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

Cristina Solé1,2,3 | Elsa Solà1,2,3 | Patricia Huelin1,2,3 | Marta Carol2,5 | Rebeca Moreira1,2,3 | Unai Cereijo6 | José-Manuel Mas6 | Isabel Graupera1,2,3 | Elsa Pose1,2,3 | Laura Napoleone1,2,3 | Gloria dePrada1,2,3 | Adrià Juanola1,2,3 | Núria Fabrellas2,5 | Ferran Torres7 | Manuel Morales-Ruiz2,3,4 | Judith Farrés6 | Wladimiro Jiménez2,3,4 | Pere Ginès1,2,3

1 Liver Unit, Hospital Clinic de Barcelona, University of Barcelona, Barcelona, Spain
2 Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
3 Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD)
4 Biochemistry and Molecular Genetics Department, Hospital Clinic de Barcelona, Barcelona, Spain
5 Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain
6 Anaxomics Biotech, Barcelona, Spain
7 Medical Statistics Core Facility, IDIBAPS, Hospital Clinic Barcelona & Biostatistics Unit, Faculty of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

Correspondence
Dr Pere Ginès, Liver Unit, Hospital Clinic de Barcelona, Barcelona, Spain.
Email: pgines@clinic.cat

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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...
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.
that includes consecutive patients with cirrhosis admitted to hospi-
tal 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) pa-
tients 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.\textsuperscript{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 informed consent.

Demographical, clinical and analytical data were collected pro-
spectively 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 cen-
trifuged 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 Clinic 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, GCSF, 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\textsuperscript{2} and CANONIC\textsuperscript{17} defi-
nitions 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 ex-
pressed as median and interquartile range (IQR). Categorical vari-
ables were expressed as number and percentage and compared with
the chi-square test or Fisher exact test. Survival curves were calcu-
lated 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 per-
formed to identify the independent factors associated with mortal-
ity. There was no specific calculation of the sample size. However,
based on previous experience studies in patients with cirrhosis,\textsuperscript{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.\textsuperscript{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 re-
sult\textsuperscript{19,20} (see Supplementary materials).

3 | RESULTS

3.1 | Baseline characteristics of patients

The baseline characteristics of patients with decompensated cirrho-
sis included in this study categorized into 3 groups (no AKI, hypovol-
aemia-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 pa-
tients with HRS-AKI compared to that of patients with hypovolea-
mia-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
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 correlate 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-\(\alpha\) and RANTES decreased significantly and MIP-1\(\alpha\) 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 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-\(\alpha\) and urinary MCP-1 and lower levels of fractalkine and MIP1-\(\alpha\) 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%)
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 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 (>10⁹/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/mL) | 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).
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 former, the impairment of kidney function in the latter is related to opposite circulatory features, namely markedly dilated vascular

### 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 (<10⁹/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 (<10⁹/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).
the existence of an inflammatory driving force that occurs with the progression of the disease. 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. 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. Two lines of evidence suggest

| 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 (×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 |
| C-reactive protein (mg/dL) | 3 (2.6-5.8) | 2.2 (1.7-6.4) | 0.358 |
| Leucocyte count (×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).
that the increased inflammatory state is not related to ACLF. Firstly, patients with HRS-AKI but without ACLF 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.

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.\textsuperscript{21} Few previous studies have shown the potential relevance of VCAM-1 as prognostic indicator of patients with cirrhosis.\textsuperscript{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-\(\alpha\) as therapeutic targets in patients with advanced cirrhosis.\textsuperscript{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,
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.

REFERENCES

1. Angeli P, Ginés P, Wong F, et al. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. J Hepatol. 2015;62(4):968-974. https://doi.org/10.1016/j.jhep.2014.12.029.
2. Ginés P, Schrier RW. Renal failure in cirrhosis. N Engl J Med. 2009;361(13):1279-1290. https://doi.org/10.1056/NEJMra0809139.
3. Angeli P, Bernardi M, Villanueva C, et al. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. J Hepatol. 2018;69(2):406-460. https://doi.org/10.1016/j.jhep.2018.03.024.
4. Ginés P, Solà E, Angeli P, Wong F, Nadim MK, Kamath PS. Hepatorenal syndrome. Nat Rev Dis Prim. 2018;4(1):23. https://doi.org/10.1038/s41572-018-0022-7.
5. Martin PY, Ginés P, Schrier RW. Nitric oxide as a mediator of hemodynamic abnormalities and sodium and water retention in cirrhosis. N Engl J Med. 1998;339(8):533-541. https://doi.org/10.1056/NEJM199808203390807.
6. Martín-Llahí M, Pépin M-N, Guevara M, et al. Terlipressin and albumin vs albumin in patients with cirrhosis and hepatorenal syndrome: a randomized study. Gastroenterology. 2008;134(5):1352-1359. https://doi.org/10.1053/j.gastro.2008.02.024.
7. Sanyal AJ, Boyer T, Garcia-Tsao G, et al. A randomized, prospective, double-blind, placebo-controlled trial of terlipressin for type 1 hepatorenal syndrome. Gastroenterology. 2008;134(5):1360-1368. https://doi.org/10.1053/j.gastro.2008.02.014.
8. Giron-Gonzalez JA, Martinez-Sierra C, Rodriguez-Ramos C, et al. Implication of inflammation-related cytokines in the natural history of liver cirrhosis. Liver Int. 2004;24(5):437-445. https://doi.org/10.1111/j.1478-3231.2004.0951.x.
9. Albillos A, Lario M, Álvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. J Hepatol. 2014;61(6):1385-1396. https://doi.org/10.1016/j.jhep.2014.08.010.
10. Cervoni J-P, Thévenot T, Weil D, et al. C-Reactive protein predicts short-term mortality in patients with cirrhosis. J Hepatol. 2012;56(6):1299-1304. https://doi.org/10.1016/j.jhep.2011.12.030.
11. Thabut D, Massard J, Gangloff A, et al. Model for end-stage liver disease score and systemic inflammatory response are major prognostic factors in patients with cirrhosis and acute functional renal failure. Hepatology. 2007;46(6):1872-1882. https://doi.org/10.1002/hep.21920.
12. Muñoz L, Albillos A, Nieto M, et al. Mesenteric Th1 polarization and monocyte TNF-α production: first steps to systemic inflammation in rats with cirrhosis. Hepatology. 2005;42(2):411-419. https://doi.org/10.1002/hep.20799.
13. Clària J, Stauber RE, Coenraad MJ, et al. Systemic inflammation in decompensated cirrhosis: characterization and role in acute-on-chronic liver failure. Hepatology. 2016;64(4):1249-1264. https://doi.org/10.1002/hep.28740.
14. Solé C, Solà E, Morales-Ruiz M, et al. Characterization of inflammatory response in acute-on-chronic liver failure and relationship with prognosis. Sci Rep. 2016;6(1):32341. https://doi.org/10.1038/srep32341.

ORCID

Cristina Solé https://orcid.org/0000-0002-6104-8345
Unai Ceretjo https://orcid.org/0000-0003-1557-8836
Pere Ginés https://orcid.org/0000-0003-4657-4504
15. Bernardi M, Moreau R, Angeli P, Schnabl B, Arroyo V. Mechanisms of decompensation and organ failure in cirrhosis: from peripheral arterial vasodilation to systemic inflammation hypothesis. *J Hepatol*. 2015;63(5):1272-1284. https://doi.org/10.1016/j.jhep.2015.07.004.

16. Arroyo V, Ginès P, Gerbes AL, et al. Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. *Hepatology*. 1996;23(1):164-176. https://doi.org/10.1002/hep.510230122.

17. Moreau R, Jalan R, Gines P, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology*. 2013;144(7):1426-1437. https://doi.org/10.1053/j.gastro.2013.02.042.

18. Fagundes C, Pépin M-N, Guevara M, et al. Urinary neutrophil gelatinase-associated lipocalin as biomarker in the differential diagnosis of impairment of kidney function in cirrhosis. *J Hepatol*. 2012;57(2):267-273. https://doi.org/10.1016/j.jhep.2012.03.015.

19. Guillén-Gómez E, Bardaji-de-Quixano B, Ferrer S, et al. Urinary proteome analysis identified nephrin and VCAM as proteins involved in diabetic nephropathy. *J Diabetes Res*. 2018;2018:1-12. https://doi.org/10.1155/2018/6165303.

20. Ibora-Egea O, Gálvez-Montón C, Roura S, et al. Mechanisms of action of sacubitril/valsartan on cardiac remodeling: a systems biology approach. *NPJ Syst Biol Appl*. 2017;3:12. https://doi.org/10.1038/s41540-017-0013-4.

21. Vestweber D. How leukocytes cross the vascular endothelium. *Nat Rev Immunol*. 2015;15(11):692-704. https://doi.org/10.1038/nri3908.

22. Lo Iacono O, Rincón D, Hernando A, et al. Serum levels of soluble vascular cell adhesion molecule are related to hyperdynamic circulation in patients with liver cirrhosis. *Liver Int*. 2008;28(8):1129-1135. https://doi.org/10.1111/j.1478-3231.2008.01763.x.

23. Vaglio A, Grayson PC, Fenaroli P, et al. Drug-induced lupus: traditional and new concepts. *Autoimmun Rev*. 2018;17(9):912-918. https://doi.org/10.1016/j.autrev.2018.03.016.

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