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Systemic Inflammation in Decompensated Cirrhosis: Characterization and Role in Acute-on-Chronic Liver Failure

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Acute-on-chronic liver failure (ACLF) in cirrhosis is characterized by acute decompensation (AD), organ failure(s), and high short-term mortality. Recently, we have proposed (systemic inflammation [SI] hypothesis) that ACLF is the expression of an acute exacerbation of the SI already present in decompensated cirrhosis. This study was aimed at testing this hypothesis and included 522 patients with decompensated cirrhosis (237 with ACLF) and 40 healthy subjects. SI was assessed by measuring 29 cytokines and the redox state of circulating albumin (HNA2), a marker of systemic oxidative stress. Systemic circulatory dysfunction (SCD) was estimated by plasma renin (PRC) and copeptin (PCC) concentrations. Measurements were performed at enrollment (baseline) in all patients and sequentially during hospitalization in 255. The main findings of this study were: (1) Patients with AD without ACLF showed very high baseline levels of inflammatory cytokines, HNA2, PRC, and PCC. Patients with ACLF showed significantly higher levels of these markers than those without ACLF; (2) different cytokine profiles were identified according to the type of ACLF precipitating event (active alcoholism/acute alcoholic hepatitis, bacterial infection, and others); (3) severity of SI and frequency and severity of ACLF at enrollment were strongly associated. The course of SI and the course of ACLF (improvement, no change, or worsening) during hospitalization and short-term mortality were also strongly associated; and (4) the strength of association of ACLF with SI was higher than with SCD. *Conclusion:* These data support SI as the primary driver of ACLF in cirrhosis. (HEPATOLOGY 2016;64:1249-1264).

Abbreviations: AAH, acute alcoholic hepatitis; ACLF, acute-on-chronic liver failure; ACLF-RF, ACLF with renal failure; AD, acute decompensation; ADH, antidiuretic hormone; AST, aspartate aminotransferase; CI, confidence interval; CLIF, chronic liver failure; CRP, C-reactive protein; Cys34, cysteine-34; DAMPs, damage-associated molecular patterns; EGF, epidermal growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; HMA, human mercaptalbumin; HNA1, reversibly oxidized human nonmercaptalbumin 1; HNA2, irreversibly oxidized human nonmercaptalbumin 2; IFNγ, interferon gamma; IP-10, IFNγ-inducible protein 10; IQR, interquartile range; IL, interleukin; LT, liver transplantation; MCP-1, macrophage chemotactic protein 1; MIP, macrophage inflammatory protein; PAMPs, pathogen-associated molecular patterns; PCC, plasma copeptin concentration; PE, precipitating event; PRC, plasma renin concentration; RR, relative risk; SCD, systemic circulatory dysfunction; SI, systemic inflammation; SIRS, systemic inflammatory response syndrome; TNFα, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor; WBC, white blood cell count.

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**These authors contributed equally to this work.*

A list of CANONIC Study Investigators is provided in the Appendix.

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Acute-on-chronic liver failure (ACLF) in cirrhosis is a highly prevalent syndrome characterized by acute decompensation (AD), organ/system failure(s), and high 28-day mortality (32%).[1] ACLF is classified in three grades of severity (ACLF-I, -II, and -III) according to the number of organ failures and may follow four different clinical courses during hospitalization: resolution, improvement (reduction in ACLF grade), steady course, or worsening.[2]

The systemic inflammation (SI) hypothesis[3] proposes that ACLF is attributed to aggravation of the SI and associated systemic circulatory dysfunction (SCD) already present in AD, which leads to organ failure(s) as a consequence of organ hypoperfusion and direct deleterious effects of inflammatory mediators on the organ microcirculation and cell physiology homeostasis. According with this hypothesis, AD would occur in the setting of chronic SI attributable to translocation of proinflammatory molecules (pathogen-associated molecular patterns; PAMPs) from the intestinal lumen to the systemic circulation and/or to the release or damage-associated molecular patterns (DAMPs) from the diseased liver or another organ. ACLF would be the result of further increase of SI in the context of precipitating events (PEs; mainly active alcoholism/acute alcoholic hepatitis [AAH], bacterial infections, or other Pes). The SI hypothesis was based on cytokine studies in small series of patients[4-7] and on the CANONIC study, in which a close relationship between blood leukocytes (white blood cell count; WBC) and C-reactive protein (CRP) levels and the presence and severity of ACLF was observed.[1]

In the current study, we assessed the SI hypothesis by two approaches. The first was aimed at examining the relationship between markers of SI, measured at enrollment and sequentially during hospitalization, and the presence, severity, and clinical course of ACLF and associated mortality in 552 patients hospitalized for AD. The second approach was aimed at assessing whether, as proposed by the SI hypothesis, ACLF in cirrhosis is not only attributed to an accentuation of the SCD and organ hypoperfusion related to SI, but also to direct deleterious effects of SI in organ function. This assessment was performed by assessing the strength of the association of SCD and SI with ACLF and mortality.

Patients and Methods

PATIENT SELECTION, BLOOD SAMPLING, DATA COLLECTION, AND DIAGNOSTIC CRITERIA OF ORGAN FAILURE AND ACLF

Two hundred thirty-seven patients with ACLF, either present at enrollment (n = 180) or developed during hospitalization (n = 57), and 285 without ACLF derived from the CANONIC investigation[1] were studied. Selection criteria were: (1) availability of

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blood samples at enrollment and (2) prospective intensive surveillance during hospitalization. Follow-up plasma samples obtained at prespecified visits during hospitalization (2, 3-7, 8-14, 15-21, and 22-28 days after enrollment) were available in 255 patients, 157 with ACLF. Sequential blood samples before ACLF, at ACLF diagnosis, and during post-ACLF follow-up were available in 20 of the 57 patients developing ACLF during hospitalization. In the remaining patients with ACLF, there were only samples at ACLF diagnosis and during follow-up. Samples were obtained in 2011 in Vacutainer ethylenedimainetetraacetic acid tubes, centrifuged at 4°C, and the plasma frozen at -90°C. Measurements were performed during 2015.

Clinical, laboratory, and follow-up data were obtained from individual electronic CRF. There was no patient with human immunodeficiency virus infection. Diagnosis of organ failure, ACLF, and ACLF grades are based on the Chronic Liver Failure (CLIF) Consortium Organ Failure score and the CANONIC study criteria[1,8] (see Supporting Information). Clinical diagnosis of AAH was based on the National Institute on Alcohol Abuse and Alcoholism Alcoholic Hepatitis Consortia criteria[9]: active alcoholism, serum bilirubin >3 mg/dL, aspartate aminotransferase (AST) >50 IU/mL, and AST/alanine aminotransferase ratio >1.5. As described,[10] systemic inflammatory response syndrome (SIRS) was defined by the presence of alterations in at least two of the following parameters: WBC count, heart and respiratory rates, and body temperature.

CHARACTERIZATION OF SI IN AD: RELATIONSHIP WITH ACLF

To characterize SI, we measured the plasma levels of 29 cytokines involved in innate and adaptive immune responses and the redox state of circulating albumin (a marker of systemic oxidative stress[11,12]). Baseline measurements were obtained at enrollment in all patients. In addition, a second baseline measurement was obtained at ACLF diagnosis in 20 of the 57 patients developing ACLF during hospitalization. To assess the role of SI in the pathogenesis of ACLF, we compared baseline plasma cytokine levels of patients with and without ACLF at enrollment and examined the relationship between these measurements and ACLF severity. Also, we assessed the chronological relationship between changes in SI and the course of ACLF during hospitalization by comparing the levels of SI markers at ACLF diagnosis with those at the last measurement during hospitalization (before discharge or liver transplantation [LT] or death). Median time from enrollment to last assessment: 15 days (percentile 25-75:7-28). Differences in baseline cytokine profiles according to the etiology (PE) were also assessed to identify potential differences in SI mechanisms. Finally, proinflammatory/anti-inflammatory cytokine ratios were determined in healthy subjects and patients with and without ACLF to assess the profile of proinflammatory and anti-inflammatory cytokine response in these patients. Cytokines were measured using a multiplexed bead-based immunoassay on a Luminex 100 Bioanalyzer (Austin, TX) (Supporting Information and Supporting Table S1).

Systemic oxidative stress was estimated by measuring the percentage of albumin oxidized at the cysteine-34 (Cys34) residue over the total albumin concentration. Free Cys34 accounts for around 80% of the antioxidant capacity of human plasma.[12,13] In the presence of systemic oxidative stress, Cys34 is converted from the reduced form with Cys34 in the free sulphydryl form (human mercaptalbumin; HMA) to mixed disulfides (human nonmercaptalbumin 1; HNA1). This process is reversible.[14-16] A smaller fraction of Cys34, however, is highly oxidized to sulfenic, sulfinic, and, finally, sulfonic forms (human nonmercaptalbumin 2; HNA2). Oxidation of albumin to HNA2 is irreversible and causes intense modifications of the protein structure.[15,16] HNA1+HNA2 as well as HNA2 were used as markers of systemic oxidative stress.[17-19] Reasons for the selection of the redox state of albumin over other markers of systemic oxidative stress and the methodology of measurement are detailed in the Supporting Information.

STRENGTH OF THE ASSOCIATION OF MARKERS OF SI AND OF SCD WITH ACLF OR ACLF WITH RENAL FAILURE

Systemic circulatory function defines a series of physiological processes influencing the cardiac output and activity of endogenous vasoactive systems that regulate arterial pressure and global organ perfusion. Microcirculatory function (which is mainly dependent on endothelial and tissue factors) regulates the blood and oxygen delivery to the cells. Given that plasma renin concentration (PRC) and the plasma concentration of copeptin (PCC) are very sensitive to changes in effective circulating blood volume,[20,21] they were selected as markers of SCD for this study. Copeptin is a stable cleavage product of the C-terminal part of the antidiuretic hormone (ADH) precursor that is produced in a 1:1 fashion with ADH. It is widely used to estimate

vasopressin release because it allows an easier, more-accurate immunological testing than ADH.[21] Other criteria used for selection of PRC and PCC as markers of systemic circulatory dysfunction and the methodology of measurements are detailed in the Supporting Information. Copeptin (as ADH) is filtered by the glomeruli and therefore may overestimate ADH secretion in patients with ACLF with renal failure (ACLF-RF).

The strength of the association of ACLF with PRC and with interleukin (IL)-8, IL-6, or HNA2 was estimated to assess whether ACLF is related not only to SCD, but also to other effects of SI in organ function. IL-8, IL-6, and HNA2 were selected because they were the inflammatory markers more strongly associated with ACLF in our patients (see Results). PRC was selected because it was not influenced, as copeptin, by glomerular filtration rate in our patients (see Results). The strength of the association of ACLF-RF with markers of SI and PRC were also compared as an outcome, given that renal failure is a paradigmatic organ failure in cirrhosis thought to be caused by severe impairment in cardiovascular function and organ hypoperfusion.[3,20,22] Four different approaches to analysis were used.

First, we visually assessed the distribution of baseline IL-8, IL-6, HNA2, and PRC according to the presence of ACLF and ACLF-RF by means of a scatterplot of patients' values. The strength of association between SI or SCD and ACLF or ACLF-RF was estimated by comparing (chi-square tests) the progression of ACLF or ACLF-RF frequency at three different levels (tertiles) of each SI marker versus PRC.

Second, we assessed the percentage of patients with ACLF or ACLF-RF showing normal levels of IL-8, IL-6, HNA2, and PRC.

The third approach included two different analyses: (1) a logistic regression model to assess which markers (IL-8, IL-6, HNA2, and/or PRC) were independently associated with the presence of ACLF or ACLF-RF and (2) a linear regression model assessing the correlation of IL-8, IL-6, HNA2, and PRC with CLIF Consortium Organ Failure score and serum creatinine as markers of ACLF and renal impairment severity.

Finally, we assessed the association of changes in markers of SI and PRC during hospitalization with changes in the clinical course of patients with and without ACLF.

SI, SCD, AND MORTALITY

We also assessed the strength of the association of baseline levels of SI and SCD markers and of changes in these markers during hospitalization with short-term (28-day and 90-day) mortality.

DATA ANALYSIS AND STATISTICAL DETAILS

Among the 29 cytokines determined, 12 were undetectable in a high percentage (more than 30%) of the patients with cirrhosis studied (Supporting Table S2). Because of this feature, two independent analyses on the association of cytokines with ACLF and mortality were performed. The main analysis, which included the 17 cytokines showing detectable plasma levels in most patients, is detailed in the main body of the article. Undetectable levels of healthy subjects in this analysis (the majority of values for some cytokines) were assigned a value equal to the lower limit of detection. The second analysis was performed with the 12 cytokines with more than 30% of undetectable values. Results with this group are briefly mentioned in the text of the article and detailed in the Supporting Information.

Discrete variables are shown as counts (percentage) and continuous variables as mean (SD). Non-normally distributed variables are summarized by the median (interquartile range; IQR) and were log-transformed for some statistical analyses and for graphical comparisons. In univariate statistical comparisons, the chi-square test was used for categorical variables, whereas the Student t test or analysis of variance were used for normal continuous variables and the Wilcoxon signed-rank test or the Kruskall-Wallis test for continuous variables not normally distributed. In all statistical analyses, significance was set at $P < 0.05$.

Results

CHARACTERIZATION OF SI IN PATIENTS WITH AD: RELATIONSHIP WITH ACLF

Patient characteristics were similar to those of the whole CANONIC cohort[1] (Supporting Table S3). Active alcoholism and bacterial infections were the most frequent PEs of ACLF, although in approximately 40% of patients no PE was identified. The presence and severity of ACLF and prognosis correlated closely with WBC and CRP levels. The prevalence of AAH (47 patients) among the 522 patients studied was only of 9% (65% among the 72 patients with active alcoholism). AAH was significantly more frequent in patients with ACLF (12.2%) than in those without ACLF (6.3%;

TABLE 1. Plasma Concentrations of Renin (PRC), Copeptin (PCC), and Cytokines and Albumin Oxidation Fractions in Healthy Subjects and in Patients With and Without ACLF

	Healthy Controls N = 40	No ACLF N = 285	ACLF N = 237	*P* Value*
Markers of SCD				
PRC (microIU/mL)	8 (6-17)	65 (17-242)	134 (36-378)	<0.001
PCC (pmol/L)	0 (0-10)	9 (3-23)	31 (13-61)	<0.0001
Proinflammatory cytokines				
TNFα (pg/mL)	9 (7-12)	20 (14-27)	29 (17-41)	<0.001
IL-6 (pg/mL)	0.3 (0.3-0.3)	21 (11-41)	39 (17-115)	<0.001
IL-8 (pg/mL)	1.6 (0.6-3.3)	37 (20-76)	84 (41-169)	<0.001
MCP-1 (pg/mL)	337 (218-413)	318 (228-436)	410 (288-713)	<0.001
IP-10 (pg/mL)	328 (234-428)	965 (558-1,676)	1,184 (665-2,157)	0.004
MIP-1β (pg/mL)	13 (6-17)	20 (13-34)	28 (19-50)	<0.001
G-CSF (pg/mL)	2.1 (1.8-11)	23 (11-50)	32 (14-83)	0.001
GM-CSF (pg/mL)	7.5 (7.5-7.5)	4.7 (2.0-9.5)	7.3 (3.5-16.8)	<0.001
Anti-inflammatory cytokines				
IL-10 (pg/mL)	1.1 (0.4-1.1)	3.4 (1.1-9.2)	8.1 (2.1-29.9)	<0.001
IL-1ra (pg/mL)	7 (3-9)	10 (5-22)	23 (9-63)	<0.001
Other cytokines				
IFNγ (pg/mL)	0.8 (0.8-4.9)	6 (2-18)	7 (3-24)	0.044
IFNα2 (pg/mL)	3 (3-3)	22 (8-56)	27 (11-63)	0.113
Eotaxin (pg/mL)	94 (55-122)	110 (81-155)	124 (89-179)	0.018
IL-17a (pg/mL)	0.7 (0.7-2.7)	3.7 (1.6-10.3)	4.5 (1.6-15.6)	0.128
IL-7 (pg/mL)	1.4 (0.1-3.9)	2.6 (1.0-8.5)	3.5 (1.6-11.1)	0.012
EGF (pg/mL)	17 (4-29)	26 (9-66)	19 (8-41)	0.046
VEGF (pg/mL)	26 (26-28)	85 (28-226)	91 (29-252)	0.745
Albumin oxidation fractions†				
HMA (%)	71 (68-74)	53 (42-62)	45 (33-56)	<0.001
HNA1+HNA2 (%)	28 (25-30)	46.4 (37.5-56.9)	51.8 (42.2-65.6)	<0.001
HNA2 (%)	1.3 (0.3-1.9)	4.5 (2.5-8.8)	9.8 (5.6-14.8)	<0.001

Data are median (IQR).
*P value between ACLF and NO ACLF.
†According to the redox state at Cys34.

$P < 0.05$). Only 78 patients (14.9%) showed SIRS. SIRS was significantly more frequent ($P < 0.05$) in patients with bacterial infection (23.6% vs. 11.9%), AAH (25.5% vs. 13.9%), or ACLF (19.9% vs. 10.9%).

Tables 1 and 2 show the results obtained with the 17 cytokines with detectable values in more than 70% of the patients. They were categorized as either cytokines with predominant inflammatory properties (tumor necrosis factor alpha [TNFα], IL-6, IL-8, macrophage chemotactic protein 1 [MCP-1], interferon gamma [IFNγ]-inducible protein 10 [IP-10], macrophage inflammatory protein [MIP]-1β, granulocyte colony-stimulating factor [G-CSF], and granulocyte macrophage colony-stimulating factor [GM-CSF]) in the innate immune system, cytokines with predominant anti-inflammatory properties (IL-10 and IL-1ra), or cytokines with other actions, including cytokines with predominant activity in the adaptive immune system (IFNγ, IFNα2, IL-17a, and IL-7), cytokines with predominant chemotactic activity on eosinophils (eotaxin), and cytokines that function as growth factors (epidermal growth factor [EGF] and vascular endothelial growth factor [VEGF]; Supporting Table S4).

As compared to healthy controls, patients with cirrhosis showed markedly increased levels of all cytokines (Table 1). Patients presenting ACLF exhibited significantly higher levels of proinflammatory cytokines than patients without ACLF. The anti-inflammatory cytokines, IL-10 and IL-1ra, followed a similar pattern. In contrast, differences in plasma levels of the remaining cytokines were slight or not significant. No significant differences in plasma cytokine levels at ACLF diagnosis were observed between patients with ACLF at enrollment and those who developed ACLF after enrollment (data not shown).

There was a clear, direct relationship between intensity of SI and severity of ACLF, as indicated by a parallel and significant increase in IL-6, IL-8, and IL-1ra from ACLF grade I to ACLF grades II and III (Table 2).

IL-8/IL-10 and IL-6/IL-10 ratios were determined as markers of proinflammatory/anti-inflammatory cytokine response (Supporting Fig. S1). The two ratios were

TABLE 2. Plasma Concentrations of Renin (PRC), Copeptin (PCC), and Cytokines and Albumin Oxidation Fractions According to the Grade of ACLF

	ACLF-I N = 126	ACLF-II N = 86	ACLF-III N = 25	P Value*
Markers of SCD				
PRC (microIU/mL)	169 (40-383)	114 (28-352)	87 (33-258)	0.771
PCC (pmol/L)	34 (16.62)	27 (13-45)	47 (11.134)	0.224
Proinflammatory cytokines				
TNFα (pg/mL)	30 (21-43)	26 (15-36)	32 (17-43)	0.029
IL-6 (pg/mL)	34 (18-96)	43 (13-106)	111 (32-355)	0.018
IL-8 (pg/mL)	62 (37-112)	97 (48-192)	144 (80-292)	<0.001
MCP-1 (pg/mL)	412 (299-633)	376 (277-646)	660 (322-1,773)	0.089
IP-10 (pg/mL)	1,218 (717-2,258)	1,162 (617-1,946)	1,689 (899-2,728)	0.267
MIP-1β (pg/mL)	27 (18-43)	28 (19-55)	46 (20-61)	0.112
G-CSF (pg/mL)	32 (15-70)	29 (14-81)	39 (15-209)	0.673
GM-CSF (pg/mL)	6.8 (3.7-15.0)	7.5 (2.7-20.1)	11.3 (5.1-29.6)	0.512
Anti-inflammatory cytokines				
IL-10 (pg/mL)	4.3 (1.1-17.9)	15.3 (5.5-41.5)	12.4 (6.6-40.8)	<0.001
IL-1ra (pg/mL)	17 (10-45)	26 (8-63)	49 (24-135)	0.019
Other cytokines				
IFNγ (pg/mL)	7 (3-24)	9 (3-25)	7 (2-16)	0.906
IFNα2 (pg/mL)	27 (11-61)	25 (12-74)	30 (12-55)	0.968
Eotaxin (pg/mL)	124 (90-178)	128 (90-180)	103 (87-176)	0.951
IL-17a (pg/mL)	3.4 (1.6-13.3)	5.5 (1.8-27.8)	8.1 (1.5-15.2)	0.623
IL-7 (pg/mL)	3.5 (1.7-8.0)	3.8 (1.4-14.6)	3.5 (2.1-6.9)	0.790
EGF (pg/mL)	20 (9-51)	19 (7-38)	14 (7-25)	0.233
VEGF (pg/mL)	95 (28-246)	77 (27-278)	89 (35-201)	0.950
Albumin oxidation fractions†				
HMA (%)	44 (33-56)	45 (32-58)	51 (42-58)	0.621
HNA1+HNA2 (%)	52.9 (42.2-64.0)	51.3 (42.2-66.9)	48.7 (41.8-58.3)	0.781
HNA2 (%)	9.5 (5.1-13.9)	9.8 (5.6-15.1)	11.1 (7.8-15.1)	0.205

Data are median (IQR).
*Comparison between ACLF grades.
†According to the redox state at Cys34.

significantly higher in patients with cirrhosis than in healthy subjects. No significant differences between patients with and without ACLF were observed.

Supporting Tables S5 and S6 show the results obtained with the cytokines showing detectable values in less than 70% of patients. All cytokines included in this analysis were also significantly higher in patients with decompensated cirrhosis with and without ACLF than in healthy controls (Supporting Table S5). However, significant differences between patients with and without ACLF were observed only in the inflammatory cytokine, MIP-1α (higher in patients with ACLF). Among the cytokines included in this analysis, only IL-15 was significantly different across the three grades of severity of ACLF (Supporting Table S6).

The profile of the 17 cytokines included in the main analysis varied according to the PEs in patients with ACLF (Table 3). ACLF precipitated by bacterial infection showed higher levels of TNFα, IL-6, and IL-1ra than ACLF precipitated by active alcoholism ($P = 0.004$, $P = 0.02$, and $P = 0.0269$, respectively) or ACLF associated with other PEs or without PE ($P = 0.04$, $P = 0.0002$, and $P = 0.02$, respectively). ACLF precipitated by active alcoholism showed higher levels of IL-8 than ACLF precipitated by bacterial infection ($P = 0.0001$) or ACLF associated with other PEs or without PE ($P = 0.001$). The cytokine profile in ACLF precipitated by both bacterial infections and active alcoholism shared the characteristics of ACLF precipitated by infection and active alcoholism alone. In ACLF patients with other PEs or without PE, no cytokine showed significantly increased levels in comparison to the other groups of patients with ACLF.

Supporting Table S7 shows that the profile associated with active alcoholism was attributed, to a great extent, to the extremely high values of IL-8 observed in patients with AAH. However, the plasma levels of IL-8 were also higher in patients with active alcoholism without AAH than in those without active alcoholism (Supporting Table S7). This increase in plasma levels of IL-8 observed in patients with alcoholic cirrhosis and active alcoholism

TABLE 3. Plasma Concentrations of Renin (PRC), Copeptin (PCC), and Cytokines and Albumin Oxidation Fractions in Patients With ACLF According to the Presence and Type of PEs

	No PEs (n = 94)	Bacterial Infection/No Active Alcoholism (n = 63)	Active Alcoholism/No Bacterial Infection (n = 28)	Bacterial Infection/ Active Alcoholism (n = 11)	Other PEs (n = 19)	*P* Value*
Markers of SCD						
PRC (microIU/mL)	151 (50-474)	164 (28-447)	92 (31-295)	205 (112-998)	69 (25-210)	0.3021
PCC (pmol/L)	31 (15-59)	38 (16-64)	24 (7-36)	33 (4-112)	34 (16-55)	0.4654
Proinflammatory cytokines						
TNFα (pg/mL)	29 (17-41)	32 (26-47)	21 (14-32)	34 (18-53)	27 (20-35)	0.0256
IL-6 (pg/mL)	30 (14-69)	72 (28-358)	37 (13-122)	83 (34-466)	34 (21-104)	0.0002
IL-8 (pg/mL)	64 (38-104)	92 (47-167)	211 (141-351)	158 (99-310)	50 (28-92)	<0.0001
MCP-1 (pg/mL)	372 (279-484)	512 (299-1,072)	515 (361-846)	832 (294-1,024)	342 (220-554)	0.0143
IP-10 (pg/mL)	1,349 (717-2,120)	1,053 (631-2,479)	882 (580-2,265)	1,596 (482-1,996)	1,153 (646-2,215)	0.7356
MIP-1β (pg/mL)	24 (16-51)	30 (21-49)	28 (18-61)	43 (32-55)	26 (15-33)	0.2005
G-CSF (pg/mL)	29 (12-69)	39 (17-89)	32 (14-64)	35 (19-434)	31 (11-179)	0.4916
GM-CSF (pg/mL)	6.1 (3.2-14.4)	11.9 (3.3-32.5)	6.7 (4.2-14.4)	9.4 (3.8-17.4)	5.4 (2.6-12.5)	0.1754
Anti-inflammatory cytokines						
IL-10 (pg/mL)	6.2 (1.9-25.8)	17.8 (4.7-55)	8.3 (1.0-22.9)	24.5 (5.9-40.2)	5.1 (1.9-12.3)	0.0790
IL-1ra (pg/mL)	19 (8-47)	41 (13-100)	16 (8-49)	25 (14-37)	16 (9-33)	0.1666
Other cytokines						
IFNγ (pg/mL)	7 (2-27)	6 (3-16)	11 (3-24)	8 (4-17)	2 (1-28)	0.4394
IFNα2 (pg/mL)	23 (9-54)	41 (20-97)	19 (5-55)	30 (11-57)	17 (8-29)	0.0447
Eotaxin (pg/mL)	122 (91-176)	130 (88-180)	131 (89-214)	91 (67-178)	93 (79-116)	0.0782
IL-17a (pg/mL)	3.7 (1.3-22.7)	4.4 (1.9-13)	2.9 (1.6-14.4)	8.1 (5.4-28.7)	2.8 (1.5-11.8)	0.5340
IL-7 (pg/mL)	3.0 (1.6-8.2)	5.8 (2.1-11.1)	2.7 (1.2-7.5)	6.4 (2.2-18.5)	2.2 (0.7-11.9)	0.0966
EGF (pg/mL)	17 (9-48)	20 (9-32)	14 (6-33)	20 (9-26)	16 (3-62)	0.9015
VEGF (pg/mL)	97 (34-286)	109 (28-240)	84 (27-127)	91 (56-148)	62 (14-299)	0.7647
Albumin oxidation fractions†						
HMA (%)	44 (32-55)	44 (32-55)	49 (39-57)	32 (19-49)	48 (35-63)	0.2151
HNA1+HNA2 (%)	53.5 (42.6-65.7)	53.6 (41.8-66.7)	50.3 (42.8-59.6)	68.2 (50.6-80.9)	51.6 (37.4-64.7)	0.2489
HNA2 (%)	9.6 (5.1-14.8)	12.3 (8.0-15.3)	8.6 (7.0-13.3)	10.3 (6.5-14)	7.0 (3.9-10.7)	0.0682

Data are median (IQR).
*P value overall.
†According to the redox state at Cys34.

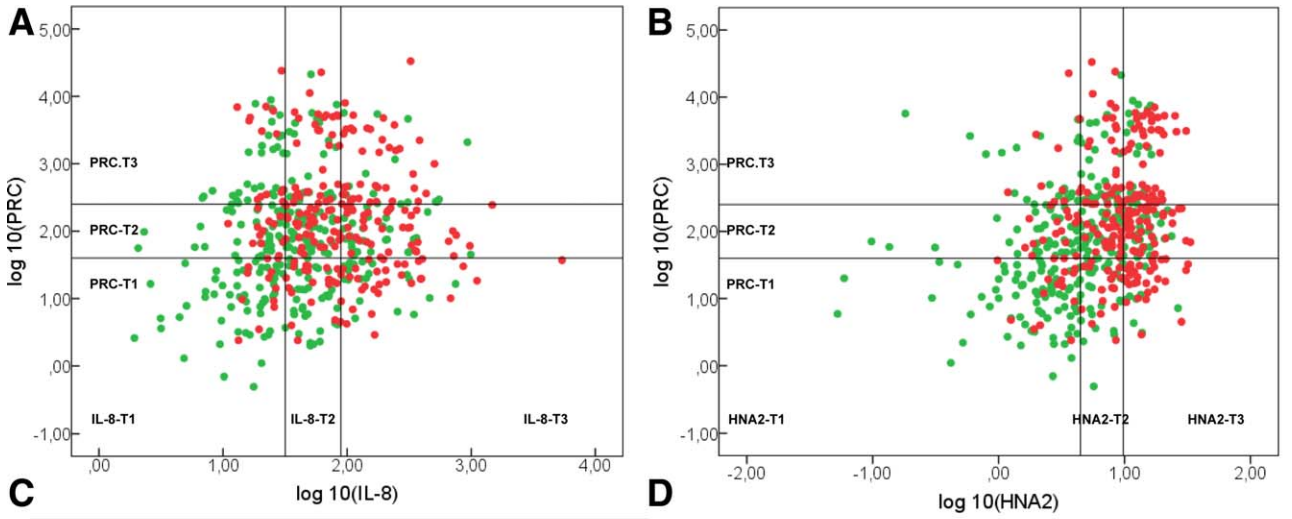
but no AAH was not detected in alcoholic patients with cirrhosis who stopped drinking (Supporting Table S7).

Supporting Table S8 shows the cytokine profile in patients with and without SIRS. Patients with SIRS showed significantly higher plasma levels of the proinflammatory cytokines, IL-6, IL-8, MCP-1, MIP-1β, and G-CSF, and of the anti-inflammatory cytokines, IL-10 and IL-1ra, than patients without SIRS.

HNA1+HNA2 and HNA2 were markedly increased ($P < 0.001$) in patients with cirrhosis with and without ACLF as compared to healthy controls (Table 1). Values for both HNA1+HNA2 and HNA2 were significantly higher in patients with ACLF than in those without (Table 1). There were no significant changes in the redox state of albumin across the three ACLF grades (Table 2).

SCD IN PATIENTS WITH AND WITHOUT ACLF

PRC and PCC were markedly above normal range in patients with and without ACLF, but values of these markers were significantly higher in the former than in the latter group (Table 1). PCC was significantly higher in patients with ACLF-RF (48.7 [19.8-81.1]) than in those with ACLF without renal failure (25.1 [10.2-44.8] pmol/L; $P < 0.001$). In contrast, no significant differences in PRC were observed between these two groups (112 [35-276] vs. 179 [33-460] microIU/mL; $P = 0.285$). These results indicate that PCC is a poor marker of SCD in patients with ACLF-RF given that it reflects not only an increased release of the ADH precursor by the neurohypophysis as a consequence of SCD, but also an impaired renal clearance of copeptin. PCC values in patients with ACLF without renal failure were significantly higher than those observed in the group of patients without ACLF (25.1 [10.2-44.8] vs. 9 [3-23] pmol/L; $P < 0.001$), indicating that ADH secretion is markedly increased in ACLF. No significant changes in PRC and PCC were observed across the three ACLF grades (Table 2).



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	IL8-T1	IL8-T2	IL8-T3	Overall PRC
PRC-T3	17/53 (32%)	35/56 (62%)	47/64 (73%)	99/173 (57%)*
PRC-T2	14/47 (30%)	27/61 (44%)	34/60 (57%)	75/168 (45%)
PRC-T1	11/67 (16%)	20/53 (38%)	30/50 (60%)	61/170 (36%)
Overall IL-8	42/167 (25%)	82/170 (48%)#	111/174 (64%)#	n=511

*p<0.05 vs previous tertile; #p<0.01 vs previous tertile

D

	HNA2-T1	HNA2-T2	HNA2-T3	Overall PRC
PRC-T3	10/33 (30%)	31/60 (52%)	56/76 (74%)	97/169 (57%)
PRC-T2	16/51 (31%)	26/59 (44%)	34/58 (59%)	76/168 (45%)
PRC-T1	14/84 (17%)	17/46 (37%)	28/36 (78%)	59/166 (36%)
Overall HNA2	40/168 (24%)	74/165 (45%)#	118/170 (69%)#	n=503

*p<0.05 vs previous tertile; #p<0.01 vs previous tertile

FIG. 1. (A,B) Scatterplots of individual patients classified by the presence (red dots) or absence (green dots) of ACLF according to individual values of PRC and IL-8 (left) or HNA2 (right). Two cut-off points for each marker divide each cohort into three tertiles (T1, lower values; T2, intermediate values; T3, higher values). Patients with ACLF are predominantly in the right part of the panels (higher degree of SI). (C,D) Presence of ACLF across the three tertiles of both PRC and IL-8 and PRC and HNA2. Differences in the prevalence of ACLF within tertiles (T2 vs. T1 and T3 vs. T2) were more significant with IL-8 and HNA2 than with PRC.

STRENGTH OF THE ASSOCIATION OF MARKERS OF SI AND OF SCD WITH THE FREQUENCY AND SEVERITY OF ACLF OR ACLF-RF AT ENROLLMENT AND THE COURSE OF ACLF DURING HOSPITALIZATION

IL-8, IL-6, and HNA2, the SI markers more closely associated with the presence and severity of ACLF at enrollment (Tables 1 and 2), and PRC were analyzed for this purpose. PCC was not included in this analysis because of its low ability for the assessment of SCD in patients with ACLF-RF.

Figure 1 shows the distribution of patients with (red dots) and without (green dots) ACLF according to baseline values of PRC and IL-8 (left) or HNA2 (right) in the whole series. Patients with ACLF were predominantly distributed in the right part of the figures (higher degree of SI; upper panels), and the strength of association of ACLF with both SI markers was greater than that with PRC (lower panels). A similar pattern of distribution was obtained with IL-6 (Supporting Fig. S2), with IL-8, IL-6 (not shown), and HNA2 after excluding patients with active alcoholism (Supporting Fig. S3) or with IL-8, IL-6 (not shown), and HNA2 when only patients with ACLF-RF were considered (Supporting Fig. S4).

Among the patients with ACLF, only 2 (1.7%) showed normal values (below the 95th percentile of the distribution in healthy subjects) of IL-6 and none had normal values of IL-8. In contrast, 17.7% showed normal PRC values ($P < 0.001$) and 8.0% presented normal HNA2 values ($P < 0.001$; Fig. 2A). The corresponding percentages in relation to patients with ACLF-RF were 0%, 0.8%, 16.4%, and 8%, respectively ($P < 0.001$; Fig. 2B). The corresponding values after excluding patients with active alcoholism are represented in Supporting Fig. S5.

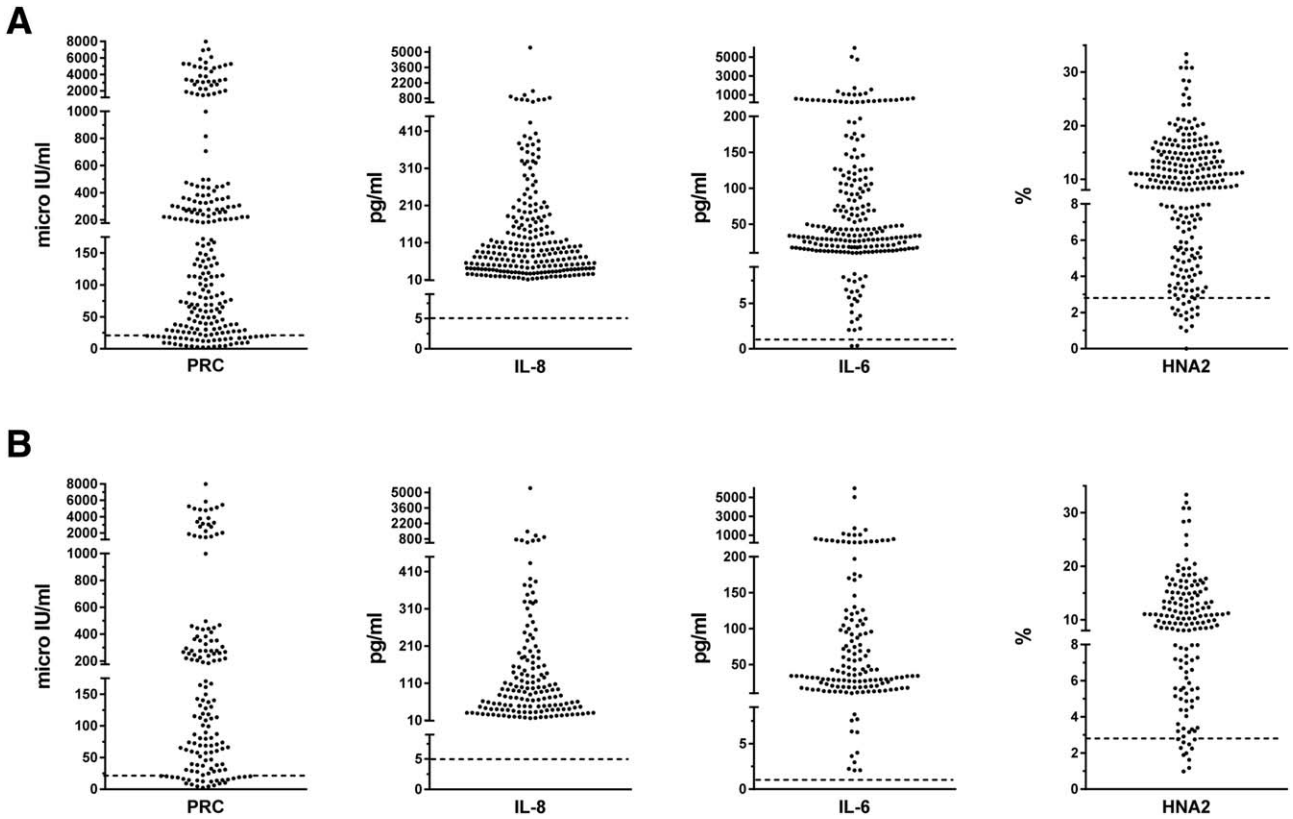


FIG. 2. Scatterplots of individual values of PRC, IL-8, IL-6, and HNA2 in patients with ACLF (A) and with ACLF associated with renal failure (B). Horizontal lines represent the upper normal limits (95% percentile of the distribution of values in healthy subjects).

The logistic regression model showed that the presence of ACLF was independently associated with IL-6, IL-8, and HNA2, but not with PRC (log PRC: relative risk [RR], 1.05; confidence interval [CI], 0.94-1.16; $P = 0.413$; log IL-8: RR, 1.73; CI, 1.40-2.14; $P < 0.001$; log IL-6: RR, 1.33; CI, 1.12-1.57; $P < 0.001$; log HNA2: RR, 2.78; CI, 2.07-3.71; $P < 0.001$). Only IL-8 and HNA2 were found to be significantly associated with the presence of ACLF-RF (RR, 1.45; 95% CI, 1.17-1.80; $P < 0.00$; and RR, 2.80; 95% CI, 1.98-3.95; $P < 0.001$, respectively).

The step-wise linear regression model fitted for CLIF-C Organ Failure Score based on the significance of the increase in model R-square allowed to select HNA2 ($P < 0.0001$) and both cytokines (IL-8, $P < 0.0001$; IL-6, $P = 0.0008$) as the markers most strongly related to severity of ACLF. Similarly, the step-wise model fitted for serum creatinine allowed to select HNA2 ($P < 0.0001$) and IL-6 ($P = 0.0114$) as the markers more strongly related to severity of renal failure. In both models, PRC did not contribute significantly to improve the final R-square.

There was a significant association between changes in markers of SI during hospitalization and the course of ACLF (Table 4). Improvement of ACLF was associated with decrease, whereas worsening of ACLF was associated with increase, in IL-6, IL-8, and HNA2. Inflammatory markers did not change in the steady form of ACLF or in patients with no ACLF throughout the study. In contrast, no relationship was observed between changes in PRC and the clinical course of ACLF.

SI AND MORTALITY

The strength of association between SI, SCD, and short-term mortality were also assessed considering IL-8, IL-6, HNA2, and PRC values. Baseline values of IL-8, IL-6 (not shown), and HNA2, but not of PRC, were strongly associated with 28-day mortality (Fig. 3) and 90-day mortality (Supporting Fig. S6).

TABLE 4. Relationship Between ACLF Course, Plasma Concentrations of Renin (PRC), and Inflammation Markers

Parameter	n	Enrollment	Last Assessment	*P* Value*
PRC (microIU/mL)				
Improvement†	71	113.5 (27.5-294.3)	82.9 (23.3-330.4)	0.9187
No change†	26	93.1 (57.6-362.5)	262.3 (87.0-1,212)	0.8450
Worsening†	40	168.6 (81.7-1,508.0)	259.7 (44.3-3,023.0)	0.7415
Non-ACLF patients‡	96	111.5 (29.0-284.1)	110.0 (59.5-251.4)	0.9188
IL-6 (pg/mL)				
Improvement	71	32.2 (13.1-104.4)	25.5 (12.2-46.7)	0.0002
No change	26	66.8 (25.2-125.5)	50.1 (19.8-117.7)	0.8589
Worsening	41	34.3 (17.7-111.2)	75.9 (20.6-134.3)	0.0744
Non-ACLF patients	87	25.0 (13.0-42.0)	16.5 (9.6-33.5)	0.0012
IL-8 (pg/mL)				
Improvement	72	89.5 (36.7-174.3)	56.7 (35.6-119.1)	0.0003
No change	26	118.8 (65.4-209.6)	186.8 (72.7-268.0)	0.8450
Worsening	41	82.7 (48.9-136.7)	116.0 (52.9-198.6)	0.1196
Non-ACLF patients	98	40.8 (22.7-75.4)	38.7 (22.4-68.7)	0.6137
HNA2 (%)				
Improvement	72	9.6 (5.3-14.1)	8.9 (5.0-13.4)	0.2215
No change	25	15.1 (11.0-20.3)	15.8 (11.2-20.5)	0.6383
Worsening	41	10.7 (8.1-13.3)	13.2 (9.6-18.4)	0.0020
Non-ACLF patients	98	6.3 (3.3-9.4)	6.1 (2.1-11.7)	0.3099

Data are median (IQR).
*Wilcoxon signed rank-sum test.
†"Improvement"/"worsening": reduction/increase of ≥1 grade from enrollment in ACLF classification. "No change": same classification as at enrollment in ACLF patients.
‡Non-ACLF patients: patients without ACLF throughout the whole study.

Similarly, there was a significant association between the course of SI during hospitalization and the 28-day and 90-day mortality (Table 5). One hundred three patients had CRP data available at enrollment and at the last follow-up assessment. Changes in CRP from enrollment in those patients who survived at 28 (median, −4.0; IQR −13.1−1.6) and 90 days (median, −2.4; IQR, −11.1−1.9) were not significantly different from those corresponding to patients who died (median, −1.3; IQR, -19.6-3.7; and median, −7.1; IQR, −20.7−0; *P* values, 0.4918 and 0.2044, respectively). Twenty-eight-day and 90-day mortality rates were also significantly associated ($P < 0.0001$) with SIRS at enrollment (23 of 78 patients with SIRS [29.5%] and 47 of 433 without SIRS [10.6%] died within 28 days after enrollment; mortality rates were 42.9% and 21.6%, respectively, at 90 days).

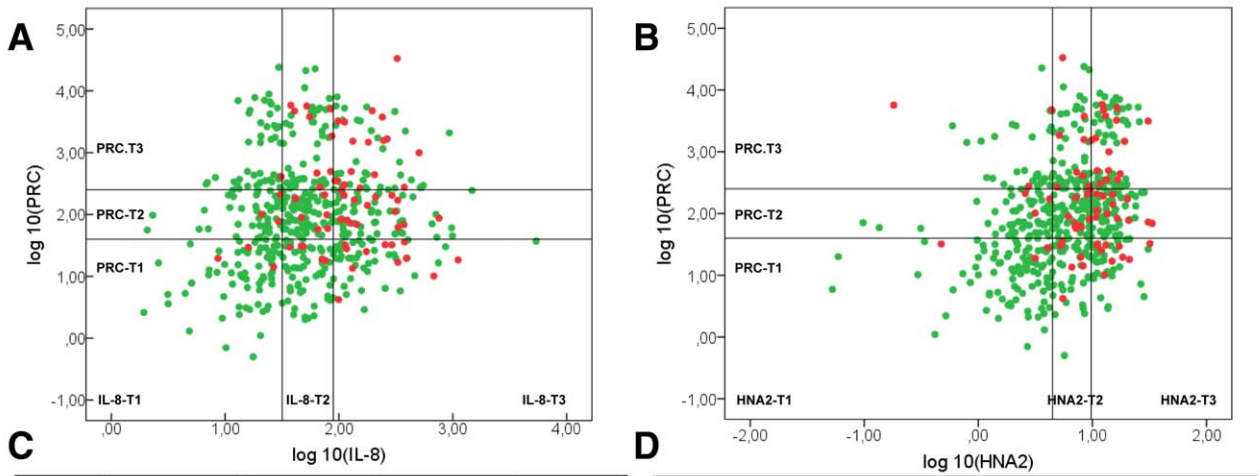
Eighteen patients showed extremely high levels of IL-8 (>800 UI/mL) and/or IL-6 (>1,000 UI/mL) at enrolment (Fig. 2). The prevalence of severe forms of ACLF (grades 2 or 3) and the 28-day mortality rate in this group of patients (39% and 28%, respectively) was higher ($P = 0.0833$ and $P = 0.0774$) than in the rest of the patients (21% and 13%, respectively).

Discussion

The discussion of the article is divided in four parts: (1) role of SI in the pathogenesis and clinical course of AD and ACLF; (2) cytokine response; (3) mechanisms of organ failure in ACLF; and (4) relationship between SI and mortality.

The first part of the analysis (Tables 1–3) strongly suggests that SI is a major mechanism of AD and ACLF and supports most of the proposals of the SI hypothesis: (1) AD of cirrhosis occurs in the setting of severe SI and oxidative stress; and (2) SI is significantly more severe in patients with ACLF than in those without ACLF. Interestingly, the reported levels of IL-6, IL-8, and TNFα in patients with severe sepsis, a condition also associated with organ failure, are similar to those found in our patients with ACLF[23-25]; (3) severity of SI correlates closely with severity of ACLF; and, finally, (4) the course of SI during hospitalization is strongly associated with the course of AD and ACLF (development of ACLF during hospitalization in patients without ACLF at enrollment or improvement, steady coursel or worsening of ACLF in patients with ACLF at enrollment).

The characterization of cytokine response in our patients (second part of the analysis; Tables 1–3 and



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	IL8-T1	IL8-T2	IL8-T3	Overall PRC
PRC-T3	3/67 (5%)	5/53 (9%)	12/50 (24%)	20/170 (12%)
PRC-T2	2/47 (4%)	5/61 (8%)	12/60 (20%)	19/168 (11%)
PRC-T1	2/53 (4%)	10/56 (18%)	20/64 (31%)	32/173 (19%)
Overall IL-8	7/167 (4%)	20/170 (12%)*	44/174 (25%)#	N=511

*p<0.05 vs previous tertile; #p<0.01 vs previous tertile

D

	HNA2-T1	HNA2-T2	HNA2-T3	Overall PRC
PRC-T3	3/84 (54%)	6/46 (13%)	10/36 (28%)	19/166 (11%)
PRC-T2	2/51 (4%)	7/59 (12%)	10/58 (17%)	19/168 (11%)
PRC-T1	3/33 (9%)	10/60 (17%)	18/76 (24%)	31/169 (18%)
Overall HNA2	8/168 (5%)	23/165 (14%)#	38/170 (22%)*	N=503

*p<0.05 vs previous tertile; #p<0.01 vs previous tertile

FIG. 3. (A,B) Scatterplots of patients who died (red dots) or survived (green dots) at 28 days after enrollment according to individual values of PRC and IL-8 (left) or human HNA2 (right). Two cut-off points for each marker divide each cohort into three tertiles (T1, lower values; T2, intermediate values; T3, higher values). Patients who died are predominantly in the right part of the panels (higher degree of SI). (C,D) Mortality rates observed within each tertile of both PRC and IL-8 and PRC and HNA2. Differences in mortality rates within tertiles (T2 vs. T1 and T3 vs. T2) were statistically significant only with IL-8 and HNA2.

Supporting Table S7; Supporting Fig. S1) disclosed interesting features. Whereas all the 17 cytokines and chemokines included in the main analysis were significantly increased in patients with AD with respect to healthy controls, only those involved in innate immune response were clearly up-regulated in patients with ACLF with respect to those without ACLF. Indeed, we found that patients with ACLF had a predominance of proinflammatory cytokines (i.e., TNFα and IL-6) and chemokines (IL-8, MCP-1, IP-10, and MIP-1β). These patients also had increased levels of G-CSF and GM-CSF, key regulatory cytokines that target committed progenitors to promote differentiation and activation of monocytes and neutrophils.[26] In contrast, among other cytokines that are involved in the activation and shaping of the adaptive immune system (IFNγ, IL-17a, and IL-7), IFNγ was only slightly higher in patients with ACLF than in those without. These findings suggest that whereas activation of both the innate and adaptive immune systems participates in the SI associated with AD, a dysregulated innate immune response might be the predominant mechanism in progression of AD to ACLF.

The assessment of the overall cytokine profile showed that not only proinflammatory molecules, but also major anti-inflammatory cytokines such as IL-10 and IL-1ra were increased in patients with AD with and without ACLF, indicating a generalized activation of the inflammatory cytokines. There was, however, a clear unbalance in favor of proinflammatory cytokines, as indicated by the significantly higher IL-6/IL-10 and IL-8/IL-10 ratios observed in patients with and without ACLF as compared to healthy subjects.

Another outstanding feature was the observation of clear differences in cytokine profile of patients with ACLF according to the type of PE. Patients with active alcoholism as PE exhibited very high IL-8 levels, as described.[27,28] Such increase in IL-8 was observed both in patients with and without AAH, although the grade of increase was greater in the former group than

TABLE 5. Changes from Enrollment (Median and IQR) in Plasma Concentrations of Renin (PRC) and Inflammation Markers According to Mortality at 28 and 90 Days After Enrollment

		28-Day Survivors				28-Day Deaths		
Marker	N	Enrollment	Last Assessment	Change	N	Enrollment	Last Assessment	Change
PRC (microIU/mL)	138	116.7 (30.1-332.5)	119.8 (27.8-407)	-6.8 (-113.6-62.2)	37	99.0 (32.5-353.1)	201.9 (57.7-1,156)	25.3 (-51.8-317.2)
IL-6 (pg/mL)	137	32.2 (15.0-106.3)	26.3 (13.5-58.8)*	-5.3 (-36.9-10.5)	38	51.5 (21.2-173.1)	63.3 (31.4-168.9)	19.3 (-43.4-68.6)§
IL-8 (pg/mL)	139	71.7 (35.6-153.7)	65.9 (36.0-125.0)*	-3.8 (-41.7-16.3)	38	107.8 (74.8-246.4)	147.3 (79.2-260.7)	6.7 (-42.1-62.2)
HNA2 (%)	139	9.5 (5.6-14.9)	10.7 (6.5-15.3)	-0.1 (-3.4-3.3)	36	12.9 (8.5-16.5)	14.1 (9.5-18.8)*	2.4 (-1.4-6.4)‡
		90-Day Survivors				90-Day Deaths		
Marker	N	Enrollment	Last Assessment	Change	N	Enrollment	Last Assessment	Change
PRC (microIU/mL)	105	108.7 (26.4-282.0)	89.6 (23.2-378.2)	-0.2 (-87.9-56.5)	66	171.3 (40.9-1,576)	246.8 (64.1-1,212)	19.7 (-171.2-204.8)
IL-6 (pg/mL)	104	40.3 (15.3-120.1)	23.6 (11.7-49.8)†	-8.4 (-57.4-3.0)	67	31.9 (17.3-117.8)	48.9 (25.5-129.2)*	14.7 (-12.2-60.9)§
IL-8 (pg/mL)	106	83.2 (35.6-157.6)	56.4 (35.3-105.4)*	-7.2 (-50.7-9.2)	67	84.7 (50.5-204.6)	124.2 (64.8-227.1)	9.6 (-717.1-77.0)§
HNA2 (%)	106	9.4 (5.6-13.6)	9.3 (5.3-15.0)	0.03 (-3.3-3.1)	65	12.5 (7.4-16.4)	13.4 (9.4-17.4)*	1.4 (-2.5-6.4)

Data are median (IQR).
*$P < 0.05$ versus enrollment.
†$P < 0.01$ versus enrollment.
‡$P < 0.05$ versus change from baseline in survivors.
§$P < 0.001$ versus change from baseline in survivors.

in the second. Patients with bacterial infection had a characteristic increase of a set of inflammatory cytokines. Finally, patients with other or without PE had no differential increase in any cytokine with respect to patients with active alcoholism or bacterial infection as PE. These data suggest specific mechanisms for SI, depending on the underlying trigger of ACLF. They also suggest that ACLF in patients with unidentifiable PE is unlikely related to unrecognized bacterial infection. Finally, they support a cause-to-effect relationship between PE, SI, and ACLF development. This suggestion, however, does not exclude other mechanisms. In fact, dysfunctional or damaged organs by itself may stimulate cytokine and reactive oxygen species production[29] and therefore contribute to development and/or progression of ACLF.

It is important to note that increased plasma levels of G-CSF, GM-CSF, TNFα, IL-6, and IFNγ have been shown to participate in the process called "emergency hematopoiesis," which develops in the context of SI.[30,31] Therefore, the activation of these cytokines found in patients with ACLF probably contributes to the leukocytosis associated with this syndrome and explains the close relationship between WBC, ACLF severity, and patient prognosis.[1,3]

The mechanism by which SI induces ACLF was explored in the third part of the analysis (Figs. 1 and 2 and Supporting Figs. S2, S3, S4, and S5, and logistic regression and step-wise linear regression models). Renal failure is a well-recognized complication in patients with cirrhosis and bacterial infections,[32] and evidences have been presented suggesting that it may be related to a sequence of events initiated by an exaggerated systemic inflammatory response that induces aggravation of splanchnic arterial vasodilation and impairment in cardiac output, homeostatic activation of endogenous neurohormonal systems to maintain arterial pressure, renal vasoconstriction and vasoconstriction in other organs (i.e., liver and brain), renal hypoperfusion, and renal failure.[20,22] Our results showing that ACLF develops in the setting of an accentuation in the SI and SCD already present in patients with AD support that this sequence of events is also important in the pathogenesis of the syndrome.

However, several other findings obtained in the four analyses assessing the strength of association of SI and SCD with ACLF strongly suggest that the mechanism of ACLF is far more complex. (1) Whereas SI at enrollment (estimated by IL-6, IL-8, and HNA2) was strongly related with frequency and severity of ACLF,

this was not true for SCD (estimated by PRC); (2) the clinical course of ACLF was associated with parallel changes in plasma levels of SI markers, but not with PRC; (3) PRC was normal or slightly increased in many patients with ACLF or with ACLF-RF. In contrast, IL-6 and IL-8 were markedly increased in almost all these patients. (4) The logistic regression model assessing those markers (IL-8, IL-6, HNA2, and/or PRC) independently associated with presence of ACLF or ACLF-RF and the linear regression model assessing the correlation of IL-8, IL-6, HNA2, and PRC with severity of ACLF and of ACLF-RF showed significant relationships with IL-6, IL-8, and HNA2, but not with PRC. These results strongly suggest that nonhemodynamic mechanisms mostly mediate the deleterious effects of SI in organ function and participate in the pathogenesis of ACLF.

Three lines of evidence support this contention.[33-38] The first includes investigations showing that the extension of SI into the organs (i.e., as a consequence of extremely high circulating plasma levels of inflammatory mediators and perhaps also of proinflammatory molecules [PAMPS and DAMPS]) may lead to organ failure through direct deleterious effects on microcirculatory homeostasis, mitochondrial function, and cell survival. The second includes investigations in patients and experimental animals with severe sepsis, a condition with many similarities with ACLF, showing that renal failure may occur in the setting of hyperdynamic circulation and normal renal perfusion. Finally, the third derives from a recent study on transjugular kidney biopsies in 65 patients with cirrhosis awaiting LT. Among the numerous glomerular, vascular, and acute or chronic tubulointerstitial lesions found in these patients, only cortical and medullary infiltration by mononuclear cells and polymorphonuclear leukocytes associated with tubular cell injury was independently associated with the presence of renal failure.[39] Therefore, as in severe sepsis, single- or multi-organ failure in cirrhosis is probably the result of a complex process involving numerous mechanisms, including impairment in systemic circulatory and local microcirculatory functions, microcirculatory thrombosis, mitochondrial and cell dysfunction, and cell death.

A final important observation was that IL-8, IL-6, and HNA2 at enrollment and their follow-up changes during hospitalization were directly associated with 28-day and 90-day mortality (fourth part of the analysis; Fig. 3 and Supporting Fig. S6; Table 5). Patients who died showed significantly higher baseline plasma levels of these mediators than those who survived. Moreover, plasma concentrations of IL-6, IL-8, and HNA further increased during hospitalization in the former and decreased in the latter group of patients. Sequential measurements of inflammatory mediators could therefore be a sensitive tool to assess treatment effectiveness and predict prognosis in patients with ACLF.

In summary, the current study supports the SI hypothesis of AD and ACLF in cirrhosis. (1) AD occurs in the setting of very high plasma levels of cytokines and oxidized albumin; (2) ACLF develops when there is a further increase in these inflammatory mediators; and (3) although ACLF frequently occurs in the setting of severe SCD, inflammatory mediators are more strongly associated with the frequency, severity, and clinical course of ACLF than PRC, indicating that both hemodynamic and nonhemodynamic mechanisms are important in the pathogenesis of ACLF. Different profiles of cytokine response were observed. Whereas SI in AD was associated with very high levels of cytokines involved in both the innate and adaptive immune systems, cytokines associated with ACLF were mainly cytokines involved in the innate immune response. Different profiles of cytokine response were also identified according to PEs of ACLF. Severity of SI at enrollment and progression or regression of SI during hospitalization were closely associated with short-term prognosis. Sequential measurement of inflammatory mediators may therefore be useful as predictors of treatment effectiveness and patient prognosis.

Appendix

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