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Published in:
ESC Heart Failure

DOI:
10.1002/ehf2.12312

ESC HEART FAILURE
ESC Heart Failure 2018; **5**: 1052–1059
Published online 25 August 2018 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.12312

ORIGINAL RESEARCH ARTICLE

Pro-gastrin-releasing peptide and outcome in patients with heart failure and anaemia: results from the RED-HF study

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Abstract

Aims Neuroendocrine activation is associated with poor outcome in heart failure (HF). The neuropeptide gastrin-releasing peptide (GRP), derived from the precursor proGRP1-125 (proGRP), has recently been implicated in inflammation and wound repair. We investigated the predictive value of proGRP on clinical outcomes in HF patients with reduced ejection fraction.

Methods and results The association between plasma proGRP (time-resolved immunofluorometric assay) and the primary endpoint of death from any cause or first hospitalization for worsening of HF was evaluated using multivariable Cox proportional hazard models in 1541 patients with systolic HF and mild to moderate anaemia, enrolled in the Reduction of Events by Darbepoetin alfa in Heart Failure (RED-HF) trial. Median proGRP levels in the RED-HF cohort were markedly increased [95 ng/L (25th, 75th percentile, 69–129 ng/L)] with 64% patients above the 80 ng/L reference limit. Baseline proGRP correlated with estimated glomerular filtration rate (r = 0.52), N terminal pro brain natriuretic peptide (r = 0.33), troponin T (r = 0.34), and haemoglobin (r = 0.16) (all $P < 0.001$). The incidence outcome increased with increasing tertiles of baseline proGRP (primary endpoint third tertile vs. the lowest tertile; hazard ratio 1.91; 95% confidence interval 1.60–2.28, $P < 0.001$). However, these associations were markedly attenuated and non-significant in adjusted models. No interaction between baseline proGRP and the effect of darbepoetin alfa treatment was detected. Moreover, no significant association between changes in proGRP during 6 month follow-up and outcome was observed.

Conclusions Pro-gastrin-releasing peptide is increased in patients with HF with reduced ejection fraction and anaemia, in particular in patients with poor renal function. However, proGRP adds little as a prognostic marker on top of conventional HF risk factors.

Keywords ProGRP; Anaemia; Heart failure; Prognosis

Received: 2 November 2017; Revised: 8 April 2018; Accepted: 28 May 2018
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Introduction

Neuroendocrine activation is a key feature in patients with certain cancers and in heart failure (HF) and is an established predictor of poor prognosis in both populations.[1,2] Neuroendocrine tumours produce proteins such as chromogranin A (CgA) that reflect neuroendocrine differentiation in various cancers[3] but are also associated with severity and outcomes in HF.[4] Conversely, neurohormones such as natriuretic peptides, adrenomedullin, endothelin, and copeptin are elevated in cancer patients and related to all-cause mortality.[5]

The neuropeptide gastrin-releasing peptide (GRP) is a mammalian bombesin homologue,[6] involved in a multitude of physiological functions with a widespread distribution in the body, particularly in neuroendocrine cells, stimulating the release of other hormones including norepinephrine.[7] Recently, GRP has also been implicated in inflammation and wound repair.[8] While GRP is highly unstable in blood and difficult to monitor, the pro-peptide pro-gastrin-releasing peptide (proGRP) is stable with a long half-life. The circulating levels of proGRP are frequently measured in the diagnostic workup of patients with pulmonary tumours.[9] In addition to CgA, the ovarian cancer marker carbohydrate antigen 125 (CA-125) and the WAP four-disulfide core domain protein HE4 have been shown to correlate with clinical severity and adverse outcome in HF patients.[4,10,11] Based on the role of GRP in inflammation and tissue repair and ability to reflect neuroendocrine activation, it is conceivable that proGRP also could be regulated during HF, but this hypothesis has not been studied.

To investigate the association between circulating proGRP and HF, we first evaluated circulating levels of proGRP in a small well-characterized population of HF patients compared with matched controls. We then assessed whether proGRP predicted clinical outcomes in 1588 patients with HF and anaemia, enrolled in the Reduction of Events by Darbepoetin alfa in Heart Failure (RED-HF) trial.[12] Based on its potential to reflect distinct pathophysiologic mechanisms in HF such as neuroendocrine activation, fibrosis, and inflammation, we hypothesized that proGRP could represent a novel biomarker for adverse outcome in these patients.

Materials and methods

Patients and study procedures

Cross-sectional cohort

One hundred and thirty-nine consecutive patients with chronic HF with reduced ejection fraction (HFrEF) referred to our tertiary hospital for evaluation were enrolled in the study. Patients with acute coronary syndromes within the last 6 months or with significant concomitant disease (e.g. malignancies, autoimmune disorders, or liver or kidney failure) were not included. The composite endpoint of all-cause mortality and heart transplantation was used as the outcome variable. The control group consisted of 32 sex-matched and age-matched healthy individuals.

RED-HF cohort

The study design, as well as baseline characteristics and results of RED-HF trial (http://clinicaltrials.gov number: NCT00358215), has been published.[13,14] Patients were eligible for the study if they had New York Heart Association (NYHA) functional class II–IV HF, left ventricular ejection fraction (LVEF) $\leq$ 40%, haemoglobin level 9.0 to 12.0 g/dL, and were receiving guideline-recommended HF therapy. Major exclusion criteria included transferrin saturation of less than 15%, evidence of bleeding or other correctable causes of anaemia, a serum creatinine level more than 3 mg/dL (265 μmol/L), and blood pressure more than 160/100 mmHg. Patients were randomly assigned in a 1:1 ratio to receive either darbepoetin alfa or placebo. The study drug was administered subcutaneously, with doses adjusted to maintain a haemoglobin level of 13.0 g/dL. The primary predefined outcome was a composite of death from any cause or first hospitalization for worsening of HF. The pre-specified adjudicated secondary outcomes were (i) the composite of death from cardiovascular (CV) causes or first hospitalization for worsening of HF, (ii) death from any cause, and (iii) CV death.

Both studies complied with the Declaration of Helsinki and were approved by the ethics committees of the participating hospitals. All patients provided written informed consent.

Biochemistry and blood sampling

Peripheral venous blood was drawn into pyrogen-free tubes with EDTA as anticoagulant, and plasma was isolated and stored at −80°C following centrifugation. The plasma from the cross-sectional population has previously been thawed once, while samples from RED-HF had not been thawed previously. ProGRP was analysed by time-resolved immunofluorometric assay.[9] N terminal pro brain natriuretic peptide (NT-proBNP), C-reactive protein, and troponin T (TnT) were assayed on a MODULAR platform (Roche Diagnostics, Basel, Switzerland). Both C-reactive protein and TnT were measured by a high-sensitivity assay. Galectin-3 levels have been determined previously in the clinical HF cohort and were determined by enzyme-linked immunosorbent assay (BG Medicine, Waltham, MA).[15]

Statistical analysis

Trends across tertiles of proGRP were tested using Kruskal–Wallis H test, one-way ANOVA, or χ^2 depending on distribution and variable type (i.e. categorical or continuous). For comparing treatment effects on proGRP, the Mann–Whitney *U*-test was used on change values, while Wilcoxon matched pairs test was used to assess longitudinal changes within groups. Stepwise linear regression was used to identify the most important predictors of proGRP. Kaplan–Meier curves were constructed to visualize and evaluate (log-rank test) differences in survival. A restricted cubic spline analysis with three knots was undertaken on the primary outcome to assess linearity of risk. Survival analyses

for RED-HF were performed using the Cox proportional hazard regression models to estimate hazard ratios (HRs) and 95% confidence intervals for the leukocyte markers included as log-transformed continuous variables at baseline, which included mainly clinical variables at Step 1 [age, gender, NYHA class, hospitalization for HF within 6 months, log serum creatinine, LVEF, aetiology, body mass index (BMI), left bundle-branch block, history of atrial fibrillation or flutter, and systolic blood pressure]. At Step 2, log-transformed serum concentrations of TnT, NT-proBNP, and C-reactive protein were included. Interaction between proGRP and BMI on outcomes was assessed in a Cox proportional hazard regression model including the proGRP, BMI, and their interaction term.

For the analysis of changes in proGRP concentrations from baseline to 6 month follow-up, a 15% relative change was used as cut-off, which is consistent with other studies.[16] Harrell's C-statistic and net reclassification improvement were calculated to evaluate the prognostic usefulness of biomarkers. A two-sided *P*-value < 0.05 was considered to be significant. All statistical analyses were performed with the use of SAS software, version 9.2.

Results

Plasma pro-gastrin-releasing peptide levels in the clinical heart failure cohort

Median plasma levels of proGRP were mildly elevated in 139 HF patients [mean age ± standard deviation 57 ± 11 years, 18% women, BMI 26.8 ± 5.7 kg/m^2, NYHA class (II 27%, III 48%, and IV 25%), 48% ischaemic aetiology, LVEF 29 ± 11, estimated glomerular filtration rate (eGFR) 76 ± 25 mL/min/1.73m^2] compared with 32 matched healthy controls (age 57 ± 11 years, 16% women): median 54 ng/L (25th, 75th percentile: 42, 75 ng/L) vs. 51 (38, 57) ng/L, $P = 0.046$, respectively. A higher proportion of HF patients had proGRP levels above the 80 ng/L reference limit compared with controls (3% vs. 20%, $P = 0.024$), and only 6% of controls had levels in the top tertile of HF patients ($P = 0.002$). Increased proGRP levels were associated with clinical severity as evaluated by NYHA (*Figure 1A*) with particularly high levels in NYHA IV ($P = 0.003$ and $P = 0.019$ vs. controls and NYHA II, respectively) but not with ischaemic aetiology ($P = 0.24$). ProGRP showed a strong inverse correlation with eGFR ($r = -0.60$, $P < 0.001$), positive correlation with galectin-3 ($r = 0.49$, $P < 0.001$), modest positive correlation with NT-proBNP ($r = 0.39$, $P < 0.001$) (*Figure 1B*), and poor and insignificant correlation with LVEF ($r = -0.16$, $P = 0.08$) and C-reactive protein ($r = 0.13$, $P = 0.134$). Kaplan–Meier analysis revealed a significant association with the composite of death/heart transplantation ($n = 47$) when evaluated as tertiles (*Figure 1C*) and according to the reference cut-off of 80 ng/L (i.e. above or below 80 ng/L, $P = 0.022$).

Plasma pro-gastrin-releasing peptide in the RED-HF cohort

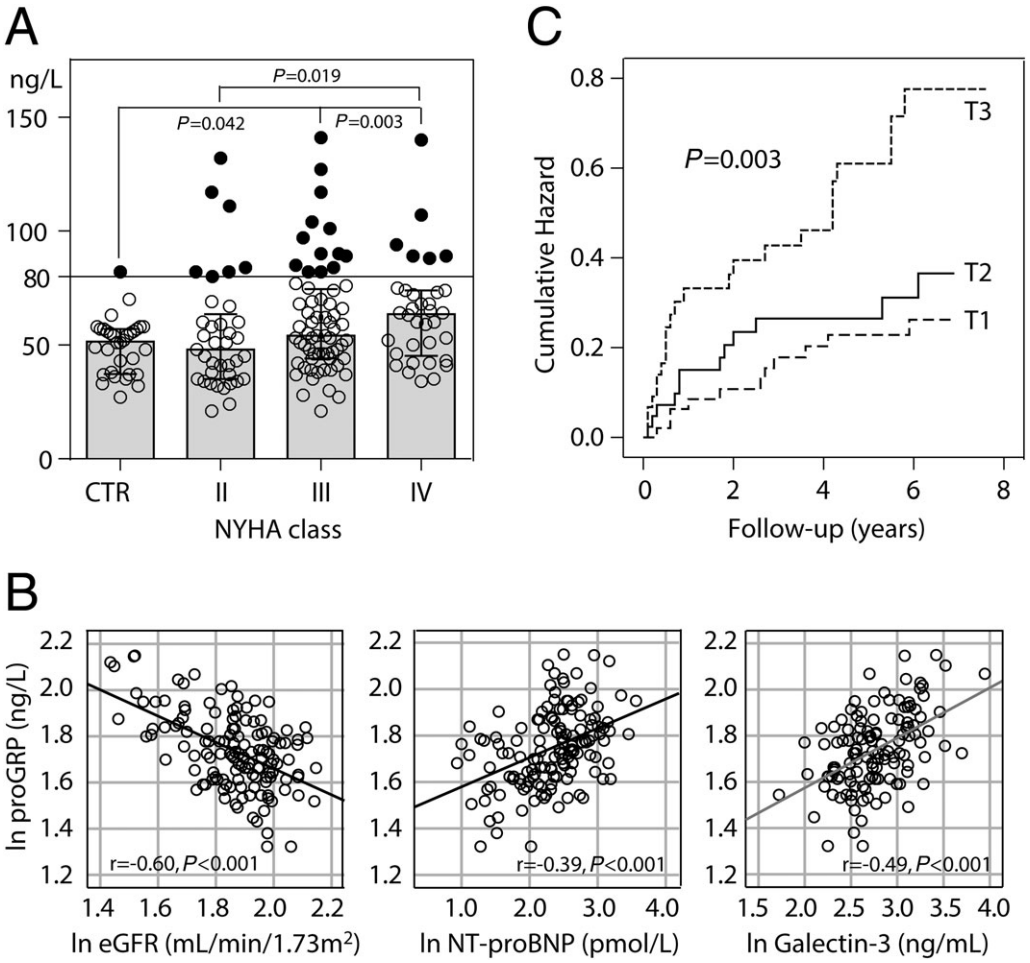
Of the 2278 patients enrolled in the RED-HF study, measurement of proGRP at baseline was available in 1541 (68%) subjects. *Table 1* shows the baseline demographic and clinical characteristics according to tertiles of proGRP. Median proGRP levels were markedly higher in the RED-HF cohort: 95 (69, 129) ng/L with 64% of patients having levels above the 80 ng/L reference limit. Elevated proGRP were associated with several features indicating more severe HF such as NYHA class, duration of disease, incidence of atrial fibrillation/flutter, poor kidney function, higher TnT and NT-proBNP levels, and relevant to this population, lower haemoglobin and transferrin saturation. Similar to the clinical HF cohort, proGRP was poorly correlated with C-reactive protein ($r = 0.05$, $P = 0.067$). Stepwise linear regression analyses revealed that the five strongest predictors of proGRP were eGFR (B ± standard error = 0.03 ± 0.1), log NT-proBNP ($B = 6.9 \pm 2.1$), creatinine ($B = 43 \pm 6$), male sex ($B = -25 \pm 4$), and age ($B = 0.66 \pm 0.16$), all $P < 0.001$.

Association between baseline pro-gastrin-releasing peptide levels and outcome in RED-HF

During a median follow-up of 28 months (range 0.03–72.4 months), 784 patients reached a primary endpoint, 703 patients reached the composite of death from CV causes or first hospitalization for worsening of HF, while 637 patients died, 532 due to CV causes. The cubic spline analysis revealed a linear increase in the primary endpoint of RED-HF within the range of first and second tertile, followed by flattening of the curve around Tertile 3 (*Figure 2A*). Kaplan–Meier estimates for the primary endpoint according to baseline tertiles of proGRP are presented in *Figure 2B* showing lower risk in Tertile 1 (≤77 ng/L), similar to the upper reference limit in our laboratory (>80 ng/L), with a stepwise increase in risk at higher tertiles (*Figure 2B*).

In unadjusted analyses, baseline proGRP by tertiles and as a continuous variable was significantly associated with all endpoints with HRs ranging from 1.86 to 2.06 for Tertile 3 compared with Tertile 1 (all $P < 0.001$) (*Table 2*). In multivariable analysis adjusting for pre-specified clinical variables (i.e. age, gender, NYHA class, hospitalization for HF within 6 months, log serum creatinine, LVEF, aetiology, BMI, left bundle-branch block, history of atrial fibrillation or flutter, and systolic blood pressure), the associations with outcome were markedly attenuated but remained significant for the

Figure 1 Circulating levels of pro-gastrin-releasing peptide (proGRP) in 139 patients with heart failure according to (A) New York Heart Association (NYHA) class and compared with age-matched and sex-matched healthy controls (CTR, *n* = 32); (B) correlation with estimated glomerular filtration rate (eGFR), N terminal pro brain natriuretic peptide (NT-proBNP), and galectin-3 levels; and (C) cumulative incidence of a composite endpoint consisting of all-cause mortality and heart transplantation (*n* = 47) shown as Kaplan–Meier curves according to tertiles of proGRP (T1: ≤46; T2: 47–65; T3: >65 ng/L).



primary endpoint and mortality when evaluated as a continuous variable and tertiles (*Table 2*). When NT-proBNP, TnT, and C-reactive protein were added to the multivariable models, however, the significance of proGRP was further attenuated and was no longer significant for any outcome with HRs around 1 (Step 2, *Table 2*).

As proGRP was inversely associated with BMI, we also assessed if there was an interaction between BMI and proGRP for the risk of adverse outcome (*Table 3*). This analysis revealed an association between low BMI and poor prognosis for the mortality outcomes but not the composite outcomes. However, no interaction between BMI and proGRP was detected.

No association between change in proGRP (i.e. decrease: <−15%; no change: 15–15%; increase: >15%) and any outcome was observed in univariate analysis (*P*-values between 0.16 and 0.28).

Effect of darbepoetin alpha on pro-gastrin-releasing peptide levels and clinical outcomes

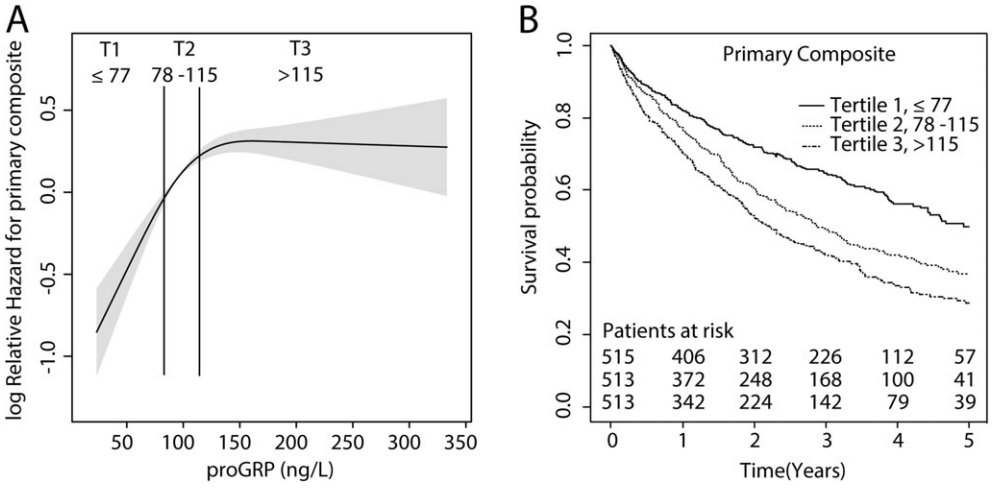
Plasma proGRP levels were similar at baseline in the placebo and darbepoetin alpha treatment groups (mean ± standard deviation 105 ± 55 ng/L vs. 110 ± 79 ng/L, *P* = 0.163, respectively). At 6 months, there was no clear effect of darbepoetin alpha therapy on proGRP levels (6 month levels 105 ± 56 and 108 ± 86 ng/L, *P* = 0.724, in placebo and darbepoetin alpha groups, respectively), although a minor difference in the change in proGRP between the groups was noted (−3 ± 33 vs. 1 ± 32 ng/L, *P* = 0.033, placebo and darbepoetin alpha, respectively). No interaction between baseline proGRP levels and darbepoetin alpha on any outcome was observed (*P*-values 0.19–0.29 in unadjusted analyses).

Table 1 Characteristics of patients from the RED-HF trial according to proGRP tertiles at baseline

Characteristic ProGRP (ng/L)	T1, *n* = 515 ≤77	T2, *n* = 513 78–115	T3, *n* = 513 >115	*P*-value
Age, years	66 ± 12	70 ± 11	73 ± 11	<0.001
Female sex	49	39	42	0.006
Race (White/Black)	63/13	65/10	72/7	
BMI (SD), kg/m^2	27.9 ± 5.7	27.1 ± 5.7	26.4 ± 5.5	<0.001
NYHA (III or IV)	65	65	71	0.039
Ischaemic HF	65	73	76	<0.001
Duration HF, years	4.9 ± 5.0	5.5 ± 5.5	5.8 ± 5.7	0.009
LVEF, %	30.6 ± 6.6	30.2 ± 6.6	29.9 ± 7.3	0.429
Medical history				
Hypertension	77	72	73	0.240
Diabetes	42	49	44	0.620
Atrial fibrillation or flutter	25	34	37	<0.001
Hospitalization WHF	33	37	39	0.168
Medication				
ACE-Inhibitors or ARB	93	90	88	0.016
Beta-blocker	85	86	85	0.881
Diuretic	88	92	94	0.001
Systolic BP	123 ± 17	120 ± 18	118 ± 19	<0.001
Heart rate, b.p.m.	73 ± 11	72 ± 11	72 ± 11	0.117
Biochemistry				
Creatinine, mg/dL	1.2 ± 0.4	1.5 ± 0.5	1.8 ± 0.6	<0.001
eGFR, mL/min/1.73m^2	64 ± 23	49 ± 19	38 ± 14	<0.001
Haemoglobin, g/dL	11.2 ± 0.7	11.0 ± 0.7	10.9 ± 0.7	<0.001
Transferrin saturation, %	27.9 ± 11.2	26.9 ± 10.6	26.4 ± 11.0	0.010
Platelets	241 ± 76	232 ± 80	222 ± 76	<0.001
WBC	6.8 ± 2.3	6.8 ± 2.3	6.7 ± 1.9	0.811
hsCRP, mg/dL	1.0 (0.7, 1.3)	1.0 (0.7, 1.5)	1.1 (0.7, 1.5)	0.188
NT-proBNP, pmol/L	0.7 (0.3, 1.3)	1.1 (0.7, 2.0)	1.5 (0.8, 2.7)	<0.001
hsTnT, ng/mL	0.6 (0.3, 1.2)	1.1 (0.6, 2.0)	1.5 (0.8, 2.7)	<0.001

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HF, heart failure; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitive troponin; LVEF, left ventricular ejection fraction; proGRP, pro-gastrin-releasing peptide; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association; SD, standard deviation; WBC, white blood cell; WHF, worsening heart failure.
Patient characteristics are given as mean ± SD for continuous variables and % of cases for categorical variables.

Figure 2 Association between baseline pro-gastrin-releasing peptide (proGRP) levels and the primary endpoint (*n* = 784) in the RED-HF cohort (*n* = 1541) during the whole study (mean follow-up: 28 months; range: 0.03–72.4 months) expressed as (A) restricted cubic spline with tertile cut-offs at enrolment shown as lines and (B) Kaplan–Meier curves showing the cumulative incidence of the primary endpoint according to tertiles at enrolment (T1: ≤77; T2: 78–115; T3: >116 ng/L).



DOI: 10.1002/ehf2.12312

Table 2 Association between baseline levels of proGRP and outcomes in the RED-HF study

	UNI	Step 1	Step 2
All-cause mortality or first hospitalization for worsening heart failure			
T2	1.52 (1.27–1.82)	1.19 (0.99–1.44)	0.93 (0.77–1.13)
T3	1.91 (1.60–2.28)	1.26 (1.03–1.54)	1.00 (0.81–1.22)
Continuous	1.35 (1.24–1.47)	1.11 (1.00–1.23)	0.97 (0.87–1.08)
P-trend/*P*-cont*	<0.001/<0.001	0.070/0.042	0.667/0.604
Cardiovascular mortality or first hospitalization for worsening heart failure			
T2	1.49 (1.23–1.81)	1.17 (0.96–1.43)	0.92 (0.75–1.13)
T3	1.86 (1.54–2.24)	1.21 (0.98–1.50)	0.96 (0.78–1.19)
Continuous	1.34 (1.23–1.47)	1.10 (0.99–1.22)	0.96 (0.86–1.08)
P-trend/*P*-cont*	<0.001/<0.001	0.169/0.087	0.715/0.490
All-cause mortality			
T2	1.66 (1.36–2.05)	1.29 (1.04–1.60)	1.05 (0.84–1.30)
T3	2.06 (1.69–2.52)	1.33 (1.06–1.67)	1.07 (0.85–1.35)
Continuous	1.40 (1.28–1.54)	1.15 (1.03–1.29)	1.02 (0.90–1.15)
P-trend/*P*-cont*	<0.001/<0.001	0.029/0.016	0.839/0.756
Cardiovascular Mortality			
T2	1.62 (1.29–2.02)	1.25 (0.99–1.57)	1.01 (0.80–1.28)
T3	1.99 (1.60–2.48)	1.27 (0.99–1.63)	1.02 (0.79–1.30)
Continuous	1.39 (1.26–1.54)	1.14 (1.01–1.30)	1.01 (0.88–1.15)
P-trend/*P*-cont*	<0.001/<0.001	0.111/0.039	0.993/0.934

proGRP, pro-gastrin-releasing peptide.
Hazard ratios and 95% confidence interval are shown for proGRP Tertiles 2 and 3 compared with Tertile 1 and for proGRP as a continuous (log) variable in univariate (UNI) analysis, when adjusted for clinical and biochemical variables (Step 1), and lastly for C-reactive protein, troponin T, and N terminal pro brain natriuretic peptide (Step 2).

Table 3 Interaction between baseline levels of proGRP and BMI on outcomes in the RED-HF study

Variables	HR	*P*
All-cause mortality or first hospitalization for worsening HF		
ProGRP	1.14 (0.82–1.58)	0.444
BMI	0.99 (0.98–1.00)	0.157
ProGRP × BMI	1.00 (0.99–1.02)	0.471
CV mortality or first hospitalization for worsening HF		
ProGRP	1.13 (0.80–1.59)	0.500
BMI	0.99 (0.98–1.01)	0.324
ProGRP × BMI	1.00 (0.99–1.02)	0.474
All-cause mortality		
ProGRP	1.10 (0.77–1.59)	0.600
BMI	0.97 (0.96–0.99)	<0.001
ProGRP × BMI	1.01 (0.99–1.02)	0.36
CV mortality		
ProGRP	1.09 (0.73–1.63)	0.677
BMI	0.97 (0.95–0.99)	<0.001
ProGRP × BMI	1.01 (0.99–1.02)	0.380

BMI, body mass index; CV, cardiovascular; HF, heart failure; HR, hazard ratio; proGRP, pro-gastrin-releasing peptide.

Discussion

The main finding in our study is that patients with HFrEF and mild or moderate anaemia had markedly increased circulating levels of the tumour marker proGRP, with a value above the upper reference limit in 64% of cases. High proGRP levels were associated with worse HF, as evaluated by clinical and biochemical indices, and with adverse long-term outcome. However, proGRP did not add independent prognostic information on outcomes after adjustment for clinical and biochemical indices of HF severity.

Levels of proGRP were only mildly elevated in our small cohort of HF patients compared with healthy controls, but the more pronounced differences in the most severely affected patients (i.e. NYHA IV) and the association with outcome in the top tertile of proGRP encouraged us to evaluate proGRP in a larger HF population. ProGRP levels were markedly elevated in a large cohort of HF patients with anaemia from the RED-HF trial. To the best of our knowledge, these observations are the first reports of proGRP in HF patients. Elevated circulating levels of tumour markers are not uncommon in HF, although the exact mechanism for their elevation is unclear and may vary depending on the marker. Increased levels of CA-125 and HE4 in HF and association with clinical severity and adverse outcome[10] have been suggested to reflect congestion[17] and immune activation,[11,18] whereas increased CgA levels in HF, a marker of neuroendocrine differentiation, have been suggested to reflect overall neuroendocrine activity.[4] We found that proGRP levels were associated with multiple clinical and biochemical markers of HF severity with kidney function and NT-proBNP as the strongest determinants in both cohorts. In addition, galectin-3, which we have previously reported in the clinical HF cohort,[15] was a strong determinant of proGRP.

Expression of both GRP mRNA and proGRP protein has been detected in experimental models and human kidney cell lines.[19] Furthermore, elevated proGRP levels correlate with the severity of renal failure in several studies with a decrease following haemodialysis indicating renal metabolism and/or excretion, limiting its use as a prognostic marker in patients with reduced renal function.[20,21] As for cardiac tissues, we found no reports on proGRP expression. However, protein

DOI: 10.1002/ehf2.12312

levels of the GRP-homologue bombesin have been detected in cardiac tissue from rats[22] and have also been shown to regulate blood pressure through peripheral α-adrenergic receptors.[6] ProGRP levels in patients from the RED-HF trial were moderately associated with features characterizing anaemia in HF such as lower haemoglobin and transferrin saturation but were not correlated with inflammation as reflected by C-reactive protein or white blood cell. However, although C-reactive protein is a reliable marker of general systemic inflammation, it does not necessarily reflect local and more specific inflammatory processes in a complex disease such as HF. Our finding that proGRP correlated strongly with galectin-3, a macrophage activation marker that is involved in the progression of HF through promotion of myocardial fibrosis and inflammation,[23,24] may suggest a role for proGRP in myocardial remodelling. We found no previous studies evaluating a link between proGRP and fibrosis; however, bombesin has been shown to promote pulmonary fibrosis.[25] Also, antagonism of the GRP receptor reduced mortality rates by limiting inflammatory infiltration in a sepsis model and attenuated TLR-4 signalling and the release of inflammatory cytokines from activated macrophages.[26,27] Taken together, as GRP is an important regulator of catecholamine secretion,[7] which may increase production of BNP,[28] proGRP levels in HF seem to reflect neurohormonal activation, worsening kidney function, and myocardial remodelling. However, we cannot exclude the possibility that the failing myocardium itself could, at least in part, contribute to the elevated levels of proGRP in HF patients.

In multivariable analysis adjusting for pre-specified clinical variables, proGRP levels were still associated with some adverse outcomes. However, when NT-proBNP, C-reactive protein, and TnT were added to the model, proGRP was not significantly associated with any of the outcomes. The strong association between proGRP and other markers for HF severity and in particular NT-proBNP in the RED-HF cohort could explain the marked attenuation of risk in patients with high proGRP levels upon multivariable adjustment for NT-proBNP, C-reactive protein, and TnT. The inverse association between BMI and all-cause and CV deaths in our study supports the well-established inverse association between BMI and risk of mortality in HFrEF.[29] While BMI and proGRP were inversely correlated in our patients, no interaction between BMI and proGRP was detected. Finally, no association between change in proGRP levels during 6 month follow-up and outcome was observed. Thus, the clinical usefulness of proGRP as a predictor of long-term outcome is likely to be limited.

In conclusion, the neuropeptide proGRP is increased in HF, in particular in patients with severe clinical disease and poor renal function. Whereas our data further support an association between tumour markers and HF, proGRP adds little as a prognostic marker on top of conventional CV risk factors

Conflict of interest

I.S.A., J.J.M., and D.J.v.V. are members of the RED-HF Executive Committee but have received no payments the last 12 months. J.J.M. has received travel and accommodation costs paid by Cytokinetics/Amgen in relation to advisory board and clinical trial meetings.

Funding

None.

References

1. Sato Y, Fujiwara H, Takatsu Y. Biochemical markers in heart failure. *J Cardiol* 2012; **59**: 1–7.
2. Zucker IH, Patel KP, Schultz HD. Neurohumoral stimulation. *Heart Fail Clin* 2012; **8**: 87–99.
3. Capdevila J, Meeker A, Garcia-Carbonero R, Pietras K, Astudillo A, Casanovas O, Scarpa A. Molecular biology of neuroendocrine tumors: from pathways to biomarkers and targets. *Cancer Metastasis Rev* 2014; **33**: 345–351.
4. Di Comite G, Morganti A. Chromogranin A: a novel factor acting at the cross road between the neuroendocrine and the cardiovascular systems. *J Hypertens* 2011; **29**: 409–414.
5. Pavo N, Raderer M, Hulsmann M, Neuhold S, Adlbrecht C, Strunk G, Goliasch G, Gisslinger H, Steger GG, Hejna M, Kostler W, Zochbauer-Muller S, Marosi C, Kornek G, Auerbach L, Schneider S, Parschalk B, Scheithauer W, Pirker R, Drach J, Zielinski C, Pacher R. Cardiovascular biomarkers in patients with cancer and their association with all-cause mortality. *Heart* 2015; **101**: 1874–1880.
6. Ohki-Hamazaki H, Iwabuchi M, Maekawa F. Development and function of bombesin-like peptides and their receptors. *Int J Dev Biol* 2005; **49**: 293–300.
7. Okubo M, Kaku K, Kaneko T, Yanaihara N. Effects of bombesin and gastrin releasing peptide on catecholamine secretion from rat adrenal gland *in vitro*. *Endocrinol Jpn* 1985; **32**: 21–27.
8. Baroni A, Perfetto B, Canozo N, Braca A, Farina E, Melito A, De Maria S, Carteni M. Bombesin: a possible role in wound repair. *Peptides* 2008; **29**: 1157–1166.
9. Nordlund MS, Warren DJ, Nustad K, Bjerner J, Paus E. Automated time-resolved immunofluorometric assay for progastrin-releasing peptide. *Clin Chem* 2008; **54**: 919–922.
10. Turgut O, Tandogan I, Yilmaz MB, Gul I, Gurlek A. CA125 levels among patients with advanced heart failure: an emerging independent predictor for survival. *Int J Cardiol* 2010; **145**: 71.
11. de Boer RA, Cao Q, Postmus D, Damman K, Voors AA, Jaarsma T, van Veldhuisen DJ, Arnold WD, Hillege HL, Sillje HH. The WAP four-disulfide cores domain protein HE4: a novel biomarker for heart

failure. *JACC Heart Failure* 2013; **1**: 164–169.
12. Swedberg K, Young JB, Anand IS, Cheng S, Desai AS, Diaz R, Maggioni AP, McMurray JJ, O'Connor C, Pfeffer MA, Solomon SD, Sun Y, Tendera M, van Veldhuisen DJ, Committees R-H, Investigators R-H. Treatment of anemia with darbepoetin alfa in systolic heart failure. *N Engl J Med* 2013; **368**: 1210–1219.
13. McMurray JJ, Anand IS, Diaz R, Maggioni AP, O'Connor C, Pfeffer MA, Polu KR, Solomon SD, Sun Y, Swedberg K, Tendera M, van Veldhuisen DJ, Wasserman SM, Young JB, Committees R-H, Investigators. Design of the Reduction of Events with Darbepoetin alfa in Heart Failure (RED-HF): a phase III, anaemia correction, morbidity-mortality trial. *Eur J Heart Fail* 2009; **11**: 795–801.
14. McMurray JJ, Anand IS, Diaz R, Maggioni AP, O'Connor C, Pfeffer MA, Solomon SD, Tendera M, van Veldhuisen DJ, Albizem M, Cheng S, Scarlata D, Swedberg K, Young JB, R-HC I. Baseline characteristics of patients in the Reduction of Events with Darbepoetin alfa in Heart Failure trial (RED-HF). *Eur J Heart Fail* 2013; **15**: 334–341.
15. Ueland T, Aukrust P, Broch K, Aakhus S, Skardal R, Muntendam P, Gullestad L. Galectin-3 in heart failure: high levels are associated with all-cause mortality. *Int J Cardiol* 2011; **150**: 361–364.
16. Masson S, Anand I, Favero C, Barlera S, Vago T, Bertocchi F, Maggioni AP, Tavazzi L, Tognoni G, Cohn JN, Latini R, Valsartan Heart Failure T, Gruppo Italiano per lo Studio della Sopravvivenza nell'Insufficienza Cardiaca-Heart Failure I. Serial measurement of cardiac troponin T using a highly sensitive assay in patients with chronic heart failure: data from 2 large randomized clinical trials. *Circulation* 2012; **125**: 280–288.
17. Turk HM, Pekdemir H, Buyukberber S, Sevinc A, Camci C, Kocabas R, Tarakcioglu M, Buyukberber NM. Serum CA 125 levels in patients with chronic heart failure and accompanying pleural fluid. *Tumor Biol* 2003; **24**: 172–175.
18. Kosar F, Aksoy Y, Ozguntekin G, Ozerol I, Varol E. Relationship between cytokines and tumour markers in patients with chronic heart failure. *Eur J Heart Fail* 2006; **8**: 270–274.
19. Dumesny C, Whitley JC, Baldwin GS, Giraud AS, Shulkes A. Developmental expression and biological activity of gastrin-releasing peptide and its receptors in the kidney. *Am J Physiol Renal Physiol* 2004; **287**: F578–F585.
20. Nakahama H, Tanaka Y, Fujita Y, Fujii M, Sugita M. CYFRA 21-1 and proGRP, tumor markers of lung cancer, are elevated in chronic renal failure patients. *Respirology* 1998; **3**: 207–210.
21. Nomura F, Koyama A, Ishijima M, Takano S, Narita M, Nakai T. Serum levels of five tumor markers for lung cancer in patients with chronic renal failure. *Oncol Rep* 1998; **5**: 389–392.
22. Ceconi C, Condorelli E, Quinzanini M, Rodella A, Ferrari R, Harris P. Noradrenaline, atrial natriuretic peptide, bombesin and neurotensin in myocardium and blood of rats in congestive cardiac failure. *Cardiovasc Res* 1989; **23**: 674–682.
23. Lin YH, Lin LY, Wu YW, Chien KL, Lee CM, Hsu RB, Chao CL, Wang SS, Hsein YC, Liao LC, Ho YL, Chen MF. The relationship between serum galectin-3 and serum markers of cardiac extracellular matrix turnover in heart failure patients. *Clin Chim Acta* 2009; **409**: 96–99.
24. Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JP, Schroen B, Andre S, Crijns HJ, Gabius HJ, Maessen J, Pinto YM. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation* 2004; **110**: 3121–3128.
25. Ashour K, Shan L, Lee JH, Schlicher W, Wada K, Wada E, Sunday ME. Bombesin inhibits alveolarization and promotes pulmonary fibrosis in newborn mice. *Am J Respir Crit Care Med* 2006; **173**: 1377–1385.
26. Dal-Pizzol F, Di Leone LP, Ritter C, Martins MR, Reinke A, Pens Gelain D, Zanotto-Filho A, de Souza LF, Andrades M, Barbeiro DF, Bernard EA, Cammarota M, Bevilaqua LR, Soriano FG, Claudio J, Moreira F, Roesler R, Schwartsmann G. Gastrin-releasing peptide receptor antagonist effects on an animal model of sepsis. *Am J Respir Crit Care Med* 2006; **173**: 84–90.
27. Petronilho F, Vuolo F, Galant LS, Constantino L, Tomasi CD, Giombelli VR, de Souza CT, da Silva S, Barbeiro DF, Soriano FG, Streck EL, Ritter C, Zanotto-Filho A, Pasquali MA, Gelain DP, Rybarczyk-Filho JL, Moreira JC, Block NL, Roesler R, Schwartsmann G, Schally AV, Dal-Pizzol F. Gastrin-releasing peptide receptor antagonism induces protection from lethal sepsis: involvement of toll-like receptor 4 signaling. *Molecular Medicine (Cambridge, Mass)* 2012; **18**: 1209–1219.
28. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. *N Engl J Med* 1998; **339**: 321–328.
29. Nagarajan V, Kohan L, Holland E, Keeley EC, Mazimba S. Obesity paradox in heart failure: a heavy matter. *ESC Heart Failure* 2016; **3**: 227–234.