ESC European Society of Cardiology

European Journal of Heart Failure (2023)
doi:10.1002/ejhf.3085

RESEARCH ARTICLE

Neuropeptide Y is elevated in heart failure and is an independent predictor of outcomes

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Received 3 August 2023; revised 17 October 2023; accepted 4 November 2023

Aims Neuropeptide Y (NPY) is the most abundant neuropeptide found in the heart and is released alongside norepinephrine following prolonged sympathetic activation, a process that is implicated in the pathophysiology of heart failure (HF). In patients with severely impaired left ventricular ejection fraction (LVEF) undergoing cardiac resynchronization therapy, higher levels of NPY measured in coronary sinus blood, are associated with poorer outcome. The aim was to examine the association of peripheral venous NPY levels and outcomes in a HF population with a range of LVEF, using a highly sensitive and specific assay.

Methods and results The association between NPY and the composite outcome of cardiovascular death or HF hospitalization, its components, and all-cause mortality was examined using Cox regression analyses among 833 patients using a threshold of elevated NPY identified through binary recursive partitioning adjusted for prognostic variables including estimated glomerular filtration rate (eGFR), ejection fraction and B-type natriuretic peptide (BNP). The mean value of NPY was 25.8 ± 18.2 pg/ml. Patients with high NPY levels (≥29 pg/ml) compared with low values were older (73 ± 10 vs. 71 ± 11 years), more often male (58.5% vs. 55.6%), had higher BNP levels (583 [261–1096] vs. 440 [227–829] pg/ml), lower eGFR (46.4 ± 13.9 vs. 52.4 ± 11.7 ml/min/1.73 m^2), and were more often treated with diuretics. There was no associated risk of HF hospitalization with NPY levels ≥29 vs. <29 pg/ml. Higher NPY levels were associated with a greater risk of cardiovascular and all-cause death (adjusted hazard ratio 1.56 [95% confidence interval 1.21–2.10], $p = 0.003$ and 1.30 [1.04–1.62], $p = 0.02$, respectively). There was no associated risk of HF hospitalization with higher NPY levels.

Conclusions Peripherally measured NPY is an independent predictor of all-cause and cardiovascular death even after adjustment for other prognostic variables, including BNP.

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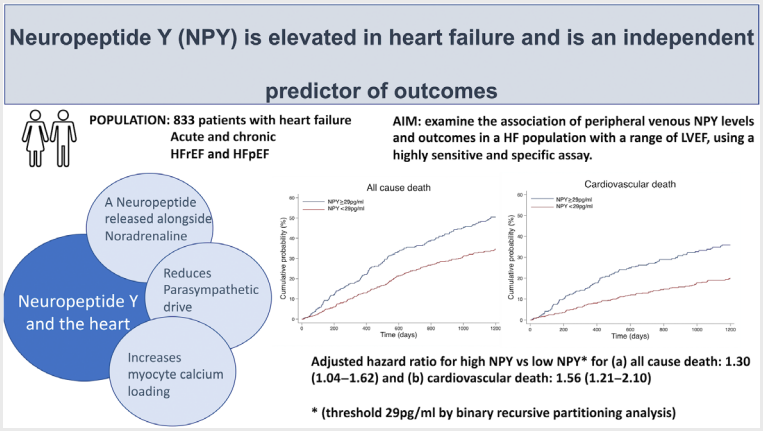
Graphical Abstract



Neuropeptide Y in heart failure and the association with outcomes

Keywords Biomarker • Heart failure • Neuropeptide Y • Sympathetic nervous system

Introduction

Activation of the sympathetic nervous system is central to the complex neurohormonal activation which occurs in heart failure (HF).[1–3] Circulating levels of catecholamines are associated with the risk of death, and inhibition of the sympathetic nervous system with beta-blockers is a central therapy for patients with HF.[4–6] Neuropeptide Y (NPY) is a 36 amino acid peptide released by the central and peripheral nervous system following prolonged sympathetic activation and plays a prominent role in many physiological functions. It is the most abundant neuropeptide in the heart,[7] found in neurons supplying the vasculature, cardiomyocytes and endocardium.[8] NPY is released by cardiac sympathetic nerve terminals alongside norepinephrine, and functions as a co-transmitter and a local modulator of cardiac function, acting as a potent vasoconstrictor whilst also reducing parasympathetic drive[9] and increasing myocyte calcium loading,[10] therefore it may be important in the pathophysiology of HF.

Neuropeptide Y has a longer half-life than norepinephrine and potentiates its vasoconstrictor effect. Functional NPY arises following cleavage of a pre-pro-NPY which is further truncated by the enzyme dipeptidyl peptidase-4. Its actions are mediated through the G protein receptors Y1R-Y6R. It is thought to be implicated in the pathogenesis of atherosclerosis,[11] maintaining cardiac contraction, and promoting ventricular hypertrophy.[8] Studies conducted before the widespread use of beta-blockers have demonstrated high baseline levels of NPY in patients with HF compared to healthy subjects using non-specific assays.[12–16] Others by comparison, have measured median peripheral venous NPY level in 303 normal adult subjects (using an assay with a similar level of detection to ours and minimal cross-reactivity) as <2 pg/ml.[17] Our own data have shown that peripheral venous levels of NPY in patients of a similar age undergoing elective coronary angiography with normal left ventricular systolic function and normal coronary arteries are 9.6 ± 1.0 pg/ml.[18] However, in patients who have had a recent myocardial infarction, NPY levels remain elevated for at least 48 h and are correlated with infarct size and subsequent left ventricular systolic dysfunction at 6 months.[19,20] More recently NPY levels measured in the coronary sinus have been shown to correlate with outcomes in HF patients with severely impaired left ventricular ejection fraction (LVEF) undergoing cardiac resynchronization therapy following adjustment for age, ejection fraction and kidney function.[21]

We examined NPY levels in a large prospective cohort of patients with HF treated with contemporary therapies, including beta-blockers, to determine if NPY levels measured from peripheral venous samples is an independent predictor of outcomes in patients with HF with a range of LVEF.

Methods

Participants

We included patients with HF from two separate cohort studies that included both ambulatory patients and those hospitalized with an episode of decompensation which have been previously described.[22,23] The ambulatory cohort was enrolled between December 2006 and 2009[22] and the cohort who were hospitalized with HF were enrolled between January 2013 and December 2014.[23] The diagnosis of HF (both acute and chronic) was made using the European Society of Cardiology definition of HF recommended at the time of recruitment.[24,25] Both cohorts were recruited from the same three hospitals.

Eligible participants for the ambulatory group were identified during a hospital admission with decompensated HF and were invited to attend a study visit at 1 month following discharge. Participants were included if they were >18 years old with a B-type natriuretic peptide (BNP) level of >100 pg/ml. The main exclusion criteria were primary presentation with myocardial infarction or a concurrent illness likely to reduce life expectancy or cognition.

The hospitalized cohort was eligible for inclusion if they had signs and symptoms of HF, a BNP >100 pg/ml and objective evidence of heart dysfunction on echocardiography (either left ventricular systolic dysfunction, elevated filling pressures, or significant valvular disease). Patients were excluded if they were unable or unwilling to provide written consent.

The BNP was assayed at the time of recruitment, within 24 h of HF hospitalization for both studies.

The studies were each approved by the West of Scotland Research Ethics Committee, and each patient consented to the measurement of potential biomarkers in their blood and urine and for use in subsequent studies. All patients provided written informed consent.

Measurement of neuropeptide Y

Whole blood samples were collected on the same day that the echocardiographic and clinical assessment was performed. Samples were processed immediately by centrifugation at 3000 *g* for 15 min and aliquoted and stored at −80°C until assay. A commercially available ELISA kit (EZHNPY-25 K, Millipore, Billerica, MA, USA) was used according to the manufacturer's instructions to measure NPY concentration, with a lower limit of detection of 3 pg/ml as described previously.[20] There is 0% cross-reactivity with structurally similar peptides (including peptide YY, pancreatic polypeptide, glucose-dependent insulinotropic polypeptide, ghrelin, proinsulin, or glucagon), and inter-assay coefficients of variation ran at 8.1% at 19.8 pg/ml and 8.0% at 201 pg/ml.

Outcomes

All patients consented to follow-up through electronic records search with the Information Services Division of the Scottish National Health Service for data on in-hospital and out-of-hospital deaths and hospitalizations, held by the General Register Office for Scotland. Participants in the ambulatory cohort were followed up from the date of study visit (between 16 January 2007 and 6 March 2009) until death or censoring on 31 August 2012. Those enrolled in the hospitalized group were followed up for a minimum of 12 months and a maximum of 21 months.

The outcomes studied included a composite of cardiovascular death or first hospitalization for HF, its components, and all-cause mortality.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation or median (interquartile range [IQR]) for non-normally distributed data. Categorical data values were expressed as frequencies and percentages.

As there are no currently accepted definitions of normal and abnormal levels of NPY, the best threshold separating low from high hazard of death was calculated using binary recursive partitioning.[26] This method determines the value of a continuous variable where the log hazard ratio (HR) is maximally far away from 1. This approach does not make a prior assumption about a specific cut-off value or whether such a value exists.

Differences in baseline characteristics according to high versus low NPY were tested using a chi-square test for categorical variables and Student's *t*-test or Mann–Whitney U test for continuous variables depending on their distribution. Event rates (per 100 patient-years of follow-up) in each NPY group were estimated and univariable and multivariable Cox proportional hazard regression models were used to compare HR with 95% confidence intervals (CI) for outcomes according to NPY group. Multivariable models were adjusted for study cohort and the following characteristics known to be associated with outcomes in HF: age, sex, diabetes, atrial fibrillation, ischaemic aetiology of HF, systolic blood pressure, heart rate, New York Heart Association classification, body mass index, history of HF hospitalization, ejection fraction, estimated glomerular filtration rate (eGFR) and BNP (log-transformed).

For analyses of NPY as a continuous outcome, restricted cubic splines (using log transformed NPY) were constructed for each outcome using the same adjusted model as above with median value (log 22.6 pg/ml) as the reference value.

To examine if the relationship between NPY and outcomes differed by study (because of differences in the time period the patients and samples were collected), we tested an interaction between NPY levels and study in a multivariable model for each outcome. We found no evidence of interaction (online supplementary *Table S1*). We tested the additional value of NPY to the MAGGIC risk score using a change in c-statistic, integrated discrimination index (IDI) and net reclassification index (NRI) as a binary and continuous variable.

All analyses were conducted using Stata version 17.1 (Stata Corp., College Station, TX, USA). A p-value < 0.05 was considered statistically significant.

Results

Of the 961 patients, 833 had a sample in which NPY could be measured. The mean concentration of NPY was 25.8 ± 18.2 pg/ml with a median (IQR) of 22.6 (14.6–32.0) pg/ml. The distribution of NPY levels is shown in *Figure 1*. Mean NPY in men was 26.3 ± 20.7 and 25.8 ± 18.2 pg/ml in women ($p = 0.34$). Median values were 23.0 (14.0–32.0) and 21.5 (14.0–31.0) pg/ml, respectively.

Patient characteristics

Patient characteristics according to high versus low NPY levels as defined by an optimal cut-off value of 29 pg/ml are shown in *Table 1* and by quartile of NPY in online supplementary *Table S2*. Patients with higher NPY were older (73 ± 10 years vs. 71 ± 11 years) and

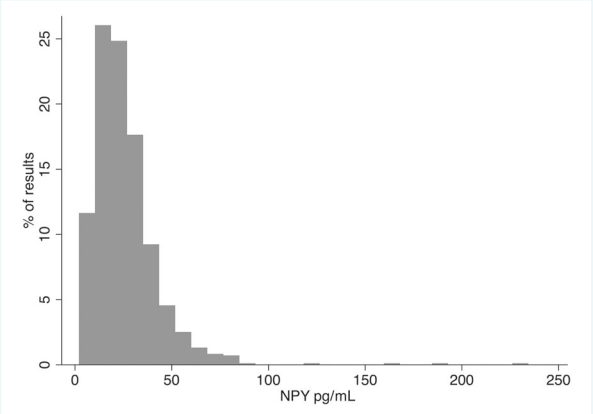


Figure 1 Distribution of neuropeptide Y (NPY) levels.

Table 1 Baseline characteristics according to neuropeptide Y level

Characteristic	Total ($n = 833$)	NPY <29 pg/ml ($n = 561$)	NPY ≥29 pg/ml ($n = 272$)	*p*-value
NPY, pg/ml	22.6 [14.6–32.0]	17.4 [12.6–23.0]	37.3 [32.4–47.7]	<0.001
	25.8 ± 18.2	17.1 ± 6.8	43.5 ± 21.1	<0.001
Demographics				
Age, years	71.7 ± 10.7	71.2 ± 11.0	72.8 ± 10.1	0.048
Women, *n* (%)	362 (43.5)	249 (44.4)	113 (41.5)	0.44
Race[a], *n* (%)				0.37
White	817 (98.1)	552 (98.4)	265 (97.4)	
Black	3 (0.4)	1 (0.2)	2 (0.7)	
South Asian	13 (1.6)	8 (1.4)	5 (1.8)	
BMI, kg/m^2	28.4 ± 6.7	28.8 ± 7.1	27.6 ± 5.8	0.014
Past medical history, *n* (%)				
Cerebrovascular disease	179 (21.5)	128 (22.8)	51 (18.8)	0.18
Previous MI	354 (42.5)	226 (40.3)	128 (47.1)	0.064
Hypertension	541 (64.9)	363 (64.7)	178 (65.4)	0.83
Atrial fibrillation	440 (52.8)	295 (52.6)	145 (53.3)	0.84
Diabetes	259 (31.1)	176 (31.4)	83 (30.5)	0.80
COPD	223 (26.8)	157 (28.0)	66 (24.3)	0.26
Rheumatoid arthritis	23 (2.8)	17 (3.0)	6 (2.2)	0.50
Connective tissues disease	16 (1.9)	13 (2.3)	3 (1.1)	0.23
Hypothyroidism	79 (9.5)	59 (10.5)	20 (7.4)	0.14
Peripheral vascular disease	129 (15.5)	85 (15.2)	44 (16.2)	0.70
Smoker	525 (63.0)	347 (61.9)	178 (65.4)	0.31
Alcohol excess	136 (16.3)	96 (17.1)	40 (14.7)	0.38
Vital signs				
Heart rate, bpm	80 ± 19	80 ± 20	79 ± 18	0.65
Systolic blood pressure, mmHg	133 ± 25	133 ± 25	132 ± 26	0.36
HF characteristics, *n* (%)				
NYHA functional class				0.23
I	24 (2.9)	20 (3.6)	4 (1.5)	
II	454 (54.5)	309 (55.1)	145 (53.3)	
III	310 (37.2)	200 (35.7)	110 (40.4)	
IV	45 (5.4)	32 (5.7)	13 (4.8)	
Previous HF admission	260 (31.2)	161 (28.7)	99 (36.4)	0.025
HF diagnosis within 2 years	246 (29.5)	152 (27.1)	94 (34.6)	0.027
Permanent pacemaker	43 (5.2)	27 (4.8)	16 (5.9)	0.51
CRT	9 (1.1)	6 (1.1)	3 (1.1)	0.97
ICD	14 (1.7)	10 (1.8)	4 (1.5)	0.74
Ischaemic aetiology	423 (50.8)	272 (48.5)	151 (55.5)	0.057
ECG/Echo				
Sinus rhythm, *n* (%)	476 (57.1)	321 (57.2)	155 (57.0)	0.95
LBBB, *n* (%)	180 (21.6)	121 (21.6)	59 (21.7)	0.97
QRS duration, ms, median [IQR]	102.0[90.0–126.0]	102.0 [90.0–126.0]	103.0 [92.0–126.0]	0.53
Ejection fraction, %	40.1 (13.6)	40.7 (13.9)	38.8 (12.8)	0.066
Heart failure category, *n* (%)				0.15
HFrEF	390 (48.2)	252 (46.2)	138 (52.2)	
HFmrEF	209 (25.8)	142 (26.0)	67 (25.5)	
HFpEF	210 (30.0)	152 (27.8)	56 (22.1)	
Medications, *n* (%)				
Diuretic	724 (86.9)	468 (83.4)	256 (94.1)	<0.001
ACE inhibitor or ARB	600 (72.0)	404 (72.0)	196 (72.1)	0.99
Beta-blocker	528 (63.4)	339 (60.4)	189 (69.5)	0.011
Mineralocorticoid receptor antagonist	100 (12.0)	61 (10.9)	39 (14.3)	0.15
Digoxin	172 (20.6)	108 (19.3)	64 (23.5)	0.15
Antiplatelet	492 (59.1)	314 (56.0)	178 (65.4)	0.01
Warfarin	303 (36.4)	202 (36.0)	101 (37.1)	0.75

Table 1 (Continued)

Characteristic	Total ($n = 833$)	NPY <29 pg/ml ($n = 561$)	NPY ≥29 pg/ml ($n = 272$)	*p*-value
Bloods				
BNP, pg/ml, median [IQR]	465.0 [239.0–931.0]	440.0 [227.0–829.0]	582.5 [261.0–1095.5]	0.004
Troponin I, ng/ml	0.1 ± 0.9	0.2 ± 1.1	0.1 ± 0.3	0.36
Sodium, mmol/L	138.5 ± 4.0	138.3 ± 4.1	138.9 ± 3.6	0.047
Bilirubin, mmol/L	13.7 ± 10.5	13.9 ± 10.9	13.2 ± 9.8	0.37
AST, mmol/L	28.5 ± 47.4	25.6 ± 17	34.4 ± 78.9	0.012
TSH, mU/L	2.3 ± 3.7	2.1 ± 3.0	2.7 ± 4.9	0.035
T4, mmol/L	13.4 ± 4.8	13.3 ± 5.2	13.7 ± 4.0	0.35
Haemoglobin, g/dl	12.4 ± 1.9	12.5 ± 1.9	12.3 ± 2.0	0.43
Lymphocytes, $\times 10^9$/L	1.8 ± 1.3	1.7 ± 1.1	1.8 ± 1.7	0.42
Urea, mmol/L	9.8 ± 5.2	9.1 ± 4.6	11.3 ± 5.9	<0.001
eGFR, ml/min/1.73 m^2	50.5 ± 12.8	52.4 ± 11.7	46.4 ± 13.9	<0.001
Albumin, mmol/L	37.3 ± 4.6	36.9 ± 4.7	38.1 ± 4.3	<0.001

Data are given as median [interquartile range], mean ± standard deviation, or *n* (%). Percentages may not total 100 due to rounding.
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; AST, aspartate transaminase; BMI, body mass index; BNP, B-type natriuretic peptide; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; ECG, electrocardiogram; Echo, echocardiography; eGFR, estimated glomerular filtration rate; HF, heart failure; HFmrEF, heart failure with mildly reduced ejection fraction (40–50%); HFpEF, heart failure with preserved ejection fraction (>50%); HFrEF, heart failure with reduced ejection fraction (<40%); ICD, implantable cardioverter-defibrillator; IQR interquartile range; LBBB, left bundle branch block; MI, myocardial infarction; NPY, neuropeptide Y; NYHA, New York Heart Association; TSH, thyroid-stimulating hormone.
[a]Race was self-reported by participants.

were more likely to be men. In general, those with higher NPY had an overall profile suggesting more advanced HF: BNP levels were higher and LVEF and eGFR levels were lower. Loop diuretic usage was greater in those with higher NPY (91.4% vs. 83.4%, $p < 0.001$). They were more likely to have an ischaemic aetiology and have a diagnosis of HF within the last 2 years. Over 70% were treated with an angiotensin-converting enzyme inhibitor (ACEi) or an angiotensin receptor blocker (ARB) and over 60% with a beta-blocker. There was no difference in the use of ACEi or ARB between patients with high or low NPY but beta-blocker use was higher in the high NPY group.

Correlations with neuropeptide Y

Increasing NPY levels correlated with BNP levels and eGFR levels ($r = 0.07$, $p = 0.03$ and $r = -0.20$, $p < 0.001$, respectively) but not LVEF ($r = -0.02$, $p = 0.59$) after adjustment for age, sex and eGFR (*Figure 2*, online supplementary *Table S3*).

Neuropeptide Y thresholds for outcomes

Binary recursive partitioning analysis was used to define an NPY threshold that best identifies patients reaching an outcome of death. This cut-off value of 29.0 pg/ml was slightly higher than the median value (22.6 [14.6–32.0]) pg/ml).

Clinical outcomes

The rates of a composite of cardiovascular death or hospitalization for HF, hospitalization for HF and deaths, cardiovascular and all-cause, according to NPY group are shown in *Table 2*. During a median follow-up of 36.2 months, 298 were hospitalized for HF, 362 patients died of which 209 had a cardiovascular cause of death. The event rates per 100 patient-years were consistently higher in those with higher NPY levels (*Table 2*, *Figure 3*).

After adjusting for known prognostic factors including BNP, ejection fraction and eGFR, the risk of cardiovascular death or hospitalization for HF was numerically higher in those with a high NPY but was not statistically significant (*Table 2*). The risk of hospitalization for HF was the same in both NPY groups. Patients in the high NPY group had a 56% greater risk of cardiovascular death (HR 1.56, 95% CI 1.21–2.1; $p = 0.003$). The risk of death from any cause was greater in those with NPY ≥29 pg/ml compared to those with NPY <29 pg/ml (HR 1.30, 95% CI 1.04–1.62; $p = 0.02$). There was no association with the risk of non-cardiovascular death (HR 1.05, 95% CI 0.74–1.50; $p = 0.788$).

As expected those with a higher N-terminal proBNP and lower ejection fraction were at higher risk of HF hospitalization (adjusted HR [95% CI] per log unit increase in BNP and per 5% decrease in ejection fraction was 1.48 [1.29–1.70], $p < 0.001$ and 1.09 [1.04–1.14], $p < 0.001$, respectively).

When modelled as a continuous variable, we observed a similar pattern, higher NPY was associated with a higher risk of all-cause death and cardiovascular death but not hospitalization for HF after multivariable adjustment (*Figure 4*). The HRs for the association between different levels of NPY and each outcome, modelled as a continuous variable are given in online supplementary *Table S4*. Addition of NPY to the MAGGIC risk score did not change the c-statistic or IDI or NRI (online supplementary *Tables S5* and *S6*).

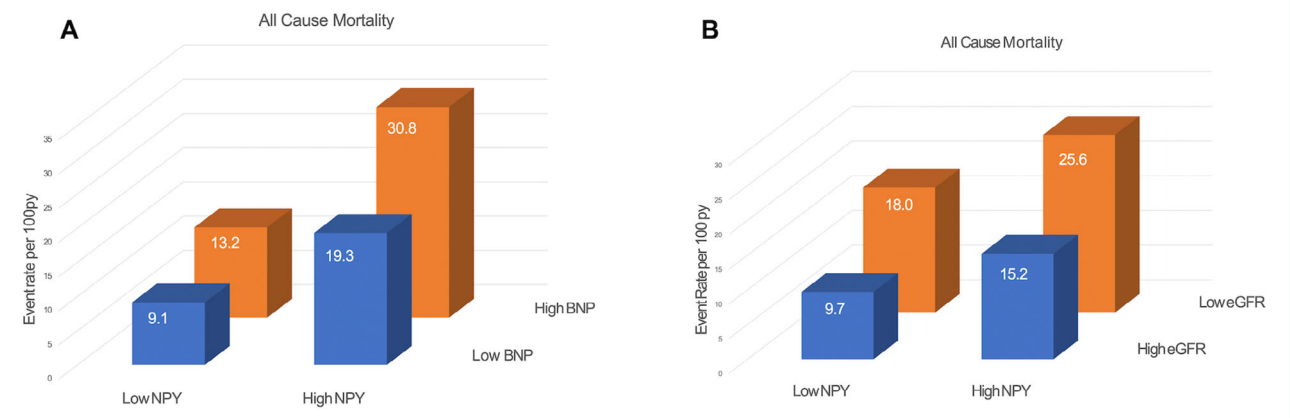


Figure 2 Rate of all-cause mortality per 100 patient-years (py) according to (*A*) neuropeptide Y (NPY) and B-type natriuretic peptide (BNP) levels and (*B*) NPY and estimated glomerular filtration rate (eGFR) levels. High BNP is defined as a value greater than the median of 465 pg/ml. High eGFR is defined as a value greater than the median value of 57 ml/min/1.73 m^2.

Table 2 Rates and risk of outcome according to baseline neuropeptide Y levels as a categorical variable

Characteristic	NPY <29 pg/ml (*n* = 561)	NPY ≥29 pg/ml (*n* = 272)	*p*-value
All-cause death			
Events	210	152	
Event rate (95% CI) per 100 pt-years	13.1 (11.5–15.1)	21.4 (18.3–25.1)	
Unadjusted HR (95% CI)	1.00 (ref)	1.63 (1.32–2.01)	<0.001
Adjusted HR[a] (95% CI)	1.00 (ref)	1.30 (1.04–1.62)	0.023
Cardiovascular death			
Events	110	99	
Event rate (95% CI) per 100 pt-years	6.9 (5.7–8.3)	13.9 (11.4–17.0)	
Unadjusted HR (95% CI)	1.00 (ref)	2.01 (1.54–2.64)	<0.001
Adjusted HR[a] (95% CI)	1.00 (ref)	1.56 (1.2–2.1)	0.003
Cardiovascular death or heart failure hospitalization			
Events	252	151	
Event rate (95% CI) per 100 pt-years	20.1 (17.8–22.8)	26.8 (22.9–31.5)	
Unadjusted HR (95% CI)	1.00 (ref)	1.32 (1.08–1.61)	0.007
Adjusted HR[a] (95% CI)	1.00 (ref)	1.14 (0.92–1.42)	0.223
Heart failure hospitalization			
Events	195	103	
Event rate (95% CI) per 100 pt-years	15.6 (13.5–17.9)	18.3 (15.1–22.2)	
Unadjusted HR (95% CI)	1.00 (ref)	1.16 (0.91–1.47)	0.223
Adjusted HR[a] (95% CI)	1.00 (ref)	1.00 (0.77–1.29)	0.973

CI, confidence interval; HR, hazard ratio; NPY, neuropeptide Y, pt, patient.
[a]Adjusted for age, sex, diabetes, atrial fibrillation, ischaemic aetiology of heart failure, systolic blood pressure, heart rate, New York Heart Association classification, body mass index, history of heart failure hospitalization, ejection fraction, estimated glomerular filtration rate and B-type natriuretic peptide (log-transformed).

Discussion

We found that NPY levels were higher in patients with HF across the range of LVEF and that higher concentrations of NPY were associated with a higher risk of death, although not of hospitalization for worsening HF. This elevated risk of death persisted despite adjustment for other prognostic variables, including BNP (*Graphical Abstract*).

Although increased peripheral and coronary venous NPY-like immunoreactivity has been previously recognized in HF, most earlier studies were small and used non-specific and insensitive assays.[12–16] Our assay is highly sensitive (lower limit of detection 2–3 pg/ml compared to >90 pg/ml) and selective (0% cross-reactivity with structurally similar peptides). The baseline NPY concentration of 22.6 (14.6–32.0) pg/ml in our patients was higher than that found in healthy controls (<2 pg/ml) and patients

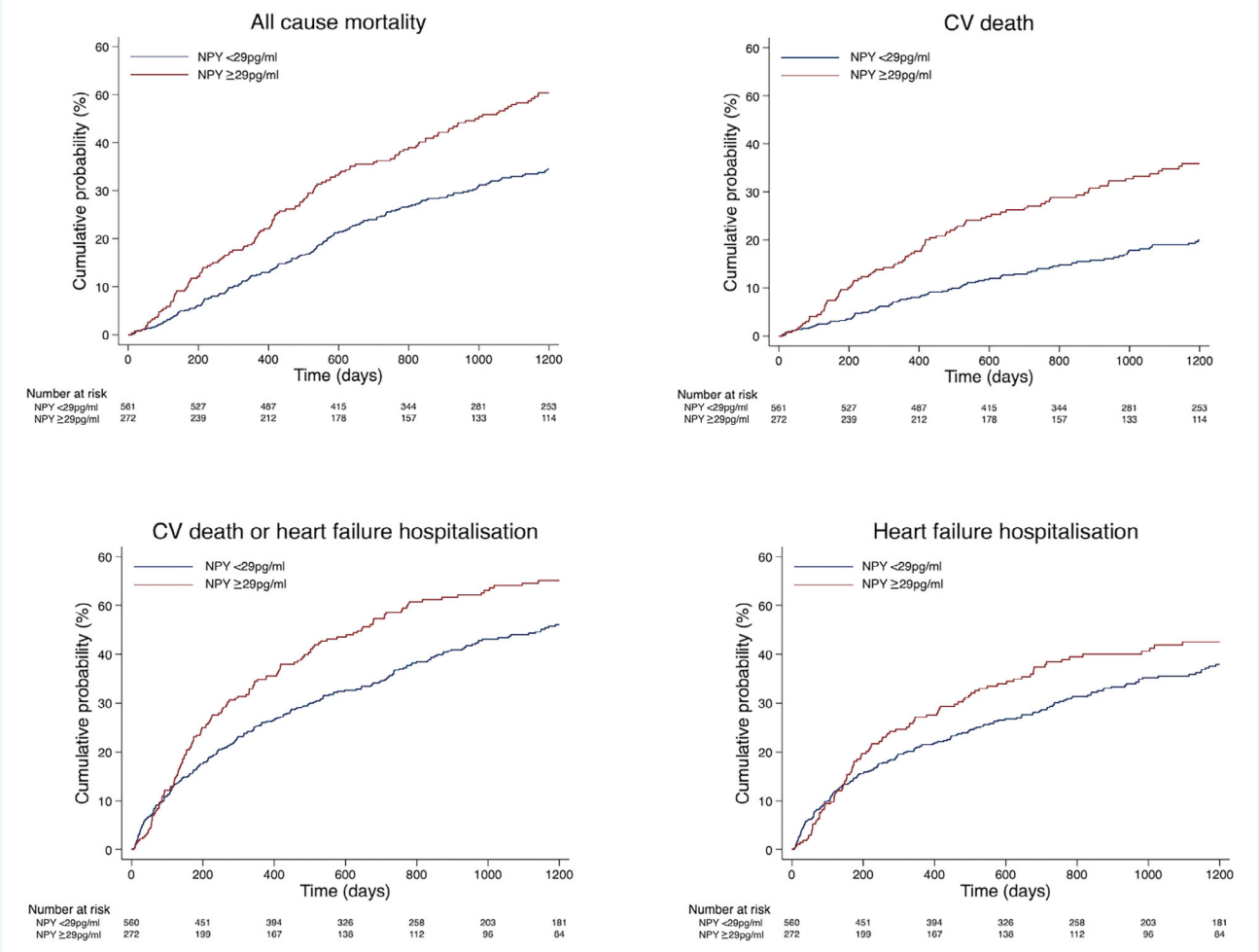


Figure 3 Cumulative incidence of outcomes of interest according to baseline neuropeptide Y (NPY). CV, cardiovascular.

with either stable angina or an acute coronary syndrome (10.0 [1.8–12.4] pg/ml), using a similar assay.[17,18]

Although some prior studies demonstrated an association between more severe HF and higher NPY levels, most of these were not large enough or had insufficient follow-up to determine whether NPY concentration was predictive of clinical outcomes. However, one recent study of 105 patients with severely impaired LVEF undergoing cardiac resynchronization therapy implantation examined the association between coronary sinus NPY levels and the composite of death, cardiac transplant, or left ventricular assist device implantation over a median follow-up of 28.8 months.[21] In that study, an NPY value of ≥130 pg/ml (reflecting the much higher coronary than peripheral venous concentrations) was associated with worse outcomes although only 20 events occurred (18 deaths and one each of the other two components of the composite). Although that analysis was adjusted for age, eGFR, and LVEF, it was not adjusted for natriuretic peptide level which is the single most powerful predictor of prognosis in HF. This is clearly crucial in determining whether NPY provides additional useful prediction of outcomes, especially as we found a greater elevation of BNP in individuals with higher NPY levels at baseline. It is therefore important that in this much larger study with a total of 362 deaths we found an NPY level ≥29 pg/ml was associated with all-cause mortality, even after adjustment for other prognostic variables including BNP.

Unusually, an NPY level ≥29 pg/ml was not associated with a higher risk of hospitalization for worsening HF. In our observational clinical cohort study, we do not have a record of the mode of death. However, the dissociation between mortality and admission raises the possibility that higher NPY is associated with sudden death as opposed to worsening pump failure, leading to hospitalization or death. Whilst the cut-off NPY value identified should be further validated in other populations, a biomarker with incremental prognostic value in relation to cardiovascular mortality may be helpful in identifying those who require more intense follow-up or who may even benefit from an implantable cardioverter-defibrillator. While we can only speculate about this, recent studies of patients with acute myocardial infarction lends some support to this hypothesis.[20,27] Patients experiencing ventricular tachycardia/fibrillation after infarction had significantly higher

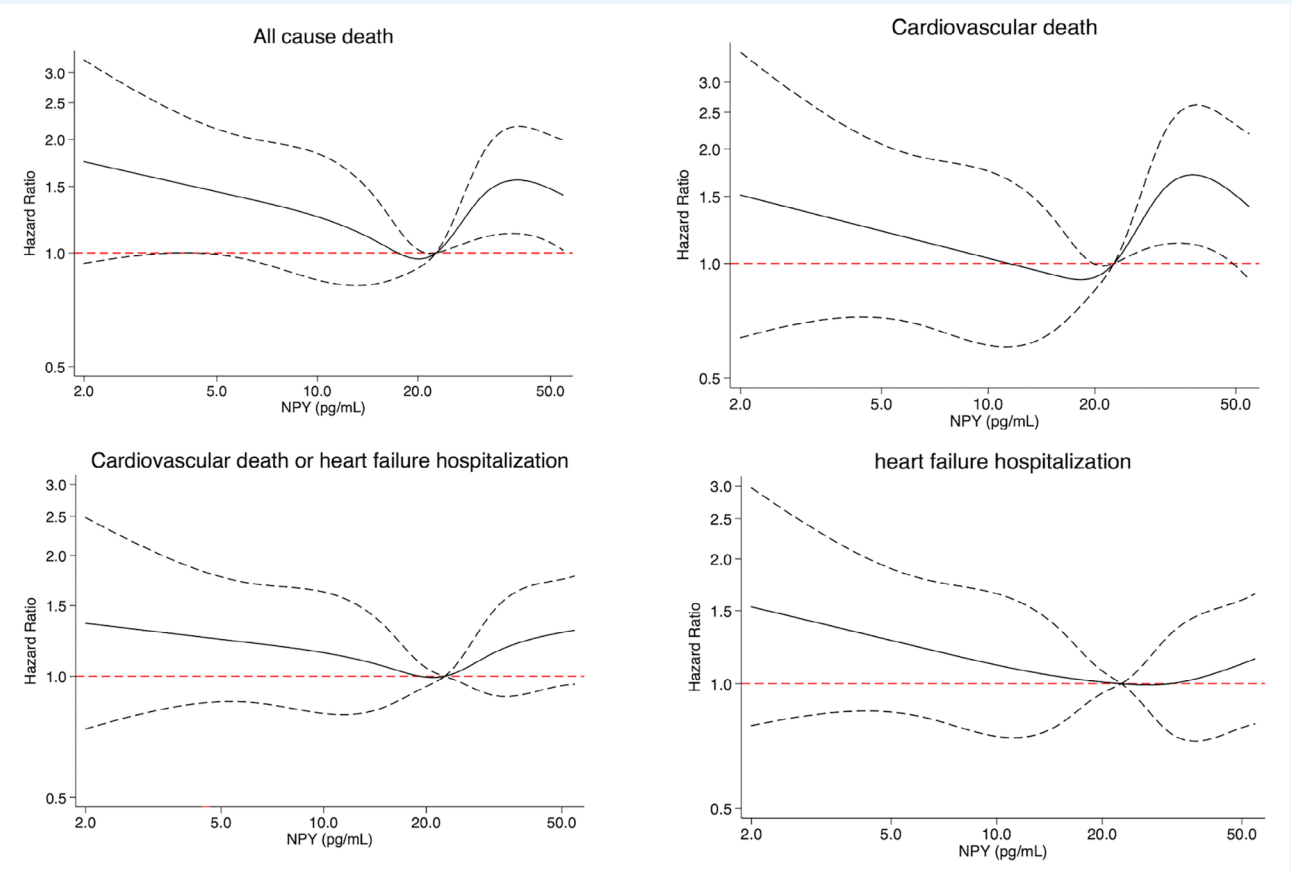


Figure 4 Association between baseline (log transformed) neuropeptide Y (NPY) and the risk of cardiovascular death or heart failure hospitalization, heart failure hospitalization, cardiovascular death and all-cause mortality (restricted cubic spline analysis). The reference value is (log) 22.6 pg/ml. Models are adjusted for age, sex, diabetes, atrial fibrillation, ischaemic aetiology of heart failure, systolic blood pressure, heart rate, New York Heart Association classification, body mass index, history of heart failure hospitalization, ejection fraction, estimated glomerular filtration rate and B-type natriuretic peptide (log transformed).

venous plasma NPY levels (31.9 [27.8–47.7]) than those who did not (17.8 [10.6–30.2] pg/ml).[20] Some recent experimental data are also of interest here. Hoang and colleagues found that beta-blockade alone did not block the electrophysiological effects of sympatho-excitation whilst the addition of an NPY Y1 receptor antagonist could.[28] It is also plausible that autonomic remodelling in HF might lead to a greater role of NPY, relative to norepinephrine in this condition. Of course, other potentially detrimental effects of NPY in HF should not be discounted. Classically NPY is a vasoconstrictor and its venoconstrictive action is enhanced in patients with HF.

The role of biomarkers in clinical practice is still to be determined but some appear to have potential promise in predicting prognosis over and above clinical and routine laboratory findings.[29] The interaction of several complex processes are responsible for the final common pathway in HF neurohormonal activation, myocyte injury, renal dysfunction, oxidative stress, inflammation and vascular remodelling.[30] The role of natriuretic peptides as a prognostic marker is well established but as the pathophysiology of HF becomes better understood, there has been an increase in number of biomarkers reflecting these different pathways. Different biomarkers reflecting separate pathological pathways may offer different prognostic insights with regard to endpoints.

The only biomarkers that currently assess neurohumoral activation (mid-regional pro-adrenomedullin and copeptin) reflect vasodilatation and fluid balance whereas NPY plays an important role in many key aspects of the pathophysiology giving it a potential advantage. These include acting directly on cardiomyocytes to cause calcium overload,[20] constricting the peripheral and coronary vasculature,[8,18] and impairing cardiac vagal tone[9] with its release being directly driven by the degree of cardiac systolic impairment,[8] whilst not being targeted by beta-blockade. Mechanistically, it is unsurprising therefore that it may offer specific insight into arrhythmic risk in terms of sudden cardiac death and why we did not observe an association with HF hospitalizations. The strength of NPY as a biomarker appears to be related to its association with cardiovascular death above and beyond natriuretic peptide.

A potential link between neuroendocrine activation and the immune system has also been postulated and in support of this NPY levels are elevated in patients with rheumatoid arthritis.[31]

It should be noted that dipeptidyl peptidase-4 inhibitors used to treat type 2 diabetes, also inhibit the breakdown of NPY and are associated with adverse effects on cardiac remodelling and a higher risk of cardiovascular hospitalization and death, for example with vildagliptin in the VIVIDD trial.[32]

Limitations

Our study has several limitations. This was a post-hoc analysis and is therefore inadequately powered for outcomes which were not adjudicated. The analysis was performed in an older cohort of HF patients not receiving sacubitril/valsartan or sodium–glucose cotransporter 2 inhibitors and with a low level of device implantation. We only were able to measure NPY at baseline and changes in NPY levels and the association with outcomes could not be assessed. As we only measured NPY peripherally, we do not know what proportion of the NPY measured was due to cardiac NPY.

We did not have a healthy cohort to compare NPY levels but previous studies have shown that levels are lower in patients without HF. Further validation of the association between NPY and outcomes is needed in other cohorts (ideally with central and peripheral samples) with differing characteristics to determine the utility of NPY as a biomarker in HF. We did not have access to mode of death, which would have been useful to determine if NPY is more closely linked to sudden cardiac death than pump failure death.

Conclusion

In patients with HF across a range of LVEF, higher NPY levels are associated with higher levels of BNP and other characteristics known to be associated with poorer outcomes. We found that after adjusting for these other variables, including BNP, NPY remained a predictor of mortality. Our findings suggest that NPY could be used to identify high-risk patients with HF and that NPY receptors may be a useful therapeutic target.

Supplementary Information

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

Funding

The study was supported by a British Heart Foundation Senior Clinical Research Fellowship (FS/SCRF/20/32005) to Neil Herring and the BHF Oxford Centre of Research Excellence (RE/13/1/30181). Carly Adamson, Mark C. Petrie, John J.V. McMurray and Pardeep S. Jhund are supported by a British Heart Foundation Centre of Research Excellence Grant (RE/18/6/34217) and the Vera Melrose Heart Failure Research Fund. Ross Campbell was supported by a project grant from the British Heart Foundation; Grant number PG/13/17/30050.

Conflict of interest: R.C. has received consultancy honoraria from Bayer and for speaking honoraria from AstraZeneca. P.W. reports grant income from Roche Diagnostics, AstraZeneca, Boehringer Ingelheim, and Novartis, outside the submitted work. M.C.P. reports research grants from Boehringer Ingelheim, Roche, SQ Innovations, AstraZeneca, Novartis, Novo Nordisk, Medtronic, Boston Scientific, Pharmacosmos; consulting fees from Boehringer Ingelheim, Novartis, AstraZeneca, Novo Nordisk, Abbvie, Bayer, Takeda, Corvia, Cardiorentis, Pharmacosmos, Siemens, Vifor; honoraria from Boehringer Ingelheim, Novartis, AstraZeneca, Novo Nordisk, Abbvie, Bayer, Takeda, Corvia, Cardiorentis, Pharmacosmos, Siemens, Vifor; he is a director of Global Clinical Trial Partners Ltd. J.J.V.M. has received payments through Glasgow University for work on clinical trials, consulting and other activities from Alnylam, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, BMS, Cardurion, Cytokinetics, Dal-Cor, GSK, Ionis, KBP Biosciences, Novartis, Pfizer, Theracos; personal lecture fees from the Corpus, Abbott, Hikma, Sun Pharmaceuticals, Medscape/Heart.Org, Radcliffe Cardiology, Servier, Director, Global Clinical Trial Partners (GCTP). P.S.J. reports speaker fees from AstraZeneca, Novartis, Alkem Metabolics, ProAdWise Communications, Sun Pharmaceuticals; advisory board fees from AstraZeneca, Boehringer Ingelheim, Novartis; research funding from AstraZeneca, Boehringer Ingelheim, Analog Devices Inc. P.S.J.'s employer the University of Glasgow has been remunerated for clinical trial work from AstraZeneca, Bayer AG, Novartis and NovoNordisk. Director, Global Clinical Trial Partners (GCTP). All other authors have nothing to disclose.

Ethics Statement

The studies were each approved by the West of Scotland Research Ethics Committee, and each patient consented to the measurement of potential biomarkers in their blood and urine and for use in subsequent studies. All patients provided written informed consent.

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