Research Article

Plasma Lipopolysaccharide Concentrations in Cardiorenal Syndrome Type 1

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Keywords

Cardiorenal syndrome · Lipopolysaccharide · Heart failure · Acute kidney injury

Abstract

Background: Cardiorenal syndrome (CRS) type 1 is characterized by a rapid worsening of cardiac function that leads to acute kidney injury (AKI). This study evaluated the role of lipopolysaccharide (LPS) in the development of AKI in patients with acute heart failure (AHF) and its relationship with renal parameters, to enable a better comprehension of the pathophysiology of CRS type 1. **Methods:** We enrolled 32 AHF patients, 15 of whom were classified as having CRS type 1. Eight of these 15 exhibited AKI at the time of admission (caused by AHF) and the other 7 developed AKI during their stay in hospital (in the first 48 h). We evaluated the plasma LPS concentrations as well as conventional (serum creatinine [sCr] and urea) and unconventional (neutrophil gelatinase-associated lipocalin [NGAL] and cystatin C) renal markers. **Results:** LPS levels were significantly higher in the CRS type 1 patients. No significant difference in LPS level was found in patients who were admitted with AKI and those developed AKI in hospital, but there was a tendency towards a higher level of LPS in CRS type 1 patients admitted with AKI. The LPS concentrations at admission were similar in CRS type 1 survivors ($n = 12$) and nonsurvivors ($n = 3$) ($p = 0.22$). We observed a positive correlation between LPS level and

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NGAL, Scr at admission and peak Scr during hospitalization and urea at admission. **Conclusion:** CRS type 1 patients present with an increased level of LPS and there is a direct correlation between LPS and renal parameters. This pilot research is the first study to explore the premise of LPS as novel pathophysiological factor in CRS type 1.

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Introduction

Cardiorenal syndrome (CRS) type 1 is a multifactorial syndrome characterized by acute deterioration of cardiac function leading to acute kidney injury (AKI). The pathophysiology of CRS 1 type is complex and involves numerous factors; hemodynamic and nonhemodynamic contributors are interrelated and connected with each other [1]. There is a growing body of evidence suggesting that oxidative stress, and immune-mediated, inflammatory, and apoptotic mechanisms could play a role in the pathogenesis of CRS type 1 [2].

Novel indications suggest a role for lipopolysaccharide (LPS) in CRS pathogenesis. LPS is increased in both patients with congestive heart failure (CHF) and chronic kidney disease [3–5].

In these patients, volume overload and subsequent mesenteric venous congestion finally cause the translocation of Gram-negative bacteria throughout the intestinal villi, with the release of LPS in systemic circulation and the consequent activation of inflammatory pathways [6]. The aim of this *in vivo* study was to evaluate the role of LPS in the development of AKI in patients with acute heart failure (AHF) and CRS type 1. Specifically, we evaluated, for the first time, the plasma level of LPS and its relationship with renal parameters, to enable a better comprehension of the pathophysiology of CRS type 1.

Materials and Methods

We performed an observational pilot study at the Internal Medicine Department of San Bortolo Hospital, Vicenza, Italy. All patients admitted were screened for inclusion in the study. Patients with AKI prior to the episode of AHF or with other potential causes of AKI were excluded from the study. Furthermore, patients with an estimated glomerular filtration rate (eGFR) <45 mL/min/1.73 m² or a history of kidney transplantation were excluded. Hypotensive patients who required inotropic support prior to the diagnosis of AKI were not included. Patients with sepsis and the source/evidence of the infection (Gram-positive and Gram-negative) during their hospitalization were also excluded. A baseline value of at least 1 serum creatinine (sCr) level from within the last 3 months before admission was required for all patients enrolled in the study; we thus excluded patients for whom this was not available (Fig. 1). AHF was defined by European Society of Cardiology (ESC) guidelines [7]. AKI was defined according to the Acute Kidney Injury Network (AKIN) criteria [8]. CRS 1 type was defined according to the current classification [1].

We enrolled 32 AHF patients, 15 of whom were classified as having CRS type 1. Eight of these 15 exhibited AKI at the time of admission (caused by AHF), and the other 7 developed AKI during their stay in hospital stay (within 48 h of admission). EDTA blood samples were collected from all patients within 8 h of admission to the Internal Medicine Department. We also collected blood samples, within 24 h of developing AKI, from patients who developed CRS type 1. For AHF patients, we used the blood sample from the time of admission; we used the blood sample within 24 h of developing AKI for patients who developed CRS type 1.

The primary end point of the study was the evaluation of LPS level in AHF patients and CRS type 1 (the AHF + AKI condition) patients. We also performed a subanalysis of the CRS type 1 group, evaluating the LPS level in patients admitted with AKI, those who developed AKI during their hospital stay (in the first 48 h), and in survivors (*n* = 12) and nonsurvivors (*n* = 3). CRS type 1 patients that died in hospital were defined as CRS type 1 nonsurvivors.

Quantitative determination of LPS and cystatin C levels in the plasma was performed by enzyme-linked immunosorbent assay (ELISA) (for LPS: Cloud-Clone Corp., Texas, TX, USA; for cystatin C: BioVendor – Laboratori medicina a.s., Brno, Czech Republic). The ELISA kit for LPS is a competitive inhibition enzyme immu-

Fig. 1. Flow chart of enrolled patients.

Table 1. Clinical and laboratory parameters of CRS type 1 and AHF patients included in the study

	CRS type 1 (n = 15)	AHF (n = 17)	p value
Age, years	75.2±9.0	73.5±10.2	0.62
Weight, kg	80 (75–90)	74 (65–100)	0.80
Male	53%	47%	0.73
Diabetes	67%	76%	0.55
Hypertension	87%	88%	0.87
Serum creatinine at baseline, mg/dL	1.04 (0.92–1.2)	0.94 (0.86–1.2)	0.11
Serum creatinine at admission, mg/dL	1.3 (1.2–1.4)	0.91 (0.83–1.1)	<0.001
Urea at admission, mg/dL	774 (58–142)	50 (41–57)	0.002
Hemoglobin at admission, g/dL	11.4 (9.7–13)	13.6 (11.1–14.3)	0.06
Albumin at admission, g/L	4.2 (3.97–4.4)	4.1 (3.9–4.3)	0.57
Peak of creatinine, mg/dL	1.6 (1.4–1.7)	0.96 (0.91–1.13)	0.04
BNP at admission, pg/mL	595 (330–1900)	524 (358–883)	0.09
Troponin I at admission, ng/mL	0.06 (0.03–0.14)	0.07 (0.05–0.1)	0.82
NGAL at admission, ng/mL	291 (213–1300)	197 (144–239)	0.02
Cystatin C at admission, ng/mL	626 (513–725)	398 (370–676)	0.04
Ejection fraction, %	41 (35–49)	38 (29–48)	0.65
Mean arterial pressure, mm Hg	103 (89–115)	107 (96–116)	0.57
Mechanical ventilation, n	0/15	0/15	1
Renal replacement therapy, n	0/15	0/15	1
Mortality, n	3/15	1/17	0.29
Length of stay, days	8 (7–11)	8 (6–10)	0.25
Lipopolysaccharide, ng/mL	106.0 (66.3–210.2)	14.0 (10.6–25.7)	0.04

noassay technique for the quantitative measurement of this molecule in the serum, plasma, and other biological fluids. A monoclonal antibody specific to LPS is precoated on a microplate. A competitive inhibition reaction is launched between biotin-labeled LPS and unlabeled LPS (standards or samples) with the LPS-specific precoated antibody. After incubation, the unbound conjugate is washed off. Avidin conjugated to horseradish peroxidase (HRP) is added to each microplate well, and then incubated. The amount of bound HRP conjugate is inversely proportionate to the concentration of LPS in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportionate to the LPS in the sample. The standard curve concentrations used were 1,000, 333.33, 111.11, 37.04, and 12.35 ng/mL. All ELISA determi-

nations were performed according to the manufacturer's protocol and instructions. For LPS and cystatin C, optical density was read by using a VICTORX4 multilabel plate reader (PerkinElmer Life Sciences, Waltham, MA, USA) at 450 nm. The concentration values for these molecules were calculated from standard curves, according to the manufacturer's protocols. Quantitative determination of neutrophil gelatinase-associated lipocalin (NGAL) was performed by means of a triage NGAL panel (Alere, San Diego, CA, USA). Statistical analysis was performed using the SPSS software package. $p < 0.05$ was considered statistically significant.

Results

Baseline Characteristics

Characteristics of patients and comparisons between CRS type 1 and AHF are reported in Table 1. No patients required mechanical ventilation or renal replacement therapy. Comorbidities and medical therapy were similar in CRS 1 type and AHF patients; specifically, the amount of diuretics administered was similar ($p = 0.87$). At admission, sCr, urea, NGAL, and cystatin C levels were significantly higher in CRS type 1 than AHF subjects (Table 1). There was no significant difference in terms of median hemoglobin between the 2 groups ($p = 0.06$). However, the p value did not reach statistical significance; there was a tendency towards a lower level of hemoglobin in CRS type 1 patients.

LPS Levels in AHF and CRS Type 1 Patients

The plasma level of LPS was measured by ELISA in AHF and CRS type 1 patients, and was significantly higher in CRS 1 type patients [106.0 [IQR 66.3–210.2] vs. 14.0 [IQR 10.6–25.7] ng/mL; $p = 0.04$; Fig. 2a). The median LPS value was >7 times higher in the CRS 1 type patients than in those with AHF only. No significant difference in LPS level was found in patients who were admitted with AKI and those who developed AKI in their first 48 h in hospital (205.3 [112.1–443.7] vs. 89.3 [36.0–101.5] ng/mL; $p = 0.09$). There was a tendency towards a higher level of LPS in CRS type 1 patients admitted with AKI (Fig. 2b). Three of 15 CRS type 1 patients died in hospital (i.e., nonsurvivors). The LPS concentration at admission was similar in CRS type 1 survivors and nonsurvivors ($p = 0.22$) (Fig. 2c).

To corroborate the LPS findings, we studied the relationship between LPS and renal markers. We observed a positive correlation between LPS level and NGAL (Spearman's $\rho = 0.37$, $p = 0.04$), Scr at admission (Spearman's $\rho = 0.61$, $p < 0.001$) and peak of Scr during hospitalization (Spearman's $\rho = 0.62$, $p < 0.001$) and urea at admission (Spearman's $\rho = 0.53$, $p = 0.002$). There was no significant correlation between cystatin C and LPS level (Fig. 2d).

Discussion

To the best of our knowledge, the biological role and level of LPS in CRS type 1 and an association with renal impairment have not been yet investigated. In this *in vivo* pilot study, we hypothesized, for the first time, that LPS could be one of the soluble molecules stimulating proinflammatory induction, and ultimately renal dysfunction, in AHF patients affected by CRS type 1 [4, 9]. We investigated the LPS level and its relationship with renal biomarkers in AHF patients with and without AKI (CRS type 1). Based on our results, we demonstrated a significantly higher plasma level of LPS in CRS type 1 patients than in those with AHF only. Furthermore, we found a positive correlation between LPS and conventional and unconventional renal markers (urea and Scr at admission and peak of Scr and NGAL). The LPS level was similar in CRS type 1 patients admitted with AKI and those who developed AKI in the first 48 h after hospitalization. We theorized that patients who developed AKI during their stay in hospital had a prior translocation of

Fig. 2. LPS level and correlation with renal parameters. **a** Plasma level of LPS in AHF and CRS type 1 patients: the LPS level was significantly higher in CRS type 1 ($p = 0.004$). **b** Plasma level of LPS in CRS type 1 patients admitted with AKI and patients who developed AKI in hospital. The p value did not reach statistical significance; there was a tendency towards a higher level of LPS in CRS type 1 patients admitted with AKI. **c** Plasma level of LPS in CRS type 1 survivors and nonsurvivors. **d** Positive correlation between LPS and renal parameters.

LPS, which culminated in AKI manifestation. This hypothesis is corroborated by the correlation that existed between LPS level and the peak of sCr.

LPS is a lipoglycan found on the outer membrane of Gram-negative bacteria and is one of the strongest inducers of immune dysregulation and cytokine production, partly through the gene transcription induction by the nuclear transcription factor NF- κ B [9].

At the molecular level, LPS is recognized by TLR4, which is constitutively expressed in cardiac and renal cells such as tubular epithelial and endothelial cells. The cellular response to LPS requires a carrier protein (LPS-binding protein, LBP) to facilitate the transfer of LPS to TLR4 [10]. NF- κ B pathway activation induces the production of cytokines and nitric oxide,

which play a role in directly destroying the tubular epithelial and cardiac cells. Indeed, TLR4 requires a coreceptor for the connection with LPS; in fact, the MD-2 protein is necessary for LPS recognition and is directly implicated in the activation of the receptor complex [11].

LPS increases peripheral vascular resistance and prothrombotic mediators and depresses left ventricular function, leading to impaired organ perfusion [12]. In the gastrointestinal tract, there is an enormous amount of endotoxin, approximately 300 mg, sufficient to kill the host [13, 14]. It is well known that hypoperfusion with consequent ischemia and congestion of the gut alter the integrity of mucosa, allowing the translocation of endotoxin and bacteria into the bloodstream [14]. These findings may play a role in the development of sepsis and multiorgan failure, such as myocardial depression and renal impairment. Indeed, heart and kidney impairments seem to be induced by the release of circulating proinflammatory and proapoptotic mediators triggered by the interaction of endotoxin with immune cells.

In the context of CRS type 1, we hypothesized that mesenteric venous congestion leads to bowel-wall edema with a translocation of Gram-negative bacteria throughout the endothelial cells of the intestinal villi, the release of LPS in the systemic circulation, and the consequent immune system activation. These findings are supported by the combination of direct and indirect effects of endotoxin, which induces the release of a huge array of pro- and anti-inflammatory mediators, implicated in the pathogenesis of cardiac and renal dysfunction [15–17].

We hypothesized that LPS causes the impairment of tubular and endothelial dysfunction, leading to renal impairment. Similar to in AKI induced by Gram-negative sepsis, we hypothesized that, at a tubular level, cellular shedding, cellular desquamation with the loss of barrier function, and high permeability result in the leakage of glomerular filtrate. In addition, at the endothelial level, endothelial cells lose their role of barrier permeability and their function of regulating vascular tone and coagulation [18]. In any case, it is not clear what role LPS plays in acute and chronic heart failure and in different types of CRS. Most studies have attributed the increase of LPS to a general inflammatory state secondary to the loss of the barrier of the gut, allowing bacterial translocation [16, 17, 19, 20]. However, this has not yet been proven and is confined to the world of hypothesis. Importantly, decontamination of the gastrointestinal tract with antimicrobial therapy in CHF or cardiopulmonary bypass patients may reduce the plasma levels of LPS and proinflammatory cytokines [21, 22].

In the light of the positive correlation between LPS and admission renal parameters, and between LPS and in-hospital peak of plasmatic creatinine, we speculated that a higher LPS level may induce, enhance, and maintain kidney injury throughout inflammation, oxidative stress, and tubular and endothelial damage. There is evidence that LPS induces a loss of the endothelial barrier, thereby affecting and damaging endothelial cells directly and indirectly throughout inflammatory activation and imbalances in the cytokine network [23].

This pilot study explored the role of LPS in the pathophysiology of CRS type 1. We acknowledge the limitation of the small sample size, especially as there is no other study in the literature with which to make a comparison. Our preliminary results can be considered as having generated a hypothesis and stimulating further studies with a better design and a larger population as well as exploration of novel pathophysiological mechanisms in CRS type 1. Investigations elucidating the paracrine signaling pathways, inflammatory activation, and the cellular and molecular response to LPS will better define the pathophysiological process. Furthermore, emerging data are expected and necessary to open up new and innovative avenues for therapies in CRS.

Statement of Ethics

This study was approved by the Ethics Committee of San Bortolo Hospital in Vicenza and the procedures were in accordance with the Helsinki Declaration.

Disclosure Statement

The authors declare no conflict of interest. This paper has not been published and is not under consideration elsewhere. The results presented in this paper have not been published previously, except in abstract form. All persons listed have contributed sufficiently to the project to be included as authors, and all those who are qualified to be authors are listed in the author byline.

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Author Contributions

Conception and design of the research: G.M.V., A.B., G.V., and C.R. Acquisition of data: G.M.V., C.B., and M.d.C. Analysis and interpretation of the data: all authors. Statistical analysis: G.M.V., A.B., and G.A. Obtaining funding and supervising the work: G.V. and C.R. Drafting the manuscript: G.M.V. and A.B. Critical revision of the final draft for important intellectual content: G.V. and C.R.

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