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RESEARCH ARTICLE

End-organ protective effect of serelaxin in patients hospitalized for heart failure: Results of the biomarker substudy of Relaxin in Acute Heart Failure-2 (RELAX-AHF-2)

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Aims Serelaxin is recombinant human relaxin-2, a hormone responsible for haemodynamic adaptations and organ protection in pregnancy. In the RELAX-AHF trial, serelaxin demonstrated reductions in cardiac, renal and hepatic damage. In RELAX-AHF-2, organ damage-related biomarkers were assessed in a biomarker substudy.

Methods and results Patients enrolled within 16 h of presentation for heart failure hospitalization were randomized to 48-h infusions of either serelaxin (30 μg/kg/day) or placebo, and plasma samples were obtained at baseline, 2, 5, and 14 days in patients participating in the biomarker substudy. Of the 6545 patients analysed in RELAX-AHF-2, 1020 (15.6%) patients (mean age 72 ± 12 years; 61% male) were enrolled in the biomarker substudy. Compared to placebo, serelaxin decreased percentage change from baseline in troponin T through day 14 (serelaxin +0.2% vs. placebo +40.3%; $p = 0.042$), as well as creatinine (−0.8% vs. +5.8%; $p = 0.002$), cystatin C (+3.8% vs. +8.3%; $p = 0.016$), and uric acid (+0.8% vs. +7.2%; $p = 0.0014$) at day 2. The decrease of N-terminal pro-B-type natriuretic peptide (NT-proBNP) from baseline to day 2 was greater in serelaxin-treated patients (−39.8% vs. −27.6%; $p = 0.002$). Early changes in NT-proBNP and troponin, but not creatinine, cystatin C and uric acid, were associated with 180-day mortality. In this substudy population, serelaxin did not reduce 180-day cardiovascular death (hazard ratio [HR] 0.78; 95% confidence interval [CI] 0.49–1.25; $p = 0.30$), but significantly reduced worsening heart failure through day 5 (HR 0.55; 95% CI 0.33–0.93; $p = 0.027$).

Conclusion In this substudy, serelaxin decreased plasma concentrations of cardiac, renal and hepatic injury markers. Changes of most of these markers were associated with cardiovascular mortality. In this pre-specified biomarker subgroup, serelaxin did not reduce 180-day cardiovascular mortality but significantly reduced worsening heart failure through day 5.

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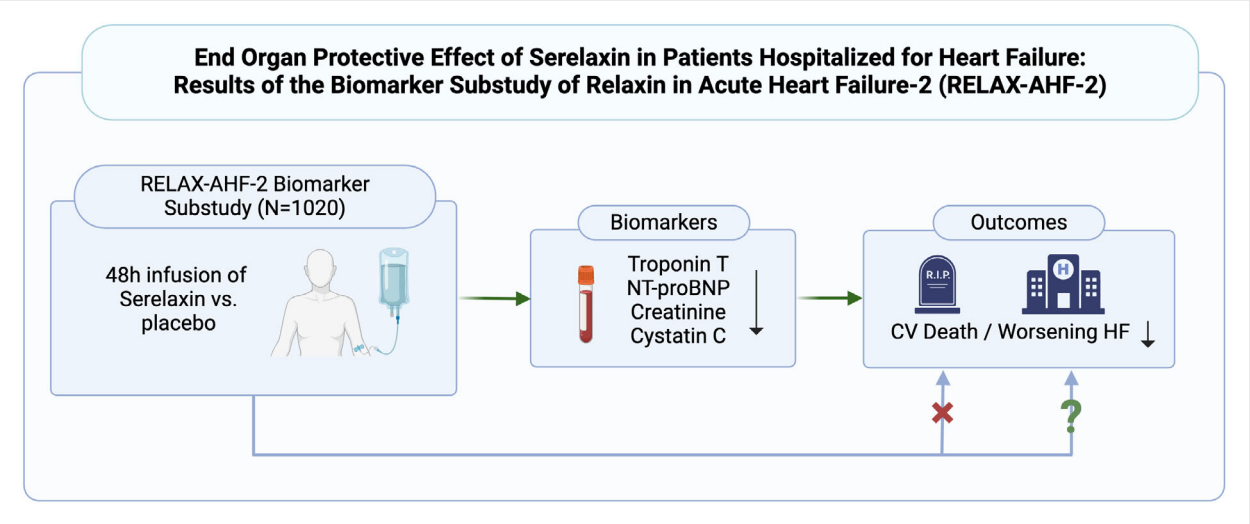
Graphical Abstract



End-organ protective effect of serelaxin in patients with acute heart failure. CV, cardiovascular; HF, heart failure; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Keywords Acute heart failure • Biomarkers • Organ protection • Pharmacotherapy

Introduction

Serelaxin is a recombinant form of human relaxin-2, a naturally occurring peptide hormone that mediates maternal systemic haemodynamic, vascular and renal adaptations to an increase in intravascular volume and stresses during pregnancy.[1] In a randomized placebo-controlled trial of 1161 patients hospitalized with acute heart failure (Relaxin in Acute Heart Failure [RELAX-AHF]), a 48-h intravenous infusion of serelaxin initiated within 16 h of presentation demonstrated significant prevention of short-term cardiac, renal and hepatic damage.[2] RELAX-AHF also demonstrated significant improvement in the primary endpoint of dyspnoea relief over 5 days, as well as reduction of the pre-specified additional endpoints of worsening heart failure at 5 days and both cardiovascular and all-cause mortality at day 180.[3] Two potentially linked hypotheses emerged from the RELAX-AHF trial. The first hypothesis was that a 48-h infusion of serelaxin could improve clinical outcomes of 180-day cardiovascular mortality and worsening heart failure at 5 days. This hypothesis was tested in 6545 patients in the RELAX-AHF-2 trial, which failed to demonstrate a beneficial effect of serelaxin on cardiovascular mortality (hazard ratio [HR] 0.98; 95% confidence interval [CI] 0.83–1.15; $p = 0.77$) or worsening heart failure (HR 0.89; 95% CI 0.75–1.07; $p = 0.19$).[4,5]

A second hypothesis was that serelaxin provides short-term end-organ protection to patients hospitalized for heart failure. In RELAX-AHF, serelaxin demonstrated beneficial effects on biomarkers, reducing troponin T, creatinine, cystatin C, aspartate aminotransferase (AST) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) within 5 days after hospital admission and a greater decrease of these biomarkers was associated with a lower risk of all-cause mortality in the first 6 months.[2] The biomarker substudy of RELAX-AHF-2 was designed to investigate whether the findings that serelaxin provides short-term end-organ protection in patients hospitalized for heart failure could be replicated.

Methods

Study design

The design and primary results of RELAX-AHF-2 have been reported elsewhere.[4,5] In summary, RELAX-AHF-2 was a multicentre, double-blind, placebo-controlled trial in 6545 patients who were hospitalized for acute heart failure. Patients had dyspnoea, signs of congestion on chest radiography, increased plasma concentrations of natriuretic peptides and a systolic blood pressure of ≥125 mmHg. Patients were randomly assigned within 16 h after presentation to receive a 48-h intravenous infusion of either serelaxin (30 µg per kilogram of body weight per day) or placebo, in addition to standard of care. The two primary endpoints were cardiovascular death at 180 days and worsening heart failure at day 5.

The present study was a pre-specified substudy of the RELAX-AHF-2 study including 1020 patients from selected centres. All patients in these selected centres were asked to participate in the substudy. Only those who consented to the main study and the separate substudy were included. In addition to the local laboratory blood samples, biomarker blood samples related to cardiac, renal

and hepatic injury or dysfunction were collected and stored and analysed centrally. These biomarkers were measured with the primary aim to establish the effects of serelaxin on changes in serum/plasma concentrations of NT-proBNP, high-sensitivity troponin T (hsTnT), creatinine, cystatin C, blood urea nitrogen (BUN), uric acid, AST, and alanine aminotransferase (ALT). Plasma hsTnT was measured using the high-sensitivity Roche Elecsys assay (Roche Diagnostics GmbH, Mannheim, Germany). The 99th percentile upper reference limit was 0.014 μg/L, and the lowest concentration with a coefficient of variation of 10% was 0.013 g/L. NT-proBNP plasma levels were measured using the Roche Elecsys proBNP assay (Roche Diagnostics GmbH), which has a reporting range of 5–35 000 ng/L. Cystatin C plasma levels were measured using the Gentian Cystatin C Immunoassay (Beckman Coulter, Brea, CA, USA), with a reporting range of 0.25–8.4 mg/L (18.7–629 nmol/L). Samples were taken at baseline, days 2, and 5, discharge, and day 14. The ethics committee at each trial centre approved the trial, and all patients provided written informed.

The trial was funded by Novartis Pharma and was registered as RELAX-AHF-2 (ClinicalTrials.gov, NCT01870778).

Statistical analysis

Analyses were conducted on the intention-to-treat principle, with patients allocated according to randomized treatment. Baseline characteristics were summarized using count (%) for categorical variables and mean ± standard deviation or median (interquartile range) for normally and non-normally distributed continuous variables, respectively. Differences in the distribution of the baseline characteristics between the two treatment groups were tested using the χ^2 test for categorical variables, the independent-samples *t*-test for normally distributed continuous variables, and the Mann–Whitney U test for non-normally distributed continuous variables.

Longitudinal percentage changes from baseline measurements for each biomarker were analysed with a linear mixed-effects model with a random intercept for subject, the day of measurement (day 2, day 5, and day 14) as a within-subject variable, and the allocated study treatment as a between-subject variable. Estimates of the mean percentage change from baseline values at each time point were calculated from the fitted models and compared between the two treatment groups using approximate Wald tests.

Non-parametric estimates of the cumulative incidence curves for death from any cause through day 180, death from cardiovascular causes through day 180, and worsening heart failure through day 5 were obtained separately for the subgroup of patients included in the biomarker substudy and the subgroup of patients not included in the biomarker substudy, and compared between the two treatment groups using log-rank tests. HRs and 95% CIs for the effect of serelaxin versus placebo on these study endpoints were estimated from Cox regression models. The associations between the percentage change from baseline values of the biomarkers and (i) 180-day all-cause mortality and (ii) 180-day cardiovascular mortality were estimated from Cox regression models adjusted for the baseline biomarker values. Note that all *p*-values are nominal and not adjusted for multiple comparisons.

Role of the funding source

The study was designed by the members of the executive committee. Data collection and analysis were performed by contract research organizations. The study databases were held by the sponsor and at the department of epidemiology of the University Medical Center Groningen, The Netherlands. The executive committee had full access to the data. The authors not employed by the sponsor had ultimate editorial authority, with no interference by the sponsor on their final interpretation.

Results

Patient characteristics

The characteristics of patients included in the substudy ($n = 1020$) and those that were not ($n = 5525$) are provided in online supplementary *Table S1*. Patients in the biomarker substudy were slightly younger (72 ± 12 vs. 73 ± 11 years; $p < 0.001$), more often from North America (17.3% vs. 9.4%; $p < 0.001$), more often black (8.3% vs. 4.5%; $p < 0.001$), and had higher mean baseline systolic blood pressure (149 ± 18 vs. 146 ± 16 mmHg; $p < 0.001$), and lower median NT-proBNP (5453 [3098–9006] vs. 6188 [3636–10 140] ng/L; $p < 0.001$) compared to those not in the substudy. Despite a higher systolic blood pressure at baseline, the blood pressure decrease during the first 5 days was not different between groups (online supplementary *Figure S1*).

Of the 1020 patients included in this biomarker substudy, 503 patients were randomly assigned to placebo and 517 patients to serelaxin. Baseline characteristics of the patients are presented in *Table 1*. Except for a slightly lower body weight and body mass index in the serelaxin group, patient characteristics were similar between groups.

Effects of serelaxin on changes in biomarkers

The mean percentage change of biomarker concentrations from baseline to day 2, 5 and 14 are presented in *Table 2* and *Figure 1*. From baseline to day 2, mean change in NT-proBNP in the placebo group was −27.6% compared to −39.8% in the serelaxin group ($p = 0.0018$). HsTnT increased during hospital admission in all patients, but serelaxin significantly decreased this troponin release by day 5 (7.4% vs. 27.4%; $p = 0.031$) through day 14 (+0.2% vs. +40.3%; $p = 0.042$). Serelaxin reduced the increase of markers related to renal damage/dysfunction during the first 2 days, but afterwards this effect diminished. From baseline to day 2 increases in both creatinine (−0.8 vs. 5.8%; $p = 0.0017$), cystatin C (+3.8 vs. +8.3%; $p = 0.016$) and uric acid (+0.8 vs. +7.2%; $p = 0.0014$) were lower in serelaxin versus placebo-treated patients. While the increase in uric acid remained significantly lower in the serelaxin group at day 5, the differences in creatinine and cystatin C were no longer present at day 5 and day 14. There was a trend for both AST and ALT to have a greater increase at day 14 in the placebo group, but only AST was significantly better in the serelaxin patients.

Association between changes in biomarkers and 180-day all-cause mortality

Continuous changes of cardiac, renal and hepatic biomarkers from baseline were associated with 180-day all-cause mortality in this

Table 1 Baseline characteristics inclusion in biomarker and laboratory substudy population stratified by study treatment

Variable	Placebo ($n = 503$)	Serelaxin ($n = 517$)	*p*-value
Age, years	71.3 ± 12.1	72.5 ± 12.3	0.11
Age group, *n* (%)			0.088
<75 years	275 (54.7)	254 (49.1)	
≥75 years	228 (45.3)	263 (50.9)	
Male sex, *n* (%)	310 (61.6)	314 (60.7)	0.82
Race, *n* (%)			0.63
White	455 (90.5)	460 (89)	
Black	40 (8)	45 (8.7)	
Other or missing data	8 (1.6)	12 (2.3)	
Geographical region, *n* (%)			0.96
Eastern Europe	224 (44.5)	219 (42.4)	
Western Europe	158 (31.4)	173 (33.5)	
North America	87 (17.3)	89 (17.2)	
Latin America	33 (6.6)	35 (6.8)	
Other	1 (0.2)	1 (0.19)	
History of heart failure, *n* (%)	368 (73.2)	378 (73.1)	1
Previous hospitalization for heart failure, *n* (%)	241/469 (51.4)	264/475 (55.6)	0.22
No. of hospitalizations for heart failure within previous year	1.2 ± 1.4	1.2 ± 1.3	0.96
Ischaemic cause of heart failure, *n* (%)	195/368 (53)	203/376 (54)	0.84
Ejection fraction at index hospitalization (%)	38 ± 14.1	38.5 ± 14.1	0.6
Ejection fraction ≤40%, *n* (%)	254/485 (52.4)	269/498 (54)	0.65
NYHA class 1 month before admission, *n* (%)			0.68
I/II	165/362 (45.6)	171/364 (47)	
III	155/362 (42.8)	158/364 (43.4)	
IV	42/362 (11.6)	35/364 (9.6)	
Intravenous nitrates at randomization, *n* (%)	32 (6.4)	34 (6.6)	0.99
Time from earlier of presentation to hospital or first IV loop diuretic to randomization (h)	6.9 [5–10.5]	7.3 [5–11.3]	0.8
Time from presentation to randomization (h)	6.9 [5–10.5]	7.3 [5–11.3]	0.77
Time from first IV loop diuretic to randomization (h)	5.4 [3.4–9.2]	5.5 [3.3–9.3]	0.96
Time from randomization to study drug administration (h)	0.5 [0.3–1.1]	0.5 [0.3–1]	0.96
Weight (kg)	86.8 ± 20.5	82.6 ± 20.5	0.0013
BMI (kg/m^2)	30.4 ± 6.3	29.5 ± 6.6	0.017
SBP (mmHg)	148.6 ± 18.6	148.3 ± 17.5	0.83
DBP (mmHg)	82.4 ± 14.9	82.9 ± 14.7	0.58
Heart rate (bpm)	83.1 ± 16.6	83.7 ± 16.8	0.58
Respiration rate (breaths/min)	22.1 ± 4.8	22.1 ± 4.6	0.97
Temperature (°C)	36.5 ± 0.4	36.5 ± 0.4	0.56
eGFR (ml/min/1.73 m^2)	51.3 ± 14.4	51.6 ± 13.7	0.74
BNP (ng/L)	1140 [743–1969]	1065 [675–1555]	0.21
NT-proBNP (ng/L)	5222 [2877–9006]	5628 [3297–9006]	0.3
Troponin T	0.04 [0.02–0.06]	0.04 [0.02–0.06]	0.48
Creatinine (md/dl)	1.24 [1.03–1.58]	1.26 [1.03–1.48]	0.40
Cystatin C (mg/dl)	1.52 [1.20–1.91]	1.52 [1.23–1.81]	0.66
Blood urea nitrogen (mg/dl)	8.60 [6.70–11.8]	8.80 [7.10–11.5]	0.47
Uric acid (μmol/L)	470 [382–560]	460 [370–540]	0.15
Aspartate aminotransferase (U/L)	23.0 [19.0–33.0]	24.0 [18.2–36.0]	0.35
Alanine aminotransferase (U/L)	19.0 [14.0–31.2]	21.0 [15.0–33.0]	0.08

Values are given as mean ± standard deviation, or median [interquartile range], unless otherwise specified.
BMI, body mass index; BNP, B-type natriuretic peptide; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; IQR, interquartile range; IV, intravenous; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; SBP, systolic blood pressure.

Table 2 Percentage change from baseline

Time	Placebo, mean (SE)	Serelaxin, mean (SE)	Mean difference (SE)	*p*-value
NT-proBNP				
Day 2	−27.6 (2.78)	−39.8 (2.75)	12.2 (3.91)	0.0018
Day 5	−38.3 (2.8)	−42.2 (2.76)	3.8 (3.93)	0.33
Day 14	−39.4 (2.86)	−42.4 (2.8)	3 (4)	0.46
Troponin T				
Day 2	27.4 (13.65)	7.4 (13.51)	20 (19.2)	0.3
Day 5	58.6 (13.75)	16.8 (13.57)	41.8 (19.32)	0.031
Day 14	40.3 (14.1)	0.2 (13.8)	40 (19.73)	0.042
Creatinine				
Day 2	5.8 (1.5)	−0.8 (1.46)	6.6 (2.09)	0.0017
Day 5	12.5 (1.54)	9.5 (1.48)	3 (2.13)	0.17
Day 14	13.1 (1.54)	13.3 (1.49)	−0.3 (2.14)	0.91
Cystatin C				
Day 2	8.3 (1.32)	3.8 (1.31)	4.5 (1.86)	0.016
Day 5	15.3 (1.33)	13.8 (1.31)	1.5 (1.87)	0.41
Day 14	17 (1.35)	15.2 (1.33)	1.8 (1.89)	0.33
BUN				
Day 2	11.7 (2.52)	5.2 (2.46)	6.5 (3.52)	0.063
Day 5	31.6 (2.59)	30.3 (2.49)	1.3 (3.59)	0.73
Day 14	36.9 (2.6)	35.6 (2.5)	1.3 (3.61)	0.72
Uric acid				
Day 2	7.2 (1.44)	0.8 (1.41)	6.4 (2.01)	0.0014
Day 5	11.2 (1.46)	6.3 (1.42)	4.8 (2.04)	0.018
Day 14	8.8 (1.47)	6.2 (1.42)	2.6 (2.04)	0.2
AST				
Day 2	−6.9 (8.17)	−13 (7.98)	6.1 (11.43)	0.59
Day 5	7.7 (8.46)	12.1 (8.1)	−4.4 (11.72)	0.7
Day 14	21.9 (8.46)	−1.5 (8.13)	23.4 (11.74)	0.046
ALT				
Day 2	−5.9 (10.17)	−12.4 (9.96)	6.5 (14.24)	0.65
Day 5	14.8 (10.54)	15.9 (10.11)	−1.1 (14.61)	0.94
Day 14	36.3 (10.57)	15.2 (10.14)	21.1 (14.65)	0.15

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SE, standard error.

trial (*Table 3*). A doubling of NT-proBNP from baseline to both day 2, day 5 and day 14 was associated with a highly statistically significant approximately twofold increase in the risk of dying within 180 days. Similarly, a doubling of hsTnT from baseline to day 2, 5 and 14 was associated with an approximately 1.5 times increased risk of 180-day mortality. In contrast, none of the renal markers showed an association between change from baseline to day 2 and 5 and 180-day mortality, but a change in creatinine and BUN between baseline and day 14 was modestly associated with 180-day mortality. Finally, changes in both AST and ALT between baseline and day 2 and 14 were strongly associated with 180-day mortality.

Effects of serelaxin on worsening heart failure and 180-day cardiovascular mortality

The cumulative incidence of worsening heart failure within 5 days was nearly identical in the placebo groups from the patients in this substudy and those not in the substudy, and there was no effect of serelaxin in those patients not in the substudy (HR 0.96; 95% CI 0.79–1.16; $p = 0.64$; *Figure 2*). However, in this biomarker substudy, serelaxin treatment was associated with a 45% reduction in worsening heart failure through 5 days (serelaxin, 22 patients [4.3%], placebo, 38 patients [7.6%]; HR 0.55; 95% CI 0.33–0.93; $p = 0.027$; *Figure 2*; *p*-value for interaction between the main study and the biomarker substudy = 0.0594) The cumulative incidences of cardiovascular death through 180 days were nearly superimposable in the placebo and serelaxin treatment groups of patients not in the substudy (HR 1.01; 95% CI 0.85–1.20; $p = 0.92$; *Figure 3*). In this substudy, death from cardiovascular causes at day 180 had occurred in 32 patients (6.2%) in the serelaxin group and in 39 patients (7.8%) in the placebo group (HR 0.78; 95% CI 0.49–1.25; $p = 0.30$; *p*-value for interaction between the main study and the biomarker substudy = 0.32).

Discussion

In the present RELAX-AHF-2 biomarker substudy, a 48-h infusion of serelaxin in patients hospitalized for heart failure had beneficial effects on the evolution of multiple biomarkers, supporting a

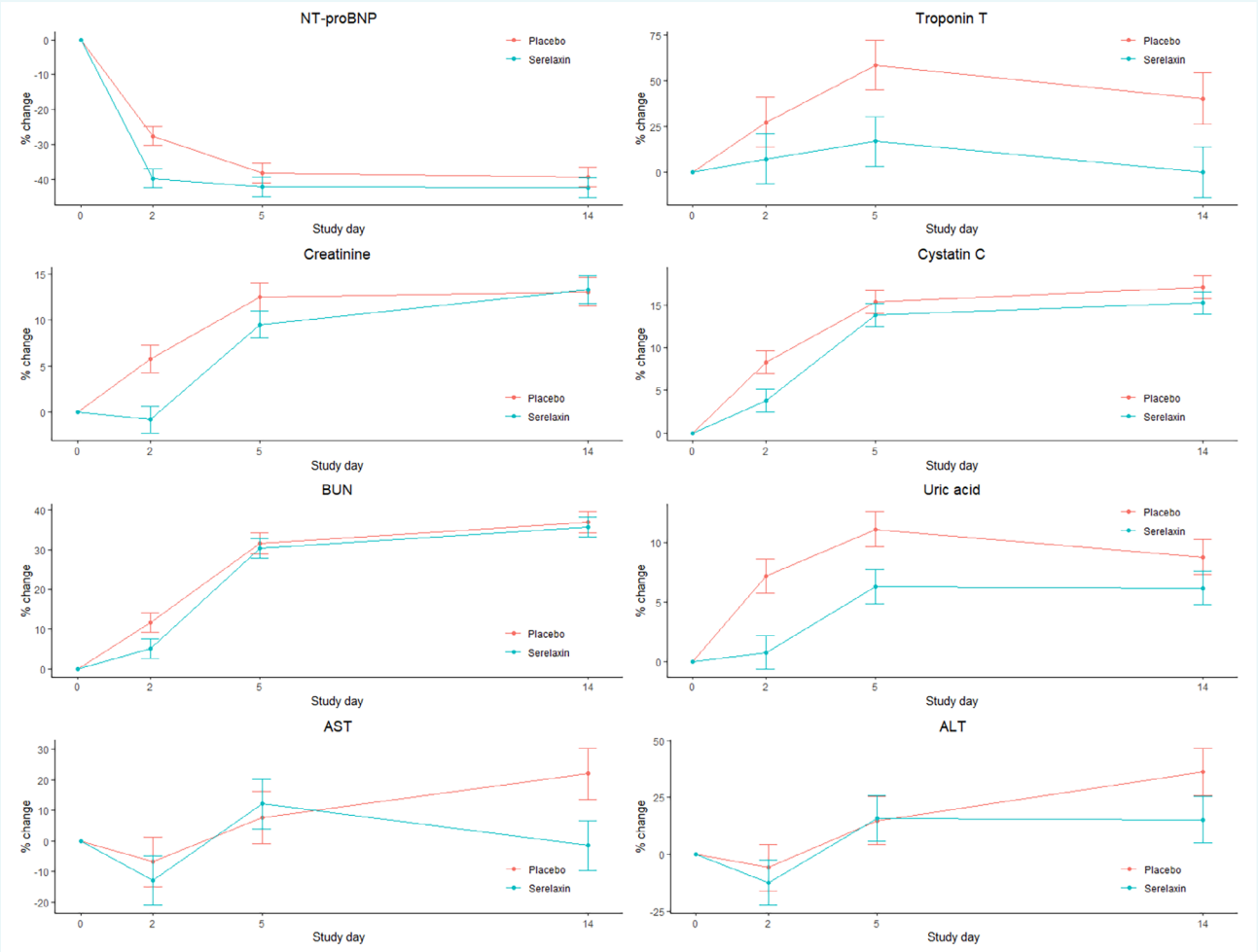


Figure 1 Percentage change from baseline (mean ± standard error) to day 2, day 5, and day 14 for the eight different biomarkers. AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

unique protective effect of serelaxin on end-organ damage. As with other studies, short-term increases in biomarkers of end-organ damage in this substudy were associated with increased 180-day mortality. Patients treated with serelaxin had significant reductions in the short-term event of worsening heart failure within 5 days, although there was no significant change in cardiovascular mortality at 180 days (*Graphical Abstract*).

Relaxin has been shown to be cardioprotective in multiple pre-clinical studies, providing the biological plausibility for such an effect in clinical trials.[6] In this substudy of 1020 patients with acute heart failure, myocardial injury was common with 95% of patients who had plasma troponin T concentration above the upper reference limit. Troponin concentrations increased significantly in the placebo group, consistent with the concept of ongoing myocardial damage during heart failure decompensation. However, serelaxin largely prevented an increase in troponins with significantly lower troponin concentrations on days 5 and 14. At days 5 and 14, patients in the placebo group had 40% greater increase from baseline in troponin compared to the serelaxin-treated patients. A similar beneficial effect on cardiac protection was seen in the 1161 patients of the RELAX-AHF trial which had nearly identical enrolment criteria as RELAX-AHF-2.[2] To our knowledge and on the basis of two independent trials of over 2000 total patients, serelaxin is the only drug to have demonstrated this cardioprotective benefit in patients with acute heart failure.

Additional end-organ protective effects were evident with serelaxin. In the RELAX-AHF trial, patients receiving serelaxin had less worsening of their cystatin C, creatinine, BUN and uric acid concentrations at day 2, suggestive of a protective effect on renal function.[2] In the current substudy of RELAX-AHF-2, there was also significant attenuation of worsening creatinine, cystatin C, BUN and uric acid concentrations at day 2. While it is known that renal congestion is a major cause of worsening renal function in the setting of acute heart failure,[7] given that the beneficial effect was only present during the 48 h of the serelaxin infusion, this renal protection is unlikely to be solely due to decongestion. In the TRUE-AHF trial with ularitide therapy in patients with acute heart failure, there was substantial decongestion, yet creatinine

Table 3 Association of percentage change from baseline with 180-day all-cause mortality

Biomarker	HR (95% CI)	*p*-value
NT-proBNP (per 10% increase)		
Day 2	1.026 (1.013–1.038)	<0.0001
Day 5	1.04 (1.029–1.052)	<0.0001
Day 14	1.094 (1.066–1.122)	<0.0001
Troponin T (per 10% increase)		
Day 2	1.011 (1.006–1.016)	<0.0001
Day 5	1.002 (0.999–1.005)	0.18
Day 14	1.003 (1–1.006)	0.097
Creatinine (per 10% increase)		
Day 2	0.974 (0.871–1.089)	0.64
Day 5	0.96 (0.879–1.048)	0.36
Day 14	1.057 (1.019–1.096)	0.003
Cystatin C (per 10% increase)		
Day 2	0.965 (0.844–1.104)	0.6
Day 5	1.006 (0.916–1.105)	0.9
Day 14	1.056 (1.016–1.098)	0.0059
BUN (per 10% increase)		
Day 2	1.024 (0.955–1.098)	0.5
Day 5	1.008 (0.963–1.055)	0.74
Day 14	1.044 (1.019–1.07)	0.00059
Uric acid (per 10% increase)		
Day 2	1.028 (0.935–1.13)	0.57
Day 5	1.001 (0.912–1.099)	0.98
Day 14	1.042 (0.986–1.101)	0.14
AST (per 10% increase)		
Day 2	1.036 (1.018–1.054)	<0.0001
Day 5	0.987 (0.95–1.026)	0.52
Day 14	1.009 (1.006–1.013)	<0.0001
ALT (per 10% increase)		
Day 2	1.036 (1.018–1.054)	<0.0001
Day 5	1.022 (1.001–1.043)	0.043
Day 14	1.008 (1.005–1.011)	<0.0001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CI, confidence interval; HR, hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

significantly worsened.[8] A study of 65 patients with chronic heart failure provides a plausible mechanism for a renal protective effect of serelaxin. In this study, serelaxin improved renal plasma flow and reduced intraglomerular pressures, possibly protecting the kidney from ischaemic or other damage during a heart failure exacerbation.[9]

Patients hospitalized for heart failure have a markedly increased risk of recurrent hospitalization and death.

One hypothesis for the risk conferred by hospitalizations for heart failure is that such decompensations are accompanied by end-organ damage that increases the likelihood of subsequent clinical worsening. This hypothesis was initially supported by the findings from the first Phase III trial with serelaxin, RELAX-AHF, where changes in markers of cardiac (hsTnT), renal (creatinine and cystatin C), and hepatic (AST and ALT) damage and of decongestion (NT-proBNP) at day 2 during admission were associated with 180-day mortality.[2] Importantly, in this biomarker substudy from RELAX-AHF-2, there was additional evidence of a relationship between changes in troponin T, creatinine, BUN, AST and ALT, and NT-proBNP with 180-day mortality. Although serelaxin had significant effects on end-organ damage in both trials and serelaxin-treated patients had increased survival in the RELAX-AHF trial, there was no beneficial effect on 180-day mortality in either the overall RELAX-AHF-2 trial[5] or this biomarker substudy of RELAX-AHF-2. Interestingly, in a meta-analysis including 11 359 patients receiving therapy within 16 h of admission for acute heart failure in the serelaxin development program, serelaxin was associated with a nominally significant 13% reduction in all-cause mortality (95% CI 0.77–0.98; $p = 0.026$).[10]

In this current substudy, serelaxin treatment was associated with a 45% reduction in in-hospital worsening heart failure. This is in contrast to the RELAX-AHF-2 main study, where serelaxin did not significantly reduce worsening heart failure through day 5 (HR 0.89; 95% CI 0.75–1.07; $p = 0.19$). We cannot clearly explain this discrepancy. However, it should be noted that this subgroup was not randomly selected from the overall RELAX-AHF-2 population, but rather represents a cohort of patients who were more frequently from sites in North America, more often of Black race, younger, had a higher baseline systolic blood pressure, and a lower NT-proBNP compared to those patients not included in this biomarker substudy. However, despite higher baseline systolic blood pressure we did not observe differences in the change in blood pressure during the first 5 days between groups. In addition, none of these parameters showed a significant interaction with serelaxin treatment effect in RELAX-AHF-2. Nonetheless, the combination of these characteristics might accidently have resulted in a profile of patients that had an augmented beneficial response to treatment with serelaxin. Importantly, this biomarker subgroup study was not powered for cardiovascular death of worsening heart failure, while the RELAX-AHF-2 main study was. On the other hand, in a meta-analysis[10] noted above pooling data of 11 359 acute heart failure patients from six randomized controlled trials,[3,5,9,11–13] worsening heart failure to day 5 was reduced in the serelaxin group by 23% (HR 0.77; 95% CI 0.67–0.89; $p = 0.0002$).

Limitations

Several limitations of this study should be acknowledged. First, patients included in RELAX-AHF-2 were selected based on elevated systolic blood pressures at baseline and thus only represent a subset of the general population of patients with acute heart failure. Second, the results of these analyses are based on a subgroup of patients from selected study sites who were willing to participate in this substudy. Third, while this substudy was prospective, pre-specified and well-powered to assess the effects of serelaxin effect on biomarkers, it was not powered to evaluate the effect of serelaxin on either cardiovascular or all-cause mortality. Fourth, while originally 1600 patients were planned to be included in this substudy, only 1020 were included due to a slower rate of inclusion than expected. Finally, some inconsistencies between

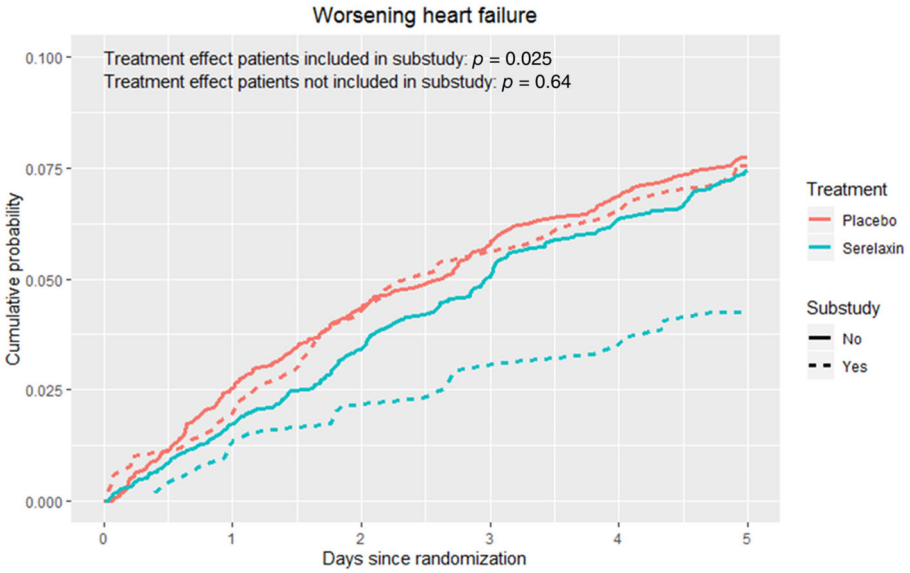


Figure 2 Cumulative incidence of worsening heart failure to day 5 in patients with serelaxin versus placebo.

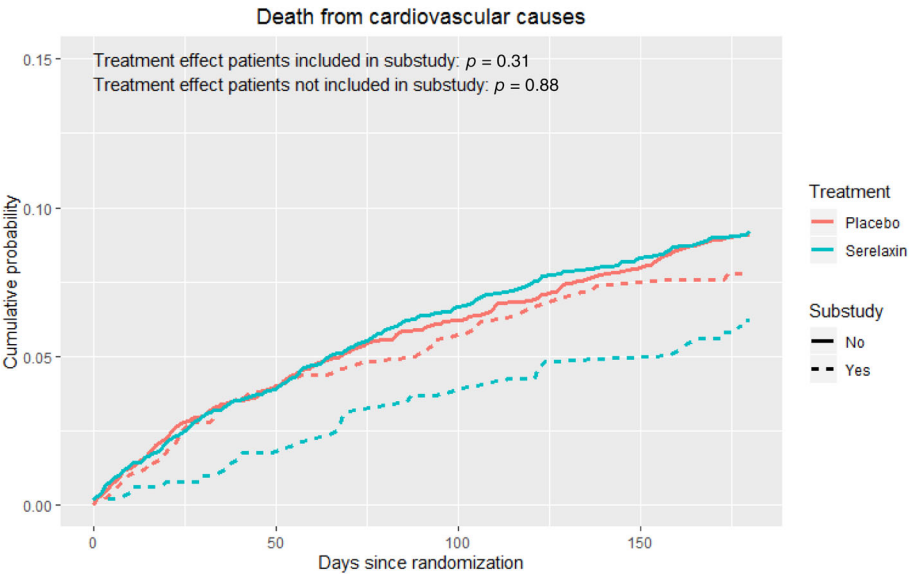


Figure 3 Cumulative incidence of 180-day cardiovascular death in patients treated with serelaxin versus placebo.

the present study and the biomarker results from RELAX-AHF, such as a reduced effect on hepatic markers and the lack of an association between change in renal markers and outcome, should be noted.

Clinical implications

Data from RELAX-AHF-2 have shown that serelaxin does not result in a significant reduction in cardiovascular mortality or in-hospital worsening heart failure. Nevertheless, the present and other studies show beneficial effects on organ function, as reflected by a reduction in several biomarkers. Most of these biomarkers return to baseline soon after stopping intravenous serelaxin. It has therefore been suggested that agents with a longer half-life or dosing for a longer period of time might provide better clinical outcomes with relaxin analogues. Interestingly, several longer acting agents are currently being developed for the treatment of patients with heart failure.

Conclusions

In two studies including a total of over 2000 patients hospitalized for acute heart failure, serelaxin demonstrated significant reduction of troponin release, as well as protection of renal and hepatic function.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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