifaximin or placebo, which was significantly superior to that seen in patients treated with norfloxacin[12]. Furthermore, the emergence of resistant bacteria has also been reported after treatment with rifaximin. Hopefully, a firm conclusion will by provided by a planned or ongoing randomized trial on the efficacy of long-term prophylaxis with rifaximin in the management of cirrhosis-related complications (NCT 01904409, www.clinicaltrials.gov).

Non-antibiotic based interventions

It appears evident that novel non-antibiotic approaches, which limit BT without contributing to a rise in bacterial virulence and multi-resistant infections, are needed (table 1). Direct or indirect evidence come from experimental and clinical studies on prebiotics and probiotics, beta-blockers, obeticholic acid, oral carbon adsorbents, and low-molecular weight heparin.

Pre- and probiotics stabilize mucosal barrier function and modulate the gut microflora, suppressing pathogenic microbial growth and favoring the preservation of a "healthier" microbioma[8,13]. Indeed, some experimental and clinical data support their positive effect in reducing BT and the downstream inflammatory responses and circulatory disturbances in patients with decompensated cirrhosis. However, their efficacy in improving relevant clinical outcomes still has to be demonstrated and adequately-powered randomized trials evaluating probiotics with a definite composition of bacterial strains as an alternative or adjunctive prophylactic treatment need to be performed.

Non-selective beta-blockers (NSBB) are the mainstay of the long-term primary and secondary pharmacological prophylaxis against variceal bleeding. They decrease portal pressure by lowering cardiac output through blockade of beta-1 adrenoceptors and increases splanchnic vasoconstriction blockade of beta-2 adrenoceptors[14]. However, the benefit on survival appears to reside not only on the direct effect on portal pressure and the resulting prevention of bleeding, but also on other biological effects. Indeed, by dumping the sympathetic hypertone seen in patients with advanced cirrhosis, NSBB accelerate intestinal transit, decrease bacterial overgrowth and intestinal permeability[8,14], thus reducing BT and markers of systemic inflammation[15]. Clinical data indicate that long-term NSBB protect against SBP and other clinical complications of the disease (HRS, HE and bacteremia)[8,14], thus suggesting that their use in patients with advanced cirrhosis can be extended beyond prophylaxis of bleeding. However, adequately-powered randomized clinical trials assessing the effect of long-term NSBB in decompensated cirrhosis having the reduction of AD, ACLF and mortality as primary end-points need to be performed before reaching a firm conclusion. Furthermore, the effect of NSBB on systemic hemodynamic may be detrimental in patients presenting a severe circulatory dysfunction, so that he Baveno VI consensus indicates that NSBB should be discontinued in patients with refractory ascites who develop arterial hypotension (systolic blood pressure < 90 mmHg) hyponatremia (serum sodium < 130 mmol/L) and acute kidney injury[16].

Other future pharmacological and non-pharmacological approaches to reduce BT include: 1) farnesoid-X receptor agonists, such as obeticholic acid, which reduces bacterial fecal load and improves intestinal dysbiosis, intestinal barrier function, and gut inflammation in experimental cirrhosis[17]; 2) low-molecular weight heparin, which has been shown to delay the occurrence of hepatic decompensation and to improve survival in a small-sized randomized clinical trial. These effects were not only associated with a reduced incidence of portal vein thrombosis, but also with evidence of a decreased BT and systemic inflammation. These effects may be related to the improvement of the intestinal barrier through the preservation of the microcirculatory blood flow by dissolving microthrombi[18]; 3) oral carbon adsorbents, capable of removing endotoxin and other bacterial metabolic toxins within the intestinal lumen, as it is currently under investigation by the "CARBALIVE" Consortium using a patented nanoporous carbon adsorbent with tailored porosity (Yaq-001) within the frame of a project funded by European Union's HORIZON 2020 Research and Innovation Programme; and 4) specific nutritional approaches, since nutrition is known to influence the microbioma and its function as well as potential toxic products derived from the interaction of the microbiota

with the diet, such as ethanol, acetaldehyde, trimethylamine, and short-chain and free fatty acids[19].

Interventions on downstream consequences of bacterial translocations

Based on its biological activities and recognized clinical effects, human albumin (HA) is at present the best commercially available candidate for a long-term treatment of patients with decompensated cirrhosis.

HA is currently used in patients with decompensated cirrhosis with the scope to counteract effective hypovolemia, based on its capacity to act as plasma-expander. HA is the main modulator of fluid distribution among the body compartments as it accounts for about 70-80% of the plasma oncotic pressure. The oncotic property derives for 2/3 from the direct osmotic effect related to its molecular mass and for 1/3 from the Gibbs-Donnan effect. The latter is related to the negative net charge of the molecule at physiological pH, which allows the protein to attract positively charged molecules (i.e. sodium and, therefore, water) into the intravascular compartment. Besides that, the long circulatory half-life and the prolonged total half-life also contribute to make HA a far more efficient plasma-expander in cirrhosis as compared to crystalloids and synthetic colloids[20-22].

HA is also provided of many other biological properties that are unrelated to the regulation of fluid compartmentalization (fig. 1). Among these non-oncotic properties, some assume particular importance in relation to the chronic inflammatory state of decompensated cirrhosis, such as antioxidant and scavenging activities, binding and transport of many endogenous and exogenous substances, and regulation of endothelial function and inflammatory/immunological responses[20-22]. HA is the major source of extracellular sulfhydryl groups, mainly located at the cysteine-34 free residue, which is mostly in the reduced state, thus acting as potent scavenger of reactive oxygen and nitrogen species (ROS; RNS). HA is also capable to bind at the N-terminal portion of the molecule several metal ions, which are therefore inhibited to catalyse ROS/RNS-generating chemical reactions[20-22]. As a result, HA is the main circulating antioxidant system in the body. Due to its peculiar and dynamic structure, HA binds and carries a great variety of hydrophobic molecules, such as endogenous (i.e. cholesterol, fatty acids, bilirubin, thyroxine) or exogenous (i.e., drugs including many antibiotics) substances, lipopolysaccharides (LPS), transition metal ions, and NO, with consequent implications on their solubilisation, transport, and metabolism[20-22]. HA also contributes to the integrity of microcirculation by binding the interstitial matrix and interacting with the sub-endothelial space, thus participating in the maintenance of the normal capillary permeability. HA may impact positively on endothelial function also by reducing oxidative damage and modulating the signalling systems between neutrophils and endothelial cells. Moreover, HA carries an anti-thrombotic effect by binding NO, preventing its rapid inactivation and thus prolonging its anti-aggregant effect on

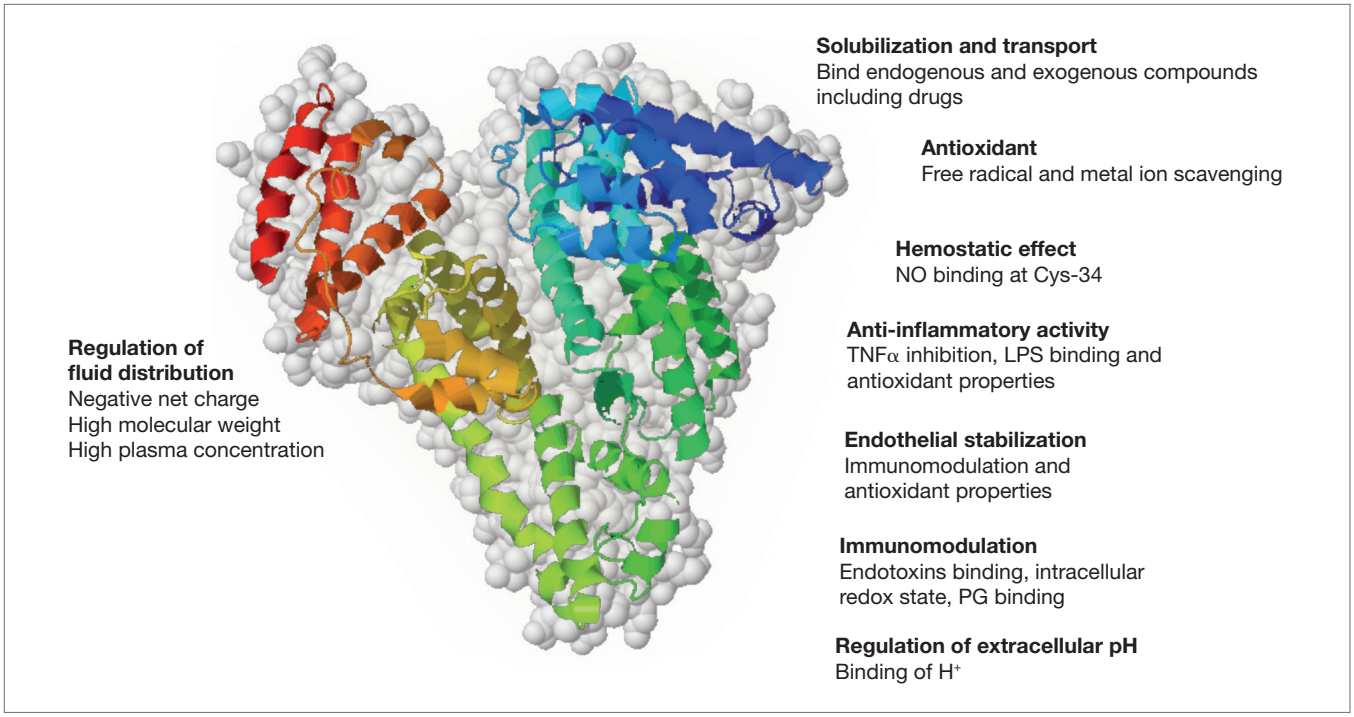

Figure 1. Oncotic (left) and non-oncotic (right) properties of human albumin. Cys-34: cysteine-34; LPS: lipopolysaccharides: NO: nitric oxide; PG: prostaglandins; TNF-α: tumour necrosis factor-alpha.

platelets[20-22]. Finally, HA is able to bind PGE_2 thus reducing its bioavailability. As PGE_2 induces immune suppression and its circulating levels are several fold elevated in patients with end-stage liver disease, HA supplementation may contribute to restore the immune competence and thus to reduce the risk of infection by lowering circulating free PGE_2[23].

Accumulating evidences indicate that the beneficial effects on effective blood volume seen after HA administration in decompensated cirrhosis are mediated not only by the capacity of HA to act as plasma expander, but are also related to its non-oncotic properties, which can indirectly improve cardiac contractility and peripheral vascular resistances. In rats with cirrhosis and ascites, HA, but not hydroxyethyl starch, improves left ventricular function ex vivo, counteracting the negative effects of oxidative stress and TNF-α activation[24]. In patients with cirrhosis and SBP, HA, but not hydroxyethyl starch, succeeds in ameliorating systemic hemodynamic, as witnessed by the significant changes of plasma renin activity (PRA) and arterial pressure[25]. Along with parameters suggesting total blood volume expansion, the concomitant striking rise in peripheral vascular resistances can only be related to the non-oncotic properties of HA. In fact, a significant decrease in the plasma levels of Factor VIII and von Willebrand-related antigen was found, suggesting that HA was able to attenuate endothelial activation. Furthermore, the significant increase in NO metabolites seen with HE was prevented by HA administration[25].

Randomized clinical trials and meta-analyses have demonstrated the efficacy of HA to treat or prevent clinical complications of cirrhosis all characterized by extreme effective hypovolemia. International guidelines recommend the use of HA for the prevention of post-paracentesis circulatory dysfunction (PPCD) or renal failure induced by SBP, and for the diagnosis and treatment of HRS in association with vasoconstrictor drugs. In all these clinical indications, HA is given as a single or dual infusion or for a short-course (maximum 2 weeks) of daily administration[26]. Based on its oncotic and non-oncotic properties, HA would appear to be an ideal therapeutic tool for long-term treatment of patients with decompensated cirrhosis, by exerting a beneficial effect at different steps of the vicious circle that links circulatory dysfunction, inflammatory response, and oxidative stress (fig. 2). Indeed, chronic HA administration could improve circulatory dysfunction directly, by acting as plasma-expander, and indirectly, by increasing cardiac contractility and systemic vascular resistances. The resulting preservation of the effective blood volume would guarantee adequate organ perfusion and avoid/delay the occurrence of organ impairment/failure. At the same time, HA could also antagonize the deleterious effects of immune dysfunction and chronic or acute inflammation through its non-oncotic properties, thus protecting target organs against tissue damage and microcirculatory disturbances.

Few data are available on long-term HA treatment in patients with cirrhosis, mainly given to control ascites. Two controlled clinical trials both conducted in Italy about two decades ago have shown that the combined treatment with diuretics plus HA is overall more effective than diuretics alone in resolving and controlling ascites[27,28]. A significant positive effect on patient survival was also seen in the more recent study, but the low sample size prevents to reach any firm conclusion[28]. Important information on the efficacy and safety of chronic HA administration will likely be provided by a no-profit open-label, multicentre, randomized clinical trial, actually ongoing in Italy, sponsored by the Italian Drug Agency (ANSWER study). The study compares the effectiveness of long-term weekly administration of HA plus diuretics (40 grams twice a week for the first 2 weeks and 40 gr once a week for a maximum of 18 months) with diuretic treatment alone in 420 patients with cirrhosis and uncomplicated ascites receiving at least 200 mg per day of an antialdosteronic drug and 25 furosemide per day (NCT 01288794, www.clinicaltrials.gov).

The results of the interim analysis on 386 patients have been presented (Annual Meeting of the Italian Association of the Study of the Liver, February 2015). In the group of patients receiving chronic HA, the incidence rate of paracentesis, refractory ascites, and need of ≥ 3 large-volume paracentesis/month was about halved. Furthermore, the HA arm also presented a significant advantage in the incidence rates of SBP, HE grade III-IV episodes and renal impairment episodes as defined by serum creatinine greater than 1.5 mg/dl. Finally, Kaplan-Meier analysis showed that intention-to-treat mortality was also reduced in patients receiving HA with a p-value of 0.045. Thus, it appears that long-term HA administration, beside a clear-cut improvement in the management of ascites, is also able to reduce the incidence of severe clinical complications of the disease and, if the interim analysis results will be confirmed, prolong survival in patients with decompensated cirrhosis. The final results of the ANSWER study will be likely available by the end of 2016.

Finally, statins, besides being a lipid lowering agent, exert a series of activities of great interest for the treatment of decompensated cirrhosis, including anti-inflammatory, immunomodulatory, anti-oxidant, anti-apoptotic[29], and portal-pressure reducing properties[30]. A very recent randomized controlled trial showed that addition of simvastatin to standard therapy (NSBB and ban ligation) did not reduce rebleeding but is associated with a survival benefit for patients with Child-Pugh class A or B cirrhosis[31]. As survival was not the primary endpoint of the study, these results require further validation, but open a novel possibility of treatment for patients with decompensated cirrhosis.

Conclusions

The advances emerged by experimental and clinical research in the last decade have designed a new pathophysiological scenario in patients with decompensated cirrhosis, identifying bacterial translocation as a “core”

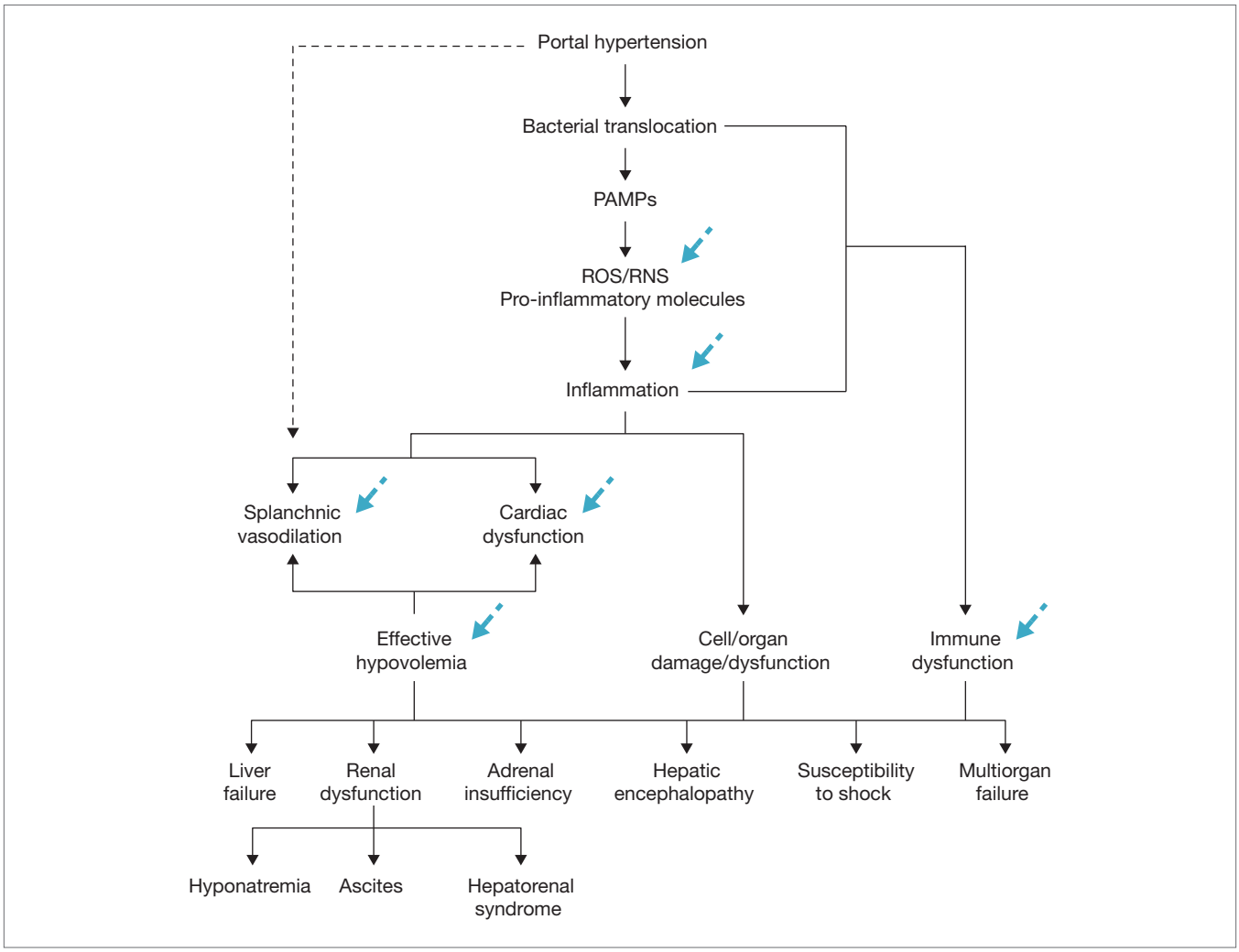

Figure 2. Major pathophysiological events in advanced liver cirrhosis. Arrows indicate the events, which can be directly antagonized by human albumin though its oncotic and non-oncotic properties. PAMP: pathogen associated molecular pattern; ROS: reactive oxygen species: RNS: reactive nitrogen species.

initiating event which produces a series of strictly interrelated consequences leading to a progressively severe circulatory dysfunction and systemic inflammation. Beside the expected deterioration of the patient overall clinical condition deriving from the exhaustion of liver function, AD and ACLF represent the systemic life-threatening manifestations of this pathophysiological scenario.

A most unmet need is, therefore, a long-term therapeutic approach that, alone or in combination with others, would be capable to antagonize BT, modulate systemic inflammation, restore the immunological response, and improve circulatory dysfunction through direct or indirect actions.

At present, the most promising commercially available agents are rifaximin, human albumin and NSBB, but adequately powered randomized clinical trials having as primary end-points the reduction of AD, ACLF, and mortality, are needed to confirm their efficacy. Other innovative approaches are also under investigation. Thus, the search for the "Holy Gral" ensuring an integrated prevention of life-threatening complications of cirrhosis has already started.

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SECTION III

THE MANAGEMENT OF THE PATIENT WITH ACLF

Faouzi Saliba

AP-HP Hôpital Paul Brousse, Centre Hépato-Biliaire, Villejuif, France

Université Paris-Sud, Faculté de Médecine, Le Kremlin-Bicêtre, France

Inserm, U785, Villejuif, France

Faouzi Saliba

Few improvements in the management of patients with advanced cirrhosis occurred during the last decade[1]. Mortality rate of cirrhotic patients admitted to the intensive care units (ICU) ranges from 34 to 69%. We recently reported the outcome and prognostic factors affecting outcome in a recent and large series of 377 cirrhotic patients admitted to the ICU[2]. We concluded that ICU scores (mainly SOFA score) when performed at admission or within 2-4 days from admission are superior to liver specific scores (model for end-stage liver disease (MELD) and Child-Pugh to determine outcome. Infections, the need for inotropic support or respiratory support are major predictors of worse outcome. The mortality rates reported in the literature of cirrhotic patients requiring mechanical ventilation, range from 59% to 93%. Mechanical ventilation has been identified as an independent factor related to ICU mortality[3]. For cirrhotic patients admitted to ICU and requiring mechanical ventilation, selecting for transplant patients with a MELD score higher than 21.5 at time of ICU discharge could decrease the one-year mortality. We also showed that the occurrence of three or more organ failures or the need for 3 organs support in cirrhotic patients has almost a fatal outcome[4].

Acute-on-chronic liver failure (ACLF) is currently characterized by acute decompensation of cirrhosis, organ failure and high short-term mortality. The first large prospective European multicenter study (the CANONIC study, > 1300 patients) performed within the EASL-CLIF consortium group has shown that merely 30% of the cirrhotic patients admitted to hospital (ward or ICU) for an acute decompensation present with ACLF at admission or develop the syndrome during hospitalization[5]. The 28-day mortality rate associated with ACLF is 30%. A CLIF-SOFA score has been proposed and three types of risk factors obtained from the CLIF-SOFA score at enrolment were found to be related to high 28-day mortality rate: 1) the presence of 2 organ failures or more, 2) the presence of one organ failure when the organ that failed was the kidney, and 3) the coexistence of a single "non-kidney" organ failure with kidney dysfunction. Based on the number of organ failure at enrolment, three grades of ACLF with different outcome were defined[5]. When analyzing in this cohort the group of patients admitted with decompensated cirrhosis without ACLF, age, serum sodium, white-cell count, creatinine and INR were found to be best predictors of mortality. This led to the creation of new CLIF-C Acute Decompensation score (ADs), which is more accurate than other liver scores in predicting prognosis in hospitalized cirrhotic patients without ACLF. The CLIF-C ADs has been validated on an external cohort and therefore may be used to identify a high-risk cohort for intensive management and a low-risk group that may be discharged early[6].

Medical management of patients with acute-on-chronic liver failure

When ACLF occurrence is related to a precipitating event (i.e. bleeding, infection, drug abuse or intoxication, alcoholic hepatitis…), management of the acute event is mandatory. Nevertheless, this often does not prevent the occurrence of ACLF, and in up to 40% of the cirrhotic patients recruited in the CANONIC study, no precipitating event was found. In addition, precipitating events may be initiators of ACLF but do not drive the outcome[7]. An important concept derived from the CANONIC study is that ACLF is associated with systemic inflammation even in patients who do not have identifiable precipitating events[7].

A recent review on the management of critically ill cirrhotic patients detailed all known aspect of treatment of a decompensated episode[1]. This included management of gastro-intestinal bleeding related to portal hypertension, spontaneous bacterial peritonitis, other infections (pulmonary, urinary…), hepato-renal syndrome type 1 and 2, hepatic encephalopathy or other neurological complications. The aim of this chapter is to focus only on treatment of patients at the stage of ACLF.

Patients with ACLF would require a transfer to an ICU liver unit or medical ICU with an intensivist experienced with liver disease and in preference within a transplant center. The management would mainly rely on the prevention and the treatment of organ failure. Early management of these patients in order to avoid reaching a stage of multiple organ failure and end-stage hepatic failure is crucial.

The optimal management of ACLF has not been defined. At this stage, it is comparable to management of non-cirrhotic patients with organ failure once these patients require organ support (mechanical ventilation, hemodialysis, inotropic support). The main key elements in patients with liver disease is optimizing drug dosage according to

degree of hepatic and renal impairment and avoid the use of hepatotoxic and nephrotoxic drugs. Therapeutic drug monitoring of antimicrobial agents (antibiotics, antiviral or antifungals mainly azoles) or others drugs often use in ICU (sedatives, anticonvulsant and cardiovascular medications). Liver transplantation has dramatically improved survival. Indication and optimal timing should be discussed by the team and considered for each patient according to patient evolution (progression, stabilization, control, worsening).

Granulocyte colony-stimulating factor in ACLF

Gaia et al. showed that multiple courses of Granulocyte colony-stimulating factor (G-CSF) in decompensated cirrhotics were associated with bone marrow stem cell mobilization. Spahr et al. have reported that G-CSF mobilizes $CD34^+$ cells, increases hepatocyte growth factor, and induces hepatic progenitor cells to proliferate within 7 days of administration in patients with alcoholic steatohepatitis[8].

In a randomized controlled trial, Garg et al. have shown that the use of G-CSF in patients with ACLF was associated with significantly improved survival of 69.6% compared with the 29% in the placebo group, along with increased recruitment of $CD34^+$ cells on histology[9]. Duan et al. have shown that G-CSF improved survival in patients with hepatitis B–related ACLF by increased neutrophil and more importantly $CD34^+$ cell counts in peripheral circulation [10. As erythropoietin has also been show to promote hepatic regeneration in animal studies, in a single centre, double blind randomized study, patients with decompensated cirrhosis were randomly assigned to groups given subcutaneous G-CSF (5 µg/kg/d) for 5 days and then every third day (12 total doses), along with subcutaneous darbopoietin α (40 mcg/week) for 4 weeks (GDP group, n = 29), or only placebo (control group, n = 26). The primary end point was survival at 12 months. A higher proportion of patients in the GDP group than controls survived until 12 months (68.6% vs. 26.9%; p = 0.003). At 12 months, Child-Pugh and MELD scores and the need for large-volume paracentesis were significantly reduced in GDP group, compared with controls. A lower proportion of patients in the GDP group developed septic shock (6.9%) during follow-up compared with controls (38.5%; p = 0.005). These interesting findings would need further evaluation in large multicenter trials[11].

Liver support in the treatment of patients with acute-on-chronic liver failure

The following extra-corporeal liver support therapies have been developed with trials conducted in patients with decompensated cirrhosis usually associated with different degrees of organ failure. No trial has been performed with these devices with the current definition of ACLF. Meanwhile, heterogeneity of cirrhotic patients, with different prognosis, disabled to select a specific group of patients who would most likely benefit from the therapy.

Bioartificial liver support devices (BAL). The ELAD system

BALs are essentially bioreactors, with embedded hepatocytes (liver cells) that perform the functions of a normal liver. Bioreactors can provide a favorable growth and metabolic environment, mass exchange and immunological isolation as a platform.

The advantages of using a BAL over other dialysis-type devices is that metabolic functions (such as lipid and plasma lipoprotein synthesis, regulation of carbohydrate homeostasis, production of serum albumin and clotting factors…), in addition to detoxification, can be replicated without the use of multiple devices. However, despite the great development of the devices in the recent years, the concept of bioartificial livers still has major limitations. The main device that is under clinical investigation in acute on chronic liver disease is the Extracorporeal Liver Assist Device (ELAD®) (Vital Therapies Inc., San Diego, CA) that uses hepatocytes derived from human liver (immortalized C3A cell line) which is derived from hepatoblastoma cell line hepG 2. The C3A cells have demonstrated experimentally capacity of synthesis of albumin, factor V, transferrin, antithrombin III, C3 complement, alpha-1 antitrypsin and alphafetoprotein as well as the ability to metabolize galactose and lidocaine.

A phase III clinical trial to determine if ELAD can increase overall survival in patients with alcoholic hepatitis (AH) has been recently presented[12]. Patients with clinical or histological diagnosis of AH, total bilirubin > 8mg/dl, Maddrey DF > 32, MELD ≤ 35, platelets ≥ 40000/mm^3, and without severe concomitant disease, uncontrolled sepsis or bleeding, hemodynamic instability or need for chronic dialysis were recruited in the study. Patients were randomized to either 3-5 days continuous ELAD therapy plus standard of care (SOC) or to SOC alone. From 2013-2015, 203 subjects were enrolled (96 ELAD and 107 SOC) at 40 sites. Comparison of baseline characteristics showed no significant differences between groups and within subgroups, including treatment with steroids or pentoxifylline. In an intent-to-treat (ITT) analysis, the study did not show any significant difference in overall survival (52.1% vs. 52.3%). Although not pre-specified, survival in subjects with a combination of MELD and age less than baseline median (MELD < 28 or age < 47years; n = 59) was significantly better in ELAD than SOC (100% vs.73%, p = 0.006) at 91 days. After 100 days, survival was fairly stable in all subject groups.

Artificial liver support devices

Artificial liver support devices are intended to provide detoxification functions to patients with acute and ACLF

until liver transplantation (LT). The best-studied artificial liver support devices are the molecular adsorbents recirculating system (MARS®) and the fractionated plasma separation and absorption system (Prometheus®), which are based upon the principles of albumin dialysis. Plasma exchange is being used mainly in eastern countries.

Clinical efficacy of MARS® in patients with ACLF

MARS® is an extracorporeal albumin dialysis method that has been assessed as a therapeutic option for the elimination of albumin-bound substances. Prospective trials have shown that albumin dialysis with MARS® is able to improve cholestasis, renal and liver functions, hepatic encephalopathy, and hemodynamic situations effectively, and might improve survival in certain situations.

MARS®, when compared to standard medical treatment (SMT), has shown in a prospective RCT a significant reduction of ammonia levels and a faster and higher improvement of grade 3 and 4 hepatic encephalopathy within five days of therapy ($p = 0.045$)[13]. Heemann and colleagues randomized 24 patients with severe cholestasis (bilirubin > 20mg/dl) not improving after 3 to 5 days of SMT and compared SMT to SMT+MARS®[14]. The determining factors for acute decompensation were infection, drug intoxication, and hemorrhage or alcohol abuse. They showed a significant improvement in the 30-days survival rate in favor of the MARS® group (91% vs. 54%; $p < 0.05$); improvement was also shown on hepatic encephalopathy, arterial pressure, total bilirubin, biliary acids, and creatinine[14].

Recently, the results of a large European multicenter RCT performed in patients with ACLF that compared MARS® to SMT were published[15]. The RELIEF trial included 189 patients with bilirubin > 5mg/dl and at least one of the following: hepatic encephalopathy grade II-IV, hepatorenal syndrome or bilirubin > 20 mg/dl. The more frequent precipitating event was alcohol abuse followed by bacterial infection. Mean number of MARS® sessions was 6.5 (3.1). No differences were observed between the SMT+MARS® and the SMT groups in 28-day transplant free survival neither in the ITT population (n = 179 patients); 60.7% vs. 58.9% $p = 0.79$) nor in the PP population (n = 156 patients) (60% vs. 59.2%; $p = 0.88$). Similarly, there were no differences regarding 90-day transplant-free survival (ITT population: 46.1% vs. 42.2%; $p = 0.71$; PP population: 44.7% vs. 43.7%; $p = 0.97$). In terms of MARS® treatment efficiency, the proportion of patients with a marked reduction of the degree of HE (from grade II-IV to grade 0-I) tended to be higher in patients treated with MARS® (MARS®: 15/24 (62.5%) vs. SMT: 13/34 (38.2%), $p = 0.07$). Also, the proportion of patients with a serum creatinine below 1.5 mg/dl at day 4 in patients with HRS at baseline, tended to be higher in patients assigned to the MARS® arm (MARS®: 16/34 (47.1%) vs. SMT: 10/38 (26. 3%); $p = 0.07$).

Fractionated Plasma Separation and Adsorption (FPSA, Prometheus®)

The Prometheus system (Fresenius Medical Care AG, Hamburg, Germany) is based on the concept of fractionated plasma separation and adsorption (FPSA). Evenepoel and colleagues treated nine patients with acute on chronic liver failure with Prometheus® for 3 consecutive days[16]. A significant decrease of urea, creatinine, total bilirubin, bile acids was observed and particularly a decrease in serum albumin concentration (30.2 ± 1.6 vs. 27.4 ± 1.7 g/L, $p = 0.055$). A large multicenter randomized trial has been performed in Europe in 145 patients with acute decompensation of a chronic liver disease[17]. The HELIOS trial defined ACLF as cirrhotic patients with Child-Pugh score > 10 and bilirubin > 5mg/dl. In an intent-to-treat analysis, the probabilities of survival on day 28 were 66% in the FPSA group and 63% in the control group ($p = 0.70$); on day 90, they were 47% and 38%, respectively ($p = 0.35$). The data show that the approach is safe and well tolerated, although there is no overall significant survival benefit at 28 days.

Plasma exchange

Plasma exchange (PE), has been applied since many years for various indication including patients with end-stage liver disease. The rationale is by causing elimination of a wide variety of substances, toxins and mediators could facilitate liver regeneration and recovery[18]. PE has been widely used in eastern countries in patients with decompensated cirrhosis and ACLF as a bridging therapy to LT. In contrast to plasmapheresis, the patient's plasma under PE is removed and exchanged with fresh frozen plasma.

Yue Meng et al. evaluated the efficacy of PE in patients with ACLF secondary to HBV decompensated cirrhosis[19]. Patients were enrolled into either a PE group (n = 38) or control group (n = 120). All patients were treated with entecavir along with the standard of care. Patients in the PE group received 2-5 sessions of PE therapy. Patients in the PE group were sicker with higher MELD scores and lower albumin levels compared to the control group. The cumulative survival rate at week 4 and week 12 in the PE group and control group were, respectively, 37% and 18%, and 29% and 14% ($p < 0.001$, by log rank test). Mean HBV DNA level and HBeAg seroconversion were not significantly different among groups. On multivariate analysis, hepatic encephalopathy, ascites, PE treatment, and MELD scores were independent factors for liver-related mortality at week 12[19].

Mao et al. analyzed 62 patients with ACLF related to HBV reactivation who received PE treatment and compared them to 131 patients treated with standard of care. The 30-day survival rate of the patients who received PE versus controls was 41.9% vs. 25.2% ($p < 0.05$). The 30-day survival rate of patients in the PE group (50%) with a MELD score from 20 to 30 was

higher than that of the control group (31.7%, $p < 0.05$) but this was not seen for patients with MELD scores > 30[20]. In another study by Ling et al., of the 126 patients with hepatitis B-related ACLF and MELD score ≥ 30, 42 received emergency LT within 72 h (ELT group) and the other 84 were given artificial liver support as salvage treatment. Of the 84 patients, 33 were found to have reduced MELD score (< 30) on the day of LT. The decrease in the MELD score after treatment with artificial liver support pre-transplantation led to improved survival post-transplantation, which was comparable to that of patients who underwent emergency liver transplantation[21]. A combination of PE treatment with continuous hemodialfiltration, plasma bilirubin adsorption or MARS® has been shown to be more effective than PE alone[18].

Overall, heterogeneity of patients, heterogeneity of definitions of ACLF, modalities and complexity of the decompensation event incrementing hepatic and extra-hepatic organ failure, the major role of SIRS and sepsis, the lack of hepatic cell regeneration in advanced cirrhosis, make extremely difficult the evaluation of these devices and these would need to be evaluated with the current definition of the syndrome. RCT re-evaluating indications, timing of treatment, or cost-effectiveness, are still justified and needed in order to perform the impact of liver support therapies in medical practice.

Liver transplantation

The use of LT in this context is hampered by the shortage of organs, as well as, by the high frequency of concomitant conditions that contraindicate the procedure. Most studies on LT in ACLF were assessed in hepatitis B endemic countries with most cirrhosis of viral etiology (mainly hepatitis B virus) and an acute exacerbation of hepatitis B. Thus, chronic hepatitis B constitutes at least 70% of the underlying liver diseases leading to ACLF, which essentially concerns Asian countries[22]. The timing of transplantation is crucial as patients with ACLF may provide a short window of opportunity. Therefore, living-donor transplantation is an attractive option, the experience of which has been reported extensively from South-East Asia. Duan and colleagues have recently reported the evolution of 100 patients who underwent LT for ACLF. They compared living donor LT to deceased donor LT and did not show difference in survival (5-year cumulative survival rate 74.1%), that was similar to those of patients with acute liver failure[22]. These data from Hong Kong suggest that although the patients with ACLF hepato-renal syndrome had stormier post-operative course, living donor transplantation could be performed safely. In another cohort reported by Bariwani et al., with 332 patients transplanted, whose median follow-up time was 37 months in the ACLF cohort versus 38.6 months in the non-ACLF cohort, ACLF before liver transplantation did not lead to worse outcomes post-transplant[23]. Moreover, the majority of them received anti-HBV therapy rapidly after the onset of ACLF.

Although LT is a curative therapy for these patients, it is important to establish selection criteria to determine which patients with ACLF would be most likely to benefit from LT because of shortage of grafts and medical expenses.

In 2002, the New York State Committee on Quality Improvement in Living Liver Donation prohibited live liver donation for potential recipients with MELD score greater than 25. Despite the paucity of evidence to support this recommendation, many centers in North America remain reluctant to offer living donor (LD) to patients with moderate to high MELD scores. LD liver transplantation can provide excellent graft function and survival rates in high MELD score recipients. Thus, when deceased donor organs are scarce, a high MELD score alone should not be an absolute contraindication to living liver donation. Acceptable outcomes are achievable after LT in patients with MELD scores of 40 or higher but come at high pre-transplantation and post-transplantation resource utilization[24]. Since then, there has been a progressive increase in the median MELD score of liver transplant recipients, with more patients waitlisted at a MELD score of 30 to 40, resulting in a large increase in the number of liver transplants performed for patients with a high MELD score. Several authors admit to use preferentially high-quality donors in this critically ill group of patients of ACLF, in order to minimize the incidence of graft dysfunction during and after operation.

Conclusion

Currently, ACLF has been well defined. Inflammation and infection are main determinants of ACLF but more pathophysiological mechanisms and pathways need to be elucidated. Early management of ACLF is essential to avoid worsening and development of multiple organ failure. Optimal management of ACLF has not been yet defined. In recent years, there has been a considerable interest in the use of newer forms of liver support that may provide a bridge until an appropriate donor is available for transplantation. Liver support therapies, mainly albumin dialysis and/or PE, aim to improve clinical, neurological and biological parameters. These improvements would allow these patients awaiting liver transplantation be transplanted in better conditions. However, randomized controlled trials evaluating these devices with the current definition of ACLF, as well as studies on indications and timing of treatment and cost-effectiveness studies are still needed to evaluate the impact of liver support therapies in medical practice. Patients with ACLF should be evaluated for a liver transplant, and indication should be reconsidered according to progression of the ACLF, the presence of active infections, psychosocial aspects, and comorbidities. These patients, due to their high MELD scores, can have a rapid access to LT. A multidisciplinary approach between hepatologists, intensivists, transplant surgeons and transplant coordinators is crucial.

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