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The relationship of circulating relaxin-2 concentrations with short-term prognosis in patients with acute heart failure: the RELAHF study

Introduction

Relaxin is a natural hormone of the insulin family which is detected only at very low blood concentrations (<10 pg/mL) in a small proportion of healthy people (<20%), with the exception of pregnant women, in whom blood concentrations usually increase to over 100 times the baseline concentration (>1000 pg/mL).[1–3] Relaxin plays a role in the regulation of vascular tone by binding to the RXFP1 receptors present in the renal and systemic vasculature.[4]

In the recent RELAX-AHF (RELAXin in Acute Heart Failure) clinical trial, serelaxin, a recombinant form of relaxin, was administered to patients with acute heart failure (AHF), resulting in an improvement in dyspnoea and a possible increase in mid-term survival.[5] However, the mechanism behind these beneficial results is not completely understood. A few studies have reported that relaxin concentrations are elevated in patients with moderate–severe dilated cardiomyopathy and are correlated with the severity of heart failure (HF),[6] but not with mortality or rehospitalization rates or with natriuretic peptide blood concentrations. In a recent study, relaxin concentrations were found to be related to clinical and echocardiographic markers of pulmonary hypertension.[7]

In view of the scarcity of data, we designed the present exploratory study in patients with AHF recruited in emergency departments (EDs). The objectives were to investigate: (i) the frequencies of patients with detectable and undetectable relaxin concentrations; (ii) the clinical factors associated with undetectable relaxin concentrations and whether undetectable relaxin concentrations are associated with short-term outcome, and (iii) the clinical factors associated with relaxin concentrations in patients with detectable values and whether there is a relationship between relaxin concentrations and short-term outcome.

Methods

The RELAHF (RELaxine blood concentrations in patients with Acute Heart Failure) study was designed as a prospective, analytical, observational, multicentre study with cohort follow-up to analyse human relaxin-2 concentrations in consecutive patients diagnosed with AHF in four Spanish EDs over 2 months (January and February 2014). The diagnostic criteria for AHF were those defined by the guidelines of the European Society of Cardiology prevailing at the time of the study.[8] The criteria for patient inclusion were identical to those in previous studies conducted by our research group.[9] Blood samples were taken at the time of the first investigation in the ED and were stored at −80°C until transfer to the reference laboratory for processing. The study was approved by the committees for ethics and clinical investigation of the participating hospitals and was developed according to the Declaration of Helsinki.

Clinical variables

We collected data on these variables: demographic characteristics; co-morbidities; baseline functional and cardiorespiratory status [Barthel index and New York Heart Association (NYHA) class, respectively]; chronic treatment, and vital signs (electrocardiographic and analytical data) on arrival at the ED. Lastly, echocardiographic data for the 6 months prior to the current ED index episode were collected.

Follow-up and events

Follow-up was carried out by telephone and referred to clinical history noted in both hospital and primary care contexts, as well as local death registries. The primary event was 30-day all-cause death.

Biochemical analyses

N-terminal pro-brain natriuretic peptide (NT-proBNP) and relaxin-2 concentrations were determined in a central laboratory blinded to clinical details. Extraction and storage conditions for the transportation of samples were predefined and standardized across all sites. Relaxin-2 quantification was performed using the enzyme-linked immunosorbent assay (ELISA) sandwich method using two polyclonal antibodies able to bind to human relaxin (Immundiagnostik AG, Bensheim, Germany) in an automated Triturus system (Grifols SA, Barcelona, Spain). The detection limit was 1.5 pg/mL, and the range of linearity was set at 1.5–250 pg/mL. Levels of inter- and intra-assay inaccuracy were less than 8%. As this is not a standardized technique, duplicate determinations were carried out in 20% of the samples to confirm the correlation.

Data collection and statistical analyses

Categorical variables are expressed as the number and percentage, and quantitative variables as the mean and standard deviation [or median and interquartile range (IQR) if not normally distributed]. Patients were dichotomized based on whether relaxin-2 levels were detectable or not. Patients with detectable concentrations were grouped by quartiles. Categorical variables were compared with the χ^2 test or Fisher's exact test. Quantitative variables were compared by analysis of variance (ANOVA) (or the Kruskal–Wallis non-parametric test if not normally distributed). Correlations among biomarkers were measured by linear regression. Survival analysis was carried out using the Cox proportional hazard method with 30-day curves; crude hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated and adjusted for differences among the groups found in the univariate study. An interaction analysis was predefined for sex, age and type of AHF (first episode of AHF vs. decompensation of known chronic HF).

Results

The cohort included 522 patients. Relaxin-2 concentrations were undetectable (<1.5 pg/mL) in 92 (17.6%) subjects, more frequently in men ($P < 0.001$) and those treated with aldosterone receptor antagonists ($P = 0.03$) (*Table 1*). Thirty-day mortality in patients with undetectable relaxin-2 concentrations was significantly lower than in those with detectable concentrations (2.2%

Table 1 Baseline characteristics of the study population and comparisons between patients with undetectable (<1.5 pg/mL) and detectable (≥1.5 pg/mL) relaxin-2 concentrations

	All subjects (*n* = 522)	Relaxin-2 undetectable (*n* = 92)	Relaxin-2 detectable (*n* = 430)	*P*-value[a]
Sociodemographic variables				
Age, years, mean ± SD	80.9 ± 9.1	80.68 ± 9.58	80.95 ± 8.95	0.79
Female sex, *n* (%)	280 (53.6%)	34 (37.0%)	246 (57.2%)	**<0.001**
Co-morbidity, *n* (%)				
Arterial hypertension	431 (82.6%)	74 (80.4%)	357 (83.0%)	0.55
Diabetes mellitus	204 (39.1%)	35 (38.0%)	169 (39.3%)	0.82
Dyslipaemia	203 (38.9%)	37 (40.2%)	166 (38.6%)	0.77
Ischaemic heart disease	170 (32.6%)	31 (33.7%)	139 (32.3%)	0.80
Heart valve disease	116 (22.2%)	12 (13.0%)	104 (24.2%)	**0.02**
Atrial fibrillation	264 (50.6%)	46 (50.0%)	218 (50.7%)	0.90
Chronic kidney disease	140 (26.8%)	25 (27.2%)	115 (26.8%)	0.94
Cerebrovascular disease	67 (12.8%)	11 (12.0%)	56 (13.0%)	0.78
COPD	105 (20.1%)	18 (19.6%)	87 (20.2%)	0.89
Peripheral artery disease	44 (8.4%)	9 (9.8%)	35 (8.2%)	0.61
First episode of HF	207 (39.7%)	37 (40.2%)	170 (39.5%)	0.96
Basal status, *n* (%)				
Basal NYHA III or IV	93 (17.8%)	19 (20.6%)	74 (17.2%)	0.39
Barthel Index <60 points[b]	59 (12.2%)	11 (13.1%)	48 (12.0%)	0.77
Type of ventricular dysfunction[b], *n* (%)				
Systolic	103 (19.7%)	23 (54.8%)	80 (48.2%)	0.45
Diastolic	105 (20.1%)	19 (45.2%)	86 (51.8%)	
Known LVEF	208 (39.8%)	42 (45.7%)	166 (38.6%)	0.21
Chronic treatment, *n* (%)				
Loop diuretics	334 (64.0%)	60 (65.2%)	274 (63.7%)	0.79
Potassium-sparing diuretics	85 (16.3%)	22 (23.9%)	63 (14.7%)	**0.03**
Thiazide diuretics	55 (10.5%)	10 (10.9%)	45 (10.5%)	0.91
Beta-blockers	184 (35.2%)	34 (37.0%)	150 (34.9%)	0.71
ACE inhibitors	136 (26.1%)	22 (23.9%)	114 (26.5%)	0.61
ARB II	121 (23.2%)	19 (20.7%)	102 (23.7%)	0.53
Nitrates	88 (16.9%)	11 (12.0%)	77 (17.9%)	0.17
Digoxin	79 (15.1%)	11 (12.0%)	68 (15.8%)	0.35
Clinical data for the acute episode				
Heart rate, b.p.m., mean ± SD	90.2 ± 26.0	87.7 ± 22.6	90.7 ± 26.7	0.33
Respiratory rate, breaths/min, mean ± SD	23.7 ± 8.3	22.3 ± 7.3	23.9 ± 8.5	0.16
SBP, mmHg, mean ± SD	142.7 ± 26.4	140.8 ± 27.9	143.1 ± 26.1	0.45
SaO_2 <90%, *n* (%)	129 (24.7%)	24 (26.7%)	105 (24.4%)	0.77
Electrocardiogram, *n* (%)				
Atrial fibrillation in the ECG	261 (50.0%)	47 (51.1%)	214 (49.8%)	0.82
LBBB/pacemaker	83 (15.9%)	12 (13.0%)	71 (16.5%)	0.42
Left ventricular hypertrophy	17 (3.3%)	2 (2.2%)	15 (3.5%)	0.52
Laboratory tests, *n* (%)				
Anaemia	292 (55.9%)	53 (57.6%)	239 (55.6%)	0.72
eGFR <60 mL/min/1.73 m^2	305 (58.4%)	58 (63.0%)	247 (57.4%)	0.32
Sodium <135 mEq/L	108 (20.7%)	19 (20.7%)	89 (20.7%)	0.99
Potassium 3.5–5.0 mEq/L	397 (76.1%)	75 (81.5%)	322 (74.9%)	0.41
Normalized troponins, mean ± SD	3.79 ± 8.0	4.97 ± 12.9	3.53 ± 6.5	0.34
NT-proBNP, pmol/L (median p25–p75)	4160 (1751–9132)	3896 (1737–7446)	4219 (1774–9345)	0.45

ACE, angiotensin-converting enzyme; ARB II, angiotensin II receptor blocker; COPD, chronic obstructive pulmonary disease; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; HF, heart failure; LBBB, left bundle branch block; LVEF, left ventricular ejection fraction; NT-proBNP, *N*-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; SaO_2, arterial oxygen saturation; SBP, systolic blood pressure; SD, standard deviation.

[a]Values in bold denote statistically significant differences.

[b]Performed in only 485 individuals.

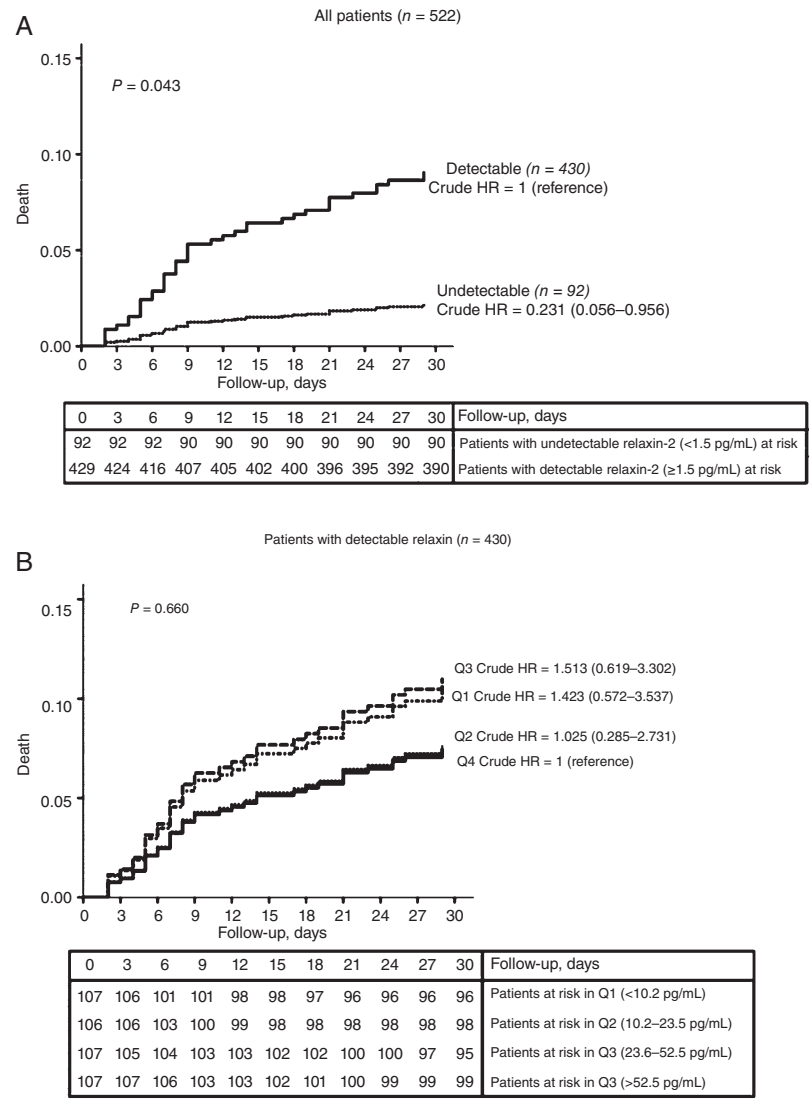

Figure 1 Survival curves for mortality at 30 days: (*A*) in patients with detectable and undetectable relaxin concentrations, and (*B*) according to relaxin concentration quartile (Q) in patients with detectable levels. HR, hazard ratio.

vs. 9.1%; $P = 0.026$). The survival curve analysis (*Figure 1*) showed a crude HR of 0.23 (95% CI 0.06–0.95; $P = 0.043$) in patients with undetectable relaxin-2 concentrations, which became 0.22 (95% CI 0.05–0.92; $P = 0.038$) after multivariate adjustment by sex, heart valve disease, type of AHF and chronic treatment with potassium-sparing diuretics.

Relaxin-2 concentrations in the 430 patients (82.4%) with detectable values did not follow a normal distribution (Kolmogorov–Smirnov statistic 0.412, $P < 0.001$) and reached a median of 30.0 pg/mL (IQR 15.2–70.5 pg/mL). Determinations of concentrations were repeated in 80 patients, obtaining a Pearson correlation coefficient of 0.74 ($P < 0.001$). No significant differences in relaxin-2 concentrations were found between patients who remained alive (median: 30.9 pg/mL, IQR 15.8–74.7 pg/mL) and those who had died (median: 30.9 pg/mL, IQR 15.6–85.2 pg/mL) at 30 days ($P = 0.89$). An analysis by quartiles (see supplementary material online, *Table S1*) demonstrated that patients with low relaxin-2 concentrations [quartile (Q) 1 and Q2] more frequently presented dyslipaemia ($P = 0.007$) and those with high concentrations (Q3 and Q4) more frequently received chronic treatment with digoxin ($P = 0.023$). An analysis of 30-day mortality by quartile showed no differences (*Figure 1*), and neither were any interactions with sex, age and type of AHF (first episode vs. decompensation of chronic HF) observed ($P = 0.20$, $P = 0.23$ and $P = 0.63$, respectively).

Analysis of correlations between relaxin-2 concentrations and other clinical and biochemical parameters revealed a significant, albeit weak ($R^2 = 0.018$), correlation only with haemoglobin values ($P = 0.005$) (*Table 2*).

Discussion

The RELAHF study achieved three main findings: (i) relaxin-2 concentrations were not detectable in a small proportion of patients with AHF and this was associated with lower 30-day mortality; (ii) there was no relationship between detectable relaxin-2 concentrations and short-term patient outcome, and (iii) relaxin-2 concentrations were not related to NT-proBNP values or to other clinical or analytical parameters (except haemoglobin).

We found 17.6% of patients with AHF to have undetectable relaxin-2 blood concentrations. This incidence lies between the 7% described by Martinez-Solano *et al.*[10] and the 25% reported by Pintalhao *et al.*[7] It is likely that differences in the baseline characteristics of the various study populations explain this variation. Remarkably, all of these figures are very much lower than those observed in healthy young people, in whom relaxin-2 concentrations are undetectable in 84% of men and 79% of women.[3] This suggests that relaxin-2 secretion may be a compensatory factor in patients with HF and may indicate partial failure of the remaining homeostatic mechanisms induced by HF. The lack of relaxin-2 detection may then identify patients who, if all other clinical and biochemical parameters associated with adverse events are equal, have a lower probability of short-term mortality.

In patients with detectable relaxin-2 concentrations, there was no relationship between these concentrations and short-term outcome. Few studies are available on this relationship in patients with AHF and the results are discordant, thereby making comparison with our results difficult. Fisher *et al.* found no relationship between relaxin concentrations and 1-year mortality in 87 patients with systolic dysfunction and hospitalized for AHF during a clinical trial.[6] Conversely, Pintalhao *et al.* reported a relationship between relaxin concentrations and in-hospital mortality in 117 patients with AHF.[7] Finally, in 115 patients with chronic HF, Xie *et al.* showed that relaxin concentrations predicted the development of severe cardiovascular events within 180 days of hospital

Table 2 Correlations between relaxin concentrations and other clinical and analytical variables

Variable	*R*	R^2	*P*-value[a]
Age	−0.039	0.002	0.42
SBP	0.058	0.003	0.24
Heart rate	−0.063	0.004	0.20
LVEF[b]	0.002	0.000	0.98
Haemoglobin	0.134	0.018	**0.005**
eGFR	0.066	0.004	0.17
Sodium	−0.035	0.001	0.47
Potassium	0.015	0.0002	0.74
Normalized troponins	−0.016	0.000	0.75
NT-proBNP	−0.064	0.004	0.19

eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; NT-proBNP, *N*-terminal pro-brain natriuretic peptide; SBP, systolic blood pressure.
[a]Values in bold denote statistically significant differences.
[b]Available in only 166 individuals.

discharge (area under the curve: 0.816, 95% CI 0.724–0.909).[11] Mazurek *et al.*[12] found higher levels of relaxin in patients with pulmonary hypertension. It is of note, however, that the populations included in these studies were very different from that of the current RELAHF study. Indeed, the RELAHF study enrolled a much larger sample of consecutively recruited patients (n = 522) with a wider variety of clinical forms of AHF, ranging from the mildest forms in patients discharged from EDs without hospitalization to the most severe forms in patients admitted to intensive care units,[9] which, in turn, led to marked differences in baseline and clinical patient characteristics between this and the previous studies. One explanation for the lack of relationship between relaxin-2 concentration and early death found in the present study may refer to the expression of relaxin receptors. Some studies have reported a reduction in the expression of these receptors in failing myocardiocytes.[13] Consequently, an increase in relaxin-2 secretion may result in insufficient response to this reduction in receptor expression. Nonetheless, this hypothesis must be investigated in future studies.

Finally, we failed to demonstrate correlations between relaxin-2 concentrations and several clinical and biochemical parameters, with the exception of a significant, albeit weak (R^2 = 0.018), correlation with haemoglobin values (P = 0.005). We have no explanation for this finding. Haemoglobin values are reduced in pregnant women as a result of the physiological increase in volaemia, a condition that, in part, reflects the adaptive increase in relaxin-2 secretion. Of note, the absence of a correlation between relaxin-2 and NT-proBNP concentrations in the RELAHF study confirms previously published results in smaller studies.[6,7] This lack of relationship may be attributable to differences in the release mechanisms and stimuli of each of these proteins.[14,15]

Limitations

We did not register the time of HF signs and symptoms evolution before ED consultation, and neither did we measure levels of testosterone or other sex hormones. Echocardiographic evaluations were available in 208 individuals (including 166 patients with detectable relaxin-2 concentrations). Although the method of relaxin-2 detection was not standardized, we observed a good correlation among determinations made in duplicate. Data on the cause of death were not available and therefore it was not possible to establish whether secretion of relaxin-2 is exclusively associated with cardiovascular mortality. Finally, as this was an exploratory hypothesis-generating study, we did not define, *a priori*, the sample size and therefore results may include a beta error. In fact, the statistical power for the differences in 30-day mortality between the groups with undetectable and detectable relaxin-2 was 66%.

Conclusions

The results of the RELAHF study suggest that relaxin-2 may play a role in the pathophysiology of AHF as the percentage of patients with undetectable concentrations is lower than in healthy people. Thus, patients not presenting measurable concentrations of relaxin-2 in blood may represent a subgroup with better short-term prognosis, perhaps because they are in a pathophysiologically early stage of disease. Further studies are required to confirm and expand these findings.

Supplementary Information

Additional Supporting Information may be found in the online version of this article:
Table S1. Comparison of patients with detectable relaxin-2 concentrations according to relaxin-2 concentration quartiles.

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