Systemic inflammation in acute cardiorenal syndrome: an observational pilot study

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Abstract

Aims Acute cardiorenal syndrome (CRS) with and without consideration of the volume state was assessed with regard to inflammatory parameters.

Methods and results Blood samples from patients with acute CRS (Ronco type 1 or 3, Group 1, n = 15), end-stage renal disease (Group 2, n = 12), hypertension (Group 3, n = 15), and, in a second cohort, with acute CRS and hypervolemia (Group 4, n = 9) and hypertension (Group 5, n = 10) were analysed with regard to lipopolysaccharide-binding protein (LBP), interleukins (ILs), and monocyte function (flow cytometry) both on admission (all groups) and on discharge (Groups 1 and 4). By discharge, one Group 1 patient died. LBP (ANOVA for Groups 1–3: P = 0.001) and IL-6 (Kruskal–Wallis for Groups 1–3: P < 0.0001) were higher in Group 1 (LBP: 11.7 ± 2.0 μg/mL; IL-6: 15.0 ± 6.1 pg/mL) and in Group 2 (LBP: 10.4 ± 1.4 μg/mL; IL-6: 14.6 ± 3.8 pg/mL) than in Group 3 (LBP: 5.8 ± 0.4 μg/mL; IL-6: 1.8 ± 0.4 pg/mL). In a direct comparison, the proportion of activated monocytes (CD14 and CD16 positive) was higher in Group 1 (6.9% ± 0.7%) vs. Group 3 (5.1% ± 0.6%; P = 0.018). Group 4 patients had higher IL-6 plasma levels (34.2 ± 10.1 pg/mL) than Group 1 patients (15.0 ± 6.1 pg/mL; P = 0.03). All other findings obtained in CRS groups (Groups 1 and 4) were comparable.

Conclusions In acute CRS, a state of systemic inflammation was found, which is comparable with the end-stage renal disease situation. In comparison with hypertensive controls, a monocytic activation was found in acute CRS regardless of volume state.

Keywords Heart failure; Cardiorenal syndrome; Monocyte function; Inflammation

Introduction

Acute cardiorenal syndrome (CRS) (Ronco type 1 or 3) requires a vigorous action to improve the outcome.¹⁻³ The classification of CRS proposed by Ronco et al.¹ has constraints regarding therapy.⁵ The multiple pathways leading to a combined heart and kidney failure include a bi-organ crosstalk in terms of neurohumoral stimulation and anaemia, which, in turn, may exacerbate the patient outcome.¹,⁵ Nevertheless, when defining the CRS as chronic heart failure (CHF) with varying degrees of accompanying renal dysfunction, the degree of renal dysfunction represents a better predictor of outcome than the degree of existing systolic cardiac dysfunction.⁵ Likewise, cardiac transplant recipients with a reduced estimated glomerular filtration rate (<40 mL/min) had a worse outcome after cardiac transplantation.⁷ Intriguingly, CHF therapies including sacubitril and aldosterone receptor antagonism rely on a preserved kidney function. Chronic kidney disease (CKD) is considered to be a risk factor for atherosclerosis.⁸ In CKD, activated CD16 positive monocytes were found to be more prevalent.⁹ Consequently, the differentiation of monocytes to macrophages may lead to a release of chemokines and growth factors favouring smooth-muscle cell sprouting, foam-cell formation, and atherosclerosis.¹⁰ In general, pro-inflammatory pathways relate to an adverse outcome in CRS.¹¹ The presence of peripheral oedema may include gut oedema facilitating enteric endotoxin translocation.¹² Endotoxins may lead to increased plasma levels of...
lipopolysaccharide-binding protein (LBP)\(^\text{13,14}\). In small studies on CRS, type 5\(^\text{1}\) due to sepsis\(^\text{15}\) and type 1\(^\text{1}\) due to acute cardiac failure\(^\text{16}\), patient plasma has been shown to transmit pro-apoptotic effects to renal tubular cells via a caspase-3 mechanism.

Nevertheless, unlike in conditions of acute heart failure\(^\text{12}\) or sepsis\(^\text{15}\), an endotoxin mechanism still has not been demonstrated in patients with acute CRS, type 1 or 3. Systemic inflammation in an acute setting of cardiac and/or renal failure with or without hypervolemia can be mediated, at least in part, by an endotoxin mechanism. The current study was set out to investigate, whether or not systemic inflammation including LBP is present in acute CRS, type 1 or 3. Hypothetically, systemic inflammation is more likely to occur in acute CRS (type 1 or 3) than in control patients.

In addition, the role of monocyte function in CRS still is unclear. CD16 expression occurring during monocyte activation correlates with inflammation\(^\text{18,19}\). Specifically, monocyte activation was shown to occur in atherosclerosis\(^\text{20}\), and is paralleled by an increased release of tumour necrosis factor (TNF)-\(\alpha\), interleukin (IL)-6 and IL-1\(\beta\).\(^\text{19,21}\) Hypothetically, hospitalized patients with acute CRS show more pronounced monocyte activation in comparison with hypertensive patients and/or end-stage renal disease (ESRD) patients. “In addition, patients with acute CRS, type 1 or 3, presenting with hypervolemia are expected to show the highest degree of monocyte activation in comparison to control groups. In analogy to acute heart failure\(^\text{12}\), an endotoxin translocation across an oedematous gut wall is postulated.” Likewise, systemic inflammation is expected to be highest in acute CRS, type 1 or 3, with peripheral oedema on admission when compared with acute CRS, type 1 or 3, without consideration of the volume state. Thus, the aim of this study is to establish a link between the volemic phenotype of acute CRS and the degree of prevalent systemic inflammation.

**Methods**

The study was designed as a cross-sectional study conducted at the University Hospital Halle, Germany, from 2012 to 2013. In addition, one follow-up visit at hospital discharge was scheduled for two of five subgroups (Group 1 and Group 4), thus covering the hospital stay as a prospective observational study where every patient served as its own control (baseline vs. follow-up exam). All study-related procedures were in accordance to Good Laboratory Practice and International Conference on Harmonization-Good Clinical Practice principles. The local ethics committee (institutional review board) approved this study consisting of two study cohorts (first cohort: Groups 1–3; second cohort: Groups 4–5). All patients provided a written informed consent prior to study participation.

**Patients**

**Study cohort 1 (2012)**

Consecutively, hospitalized patients with acute CRS (type 1 or 3) were considered as Group 1, with ESRD as Group 2 and with hypertensive emergency as Group 3.

**Study cohort 2 (2013)**

Consecutively, hospitalized patients with acute CRS (type 1 or 3) and signs of hypervolemia on admission were considered as Group 4, with hypertensive emergency as Group 5.

Group 6: Fully documented Group 4 patients were excluded from the analysis due to the absence of peripheral oedema on admission.

**Inclusion criteria**

Inclusion criteria are as follows:

1. Group 1: Acute CRS (type 1), that is, acute cardiac failure with reduced or preserved ejection fraction and an accompanying CKD, stage G3a, or higher according to Kidney Disease: Improving Global Outcomes\(^\text{22}\) or with acute CRS (type 3), that is, acute kidney injury (Acute Kidney Injury Network 1 or higher\(^\text{23}\)) and CHF with reduced or preserved ejection fraction on admission.
2. Group 2: ESRD with ongoing renal replacement therapy for more than 1 year (either chronic haemodialysis thrice a week or chronic ambulatory peritoneal dialysis) and hospitalization for an elective diagnostic procedure, elective minor surgery, or elective intervention. Blood samples were taken on admission before the scheduled diagnostic or interventional measure was performed. In haemodialysis patients, blood sampling was performed right before haemodialysis procedure was started (after venous puncture or flushing of dialysis catheter, prior to connection to dialysis tubes).
3. Groups 3 and 5: Known arterial hypertension with a hypertensive emergency leading to hospitalization.
4. Group 4: Acute CRS (type 1 or type 3) with accompanying peripheral oedema.

**Exclusion criteria**

Age less than 18 years or more than 99 years, a known malignoma, a sepsis or a relevant infection on admission, or a psychiatric or neurologic disorder with an inability to provide the informed consent were considered as exclusion criteria.

**Group-specific exclusion criteria**

Group-specific exclusion criteria are as follows:

1. Group 1 and Group 4: a chronic CRS condition (types 2, 4, and 5),
2. Group 4: absence of peripheral oedema,
3. Group 2: an acute CRS condition (type 1 or 3),

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(4) Group 3 and Group 5: known kidney disease; known heart disease other than left ventricular hypertrophy; diabetes.

**Echocardiography**

A transthoracic echocardiography exam was performed in each study participant. For Group 1 and Group 4 patients, echocardiography results were used to ascertain the diagnosis of CRS by proving either a condition of CHF with reduced or preserved ejection fraction. In ESRD patients, a structured echocardiographic exam was obtained adhering to relevant guidelines.

**Laboratory parameters**

Laboratory workup included the determination of plasma brain natriuretic peptide (BNP), creatinine, C-reactive protein, LBP, IL-6 (Central Laboratory unit of University Hospital Halle), IL-1β, and TNF-α (amedes, Halle/Leipzig GmbH, Halle, Germany). The determination of endotoxin plasma levels was carried out by the accredited laboratory Dr. Michael Lohmeyer GmbH, Mendelstr. 11, Münster, Germany, using a commercial Limulus Amebocyte Lysate Test (turbidimetric method, Pyrotell-T®, Associates of Cape Cod, Inc.; reagents by Haemochrom, Essen, Germany). Plasma samples (0.5 mL) were stored in 1.5 mL Eppendorf® Safe-Lock microcentrifuge tubes at −70°C until analysis was performed.

**Flow cytometric characterization of monocytes and neutrophilic granulocytes**

Venous blood samples were obtained in a fasted condition and processed in a research center within 24 h. Using MACS Quant (Miltenyi Biotec, Bergisch Gladbach, Nordrhein-Westfalia, Germany), flow cytometry was performed using the following monoclonal antibodies (clone): Anti-CD15eFluor450, (HI98), Beckman Coulter, Krefeld, Germany; Anti-CD14 Pe-Cy7 (5B1), BD Biosciences, Heidelberg, Germany; Anti-CD45 (J33), eBioscience, Frankfurt/Main, Germany; Anti-CD45 APC-Vio770 (61D3), eBioscience; Anti-CD16 APC (3G8), BD Biosciences; Anti-CD14 APC (L243), BD Biosciences. The ratio of inactivated monocytes (CD14-positive/CD16-negative) vs. activated (CD14-positive/CD16-positive) monocytes was determined allowing for relative quantification as percentage of whole white blood cell count. Three groups of monocytes were considered depending on the phenotype: Mo-1 (CD14++CD16−), Mo-2 (CD14++CD16+), and Mo-3 (CD14+CD16++). Functional monocyte activation and functional neutrophilic granulocyte activation were determined using a commercial assay (Phagoburst® by Glycotope, Berlin, Germany). Using flow cytometry, the production of reactive oxidants following incubation with opsonized *Escherichia coli* bacteria was quantified via conversion of the fluorogenic substrate dihydrorhodamine. In short, 100 μL heparinized whole blood was incubated with opsonized bacteria (*E. coli*, 20 μL, 1–2 × 10⁸ bacteria per millilitre) as a stimulant over 10 min at 37°C. In both assays, activated monocytes and activated neutrophilic granulocytes displaying an oxidative burst were given as percentage of all monocytes and neutrophilic granulocytes. In addition, the spontaneous activation was determined in a control assay without opsonized bacteria to account for background activation.

**Statistics**

Analysis was performed among groups and within groups (baseline vs. follow-up); results were given as medians ± standard error using Graphpad Prism, version 7.04 (San Diego, California, USA). Kolmogorov–Smirnov test was performed to test for normality. Parametric tests (Student’s unpaired or paired, two-tailed t-test or ANOVA with Newman–Keuls post hoc test) or non-parametric test (Mann–Whitney test or paired Wilcoxon signed-rank test or Kruskal–Wallis) were used, where appropriate.

**Results**

Fifteen patients (Group 1) with an acute CRS (type 1 or 3), 12 ESRD patients (Group 2: 11 patients on haemodialysis and 1 on peritoneal dialysis), and 15 hypertensive patients (Group 3) entered this study. Study flow is displayed in Figure 1. Baseline characteristics were provided in Tables 1 and 2. In addition, in a second time period, nine patients with acute CRS presenting with hypervolemia were recruited as Group 4. Ten otherwise healthy hypertensive patients with hypertensive emergency on admission served as controls (Group 5). Baseline characteristics were provided in Tables 3 and 4.

Thirteen (86.7%) Group 1 patients, 10 (83.3%) Group 2 patients, six (40%) Group 3 patients, all (100%) of Group 4 patients, and five (50%) Group 5 patients were male. Ten (66.7%) Group 1 patients, five (50%) Group 2 patients, and five (55.5%) Group 4 patients had diabetes mellitus. As an exclusion criterion, none of Group 3 and Group 5 patients had diabetes. The hospital stay was longer in surviving CRS patients of Group 1 and Group 4 than in control groups. CRS patients (Group 1) and ESRD patients (Group 2) were older than hypertensive controls (Group 3). Likewise, patients with acute CRS with hypervolemia (Group 4) were older than hypertensive controls (Group 5).

During the hospital stay, one Group 1 patient died and three patients of Group 1 withdrew their consent for study participation (Figure 1). One Group 4 patient who was transferred to the cardiac surgery department was lost for follow-up after discharge (Figure 2).
Elevated pro-inflammatory parameters in acute cardiorenal syndrome and in end-stage renal disease

Laboratory parameters related to inflammation including C-reactive protein, IL-6, procalcitonin, and LBP were elevated, while serum albumin and haemoglobin were decreased both in Group 1 (acute CRS) and in Group 2 (ESRD) when compared with hypertensive controls of Group 3 (Table 1). Lymphocyte count was less both in Group 1 (acute CRS) and in Group 2 (ESRD) than in hypertensive control patients of Group 3 (Table 2). Neutrophilic granulocyte count was higher in CRS patients of Group 1 compared with Groups 2 and 3 in post hoc tests. TNF-α (data not shown), basophilic granulocyte (data not shown), leucocyte, monocyte, and platelet count were not different among groups (Table 2). IL-1β and endotoxin plasma levels were below threshold in all study participants. After exclusion of patients on renal replacement therapy, pre-specified laboratory parameters did not change in patients with acute CRS (Group 1) on hospital admission vs. discharge (data not shown).

Taken together, on hospital admission, patients with acute CRS (Group 1) and with ESRD (Group 2) display a pro-inflammatory phenotype.

End-stage renal-disease patients show diastolic cardiac dysfunction

The transthoracic echocardiography revealed a comparable left ventricular ejection fraction (LVEF) among ESRD (Group 2) and hypertensive control patients (Group 3). However, BNP levels were as high in CRS patients (Group 1) as in ESRD patients (Group 2) indicative for CHF with preserved ejection fraction among ESRD patients (Group 2). Acute CRS patients (Group 1) had a significantly lower LVEF in comparison with either control group (Group 2 or 3). Specifically, eight (53%) Group 1 patients had heart failure with reduced ejection fraction, while two (17%) of Group 2 patients and none of Group 3 patients had a heart failure condition with reduced ejection fraction (P = 0.005). Interestingly, pre-specified parameters among CRS patients (Group 1) and ESRD patients (Group 2) showed a notable concordance, except for serum urea, creatinine, and eosinophilic granulocyte count being highest in Group 2, neutrophilic granulocyte count being highest in Group 1, and LVEF being lowest in Group 1 patients. Of note, serum phosphate was not different between patients with ESRD (Group 2) and acute CRS (Group 1). In short, based on BNP and LVEF data, CHF with preserved ejection fraction appears to be highly prevalent among ESRD patients (Group 2), thus presenting as a CRS, type 4.1

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**Figure 1** Study flow of study participants of Group 1 (cardiorenal syndrome patients, type 1 or 3), Group 2 (end-stage renal disease patients), and Group 3 (hypertensive patients). FACS, fluorescence-activated cell scanning.
In vivo activation of peripheral blood monocytes in patients with an acute cardiorenal syndrome in comparison with hypertensive control patients

As shown in Table 2, the percentage of MO-2-type activated monocytes was not different among groups \((P = 0.08)\). In a direct subgroup comparison Group 1 (acute CRS) and Group 3 (hypertensive controls), the proportion of activated monocytes belonging to the subgroup MO-2 was higher in patients with acute CRS (Group 1: 6.9\% ± 0.7\%; Group 3: 5.1\% ± 0.6\%; \(P = 0.018\)).

Using opsonized \(E.\) coli bacteria as a stimulus, monocyte stimulation was found to be reduced in CRS (Group 1) vs. hypertensive patients (Group 3). When directly comparing CRS (Group 1) and hypertensive patients (Group 3) using the two-tailed Mann–Whitney test, this difference was confirmed (Group 1: 45.3\% ± 8.0\%; Group 3: 94.2\% ± 5.1\%; \(P = 0.01\)) being indicative for a pre-test monocytic stimulation in vivo. After accounting for spontaneous activation in a negative control, the difference in monocyte activation prevailed (data not shown).

Taken together, monocytic activation was found in CRS (Group 1) vs. hypertensive patients (Group 3). The finding of less in vitro stimulation of monocytes in CRS patients (Group 1) is in line with a higher degree of in vivo activation.
Cardiorenal syndrome with hypervolemia on admission

The baseline characteristics of CRS patients of Group 1 (Tables 1 and 2) and Group 4 (Tables 3 and 4) were comparable, except for the IL-6 data. In a direct comparison, the IL-6 level was elevated in acute CRS with hypervolemia (Group 4: 34.2 ± 10.1 pg/mL) in comparison with CRS patients of Group 1 (15.0 ± 6.1 pg/mL; \( P = 0.03 \)). Pertinent inflammation parameters of all patient groups are displayed in Figure 3.

In comparison with hypertensive controls (Group 5), the proportion of activated monocytes belonging to the subgroup MO-2 was higher in CRS patients of Group 4 (Table 4). Similar to the results obtained from Group 1 patients, \( E. \) coli stimulation showed less monocyte activation in acute CRS patients with hypervolemia (Group 4) in comparison with hypertensive control patients (Group 5). After accounting for baseline stimulation, these results were confirmed (data not shown). All pre-specified laboratory parameters did not change by discharge in comparison with the admission exam in Group 4 patients, when excluding patients on renal replacement therapy (data not shown). Taken together, inflammatory parameters appear to be more increased in acute CRS with hypervolemia in comparison with hypertensive controls. Except for IL-6 data, these results replicate the ones obtained in acute CRS without consideration of volume state.

### Table 3: Patient characteristics, laboratory, and clinical parameters (median ± standard error) in patients with acute CRS (type 1 or 3') and hypervolemia on admission (Group 4) and in hypertensive patients (Group 5)

| Parameter (unit) | Group 4 \((n = 9)\) | Group 5 \((n = 10)\) | \( P \) |
|------------------|---------------------|---------------------|------|
| Age (years)      | 76.8 ± 3.1          | 55.7 ± 4.8          | 0.0033 |
| Hospital stay (days) | 20.0 ± 9.0      | 3.0 ± 0.5           | <0.0001 |
| LVEF (%)         | 43 ± 3.9            | 60 ± 2.4            | 0.0018 |
| eGFR             | 23.5 ± 3.8          | 97.0 ± 4.8          | <0.0001 |
| Creatinine (\(\mu\)mol/L) | 224.0 ± 23.4  | 68.5 ± 5.4          | <0.0001 |
| Urea (mmol/L)    | 25.1 ± 3.4          | 4.1 ± 0.4           | <0.0001 |
| Phosphate (mmol/L) | 1.3 ± 0.1        | 1.1 ± 0.1           | 0.0336 |
| BNP (ng/mL)      | 1383.0 ± 962.4      | 19.5 ± 10.5         | <0.0001 |
| C-reactive protein (mg/dL) | 38.9 ± 8.9  | 0.0 ± 0.5           | <0.0001 |
| LBP (\(\mu\)g/mL) | 12.0 ± 2.2         | 6.1 ± 0.4           | <0.001 |
| Procalcitonin (ng/mL) | 0.3 ± 0.1      | 0.0 ± 0.0           | <0.0001 |
| Interleukin-6 (pg/mL) | 34.2 ± 10.1  | 1.2 ± 0.4           | 0.0002 |
| Albumin (mg/dL)  | 29.0 ± 2.1          | 41.5 ± 1.1          | <0.0001 |
| Haemoglobin (mmol/L) | 6.2 ± 0.4        | 9.3 ± 0.4           | <0.0001 |

CRS, cardiorenal syndrome; eGFR, estimated glomerular filtration rate; LBP, lipopolysaccharide-binding protein; LVEF, left ventricular ejection fraction.

### Table 4: Laboratory parameters (median ± standard error) of leucocytes including results of functional monocyte activation assay using opsonized \( Esherichia \) coli bacteria in patients with acute CRS (type 1 or 3') and hypervolemia on admission (Group 4) and in hypertensive patients (Group 5)

| Parameter (unit) | Group 4 \((n = 9)\) | Group 5 \((n = 10)\) | \( P \) |
|------------------|---------------------|---------------------|------|
| Total leukocyte count (Gpt/L) | 7.2 ± 0.6        | 6.7 ± 0.5           | 0.49 |
| Neutrophilic granulocytes (%) | 74.0 ± 2.4       | 60.0 ± 2.4          | 0.0021 |
| Lymphocytes (%) | 11.0 ± 2.1          | 30.5 ± 2.6          | <0.0001 |
| Eosinophilic granulocytes (%) | 2.5 ± 0.7        | 2.5 ± 0.5           | 0.43 |
| Mo-1: CD14+/CD16– as % of all monocytes | 84.9 ± 2.5       | 87.2 ± 0.6          | 0.0379 |
| Mo-2: CD14++/CD16+ as % of all monocytes | 9.2 ± 1.8        | 5.0 ± 0.3           | 0.0017 |
| Mo-3: CD14+/CD16++ as % of all monocytes | 6.3 ± 1.2        | 8.0 ± 0.5           | 0.55 |
| % of monocyte activation (\(E. \) coli) | 99.9 ± 0.8       | 100 ± 0.01          | 0.0351 |

CRS, cardiorenal syndrome. Mo-1, Mo-2, and Mo-3 represent monocyte fractions depending on the CD16 expression: CD16-negative: inactivated monocytes; CD14+/CD16+ and CD14+/CD16++: activated monocytes.

Monocytic differentiation on admission in patients with acute cardiorenal syndrome (type 1 or 3) with hypervolemia and in control patients.
Acute cardiorenal syndrome without signs of hypervolemia on admission is not associated with systemic inflammation

For hypothesis generation, five dropout patients of Group 4 (exclusion criterion: no signs of hypervolemia) were referred to as Group 6.

In a direct comparison between Group 4 and Group 6 patients, age was not different. Four of five (80%) Group 6 patients were diabetics; length of hospital stay (15 ± 8.1 days) and LVEF (55 ± 6.2%) were not different in comparison with Group 4. Serum creatinine (Group 4: 224.0 ± 23.4 μmol/L; Group 6: 122 ± 60.2 μmol/L; P = 0.056) tended to be less in Group 6 patients, and serum BNP (Group 4: 1383.0 ± 962.4 ng/mL; Group 6: 209.0 ± 115.8 ng/mL; P = 0.001) was less in Group 6 vs. Group 4 patients on admission. Most strikingly, inflammation parameters on admission were less in Group 6 vs. Group 4 (C-reactive protein: Group 4: 38.9 ± 8.9 mg/dL; Group 6: 4.4 ± 1.8 mg/dL; P = 0.01; LBP: Group 4: 12.0 ± 2.2 μg/mL; Group 6: 8.0 ± 1.3 μg/mL; P = 0.048; procalcitonin: Group 4: 0.3 ± 0.1 ng/mL; Group 6: 0.1 ± 0.1 ng/mL; P = 0.03; IL-6: Group 4: 34.2 ± 10.1 pg/mL; Group 6: 6.8 ± 1.7 pg/mL; P = 0.007). These data suggest that systemic inflammation is increased in acute CRS with hypervolemia in comparison with acute CRS without hypervolemia on admission.

Role of haemodialysis for the recovery of patients with an acute cardiorenal syndrome

One (6.7%) Group 1 patient was on maintenance haemodialysis before hospitalization.

Five Group 1 patients (33.3%) including the one who died had to be started on haemodialysis via an implantable haemodialysis catheter. Three or 21.4% of all surviving Group 1 patients became chronic, de novo haemodialysis patients with a thrice-a-week schedule. In five (55.6%) Group 4 patients, a haemodialysis treatment via an implantable permanent haemodialysis catheter was initiated and maintained after discharge. In summary, an acute renal replacement therapy was necessary in one-third of acute CRS patients without consideration of volemic state on admission (Group 1) and in half of acute CRS patients with hypervolemia on admission (Group 4).
Discussion

As a main finding, parameters of systemic inflammation were increased in patients with acute CRS (Groups 1 and 4), regardless of consideration of volume state (Group 4) or not (Group 1). Likewise, in a direct comparison, more prevalent monocytic activation was found in patients with acute CRS both with (Group 4) and without consideration of volume state (Group 1) vs. hypertensive controls (Groups 3 and 5). In an animal model of acute kidney injury, a microbiota-depleted gut was associated with a reduced cellular inflammation within ischaemic kidneys and with a protection against renal ischaemia–reperfusion injury.\textsuperscript{26} Conversely, hypervolemia in CHF was found to be associated with increased lipopolysaccharide plasma levels and systemic inflammation.\textsuperscript{27} There, an increase in venous congestion led to increased IL-6 plasma levels, while TNF-\(\alpha\) levels were not affected. The findings of absent TNF-\(\alpha\) plasma increase and elevated IL-6 plasma levels were replicated in the current study in patients with acute CRS and hypervolemia (Group 4).

In fact, the IL-6 elevation was more pronounced in acute CRS with hypervolemia than in acute CRS without consideration of the volume state. In addition, the diagnosis of acute CRS with hypervolemia on admission was associated with a
50% likelihood to become a maintenance haemodialysis patient by discharge. When including dropout patients with acute CRS without hypervolemia on admission (Group 6), these patients showed less systemic inflammation in terms of LBP and CRS plasma levels in comparison with patients with acute CRS and hypervolemia (Group 4). Conversely, in a direct comparison of Group 6 vs. Group 1 (acute CRS without consideration of the volume state), there was no statistical difference for LBP and C-reactive protein. These data, derived from a small patient cohort, need to be reassessed in randomized clinical trials including patients with acute CRS and consideration of the volume state. Here, a modified classification of CRS involving the phenotype, that is, the volume state, is suggested (Figure 4). The CRS diagnosis derived from a classification based on acuity and phenotype may provide a rationale for interventions. The ultimate goal is to provide specific therapies such as the correction of hypervolemia in acute CRS. In addition, the descriptive data from the current pilot study may provide a rationale to further investigate additional therapeutic targets in acute CRS. If the hypothesis of microbiota-associated inflammation due to enteric-wall oedema holds true for the acute CRS condition, renal replacement therapy may improve both volume control and systemic inflammation in patients with acute CRS with hypervolemia. In direct comparisons between patients with an acute CRS, regardless of volume state on admission (Groups 1 and 4) and hypertensive controls (Groups 3 and 5), a higher proportion of activated monocytes (Mo-2 type) was found in CRS patients. In addition, the reduced ability of E. coli-induced monocyte activation among CRS patients (Groups 1 and 4) in comparison with hypertensive control patients (Groups 3 and 5) implies a higher degree of activated monocytes in vivo, as lipopolysaccharide is known to activate CD14 expression in monocytes.28 In fact, a previous study demonstrated that an enhanced expression of the membrane-bound adhesion molecule CD11b reduces the ability for in vitro stimulation of monocytes and vice versa.29 In our study, elevated endotoxin levels were not detected. However, an underestimation could be due to a lipopolysaccharide insertion into the cell membranes.30 Therefore, alternative diagnostic strategies for endotoxin detection and quantification in biological samples are needed.

From the literature, hypervolemic acute heart failure was shown to be associated with endotoxin-mediated inflammation.12 A selective decontamination of the digestive tract was shown to improve outcome among critically ill surgical and medical patients, in whom 9% survived a cardiopulmonary resuscitation prior to the intervention.31 Lastly, a probiotic intervention was shown to maintain gut homeostasis and to diminish endotoxin effects on rat-derived ileum-epithelial cells.32 Once the gut epithelial barrier leaks and endotoxemia occurs, the deleterious consequences may include a monocytic activation and direct renotubular, proapoptotic effects.15–17

Even if the detailed mechanism of monocyte activation found in this pilot study remains obscure, monocyte

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**Figure 4** Take-home figure: typical clinical findings and suggested interventions in acute cardiorenal syndrome (CRS) accompanied by hypervolemia or hypovolemia.

| Hypervolemia and/or hypertensive emergency in CRS | Hypovolemia and/or arterial hypotension in CRS |
|--------------------------------------------------|-----------------------------------------------|
| Increased left-ventricular preload, decreased cardiac performance | Decreased left-ventricular preload, decreased cardiac performance |
| Secondary pulmonary hypertension with (interstitial) pulmonary edema, acute kidney injury, chemoreflex-mediated sympathoactivation, if pCO2↑ | Baroreflex-mediated sympathoactivation as a response to hypotension, (interstitial) pulmonary edema, acute kidney injury. |
| **All CRS patients:** exclusion of relevant valvular heart disease, therapy of pulmonary edema, acute kidney injury, if applicable. | |
| **CRS with hypervolemia:** correction of intravascular fluid overload; antihypertensive treatment, if applicable. | **CRS with hypovolemia:** correction of hypovolemia; correction of arterial hypotension, if applicable. |
activation in acute, hypervolemia-associated CRS may be considered as a proatherosclerotic event. Therefore, the role of monocyte activation in CRS should be further investigated. In addition, the gender difference as well as the preponderance of type-2 diabetes among patients with acute CRS (Groups 1 and 4) necessitates further investigation. Hypothetically, the gut epithelial and/or endothelial barrier function may be impaired by repeat hyperglycaemia, thus promoting oedema formation in diabetics with acute CRS.

Taken together, the presented data point at a prevalent multifaceted systemic inflammation and monocyte activation in acute CRS. The clinical, observational data hint at early renal replacement therapy as a promising therapy option in hospitalized patients with acute CRS. More studies are needed to clarify the detailed mechanism of inflammation in acute CRS.

Limitations

As this study is considered as a pilot study, the patient numbers are small. In addition, the putative mechanism of lipopolysaccharide stimulation in hypervolemia-associated acute CRS was not shown due to sensitivity issues of the laboratory assay used.

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Conflict of interest

C.L., C.U., D.G., S.D., A.W., M.G., and R.P. declare that they have no conflict of interest.

Declaration of Helsinki

All authors complied with the Declaration of Helsinki. The local ethics committee has approved the research protocol, and informed consent was obtained from all subjects in this study.

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