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Renal renin-angiotensin system activation in acute heart failure and its association with endothelial dysfunction, inflammation and oxidative stress.

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Renal renin-angiotensin system activation in acute
heart failure and its association with endothelial
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TÍTULO DISSERTAÇÃO

Renal renin-angiotensin system activation in acute heart failure and its association with endothelial dysfunction, inflammation and oxidative stress.

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ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTES TRABALHOS APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTES TRABALHOS (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
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Obrigado!

Title: Renal renin-angiotensin system activation in acute heart failure and its association with endothelial dysfunction, inflammation and oxidative stress.

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Abstract:

Introduction and objectives: Acute heart failure (AHF) and cardiogenic shock (CS) are associated with high hospitalization and mortality rates. Renin-angiotensin-aldosterone system (RAAS) activation and redox dysfunction contribute to cardiac and renal dysfunction, but their interplay in human AHF and CS has not been explored. We aimed to investigate plasma and urinary angiotensinogen (P-AGT; U-AGT, an intrarenal RAAS marker) and urinary H₂O₂ (U-H₂O₂) in AHF and CS patients. Furthermore, we evaluated the correlations between them, as well as their association with endothelial dysfunction, inflammation, oxidative stress and cardiac and renal impairment.

Methods: Patients with AHF (n=23) or CS (n=25) and healthy controls (n=22) were included. U-AGT, P-AGT, U-H₂O₂, endothelial dysfunction, inflammation, oxidative stress and cardiac/renal impairment markers were evaluated at days 1-2 (admission), days 3-4 and days 5-8 in AHF and CS patients and at a single time point in controls.

Results: At admission, AHF and CS patients had similar P-AGT, higher U-AGT and lower U-H₂O₂ than controls. There were no changes in P-AGT, U-AGT or U-H₂O₂ along hospitalization. P-AGT and U-AGT positively correlated with endothelial dysfunction, inflammation and oxidative stress, while U-H₂O₂ negatively correlated with these biomarkers and with U-AGT in the overall study population. Among patients, U-H₂O₂ positively correlated with inflammation, oxidative stress and P-AGT, and both U-AGT and U-H₂O₂ were associated with renal impairment.

Conclusions: AHF and CS patients appear to have intrarenal RAAS activation and increased H₂O₂ consumption, probably by myeloperoxidase. Furthermore, their association with renal dysfunction is likely related to endothelial injury, inflammation and oxidative stress.

Keywords: Acute heart failure; cardiogenic shock; urinary angiotensinogen; urinary hydrogen peroxide; endothelial dysfunction; inflammation.

Título: Sistema renina-angiotensina renal en la insuficiencia cardíaca aguda y su asociación con disfunción endotelial, inflamación y estrés oxidativo

Resumen:

Introducción y objetivos: Insuficiencia cardíaca aguda (ICA) y shock cardiogénico (SC) causa elevada hospitalización y mortalidad. La activación del sistema renina-angiotensina-aldosterona (SRAA) y la disfunción redox favorecen la disfunciones cardíaca/renal, pero no se ha explorado su interacción con ICA o SC. Nuestro objetivo fue investigar el angiotensinógeno urinario y plasmático (P-AGT; U-AGT, marcador del RAAS intrarrenal) y el H₂O₂ urinario (U-H₂O₂) en pacientes con ICA o SC. También, evaluamos las correlaciones entre estos, y su asociación con disfunción endotelial, inflamación, estrés oxidativo e insuficiencias cardíaca y renal.

Métodos: Se incluyeron pacientes con ICA (n=23) o SC (n=25) y controles sanos (n=22). Se evaluaron U-AGT, P-AGT, U-H₂O₂, y marcadores de inflamación, estrés oxidativo y disfunción endotelial/cardíaca/renal en los días 1-2 (ingreso), 3-4 y 5-8 en los enfermos, y en un único momento en los controles.

Resultados: Al ingreso, los pacientes tenían P-AGT similar, mayor U-AGT y menor U-H₂O₂ que los controles. No hubo cambios en P-AGT, U-AGT o U-H₂O₂ durante la hospitalización. P-AGT y U-AGT correlacionaron positivamente con disfunción endotelial, inflamación y estrés oxidativo, mientras que U-H₂O₂ correlacionó negativamente con estos biomarcadores y con U-AGT en la población general del estudio. Entre los enfermos, U-H₂O₂ correlacionó positivamente con inflamación, estrés oxidativo y P-AGT, y tanto U-AGT como U-H₂O₂ se asociaron con insuficiencia renal.

Conclusiones: Los pacientes con ICA o SC tienen activación del SRAA intrarrenal y aumento del consumo de H₂O₂, probablemente por la mieloperoxidasa. Su asociación con la disfunción renal probablemente esté relacionada con lesión endotelial, inflamación y estrés oxidativo.

Palabras-clave: Insuficiencia cardiaca aguda; shock cardiogénico; angiotensinógeno urinario; peróxido de hidrógeno urinario; disfunción endotelial; inflamación

Resumo:

Introdução e objetivos: A insuficiência cardíaca aguda (ICA) e o choque cardiogénico (CC) são importantes causas de internamento e mortalidade. A ativação do sistema renina-angiotensina-aldosterona (SRAA) e a disfunção redox contribuem para disfunção cardíaca/renal, mas a sua associação na ICA e CC não foi explorada. Pretendemos quantificar o angiotensinogénio plasmático e urinário (P-AGT; U-AGT, marcador do SRAA renal) e o H_2O_2 urinário (U- H_2O_2) em doentes com ICA e CC, e avaliar correlações entre eles e com disfunção endotelial, inflamação, stresse oxidativo, e compromisso cardíaco/renal.

Métodos: O estudo incluiu controlos saudáveis (n=22) e doentes com ICA (n=23) e CC (n=25). Quantificámos P-AGT, U-AGT, U- H_2O_2 e marcadores de disfunção endotelial, inflamação, stresse oxidativo e disfunção renal e cardíaca nos dias 1-2 (admissão), 3-4 e 5-8 nos doentes, e num só momento nos controlos.

Resultados: Na admissão, os doentes com ICA e CC apresentavam P-AGT semelhante, U-AGT superior e U- H_2O_2 inferior aos controlos. Não houve alteração nestes marcadores durante o internamento. Na população global do estudo, o P-AGT e U-AGT correlacionaram-se positivamente com disfunção endotelial, inflamação e stresse oxidativo, mas o U- H_2O_2 correlacionou-se negativamente com estes marcadores. Nos doentes, o U- H_2O_2 correlacionou-se positivamente com inflamação, stresse oxidativo e P-AGT, e quer o U- H_2O_2 , quer o U-AGT apresentaram correlação positiva com disfunção renal.

Conclusões: Nos doentes com ICA e CC parece haver ativação do SRAA renal e maior consumo de H_2O_2 , provavelmente pela mieloperoxidase. A associação do U-AGT e U- H_2O_2 com a disfunção renal está provavelmente relacionada com lesão endotelial, inflamação e stresse oxidativo.

Palavras-chave: Insuficiência cardíaca aguda; choque cardiogénico; angiotensinogénio urinário; peróxido de hidrogénio urinário; disfunção endotelial; inflamação.

Abbreviations:

AHF – acute heart failure

CS – cardiogenic shock

RAAS – renin-angiotensin-aldosterone system

ROS – reactive oxygen species

AGT – angiotensinogen

H₂O₂ – hydrogen peroxide

Abreviaturas:

ICA – insuficiencia cardíaca aguda

SC – shock cardiogénico

SRAA – sistema renina-angiotensina-aldosterona

ROS – especies reactivas de oxígeno

AGT – angiotensinogénico

H₂O₂ – peróxido de hidrógeno

INTRODUCTION

Acute heart failure (AHF) is caused by the rapid onset or aggravation of a cardiac incapacity to pump the enough amount of blood per minute in order to supply the needs of the body's organs [1]. Ultimately, it may present as cardiogenic shock (CS), its most severe manifestation, which frequently results in multi-organ failure and consequent death [2]. AHF has an estimated incidence of 5.7 per 1000 person-years [3], being accountable for more than 1 million hospitalizations per year in Europe [4]. In Portugal, recent data point to an in-hospital mortality rate of 13% for AHF and a 30-day readmission rate between 9 and 14% [5]. In what concerns CS, it represents 2-5% of AHF presentations, with an in-hospital mortality that goes up to 50% [2].

The context in which AHF develops is of high complexity, encompassing an interplay among the dysfunction of the cardiac, pulmonary, renal and vascular systems. Several mechanisms, including neurohormonal activation (sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS) and inflammation are part of this intricate pathophysiology, being accompanied by an increase in reactive oxygen species (ROS). These mechanisms are interlinked and perpetuate each other, which leads to the aggravation of cardiac and renal damage, resulting in compromised function [6, 7].

The RAAS integrates the communication between the cardiovascular, renal and central nervous systems, regulating circulation volume and arterial pressure. Briefly, renin converts angiotensinogen (AGT) to angiotensin I (Ang I), which is consequently converted to angiotensin II (Ang II) by angiotensin-converting enzyme (ACE).

Physiologically, Ang II stimulates the angiotensin II type 1 (AT_1) receptor, triggering aldosterone secretion from the adrenal glands, renal tubular sodium and water reabsorption, systemic arterial and renal arteriolar constriction, vascular smooth muscle contraction and, locally, myocyte hypertrophy [8]. Besides acting as an endocrine regulator, the RAAS is now seen as a paracrine or autocrine system, given the production of AGT and Ang II within tissues as the myocardium and the kidney [9, 10, 11]. In heart failure (HF), sustained activation of the RAAS contributes to pathological left ventricle remodelling characterized by hypertrophy, myocyte loss and fibrosis [11] and might contribute to stratify AHF patients at increased cardiovascular risk [12].

Accordingly, RAAS blockade is the first line therapy for HF with reduced ejection fraction (HFrEF), mainly accomplished through ACE inhibitors (ACEi), angiotensin receptor blockers (ARB) or angiotensin receptor-neprilysin inhibitors (ARNi) [13].

Oxidative stress results from of an imbalance between the formation of ROS and/or reactive nitrogen species and their clearance by endogenous antioxidant defense mechanisms [14]. Major sources of ROS include mitochondrial oxidases and other prooxidant enzymes, such as NADPH oxidases (NOX), xanthine oxidase (XO), uncoupled endothelial nitric oxide synthase (eNOS), myeloperoxidase (MPO) and monoamine oxidases (MAO) [15]. MPO is one of the most studied enzymes in human HF, being considered an important link between oxidative stress and inflammation. Under inflammatory conditions, the MPO released by activated neutrophils and monocytes uses hydrogen peroxide (H_2O_2) derived by leukocyte oxidative burst and other ROS sources (e.g. vascular NOX) to produce HClO and other potent oxidant species that can cause significant damage in cardiovascular and renal tissues. Importantly, MPO seems to be an incremental prognostic biomarker for HF or cardiovascular death among patients with acute myocardial infarction. In addition, it appears to help in identifying forms of AHF with cardiorenal syndrome [15-17].

In physiological conditions, ROS are involved in signalling processes regulating cell and organ function. However, persistently elevated ROS concentrations are potentially pathological by promoting not only macromolecule damage, including the oxidation of proteins, lipids and DNA, but also the dysregulation of redox-controlled processes, thus contributing to a vast array of human diseases [15, 18]. In the heart, ROS excess elicits mitochondrial damage and dysfunction, contractile dysfunction, adverse myocardial remodelling, inflammation, endothelial dysfunction and ischemic cardiomyopathy [15, 19, 20]. Regarding the kidney, ROS regulate renal haemodynamics, diuresis and natriuresis, cell growth and differentiation, production and breakdown of the extracellular matrix, inflammation and integrate the immune system defense. At the same time, abnormal accumulation of ROS promote renal dysfunction and tissue damage, contributing to acute kidney injury and pathogenesis of chronic kidney disease [21-24].

Ang II stimulates the production of ROS, which mediate many of the RAAS deleterious effects in cardiovascular and renal function. Noteworthy, besides being downstream mediators of RAAS, there is evidence that ROS also regulate RAAS activation and that, in pathological conditions such as HF and/or renal disease, the interrelationship between these pathways creates a vicious cycle that amplifies their deleterious impact, perpetuating the cardiac and renal damage [18]. Interestingly, we recently reported that chronic HF (CHF) patients with preserved renal function showed mostly inverse

correlations between oxidative stress and RAAS markers, while CHF patients with impaired renal function (iRF) exhibited several positive correlations between these pathways, including significant correlations of urinary H₂O₂ (U-H₂O₂) with plasma renin concentration or plasma AGT (P-AGT) concentration, of urinary isoprostanes with P-AGT or serum ACE activity, and a borderline significant correlation between U-H₂O₂ and urinary AGT (U-AGT) [25].

U-AGT has been used as a biomarker of intrarenal RAAS activity [10]. Yokoyama *et al* suggested that the time-course changes in U-AGT/creatinine ratio can be used as a monitoring tool for AHF patients [26]. In AHF, U-AGT predicts prognosis at 1 year and a higher risk of development of acute kidney injury (AKI) in this circumstance [27], defined as cardiorenal syndrome type 1 [28]. Also, Chen *et al* underlined that U-AGT values at the time of AKI diagnosis in AHF were an indicator of AKI progression and death [29].

Oxidative stress and RAAS are also implicated in endothelial dysfunction and inflammation, two other important mechanisms involved in HF pathophysiology. ROS directly contribute to endothelial dysfunction by inactivating nitric oxide (NO) and by promoting eNOS uncoupling, which further elevates O₂^{•-} [30]. Moreover, increased production of ROS leads to the upregulation of chemokines, activation of neutrophil integrins, and expression of surface adhesion molecules by endothelial cells, which are inflammatory mechanisms promoting pathological cardiac remodelling. Furthermore, inflammatory molecules, such as TNF- α , interleukin 6 (IL-6) and TGF- β increase ROS generation [31]. On the other hand, decreased NO concentration in the context of endothelial dysfunction favors the profibrotic signalling induced by Ang II and aldosterone in the myocardium [30]. Additionally, the RAAS also stimulates the production of adhesion molecules and cytokines and the polarization of macrophages to the M1 pro-inflammatory subtype, contributing to inflammation [31].

Although important research has been conducted with the intent to characterize the systemic [12, 32] and the intrarenal [33] RAAS in AHF, there are no studies on the relationship between these two systems and oxidative stress in AHF or CS. This could lead to a better understanding of AHF pathophysiology, helping to improve the already existing risk scores [34] and bringing new insights into the management of the disease. Therefore, the present study aimed to evaluate U-AGT and P-AGT and U-H₂O₂ at admission and during hospitalization, as well as their relationship, both in AHF and CS

patients. Moreover, we also assessed their association with markers of inflammation, endothelial dysfunction, oxidative stress, cardiac and renal dysfunction.

METHODS

Study design and population

This study is part of a larger research project (RIFF-HEART) involving patients from the Intermediate & Intensive Care Units (ICU) of the Department of Intensive Care Medicine with the diagnosis of AHF and CS.

We performed a single-center cohort study and consecutively recruited patients with a single diagnosis of AHF (n=23) or CS (n=25) admitted to the ICU of Department of Intensive Care Medicine of Centro Hospitalar Universitário São João (CHUSJ), from January 2017 to December 2019. Controls (n=22) were recruited among healthy blood donors volunteers from the Department of Immunohemotherapy of CHUSJ, from September 2017 to October 2017. All eligible patients (or their legal representative) provided written informed consent to participate in the study. Blood donor volunteers provided oral informed consent. The study protocol conforms to the Guidelines for Good Clinical Practice and the ethical guidelines of the 1975 Declaration of Helsinki. The study was approved by the institution's ethics committee. Patients were followed during their stay in the ICU by the medical team of the project.

Clinical data and sample collection

Illness severity was assessed by the Acute Physiology And Chronic Health Evaluation II (APACHE II) and Simplified Acute Physiology Score II (SAPS II) scoring systems at ICU admission.

Physical examination of the patients was performed during their ICU stay, and a record of demographic and clinical data was completed by the medical team and anonymously coded to the project database, along with laboratory data, guaranteeing confidentiality. Blood and spot urine samples were collected in patients (AHF and CS groups) at 3 different time points during ICU stay, whenever possible: up to 48 hours (days 1-2, admission), on days 3 or 4 and on days 5 to 8 after ICU admission. Samples (blood and spot urine) from controls were collected at a single time point. All samples were processed within 1-2 hours of collection and stored at -80 °C until assayed.

Routine clinical haematological, biochemical and cardiac markers

Plasma B-type natriuretic peptide (P-BNP) and high-sensitivity troponin I (P-hs-trop I) were measured by chemiluminescent microparticle immunoassays using an Abbot® Architect i2000 automated analyser (Abbott® Diagnostics, Lake Forest, IL, USA). An Olympus Beckman Coulter® AU5400 automated clinical chemistry analyser (Olympus, Hamburg, Germany) was used for the quantification of serum C-reactive protein (S-CRP) by an immunoturbidimetric assay, serum adenosine deaminase activity (ADA) by an enzymatic assay, serum urea (S-Urea) concentration by a kinetic urease/glutamate dehydrogenase method, plasma and urine creatinine by the colorimetric Jaffe method and urinary protein by the pyrogallol red method.

Quantification of H₂O₂ and angiotensinogen

We measured H₂O₂ in spot urine samples (U-H₂O₂) using a microplate fluorimetric assay (Amplex® Red Hydrogen Peroxide/Peroxidase Assay Kit; Molecular Probes, Invitrogen, Thermo Fisher Scientific, Massachusetts, USA), following the manufacturer's instructions. U-H₂O₂ values were further normalized to urinary creatinine concentrations. Angiotensinogen was quantified in plasma (P-AGT) and spot urine (U-AGT) samples using an enzyme-linked immunosorbent assay test (Human Total Angiotensinogen Assay Kit, Immuno-Biological Laboratories Co., Gunma, Japan), following the manufacturer's instructions. U-AGT values were corrected for urinary creatinine content.

Evaluation of biomarkers of vascular/endothelial dysfunction, inflammation and oxidative stress

Serum endothelium activation markers (intercellular adhesion molecule 1, S-ICAM-1; vascular cell adhesion molecule 1, S-VCAM-1; E-Selectin, S-E-Selectin), and proinflammatory cytokines (tumour necrosis factor alpha, S-TNF- α ; interleukin 1 beta, S-IL-1 β ; interleukin 6, S-IL-6) were evaluated by multiplex immunoassays using with a Luminex 200 analyzer (Luminex Corporation, Austin, TX, USA), according to the protocols of Luminex Human Magnetic Assay (R&D Systems, Inc., Minneapolis, USA) and MILLIPLEX® MAP Human High Sensitivity T Cell Magnetic Bead Panel (Millipore Corporation, Billerica, MA, USA), respectively. Raw data analysis (mean fluorescence intensity) was performed using ISTM 2.3 software (Luminex Corporation). Serum interleukin 10 (S-IL-10) and serum myeloperoxidase (S-MPO) were quantified by enzyme immunoassays using commercial kits (Thermo Scientific™ Human Interleukin-

10 (IL-10) ELISA Kit, Thermo Scientific, Rockford, Illinois, USA; BioCheck MPO Enzyme Immunoassay, Oxis International Inc., Foster City, CA, USA). Urinary cysteinyl leukotrienes (U-CysLT) were measured in unextracted spot urine samples (U-CysLT) by a competitive enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Cysteinyl Leukotriene ELISA kit, Cayman Chemical Company, Ann Arbor, MI, USA). U-CysLT values were further normalized to urinary creatinine content. Urinary isoprostanes (U-Isop) were quantified by a competitive enzyme immunoassay (Urinary Isoprostane ELISA Kit; Oxford Biomedical Research ® Inc., Oxford, MI, USA) in non-extracted spot urine containing the preservative butylated hydroxy toluene (BHT, 0.005%, w/v) added before storage. U-Isop values were corrected for urinary creatinine concentrations.

Data and statistical analysis

Results are expressed as mean±standard error of the mean (SEM) or as median (25th percentile; 75th percentile) for data with normal or non-normal distribution, respectively, or as percentage, and are graphically represented as Box and Whiskers plots. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [35]. Statistical analysis was performed using unpaired Student's t-test or Mann–Whitney U-test, for comparisons between two groups, or by one-way ANOVA followed by a Tukey's multiple comparison test or a Kruskal Wallis test followed by a Dunn's post hoc test, where appropriate, for comparison between three groups. Categorical variables were analysed by the Fisher's exact test or by the Chi Square test. U-AGT, P-AGT and U-H₂O₂ evolution throughout the hospitalization was evaluated by a paired analysis using a mixed-effects model (Restricted Maximum Likelihood, REML), after applying a base 10 log transformation whenever appropriate, and also by an unpaired Kruskal Wallis test to compare the medians in each time point. *P* values of <0.05 were considered significant. Spearman's correlation analysis was used to estimate correlations between sets of nonparametric data in the study population (Controls; AHF and CS patients throughout hospitalization).

To prevent possible bias in clinical evaluation, all the patients were examined by the same medical team included in the project. However, we cannot exclude bias from eventual patients' selection (severe patients) because acute cardiac patients with non-invasive ventilation can also be distributed to the cardiac unit of Cardiology Department in the Hospital.

To assure comparability of biomarkers assessment, samples from Controls, AHF and CS groups were evenly distributed in each assay plate. There were missing values in some biomarkers due to insufficient sample volume of samples or reagents to perform sample processing, dilution tests and assays. In addition, some routine clinical biomarkers could only be assessed at a single time point during hospitalization due to hospital's internal policies, and we had not permission to measure routine clinical biomarkers in controls (blood donor volunteers), with the exception of creatinine, or to have access to their hospital laboratory reports. The final number per group for the biomarkers/parameters evaluated at admission was as following: P-BNP, n=20 vs 17 (AHF vs CS); P-hs-trop I, n=23 vs 23 (AHF vs CS); eGFR, n=22 vs 23 vs 23 (Controls vs AHF vs CS); S-Urea, n=23 vs 23 (AHF vs CS); U-protein/creatinine ratio, n=22 vs 15 (AHF vs CS); S-ICAM-1, n =22 vs 23 vs 24 (Controls vs AHF vs CS); S-VCAM-1, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-E-Selectin, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-TNF- α , n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-IL-1 β , n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-IL-6, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-MPO, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-IL-10, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-CRP, n= 23 vs 23 (AHF vs CS); S-ADA, n=13 vs 12 (AHF vs CS) U-AGT, n=22 vs 23 vs 18 (Controls vs AHF vs CS); P-AGT, n=22 vs 23 vs 24 (Controls vs AHF vs CS), U-H₂O₂, n=22 vs 23 vs 19 (Controls vs AHF vs CS); U-CysLT, n=22 vs 22 vs 19 (Controls vs AHF vs CS); U-Isop, n=22 vs 21 vs 17 (Controls vs AHF vs CS). To avoid biasing the results, no imputation for missing values was used.

Sample size was defined according to the RIFF-HEART project's primary objectives that consisted in characterizing resolution of inflammation and endothelitis at admission and during hospitalization.

Reporting of the study conforms to STROBE statement along with references to STROBE and the broader EQUATOR guidelines [36].

RESULTS

Population demographical, clinical and biochemical characterization

General demographic characteristics, clinical and biochemical parameters are shown in [Table 1](#).

In this study, 22 healthy controls and 48 patients, 23 with AHF and 25 with CS, were assessed. The CS group had predominantly male patients, compared to AHF, although no significant differences were found among the studied groups. Patients in the AHF group

were significantly older than controls and CS patients. APACHE II and SAPS II scores were significantly higher in the CS group, in which almost half the patients were on VA-ECMO.

Precipitating factors on admission were identical for AHF and CS groups, with acute coronary syndrome being the predominant trigger in both groups, followed by acute decompensated heart failure in AHF and infection in CS. Arterial hypertension, diabetes, dyslipidemia and anemia were the most prevalent comorbidities in both groups, but there was a significantly higher number of patients presenting arterial hypertension or dyslipidemia in AHF than in CS.

CS patients had significantly higher concentration of P-hs-trop I at admission, but no differences were found in P-BNP values when compared to AHF patients. All groups, including controls, presented a mild reduction of eGFR compared to the normal reference range. Also, both patient groups presented higher than normal serum urea and proteinuria. CS patients had a longer length of stay in the ICU than patients with AHF, but there were no differences between these groups regarding the hospital total length of stay and mortality outcomes.

Vascular/endothelial dysfunction, inflammation and oxidative stress biomarkers

Concerning vascular and endothelial dysfunction biomarkers, we found significantly higher concentration of S-ICAM-1 in CS patients compared to AHF patients and controls ($p < 0.05$) (Table 2). S-VCAM-1 was increased by 2-fold in AHF and almost by 4-fold in CS patients compared to controls ($p < 0.001$), but no differences were found in S-E-Selectin values among the groups (Table 2). U-CysLT were significantly increased in AHF ($p < 0.001$) and CS ($p < 0.01$) groups relative to controls (Table 2).

Patients with AHF or CS exhibited higher values of inflammatory markers such as S-TNF- α (overall p value = 0.0305), S-IL-6 ($p < 0.001$) and S-MPO ($p < 0.001$) compared to controls (Table 2). A significant increase in S-IL-10 concentration was observed only in CS patients ($p < 0.01$ vs controls) (Table 2). There were no significant differences in S-CRP or S-ADA values between AHF and CS groups (Table 2).

AHF and CS patients had higher oxidative stress than controls as demonstrated by significantly raised U-Isop values ($p < 0.001$) (Table 2).

U-AGT, P-AGT and U-H2O2 at admission

U-AGT was significantly higher and U-H2O2 was markedly lower in AHF and CS at ICU admission, with these changes being more marked in CS ([Figure 1A and 1C](#)). No significant differences between groups were seen for P-AGT values ([Figure 1B](#)).

U-AGT, P-AGT and U-H2O2 profile during hospitalization

There were no significant changes in U-AGT values throughout hospitalization in AHF and CS groups whether considering a paired ([Figure 2A and 2C](#)) or unpaired analysis ([Figure 2B and 2D](#)). Also, there were no significant changes in P-AGT values throughout hospitalization in AHF patients ([Fig. 3A for paired and 3B for unpaired analysis](#)) or CS patients ([Fig. 3C for paired and 3D for unpaired analysis](#)). Moreover, we did not observe significant changes in U-H2O2 values throughout hospitalization in AHF and CS patients whether considering a paired ([Figure 4A and 4C](#)) or unpaired analysis ([Figure 4B and 4D](#)).

Correlation analysis in the overall study population

Both U-AGT and P-AGT showed significant positive correlations with markers of vascular and endothelial dysfunction (U-AGT vs S-VCAM-1; U-AGT vs U-CysLT; P-AGT vs S-VCAM-1) and inflammation (U-AGT vs S-TNF- α ; U-AGT vs S-IL-6; U-AGT vs IL-10; U-AGT vs S-MPO; P-AGT vs S-IL-6; P-AGT vs S-MPO) ([Table 3](#)). U-H2O2 presented inverse correlations with endothelial dysfunction (U-H2O2 vs S-VCAM-1) and inflammation markers (U-H2O2 vs S-IL-6; U-H2O2 vs S-MPO) ([Table 3](#)).

Regarding the correlations within and between RAAS and ROS/oxidative stress markers, there were significant positive correlations for U-AGT with P-AGT and for P-AGT with U-Isop, but an inverse correlation was observed between U-AGT and U-H2O2 ([Table 3](#)).

Correlation analysis within patients

When analysing correlations only in patients, we still observed significant positive correlations of U-AGT with biomarkers of vascular and endothelial dysfunction (U-AGT vs S-VCAM-1; U-AGT vs U-CysLT) and inflammation (U-AGT vs S-IL-6) ([Table 3](#)). P-AGT was also positively correlated with inflammation (P-AGT vs S-CRP) ([Table 3](#)). The inverse correlations between U-H2O2 and inflammatory status were not observed when excluding the control group from the analysis. Instead, U-H2O2 showed significant

positive correlations with inflammatory markers such as S-TNF- α and S-ADA (Table 3). U-H₂O₂ also presented a positive correlation with U-Isop, which in turn were positively correlated with P-AGT (Table 3).

There was a significant positive correlation between P-AGT and P-BNP and an inverse correlation between U-H₂O₂ and P-hs-troponin I (Table 3).

Both U-AGT and U-H₂O₂ were related with renal dysfunction, as evidenced by the positive correlations of U-AGT with U-protein/creatinine and U-H₂O₂ with S-Urea and by the inverse correlation between U-H₂O₂ and eGFR (Table 3).

DISCUSSION

The major findings of our study are that both AHF and CS patients present intrarenal RAAS activation and oxidative stress, which are associated with renal dysfunction, inflammation and endothelial injury. Moreover, our results also point to a marked H₂O₂ consumption in these patients, probably by MPO.

Patients with AHF and CS showed activation of the intrarenal RAAS, as evaluated by U-AGT, which is in accordance with previous studies in cardiovascular diseases as hypertension [37] and chronic kidney disease [38] as well as HFrEF [11]. U-AGT was positively correlated with several markers of vascular/endothelial dysfunction and inflammatory status and also with markers of renal dysfunction within AHF and CS. Moreover, in the overall study population, U-AGT was also positively correlated with P-AGT values, probably reflecting the connection between systemic and renal AGT [10]. In addition, P-AGT was positively associated with both U-Isop and markers of vascular/endothelial dysfunction and inflammation. Collectively, the positive correlations between U-AGT or P-AGT and markers of vascular/endothelial dysfunction and inflammation further reinforce the pathophysiological relevance of RAAS in HF [8, 10, 30, 31].

The correlation analysis involving all the studied groups revealed a negative correlation of U-AGT with U-H₂O₂ values, which were shown to be diminished in AHF and CS patients when compared with controls, although U-Isop concentrations were higher, reflecting increased oxidative stress. This negative correlation between U-AGT and U-H₂O₂ levels was somehow intriguing since one would expect that the activation of intrarenal RAAS observed in AHF and CS patients contributed to their increased

inflammatory status and oxidative stress. Indeed, as we have recently reviewed, RAAS and ROS are interlinked and in pathological conditions perpetuate a feedforward mechanism that contributes to disease progression and severity [18]. Also, in our previous study with CHF patients, we observed a positive correlation between U-AGT and U-H₂O₂ (25). The overall redox state of AHF and CS patients is imbalanced, as indicated by their higher U-Isop levels. Noteworthy, in the overall study population, U-H₂O₂ also presented significant negative correlations with systemic inflammatory markers, including S-MPO, S-IL-6 and also the endothelial adhesion molecule, S-VCAM-1, which were all significantly increased in AHF and CS when compared to controls. Therefore, the marked inflammation and oxidative stress exhibited by these patients is more compatible with the view that diminished values of U-H₂O₂ result from increased consumption, probably by MPO which is known to use H₂O₂ as a substrate to produce potent oxidants that contribute to tissue damage in HF.

U-AGT, P-AGT and U-H₂O₂ levels were not altered along hospitalization, from admission (days 1-2), to days 3-4 and days 5-8. Current evidence suggests that U-AGT remain high during hospitalization in patients with AHF who develop AKI, despite a trend for a decrease. In contrast, AHF patients who do not develop AKI exhibit constantly lower values during hospitalization in those AHF patients who do not develop AKI [27]. Furthermore, in acute decompensated HF, U-AGT is a strong predictor of cardiorenal syndrome diagnosis [27] and progression [29] and of 1-year prognosis [27]. With respect to P-AGT, previous results also show stable concentrations during hospitalization of AHF patients, both in those with and without AKI [27]. Furthermore, as far as we know, our study is the first to evaluate U-H₂O₂ values during the hospitalization period of AHF and CS patients. In agreement with data showing that U-AGT predicts the decline in kidney function, we found a positive correlation between U-AGT and U-Protein/creatinine on admission. Also, U-H₂O₂ was negatively correlated to eGFR and positively correlated to S-Urea which corroborates current evidence on the deleterious effect of ROS on renal function [5]. As pointed out above, increased consumption of U-H₂O₂ by MPO might produce other ROS, like HClO [39], which can contribute to the deleterious effect on renal function [40, 41]. Indeed, in patients with AHF and CS, we observed positive correlations between U-H₂O₂ and oxidative stress (U-Isop) and inflammatory markers (S-TNF- α). Also, U-AGT was positively correlated with markers of endothelial dysfunction and inflammation. Overall, these results

suggest that U-AGT and U-H₂O₂ association with renal dysfunction involves endothelial injury, inflammation and oxidative stress.

Regarding cardiac biomarkers, we observed that P-AGT and P-BNP were positively correlated, expanding the already described relationship between the RAAS and P-BNP in AHF patients [12].

Our study presents as a major strength the fact that we could study AHF and CS patients in the “real-world conditions” that represent the exact clinical setting in which patients are treated; a situation by far very different from the controlled and selected environment of clinical trials [42]. This major strength might also be seen as a limitation of the study since the multiplicity of etiologies and comorbidities, as well as drug treatments, makes the analysis of data much more complex. However, we endorse the view that “real-world conditions” have a higher potential for fulfil the needs of patients with AHF and/or CS. As any, our study has some limitations, particularly its retrospective single-center design advises the translation of results to be cautious.

CONCLUSIONS

In conclusion, AHF and CS patients have increased intrarenal RAAS activity and increased H₂O₂ consumption, probably by MPO. The association between these markers with renal dysfunction likely involves inflammation, oxidative stress and endothelial injury.

What is known about the topic?

- AHF and CS (its most severe form) are associated with dysfunction of the cardiac, pulmonary, renal and vascular systems.
- The RAAS and inflammation participate in the pathophysiology of AHF and CS, as well as ROS.
- The association between the RAAS and oxidative stress, and markers of inflammation, endothelial, cardiac and renal dysfunction is not yet clear in AHF and CS.

What does this study add?

- AHF and CS patients show activation of intrarenal RAAS, as evaluated by U-AGT levels, and increased oxidative stress, when compared with healthy controls.
- Oxidative stress in AHF and CS is associated with increased MPO activity that increases U-H₂O₂ consumption.
- RAAS and oxidative stress (namely MPO-derived ROS) contribute to inflammation, endothelial and renal dysfunction, in AHF and CS.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- [1] Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail.* 2016;18(8):891-975. doi:10.1002/ejhf.592
- [2] Chioncel O, Parissis J, Mebazaa A, et al. Epidemiology, pathophysiology and contemporary management of cardiogenic shock - a position statement from the Heart Failure Association of the European Society of Cardiology [published correction appears in *Eur J Heart Fail.* 2021 Feb;23(2):345]. *Eur J Heart Fail.* 2020;22(8):1315-1341. doi:10.1002/ejhf.1922
- [3] Sinnenberg L, Givertz MM. Acute heart failure. *Trends Cardiovasc Med.* 2020;30(2):104-112. doi:10.1016/j.tcm.2019.03.007
- [4] Arrigo M, Jessup M, Mullens W, et al. Acute heart failure. *Nat Rev Dis Primers.* 2020;6(1):16. Published 2020 Mar 5. doi:10.1038/s41572-020-0151-7
- [5] Marques-Alves P, Marinho AV, Almeida JP, et al. Real-world analysis of acute decompensated heart failure outcomes in Portugal. *ESC Heart Fail.* 2020;7(2):551-558. doi:10.1002/ehf2.12599
- [6] Sabbah HN. Pathophysiology of acute heart failure syndrome: a knowledge gap. *Heart Fail Rev.* 2017;22(6):621-639. doi:10.1007/s10741-017-9651-2
- [7] Tomasoni D, Lombardi CM, Sbolli M, Cotter G, Metra M. Acute heart failure: More questions than answers. *Prog Cardiovasc Dis.* 2020;63(5):599-606. doi:10.1016/j.pcad.2020.04.007
- [8] Sayer G, Bhat G. The renin-angiotensin-aldosterone system and heart failure. *Cardiol Clin.* 2014;32(1):21-vii. doi:10.1016/j.ccl.2013.09.002
- [9] Xu Y, Rong J, Zhang Z. The emerging role of angiotensinogen in cardiovascular diseases. *J Cell Physiol.* 2021;236(1):68-78. doi:10.1002/jcp.29889
- [10] Nishiyama A, Kobori H. Independent regulation of renin-angiotensin-aldosterone system in the kidney. *Clin Exp Nephrol.* 2018;22(6):1231-1239. doi:10.1007/s10157-018-1567-1
- [11] Hartupee J, Mann DL. Neurohormonal activation in heart failure with reduced ejection fraction. *Nat Rev Cardiol.* 2017;14(1):30-38. doi:10.1038/nrcardio.2016.163

- [12] Nakada Y, Takahama H, Kanzaki H, et al. The predictability of renin-angiotensin-aldosterone system factors for clinical outcome in patients with acute decompensated heart failure. *Heart Vessels*. 2016;31(6):925-931. doi:10.1007/s00380-015-0688-7
- [13] Arendse LB, Danser AHJ, Poglitsch M, et al. Novel Therapeutic Approaches Targeting the Renin-Angiotensin System and Associated Peptides in Hypertension and Heart Failure. *Pharmacol Rev*. 2019;71(4):539-570. doi:10.1124/pr.118.017129
- [14] van der Pol A, van Gilst WH, Voors AA, van der Meer P. Treating oxidative stress in heart failure: past, present and future. *Eur J Heart Fail*. 2019;21(4):425-435. doi:10.1002/ejhf.1320
- [15] Sousa T, Reina-Couto M, Gomes P. Role of Oxidative Stress in the Pathophysiology of Arterial Hypertension and Heart Failure. In: Chakraborti S, Dhalla N, Ganguly N, Dikshit M. *Oxidative Stress in Heart Diseases*. Springer, Singapore. 2019. p. 509-537. https://doi.org/10.1007/978-981-13-8273-4_23
- [16] O'Donoghue ML, Morrow DA, Cannon CP, et al. Multimarker Risk Stratification in Patients With Acute Myocardial Infarction. *J Am Heart Assoc*. 2016;5(5):e002586. Published 2016 May 20. doi:10.1161/JAHA.115.002586
- [17] Virzì GM, Breglia A, Brocca A, et al. Levels of Proinflammatory Cytokines, Oxidative Stress, and Tissue Damage Markers in Patients with Acute Heart Failure with and without Cardiorenal Syndrome Type 1. *Cardiorenal Med*. 2018;8(4):321-331. doi:10.1159/000492602
- [18] Morato M, Reina-Couto M, Pinho D, Albino-Teixeira A, Sousa T. Regulation of the Renin-Angiotensin-Aldosterone System by Reactive Oxygen Species. In: Tolekova AN, *Renin-Angiotensin System - Past, Present and Future*, IntechOpen, 2017. p. 120-157 DOI: 10.5772/67016. Available at: <https://www.intechopen.com/books/renin-angiotensin-system-past-present-and-future/regulation-of-the-renin-angiotensin-aldosterone-system-by-reactive-oxygen-species>
- [19] D'Oria R, Schipani R, Leonardini A, et al. The Role of Oxidative Stress in Cardiac Disease: From Physiological Response to Injury Factor. *Oxid Med Cell Longev*. 2020;2020:5732956. Published 2020 May 14. doi:10.1155/2020/5732956
- [20] Münzel T, Camici GG, Maack C, Bonetti NR, Fuster V, Kovacic JC. Impact of Oxidative Stress on the Heart and Vasculature: Part 2 of a 3-Part Series. *J Am Coll Cardiol*. 2017;70(2):212-229. doi:10.1016/j.jacc.2017.05.035
- [21] Gyurászová M, Gurecká R, Bábíčková J, Tóthová Ľ. Oxidative Stress in the Pathophysiology of Kidney Disease: Implications for Noninvasive Monitoring and

Identification of Biomarkers. *Oxid Med Cell Longev*. 2020;2020:5478708. Published 2020 Jan 23. doi:10.1155/2020/5478708

[22] Nistala R, Whaley-Connell A, Sowers JR. Redox control of renal function and hypertension. *Antioxid Redox Signal*. 2008;10(12):2047-2089.

doi:10.1089/ars.2008.2034

[23] Cowley AW Jr. Renal medullary oxidative stress, pressure-natriuresis, and hypertension. *Hypertension*. 2008;52(5):777-786.

doi:10.1161/HYPERTENSIONAHA.107.092858

[24] Xu N, Jiang S, Persson PB, Persson EAG, Lai EY, Patzak A. Reactive oxygen species in renal vascular function. *Acta Physiol (Oxf)*. 2020;229(4):e13477.

doi:10.1111/apha.13477

[25] Reina-Couto M, Afonso J, Carvalho J, et al. Interrelationship between renin-angiotensin-aldosterone system and oxidative stress in chronic heart failure patients with or without renal impairment. *Biomed Pharmacother*. 2021;133:110938.

doi:10.1016/j.biopha.2020.110938

[26] Yokoyama S, Kawakami R, Tobiume A, et al. Time Course Changes in Urinary Angiotensinogen and Circulating N-Terminal Pro-B-Type Natriuretic Peptide in Patients Hospitalized with Acute Heart Failure. *Intern Med*. 2020;59(22):2839-2847.

doi:10.2169/internalmedicine.5212-20

[27] Yang X, Chen C, Tian J, et al. Urinary Angiotensinogen Level Predicts AKI in Acute Decompensated Heart Failure: A Prospective, Two-Stage Study. *J Am Soc Nephrol*. 2015;26(8):2032-2041. doi:10.1681/ASN.2014040408

[28] Rangaswami J, Bhalla V, Blair JEA, et al. Cardiorenal Syndrome: Classification, Pathophysiology, Diagnosis, and Treatment Strategies: A Scientific Statement From the American Heart Association. *Circulation*. 2019;139(16):e840-e878.

doi:10.1161/CIR.0000000000000664

[29] Chen C, Yang X, Lei Y, et al. Urinary Biomarkers at the Time of AKI Diagnosis as Predictors of Progression of AKI among Patients with Acute Cardiorenal Syndrome.

Clin J Am Soc Nephrol. 2016;11(9):1536-1544. doi:10.2215/CJN.00910116

[30] Zuchi C, Tritto I, Carluccio E, Mattei C, Cattadori G, Ambrosio G. Role of endothelial dysfunction in heart failure. *Heart Fail Rev*. 2020;25(1):21-30.

doi:10.1007/s10741-019-09881-3

- [31] Aimo A, Castiglione V, Borrelli C, et al. Oxidative stress and inflammation in the evolution of heart failure: From pathophysiology to therapeutic strategies. *Eur J Prev Cardiol.* 2020;27(5):494-510. doi:10.1177/2047487319870344
- [32] Verbrugge FH, Tang WH, Mullens W. Renin-Angiotensin-aldosterone system activation during decongestion in acute heart failure: friend or foe?. *JACC Heart Fail.* 2015;3(2):108-111. doi:10.1016/j.jchf.2014.10.005
- [33] Wysocki J, Batlle D. Urinary Angiotensinogen: A Promising Biomarker of AKI Progression in Acute Decompensated Heart Failure: What Does It Mean?. *Clin J Am Soc Nephrol.* 2016;11(9):1515-1517. doi:10.2215/CJN.07780716
- [34] Zhou LZ, Yang XB, Guan Y, et al. Development and Validation of a Risk Score for Prediction of Acute Kidney Injury in Patients With Acute Decompensated Heart Failure: A Prospective Cohort Study in China. *J Am Heart Assoc.* 2016;5(11):e004035. Published 2016 Nov 16. doi:10.1161/JAHA.116.004035
- [35] Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate [published correction appears in *Ann Intern Med.* 2011 Sep 20;155(6):408]. *Ann Intern Med.* 2009;150(9):604-612. doi:10.7326/0003-4819-150-9-200905050-00006
- [36] Simera I, Moher D, Hoey J, Schulz KF, Altman DG. A catalogue of reporting guidelines for health research. *Eur J Clin Invest.* 2010 Jan;40(1):35-53. doi: 10.1111/j.1365-2362.2009.02234.x. PMID: 20055895.
- [37] Kobori H, Alper AB Jr, Shenava R, et al. Urinary angiotensinogen as a novel biomarker of the intrarenal renin-angiotensin system status in hypertensive patients. *Hypertension.* 2009;53(2):344-350. doi:10.1161/HYPERTENSIONAHA.108.123802
- [38] Kobori H, Navar LG. Urinary Angiotensinogen as a Novel Biomarker of Intrarenal Renin-Angiotensin System in Chronic Kidney Disease. *Int Rev Thromb.* 2011;6(2):108-116.
- [39] Anatoliotakis N, Deftereos S, Bouras G, et al. Myeloperoxidase: expressing inflammation and oxidative stress in cardiovascular disease. *Curr Top Med Chem.* 2013;13(2):115-138. doi:10.2174/1568026611313020004
- [40] Matthijssen RA, Huugen D, Hoebers NT, et al. Myeloperoxidase is critically involved in the induction of organ damage after renal ischemia reperfusion. *Am J Pathol.* 2007;171(6):1743-1752. doi:10.2353/ajpath.2007.070184

- [41] Lehnert A, Lange S, Niemann G, et al. Myeloperoxidase deficiency ameliorates progression of chronic kidney disease in mice. *Am J Physiol Renal Physiol*. 2014;307(4):F407-F417. doi:10.1152/ajprenal.00262.2014
- [42] DeVore AD, Hernandez AF. From Theory to Practice: The Use of Real-World Data to Evaluate New Heart Failure Therapies. *JACC Heart Fail*. 2020;8(1):55-56. doi:10.1016/j.jchf.2019.09.010

Table 1.

Demographic, clinical and biochemical characteristics of study population at admission.

Demographic, clinical and biochemical parameters	Controls (n=22)	AHF (n=23)	Cardiogenic shock (n=25)	P value
Age (Years)	56±1	70±3**	58±4 ^{##}	0.0006
Gender: Men, n (%)	13 (59)	11 (48)	20 (80)	0.0637
Gender: Women, n (%)	9 (41)	12 (52)	5 (20)	0.0637
APACHE II Score	n/a	17±2	24±2	0.0075
SAPS II Score	n/a	37 (27; 41)	50 (40; 69)	0.0013
VA-ECMO, n (%)	n/a	0 (0)	11 (44)	0.0003
Precipitating factors on admission, n (%)				
Acute coronary syndrome	n/a	9 (39)	10 (40)	>0.9999
Arrhythmia	n/a	3 (13)	4 (16)	>0.9999
ADHF	n/a	5 (22)	3 (12)	0.3657
Valvular disease	n/a	2 (9)	1 (4)	0.5020
Infection	n/a	1 (4)	5 (20)	0.1014
Other	n/a	3 (13)	1 (4)	0.2574
Unknown	n/a	0 (0)	1 (4)	0.3324
Comorbidities, n (%)				
Arterial Hypertension	n.d.	18 (78)	12 (48)	0.0305
Diabetes	n.d.	13 (57)	8 (32)	0.0871
Obesity	n.d.	3 (13)	1 (4)	0.2574
Dyslipidemia	n.d.	13 (57)	4 (16)	0.0050
Chronic pulmonary disease	n.d.	3 (13)	2 (8)	0.5677
Chronic kidney disease	n.d.	4 (17)	1 (4)	0.1292
Cancer	n.d.	1 (4)	0 (0)	0.2921
Anemia	n.d.	12 (52)	17 (68)	0.2627
Cardiac biomarkers				
P-BNP (pg/mL)	n.d.	897 (417; 1542)	869 (321; 2691)	>0.9999
P-hs-trop I (ng/L)	n.d.	423 (114; 11586)	9311 (209;105413)	0.0304
Renal function				
eGFR (mL/min per 1.73 m ²)	77±4	71±6	70±5	0.5662
S-Urea (mg/dL)	n.d.	54 (37;77)	66 (44; 73)	0.4355
U-protein/creatinine (mg/mg)	n.d.	0.3 (0.1; 0.7)	0.4 (0.2; 1.1)	0.4575
Outcomes				
ICU length of stay (days)	n/a	4 (3;7)	10 (5; 21)	0.0021
Total hospital length of stay (days)	n/a	12 (9;18)	14 (6; 22)	0.7156
In-hospital mortality, n (%)	n/a	5 (22)	11 (44)	0.1323
Mortality post discharge (<12 months), n (%)	n/a	3 (17)	4 (29)	0.6691

ADHF, acute decompensated heart failure; APACHE II, acute physiology and chronic health evaluation II; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; n/a, not applicable; n.d., not determined; P-BNP, plasma B-type natriuretic peptide; P-hs-trop I, plasma high-sensitivity troponin I; SAPS II, Simplified Acute Physiology Score II; S-urea; serum urea; U-protein/creatinine, urinary protein/creatinine; VA-ECMO, venoarterial extracorporeal membrane oxygenation. Results are expressed as number (%), mean±SEM or as median (25th percentile; 75th percentile) for data with normal or non-normal distribution, respectively. **p<0.01 vs Controls; ^{##}p<0.01 vs AHF.

Table 2.

Admission values of biomarkers of vascular/endothelial dysfunction, inflammation and oxidative stress in Controls, AHF and CS.

Biomarkers	Controls	AHF	CS	<i>P</i> value
S-ICAM-1 (ng/mL)	300.6 (203.4; 759.3)	329.1 (264.3; 443.7)	559.5 (354.3; 990.7)* [#]	0.0056
S-VCAM-1 (ng/mL)	705.1 (516.2; 827.7)	1441 (1004;2257)***	2640 (1378; 4000)***	<0.0001
S-E-Selectin (ng/mL)	27.7 (22.6; 34.1)	31.0 (26.1; 42.7)	32.9 (23.2; 52.3)	0.2029
U-CysLT (pg/mL)	494.7 (373.2; 693.0)	794.9 (648.7; 1338.0)***	772.3 (632.3;1248.0)**	0.0002
S-TNF- α (pg/mL)	4.8 (3.6; 6.2)	7.2 (4.3; 13.5)	7.0 (5.1; 11.9)	0.0305
S-IL-1 β (pg/mL)	0.05 (0.00; 0.18)	0.07 (0.01; 0.32)	0.11 (0.02; 0.35)	0.2881
S-IL-6 (pg/mL)	0.7 (0.0; 2.4)	10.9 (5.3; 30.6)***	38.6 (20.1; 102.8)***	<0.0001
S-IL-10 (pg/mL)	0.0 (0.0; 1.2)	0.0 (0.0; 28.6)	21.1 (0.0; 72.6)**	0.0085
S-CRP (mg/L)	n.d.	39.8 (10.1; 77.0)	75.1 (46.5; 227.1)	0.0606
S-ADA (U/L)	n.d.	25.2 $\pm$ 1.9	21.0 $\pm$ 2.1	0.1425
S-MPO (ng/mL)	116.1 (75.9; 148.5)	335.9 (222.5; 512.7)***	379.5 (175.4; 536.9)***	<0.0001
U-Isop (ng/mg creatinine)	1.1 (0.7; 1.7)	2.2 (1.4; 2.6)***	1.9 (1.4; 2.5)***	0.0007

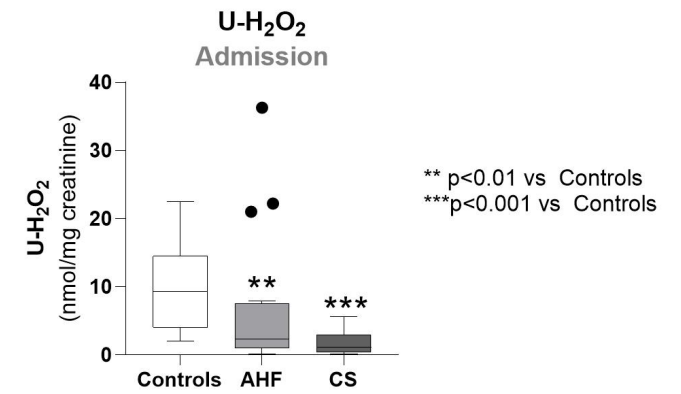
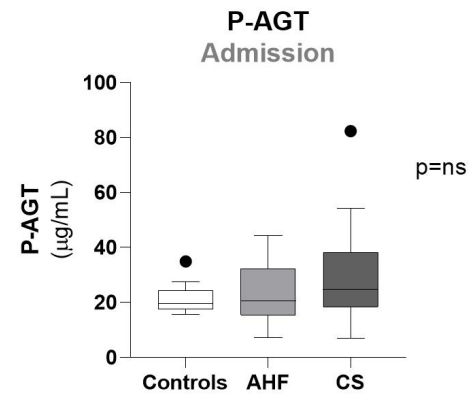
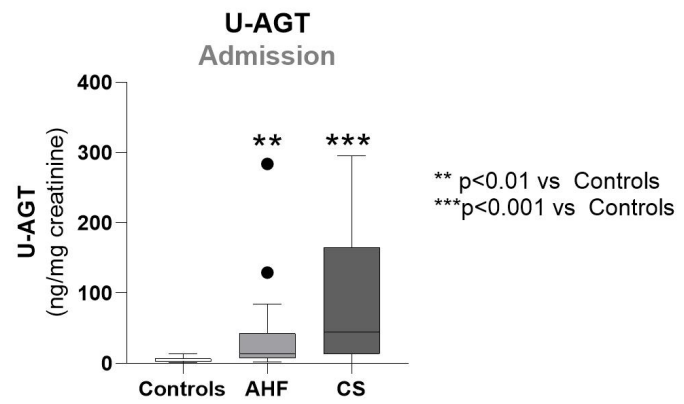
n.d., not determined; S-ADA, serum adenosine deaminase; S-CRP, serum C-reactive protein; S-ICAM-1, serum intercellular adhesion molecule 1; S-IL-1 β , serum interleukin 1 beta; S-IL-6, serum interleukin 6; S-IL-10, serum interleukin 10; S-MPO, serum myeloperoxidase; S-TNF- α , serum tumour necrosis factor alpha; S-VCAM-1, serum vascular cell adhesion molecule 1; U-CysLT, urinary cysteinyl leukotrienes; U-Isop, urinary isoprostanes. Results are expressed as mean $\pm$ SEM or as median (25th percentile; 75th percentile) for data with normal or non-normal distribution, respectively. **P*<0.05 vs Controls; ***P*<0.01 vs Controls; ****P*<0.001 vs Controls; [#]*P*<0.05 vs AHF.

Table 3.

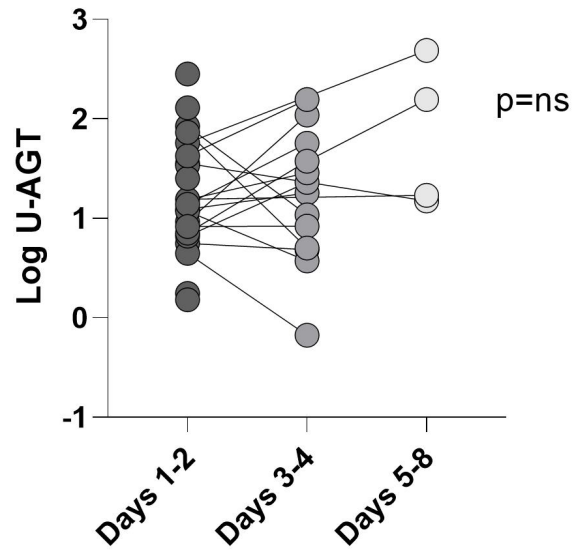
Spearman correlations for U-AGT, P-AGT and U-H₂O₂ in all groups or within patients (AHF and CS).

Biomarkers	Correlations Controls+AHF+CS		Biomarkers	Correlations Within patients	
	r Spearman	P value		r Spearman	P value
Vascular/Endothelial dysfunction & Inflammatory status			Vascular/Endothelial dysfunction & Inflammatory status		
U-AGT vs S-VCAM-1	0.444	<0.001	U-AGT vs S-VCAM-1	0.228	0.032
U-AGT vs U-CysLT	0.435	<0.001	U-AGT vs U-CysLT	0.288	0.010
U-AGT vs S-TNF- α	0.272	0.004	U-AGT vs S-IL-6	0.218	0.041
U-AGT vs S-IL-6	0.446	<0.001			
U-AGT vs S-IL-10	0.298	0.039			
U-AGT vs MPO	0.447	<0.001			
P-AGT vs S-VCAM-1	0.234	0.008	P-AGT vs S-CRP	0.319	0.001
P-AGT vs S-IL-6	0.208	0.020			
P-AGT vs S-MPO	0.211	0.017			
U-H ₂ O ₂ vs S-VCAM-1	-0.360	<0.001	U-H ₂ O ₂ vs S-TNF- α	0.214	0.049
U-H ₂ O ₂ vs S-IL-6	-0.387	<0.001	U-H ₂ O ₂ vs S-ADA	0.357	0.049
U-H ₂ O ₂ vs S-MPO	-0.357	<0.001			
ROS/Oxidative stress/RAAS			ROS/Oxidative stress/RAAS		
U-AGT vs U-H ₂ O ₂	-0.344	<0.001	P-AGT vs U-H ₂ O ₂	0.225	0.041
P-AGT vs U-Isop	0.199	0.047	U-H ₂ O ₂ vs U-Isop	0.275	0.016
U-AGT vs P-AGT	0.256	0.007			
Cardiac dysfunction/injury			Cardiac dysfunction/injury		
			P-AGT vs P-BNP	0.267	0.049
			U-H ₂ O ₂ vs P-hs-trop I	-0.254	0.021
Renal dysfunction			Renal dysfunction		
			U-AGT vs U-protein/creatinine	0.555	<0.001
			U-H ₂ O ₂ vs eGFR	-0.269	0.013
			U-H ₂ O ₂ vs S-Urea	0.286	0.009

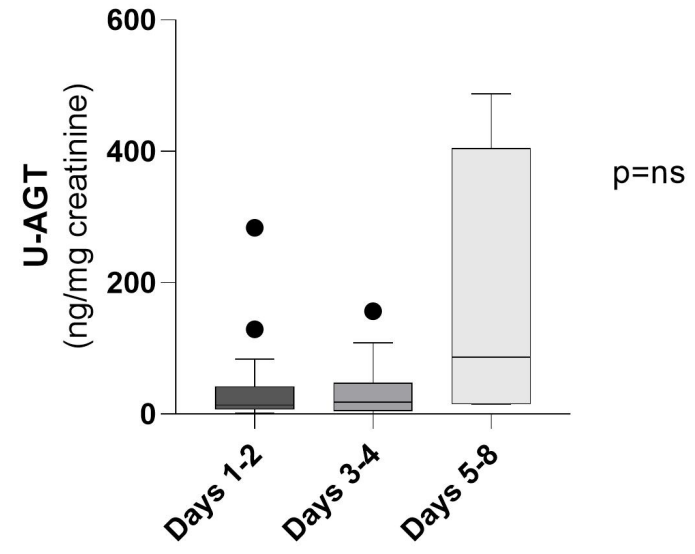
eGFR, estimated filtration glomerular rate; P-AGT, plasma angiotensinogen; P-BNP, plasma B-type natriuretic peptide; P-hs-trop I, high sensitivity troponin I; S-ADA, serum adenosine deaminase; S-CRP, serum C-reactive protein; S-IL-6, serum interleukin 6; S-IL-10, serum IL-10; S-MPO, serum myeloperoxidase; S-TNF- α , serum tumour necrosis factor alpha; S-Urea, serum urea; S-VCAM-1, serum vascular cell adhesion molecule 1; RAAS, renin-angiotensin aldosterone system; ROS, reactive oxygen species; U-AGT, urinary angiotensinogen; U-CysLT, urinary cysteinyl leukotrienes; U-H₂O₂; U-Isop, urinary isoprostanes.



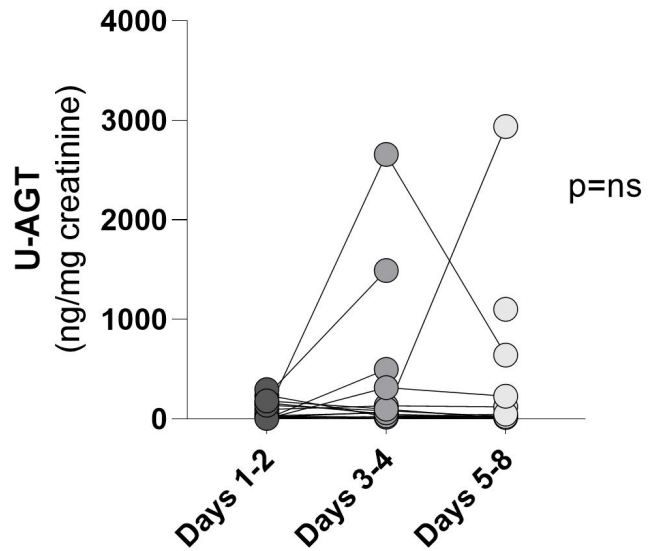
A AHF
Log U-AGT during hospitalization



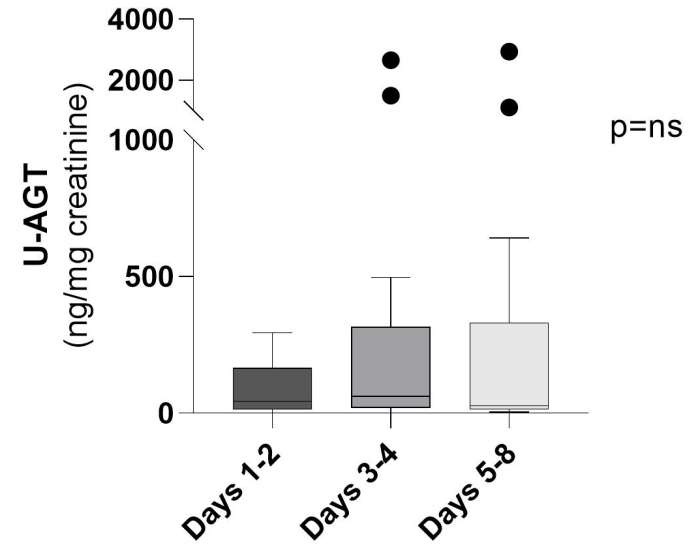
B AHF
U-AGT during hospitalization

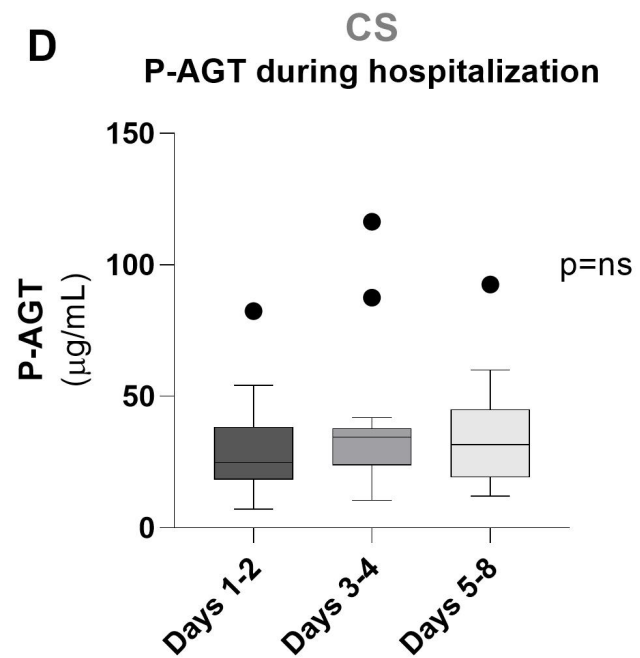
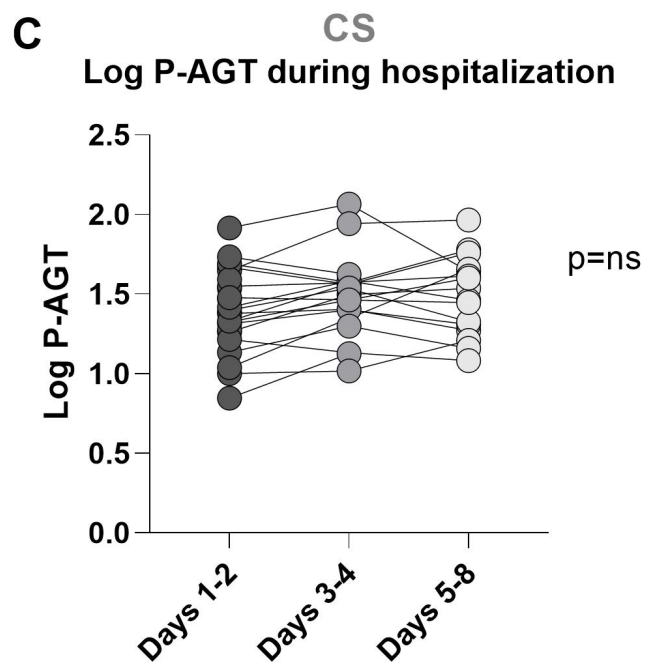
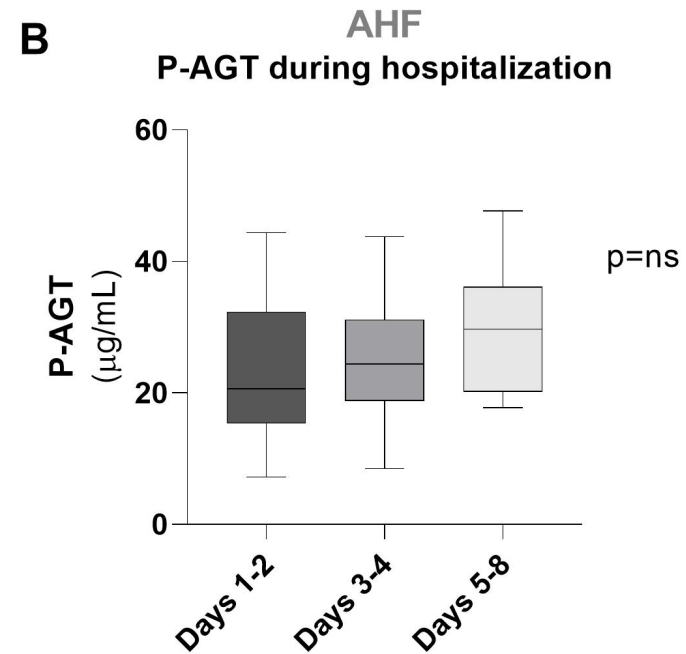
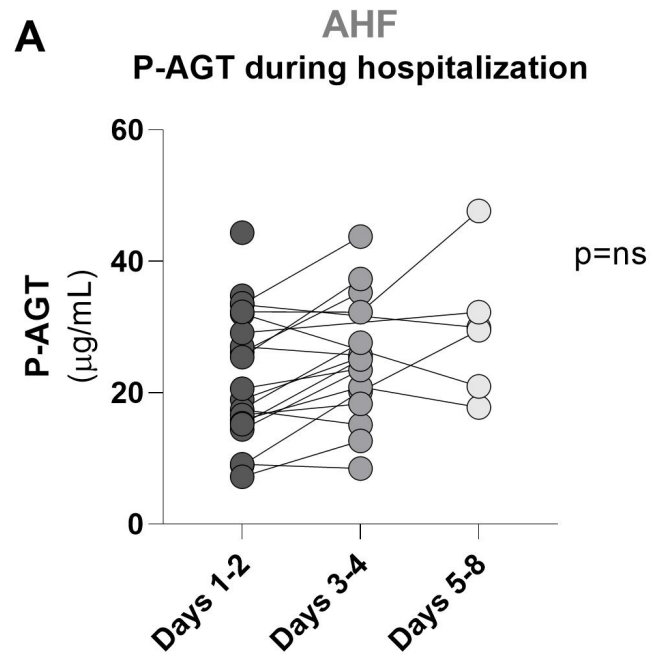


C CS
U-AGT during hospitalization

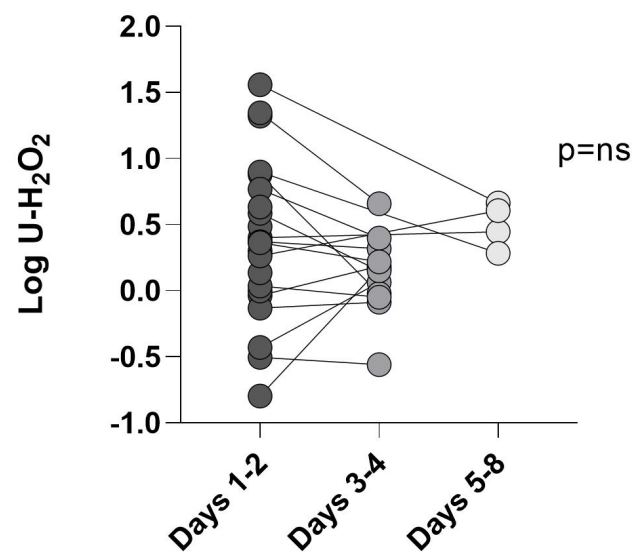


D CS
U-AGT during hospitalization

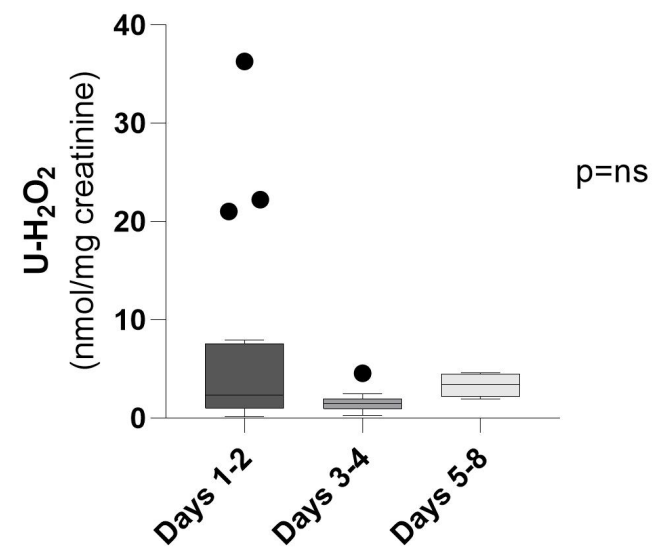




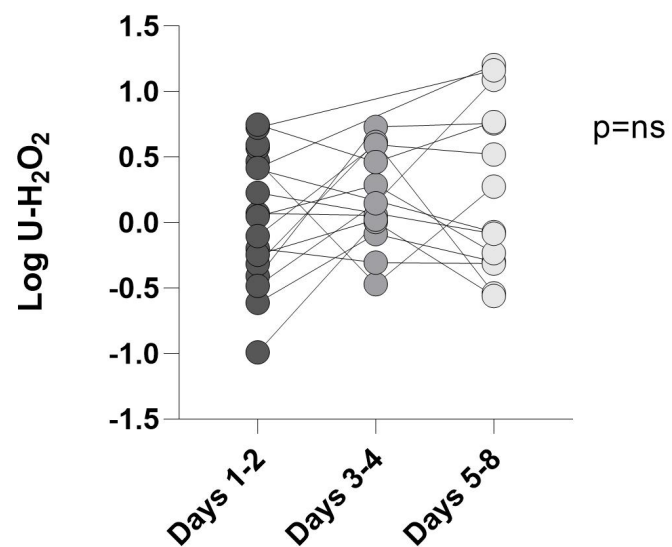
A AHF
Log U-H₂O₂ during hospitalization



B AHF
U-H₂O₂ during hospitalization



C CS
Log U-H₂O₂ during hospitalization



D CS
U-H₂O₂ during hospitalization

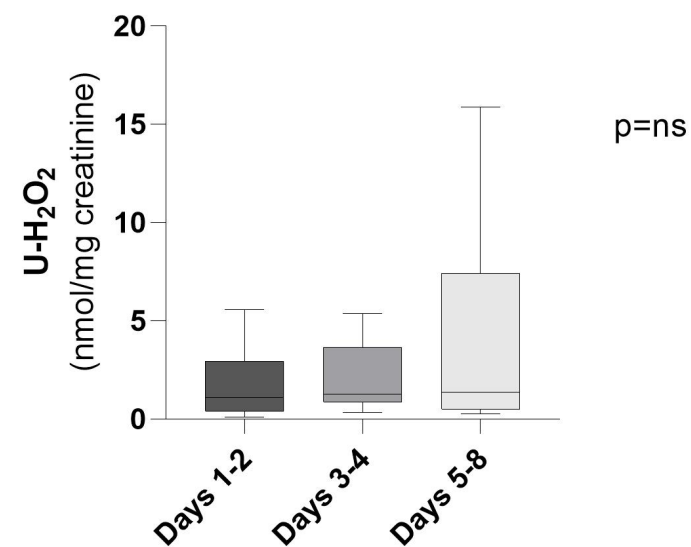


Figure Legends

Figure 1. Admission values of U-AGT, P-AGT and U-H₂O₂ in controls and in patients with AHF and CS. Results are presented in Box and Whiskers plots. **p<0.01 vs controls; ***p<0.001 vs controls.

Figure 2. U-AGT profile during hospitalization: (A) Evolution for each AHF patient; (B) Box and Whiskers plot for each time period in AHF patients; (C) Evolution for each CS patient; (D) Box and Whiskers plot for each time period in AHF patients.

Figure 3. P-AGT profile during hospitalization: (A) Evolution for each AHF patient; (B) Box and Whiskers plot for each time period in AHF patients; (C) Evolution for each CS patient; (D) Box and Whiskers plot for each time period in AHF patients.

Figure 4. U-H₂O₂ profile during hospitalization: (A) Evolution for each AHF patient; (B) Box and Whiskers plot for each time period in AHF patients; (C) Evolution for each CS patient; (D) Box and Whiskers plot for each time period in AHF patients.

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 3: “...We aimed to investigate plasma and urinary angiotensinogen (P-AGT; U-AGT, an intrarenal RAAS marker) and urinary H₂O₂ (U-H₂O₂) in AHF and CS patients. Furthermore, we evaluated the correlations between them, as well as their association with endothelial dysfunction, inflammation, oxidative stress and cardiac and renal impairment. Methods: Patients with AHF (n=23) or CS (n=25) and healthy controls (n=22) were included. U-AGT, P-AGT, U-H₂O₂, endothelial dysfunction, inflammation, oxidative stress and cardiac/renal impairment markers were evaluated at days 1-2 (admission), days 3-4 and days 5-8 in AHF and CS patients and at a single time point in controls. Results: At admission, AHF and CS patients had similar P-AGT, higher U-AGT and lower U-H₂O₂ than controls. There were no changes in P-AGT, U-AGT or U-H₂O₂ along hospitalization. P-AGT and U-AGT positively correlated with endothelial dysfunction, inflammation and oxidative stress, while U-H₂O₂ negatively correlated with these biomarkers and with U-AGT in the overall study population. Among patients, U-H₂O₂ positively correlated with inflammation, oxidative stress and P-AGT, and both U-AGT and U-H₂O₂ were associated with renal impairment. Conclusions: AHF and CS patients appear to have intrarenal RAAS activation and increased H₂O₂ consumption, probably by myeloperoxidase. Furthermore, their association with renal dysfunction is likely related to endothelial injury, inflammation and oxidative stress.”
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Pages 6 to 8: “Acute heart failure (AHF) is caused by the rapid onset or aggravation of a cardiac incapacity to pump the enough amount of blood per minute in order to supply the needs of the body's organs [1]. Ultimately, it may present as cardiogenic shock (CS), its most severe manifestation, which frequently results in multi-organ failure and consequent death [2]. AHF has an estimated incidence of 5.7 per 1000 person-years [3], being accountable for more than 1 million hospitalizations per year in Europe [4]. In Portugal, recent data point to an in-hospital mortality rate of 13% for AHF and a 30-day readmission rate between 9 and 14% [5]. In what concerns CS, it represents 2-5% of AHF presentations, with an in-hospital mortality that goes up to 50% [2]. The context in which AHF develops is of high complexity, encompassing an interplay among the dysfunction of the cardiac, pulmonary, renal and vascular systems. Several mechanisms, including neurohormonal activation (sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS) and inflammation are part of this intricate pathophysiology, being accompanied by an increase in reactive oxygen species (ROS). These mechanisms are interlinked and perpetuate each other, which leads to the aggravation of cardiac and renal damage, resulting in compromised function [6, 7]. The RAAS integrates the communication between the cardiovascular, renal and central nervous systems, regulating circulation volume and arterial pressure. Briefly, renin converts angiotensinogen (AGT) to angiotensin I (Ang I), which is consequently converted to angiotensin II (Ang II) by angiotensin-converting enzyme (ACE). Physiologically, Ang II stimulates the angiotensin II type 1 (AT₁) receptor, triggering aldosterone secretion from the adrenal glands, renal tubular sodium and water reabsorption, systemic arterial and renal arteriolar constriction, vascular smooth muscle contraction and, locally, myocyte

hypertrophy [8]. Besides acting as an endocrine regulator, the RAAS is now seen as a paracrine or autocrine system, given the production of AGT and Ang II within tissues as the myocardium and the kidney [9, 10, 11]. In heart failure (HF), sustained activation of the RAAS contributes to pathological left ventricle remodelling characterized by hypertrophy, myocyte loss and fibrosis [11] and might contribute to stratify AHF patients at increased cardiovascular risk [12]. Accordingly, RAAS blockade is the first line therapy for HF with reduced ejection fraction (HFrEF), mainly accomplished through ACE inhibitors (ACEi), angiotensin receptor blockers (ARB) or angiotensin receptor-neprilysin inhibitors (ARNi) [13].

Oxidative stress results from an imbalance between the formation of ROS and/or reactive nitrogen species and their clearance by endogenous antioxidant defense mechanisms [14]. Major sources of ROS include mitochondrial oxidases and other prooxidant enzymes, such as NADPH oxidases (NOX), xanthine oxidase (XO), uncoupled endothelial nitric oxide synthase (eNOS), myeloperoxidase (MPO) and monoamine oxidases (MAO) [15]. MPO is one of the most studied enzymes in human HF, being considered an important link between oxidative stress and inflammation. Under inflammatory conditions, the MPO released by activated neutrophils and monocytes uses hydrogen peroxide (H₂O₂) derived by leukocyte oxidative burst and other ROS sources (e.g. vascular NOX) to produce HClO and other potent oxidant species that can cause significant damage in cardiovascular and renal tissues. Importantly, MPO seems to be an incremental prognostic biomarker for HF or cardiovascular death among patients with acute myocardial infarction. In addition, it appears to help in identifying forms of AHF with cardiorenal syndrome [15-17].

In physiological conditions, ROS are involved in signalling processes regulating cell and organ function. However, persistently elevated ROS concentrations are potentially pathological by promoting not only macromolecule damage, including the oxidation of proteins, lipids and DNA, but also the dysregulation of redox-controlled processes, thus contributing to a vast array of human diseases [15, 18]. In the heart, ROS excess elicits mitochondrial damage and dysfunction, contractile dysfunction, adverse myocardial remodelling, inflammation, endothelial dysfunction and ischemic cardiomyopathy [15, 19, 20]. Regarding the kidney, ROS regulate renal haemodynamics, diuresis and natriuresis, cell growth and differentiation, production and breakdown of the extracellular matrix, inflammation and integrate the immune system defense. At the same time, abnormal accumulation of ROS promote renal dysfunction and tissue damage, contributing to acute kidney injury and pathogenesis of chronic kidney disease [21-24].

Ang II stimulates the production of ROS, which mediate many of the RAAS deleterious effects in cardiovascular and renal function. Noteworthy, besides being downstream mediators of RAAS, there is evidence that ROS also regulate RAAS activation and that, in pathological conditions such as HF and/or renal disease, the interrelationship between these pathways creates a vicious cycle that amplifies their deleterious impact, perpetuating the cardiac and renal damage [18]. Interestingly, we recently reported that chronic HF (CHF) patients with preserved renal function showed mostly inverse correlations between oxidative stress and RAAS markers, while CHF patients with impaired renal function (iRF) exhibited several positive correlations between these pathways, including significant correlations of urinary H₂O₂ (U-H₂O₂) with plasma renin concentration or plasma AGT (P-AGT) concentration, of urinary isoprostanes with P-AGT or serum ACE activity, and a borderline significant correlation between U-H₂O₂ and urinary AGT (U-AGT) [25].

U-AGT has been used as a biomarker of intrarenal RAAS activity [10]. Yokoyama et al suggested that the time-course changes in U-AGT/creatinine ratio can be used as a monitoring tool for AHF patients [26]. In AHF, U-AGT predicts prognosis at 1 year and a higher risk of development of acute kidney injury (AKI) in this

			circumstance [27], defined as cardiorenal syndrome type 1 [28]. Also, Chen et al underlined that U-AGT values at the time of AKI diagnosis in AHF were an indicator of AKI progression and death [29]. Oxidative stress and RAAS are also implicated in endothelial dysfunction and inflammation, two other important mechanisms involved in HF pathophysiology. ROS directly contribute to endothelial dysfunction by inactivating nitric oxide (NO) and by promoting eNOS uncoupling, which further elevates O₂•- [30]. Moreover, increased production of ROS leads to the upregulation of chemokines, activation of neutrophil integrins, and expression of surface adhesion molecules by endothelial cells, which are inflammatory mechanisms promoting pathological cardiac remodelling. Furthermore, inflammatory molecules, such as TNF-α, interleukin 6 (IL-6) and TGF-β increase ROS generation [31]. On the other hand, decreased NO concentration in the context of endothelial dysfunction favors the profibrotic signalling induced by Ang II and aldosterone in the myocardium [30]. Additionally, the RAAS also stimulates the production of adhesion molecules and cytokines and the polarization of macrophages to the M1 pro-inflammatory subtype, contributing to inflammation [31].”
Objectives	3	State specific objectives, including any prespecified hypotheses	Pages 8 and 9: “Although important research has been conducted with the intent to characterize the systemic [12, 32] and the intrarenal [33] RAAS in AHF, there are no studies on the relationship between these two systems and oxidative stress in AHF or CS. This could lead to a better understanding of AHF pathophysiology, helping to improve the already existing risk scores [34] and bringing new insights into the management of the disease. Therefore, the present study aimed to evaluate U-AGT and P-AGT and U-H₂O₂ at admission and during hospitalization, as well as their relationship, both in AHF and CS patients. Moreover, we also assessed their association with markers of inflammation, endothelial dysfunction, oxidative stress, cardiac and renal dysfunction.”
Methods			
Study design	4	Present key elements of study design early in the paper	Page 9: “This study is part of a larger research project (RIFF-HEART) involving patients from the Intermediate & Intensive Care Units (ICU) of the Department of Intensive Medicine with the diagnosis of AHF and CS. We performed a single-center cohort study...”
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 9: “...consecutively recruited patients with a single diagnosis of AHF (n=23) or CS (n=25) admitted to the ICU of Department of Intensive Medicine of Centro Hospitalar Universitário São João (CHUSJ), from January 2017 to December 2019. Controls (n=22) were recruited among healthy blood donors volunteers from the Department of Immunohemotherapy of CHUSJ, from September 2017 to October 2017.”
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (b) For matched studies, give matching criteria	Page 9: “...consecutively recruited patients with a single diagnosis of AHF (n=23) or CS (n=25) admitted to the ICU of Department of Intensive Medicine of Centro Hospitalar Universitário São João (CHUSJ), from January 2017 to December 2019. Controls (n=22) were recruited among healthy blood donors volunteers from the Department of Immunohemotherapy of CHUSJ, from September 2017 to October 2017.”

		and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Define all outcomes, exposure, predictors (...): Not applicable. Diagnostic criteria: Page 9 : "recruited patients with a single diagnosis of AHF (n=23) or CS (n=25) admitted to the ICU of Department of Intensive Care Medicine of Centro Hospitalar Universitário São João (CHUSJ) (...) Controls (n=22) were recruited among healthy blood donors volunteers from the Department of Immunohemotherapy of CHUSJ".
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Pages 9 to 11: "Blood and spot urine samples were collected in patients (AHF and CS groups) at 3 different time points during ICU stay, whenever possible: up to 48 hours (days 1-2, admission), on days 3 or 4 and on days 5 to 8 after ICU admission. Samples (blood and spot urine) from controls were collected at a single time point. All samples were processed within 1-2 hours of collection and stored at -80 oC until assayed. Routine clinical biomarkers Plasma B-type natriuretic peptide (P-BNP) and high-sensitivity troponin I (P-hs-trop I) were measured by chemiluminescent microparticle immunoassays using an Abbot® Architect i2000 automated analyser (Abbott® Diagnostics, Lake Forest, IL, USA). An Olympus Beckman Coulter® AU5400 automated clinical chemistry analyser (Olympus, Hamburg, Germany) was used for the quantification of serum C-reactive protein (S-CRP) by an immunoturbidimetric assay, serum adenosine deaminase activity (ADA) by an enzymatic assay, serum urea (S-Urea) concentration by a kinetic urease/glutamate dehydrogenase method, plasma and urine creatinine by the colorimetric Jaffe method and urinary protein by the pyrogallol red method. Quantification of H2O2 and angiotensinogen We measured H2O2 in spot urine samples (U-H2O2) using a microplate fluorimetric assay (Amplex® Red Hydrogen Peroxide/Peroxidase Assay Kit; Molecular Probes, Invitrogen, Thermo Fisher Scientific, Massachusetts, USA), following the manufacturer's instructions. U-H2O2 values were further normalized to urinary creatinine concentrations. Angiotensinogen was quantified in plasma (P-AGT) and spot urine (U-AGT) samples using an enzyme-linked immunosorbent assay test (Human Total Angiotensinogen Assay Kit, Immuno-Biological Laboratories Co., Gunma, Japan), following the manufacturer's

			instructions. U-AGT values were corrected for urinary creatinine content. Evaluation of biomarkers of vascular/endothelial dysfunction, inflammation and oxidative stress Serum endothelium activation markers (intercellular adhesion molecule 1, S-ICAM-1; vascular cell adhesion molecule 1, S-VCAM-1; E-Selectin, S-E-Selectin), and proinflammatory cytokines (tumour necrosis factor alpha, S-TNF-α; interleukin 1 beta, S-IL-1β; interleukin 6, S-IL-6) were evaluated by multiplex immunoassays using with a Luminex 200 analyzer (Luminex Corporation, Austin, TX, USA), according to the protocols of Luminex Human Magnetic Assay (R&D Systems, Inc., Minneapolis, USA) and MILLIPLEX[®] MAP Human High Sensitivity T Cell Magnetic Bead Panel (Millipore Corporation, Billerica, MA, USA), respectively. Raw data analysis (mean fluorescence intensity) was performed using ISTM 2.3 software (Luminex Corporation). Serum interleukin 10 (S-IL-10) and serum myeloperoxidase (S-MPO) were quantified by enzyme immunoassays using commercial kits (Thermo Scientific[™] Human Interleukin-10 (IL-10) ELISA Kit, Thermo Scientific, Rockford, Illinois, USA; BioCheck MPO Enzyme Immunoassay, Oxis International Inc., Foster City, CA, USA). Urinary cysteinyl leukotrienes (U-CysLT) were measured in unextracted spot urine samples (U-CysLT) by a competitive enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Cysteinyl Leukotriene ELISA kit, Cayman Chemical Company, Ann Arbor, MI, USA). U-CysLT values were further normalized to urinary creatinine content. Urinary isoprostanes (U-Isop) were quantified by a competitive enzyme immunoassay (Urinary Isoprostane ELISA Kit; Oxford Biomedical Research [®] Inc., Oxford, MI, USA) in non-extracted spot urine containing the preservative butylated hydroxy toluene (BHT, 0.005%, w/v) added before storage. U-Isop values were corrected for urinary creatinine concentrations.”
Bias	9	Describe any efforts to address potential sources of bias	Page 11: “To prevent possible bias in clinical evaluation, all the patients were examined by the same medical team included in the project. However, we cannot exclude bias from eventual patients’ selection (severe patients) because acute cardiac patients with non-invasive ventilation can also be distributed to the cardiac unit of Cardiology Department in the Hospital.” Page 12: “To avoid biasing the results, no imputation for missing values was used.”

Study size	10	Explain how the study size was arrived at	Page 12: “Sample size was defined according to the RIFF-HEART project’s primary objectives that consisted in characterizing resolution of inflammation and endothelitis at admission and during hospitalization.”
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 11: “Results are expressed as mean±standard error of the mean (SEM) or as median (25th percentile; 75th percentile) for data with normal or non-normal distribution, respectively, or as percentage, and are graphically represented as Box and Whiskers plots. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [35].”
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, explain how loss to follow-up was addressed (e) Describe any sensitivity analyses	Pages 11 and 12: “Statistical analysis was performed using unpaired Student’s t-test or Mann–Whitney U-test, for comparisons between two groups, or by one-way ANOVA followed by a Tukey’s multiple comparison test or a Kruskal Wallis test followed by a Dunn’s post hoc test, where appropriate, for comparison between three groups. Categorical variables were analysed by the Fisher’s exact test or by the Chi Square test. U-AGT, P-AGT and U-H2O2 evolution throughout the hospitalization was evaluated by a paired analysis using a mixed-effects model (Restricted Maximum Likelihood, REML), after applying a base 10 log transformation whenever appropriate, and also by an unpaired Kruskal Wallis test to compare the medians in each time point. P values of <0.05 were considered significant. Spearman’s correlation analysis was used to estimate correlations between sets of nonparametric data in the study population (Controls; AHF and CS patients throughout hospitalization). To prevent possible bias in clinical evaluation, all the patients were examined by the same medical team included in the project. However, we cannot exclude bias from eventual patients’ selection (severe patients) because acute cardiac patients with non-invasive ventilation can also be distributed to the cardiac unit of Cardiology Department in the Hospital. To assure comparability of biomarkers assessment, samples from Controls, AHF and CS groups were evenly distributed in each assay plate. There were missing values in some biomarkers due to insufficient sample volume of samples or reagents to perform sample processing, dilution tests and assays. In addition, some routine clinical biomarkers could only be assessed at a single time point during hospitalization due to hospital’s internal policies, and we had not permission to measure routine clinical biomarkers in controls (blood donor volunteers), with the exception of creatinine, or to have access

			to their hospital laboratory reports. The final number per group for the biomarkers/parameters evaluated at admission was as following: P-BNP, n=20 vs 17 (AHF vs CS); P-hs-trop I, n=23 vs 23 (AHF vs CS); eGFR, n=22 vs 23 vs 23 (Controls vs AHF vs CS); S-Urea, n=23 vs 23 (AHF vs CS); U-protein/creatinine ratio, n=22 vs 15 (AHF vs CS); S-ICAM-1, n =22 vs 23 vs 24 (Controls vs AHF vs CS); S-VCAM-1, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-E-Selectin, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-TNF-α, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-IL-1β, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-IL-6, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-MPO, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-IL-10, n=22 vs 23 vs 24 (Controls vs AHF vs CS); S-CRP, n= 23 vs 23 (AHF vs CS); S-ADA, n=13 vs 12 (AHF vs CS) U-AGT, n=22 vs 23 vs 18 (Controls vs AHF vs CS); P-AGT, n=22 vs 23 vs 24 (Controls vs AHF vs CS), U-H2O2, n=22 vs 23 vs 19 (Controls vs AHF vs CS); U-CysLT, n=22 vs 22 vs 19 (Controls vs AHF vs CS); U-Isop, n=22 vs 21 vs 17 (Controls vs AHF vs CS).”
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	Page 9: “Study design and population This study is part of a larger research project (RIFF-HEART) involving patients from the Intermediate & Intensive Care Units (ICU) of the Department of Intensive Care Medicine with the diagnosis of AHF and CS. We performed a single-center cohort study and consecutively recruited patients with a single diagnosis of AHF (n=23) or CS (n=25) admitted to the ICU of Department of Intensive Care Medicine of Centro Hospitalar Universitário São João (CHUSJ), from January 2017 to December 2019. Controls (n=22) were recruited among healthy blood donors volunteers from the Department of Immunohemotherapy of CHUSJ, from September 2017 to October 2017.”
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic,	Pages 12 and 13: “General demographic characteristics, clinical and biochemical parameters are shown in Table 1. In this study, 22 healthy controls and 48 patients, 23 with AHF and 25 with CS, were assessed. The CS group had predominantly male patients, compared to AHF, although no significant differences were found among the studied groups. Patients in the AHF group were

		clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	significantly older than controls and CS patients. APACHE II and SAPS II scores were significantly higher in the CS group, in which almost half the patients were on VA-ECMO.”
Outcome data	15*	Report numbers of outcome events or summary measures over time	Page 13: “CS patients had a longer length of stay in the ICU than patients with AHF, but there were no differences between these groups regarding the hospital total length of stay and mortality outcomes.” Page 14: “There were no significant changes in U-AGT values throughout hospitalization in AHF and CS groups whether considering a paired (Figure 2A and 2C) or unpaired analysis (Figure 2B and 2D). Also, there were no significant changes in P-AGT values throughout hospitalization in AHF patients (Fig. 3A for paired and 3B for unpaired analysis) or CS patients (Fig. 3C for paired and 3D for unpaired analysis). Moreover, we did not observe significant changes in U-H ₂ O ₂ values throughout hospitalization in AHF and CS patients whether considering a paired (Figure 4A and 4C) or unpaired analysis (Figure 4B and 4D).”
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Pages 13-15: “Vascular/endothelial dysfunction, inflammation and oxidative stress biomarkers Concerning vascular and endothelial dysfunction biomarkers, we found significantly higher concentration of S-ICAM-1 in CS patients compared to AHF patients and controls (p<0.05) (Table 2). S-VCAM-1 was increased by 2-fold in AHF and almost by 4-fold in CS patients compared to controls (p<0.001), but no differences were found in S-E-Selectin values among the groups (Table 2). U-CysLT were significantly increased in AHF (p<0.001) and CS (p<0.01) groups relative to controls (Table 2). Patients with AHF or CS exhibited higher values of inflammatory markers such as S-TNF- α (overall p value= 0.0305), S-IL-6 (p<0.001) and S-MPO (p<0.001) compared to controls (Table 2). A significant increase in S-IL-10 concentration was observed only in CS patients (p<0.01 vs controls) (Table 2). There were no significant differences in S-CRP or S-ADA values between AHF and CS groups (Table 2). AHF and CS patients had higher oxidative stress than controls as demonstrated by significantly raised U-Isop values (p<0.001) (Table 2). U-AGT, P-AGT and U-H ₂ O ₂ at admission U-AGT was significantly higher and U-H ₂ O ₂ was markedly lower in AHF and CS at ICU admission, with these changes being more marked in CS (Figure 1A and 1C). No significant differences between groups were seen for P-AGT values (Figure 1B). U-AGT, P-AGT and U-H ₂ O ₂ profile during hospitalization There were no significant changes in U-AGT values throughout hospitalization in AHF and CS groups whether considering a paired

			(Figure 2A and 2C) or unpaired analysis (Figure 2B and 2D). Also, there were no significant changes in P-AGT values throughout hospitalization in AHF patients (Fig. 3A for paired and 3B for unpaired analysis) or CS patients (Fig. 3C for paired and 3D for unpaired analysis). Moreover, we did not observe significant changes in U-H2O2 values throughout hospitalization in AHF and CS patients whether considering a paired (Figure 4A and 4C) or unpaired analysis (Figure 4B and 4D). Correlation analysis in the overall study population Both U-AGT and P-AGT showed significant positive correlations with markers of vascular and endothelial dysfunction (U-AGT vs S-VCAM-1; U-AGT vs U-CysLT; P-AGT vs S-VCAM-1) and inflammation (U-AGT vs S-TNF-α; U-AGT vs S-IL-6; U-AGT vs IL-10; U-AGT vs S-MPO; P-AGT vs S-IL-6; P-AGT vs S-MPO) (Table 3). U-H2O2 presented inverse correlations with endothelial dysfunction (U-H2O2 vs S-VCAM-1) and inflammation markers (U-H2O2 vs S-IL-6; U-H2O2 vs S-MPO) (Table 3). Regarding the correlations within and between RAAS and ROS/oxidative stress markers, there were significant positive correlations for U-AGT with P-AGT and for P-AGT with U-Isop, but an inverse correlation was observed between U-AGT and U-H2O2 (Table 3). Correlation analysis within patients When analysing correlations only in patients, we still observed significant positive correlations of U-AGT with biomarkers of vascular and endothelial dysfunction (U-AGT vs S-VCAM-1; U-AGT vs U-CysLT) and inflammation (U-AGT vs S-IL-6) (Table 3). P-AGT was also positively correlated with inflammation (P-AGT vs S-CRP) (Table 3). The inverse correlations between U-H2O2 and inflammatory status were not observed when excluding the control group from the analysis. Instead, U-H2O2 showed significant positive correlations with inflammatory markers such as S-TNF-α and S-ADA (Table 3). U-H2O2 also presented a positive correlation with U-Isop, which in turn were positively correlated with P-AGT (Table 3). There was a significant positive correlation between P-AGT and P-BNP and an inverse correlation between U-H2O2 and P-hs-troponin I (Table 3). Both U-AGT and U-H2O2 were related with renal dysfunction, as evidenced by the positive correlations of U-AGT with U-protein/creatinine and U-H2O2 with S-Urea and by the inverse correlation between U-H2O2 and eGFR (Table 3)."
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 15: "The major findings of our study are that both AHF and CS patients present intrarenal RAAS activation and oxidative stress, which are associated with renal dysfunction, inflammation and endothelial injury. Moreover, our results also point to a marked H2O2 consumption in these patients, probably by MPO." Page 15: "The correlation analysis involving all the studied groups revealed a negative correlation of U-AGT with U-H2O2 values, which were shown to be diminished in AHF and CS patients when compared with controls, although U-Isop concentrations were higher, reflecting increased oxidative stress. This negative correlation between U-AGT and U-H2O2 levels was somehow intriguing since one would expect

			that the activation of intrarenal RAAS observed in AHF and CS patients contributed to their increased inflammatory status and oxidative stress.”
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 17: “Our study presents as a major strenght the fact that we could study AHF and CS patients in the “real-world conditions” that represent the exact clinical setting in which patients are treated; a situation by far very different from the controlled and selected environment of clinical trials [42]. This major strenght might also be seen as a limitation of the study since the multiplicity of etiologies and comorbidities, as well as drug treatments, makes the analysis of data much more complex. However, we endorse the view that “real-world conditions” have a higher potential for fulfil the needs of patients with AHF and/or CS. As any, our study has some limitations, particularly its retrospective single-center design advises the translation of results to be cautious.”
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 15 to 17: “Patients with AHF and CS showed activation of the intrarenal RAAS, as evaluated by U-AGT, which is in accordance with previous studies in cardiovascular diseases as hypertension [37] and chronic kidney disease [38] as well as HFrEF [11]. U-AGT was positively correlated with several markers of vascular/endothelial dysfunction and inflammatory status and also with markers of renal dysfunction within AHF and CS. Moreover, in the overall study population, U-AGT was also positively correlated with P-AGT values, probably reflecting the connection between systemic and renal AGT [10]. In addition, P-AGT was positively associated with both U-Isop and markers of vascular/endothelial dysfunction and inflammation. Collectively, the positive correlations between U-AGT or P-AGT and markers of vascular/endothelial dysfunction and inflammation further reinforce the pathophysiological relevance of RAAS in HF [8, 10, 30, 31]. The correlation analysis involving all the studied groups revealed a negative correlation of U-AGT with U-H2O2 values, which were shown to be diminished in AHF and CS patients when compared with controls, although U-Isop concentrations were higher, reflecting increased oxidative stress. This negative correlation between U-AGT and U-H2O2 levels was somehow intriguing since one would expect that the activation of intrarenal RAAS observed in AHF and CS patients contributed to their increased inflammatory status and oxidative stress. Indeed, as we have recently reviewed, RAAS and ROS are interlinked and in pathological conditions perpetuate a feedforward mechanism that contributes to disease progression and severity [18]. Also, in our previous study with CHF patients, we observed a positive correlation between U-AGT and U-H2O2 (25). The overall redox state of AHF and CS patients is imbalanced, as indicated by their higher U-Isop levels. Noteworthy, in the overall study population, U-H2O2 also presented significant negative correlations with systemic inflammatory markers, including S-MPO, S-IL-6 and also the endothelial adhesion molecule, S-VCAM-1, which were all significantly increased in AHF and CS when compared to controls. Therefore, the marked inflammation and oxidative stress exhibited by these patients is more compatible with the view that diminished values of U-H2O2 result from increased consumption, probably by MPO which is know to use H2O2 as a substrate to produce potent oxidants that contribute to tissue damage in HF. U-AGT, P-AGT and U-H2O2 levels were not altered along hospitalization, from admission (days 1-2), to days 3-4 and days 5-8. Current evidence suggests that U-AGT remain high during hospitalization in patients with AHF who develop AKI, despite a trend for a decrease. In contrast, AHF patients who do not develop AKI exhibit constantly lower values during hospitalization in those AHF patients who do not develop AKI [27]. Furthermore, in acute decompensated HF, U-AGT is a strong predictor of cardiorenal syndrome diagnosis [27] and progression [29] and of 1-year prognosis

			[27]. With respect to P-AGT, previous results also show stable concentrations during hospitalization of AHF patients, both in those with and without AKI [27]. Furthermore, as far as we know, our study is the first to evaluate U-H2O2 values during the hospitalization period of AHF and CS patients. In agreement with data showing that U-AGT predicts the decline in kidney function, we found a positive correlation between U-AGT and U-Protein/creatinine on admission. Also, U-H2O2 was negatively correlated to eGFR and positively correlated to S-Urea which corroborates current evidence on the deleterious effect of ROS on renal function [5]. As pointed out above, increased consumption of U-H2O2 by MPO might produce other ROS, like HClO [39], which can contribute to the deleterious effect on renal function [40, 41]. Indeed, in patients with AHF and CS, we observed positive correlations between U-H2O2 and oxidative stress (U-Isop) and inflammatory markers (S-TNF-α). Also, U-AGT was positively correlated with markers of endothelial dysfunction and inflammation. Overall, these results suggest that U-AGT and U-H2O2 association with renal dysfunction involves endothelial injury, inflammation and oxidative stress. Regarding cardiac biomarkers, we observed that P-AGT and P-BNP were positively correlated, expanding the already described relationship between the RAAS and P-BNP in AHF patients [12].”
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 17: “Our study presents as a major strenght the fact that we could study AHF and CS patients in the “real-world conditions” that represent the exact clinical setting in which patients are treated; a situation by far very different from the controlled and selected environment of clinical trials [42].”
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 18: “FUNDING This work was supported by FEDER funds via COMPETE (Portugal 2020) and by national funds through the Portuguese Foundation for Science and Technology (FCT) (RIFF-HEART project: PTDC/MEC-CAR/32188/2017).”

*Give information separately for exposed and unexposed groups.

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INSTRUCTIONS FOR AUTHORS

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1. Revista Española de Cardiología

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3.1. BEFORE YOU BEGIN

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Web page. Example: National Institute for Health and Clinical Excellence. Chronic heart failure in adults: management. 2010. Available at: <http://www.nice.org.uk/Guidance/cg108>. Accessed 6 Dec 2011.

Unpublished conference abstract. Veronesi F, Korfiati A, Buffat R, Raubal M. Assessing accuracy and geographical transferability of machine learning algorithms for environmental modelling (8). In: Agile 2017. 20th Conference on Geo-Information Science; 2017 May 9-12; Wageningen, The Netherlands. Available at: <https://agile-online.org/index.php/programme-2017/accepted-papers-and-posters-2017>. Accessed 15 Jun 2017.

Supplement. Example: Malecka-Tendera E, Mazur A. Childhood obesity: a pandemic of the twenty-first century. *Int J Obes (Lond)*. 2006;30(Suppl 2):S1-3.

Database. Example: Base de Datos Clínicos de Atención Primaria (BDCAP). Morbilidad registrada en Atención Primaria. Madrid: Ministerio de Sanidad, Servicios Sociales e Igualdad; 2013. <http://www.msssi.gob.es/estadEstudios/estadisticas/estadisticas/estMinisterio/SIAP/home.htm>. Accessed 1 Jun 2016.

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3.3. TYPES OF ARTICLES

Original articles

- These articles should not exceed 5000 words and should contain a title of less than 150 characters and spaces
- The manuscript should be arranged in the following order:
 1. Structured abstract (maximum 250 words) and key words (from 3 to 10). The abstract should be structured in 4 sections: *a)* Introduction and objectives; *b)* Methods; *c)* Results; and *d)* Conclusions. The abstract should be self-explanatory and should not contain references. Up to 3 abbreviations will be accepted, as long as each is used at least 3 times. Define all abbreviations on first mention in the text, except commonly used units of measure (apart from effect units). Acronyms for the names of studies, trials, registries, and scales can be used without definition on first mention, as long as they are widely used in the literature
 2. Table of abbreviations in Spanish and English (no more than 6 of the most frequently used in the text)
 3. Text. The body of the article should consist of the following sections: *a)* Introduction; *b)* Methods; *c)* Results; *d)* Discussion; and *e)* Conclusions. Sections should be appropriately subdivided and subtitled
 4. Key points. Include the following information at the end of the article:
 - What is known about the topic?
Include 3 or 4 sentences that summarize key points on what is known about the research topic (do not exceed 100 words).
 - What does this study add?
Include 3 or 4 sentences summarizing key points on the contribution of the study (do not exceed 100 words)
 5. References
 6. Tables (optional)
 7. Figure captions and figures (optional)

In addition, articles on study protocols will be considered for publication if they comply with the following:

- Randomized clinical trial
- Approval of the institutional review board or local ethics committee
- Identification of funding source and proof of funding approval
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Scientific letters

This section publishes articles that include research involving patients or basic science, or case reports (individual or case-series) of special clinical interest.

- No more than 6 authors
- No more than 1000 words (excluding tables) and a title (less than 150 characters and spaces)
- Without abstract, key words, or a hierarchy of headings and subheadings

- No more than 6 references
- No more than 2 elements (figures or tables)

Letters to the Editor

This section includes correspondence related to articles published in *Rev Esp Cardiol* or concerning editorial matters. Letters in response to articles published in the journal should be submitted within 12 weeks of the publication of the article and cannot include original data.

- No more than 4 authors
- No more than 800 words and a title of less than 150 characters and spaces
- No more than 2 figures and no tables

Images in cardiology

- No more than 3 authors
- The title must contain less than 8 words
- The accompanying text should not exceed 250 words and should contain information of clear relevance, with no bibliographic references or figure captions. All symbols evident in the images should be adequately explained in the accompanying text
- No more than 3 figures

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