Relaxin serum levels in acute heart failure are associated with pulmonary hypertension and right heart overload

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Aims
Despite the promising results of serelaxin as a new potential acute heart failure (HF) therapy, its clinical use preceded the understanding of the endogenous relaxin system in HF. We aimed to evaluate relaxin circulating levels in a population of acute HF and their association with clinical and echocardiographic parameters.

Methods and results
We included 117 patients from a registry of acute HF. Admission serum relaxin was measured using an enzyme-linked immunosorbent assay (ELISA) kit. Clinical, analytical, and echocardiographic parameters were compared between patients with relaxin levels above and below the median. Median age was 82 years [interquartile range (IQR) 72–87], 41% of the patients were male, and 63% had systolic dysfunction. Median serum relaxin was 31.4pg/mL (IQR 0.6–89.8). Patients with relaxin levels above the median had more peripheral oedema (89.8% vs. 68.4%, P = 0.004) and a significantly higher sodium retention score (mean 4.8 ± 1.5 vs. 3.6 ± 2.0, P < 0.001). These patients also had significantly higher systolic pulmonary arterial pressure [median 47.0 (IQR 36.0–61.0) vs. 34.5 (IQR 25.0–51.0) mmHg, P = 0.002], higher prevalence of right ventricular (RV) systolic dysfunction (28.1% vs. 10.3%, P = 0.02), RV dilation (31.0% vs. 5.3%, P < 0.001), and right atrial dilation (66.1% vs. 36.5%, P = 0.002), and less inferior vena cava diameter variability (40% vs. 60%, P = 0.009). No differences were noted regarding admission blood pressure, left chamber dimensions, or LV function.

Conclusion
In our population of acute HF patients, admission relaxin serum levels were associated with clinical and echocardiographic markers of pulmonary hypertension, RV dysfunction, and overload, suggesting a role for circulating relaxin as a biomarker in this setting.

Keywords
Relaxin • Circulating biomarker • Acute heart failure • Pulmonary hypertension • Right ventricular dysfunction

Introduction
Relaxin is a hormone associated with the growth and differentiation of the reproductive tract during pregnancy.¹ However, it has recently been implicated in many cardiovascular physiological and pathological processes, where it mediates vasodilation, natriuresis, and anti-inflammatory, antihypertrophic, anti-fibrotic, and anti-ischaemic effects.¹ ² The coupling of relaxin to the RXFP-1 receptor activates a complex series of signal transduction pathways, which culminate in nitric oxide (NO) synthesis
and endothelin (ET) system antagonism, inhibiting ET-1 gene expression and favouring endothelial ET$_{A}$ receptor signalling.$^{2,3}$ Despite the significant advances in our understanding of the physiological roles of relaxin in recent years$^{4}$ and the encouraging results regarding the clinical use of serelaxin in the treatment of heart failure (HF),$^{4-7}$ there are still significant knowledge gaps regarding the role and modulation of the endogenous relaxin system in the cardiovascular system and, in particular, in HF.

The involvement of the endogenous relaxin system in acute HF was first suggested by Dschietzig et al.$^{8}$ who described increased relaxin expression in the left ventricle and right atrium of patients with acute HF. This increased cardiac production correlated with HF severity and LV filling pressures and translated into increased relaxin levels in plasma. These data were initially supported by Fisher et al.$^{9}$ and by the analysis of relaxin transcardiac gradients.$^{8,10,11}$ However, some studies, focusing on chronic left HF, provided contradictory results.$^{10,12,13}$

More recently, an association between relaxin, pulmonary vasculature modulation, and right ventricular (RV) function has been suggested. Mazurek et al.$^{14}$ described increased relaxin serum levels in patients with pulmonary arterial hypertension (PAH) and found a correlation between relaxin levels, pulmonary vascular resistance (PVR), and the degree of RV dysfunction. The idea of a compensatory rise in endogenous relaxin in response to pulmonary hypertension (PH) and RV dysfunction was further strengthened by serelaxin clinical trials. Serelaxin administration to acute HF patients resulted in a pronounced decrease in pulmonary artery pressure (PAP) even before PCWP reduction, suggesting a vasodilator effect on the pulmonary bed.$^{15}$

In this study, we aimed to characterize the role of endogenous relaxin in acute HF, evaluating relaxin circulating levels in a population of acute HF patients and their association with clinical and echocardiographic parameters of left and right heart dimensions and function. We hypothesized that, in acute HF, relaxin circulating levels are increased in patients with signs of right heart overload.

## Methods

### Study population

During a 21-month period, a registry of acute HF was conducted at the Internal Medicine Department of São João Hospital Centre, Porto, Portugal, including a total of 659 patients. Patients admitted with the primary diagnosis of acute HF were eligible for registry inclusion. Patients with acute coronary syndromes and patients whose symptoms were explained by conditions other than HF were excluded from this registry. The diagnosis of HF was made according to the European Society of Cardiology guidelines.$^{15}$ All patients underwent detailed clinical history, physical examination, and venous blood sample analysis at admission and discharge. Clinical data collection and physical examination were performed by the investigators of this registry following a standardized clinical form. An echocardiogram was performed within 72 h after admission. Patients’ treatment and discharge time were decided by the attending physician. The investigation conforms with the principles outlined in the Declaration of Helsinki$^{16}$ and was approved by the local ethics committee. Patients provided informed consent.

From this registry, 117 acute HF patients were analysed, either with reduced (HFrEF) or with preserved ejection fraction (HfPEF).

Exclusion criteria were: (i) HF due to severe valve disease; (ii) rare causes of HF (such as active myocarditis, congenital heart disease, or pericardial disease); (iii) acute coronary syndrome in the past 3 months; and (iv) severe chronic kidney disease, as defined by a glomerular filtration rate <15 mL/min/1.73 m$^2$ estimated by the MDRD (Modification of Diet in Renal Disease) formula.

In order to assess patients’ admission volaemia status, we calculated the sodium retention score, as described by Cody.$^{17}$ This score incorporates physical examination findings such as peripheral oedema, rales, hepatomegaly, third sound gallop, jugular venous distention, and weight change, with higher values suggesting higher volume overload. Since there was no previous weight for comparison, weight change was assigned as zero in all patients.$^{17}$

### Echocardiographic evaluation

Images were obtained with a standard ultrasound machine (Vivid S6; GE Vingmed, Horten, Norway) using a multifrequency matrix probe (2.0–3.6 MHz). Standard techniques were used to obtain M-mode, 2D, and Doppler measurements in accordance with the American Society of Echocardiography and the European Association of Cardiovascular Imaging guidelines.$^{18}$

Left ventricular (LV) end-systolic and end-diastolic volumes along with the LVEF were calculated by the biplane Simpson’s method from apical four- and two-chamber views. LV systolic function was considered preserved if LVEF was $\geq 50\%$. Left atrial volume was calculated with the biplane disk summation technique. The E/E’ ratio was calculated from the relationship of the E atrial wave measured by pulsed-wave transmitral Doppler to the early diastolic velocity $E’$ (average from the medial and lateral mitral annular $E’$) by pulsed-wave tissue Doppler imaging.

Right atrial (RA) dimensions were estimated from an apical four-chamber view using the disk summation technique. RA volume indexes $>32$ mL/m$^2$ in men and $>27$ mL/m$^2$ in women were used to define RA dilation.$^{18}$ RV dimensions were estimated at end-diastole from an RV-focused apical four-chamber view. Diameter $>41$ mm at the base and $>35$ mm at the mid-level were used to define RV dilation.$^{18}$ RV systolic function was evaluated by tricuspid annular plane systolic excursion (TAPSE). TAPSE $<17$ mm was used to define RV systolic dysfunction. Systolic PAP (sPAP) was estimated from the peak tricuspid regurgitation (TR) velocity in 96 patients, using the simplified Bernoulli equation.$^{19}$ In patients in which TR velocity was not measurable and there were no signs of PH ($n=21$), sPAP was set at 25 mmHg for data analysis.

### Sample collection and biomarker measurement

Fasting venous blood samples were collected within the first 48 h of hospital admission into a serum separator tube from Venosafe$^{16}$. Blood was allowed to clot on ice for 30 min and then centrifuged at 4500 rpm during 15 min. Serum was transferred to coded aliquots and stored at $-80\,^\circ$C.

Plasma B-type natriuretic peptide (BNP) was measured using an Architect i2000 automated analyser (Abbott, Lisbon, Portugal). Serum biochemical parameters were measured using conventional methods with an Olympus AU5400 automated clinical chemistry analyser (Beckman-Coulter, Izasa, Porto, Portugal).

Relaxin serum levels were quantified with a commercial highly selective enzyme-linked immunosorbent assay (ELISA) kit for human relaxin.

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(Immundiagnostik, Bensheim, Germany) with no cross-reactivity with insulin. The detection limit of this assay is 0.50 pg/mL, calculated from the mean optical density of the zero standard plus 2 standard deviations (SDs). According to the manufacturer's specifications, the intra-assay coefficient of variation was 5.1% at 23.9 pg/mL (n = 20) and 5.2% at 66.0 pg/mL (n = 20), and the interassay coefficient of variation was 4.9% at 28.0 pg/mL (n = 20) and 7.9% at 42.0 pg/mL (n = 20). Briefly, assay standards, controls, and pre-diluted samples were added into the wells of a microplate coated with a polyclonal anti-relaxin antibody. Then, a detection antibody, biotin-labelled anti-relaxin, was added. Afterwards a peroxidase conjugate was added into each microtitre well, forming a 'sandwich' of capture antibody–relaxin–detection antibody–conjugate. Tetramethylbenzidine was used as peroxidase substrate. A dose–response curve was generated using the standards' values. Relaxin levels were determined directly from this standard curve. Samples were run in duplicate. If relaxin levels were below the detection limit, they were set at 0.4 pg/mL for data analysis.

**Statistical analysis**

Continuous variables are presented as mean ± SD if normally distributed or as median [interquartile range (IQR)] if non-normally distributed; categorical variables are presented as counts and proportions. Patients were dichotomized according to the median value of relaxin distribution and the two groups were compared regarding clinical, echocardiographic, and analytical parameters. Comparison between groups was performed using the \( \chi^2 \) test for categorical variables, independent samples \( t \)-test for normally distributed continuous variables, and Mann–Whitney U-test when distribution was skewed. Additionally, the association between relaxin serum levels analysed as a continuous variable and ordinal or continuous variables was analysed using Spearman correlation test. Univariable logistic regression was used to estimate the effect size of relaxin (as a dichotomous variable) on RV systolic dysfunction risk. Multivariable logistic regression was used to assess whether this association was independent of BNP admission levels. Crude and adjusted odds ratio (ORs) and the corresponding confidence intervals (95% CIs) were obtained for the association between relaxin groups and RV systolic dysfunction. Statistical analysis was conducted using STATA 12.1 (StataCorp LP). A P-value <0.05 was considered to be statistically significant.

**Results**

Relaxin concentrations were measured in admission serum samples from 117 acute HF patients. Patients’ characteristics, demographic data, and admission laboratory parameters are presented in Table 1. Median age was 82 (72–87) years and 41% of the patients were male. Ischaemic heart disease was the most frequent aetiology of HF (50.4%). Seventy-four patients (63.2%) had HFrEF.

Figure 1 represents the distribution of relaxin serum levels in our population. Median admission relaxin concentration was 31.4 pg/mL (range 0.4–1689.2 pg/mL; IQR 0.6–89.8 pg/mL); 29 patients had relaxin levels below the detection limit of the assay. When patients were dichotomized according to the relaxin median (Table 1), no differences were observed between groups regarding age, sex, smoking status, cardiovascular risk factors, HF aetiology, medication, or functional class at admission. No correlation was found between relaxin plasma levels and age (Table 2). Concerning physical examination, although no significant differences were observed in admission blood pressure or heart rate between relaxin groups, patients with relaxin levels above the median had more clinical signs of right heart overload, such as more pronounced peripheral oedema and a significantly higher sodium retention score (Table 1). These data were confirmed by a positive correlation between circulating relaxin, the extent of peripheral oedema, and sodium retention score (Table 2). Although no differences were found in lung congestion between the two relaxin groups, we found a positive correlation between relaxin circulating levels and extension of rales (Table 2). Regarding the analytical data, no significant differences were observed between relaxin groups in terms of admission laboratory parameters, including BNP (Table 1). No significant correlation was observed between relaxin and admission BNP (Table 2).

The physical examination findings suggestive of volume overload in patients with higher relaxin levels were supported by the echocardiographic data (Tables 2 and 3). Patients with higher relaxin levels had a significantly higher sPAP, a higher prevalence of systolic RV dysfunction, RV dilation, and RA dilation, and less inferior vena cava (IVC) diameter variability with inspiration, suggesting an association between relaxin circulating levels and right heart overload (Table 3). When analysing relaxin as a continuous variable, patients with RV systolic dysfunction had significantly higher relaxin circulating levels [median relaxin 73.5 (IQR 8.3–182.8) vs. 27.1 (IQR 0.4–79.3) mmHg, \( P = 0.02 \)]. The same was observed regarding RV dilation [median relaxin 67.4 (IQR 41.9–143) vs. 25.3 (IQR 0.4–83.9) mmHg, \( P = 0.008 \)]. There were no differences between the two relaxin groups regarding left chamber dimensions or LV systolic and diastolic function (Table 3). Also, relaxin concentrations did not differ between HFrEF and HFP EF patients (Figure 2).

Patients with relaxin concentrations above the median had an OR for RV systolic dysfunction of 3.38 (95% CI 1.21–9.41). BNP admission levels were also positively associated with RV dysfunction (OR 1.03 per 100 ng/mL increase in BNP, 95% CI 1.01–1.06; \( P = 0.02 \)). However, relaxin was able to predict RV dysfunction independently of BNP (adjusted OR 4.10; 95% CI 1.35–12.44; \( P = 0.01 \)).

There were no significant differences in the length of stay between relaxin groups [median 8 (5–13) days in patients with higher relaxin vs. 7.5 (6–10) days in patients with lower relaxin levels, \( P = 0.36 \)]. Five patients died during their hospital stay (three in the higher relaxin group and two in the lower relaxin group). In patients discharged alive (Table 4), those with higher relaxin admission levels had significantly lower levels of haemoglobin and albumin at discharge (Tables 2 and 4). Although there was a small difference regarding discharge sodium levels between relaxin groups (Table 4), we could not find a significant correlation between these two variables (Table 2). No differences were observed regarding discharge BNP levels, BNP variation during hospital stay, or discharge uric acid, creatinine, C-reactive protein, or troponin I (data not shown).

**Discussion**

In this study, we described, for the first time, an association between relaxin serum levels and clinical and echocardiographic parameters.
### Table 1 Patients' characteristics at admission according to relaxin groups

|                          | All patients (n = 117) | Relaxin < 31.4 pg/mL (n = 58) | Relaxin ≥ 31.4 pg/mL (n = 59) | P-value* |
|--------------------------|------------------------|--------------------------------|--------------------------------|----------|
| Male sex, n (%)          | 48 (41.0)              | 23 (39.7)                      | 25 (42.4)                      | 0.76     |
| Age, median (IQR)        | 82 (72–87)             | 80 (72–86)                     | 82 (72–87)                     | 0.48     |
| Atrial fibrillation, n (%) | 59 (50.4)             | 28 (48.3)                      | 31 (52.5)                      | 0.64     |
| History of hypertension, n (%) | 89 (76.1)        | 43 (74.1)                      | 46 (78.0)                      | 0.74     |
| Diabetes mellitus, n (%) | 50 (42.7)              | 26 (44.8)                      | 24 (40.7)                      | 0.65     |
| Smoking status, n (%)    |                        |                                |                                |          |
| Non-smoker               | 82 (70.7)              | 39 (67.2)                      | 43 (74.1)                      | 0.69     |
| Former smoker            | 4 (3.4)                | 2 (3.5)                        | 2 (3.5)                        |          |
| Current smoker           | 30 (25.9)              | 17 (29.3)                      | 13 (22.4)                      |          |
| NYHA IV (vs. II/III), n (%) | 69 (59.0)            | 37 (63.8)                      | 32 (54.2)                      | 0.29     |
| HF aetiology, n (%)      |                        |                                |                                |          |
| Ischaemic heart disease  | 59 (50.4)              | 30 (51.7)                      | 29 (49.2)                      | 0.78     |
| Hypertension             | 26 (22.2)              | 14 (24.1)                      | 12 (20.3)                      | 0.62     |
| Idiopathic               | 8 (6.8)                | 3 (5.2)                        | 5 (8.5)                        | 0.48     |
| Other                    | 18 (15.4)              | 8 (13.8)                       | 10 (16.7)                      | 0.64     |
| Medication at admission, n (%) |                  |                                |                                |          |
| ACE inhibitor or ARB     | 80 (68.4)              | 41 (70.7)                      | 39 (66.1)                      | 0.59     |
| Beta-blocker             | 59 (50.4)              | 32 (55.2)                      | 27 (45.8)                      | 0.31     |
| Spironolactone           | 14 (12.0)              | 7 (12.1)                       | 7 (11.9)                       | 0.97     |
| Statin                   | 61 (52.1)              | 34 (58.6)                      | 27 (45.8)                      | 0.11     |
| Loop diuretic            | 86 (73.5)              | 41 (70.7)                      | 45 (76.3)                      | 0.49     |
| Physical examination at admission |                      |                                |                                |          |
| Systolic BP (mmHg), median (IQR) | 128 (107–144)   | 132 (110–144)                  | 123 (103–143)                  | 0.34     |
| Diastolic BP (mmHg), mean ± SD | 68 ± 14              | 69 ± 15                        | 68 ± 14                        | 0.95     |
| Heart rate, median (IQR) | 81.0 (69.0–91.0)       | 81.5 (69.5–94.5)               | 80.5 (69.0–90.0)               | 0.39     |
| Rales, n (%)             |                        |                                |                                | 0.36     |
| No rales                 | 8 (6.9)                | 4 (7.0)                        | 4 (6.8)                        |          |
| Lower one-third of the lungs | 60 (51.7)            | 34 (59.7)                      | 26 (44.1)                      |          |
| Lower two-thirds of the lungs | 37 (31.9)           | 15 (26.3)                      | 22 (37.3)                      |          |
| Throughout lungs         | 11 (9.5)               | 4 (7.0)                        | 7 (11.9)                       |          |
| Jugular venous distension, n (%) | 47 (42.3)        | 22 (40.0)                      | 25 (44.6)                      | 0.62     |
| Hepatomegaly, n (%)      | 16 (14.4)              | 5 (9.1)                        | 11 (19.6)                      | 0.114    |
| Peripheral oedema, n (%) |                        |                                |                                |          |
| No oedema                | 24 (20.7)              | 18 (31.6)                      | 6 (10.2)                       | 0.0008   |
| Ankle                    | 30 (25.9)              | 17 (29.8)                      | 13 (22.0)                      |          |
| Leg                      | 52 (44.8)              | 18 (31.6)                      | 34 (57.6)                      |          |
| Above leg                | 10 (8.6)               | 4 (7.0)                        | 6 (10.2)                       |          |
| Sodium retention score, mean ± SD | 4.2 ± 1.8          | 3.6 ± 2.0                      | 4.8 ± 1.5                      | <0.001   |
| Admission laboratory parameters |                      |                                |                                |          |
| Haemoglobin (g/dL), mean ± SD | 11.6 ± 2.2          | 11.7 ± 2.3                     | 11.4 ± 2.2                     | 0.58     |
| Sodium (mEq/L), median (IQR) | 139 (137–143)    | 140 (137–143)                  | 139 (137–142)                  | 0.44     |
| Uric acid (mg/dL), mean ± SD | 8.6 ± 2.8           | 8.3 ± 2.5                      | 8.8 ± 3.1                      | 0.30     |
| Creatinine (mg/dL), median (IQR) | 1.4 (1.2–2.0)    | 1.4 (1.2–1.9)                  | 1.5 (1.2–2.1)                  | 0.54     |
| GFR (MDRD, ml/min), mean ± SD | 42.1 ± 17.4         | 42.8 ± 16.6                    | 41.4 ± 18.3                    | 0.66     |
| CRP (mg/L), median (IQR) | 23.3 (10.1–57.2)     | 21.6 (8.3–57.2)                | 25.3 (11.7–58.9)               | 0.53     |
| Albumin (g/L), mean ± SD | 35.2 ± 4.8           | 35.7 ± 4.7                     | 34.7 ± 4.8                     | 0.26     |
| Troponin I (ng/mL), median (IQR) | 0.07 (0.03–0.18)   | 0.08 (0.04–0.18)               | 0.06 (0.03–0.17)               | 0.66     |
| BNP (pg/mL), median (IQR) | 1212 (604–2531)      | 1037 (604–2201)                | 1663 (620–2541)                | 0.50     |

BP, blood pressure; CRP, C-reactive protein; GFR, glomerular filtration rate; HF, heart failure; HFrEF, heart failure with reduced ejection fraction (LVEF <50%); IQR, interquartile range; MDRD, Modification of Diet in Renal Disease; SD, standard deviation.

*P-value for the difference between patient groups created according to the median value of relaxin distribution.
markers of volume overload, PH, and right heart dysfunction and overload in acute HF. Namely, patients in the upper half of relaxin distribution had a mean sodium retention score 1.2 points higher, a median sPAP 12.5 mmHg higher, and an odds of RV systolic dysfunction 3.4 times higher than patients with lower relaxin levels. In non-pregnant subjects, there is evidence of local relaxin synthesis in many tissues, including the cardiovascular system, where it may act as a paracrine or autocrine factor to exert multiple physiological roles. This may explain its usually low or undetectable circulating levels in these subjects. However, increasing evidence suggests relaxin secretion into the circulation in many disease states, such as HF. While both animal and human studies described increased relaxin circulating levels in HF, others could not confirm this finding. Many reasons may explain these discrepancies: (i) study populations were highly heterogeneous concerning gender, age, HF presentation, aetiology, and LV function; (ii) there was a high variability in relaxin circulating levels in both HF and control groups, and most studies evaluated small sample sizes, which decreased statistical power; and (iii) studies used different antiserum batches and different immunoassays with different detection limits.

In our study, we indeed found great variability in relaxin serum levels, with ~25% of patients having relaxin levels below the detection limit. Our results favour the evidence of relaxin secretion into the circulation in acute HF but suggest that relaxin levels are probably not equally elevated in all HF patients, possibly identifying patients with higher sPAP and right heart overload. This could explain part of the variability and inconsistency of previous studies, which focused mainly on left heart function and load. Evidence in the literature supports the association between relaxin, PH, and RV function. In PAH, relaxin serum levels are increased and correlate with PVR and the degree of RV dysfunction, suggesting a compensatory up-regulation in response to increased inflammation and vascular remodelling. Also, serelaxin infusion in acute HF patients induced rapid haemodynamic changes, characterized by significant reductions in PCWP, PVR, PA, and RA pressure, without major changes in cardiac index. Interestingly, changes in PA were seen earlier and were more pronounced than changes in PCWP, suggesting that they were probably dependent on a vasodilator effect on the pulmonary pre-capillary and capillary bed. These data support our findings of increased relaxin synthesis as a compensatory mechanism to oppose pulmonary pressure increase. The lack of association between relaxin and surrogates of LV filling pressures and LVEF probably reflects that relaxin synthesis may be more dependent on PAP and right chamber pressures and function than on LV function and load. Also, the lack of association between BNP and relaxin levels and their independence on the prediction of RV dysfunction may likewise signify different release mechanisms, favouring this hypothesis. Indeed, previous studies also found no significant association between relaxin and BNP. Taken together, these data generate the hypothesis that relaxin levels could possibly be a useful tool to identify which acute HF patients would benefit more from pulmonary vasodilators, such as serelaxin itself.

The precise site of relaxin synthesis and relaxin receptor RXFP-1 expression in the right heart and pulmonary vasculature is still largely unknown. Relaxin is constitutively expressed in myocytes and interstitial cells of the right atrium and left ventricle of healthy humans and in rodents’ atria and left ventricle. Similarly, RXFP-1 was also detected in the human and rat myocardia. In HF, cumulative evidence shows increased relaxin gene and peptide expression both in different animal models and in humans, and this increased cardiac production translated into

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**Table 2: Correlation between relaxin serum levels and clinical and echocardiographic variables**

| Variable                              | r     | P-value |
|---------------------------------------|-------|---------|
| Age, years                            | 0.06  | 0.53    |
| Systolic blood pressure, mmHg         | −0.04 | 0.70    |
| Diastolic blood pressure, mmHg        | 0.02  | 0.83    |
| Rales*                                | 0.22  | 0.02    |
| Sodium retention score                | 0.28  | 0.004   |
| Haemoglobin at admission, g/dL        | −0.06 | 0.48    |
| Sodium at admission, g/dL             | −0.03 | 0.76    |
| GFR at admission (MDRD), mL/min      | 0.01  | 0.90    |
| Albumin at admission, g/L             | −0.13 | 0.17    |
| BNP at admission, pg/mL               | 0.12  | 0.20    |
| LVEF, %                               | −0.03 | 0.72    |
| E/E' ratio                            | −0.07 | 0.47    |
| sPAP, mmHg, median (IQR)              | 0.18  | 0.05    |
| IVC collapse at inspiration, %        | −0.21 | 0.03    |
| Haemoglobin at discharge, g/dL        | −0.26 | 0.005   |
| Sodium at discharge, mEq/L            | −0.16 | 0.10    |
| GFR (MDRD) at discharge, mL/min       | −0.03 | 0.72    |
| Albumin at discharge, g/L             | −0.20 | 0.03    |
| BNP at discharge, pg/mL               | 0.13  | 0.18    |

*P*-values are for Spearman’s correlation coefficient (r). E/E' ratio, relationship of the E atrial wave measured by pulsed-wave transmural Doppler and early diastolic velocity E' by pulsed-wave tissue Doppler imaging; GFR, glomerular filtration rate; IVC, inferior vena cava; MDRD, Modification of Diet in Renal Disease; sPAP, systolic pulmonary artery pressure.

*Categories as presented in Table 1.
Curiously, previous reports described a decrease in RXFP-1 expression in the failing myocardium, which corresponded to a reduced myocardium response to relaxin. Therefore, relaxin secretion in HF may increase in response to reduced RXFP-1 expression. Regarding the pulmonary circulation, bovine pulmonary artery endothelial cells secrete relaxin in vitro and respond to exogenous relaxin, suggesting that they express RXFP-1 receptors. Despite this knowledge, evidence is lacking regarding RXFP-1 expression and regulation in the right ventricle, and the main stimuli for relaxin secretion and RXFP-1 regulation in HF are still to be determined.

In a flow-chamber model of bovine pulmonary endothelial cells, these cells, relaxin administration blunted ET-1 secretion, suggesting that the pulmonary endothelium is probably not a major source of increased relaxin in HF. Yet, in these cells, relaxin administration blunted ET-1 secretion, suggesting a strong potential for suppression of the ET system in vitro. Also, a strong inverse correlation between circulating ET-1 and relaxin in patients with severe HF has been described, supporting these findings. The activation of the ET system has been demonstrated both in PAH and in congestive HF, where it is associated with vasoconstriction and vascular remodelling. Therefore, we may hypothesize that relaxin is elevated in acute HF patients with PH and right heart overload as a counter-regulatory hormone opposing ET system activation in the pulmonary endothelium. The apparent paradox of relaxin increase with disease severity is observed in other counter-regulatory systems, such as the natriuretic peptides, and probably reflects an inability of the endogenous system to overcome classical neurohumoral activation in HF.

Indeed, relaxin circulating concentrations in our study are much lower than those achieved by the 30 μg/kg RELAX-AHF serelaxin dose (Cmax 11.4 ng/mL). Our study population was very old and had multiple co-morbidities, in consonance with previous population-based HF registries. This may reflect the high prevalence of HF in elderly populations and the characteristics of acute HF patients.
Table 4 Discharge medication and laboratory parameters of patients who survived hospital stay

|                                | All patients (n = 112) | Relaxin <31.4 pg/mL (n = 56) | Relaxin ≥31.4 pg/mL (n = 56) | P-value* |
|--------------------------------|------------------------|-----------------------------|----------------------------|----------|
| Discharge medication, n (%)    |                        |                             |                            |          |
| ACE inhibitor or ARB           | 91 (81.2)              | 46 (82.1)                   | 45 (80.4)                  | 0.81     |
| Beta-blocker                   | 83 (74.1)              | 43 (76.8)                   | 40 (71.4)                  | 0.52     |
| Spironolactone                 | 26 (23.2)              | 16 (28.6)                   | 10 (17.9)                  | 0.18     |
| Statin                         | 73 (65.2)              | 36 (64.3)                   | 37 (66.1)                  | 0.84     |
| Loop diuretic                  | 103 (92.0)             | 51 (91.1)                   | 52 (92.9)                  | 0.60     |
| Discharge laboratory parameters|                        |                             |                            |          |
| Haemoglobin (g/dL), median (IQR)| 11.4 (10.4–13.4)      | 12.0 (10.8–13.8)            | 11.0 (9.5–12.4)            | 0.005    |
| Sodium (mEq/L), median (IQR)   | 138 (136–141)          | 139 (137–141)               | 138 (134–140)              | 0.04     |
| GFR (MDRD, mL/min), mean ± SD  | 44.6 ± 17.8            | 46.3 ± 18.2                 | 42.8 ± 17.5                | 0.31     |
| Albumin (g/L), mean ± SD       | 3.48 ± 0.4             | 3.60 ± 4.5                  | 33.7 ± 4.9                 | 0.01     |
| BNP (pg/mL), median (IQR)      | 674 (300–1289)         | 593 (256–918)               | 726 (338–1659)             | 0.18     |
| BNP decrease of ≥30% admission| 79.0 (70.5)            | 42.0 (75.0)                 | 37.0 (66.1)                | 0.30     |

GFR, glomerular filtration rate; IQR, interquartile range; MDRD, Modification of Diet in Renal Disease; SD, standard deviation.

*P-value for the difference between patient groups created according to the median value of relaxin distribution.

admitted to Internal Medicine wards. Yet, similarly to previous studies, we found no correlation between age and relaxin levels. Some concerns were raised regarding sex differences and the role of renal function in relaxin clearance. We found no differences between genders and no correlation between renal dysfunction and relaxin. Finally, patients with higher relaxin admission levels had lower haemoglobin and albumin at discharge. The significance of this association is unknown but it can mirror the severity of HF as patients with lower haemoglobin and albumin are known to have more severe HF and worse prognosis.

Our study presents some limitations. The relatively small sample size and the variability of relaxin concentrations may have prevented the identification of some weak association between relaxin levels, BNP, and parameters of LV function and load. Yet, it allowed the identification of an association between relaxin serum levels and right heart overload. Also, the exclusion criteria may limit generalization of the results to all HF aetiologies. Although the time window allowed for echocardiogram performance was 72 h, it was performed within 24 h in 73% patients, within 48 h in 15%, and thereafter in only 12%. The only parameters significantly associated with time to echocardiogram performance were IVC diameter (negative association) and IVC diameter variability (positive association). After adjusting the association between relaxin and these variables for time to echocardiogram, the difference in IVC diameter between relaxin groups became statistically significant (P = 0.03) and the difference in IVC variability remained highly significant (P = 0.003). Hence, this design led at most to an underestimation of these results. TR velocity was not measurable in 21 patients, in whom we attributed a value of 25 mmHg to sPAP. Although this value may have underestimated the real sPAP of these patients, none of them had signs of PH, and the analysis of sPAP as an ordinal variable (including these patients in the lower sPAP group) yielded similar results. Also, RV function was evaluated using TAPSE, which only reflects RV longitudinal function; however, TAPSE is easily obtainable and has shown good correlations with techniques estimating RV global systolic function. Finally, relaxin circulating levels may not entirely reflect local tissue modulation of the relaxin system, and the cross-sectional design of this study precludes the establishment of a causal association between relaxin, PH, and RV dysfunction.

In conclusion, in this acute HF population, relaxin serum levels at admission were associated with clinical and echocardiographic markers of right heart overload. These results provide a new insight into the endogenous relaxin system in acute HF, suggesting a compensatory role in the modulation of the pulmonary vasculature and right heart function. Unravelling relaxin’s role in HF will further support its clinical use as a therapeutic target and possibly as a circulating biomarker, helping to implement a patient-based approach, directing specific HF therapies to specific patient groups.

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