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Characterization of inflammatory response in hepatorenal syndrome: Relationship with kidney outcome and survival

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Abstract

Background: Several lines of evidence indicate that decompensated cirrhosis is characterized by the presence of systemic inflammation. Hepatorenal syndrome (HRS-AKI) is a unique type of renal failure that occurs at late stages of cirrhosis. However, confirmation of the presence and significance of such inflammatory response in HRS-AKI is lacking.

Aim and Methods: To characterize the systemic inflammatory response, as estimated by measuring a large number of cytokines, in 161 patients hospitalized for an acute decompensation of cirrhosis: 44 patients without acute kidney injury (AKI), 63 patients with hypovolaemia-induced AKI and 58 patients with HRS-AKI.

Results: HRS-AKI was characterized by an altered cytokine profile compared to the other two groups, particularly IL-6, IL-8, TNF-α, VCAM-1, fractalkine and MIP-1α. The inflammatory response was not related to presence of bacterial infection, concomitant acute-on-chronic liver failure or severity of renal dysfunction. Patients who responded to terlipressin and albumin had only a decrease in TNF-α and RANTES after treatment without changes in other cytokines. Interestingly, patients with persistent HRS-AKI had higher levels of IP-10 and VCAM-1 compared to those with

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Abbreviations: CCL11, Eotaxin; CX3CL1, fractalkine; G-CSF, granulocyte colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon gamma; IL-10, interleukin-10; IL-1RA, interleukin-1 receptor antagonist; IL-1β, interleukin-1 beta; IL-6, interleukin-6; IL-8, interleukin-8; IP-10, interferon-inducible protein-10; MCP-1, monocyte chemoattractant protein-1; MELD, model of end-stage liver disease; MIP-1α, macrophage inflammatory protein 1-alpha; MIP-1β, macrophage inflammatory protein 1-beta; RANTES, regulated on activation normal T cell expressed and secreted; TNF-α, tumour necrosis factor-alpha; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor.

resolution of HRS-AKI. VCAM-1 was also an independent predictor of 3-month mortality. A systems biology analysis approach showed that the inflammatory status of HRS-AKI was similar to that of chronic nonhepatic inflammatory conditions, such as lupus erythematosus or inflammatory bowel disease.

Conclusion: Hepatorenal syndrome is characterized by a marked systemic inflammatory state, reminiscent of that of nonhepatic inflammatory diseases, that correlates with patient outcomes.

KEYWORDS

AKI, cirrhosis, hepatorenal syndrome, inflammation

1 | INTRODUCTION

Hepatorenal syndrome (HRS) is a unique type of acute kidney injury (AKI), currently known as (HRS-AKI), which develops in patients with decompensated cirrhosis and is considered a very severe complication of the disease at the end of the spectrum of complications of cirrhosis.[1,2]

A large body of evidence demonstrating that circulatory dysfunction plays a key role in the pathophysiology of HRS-AKI.[2-4] It has been shown that HRS-AKI occurs as a consequence of an intense renal vasoconstriction with marked reduction in renal blood flow and glomerular filtration rate (GFR), which is pathogenically related to a striking splanchnic arterial vasodilatation with activation of major vasoconstrictor systems, as a consequence of portal hypertension.[4,5] Administration of vasoconstrictors, particularly terlipressin, in association with albumin, improves circulatory function and is able to return renal function to baseline values in many patients, which confirms the major role of circulatory dysfunction with arterial vasodilation in the pathogenesis of kidney impairment in HRS-AKI.[6,7]

In recent years, it has become increasingly evident that cirrhosis is a condition with marked systemic inflammatory state, which appears to increase with disease progression, from compensated to decompensated cirrhosis, and is related to patient outcome.[8,9] Some clinical studies have assessed inflammation by measuring leucocyte count or C-reactive protein (CRP) levels,[10] while others have assessed the prevalence of systemic inflammatory response syndrome (SIRS)[11]; however, these methods are not very accurate in the evaluation of inflammation. Besides, studies in experimental animals have also demonstrated the existence of systemic inflammation that is more conspicuous in animals with ascites compared to those without.[12] Finally, few studies have evaluated a large number of inflammatory cytokines and confirmed the presence of systemic inflammation, as estimated by increased plasma levels of relevant cytokines involved in inflammation, which increase with disease progression.[8,13,14] The hypothesis, therefore, has been raised that cirrhosis is a disease characterized by marked and progressive systemic inflammation that may play a role in the development of complications of the disease.[15] However, so far there is lack of information regarding the presence, extent and significance of inflammation in patients with HRS-AKI. Given that HRS-AKI is considered the hallmark of the disease, this information may be relevant not only with respect to pathogenesis but also for identification of potential targets of therapy to prevent disease progression.

On this background, the aim of our study was to assess the existence of systemic inflammation, as estimated by plasma levels of a large number of inflammatory cytokines, in patients with HRS-AKI and investigate the relationship between inflammation and kidney and patient outcomes. Because systemic inflammation is common in ACLF and patients with HRS-AKI frequently meet diagnostic criteria of ACLF, the role of this latter condition as potential cause of inflammation in HRS-AKI was also assessed.

LAY SUMMARY

- Hepatorenal syndrome, a type of renal dysfunction that occurs at the end stage of cirrhosis, is characterized by a marked systemic inflammatory state.
- Lack of resolution of renal impairment and short-term mortality are associated with increased levels of some inflammatory markers; IL-6, IL-8, TNF-α, ICAM-1 and particularly, VCAM-1.
- A systems biology analysis approach showed that the inflammatory status of hepatorenal syndrome was similar to that found in chronic nonhepatic inflammatory diseases, such as lupus erythematosus or inflammatory bowel disease.

2 | PATIENTS AND METHODS

2.1 | Patient population and study design

One-hundred and sixty-five episodes of AKI occurring in 161 patients with decompensated cirrhosis admitted to the Liver Unit of Hospital Clinic were investigated in the current study. These patients were selected from a prospective database with Biobank collection

that includes consecutive patients with cirrhosis admitted to hospital for treatment of an acute decompensation of the disease. Three groups of subjects were identified randomly from the database: (1) patients with decompensated cirrhosis without AKI (*N* = 44), (2) patients with hypovolaemia-induced AKI (*N* = 63) and (3) patients with HRS-AKI (*N* = 58). Forty-one of the 58 patients (71%) with HRS-AKI met the classical criteria of type-1 HRS.[16] Exclusion criteria were previous kidney/liver transplantation, chronic haemodialysis before admission, hepatocellular carcinoma outside the Milan criteria or any other advanced malignancy, and lack of inform consent.

Demographical, clinical and analytical data were collected prospectively at admission and at regular intervals during hospitalization, and patients were followed up for at least 3 months after discharge. Blood and urine samples were collected at the time of inclusion in this study. Moreover, in 27 patients with type 1-HRS, samples were also collected after treatment with terlipressin and albumin (median time between the two sample collections was of 7 days). Blood was centrifuged at 2000 *g*, at 4°C, for 10 min, and plasma was stored at −80°C until analysis. All samples were stored at the Biobank as required by Spanish legislation. This study was approved by the Institutional Review Board of our centre, the research Ethic Committee of the Hospital Clínic of Barcelona (HCB/2017/0285), and all patients signed a written informed consent for participation in this study.

2.2 | Multiplex cytokine assay

The following 18 cytokines and vascular adhesion molecules were determined in plasma with the Luminex® Immunoassay Kit (Panomics®, Affymetrix Inc, Santa Clara, CA, USA): eotaxin, G-CSF, fractalkine, IFN-γ, IL-10, IL-1RA, IL-1β, IL-6, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, TNF-α, VEGF, ICAM-1, VCAM-1 and RANTES (see Supplementary materials). Urine MCP-1 was also analysed (R&D® Systems, Minneapolis, MN, USA). Cytokines IL-10, IL-1RA and IL-1β were excluded from statistical analyses because their levels were out of the detection limit in more than 30% of the samples.

2.3 | Definitions

AKI and ACLF were defined according to ICA[1] and CANONIC[17] definitions respectively (see Supplementary materials).

2.4 | Statistical analysis

Comparisons of normally distributed continuous variables were made with Student's *t* test or ANOVA. Comparisons of non-normally distributed continuous variables were made with Mann-Whitney U or Kruskal-Wallis tests. Results for continuous variables are expressed as median and interquartile range (IQR). Categorical variables were expressed as number and percentage and compared with the chi-square test or Fisher exact test. Survival curves were calculated with Kaplan and Meier method and compared with log-rank test. Due to the lack of prior knowledge on the relevance of cut-off levels for quantitative variables, we decided to use the unsupervised criterion of the median value. Multivariate Cox regression was performed to identify the independent factors associated with mortality. There was no specific calculation of the sample size. However, based on previous experience studies in patients with cirrhosis,[14,18] it was considered that more than 100 patients had to be included in this study to achieve a significant number of outcomes in terms of lack of AKI resolution and 3-month mortality. All statistical analyses were performed using SPSS statistical package, version 23.0. The significance level for all tests was set at 0.05 two-tailed.

2.5 | Systems biology analysis

We used a systems biology approach based on artificial neural networks supervised algorithm (ANN algorithm) that measures the strength of relationships between groups of human proteins.[19] Measures are based on the topology of the network, and the training set is derived mostly from drug effects. The conditions used were those defined in BED (Biological Effectors Database, Anaxomics Biotech, Barcelona, Spain) a hand-curated collection of scientific knowledge relating biological processes to their molecular effectors. The ANN algorithm provides a predictive score (from 0% to 100%) that quantifies the amount and strength of relationships between the evaluated proteins. Each score is associated with a *P* value that describes the probability of the results being a true positive result[19,20] (see Supplementary materials).

3 | RESULTS

3.1 | Baseline characteristics of patients

The baseline characteristics of patients with decompensated cirrhosis included in this study categorized into 3 groups (no AKI, hypovolaemia-induced AKI and HRS-AKI) are shown in Table 1. Of interest, bacterial infections were more common in patients with HRS-AKI compared to the other two groups, a finding consistent with the known role of bacterial infections as triggering factors of HRS-AKI. As expected, the frequency and severity of ACLF were higher in patients with HRS-AKI compared to that of patients with hypovolaemia-induced AKI.

3.2 | Systemic inflammatory response and cytokine levels

The prevalence of SIRS, leucocyte count and serum CRP levels were higher in patients with HRS-AKI compared to the other two groups (Table 2). With respect to cytokine levels, patients with HRS-AKI had a cytokine profile different from that in the other two groups, with statistically significant differences in the levels of several cytokines, including higher urinary levels of MCP-1 and plasma IL-6, TNF-α, VCAM-1, and IL-8, and lower levels of MIP1-α and fractalkine.

To further explore the cytokine profile of HRS-AKI, we assessed whether the increased cytokine levels found in these patients could be related to concomitant bacterial infections that were more

TABLE 1 Baseline demographical, clinical and laboratory characteristics of patients included in this study

	No AKI (*N* = 44)	Hypovolaemia-induced AKI (*N* = 63)	HRS-AKI (*N* = 58)	*P* value
Age, years	62 (56-70)	62 (54-67)	59 (53-66)	0.556
Diabetes mellitus	14 (32)	21 (33)	16 (28)	0.783
Male gender	30 (68)	43 (68)	45 (78)	0.445
Aetiology of cirrhosis: Alcohol/ hepatitis C	24 (55)/10 (23)	26 (41)/15 (24)	28 (48)/12 (21)	0.558
Ascites	23 (52)	44 (67)	58 (100)	<0.001
Hepatic encephalopathy	3 (7)	12 (19)	28 (48)	<0.001
Bacterial infection	22 (50)	20 (32)	42 (72)	<0.001
Serum creatinine (mg/dL)	0.8 (0.6-1.0)	1.6 (1.3-1.9)	2.6 (1.9-3.1)	<0.001
Serum bilirubin (mg/dL)	2.5 (1.4-3.9)	2.6 (1.6-5)	4.2 (2.4-11.2)	0.004
INR	1.5 (1.3-1.6)	1.5 (1.3-1.9)	1.9 (1.5-2.2)	<0.001
Serum sodium (mEq/L)	137 (134-140)	135 (132-139)	131 (127-135)	<0.001
Platelet count ($\times 10^9$/L)	85 (56-140)	77 (49-110)	76 (50-103)	0.441
Mean arterial pressure (mm Hg)	82 (76-92)	77 (69-87)	74 (68-84)	0.010
MELD score	14 (12-18)	19 (15-24)	28 (23-34)	<0.001
Child-Pugh score	8 (6-9)	8 (7-10)	11 (9-12)	<0.001
ACLF				
Frequency	0	27 (43)	49 (84)	<0.001
Grade 1 vs 2/3	–	21/6	26/23	<0.001

INR, International normalized ratio; MELD, model for end-stage liver disease.
Values are number and percentages (in brackets) or median and interquartile range (in brackets).

common in this group of subjects. With this objective, we compared cytokine levels in patients with HRS-AKI categorized according to presence or absence of infection (42 and 16 patients, respectively) (Supplementary Table 1). Out of the 5 cytokines that were differentially increased in patients with HRS-AKI, only plasma IL-6 was significantly higher in patients with HRS-AKI associated with infections compared to those without infections (59 (21-180) vs 23 (12-55) pg/mL, respectively, $P = 0.02$). However, it is possible that a further aggravation of the increased systemic inflammation during HRS-AKI associated with bacterial infections could not be detected due to the low number of patients with HRS-AKI without infections.

Next, we analysed whether severity of kidney impairment in HRS-AKI correlated with the intensity of systemic inflammatory response. As shown in Supplementary Table 2, categorization of patients with HRS-AKI in those who met the diagnostic criteria of type-1 HRS, the most severe form of HRS-AKI, and those who did not meet these criteria, showed no significant differences with respect to frequency of SIRS, leucocyte count and CRP, as well as cytokine levels. In the HRS-AKI group, serum creatinine levels did not correlated with inflammatory biomarkers, except for urinary levels of MCP-1 ($r = 0.420$, $P = 0.002$).

We also investigated whether reversal of kidney impairment in patients with HRS-AKI was associated with changes in cytokine profile. To this purpose, we compared cytokine levels before and after kidney function improvement in 24 patients with type-1 HRS treated with vasoconstrictors and albumin. As expected, serum creatinine decreased significantly after treatment (from 2.9 to 1.3 mg/dL; $P < 0.001$). Out of all cytokines evaluated, only plasma TNF-α and RANTES decreased significantly and MIP-1α increased with reversal of kidney dysfunction (Table 3).

Since many patients with HRS-AKI meet the criteria of ACLF, we investigated whether the increased levels of inflammatory cytokines found in HRS-AKI were potentially related to the presence of concomitant ACLF. To this aim, we categorized patients with HRS-AKI according to the presence or absence of ACLF. As shown in Table 4, patients with HRS-AKI with associated ACLF had levels of cytokines that were similar to those of patients with HRS-AKI without ACLF. Moreover, the levels of cytokines did not correlate with ACLF severity, except for higher levels of IL-8 and ICAM-1 in patients with ACLF grades 2-3 vs those of patients with grade 1 (Table 5). To further explore the relationship between AKI and ACLF, we compared inflammatory cytokine levels in patients with ACLF categorized according to AKI type, either HRS-AKI or hypovolaemia-induced AKI. Patients with ACLF associated with HRS-AKI had higher levels of IL-6, TNF-α and urinary MCP-1 and lower levels of fractalkine and MIP1-α compared to the hypovolaemia-induce AKI counterparts (data not shown).

3.3 | Relationship of systemic inflammatory response and cytokine levels with kidney outcome and patient survival

Out of the 121 patients with AKI, resolution of kidney function impairment was observed in 86 (71%), whereas the remaining 35 (29%)

TABLE 2 Comparison of systemic inflammatory markers and plasma and urine cytokine levels between the 3 groups of patients

	No AKI (*N* = 44)	Hypovolaemia-induced AKI (*N* = 63)	HRS-AKI (*N* = 58)	*P* value
C-reactive protein (mg/dL)	1.6 (0.5-3.9)	1.0 (0.4-2.2)	3 (1.7-6.1)	**<0.001**
Leucocyte count (×10^9/L)	5 (4-7)	5 (4-8)	7 (4-11)	**0.046**
SIRS	7 (16)	10 (16)	17 (33)	**0.053**
Plasma cytokines				
IL-6 (pg/mL)	14 (3-46)	14 (3-46)	45 (19-104)	**<0.001**
TNF-α (pg/mL)	22 (17-39)	30 (19-50)	47 (35-61)	**<0.001**
Fractalkine (pg/m)	111 (19-559)	363 (88-21924)	111 (29-377)	**0.004**
MIP1-α (pg/mL)	2048 (12-3970)	41 (13-3970)	14 (6-52)	**0.005**
VCAM-1 (ng/mL)	1816(1249-2662)	2184(1659-2866)	2410(1850-3731)	**0.006**
IL-8 (pg/mL)	25 (12-75)	45 (18-90)	56 (37-92)	**0.009**
VEGF (pg/mL)	453 (75-19,715)	179 (104-1681)	166 (71-539)	0.088
G-CSF (pg/mL)	30 (3-82)	14 (3-41)	30 (6-58)	0.132
MCP-1 (pg/mL)	269 (175-376)	284 (214-444)	340 (212-472)	0.370
RANTES (pg/mL)	7458 (1937-23,962)	4515 (2040-17,985)	4399 (1293-20,774)	0.449
INF-γ (pg/mL)	29 (5-88)	37 (14-129)	35 (13-122)	0.454
IP-10 (pg/mL)	961 (592-1671)	1000 (594-2067)	1206 (690-2014)	0.556
ICAM-1 (ng/mL)	269 (215-500)	279 (215-377)	306 (234-443)	0.596
Eotaxin (pg/mL)	115 (78-157)	101 (68-161)	109 (84-143)	0.772
MIP1-β (pg/mL)	40 (15-91)	30 (21-72)	36 (23-80)	0.779
Urinary cytokines				
uMCP-1 (pg/mL)	408 (207-1257)	443 (193-1340)	1292 (689-3356)	**<0.001**

SIRS; Systemic inflammatory response.
Values are number and percentages (in brackets) or median and interquartile range (in brackets).

patients had persistent AKI. Patients with persistent AKI had significantly higher baseline leucocyte count and CRP levels and plasma IL-6 and VCAM-1 compared to those of patients with AKI resolution. In the group of patients with HRS-AKI, those with persistent AKI (27 patients, 47%) showed significantly higher leucocyte count and plasma levels of IP-10 and VCAM-1 compared to those who had resolution of HRS-AKI (31 patients, 53%), yet differences of these latter two cytokines were barely significant (Table 6).

At the end of the 3-month follow-up period, 110 patients (69%) were alive, 34 (21%) had died, and 15 (9%) had been transplanted and 2 were lost to follow-up. Interestingly, a number of inflammatory parameters and cytokines correlated with 3-month mortality: leucocyte count, CRP levels and plasma IL-6, IL-8, TNF-α, ICAM-1 and VCAM-1. In multivariate analysis, including those inflammatory variables that were associated with mortality in the univariate analysis, only plasma VCAM-1 was an independent predictive factor of 3-month mortality. Plasma VCAM-1 was also the only inflammatory marker independently predictive of 3-month mortality in the group of patients with HRS-AKI (3156 (2029-4349) vs 1944 (1535-2829) ng/mL, for patients who died and survived at 3 months, respectively; $P < 0.01$), even when adjusted for the presence and severity of ACLF. Survival probability in patients with HRS-AKI according to VCAM-1 levels is shown in Figure 1.

3.4 | Comparison of cytokine profile in HRS-AKI with other chronic conditions using with systems biology approach

We used a systems biology approach based on artificial neural networks that measures the strength of relationships between groups of human proteins in order to compare the extent of systemic inflammation in HRS-AKI with that of other conditions characterized by well-defined and marked systemic inflammatory status. Interestingly, the cytokine pattern of HRS-AKI was related to that of a number of inflammatory conditions, including cystic fibrosis, rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease and ulcerative colitis (predictive value > 70) (Figure 2).

4 | DISCUSSION

The results of the current study show that patients with HRS-AKI have marked systemic inflammation with altered cytokine profile compared to that of patients with decompensated cirrhosis without AKI and, most interestingly, to patients with AKI due to hypovolaemia. The systemic inflammatory response in HRS-AKI does not seem to be related to presence of bacterial infections, concomitant ACLF or intensity of kidney dysfunction and is not

TABLE 3 Systemic inflammatory markers and plasma and urine cytokine levels in patients with type 1 HRS-AKI before and after effective treatment with terlipressin and albumin

	Baseline (*N* = 24)	Terlipressin and Albumin (*N* = 24)	*P* value
Serum creatinine (mg/dL)	2.9 (2.6-3.6)	1.3 (1.2-1.5)	**<0.001**
C-reactive protein (mg/dL)	4.9 (2.5-6.7)	1.3 (1.2-1.5)	**0.008**
Leucocyte count (×10^9/L)	7 (2.9-11)	5 (3-9)	0.246
Plasma cytokines			
Eotaxin (pg/mL)	104 (78-130)	115 (85-144)	0.909
G-CSF (pg/mL)	31 (9-68)	18 (3-41)	0.259
Fractalkine (pg/mL)	140 (13-679)	79 (13-835)	0.475
INF-γ (pg/mL)	73 (25-165)	39 (18-111)	0.230
IL-6 (pg/mL)	73 (16-95)	32 (7-58)	0.128
IL-8 (pg/mL)	62 (38-78)	54 (38-94)	0.648
IP-10 (pg/mL)	1457 (563-2067)	884 (519-1258)	0.116
MCP-1 (pg/mL)	337 (189-401)	283 (222-384)	0.753
MIP1-α (pg/mL)	10 (6-47)	46 (9-3970)	**0.030**
MIP1-β (pg/mL)	50 (18-93)	31 (14-52)	0.073
TNF-α (pg/mL)	48 (33-67)	30 (22-52)	**0.007**
VEGF (pg/mL)	181 (87-485)	163 (58-961)	0.223
ICAM-1 (ng/mL)	296 (227-403)	326 (219-370)	0.775
VCAM-1 (ng/mL)	2054 (1748-3009)	2162 (1604-2598)	0.278
RANTES (pg/mL)	3897 (1321-13453)	2158 (974-5004)	**0.013**
Urinary cytokines			
uMCP-1 (pg/mL)	3293 (1113-5204)	2193 (1218-4657)	0.193

Values are number and percentages (in brackets) or median and interquartile range (in brackets).

TABLE 4 Comparison of systemic inflammatory markers and plasma and urine cytokine levels in patients with HRS-AKI categorized according to the presence of ACLF

	HRS-AKI without ACLF (*N* = 9)	HRS-AKI with ACLF (*N* = 49)	*P* value
C-reactive protein (mg/dL)	3 (2-4)	3 (2-6)	0.552
Leucocyte count (×10^9/L)	7 (5-9)	7 (4-11)	0.991
SIRS	3 (33)	14 (33)	1.000
Plasma cytokines			
Eotaxin (pg/mL)	114 (85-269)	108 (82-134)	0.439
G-CSF (pg/mL)	30 (4-102)	30 (8-56)	0.880
Fractalkine (pg/L)	133 (44-662)	106 (24-307)	0.805
INF-γ (pg/mL)	97 (22-302)	31 (12-94)	0.179
IL-6 (pg/mL)	21 (19-76)	47 (18-137)	0.629
IL-8 (pg/mL)	46 (35-83)	60 (37-99)	0.599
IP-10 (pg/mL)	1096 (816-2933)	1232 (671-1957)	0.660
MCP-1 (pg/mL)	265 (148-656)	344 (216-463)	0.755
MIP1-α (pg/mL)	41 (12-2011)	13 (5-52)	0.218
MIP1-β (pg/mL)	30 (20-87)	39 (24-75)	0.540
TNF-α (pg/mL)	51 (38-53)	46 (33-62)	0.755
VEGF (pg/mL)	146 (79-10,184)	168 (64-394)	0.547
ICAM-1 (ng/mL)	285 (217-449)	314 (239-439)	0.739
VCAM-1 (ng/mL)	2242 (1559-3132)	2459 (1825-3895)	0.512
RANTES(pg/mL)	5706 (2949-69,961)	3369 (1241-17,941)	0.084
Urinary cytokines			
uMCP-1 (pg/mL)	736 (647-1364)	1382 (810-3543)	0.213

SIRS; systemic inflammatory response.
Values are number and percentages (in brackets) or median and interquartile range (in brackets).

normalized by improvement of kidney function with pharmacological therapy. Interestingly, the intensity of the inflammatory response is correlated with kidney and patient outcomes in such a way that increased levels of some inflammatory markers, particularly VCAM-1, are associated with lack of resolution of AKI and mortality.

In the current study, a large number of consecutive patients with cirrhosis and HRS-AKI were investigated for the presence of systemic inflammatory response as assessed by a large number of inflammatory and anti-inflammatory cytokines using multiplex technology. A control group of patients with decompensated cirrhosis without AKI was included for comparison. A group of patients with decompensated cirrhosis with AKI due to hypovolaemia was also studied. This type of AKI was selected as comparator for HRS-AKI because in both conditions AKI is of prerenal origin, yet the underlying pathogenic cause is very different. While a contracted blood volume is the cause of renal hypoperfusion in the former, the impairment of kidney function in the latter is related to opposite circulatory features, namely markedly dilated vascular bed, particularly in the splanchnic circulation.[2-4] The results of the current study clearly show that as decompensated cirrhosis progresses towards HRS-AKI, there is progressive increase in inflammatory status with significantly increased levels of some powerful inflammatory cytokines. Previous studies have shown that plasma levels of inflammatory cytokines are significantly increased in decompensated compared to compensated cirrhosis, suggesting

TABLE 5 Comparison of systemic inflammatory markers and plasma and urine cytokine levels in patients with HRS-AKI associated with ACLF classified according to ACLF severity

	ACLF 1 (*N* = 26)	ACLF 2 and 3 (*N* = 23)	*P* value
C-reactive protein (mg/dL)	3 (1-6)	3 (2-6)	0.855
Leucocyte count ($\times 10^9$/L)	6 (3-11)	7 (4-12)	0.400
SIRS	7 (32)	7 (33)	0.993
Plasma cytokines			
Eotaxin (pg/mL)	101 (79-127)	118 (100-161)	0.123
G-CSF (pg/mL)	39 (12-75)	21 (6-43)	0.212
Fractalkine (pg/L)	90 (3-428)	155 (29-280)	0.595
INF-γ (pg/mL)	35 (14-94)	19 (11-124)	0.582
IL-6 (pg/mL)	45 (12-80)	56 (21-255)	0.207
IL-8 (pg/mL)	44 (27-69)	91 (43-202)	**0.006**
IP-10 (pg/mL)	898 (634-1941)	1267 (778-2162)	0.417
MCP-1 (pg/mL)	337 (198-397)	411 (234-712)	0.089
MIP1-α (pg/mL)	10 (6-29)	25 (3-3970)	0.701
MIP1-β (pg/mL)	37 (23-65)	42 (28-84)	0.548
TNF-α (pg/mL)	46 (31-61)	49 (34-63)	0.749
VEGF (pg/mL)	169 (69-323)	144 (44-1675)	0.936
ICAM-1 (ng/mL)	273 (221-344)	411 (251-536)	**0.019**
VCAM-1 (ng/mL)	2050 (1750-3279)	3040 (2120-4044)	0.200
RANTES (pg/mL)	3835 (1256-26,358)	3369 (890-16,162)	0.417
Urinary cytokines			
uMCP-1 (pg/mL)	1624 (824-4849)	1250 (752-2879)	0.274

SIRS; systemic inflammatory response.
Values are number and percentages (in brackets) or median and interquartile range (in brackets).

TABLE 6 Comparison of systemic inflammatory markers and plasma and urine cytokine levels in patients with HRS-AKI calculated according to outcome of AKI

	AKI resolution (*N* = 31)	AKI persistent (*N* = 27)	*P* value
	3.9 (2.6-5.8)	2.2 (1.7-6.4)	0.358
Leucocyte count ($\times 10^9$/L)	5.6 (3.0-9.2)	7.8 (6.4-11.6)	0.016
SIRS	11 (41)	6 (24)	0.199
Plasma cytokines			
Eotaxin (pg/mL)	103 (79-138)	110 (96-156)	0.761
G-CSF (pg/mL)	27 (4-57)	39 (6-60)	0.742
Fractalkine (pg/mL)	117 (28-4022)	97 (29-223)	0.382
INF-γ (pg/mL)	38 (13-122)	32 (11-124)	0.483
IL-6 (pg/mL)	41 (17-96)	57 (21-145)	0.459
IL-8 (pg/mL)	60 (41-80)	53 (36-152)	0.714
IP-10 (pg/mL)	915 (538-1875)	1267 (807-2564)	**0.045**
MCP-1 (pg/mL)	330 (160-402)	396 (215-550)	0.293
MIP1-α (pg/mL)	14 (8-49)	18 (5-3970)	0.784
MIP1-β (pg/mL)	33 (20-71)	43 (25-84)	0.370
TNF-α (pg/mL)	47 (32-62)	46 (35-59)	0.726
VEGF (pg/mL)	172 (67-1311)	157 (75-291)	0.606
ICAM-1 (ng/mL)	278 (228-367)	345 (236-508)	0.129
VCAM-1 (ng/mL)	2132 (1747-2871)	3139 (2005-4266)	**0.044**
RANTES (pg/mL)	4668 (2071-16,146)	3369 (1101-26,588)	0.533
Urinary cytokines			
uMCP-1 (pg/mL)	1148 (716-5069)	1164 (661-2007)	0.123

SIRS; systemic inflammatory response.
Values are number and percentages (in brackets) or median and interquartile range (in brackets).

the existence of an inflammatory driving force that occurs with the progression of the disease.[8,9] Whether this inflammatory driving force is cause or consequence of progression of liver disease is not known. Our data confirm that this inflammatory status across decompensated cirrhosis increases even more as the disease progresses towards HRS-AKI which is considered one of the latest stages of cirrhosis, given its high mortality rate. Our data therefore are in agreement with the recently proposed theory of systemic inflammation driving the complications of cirrhosis.[15] Further support to this theory comes from our systems biology analyses showing that systemic inflammation in cirrhosis is similar to that found in some key chronic inflammatory conditions, such as inflammatory bowel diseases, rheumatoid arthritis or systemic lupus erythematosus.

One relevant issue of the current study is whether the increased inflammatory state observed in patients with HRS-AKI was due to 'hepatorenal syndrome' itself or the presence of concomitant ACLF, since it has been shown that the latter condition is characterized by a marked systemic inflammation.[13,14] Two lines of evidence suggest

that the increased inflammatory state is not related to ACLF. Firstly, patients with HRS-AKI but without ALCF had plasma cytokine levels that were not significantly different from those found in patients with HRS-AKI with ACLF. Moreover, cytokine levels were largely unrelated to ACLF grade. On the other hand, cytokine profile of patients with HRS-AKI was noticeably different from that of patients with ACLF associated with hypovolaemia-induced AKI, suggesting that cytokine profile was mostly related to 'hepatorenal syndrome' and not to ACLF. Nevertheless, these findings should be taken with caution because of the relatively low number of patients included which prevented performing a propensity score matching analysis. Further studies are needed to try to dissect out whether systemic inflammation is due to hepatorenal syndrome 'per se' or to ACLF, or both.

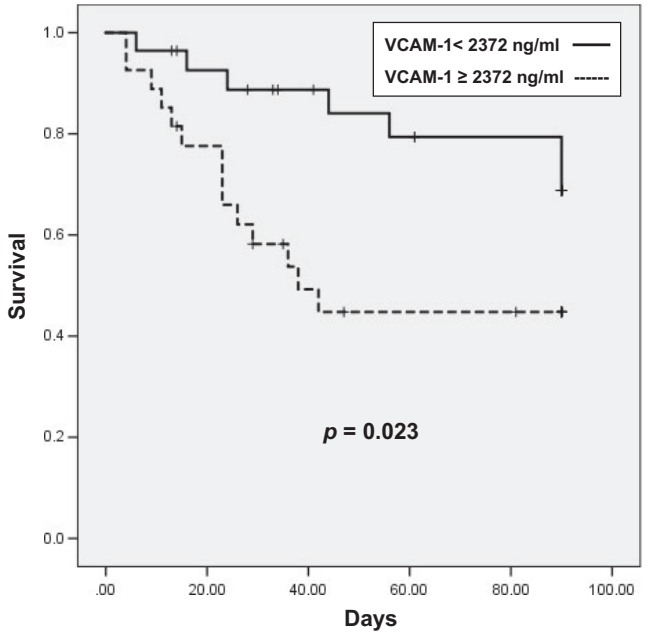

FIGURE 1 Probability of 3-month survival in patients with HRS-AKI categorized according to median levels of VCAM-1

A final issue that deserves discussion is that patients in whom HRS-AKI persisted showed higher levels of some inflammatory markers, the most important of which appears to be VCAM-1. Moreover, VCAM-1 was also an independent predictive factor of survival in the whole series of patients. VCAM-1 is an inflammatory mediator that plays a central role in triggering the process of systemic inflammation in response to several stimuli by helping recruit inflammatory cells outside of the systemic circulation.[21] Few previous studies have shown the potential relevance of VCAM-1 as prognostic indicator of patients with cirrhosis.[22] Our results extend these observations by showing that among a large number of inflammatory markers, VCAM-1 plasma levels are associated with lack of resolution of HRS-AKI and poor survival. These results together with findings of a similar inflammatory profile compared to that of some inflammatory diseases shed light on the potential role of VCAM-1 and TNF-α as therapeutic targets in patients with advanced cirrhosis.[23]

The current study has some limitations that should be acknowledged. Firstly, some cytokines, such as IL-10 and IL-1RA, were outside of the range detection limit established for the multiplex assay and could not be evaluated. The multiplex methodology allows the measurement of cytokines in little volume and in the same sample well. To achieve this multiplexing, the quantification of analytes is performed with serial dilutions of a common calibrator. Therefore, the individual adjustment of the detection limit for each analyte is not possible with the consequent lost of quantification in the case of some cytokines included in the multiplexing. This problem could be solved by performing high sensitivity immunoanalytical methods for single analytes, but due to limitations in the available sample volume,

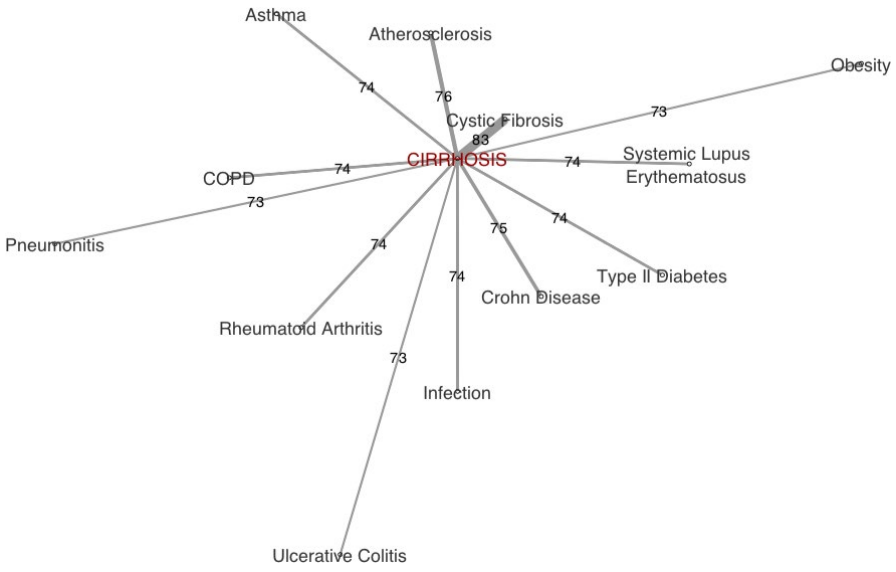

FIGURE 2 Relationship between systemic inflammatory pattern in patients included in this study and different pathological conditions based on a network analysis. Link width and distance to the central point is proportional to the predictive score of the network analysis that quantifies the network relationships between the evaluated proteins. Only medically relevant conditions with a predictive score corresponding to $P \leq 0.1$ are displayed. Conditions as defined in BED (Biological Effectors Database, Anaxomics Biotech)

this alternative strategy was not possible. Secondly, given the relative small sizes of our study populations and the exploratory nature of this study, we decided not to implement multiplicity adjustment strategies. This study will require future replication and the findings in general and the *P* values in particular should be interpreted with caution. Thirdly, although the number of patients studied may appear relatively low, it is a quite large sample of patients considering the difficulty to perform clinical studies in decompensated cirrhosis and the sample size of previous pathogenic and therapeutic studies in patients with HRS-AKI. Finally, levels of cytokines in plasma may not reflect the actual concentrations of the same cytokines in tissues. However, this is a limitation that can barely be addressed in human studies.

In conclusion, patients with HRS-AKI have marked increase in systemic inflammatory profile compared to that of patients without AKI and hypovolaemia-induced AKI, which appears to be independent of associated ACLF, and similar to inflammation observed in key systemic inflammatory diseases, such as lupus erythematosus or inflammatory bowel disease. Interestingly, in patients with HRS-AKI, lack of AKI resolution and survival is linked to some specific cytokines, particularly, VCAM-1.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

The authors have all contributed to this manuscript and approve the version of this submission. CS contributed to the conception and design of this study, acquisition of data, the analysis and interpretation of the data and drafting the manuscript; PH, MC, RM, UC, JMM, IG, EP, LN, GDP, AJ, NF, MMR, JF, WJ, participated in the analyses of the results, interpretation of data, and/or critical revision of the manuscript. FT contributed to statistical analysis and interpretation of data. PG and ES participated in this study concept, interpretation of the data, drafting the manuscript, critical revision of the manuscript for important intellectual content, obtained funding and study supervision.

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