Acute-on-chronic liver failure in cirrhosis

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Abstract

The definition of acute-on-chronic liver failure (ACLF) remains contested. In Europe and America, the term is generally applied according to the European Association for the Study of the Liver-Chronic Liver Failure (EASL-CLIF) Consortium guidelines to a syndrome that develops in patients with cirrhosis and is characterized by acute decompensation, organ failure and high short-term mortality. One-third of patients who are hospitalized for acute decompensation present with ACLF at admission or develop the syndrome during hospitalization. ACLF frequently occurs in closed temporal relationship to a precipitating event, such as bacterial infection or acute alcoholic, drug-induced or viral hepatitis. However, in approximately 40% of patients no precipitating event can be identified. The mechanism of ACLF involves systemic inflammation due to infections, acute liver damage, and, in cases without precipitating events, probably

intestinal translocation of bacteria or bacterial products. ACLF is graded into three stages (ACLF grades 1-3) on the basis of the number of organ failures, with higher grades associated with increased mortality. Liver and renal failure are the most common organ failures, followed by coagulation, brain, circulatory and respiratory failure. The 28-day mortality rate associated with ACLF is 30%. Depending on the grade, ACLF can be reversed using standard therapy in only 16-51% of patients, leaving a considerable proportion of patients with ACLF that remains steady or progresses. Liver transplantation in selected patients with ACLF grade 2 and ACLF grade 3 increases the 6-month survival from 10% to 80%.

[H1] Introduction

Cirrhosis is a progressive chronic liver disease characterized by diffuse fibrosis, severe disruption of the intrahepatic venous flow, portal hypertension and liver failure. The course of cirrhosis is divided into two stages[1] (**Figure 1**). Compensated cirrhosis defines the period between the onset of cirrhosis and the first major complication. During this period, which is relatively long in most patients (>10 years), symptoms are absent or minor, but liver lesions and portal pressure steadily progress. The term decompensated cirrhosis defines the period following the development of ascites (that is, the accumulation of large amounts of fluid within the peritoneal cavity), variceal haemorrhage and/or hepatic encephalopathy[2–4]. This period is associated short-term survival (3-5 years).

Concepts about cirrhosis are rapidly changing. First, cirrhosis is no longer considered to be an irreversible progressive disease. Indeed, decompensated cirrhosis may return to compensated cirrhosis or even to pre-cirrhotic phases if the cause of the disease is removed[5]. Second, the list of organ dysfunctions in cirrhosis (hepatic, circulatory, renal and cerebral) has been expanded to include the immune system, intestine, heart, lung, adrenal glands, muscles and thyroid gland. Third, new mechanisms involved in the

pathogenesis of cirrhotic complications, such as dysbiosis of the microbiota[6] and systemic inflammation[7] have been recognized. Finally, it is increasingly evident that patients rarely die as a consequence of an end-stage irreversible destruction of the liver. Rather, in most patients the cause of death is an acute deterioration in their clinical condition promoted by a precipitating event — a syndrome termed acute-on-chronic liver failure (ACLF)[8].

More than 13 distinct definitions of ACLF have been proposed. These definitions are generally based on personal experience or consensus agreements[9–15] (**Box 1**).

The Asia-Pacific Association for the Study of the Liver (APASL) definition has received major attention (**Box 1**)[11,12]. This definition is based on positive and negative criteria. Main positive criteria are: prior diagnosis of chronic liver disease (cirrhotic or non-cirrhotic, excluding isolated steatosis); a precipitating event that has a direct effect on the liver; acute hepatic insult; causing acute liver failure. Main negative criteria in the APASL definition are: no prior history of acute decompensation in patients with cirrhosis (decompensated cirrhosis would represent the presence of end-stage progressive liver disease); and no extra-hepatic precipitating event such as bacterial infection. The APASL definition was based on a consensus conference.

The APASL proposal did not reach wide diffusion in Europe and America for several reasons. First, the most common form of ACLF in these areas occurs in patients with decompensated cirrhosis in closed chronological relationship with bacterial infections or active alcoholism[8,13] (**Figure 1**) and these patients are not included in the APASL definition. Second, the concept that decompensated cirrhosis represents a terminal phase of the disease is not the experience of European centres. Third, extrahepatic organ failure is the most characteristic differential feature between patients with ACLF vs those with acute decompensation in the European patients. Finally, ACLF in patients

with non-cirrhotic chronic liver disease is exceptional in Europe and America due to the low prevalence of hepatitis A, B and C.

For these reasons, in 2009 the European Association for the Study of the Liver-Chronic Liver Failure (EASL-CLIF) Consortium started a prospective multicentre European observational study in 1,343 patients who were hospitalized for acute decompensation of cirrhosis (the CANONIC study). This study was aimed to define ACLF in cirrhosis, to assess the prevalence and clinical course of the syndrome and to improve the accuracy of the prognostic scores currently available [8,16–20] through an evidence-based pragmatic approach. The analysis of this study lead to a new definition with three major characteristics (**Box 1**): Acute decompensation of cirrhosis; the presence of organ failure(s) (either hepatic or extrahepatic); and a high probability of short-term (28-day) mortality. Following the publication of the main articles derived from the CANONIC study, the definition, grading of severity of ACLF and prognostic scores proposed are widely used in Europe, Asia and America for the assessment/treatment of patients with decompensated cirrhosis [21–32].

It has been suggested that differences between APASL and EASL-CLIF Consortium definitions are logical consequences of the distinct epidemiology of liver diseases between the East and in the West[28,33]. However, the differences are more likely to be related to the distinct objectives by which both definitions were designed. The ACLF definition by the APASL consensus group was designed to identify a condition that anticipates the development of extrahepatic or multi-organ failure and death[11,12]. By contrast, the goal of the CANONIC study was to characterize a syndrome in which organ failure(s) and high short-term mortality are central features[8].

Attempts to unify both definitions have been not successful[34]. Recent investigations from Asia have compared the two definitions in large series of patients with cirrhosis[22,26,35]. **Figure 2** compares the results of the largest Asian series[35] with those in

the CANONIC study[8]. A coincident diagnosis by the APASL and the EASL-CLIF Consortium definitions was observed in only a minority of patients in both series, indicating that the two ACLF definitions selected different patient populations (**Figure 2a**). The EASL-CLIF Consortium definition was significantly more accurate in predicting prognosis than the APASL definition both in the East and the West. The EASL definition was also better to predict prognosis (**Figure 2b**). Significant differences in mortality depending on the diagnostic criteria were also observed in two other cohorts of patients from China and India[23,26]. This Primer on ACLF in cirrhosis uses the EASL-CLIF Consortium definition. The reader is referred to a Review by Sarin and Choudhury[36] for a discussion of ACLF that is based the APASL criteria.

[H1] Epidemiology

[H2] Worldwide prevalence and mortality

ACLF is a major worldwide medical problem, with prevalence rates in at-risk populations in the region of 20-35% (**Table 1**). The worldwide reported mortality of ACLF according to the EASL-CLIF Consortium definition ranges between 30% and 50% and correlates closely with the number of organ failures. In Europe, the average 28-day mortality rate without liver transplant reported by the CANONIC study was 1.9% in patients with decompensated cirrhosis without ACLF and 32.8% in patients with ACLF (23% in patients with ACLF grade 1, 31% in patients with ACLF grade 2 and 74% in patients with ACLF grade 3; see **Box 2** for details)[8].

In the United States, a study using the North-American Consortium for the Study of End Stage Liver Disease (NACSELD criteria) (**Box 1**) reported that the 30-day mortality rate associated with infected decompensated cirrhosis without ACLF was 8% and this

rate increased to 27%, 49%, 64% and 77% in patients with one, two, three and four organ failures, respectively[13]. In addition, in the United States, no significant reduction in mortality in patients with ACLF has been observed over the past two decades, with mortality in the nationwide sample approaching 50%[32].

In China, the average 28-day transplant free mortality reported by Li *et al.* in patients with decompensated cirrhosis due to chronic hepatitis B virus (HBV) infection was 2.6% in patients without ACLF and 44% in patients with ACLF (EASL-CLIF Consortium definition) (23.6%, 40.8% and 60.2% in patients with ACLF grade 1, 2 and 3 respectively, **Box 2** for details)[37]. Zang *el al.* reported similar findings in Chinese patients with decompensated cirrhosis of different aetiologies[23]. The 90-day mortality rate in patients with no ACLF and with ACLF grade 1, 2 and 3 were 2.1% 39.9% 54.1% and 84.7% (EASL-CLIF Consortium definition), respectively[23].

[H2] Precipitating events

Precipitating events of ACLF vary according to geographical areas, and can be classified as hepatic or extra-hepatic depending on their site of origin **(Figure 1)** [14,34,37–39]. Reactivation of chronic hepatitis B, acute hepatitis A or hepatitis E virus infection[38], acute alcoholic hepatitis and acute bacterial infection are the most frequent precipitating events of ACLF in Asia[23]. In the West, the most common precipitating events are active alcoholism and bacterial infections, although in a considerable proportion of patients there is no recognizable precipitating event[8]. The potential role of drug-induced liver injury as precipitating event in ACLF has been insufficiently explored both in the East and in the West.

[H2] Organ failures

In the CANONIC study, among the different organ and system failures in ACLF, the most frequently affected organs or systems were kidneys (55.8% of patients) followed by liver (43.6% of patients), coagulation (27.7% of patients), brain (24.1% of patients), circulation(16.8% of patients) and lungs(9.2% of patients). At first glance, it might be surprising that not all patients with ACLF had liver failure, but there are two important issues that should be taken into account. First, the level of bilirubin used to define liver failure was very high (≥12 mg per dL), and most (if not all) patients without liver failure also have abnormal bilirubin values, which implies a variable degree of impairment of liver function in these patients. Second, it is important to note that definition of ACLF goes beyond the classical concept of decompensation of cirrhosis and includes the consequences of cirrhosis on the function of other organs[7].

[H1] Mechanisms/pathophysiology

[H2] ACLF during course of Cirrhosis

As indicated, cirrhosis is a progressive disease that inevitably leads to death unless the aetiological mechanism is suppressed by appropriate treatment or a liver transplant is performed. Indeed, there is good evidence that discontinuation of alcohol ingestion in alcoholic cirrhosis, antiviral treatment in chronic hepatitis B and C cirrhosis and immune suppressive therapy in autoimmune cirrhosis may transform decompensated cirrhosis to compensated cirrhosis or even to pre-cirrhotic phases[5]. In contrast, if the etiological mechanisms persists in patients with compensated cirrhosis, hepatic fibrosis increases progressively as a consequence of continuous liver cell necrosis and inflammation, giving rise to progressive distortion of the liver architecture, reduction in liver parenchyma cells, increase in the intrahepatic resistance to the portal venous flow, portal hypertension, liver insufficiency and acute decompensation of the disease (**Figure 1**).

The development of complications, mainly ascites and, less frequently, variceal haemorrhage or hepatic encephalopathy, marks the onset of decompensated cirrhosis., which is characterized by impairment in the function of the liver and extrahepatic organs and systems, including the brain (disturbances affecting cognitive, psychiatric and motor functions ranging from subclinical alterations to severe stupor and coma), kidney (impairment in renal sodium and free water excretion, intrarenal haemodynamics, renal perfusion and glomerular filtration rate), circulation (splanchnic arterial vasodilation leading to reduction in systemic vascular resistances and high cardiac output), lungs (impairment in the ventilation/perfusion ratio leading to hypoxia and hypocapnia), heart (impairment in chronotropic and left ventricular systolic and diastolic functions), coagulation (due to impairment in the hepatic synthesis of coagulant and anticoagulant factors and increased fibrinolysis), adrenal glands (impaired ability to provide adequate cortisol release in response to stress), intestines (reduced motility, bacterial overgrowth and increased permeability of the mucosal barrier leading to increased translocation of bacteria and/or bacterial products from the intestinal lumen to the systemic circulation), immune system (systemic inflammation and impaired function of polymorphonuclear leukocytes and monocytes), thyroid glands (impaired hormonal secretion) and muscles (sarcopenia) (**Figure 1**).

ACLF may develop at any phase of the disease from compensated to early or late decompensated cirrhosis (**Figure 1**). It is, therefore, not a terminal event of a long-standing decompensated cirrhosis. As indicated above (**Box 2**), organ failure as defined by an intense impairment in the function of six specific organs or systems that are important in determining prognosis (the liver, kidneys and brain and the coagulation, circulatory and respiratory systems[8] is the differential feature of ACLF versus decompensated cirrhosis without ACLF. By contrast, organ dysfunction, which defines a less severe impairment in the function of these (and other) organs and systems, is the differential feature of decompensated cirrhosis versus compensated

cirrhosis. For instance, according to the CANONIC study brain failure is defined by a hepatic encephalopathy grade 3 or 4 of the West Haven classification whereas brain dysfunction is defined by a hepatic encephalopathy grade 1 or 2. Renal dysfunction is defined by a serum creatinine of 1.5-1.9 mg/dl whereas renal failure is defined be a serum creatinine ≥ 2 mg/dl.

[H2] Inflammation in ACLF

ACLF is associated with features of systemic inflammation. For example, white blood cell count and plasma levels of C-reactive protein (CRP) and pro-inflammatory cytokines and chemokines such as interleukin (IL)-6, IL-1β, IL-8 are higher in patients with ACLF compared to patients with cirrhosis but not ACLF[8,22]. Moreover, among patients with ACLF, the higher the ACLF severity, as estimated by the number of organ failures, the higher plasma pro-inflammatory cytokine/chemokine levels (V. Arroyo, unpublished results, CANONIC study). The excessive systemic production of pro-inflammatory cytokines and chemokines — or 'cytokine storm' — by the patient's immune system might cause collateral tissue damage[40], a process termed immunopathology[41]. As such, a cytokine storm might also be a prominent contributor to the development of organ failures in patients with cirrhosis. Of note, in with patients with ACLF, a subset of CD14-positive monocytes exhibit overexpression of the tyrosine-protein kinase Mer (encoded by *MERTK*) which results in the inhibition of the production of inflammatory cytokines by these cells[22], suggesting that a form of compensatory immune suppression develops in parallel to the systemic inflammatory response.

There are two categories of ACLF: those in which the inducer(s) of inflammation (for example, bacterial infection or excessive alcohol intake) are identified and those in which there is no clinically identifiable trigger(s)[8]. Here, the latter category is called 'ACLF with no clinically identifiable trigger' Inducers of inflammation are either

exogenous or endogenous[42]. Among exogenous inducers we will discuss only bacterial inducers because the others are beyond the scope of this Primer and have been described elsewhere[42]. Although much of the molecular detail of how inflammation triggers ACLF remains to be elucidated, it is likely that the following general processes play a key part. “Bacterial inducers of inflammation” and “endogenous inducers of inflammation” are potential mechanisms of inflammation in ACLF.

[H3] Bacterial inducers of inflammation.

Bacterial pathogens can induce inflammation through two distinct classes of molecules: pathogen-associated molecular patterns (PAMPs)[42–44] and virulence factors[42,45]. PAMPs are recognized by the host via dedicated receptors called pattern-recognition receptors (PRRs), and examples of PRRs for bacterial ligands are detailed in **Figure 3A**[42–44]. The engagement of PRRs results in the stimulation of signalling cascades that activate transcription factors [43]. PRR-activated transcription factors can induce an array of genes encoding molecules involved in inflammation, including pro-inflammatory cytokines (**Figure 3B**) [43,45,46].

The second class of bacterial inducers of inflammation includes a large number of virulence factors[42,44]. Unlike PAMPs, most of these factors are generally not recognized by dedicated receptors but can be sensed via the effects of their activity (a process called functional feature recognition)[38,46–48].

[H3] Endogenous inducers of inflammation.

Endogenous inducers are released by necrotic cells or produced by extracellular matrix (ECM) breakdown in an injured tissue (such as the diseased liver in the case of ACLF[42,43], and are called danger-associated molecular patterns (DAMPs)[49]. DAMPs can be recognized by certain receptors of the host, with this recognition resulting in ‘sterile’ inflammation. For example, high mobility group box 1 protein (HMGB1)

engages the advanced glycation end-product-specific receptor (RAGE), which cooperates with Toll-like receptors (TLRs, a class of PRR) to induce an inflammatory response[42,43,49]. Additional factors that might also be involved in ACLF include necrotic cells, which may release members of the IL-1 family such as IL-1α and IL-33 that trigger inflammation though their respective MyD88-coupled cognate receptors[50].

[H3] Outcomes of the inflammatory response.

The purpose of the inflammatory response to bacterial infection is to promote host resistance by reducing bacterial burden while that of sterile inflammation is to promote tissue repair[51–54]. However, when these two categories of inflammatory responses are excessive, they may induce tissue damage[52]. During bacterial infection, the acute phase of the inflammatory response can be excessive and cause immunopathology. For example, effectors of the immune response such as recruited neutrophils and inflammatory monocytes, activated Th1 and Th17 cells, and cytotoxic T cells are known to be associated with high risk of immunopathology[44]. There are also some examples of DAMP-induced excessive inflammatory response causing major tissue damage. Mice deficient for *Ripk1* develop Ripk3-Mlkl-mediated necroptosis resulting in systemic inflammation, multiorgan injury and death within 3 days of birth[50]. In this model, the IL-33 (a DAMP) drives systemic inflammation and severity. Therefore, the initial tissue injury caused by necroptosis may result in further tissue damage. In the context of severe bacterial infection, cell necrosis can occur (as a feature of immunopathology) and result in DAMP release. In this case released DAMPs can perpetuate or accentuate inflammation originally triggered by bacterial inducers (PAMPs and virulence factors)[51].

[H2] ACLF with identified inducers of inflammation

The relative contribution of these inflammatory processes to ACLF likely differ depending on the trigger, and considerable research is still needed to fully elucidate the aetiological pathways of this syndrome. Of all the recognised precipitating events in ACLF, the mechanisms underlying two — sepsis and severe alcoholic hepatitis — are best-characterized and will be detailed below.

[H3] Sepsis-induced ACLF.

Organ-dysfunction caused by a dysfunctional host immune response to bacterial infection defines sepsis-induced ACLF**”.** Thirty percent of patients with cirrhosis and ACLF have bacterial sepsis as an identifiable trigger of the syndrome[8]. However, ACLF can also predispose to bacterial infection: indeed, a proportion of patients with ACLF develop bacterial infection during the course of the syndrome[8]. Among bacterial infections, spontaneous bacterial peritonitis (SBP), sepsis and pneumonia were more frequently associated with ACLF than other infections in the CANONIC study. In patients with cirrhosis and ascites, viable intestinal bacteria can cross the intestinal barrier and migrate to the general circulation and colonize the ascitic fluid[55,56].

During the first hours of bacterial infection, patients with cirrhosis have higher plasma levels of pro-inflammatory cytokines than patients without cirrhosis. This finding suggests the existence of excessive inflammation in cirrhosis[57,58]. The mechanisms that underlie this excessive inflammatory response to bacterial infection are incompletely understood[59]. In fact, most of our knowledge is based on experiments investigating the innate immune response to lipopolysaccharide (LPS), a PAMP recognized by TLR4[59–61] (**Figure 3**). The response to LPS has been studied in *ex vivo* studies performed in freshly isolated monocytes or peripheral blood mononuclear cells (PBMCs) from patients with and without cirrhosis. LPS-stimulated production of pro-inflammatory cytokines and chemokines is higher in cells from patients with cirrhosis

than in control cells[62–66]. The mechanisms of the LPS-induced "cytokine storm" associated with cirrhosis are poorly understood. *Ex-vivo* experiments have shown that PBMCs or monocytes from patients with cirrhosis exhibit defects in the following negative-feedback mechanisms of TLR4 signalling: the activation of the phosphatidylinositide3-kinase (PI3K)/ RAC-alpha serine/threonine-protein kinase (AKT) pathway[61,65]; inhibition of glycogen synthase kinase 3 activity[66]; and the induction of IL-1 receptor-associated kinase M (IRAK-M)[62] and of the anti-inflammatory cytokine IL-10[61,65]. Nevertheless, several other crucial mechanisms known to downregulate the TLR-mediated inflammatory response under non-cirrhotic conditions (in particular the induction of tumour necrosis factor, alpha-induced protein 3 (A20)) have not yet been investigated in the context of cirrhosis.

Following *in vivo* LPS challenge, plasma TNF levels are significantly higher in cirrhotic than in non-cirrhotic animals[67–71]. Moreover, in this setting, animals with but not without cirrhosis develop hepatocyte apoptosis and necrosis[70]. In addition, compared with normal livers, in cirrhotic livers LPS also elicits prolonged endoplasmic reticulum (ER) stress and a subsequent unfolded protein response that is responsible for sustained phosphorylation of eukaryotic translation initiation factor 2 subunit α (eIF-2-α) [70]. EIF-2-α phosphorylation is known to attenuate the translation of most RNAs [72]. In this context, hepatocyte TNF-mediated cell death might occur in cirrhotic livers because of the lack of translation of NF-κB-dependent survival mRNAs into proteins. In support of this hypothesis, normal hepatocytes exposed to high levels of TNF are protected against cell death because of the induction of NF-κB-dependent pro-survival proteins[73]. Together, these findings led to the theory that, in cirrhosis, LPS recognition might result in severe liver damage which is due not only to an excessive innate immune response but also to the impairment of mechanisms involved in hepatocyte ER homeostasis.

Future studies should investigate the inflammatory response and tissue damage induced by the recognition of PAMPs other than LPS. It should also be noted that the role of inducers of inflammation, other than PAMPs, such as virulence factors and DAMPs have not yet been studied in the context of sepsis-induced ACLF.

[H3] Severe alcoholic hepatitis.

Results of the CANONIC study suggest that 20% of cases of ACLF are caused by severe alcoholic hepatitis[8]. In alcoholic hepatitis, livers exhibit features of cell death and inflammation[74,75]. However, the underlying mechanisms that explain these features are still poorly understood[75], and most of the following mechanisms commented hereafter require confirmation.

Excessive alcohol consumption alters the gut microbiota and increases intestinal permeability[75]. In addition, chronic and excessive systemic inflammation causes damage to the intestinal barrier. These alterations might favour the translocation of bacteria into the bloodstream [76–78] (**Figure 4**). Regardless of whether these bacteria cause infection, they release PAMPs (such as LPS) which can reach the liver where they are recognized by TLRs expressed in resident macrophages (called Kupffer cells). This recognition stimulates the production of pro-inflammatory CXCL chemokines such as IL-8[79] that attract and activate neutrophils[80]. Neutrophil infiltration is a hallmark of alcoholic hepatitis[75]. Hepatocyte necrosis, which has been documented in severe alcoholic hepatitis[81], might result in the release of DAMPs that would be recognized by different receptors mediating an inflammatory response, as described above.

Mitochondrial DNA (mtDNA) is a type of DAMP, and mtDNA stress might also contribute to inflammation in the context of alcoholic hepatitis. Acetaldehyde metabolism results in hepatocyte reactive oxygen species (ROS) production[68]. ROS

production is also stimulated by TNF[65]. In the context of chronic alcohol consumption[82] or after LPS challenge[83], ROS overproduction induces mtDNA stress. In a mouse model of moderate mtDNA stress, mtDNA was shown to escape to the cytosol where it engaged a cell-intrinsic response involving the innate cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS) (**Figure 3A**). cGAS engagement with mtDNA, in turn, mediates type 1 IFN production and subsequent autocrine and paracrine induction of IFN target genes[84]. Therefore, a cell-intrinsic response to mtDNA stress might become an inflammatory response at tissue level and thereby contribute to liver failure.

Recent results suggest that the inhibition of liver regeneration might be involved in liver failure associated with severe alcoholic hepatitis[85]. Although hepatic progenitor cells are activated in livers with severe alcoholic hepatitis, these cells are committed to differentiate into cholangiocytes (epithelium lining the bile duct) instead of hepatocytes[85]. As a result, there might be no replacement of hepatocytes that die owing to alcoholic hepatitis. Together these findings suggest that severe alcoholic hepatitis might be caused by both immunopathology and impaired hepatocyte regeneration.

[H2] ACLF with no identifiable trigger.

The trigger of ACLF is unknown in approximately 40% of cases[8]. Although these patients show features of systemic inflammation[8], one cannot clearly explain how the systemic inflammation is stimulated. Three hypotheses might explain inflammation in ACLF with no clinically identifiable trigger. These hypotheses try to explain the mechanisms of inflammation in patients in whom ACLF develop in the absence of any obvious trigger

The first hypothesis is based on the existence of dysbiosis of the gut microbiota in patients with cirrhosis (**Figure 4**). Dysbiosis associated with cirrhosis is typically

characterized by a decrease in diversity, a decrease in *Lachnospiraceae, Ruminococcaceae, Bacteroidaceae,* Family XIV *Incertae Sedis*at the family level*,* and in *Bacteroides* at the genus level[6,86–89]. In addition, dysbiosis associated with cirrhosis involves an increase in Gram-negative *Enterobacteriaceae, Fusobacteriaceae and Porphyromonadaceae,* and Gram-positive *Streptococcaceae* at the family level[6,86–89]. Decompensation of cirrhosis results in additional distinct compositional changes of the microbiota when compared to compensated stages of liver disease[87]. There is a robust positive correlation between the abundance of certain bacterial family members and **plasma levels of** inflammatory cytokines (including IL-6 and TNF in patients with ACLF[90]. These findings suggest that metabolites produced by gut microbiota might contribute to systemic inflammation (**Figure 4**).

The second hypothesis is that some patients might have intestinal translocation of PAMPs such as LPS or bacterial CpG DNA[91] (**Figure 3A**). These ligands might reach the liver and systemic circulation and then be recognized by TLRs. Thus, TLR recognition is generally not dependent on microbial viability or invasiveness. During the peak phase of ACLF, systemic levels of LPS are higher than prior to the onset of ACLF and during remission of survivors[87,91], suggesting that higher systemic LPS levels correlate with disease severity. Interestingly, small intestinal bacterial overgrowth is a risk factor for increased systemic LPS levels in patients with cirrhosis[92]. Increased systemic levels of CpG DNA are found in patients with decompensated cirrhosis and without overt bacterial infection and correlate with mortality[93], suggesting that CpG DNA rises may be involved in the development of ACLF in patients with acutely decompensated cirrhosis**.**

The third mechanism explaining inflammation in ACLF with no clinically identifiable trigger might be the release of DAMPs, for example by necrotic hepatocytes. In

patients and animals with acute liver failure, a variety of DAMPs such as HMGB1 that might contribute to inflammation are released [83], but nothing is known about DAMPs in patients with ACLF.

Finally, another possible explanation for a failure to identify a precipitating event in ACLF could relate to a failure of current diagnostic tests or the testing protocol to identify infection or DILI.

[H1] Diagnosis, screening and prevention

[H2] Defining organ failure and ACLF

[H3] Diagnostic criteria of organ failure.

One of the assumptions made to define the EASL-CLIF criteria is that extrahepatic organ failure(s) is a major differential feature of ACLF. The Chronic Liver Failure Sequential Organ Failure Assessment (CLIF-SOFA) score was the original scale used to define organ failure in the CANONIC study[8]. It was derived from the SOFA score, a scale widely used in intensive care[16–19], which was then adapted to patients with chronic liver disease on the basis of published studies. Cut-off values were established after assessing the risk increase of 28-day mortality rates in each of the last four CLIF-SOFA score categories compared to that of the previous one in the patients enrolled in the CANONIC study. A simplified version of the CLIF-SOFA score, the CLIF Consortium Organ Failure (CLIF-C OF) score (**Table 2**), with identical criteria to diagnose organ failure and similar prognostic accuracy has been developed[20].

[H3] Mortality criteria.

Another pre-defined criterion for the diagnosis of ACLF in the development of the EASL-CLIF criteria was an expected 28-day mortality rate ≥15%. In the CANONIC series, this criterion was present in patients with ≥2 organ failures, but not in patients with one organ failure (28-day mortality 14.6%). Additional risk factors were used to further categorize patients in this low-risk subgroup. This analysis produced subgroups of patients that fulfilled the three predefined criteria for ACLF (**Figure 5**): Patients with ≥2 organ failures; patients with one organ failure (specifically kidney failure); and patients with non-renal single organ failure if these failures are associated with renal and/or cerebral dysfunction.

[H2] Grades of severity of ACLF

Patients with decompensated cirrhosis can be stratified into four groups of severity — no ACLF or ACLF grades 1-3 — on the basis of the type and number of organ failures they exhibit (**Box 1**). Kidney failure is the most prevalent organ failure in ACLF grade 1. For ACLF grade 2, liver failure is the most prevalent followed by kidney, cerebral and coagulation failure. For ACLF grade 3, the prevalence of all organ failures is high.

In the CANONIC study, 23% of patients admitted to hospital had ACLF at admission. Furthermore, 11% of patients developed the syndrome during hospitalization, which gives a total prevalence of ACLF of 31%. Among patients with ACLF, 51% had ALCF grade 1, 35% ACLF grade 2 and 13% ACLF grade 3. Besides providing the diagnosis of the syndrome, these criteria also provide data for rapid prognostic information, with ACLF grade associated with different rates of mortality (**Table 3**). The usefulness of these classification criteria as well as that of CLIF-SOFA and CLIF-C OF scores in assessing prognosis have been validated in independent series of patients[26,29–31,94,95].

[H2] Natural history of ACLF

ACLF is a syndrome that has potential for reversibility[96]. However, data from the CANONIC study clearly show that, despite this feature, mortality of ACLF patients increases cumulatively even after these patients are discharged from the hospital such that the mortality increases from approximately 20% at 28 days to over 35% at 90-days in patients with ACLF grade 1 and 30% to about 50% at 90-days in patients with ACLF grade 2[8]. Although there is considerable variability between patients [20,21], some broad principles regarding the course of the condition can be put forward. In general, at day 3-7 from presentation, approximately 50% patients with ACLF grade 1 will improve to having no ACLF, with a consequent 28-day mortality rate of approximately 7%. In 25% ACLF grade 1 will remain unchanged with a 28-day mortality rate of 24%. By contrast, approximately 25% patients with ACLF grade 1 progress to ACLF grade 2 or ACLF grade 3; their 28-day mortality is 53% and 88%, respectively. In patients presenting with ACLF grade 2, only 35% improve to having no ACLF or ACLF-1 at day 3-7 post-presentation. Those that do improve have low 28-day mortality rates of approximately 5%. In addition, approximately 50% of patients with ACLF grade 2 deteriorate to ACLF grade 3 (28-day mortality: 90%) or remain the same (28 day mortality: 26%). In patients presenting with ACLF grade 3, mortality rates remain very high with only approximately 13% improving to no ACLF or ACLF grade 1. The factors that were independently related to progression to more advanced grades were the CLIF-C ACLF score (discussed below) and the presence of liver failure. These data indicate that the syndrome is indeed very dynamic and that early intervention is crucial to minimize the risk of death.

[H2] Clinical risk factors

There are evidences that clinical course of ACLF outlined above is true for all aetiologies of ACLF. In a retrospective study from China in patients who predominantly had hepatitis-B related cirrhosis, short-term outcomes, the most closely associated with ACLF, were not affected by the nature of the precipitating illness[97]. Patients with hepatic precipitants such as reactivation of HBV or flares had a short-term (28-day and 90-day) mortality similar than patients with an extrahepatic precipitant such as infection. This was also observed in a second Chinese study in HBV cirrhotic patients who developed ACLF. The 28-day and 90-day mortality rates for any given grade of ACLF in this study [24] were similar to those reported by the CANONIC study and unrelated with the presence and type of precipitating events (**Table 4**). These studies, therefore, suggest that it is the number of organ failures and not the aetiology of cirrhosis or precipitating event the main risk factor of mortality.

A previous history of episodes of acute decompensation was absent in 23% of patients with ACLF in the CANONIC study, indicating that development of ACLF as the initial manifestation of decompensated cirrhosis is a relatively common feature. These patients without prior history of decompensation were younger, more frequently alcoholics, had more severe systemic inflammation and grade of ACLF and higher short-term mortality (42% vs 30%) than patients with ACLF and prior history of acute decompensation.

[H2] Prediction of prognosis

Since ACLF is a dynamic syndrome, prognostic scores need to be dynamic so that they can be updated sequentially on a daily basis, which would allow assessment of response to intervention, escalation for the need for urgent liver transplantation and determination of futility of ongoing treatment. The CANONIC study indicates that follow-up data within the first 3-7 days following diagnosis of ACLF are extremely important to

predict clinical course since resolution; improvement or worsening of ACLF following standard medical therapy occur within this early time period in most patients[96].

A prognostic model was developed and validated for patients with ACLF, referred to as the CLIF-C ACLF score, and for patients with acute decompensation who did not fulfil criteria for the diagnosis of ACLF, which is called the CLIF-C Acute Decompensation (CLIF-C AD; www.clifconsortium.com) score. These two scores were designed because a single score was insufficient to satisfactorily delineate the prognosis associated with acute decompensation and ACLF[8].

The CLIF-C ACLF score comprises the CLIF-C OF score, age and white blood cell count[20,98]. The score is a number from 0-100; the higher the number, the greater the risk of death. The score was validated using prospectively collected data from a series of patients not included in the CANONIC study. The CLIF-C ACLF score provided a significantly better estimate of the risk of death at 28-days, 90-days, 6-months and 12-months post-presentation compared with the Model for End-Stage Liver Disease (MELD) score, the MELD-Sodium score and the Child Pugh score [24,32,95,99–101]. Compared with the CLIF-C ACLF score, the MELD score underestimated the risk of death of patients by 20-30%, implying that organ allocation for transplants using the MELD score seriously disadvantages the patient with ACLF[20]. The performance of the CLIF-C ACLF score improved over the period of follow up suggesting that it should be updated daily[20] (20).

The CLIF-C AD score was developed in patients with acute decompensation without ACLF[98]. Variables that were found to be independently associated with survival were age, serum sodium level, serum creatinine level, white blood cell count and INR. These generated a score between 0-100 which was also significantly more accurate in predicting prognosis than the MELD, MELD-sodium and Child-Pugh scores[24,98]. Patients with a CLIF-C AD score of <45% had 28-day mortality of <3% and this

category might identify a group of patients that can be discharged early from the hospital. On the other hand, patients with CLIF-C AD score >60 were at high risk of progression to full-blown ACLF and had a 28-day mortality of approximately 20%, indicating that this is probably a 'Pre-ACLF' group. The CLIF-C AD score was also validated for sequential use.

[H2] Prevention

Early diagnosis and treatment of potential precipitating events are essential in the prevention of ACLF, and several preventative measures have been shown to be effective[5,55,102–105]. These all involve treating infections before they can go on to trigger ACLF and include: prompt administration of antibiotics tailored according to local epidemiological pattern of resistance in patients with suspected infections; long-term term suppression of HBV infection or sustained eradication of hepatitis C virus infection in patients with compensated or decompensated cirrhosis; and intravenous administration of albumin at infection diagnosis in patients with SBP. Albumin is highly effective preventing the development of type 1 Hepatorenal syndrome (HRS), a special form of ACLF characterized by rapidly progressive renal failure, in patients with SBP probably as a consequence of plasma volume expansion and also through a modulatory effect of albumin on the systemic inflammation associated with PAMPS (i.e. LPS) [104,105]. There is no evidence that intravenous albumin is effective in other bacterial infections[106,107].

There is also indirect evidence in support of other potential preventive measures for ACLF. For example, long-term oral norfloxacin administration reduces the rate of SBP (and of other bacterial infections) and type-1 hepatorenal syndrome (HRS) in patients

with decompensated cirrhosis[55,102,108–110]. Norfloxacin acts by selectively reducing the Gram-negative microbiota, decreasing the permeability of the gut barrier via stimulation of IL-10 release and modulating the immune response to bacterial translocation[111–113]. In addition, treatment of patients with severe acute alcoholic hepatitis with pentoxifylline, an inhibitor of macrophage production of TNF, or with the combination of prednisolone and intravenous N-acetylcysteine has been shown to reduce the incidence of type-1 HRS in some studies[114,115], presumably by modulating hepatic inflammation, but this has not confirmed in a recent investigation[116]. Finally, short-term administration of the combination of granulocyte colony-stimulating factor (G-CSF) plus darbopoietin (a synthetic analogue of erythropoietin has been shown to improve liver function, reduce the incidence of severe sepsis and increase 1-year survival in comparison to placebo in patients with decompensated cirrhosis[117].

[H1] Management

[H2] Medical Management

Medical management of ACLF consists of early recognition, treatment of the precipitating event and supportive care[8,14,118,119]. Early treatment of the trigger is proven to reduce mortality, for example in treatment of reactivated hepatitis B with tenofovir or alcoholic hepatitis with steroids[75,117,119–121]. However, most of ACLF management is focused on supportive care[118]

[H3] Anti-bacterial therapy.

As discussed above, bacterial infections are the precipitating event of ACLF in approximately 35% of patients **(Table 4)**[14]. As such, there should be a low threshold for

early initiation of antibiotics in patients with cirrhosis who have a bacterial infection. In patients with septic shock, every hour delay beyond presentation is associated with an adjusted odds of overall death of 1.1[122,123]. Broad spectrum antibiotics should be used, particularly in those with nosocomial or healthcare associated infections or in those with septic shock, as inappropriate initial antimicrobial therapy increases the adjusted odds of death by 10-fold[122,123]. Equally important to early initiation of antibiotics is prompt de-escalation of antibiotics once an organism is identified and/or the patient shows clinical improvement. If no organism is identified and there is persistent clinical deterioration in the setting of broad spectrum antibiotics, antifungals should be considered[123]. Measures to prevent superinfections (secondary infections that occur on top of a primary infection) should be implemented in patients with ACLF, including bundles of prevention and control of ventilator-associated pneumonia and catheter-related bactereamia and urinary tract infections, hand hygiene, barrier precautions and avoiding unnecessary instrumentation[124].

Infected and non-infected patients admitted with ACLF are highly predisposed to developing new bacterial infections during hospitalization (J. Fernández, unpublished, CANONIC study). These infections act as ‘second hit’ of the syndrome. Prevention, early diagnosis and treatment of these second infections, therefore, is a major issue in ACLF.

[H3] Anti-HBV therapy

Reactivation of HBV is a frequent precipitating event of ACLF in cirrhotic patients in Asia. Antiviral treatment in patients with hepatitis B related ACLF improves liver function and increases short and long-term survival[119–121,125,126]. Therefore, early treatment with the antiviral agents (lamivudine, tenofovir, entecavir or telbuvidine)

should be started as soon as possible[121,127].

[H3] Immunomodulation

Patients with ACLF might benefit from treatments aimed at restoring immune function, such as albumin, N-acetylcysteine and G-CSF[117,128]. Indeed, results from a recent randomized-controlled trial suggest that the administration of G-CSF prevents the development of sepsis and improves short-term survival in patients without severe forms of ACLF, who did not have sepsis, cerebral failure or multi-organ failure[128]. G-CSF is thought to act by mobilizing stem cells from the bone marrow to the periphery, including the liver, thus improving liver regeneration.

[H3] Renal dysfunction and renal failure.

Acute kidney injury (AKI) is the most frequent organ failure in ACLF[8,129]. Common causes of AKI include pre-renal, intrinsic causes and HRS[129]. Management of AKI differs depending on the underlying aetiology, and urinary biomarkers are helpful in identifying the cause of AKI[129,130]. Volume resuscitation with crystalloids and/or albumin should be used in patients with pre-renal AKI (impairment in renal function related to hypovolemia, i.e. excessive diuretic treatment). Terlipressin or norepinephrine are first choice treatment for HRS combined with volume expansion with albumin[129]. Terlipressin or norepinephrine are given to reduce the splanchnic arterial vasodilation causing systemic circulatory dysfunction and renal vasoconstriction in HRS. The effect of albumin was initially thought to be due to plasma volume expansion. However, a potential effect of albumin modulating the systemic inflammation of patients with ACLF in has recently been proposed[7,105]. Renal replacement therapy is used as a bridge to liver transplant or liver-kidney transplant in patients with severe AKI, although the dose and timing of dialysis has not been fully studied [129] .

[H3] Cardiovascular failure.

As in management of sepsis, aggressive volume resuscitation and initiation of vasoconstrictor agents (i.e. norepinephrine) to maintain an adequate blood pressure for organ perfusion is critical to counter the vasodilatory state that occurs with ACLF[14,131]. The goal mean arterial pressure is >60mmHg, and careful attention should be made to volume administration with crystalloids given the predisposition of volume overload in cirrhotic patients[118]. Colloids, including albumin, may also trigger volume overload. Terlipressin or vasopressin can be used as an adjunctive agent. There is also growing evidence that adrenal insufficiency in ACLF can further compromise haemodynamics[132]. Although still controversial, evaluation for adrenal insufficiency can be done by measuring random cortisol levels in the morning. If these levels are indeterminate, adrenal insufficiency can be confirmed with a ACTH test, which estimates the response of the adrenal glands to stress.

[H3] Brain dysfunction and brain failure.

Treatment of encephalopathy with tap water enemas, lactulose and oral non-absorbable antibiotics (rifaximin, neomycin), by improving the level of consciousness, can prevent bronchial aspiration, aspiration pneumonias and respiratory failure[14]. It is important to titrate lactulose dose and enemas appropriately to prevent hypovolaemia that results from diarrhoea and to prevent hypernatremia (rise in serum sodium) that results from lactulose. Goal stool output in a day should be 3-4 bowel movements[133]. Lower stool output is insufficient to reduce the intestinal production of ammonia and to increase ammonia clearance from blood. Higher stool output may induce hypernatremic dehydration. Patients with grade III-IV encephalopathy should be

intubated, as they have a high risk of experiencing bronchial aspiration (i.e. of saliva or gastric fluid). Intra-craneal pressure monitoring and the use of mannitol is not recommended in these patients as cerebral oedema and intra-cranial hypertension are exceptional in patients with hepatic encephalopathy associated to ACLF[12].

[H3] Coagulopathy.

Coagulopathy in patients with ACLF is often difficult to manage in the setting of fluctuations between a prothrombotic and an ineffective haemostatic state[134,135]. In the setting of active bleeding and severe coagulopathy, transfusions of platelets, cryoprecipitate (a frozen blood product prepared from plasma used to elevate fibrinogen levels) and blood should be considered. However, patients should not be prophylactically transfused with plasma for an elevated INR. Patients with portal vein thrombosis may require anticoagulant therapy to prevent recurrent variceal bleeding.

[H2] Intensive care and extracorporeal liver support devices

Admission to critical care units is mandatory in case of vascular, respiratory or brain failure and recommended in those with renal failure. Patients with liver and coagulation failure can still be treated in regular wards, but require strict clinical monitoring. In the CANONIC study[8], 50% of the patients with ACLF were admitted to the intensive care unit (86% of those had ACLF grade 3). Owing to the high mortality rate in patients with ACLF, treatments able to bridge the time between admission of patients with severe disease (ACLF grades 2 or 3 at 3-7 days following admission) to liver transplantation are, therefore, clearly needed.

Extracorporeal liver support systems are potential treatments for ACLF[136,137].

Bioartificial liver support systems use hollow-fibre bioreactors containing hepatic cells to support the metabolic and synthetic function of the diseased liver. Currently, only tumour hepatocyte or porcine hepatocyte lines (the ELAD® and the HepAssist® 2000 systems) are available. A recent randomized trial comparing ELAD versus standard medical treatment did not find any significant effect in survival[136]. Non-biological systems consist in albumin dialysis techniques and are based on the capacity of this molecule to remove water insoluble substances and pro-inflammatory molecules (such as PAMPs and reactive oxygen species) retained in plasma as a consequence of liver failure and systemic inflammation[105]. Moreover, the physiology albumin function is markedly impaired in patients with decompensated cirrhosis due to severe oxidation of the molecule by endogenous reactive oxygen species, alterations in its molecular structure, and saturation of other binding sites by water insoluble substances produced and/or retained as consequence of liver failure (i.e. bilirubin, bile salts, drugs). Three different albumin dialysis systems are currently available: the molecular adsorbent and recirculating system (MARS®), the fractionated plasma separation and absorption (FPSA®) system and single-pass albumin dialysis (SPAD®). MARS, the system most extensively evaluated in ACLF, improves systemic haemodynamics and severe hepatic encephalopathy[136,137]. Two large randomized multicentre studies, however, failed to show an improvement in survival using MARS[138,139]Finally, an artificial liver device (called the University College London-Liver Dialysis Device) aimed at removing and replacing the dysfunctional albumin of patients with cirrhosis and reducing circulating endotoxemia is currently under evaluation[140] . Plasma exchange, a detoxification system that increases survival in patients with acute liver failure[141], improves hepatic encephalopathy and liver function in non-randomized studies in patients with ACLF.

[H2] Liver transplantation

Liver transplantation represents the only definitive therapeutic option for patients with ACLF. Very few studies, however, have assessed its feasibility, selection criteria (indications and contraindications), timing and efficacy[96,142–148]. In contrast to patients with acute liver failure, patients with ACLF cannot currently be included in the high-urgency transplantation list. Moreover, since the clinical course of ACLF evolves rapidly, the timeframe for evaluation and listing is frequently very short. Advanced age, active alcoholism, uncontrolled infections and multi-organ failure are the main reasons for contraindication to transplantation or delisting. There is general agreement in considering that transplantation must be avoided in patients with severe circulatory or respiratory failure and ongoing sepsis. By contrast, for the majority of experienced centres, organ support (renal replacement therapy and mechanical ventilation) does not contraindicate transplantation in ACLF. Current data indicate that less than half of patients with ACLF are listed and that the procedure is feasible in only 10-25% of patients, since >50-70% of the listed patients die on the waiting list[147]. A recent US study showed that patients with cirrhosis, ACLF and high MELD score (>40) have higher waiting-list mortality (almost two-fold higher) than status 1A candidates (patients with acute liver failure) [145] . Defining criteria to select and prioritize patients with ACLF on the waiting list will help to improve outcomes by providing timely liver transplantation. Several studies show that both living donor liver transplantation and deceased donor transplantation offer similar results in this setting[146–148]. The reported outcome of patients transplanted for ACLF is good (**Figure 6**), ranging between 74% and 90% at 5 years, a number similar to that observed in patients transplanted for other indications[96,144–148].

[H2] Management algorithm

The three new scoring systems derived from the CANONIC study, the CLIF-C OF score (or CLIF-SOFA score), CLIF-C ACLF score and CLIF-C AD score, can be used to risk stratify cirrhotic patients with acute decompensation, to indicate early liver transplantation and to assess intensive care unit treatment futility (**Figure 7**)[20,98]. The prognosis in ACLF depends not only on the number of organ failures or the CLIF-C ACLF score at diagnosis, but also on the early response to treatment[96]. As nearly 20% of patients with ACLF grade 3 improve after treatment, patients with ≥3 organ failures should be admitted to the intensive care unit and receive unrestricted organ support for a short period of time (3-7 days). The persistence of ≥3 organ failures after this intervention may lead to the need to consider a limitation in life sustaining treatments since a fatal outcome is almost invariable in the absence of 'salvage' liver transplantation[19]. A high CLIF-C ACLF score (>64 points) after initial intervention (at day 3-7) has also been suggested as a potential futility rule in patients without possibilities of early liver transplantation[96](. However, these criteria require further validation. Patients who are potential candidates for early liver transplantation, including living donor liver transplantation, must not be limited in their treatment. In those without options for transplantation, scores and a pragmatic case-by-case evaluation should be used for the decision.

[H2] Regenerative therapy

A few studies have evaluated the effect of G-CSF therapy in small groups of patients with ACLF[128,149,150].This cytokine mobilizes bone marrow-derived stem cells, restores neutrophil function and promotes hepatic regeneration. Its administration in non-severe forms of ACLF reduces the risk of developing organ failure(s) and sepsis and improves survival. G-CSF therapy seems to be ineffective in patients with sepsis and in those

with more severe forms of ACLF. Hepatocyte and stem cell transplantation have also been proposed as potential treatments in ACLF[151].

[H1] Quality of life

Following dismissal from hospital, patients recovering from ACLF may return to functioning in their community, receive a liver transplant, be sent to intermediate care facilities like a nursing home, or be re-hospitalized. The 30-day hospital readmission rate is approximately 25%[152]. In the long-term follow-up of 6 months in the NACSELD study, 27% of patients died, 14% were transplanted and 59% were alive without liver transplantation[153]. After discharge, 45% of patients had subsequent infections. Patients who had repeat infections were older and were more likely to use proton pump inhibitors, rifaximin or prophylactic therapy for SBP with norfloxacin[154,155]. In these last three circumstances predisposition to bacterial infections are probably related to gut dysbiosis or colonization by drug multi-resistant bacteria. Of critical importance to note, patients with infection related ACLF were more likely to be delisted for liver transplantation.

[H1] Outlook

[H2] ACLF definition

The challenge of obtaining a universal definition of ACLF is an important issue. Differences between the APASL and Western definitions, however, are too important to be solved by consensus agreements alone. These definitions differ not only in terms of the characteristics of the patients, diagnostic criteria and clinical course but also, and most importantly, in the conceptual view of the disease. The APASL conference

definition postulates that the sequence of events in ACLF starts with a hepatic insult that causes acute liver failure and, as a consequence, extra-hepatic organ failure(s). By contrast, the Western definition relies on the concept that the acute impairment in liver function — which, if intense is defined as liver failure — develops simultaneously to an impairment in the function of other organs — which if intense are defined also as organ failures — as a consequence of an extrahepatic mechanism. These mechanisms could include intense systemic inflammation related to a massive release of DAMPS from the diseased liver (in the case of acute alcoholic hepatitis, viral hepatitis or DILI) or of PAMPs (by invading bacteria in the case of sepsis, or from the intestinal microbiota in patients without clear precipitating events). Such controversy can only be solved by promoting research in this outstanding new syndrome.

[H2] Clinical challenges

Investigations of ACLF have generally been performed after the diagnosis of the syndrome. As such, there are few data within the critical period prior to ACLF development. Prospective observational studies within this period are, therefore, essential, particularly those assessing biomarkers or panels of biomarkers of systemic inflammation that could be of value as predictors of treatment response and survival. Liver pathology in ACLF has also been insufficiently investigated. In patients with cirrhosis due to HBV infection, ACLF occurs in the setting of sub-massive hepatic necrosis[27]. In patients with alcoholic cirrhosis and active alcoholism, severe alcoholic hepatitis superimposed on cirrhosis is probably the predominant liver histology. Finally, two recent studies have reported severe ductular bilirubinostasis and cholestasis, a lesion also seen in non-cirrhotic patients with sepsis, as an specific lesion in ACLF[156,157].

[H2] Insights into pathophysiology

Sequential studies of the innate and adaptive immune system function prior to and after ACLF are lacking. Such studies are essential to understand the mechanism of ACLF. Moreover, the immune system function might change during the clinical course of the syndrome. As it occurs in sepsis[158], an initial activation of the immune system in ACLF might be followed by a period of immunosuppression which would favour further bacterial translocation and progression of organ failure(s).

The mechanism of organ failure in ACLF is of major interest. Renal failure in cirrhosis is considered to be secondary to systemic circulatory dysfunction and impaired renal perfusion. However, recent evidence from studies in sepsis suggest that renal failure might also be a consequence of a direct effect of renal inflammation, which impairs renal microcirculation and cell function[159]. In fact, there is evidence that inflammation might be involved in the pathogenesis of cardiac dysfunction, encephalopathy, relative adrenal insufficiency and pulmonary dysfunction in cirrhosis[7].

A major difficulty for research in ACLF is the lack of appropriate animal models. Carbon-tetrachloride induced cirrhosis in rats is an excellent model of cirrhosis but animals die prior the development of extra-hepatic organ failure[160]. Bile-duct ligated rats represent an acute model of liver failure and ascites and, in combination with the acute intra-peritoneal administration of LPS, have been used as a model of ACLF[161]. However, this model differs markedly from human cirrhosis and no extra-hepatic organ failure has been documented.

[H2] Treatment

The recognition of systemic inflammation as the main mechanism of ACLF opens new fields in the design of new therapeutic procedures. This knowledge will promote the development of new artificial liver support systems capable of removing not only potentially harmful molecules retained as a consequence of organ failure but also pro-inflammatory molecules that cause ACLF. Total plasma exchange[141] is clearly alternative method to remove PAMPs, DAMPs and free radicals.

A major issue in the management of ACLF, however, is prevention. There are three potential effective treatments that should be explored. The first consists in the prevention of bacterial translocation by long-term oral administration of poorly absorbable antibiotics[107,110,162]. Long-term weekly administration of intravenous albumin is the second approach. Preliminary data indicate that this technique prevented bacterial infections, AKI and hepatic encephalopathy and improved survival in a large Italian randomized controlled trial in patients with decompensated cirrhosis[163]. Finally, recent investigations have suggested a central role for defective bile acid receptor (also called farnesoid X-activated receptor, FXR) signalling in hepatic inflammation and intestinal bacterial translocation, factors which are known to shape ACLF[164–166]. Obeticholic acid is a potent FXR-agonist. Recent studies in animals have demonstrated that it lowers portal hypertension and improves bacterial translocation [165,166] suggesting that it might be of potential benefit in patients with ACLF.

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ToC

Patients with cirrhosis can develop acute-on-chronic liver failure (ACLF), a syndrome characterized by acute decompensation, organ failure and high short-term mortality. Arroyo et al. discuss the mechanisms, diagnosis and management of this evolving concept in the field of liver disease.

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Sensitive images

None.

Box 1. The main definitions of ACLF.

[H1] The Asian Pacific Association for the Study of the Liver (APASL) definition.

For patients with compensated cirrhosis or with any kind of non-cirrhotic chronic liver disease, except isolated steatosis (definition first made in 2004 and revised in 2009)[11,12]: Acute-on-chronic liver failure (ACLF) is the result of an acute direct hepatic insult (hepatotropic viral infections, active alcohol consumption or drug-induced liver injury) that causes liver failure. Liver failure is defined as jaundice (serum bilirubin ≥5 mg per dl) and coagulopathy (international normalized ratio ≥1.5 or prothrombin activity

<40%). This liver failure is complicated within 4 weeks by clinical ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease (including cirrhosis). Both compensated cirrhosis and non-cirrhotic chronic liver disease (non-alcoholic fatty liver disease-related chronic hepatic injury or chronic hepatitis with fibrosis or fibrosis due to other reasons) qualify as chronic liver disease. Bacterial infections are not considered hepatic insults. Patients with cirrhosis and known prior decompensation (jaundice, encephalopathy or ascites) who develop acute deterioration of their clinical status that is either related or unrelated to precipitating events are considered to have acute decompensation but not ACLF.

[H1] The European Association for the Study of the Liver-Chronic Liver Failure (EASL-CLIF) Consortium definition

For patients with cirrhosis (2013)[8]**:** ACLF is the development of acute decompensation of cirrhosis (defined by the development of ascites, encephalopathy, gastrointestinal haemorrhage and/or bacterial infection) associated with either a single organ failure (single renal failure or other single non-renal organ failure if associated with renal and/or cerebral dysfunction) or multiple organ failures.

[H1] Other definitions

- R. Jalan and R. Williams definition (2002)[10]
- The Chinese Medical Association (CMA) definition (2013)[15]
- The American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) definition (2014)[14]
- North-American Consortium for the Study of End Stage Liver Disease (NACSELD) definition (2014)[13]

Box 2 | ACLF grades

[H1] No ACLF

This category includes patients who either:

- Do not have any organ failure
- Have a single organ failure that does not involve the kidneys with serum creatinine <1.5 mg per dL and no hepatic encephalopathy
- Have single cerebral failure with serum creatinine <1.5 mg per dL

[H1] ACLF grade 1

ACLF grade 1 is diagnosed with one of the following:

- Single kidney failure
- Single liver, coagulation, circulatory or lung failure that is associated with serum creatinine of 1.5-1.9 mg/dL and/or hepatic encephalopathy grades 1 or 2
- Single cerebral failure with serum creatinine between 1.5 and1.9 mg per dL

[H1] ACLF grade 2

ACLF grade 2 is diagnosed when there are two organ failures of any combination

[H1] ACLF grade 3

ACLF grade 3 is diagnosed when there are three or more organ failures.

Figure 1 | The clinical course of cirrhosis Acute-on-chronic liver failure (ACLF) can develop at any stage from compensated to decompensated cirrhosis, and can involve hepatic or extrahepatic precipitating events, although a considerable proportion of patients have no identifiable triggering event. In this figure, paracentesis means ‘large volume paracentesis (>5 litres)’. Acute decompensation of cirrhosis defines the acute development of clinically evident ascites, hepatic encephalopathy, gastrointestinal haemorrhage or any combination of these in patients with or without prior history of these complications. Although bacterial infections are not specific complications of cirrhosis, they are considered as such in patients with prior history of ascites,

haemorrhage or encephalopathy because of their high prevalence and their association with abnormalities related to cirrhosis, including bacterial translocation and impaired leukocyte function[1–8] . DILI, Drug-induced liver injury; LT, liver transplantation; TIPS, transjugular intrahepatic portosystemic shunt. Figure courtesy of Jordi Bozzo (Scientific Publication Manager. Grifols SA **]**

Figure 2 | Different ACLF definitions capture different patient populations. A) The proportion of patients diagnosed with acute-on-chronic liver failure (ACLF) according to the Asia-Pacific Association for the Study of the Liver (APASL) definition (orange), the European Association for the Study of the Liver-Chronic Liver Failure (EASL-CLIF) Consortium definition (red), and both definitions (blue). Data are from a large series of patients from Korea [35] (1,470 patients of which 1,352 with cirrhosis with or without prior history of decompensation) and from the CANONIC study conducted in Europe [8]. B) The 90-day probability of survival in both series of patients depending on the ACLF diagnosis. Figures with European data derive from unpublished results of the CANONIC study provided by the Data Management Center of the European Foundation for the Study of Chronic Liver Failure. Figures with the Korean data adapted from *PLoSOne*, 2016 Jan 20;11(1):e0146745. doi: 10.1371. Kim TH et al, Characteristics and discrepancies in acute-on-chronic liver failure: need for a unified definition. Copyright (2015).

Figure 3 | Structural feature recognition of bacteria and induction of the inflammatory response. A) Examples of pattern-recognition receptors (PRRs) that detect unique molecular structures of bacterial pathogen-associated molecular patterns (PAMPs) and their sub-cellular localizations. PRRs for bacterial ligands include 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) and cytosolic DNA sensors (including interferon, gamma-inducible protein 16 (IFI16), absent in melanoma 2 (AIM2), and cyclic GMP-AMP synthase (cGAS)). [43–48,167]. B) An example of PRR-mediated inflammation is the activation of inflammatory signalling pathways by extracellular and intracellular lipopolysaccharide (LPS). Detection of extracellular LPS by TLR4 engages two intracellular signalling conduits: the MyD88 pathway (purple), involving the adaptor Toll/interleukin-1 receptor domain-containing adapter protein (TIRAP); and the TIR-domain-containing adaptor protein inducing interferon (IFN)-β (TRIF) pathway (green), involving the adaptor TIR domain-containing adapter molecule 2 (TICAM2) [43]. The MyD88 pathway via TNF receptor-associated factor 6 (TRAF6) stimulates different kinases (grey), inhibitor of nuclear factor (NF)-κB (IκB) kinase (IKK) and mitogen-activated protein kinases (MAPKs) that activate master transcription factors (TFs), NF-κB and activator protein-1 (AP-1), respectively. The MyD88 pathway also activates the TF interferon (IFN) regulatory factor 5 (IRF5). These activated TFs contributes to the induction of an array of pro-inflammatory genes such *as tumour necrosis factor (TNF)* and *interleukin 6 (IL6)*, and anti-inflammatory genes such as *IL10, IL1RN*. The TRIF pathway involves TRAF3 to activate the TF, IRF3, which then contributes with other TFs to the induction of type 1 IFNs [43] . Intracellular LPS is recognized by the inflammatory caspases (caspase-4/5 in humans and caspase-11 in mice) that stimulate the non-canonical NOD-, leucine-rich repeat (LRR)- and pyrin domain (PYD)-containing protein 3 (NLRP3) inflammasome [167,168]. This results in the activation of another inflammatory caspase-1 (not shown) which promotes cleavage of IL-1β and IL-18 [167,168] . Activation of caspase-4/5 by intracellular LPS can trigger a programmed cell death called pyroptosis [169].

Figure 4 | Intestinal dysbiosis and bacterial translocation. Cirrhosis is associated with quantitative differences (bacterial overgrowth) and compositional changes of the

gut microbiota, so called dysbiosis. Several factors might contribute to dysbiosis of the gut microbiota during cirrhosis including diet, use of antibiotics, decreased bile flow and intestinal motility, changes in gastric pH and impaired mucosal immunity. A second important feature of that patients with cirrhosis also show is translocation of bacteria. Disruption of tight junctions allows pathogen-associated molecular patterns (PAMPs) and possibly other microbial metabolites to use the paracellular route between adjacent intestinal epithelial cells for translocation. Intestinal permeability is already increased in pre-cirrhotic stages, whereas translocation of viable bacteria is a characteristic of cirrhosis, in particular during decompensation. Bacteria most likely use the transcellular route (transcytosis) through epithelial cells. PAMPs might also activate immune cells including monocytes, macrophages and T-cells in the lamina propria of the intestine leading to secretion of inflammatory mediators. Cytokines such as tumour necrosis factor (TNF), interleukin (IL)-6, IL-17, nitric oxide (NO) and interferon (IFN)-γ are increased in the intestine of patients or animal models with cirrhosis [76–78]. Several of these mediators are known to contribute to a dysfunction of tight junctions. On the other hand, the intestinal immune surveillance response might be impaired to remove translocated bacteria in the lamina propria.

Figure 5 | Relationship between organ failure and mortality in ACLF. 28-day mortality rates of patients with decompensated cirrhosis with (red bars) and without (green bars) acute-on-chronic liver failure (ACLF) according to the diagnostic criteria proposed in the CANONIC study[8]. Patients are divided into the following categories: Patients with no organ failure (OF); patients with single non-kidney organ failure without kidney dysfunction (KD,serum creatinine 1.5-1.9 mg per dL) or cerebral dysfunction (CD, grade 1-2 hepatic encephalopathy); patients with single kidney failure; patients with single non-kidney organ failure with KD and/or CD; patients with two

organ failures; and patients with ≥3 organ failures. Figure derived from the CANONIC study, adapted from *Gastroenterology,* **144** Moreau, R et al., Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis., 1426-1437, Copyright (2013).

Figure 6 | Liver transplant increases survival of patients with. Survival probability curves of patients with grades 2 or 3 ACLF at day 3-7 following diagnosis who were submitted to early (less than 28-day) liver transplantation (red curve; n=21) and patients with the same ACLF grades who did not receive a liver transplant (blue curve; n=120)[96]. Figures over the curves show the 28-day, 90-day and 6-month probability of survival. Reprinted from *Hepatology*, **62** Gustot T et al., Clinical course of acute-on-chronic liver failure syndrome and effects on prognosis., 243-252, Copyright (2015).

Figure 7 | Proposed algorithm for management of patients with ACLF or decompensated cirrhosis. A proposed management strategy for patients with acute-on-chronic liver failure (ACLF) based on mortality rate data from the CANONIC study[96] .The first step is the assessment of ACLF grade at days 3-7 after initiation of medical management, including organ support(s). Liver transplantation should be assessed in all patients with ACLF because of high 90-day mortality rates (>20%). Liver transplantation should be performed as early as possible in patients with ACLF grade 2 and grade 3 since they are at great risk of short-term (28-day) mortality. In the case of contraindication of liver transplantation the presence of four or more organ failures (OFs) or Chronic Liver Failure (CLIF)-C ACLF score >64 at days 3-7 after diagnosis could indicate the futility of care. Reprinted from *Hepatology*, **62** Gustot T et al., Clinical course of acute-on-chronic liver failure syndrome and effects on prognosis., 243-252, Copyright (2015).

Table 1 | Selected studies on the prevalence of ACLF

Country or Region	Diagnostic criteria	Population	Prevalence of ACLF	Refs
China	EASL-CLIF	890 patients hospitalized with decompensated cirrhosis due to chronic hepatitis B	34%§	[24]
China	EASL-CLIF	1,397 patients hospitalized with decompensated cirrhosis due to chronic hepatitis B	30%§	[97]
North America	NACSELD*	Patients with decompensated cirrhosis and acute bacterial infections	24%	[13]
Scandinavia	EASL-CLIF	Patients with cirrhosis (from a population of 600,000)	24%‡	[170]
Europe	EASL-CLIF	1,343 CANONIC study participants	30.9%§	[8]

*Patients with decompensated cirrhosis and bacterial infections who develop two organ failures. §At enrolment and during hospitalization.‡ Infection-related ACLF diagnosed between 2001 and 2010. EASL-CLIF, European Association for the Study of the Liver-Chronic Liver Failure; NACSELD, North -American Consortium for the Study of End Stage Liver Disease.

Table 2 | CLIF-C OF score[20].

Organ or system	**Sub-score = 1**	**Sub-score = 2 (OR vs sub-score 1 (95%CI)**	**Sub-score = 3§ (OR vs sub-score 1 (95%CI)**
Liver	Bilirubin <6mg per dL	6≤ bilirubin ≤12mg per dL (OR: 2.6 (1·6 – 4.3))	Bilirubin >12mg per dL§ (OR: 7.1 (4.7 – 10.7))
Kidney	Creatinine <2 mg per dL	2≤ creatinine <3.5 mg per dL § (OR: 3.8 (2.3 – 6·3))§	Creatinine ≥3.5 mg per dL or renal replacement§ (OR: 15.5 (8.9 – 26.8))
Brain	West-Haven HE[171] grade 0	West-Haven HE grade 1-2(OR: 2·1 (1·4 – 3·2))	West-Haven HE grade 3-4*§ (OR: 9·7 (5·9 – 16·1))
Coagulation	INR <2·0	2·0 ≤ INR <2·5 (OR: 5·2 (3·4 – 7·9))	INR ≥2·5§ OR: 7·5 (4·6 – 12·3)
Circulation	MAP ≥70 mmHg	MAP <70 mmHg (OR: 2·6 (1·6 – 4·3))	Use of vasopressors§ OR: 9·2 (5·2 – 16·4)
Respiratory system	PaO_2/FiO_2 >300 or SpO_2/FiO_2 >357	200< PaO_2/FiO_2 ≤300 or >214 SpO_2/FiO_2 ≤357 (OR: 2·7 (1·7 – 4·2))	PaO_2/FiO_2 ≤200# or SpO_2/FiO_2 ≤214#§ (OR: 6·4 (3·1 – 13·2))

The odds ratio (OR) describes the increase in the risk of 28-day mortality compared with the low risk category for each organ system and sub-score. HE, Hepatic encephalopathy; INR, International normalized ratio; FIO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; SpO_2, pulse oximetric saturation; MAP, Mean arterial pressure. [§]Criteria for diagnosing organ failures.*Patients submitted to mechanical ventilation due to HE and not to a respiratory failure were considered as presenting a cerebral failure (cerebral sub-score=3). # Other patients enrolled in the study with MV were considered as presenting a respiratory (respiratory sub-score=3). Adapted from *J Hepatol*, **61** Jalan R et al., Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure., 1038-1047, Copyright (2014).

CLIF-C OF, CLIF-C Organ failure

Table 3 | ACLF grade and mortality in the CANONIC study[8]

Category	28-day mortality	90-day mortality
No ACLF	1.9%	10%
ACLF (total)	33%	51%
ACLF grade 1	23%	41%
ACLF grade 2	31%	55%
ACLF grade 3	74%	78%

Adapted from *Gastroenterology,* **144** Moreau, R et al., Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis., 1426-1437, Copyright (2013).

Table 4 | Proportion of patients with potential precipitating events at enrolment in the CANONIC Study[8] .

Precipitating event	Proportion without ACLF (*n*=1040)	Proportion with ACLF (*n*=303)	*P* value[§]
Bacterial infection	21.8%	32.6%	<0.0001
Gastrointestinal bleeding	17.3%	13.2%	ns
Active alcoholism*	14.9%	24.5%	0.0002
Other event **	3.5%	8.6%	0.0002
No event ***	58.9%	43.6%	<0.0001
Any event ***	41.1%	56.4%	<0.0001
>1 event ***	5.7%	13.5%	<0.0001

§ P value compares (Chi-square test) the prevalence of potential precipitating events between patients with and without ACLF at enrolment in the CANONIC study. Bacterial infection and active alcoholism were significantly more frequent in patients with than in those without ACLF, suggesting that they were associated with the development of the syndrome. This was not the case for gastrointestinal bleeding*Within 3 months prior to inclusion. **Other precipitating events include large volume paracentesis without i.v. administration of albumin (to prevent post-paracentesis circulatory dysfunction), transjugular intrahepatic portosystemic shunt (which is used to treat portal hypertension), major surgery, acute hepatitis (caused by viral infection, ischemia, or drug-induced liver injury) and acute alcoholic hepatitis. Liver biopsy was required for the diagnosis of acute alcoholic hepatitis in the CANONIC protocol but many patients with active alcoholism had a clinical picture suggestive of

this diagnosis.*** Bacterial infections, active alcoholism or other precipitating events. ns, not significant. Adapted from *Gastroenterology,* **144** Moreau, R et al., Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis., 1426-1437, Copyright (2013).

Third party rights table

Category	Reference number	
Figure 2	[35]	*PLoSOne*, 2016 Jan 20;11(1):e0146745. doi: 10.1371. Kim TH et al, Characteristics and discrepancies in acute-on-chronic liver failure: need for a unified definition.Copyright (2015
Figure 5	[8]	*Gastroenterology,* **144** Moreau, R et al., Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. 1426-1437, Copyright (2013).
Figure 6	[96]	*Hepatology*, **62** Gustot T et al., Clinical course of acute-on-chronic liver failure syndrome and effects on prognosis., 243-252, Copyright (2015).
Figure 7	[96]	*Hepatology*, **62** Gustot T et al., Clinical course of acute-on-chronic liver failure syndrome and effects on prognosis., 243-252, Copyright (2015).
Table 2	[20]	*J Hepatol*, **61** Jalan R et al., Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure., 1038-1047, Copyright (2014).
Table 3	[8]	*Gastroenterology,* **144** Moreau, R et al., Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis., 1426-1437, Copyright (2013).
Table 4	[8]	*Gastroenterology,* **144** Moreau, R et al., Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis., 1426-1437, Copyright (2013).

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