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Sympathetic and renin-angiotensin-aldosterone system activation in heart failure with preserved, mid-range and reduced ejection fraction

Giuseppe Vergaro [a, b, *, 1, 2], Alberto Aimo [b, c, 1, 2], Concetta Prontera [b, 2], Nicolò Ghionzoli [b, 2], Chiara Arzilli [b, 2], Luc Zyw [b, 2], Claudia Taddei [b, 2], Alessandra Gabutti [b, 2], Roberta Poletti [b, 2], Alberto Giannoni [a, b, 2], Chiara Mammini [b, 2], Valentina Spini [b, 2], Claudio Passino [a, b, 2], Michele Emdin [a, b, 2]

[a] Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy
[b] Fondazione Toscana Gabriele Monasterio, Pisa, Italy
[c] Cardiology Division, University Hospital of Pisa, Pisa, Italy

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ABSTRACT

Background: Evidence of sympathetic and renin-angiotensin-aldosterone system activation provided a rationale for neurohormonal antagonism in heart failure with reduced ejection fraction (HFrEF), while no data are available in patients with milder degree of systolic dysfunction. We aimed to investigate neurohormonal function in HF with preserved and mid-range EF (HFpEF/HFmrEF).

Methods: Three cohorts (n = 189/each) of stable HFpEF, HFmrEF and HFrEF patients were selected (median age 70, 67 and 67 years; male 56%, 73% and 74%, respectively). Patients received a baseline clinical assessment including plasma renin activity (PRA), aldosterone, catecholamines, and N-terminal fraction of pro-B-type natriuretic peptide (NT-proBNP) assays, and were followed-up for all-cause death.

Results: Neuroendocrine profile was similar between HFpEF and HFmrEF, while all neurohormones except epinephrine were higher in HFrEF than in HFmrEF (NT-proBNP 2332 ng/L, IQR 995–5666 vs 575 ng/L, 205–1714; PRA 1.7 ng/mL/h, 0.4–5.6 vs 0.6 ng/mL/h, 0.2–2.6; aldosterone 153 ng/L, 85–246 vs 113 ng/L, 72–177; norepinephrine 517 ng/L, 343–844 vs 430 ng/L, 259–624; all $p < 0.001$, epinephrine 31 ng/L, 10–63 vs 25 ng/L, 10–44; $p = 0.319$). These findings were unrelated to treatment heterogeneity. Ten percent of HFpEF patients had elevated PRA, aldosterone and norepinephrine vs. 8% in HFmrEF and 21% in HFrEF. During a 5-year follow-up, survival decreased with the number of neurohormones elevated (HFpEF: log-rank 7.8, $p = 0.048$; HFmrEF: log-rank 11.8, $p = 0.008$; HFrEF: log-rank 8.1, $p = 0.044$).

Conclusions: Neurohormonal activation is present only in a subset of patients with HFpEF and HFmrEF, and may hold clinical significance. Neurohormonal antagonism may be useful in selected HFpEF/HFmrEF population.

1. Introduction

Heart failure (HF) is a leading cause of morbidity and mortality worldwide. It is also a growing public health issue because of population ageing and the increasing prevalence of HF in the elderly. This outlines the need for treatments impacting on the natural history of the disease [1].

Pharmacologic modulation of the sympathetic nervous system and renin-angiotensin-aldosterone system (SNS/RAAS) has greatly improved the prognosis of patients with systolic HF. As a result, treatment with an angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (ACEi/ARB) or angiotensin receptor-neprilysin inhibitor, a beta-blocker, and a mineralocorticoid receptor antagonist (MRA) is strongly recommended for patients with HF and reduced ejection fraction (HFrEF), defined as left ventricular ejection fraction (EF) <40% [2–4]. With the exception of MRAs, the same therapies are usually prescribed to patients with HF and mid-range EF (HFmrEF; EF 40–49%). Many clinical trials

* Corresponding author at: Scuola Superiore Sant'Anna and Fondazione Toscana Gabriele Monasterio, Via Moruzzi 1, 56124 Pisa, Italy.
E-mail address: vergaro@ftgm.it (G. Vergaro).
[1] Giuseppe Vergaro and Alberto Aimo contributed equally.
[2] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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have assessed whether the same therapeutic paradigm can be translated to patients with HF and preserved EF (HFpEF; EF ≥50%). Such trials have consistently shown that drugs for neurohormonal modulation do not improve patient prognosis, with the partial exception of spironolactone in patients enrolled in the Americas [5]. As a result, no cardiac-specific treatment is currently available for patients with HFpEF, which account for around half of the whole HF population.

Several possible explanations for the systematic failure of RAAS and adrenergic antagonists in HFpEF have been proposed. Besides methodological considerations on single studies, a commonly held belief is that substantial differences in pathophysiology exist, with HFpEF patients displaying a greater burden of comorbidities, and a lower degree of activation of the SNS/RAAS axes. Nevertheless, to our knowledge, neurohormonal activation has never been specifically assessed either in HFpEF or in HFmrEF. Clarifying this point would be important to predict whether ongoing [6] and future studies on neurohormonal modulation may yield positive results in these categories. We therefore aimed to compare circulating biomarkers related to the SNS and RAAS status across all categories of left ventricular systolic function.

2. Methods

2.1. Patient population and study protocol

The present study is a retrospective analysis of all patients with stable chronic HF (disease duration ≥6 months, unchanged therapy from ≥3 months) assessed at a tertiary referral center in Pisa, Italy from 2000 to 2017. These patients underwent a standardized protocol including complete clinical assessment (with attribution of the New York Heart Association class), 12-lead electrocardiogram, transthoracic echocardiogram, and biohumoral characterization (see below) within 3 days. This protocol conformed to the 1975 Declaration of Helsinki [7], as reflected in a priori approval by the Institution's human research committee.

2.2. Echocardiographic examination

Standard, 2-dimensional transthoracic images were obtained using a Philips IE33 Ultrasound machine, with X5-1 transducer (Philips Medical Systems, Palo Alto, California, USA). Standard techniques were used to assess wall thickness, chamber volumes, and indices of systolic and diastolic function, and volumes were measured using the biplane method of disks (modified Simpson's rule) [8–11]. The reading protocol was standardized and consistent across years.

2.3. Biohumoral evaluation

Blood samples were drawn at 8 A.M. after an overnight fasting period and a 20-minute supine rest [12]. Samples were stored at −80 °C and assays run within 7 days. Plasma renin activity (PRA) and aldosterone were measured using a radioimmunoassay (RIA) method (DiaSorin S.r.l., Saluggia, Italy) [13,14]. Plasma norepinephrine and epinephrine were evaluated by means of high-performance liquid chromatography technique using the electrochemical detector CLC 100 (Chromsystems GmbH, Munchen, Germany) [15]. N-terminal fraction of pro-B-type natriuretic peptide (NT-proBNP) was measured with the ECLIA monoclonal method using the Cobas e411 platform (Roche Diagnostics Italia, Monza, Italy) [16]. All these assays were run according to manufacturer instructions. Upper reference levels were: NT-proBNP, 150 ng/L; PRA, 2.2 ng/mL/h; aldosterone, 150 ng/L; norepinephrine, 500 ng/L; epinephrine, 90 ng/L [12–17]. Information about each biomarker was available for all patients.

2.4. Patient selection

Patients were classified as having HFpEF, HFmrEF and HFrEF based on current European Society of Cardiology Guidelines [2]. Data from 1661 patients were retrieved, distributed as follows: HFpEF, n = 189 (11%); HFmrEF, n = 345 (21%); HFrEF, n = 1127 (68%). To compare groups of equal size, populations of 189 patients from the latter 2 categories were derived, matched with the HFpEF category based on the year of characterization.

2.5. Follow-up

The follow-up lasted until March 2018. Independent interviewers obtained information from electronic health records or from patients, cardiologists or general practitioners in charge of the patient. Information on all-cause mortality was collected.

2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics (version 22, 2013). Normal distribution was assessed through the Kolmogorov-Smirnov test; variables with normal distribution were presented as mean ± standard deviation, while those with non-normal distribution as median and interquartile interval. Differences between groups were evaluated through the analysis of variance with Bonferroni correction or the Chi-square test, as appropriate. The interaction between biomarker levels and neurohormonal treatments was assessed separately in each EF category through the analysis of variance for multiple factors. The log-rank test (Mantel-Cox) was used to compare survival times on Kaplan-Meier curves. Two tailed p values <0.05 were considered significant.

3. Results

3.1. Patient population and neurohormones

Patient characteristics across the EF categories (HFpEF, n = 189; HFmrEF, n = 189; HFrEF, n = 189) are reported in Table 1. Patients with HFmrEF resembled more closely those with HFpEF than those with HFrEF. Compared with patients with HFpEF or HFmrEF, those with HFrEF were also more often on MRA and device therapy, had more often ischemic HF, had lower body mass index and worse renal function, and were more symptomatic for dyspnea (Table 1).

In the whole population, circulating neurohormones tended to increase in parallel with symptom severity, quantified through the New York Heart Association class (Fig. 1). Circulating levels NT-proBNP, PRA, aldosterone, norepinephrine, but not epinephrine, were similar between HFpEF and HFmrEF. All neurohormones except for epinephrine were higher in HFrEF than HFmrEF and HFpEF (Table 1 and Fig. 1). Notably, neurohormone levels in HFmrEF and HFpEF were often below reference values, previously measured in normal subjects over 50 years of age [12] (Fig. 1). The difference in neurohormonal activation across EF categories was confirmed after accounting for beta-blocker, ACEi/ARB, MRA, and furosemide therapy (Supplemental Table 1).

3.2. Profiles of neurohormonal activation

When considering upper reference levels as cut-offs, patients with HFpEF, HFmrEF and HFrEF showed elevation of NT-proBNP in 85, 80 and 97% of cases, PRA in 19, 29 and 47%, aldosterone in 43, 36 and 50%, norepinephrine in 40, 38 and 53%, and epinephrine in 10, 11 and 16%, respectively (Supplemental Fig. 1). As NT-proBNP was increased in the vast majority of cases, and epinephrine in a minority, patients were stratified according to the other neurohormones. Patients with PRA, aldosterone and norepinephrine increased were 10% in HFpEF and 8% in HFmrEF, compared to 21% in HFrEF. Additionally, 16% of patients with HFpEF and 22% of those with HFmrEF had at least 2 biomarkers elevated (Fig. 2). Notably, among both HFpEF and HFmrEF patients, the percentages of patients receiving ACEi/ARB or beta-blocking therapy did not differ significantly across groups (Tables 2 and 3). Renal function declined in parallel with the number of biomarkers elevated, and the percentages of HFpEF patients with moderate-to-severe dyspnea tended to increase (Tables 2 and 3).

3.3. Neurohormonal activation and outcome

Median follow-up durations were 5 years [3–10] for HFpEF, 6 years [3–9] for HFmrEF, and 5 years [2–9] for HFrEF. Fifty-nine patients with HFpEF died (31%), compared to 71 patients with HFmrEF (38%), and 92 with HFrEF (49%). In all EF categories, the number of patients dying during follow-up tended to increase in parallel with the number of biomarkers elevated (Fig. 2). Accordingly, patients with no biomarker increased had the longest survival, and those with 3 biomarkers increased the shortest (Supplemental Fig. 2). Patient classification according to their biomarker profile (Fig. 2) predicted all-cause mortality regardless of age, sex, HF category (HFrEF/HFmrEF/HFpEF), ischemic etiology,

Table 1
Patient characteristics across categories of systolic dysfunction.

	HFpEF n = 189	HFmrEF n = 189	HFrEF n = 189	*p* (all)	*p* (HFpEF vs. HFrEF	*p* (HFpEF vs. HFmrEF)	*p* (HFmrEF vs. HFrEF)
Age (years)	70 (59–78)	67 (58–75)	67 (58–75)	0.277	0.178	0.113	0.604
Men (n, %)	105 (56)	138 (73)	139 (74)	**<0.001**	**<0.001**	**<0.001**	0.947
BMI (kg/m^2)	28.0 (24.5–31.2)	27.0 (24.8–29.7)	25.5 (23.2–29.1)	**<0.001**	**<0.001**	0.198	**0.002**
LVEF (n, %)	50 (50–52)	43 (41–45)	24 (20–30)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
LVEDD (mm)	48.2 (6.8)	55.5 (6.1)	64.8 (7.4)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
LVESD (mm)	37.1 (5.3)	41.8 (6.9)	55.4 (8.3)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
LVMI (g/m^2)	122 (37)	131 (31)	150 (33)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
NYHA I–II/III–IV, n (%)	127/62 (67/33)	139/50 (74/26)	105/84 (56/44)	**<0.001**	**0.001**	0.191	**<0.001**
Ischemic etiology (n, %)	44 (23)	77 (41)	97 (51)	**<0.001**	**<0.001**	**<0.001**	**0.039**
Creatinine (mg/dL)	1.13 (0.49)	1.14 (0.51)	1.28 (0.57)	**0.006**	**0.015**	0.832	**0.021**
eGFR (mL/min/1.73 m^2)	67 (50–88)	74 (54–91)	62 (44–82)	**<0.001**	**0.021**	0.195	**<0.001**
Serum sodium (mEq/L)	139 (3)	139 (3)	140 (4)	0.460	0.832	0.914	0.856
Serum potassium (mEq/L)	3.99 (0.53)	4.03 (0.51)	4.01 (0.46)	0.743	0.977	0.840	0.912
Hemoglobin (g/dL)	13.3 (1.9)	13.2 (1.8)	13.4 (1.8)	0.553	0.921	0.965	0.829
C-reactive protein (mg/dL)	0.33 (0.16–0.77)	0.30 (0.12–0.70)	0.36 (0.15–0.99)	0.266	0.392	0.627	0.987
Resting heart rate (bpm)	69 (9)	75 (10)	79 (11)	**0.001**	**0.001**	0.102	0.618
Systolic BP (mmHg)	126 (11)	131 (14)	119 (13)	**<0.001**	**0.033**	0.178	**<0.001**
Atrial fibrillation (n, %)	61 (32)	iojs3 (28)	57 (30)	0.733	0.777	0.430	0.861
Hypertension (n, %)	119 (63)	121 (64)	92 (49)	**0.002**	**0.027**	0.548	**0.014**
Diabetes (n, %)	55 (31)	60 (32)	56 (31)	0.823	0.876	0.323	0.765
COPD (n, %)	34 (18)	27 (14)	22 (12)	0.211	0.139	0.345	0.432
Beta-blockers (n, %)	91 (48)	124 (66)	143 (76)	**<0.001**	**<0.001**	**0.003**	0.082
ACEi/ARB (n, %)	126 (67)	148 (78)	138 (73)	**<0.001**	0.050	**0.020**	0.570
MRA (n, %)	47 (25)	49 (26)	105 (56)	**<0.001**	**<0.001**	0.993	**<0.001**
Furosemide (n, %)	107 (57)	107 (57)	147 (78)	**<0.001**	**<0.001**	0.626	**<0.001**
Digoxin (n, %)	37 (20)	34 (18)	60 (32)	**<0.001**	**<0.001**	0.567	**<0.001**
CRT (n, %)	4 (2)	10 (5)	19 (10)	**<0.001**	**<0.001**	**0.001**	**0.027**
ICD (n, %)	4 (2)	5 (3)	21 (11)	**<0.001**	**<0.001**	0.111	**<0.001**
NT-proBNP (ng/L)	645 (235–1612)	575 (205–1714)	2332 (995–5666)	**<0.001**	**<0.001**	0.471	**<0.001**
PRA (ng/mL/h)	0.6 (0.2–1.6)	0.6 (0.2–2.6)	1.7 (0.4–5.6)	**<0.001**	**<0.001**	0.192	**<0.001**
Aldosterone (ng/L)	130 (74–186)	113 (72–177)	153 (85–246)	**<0.001**	**0.006**	0.395	**<0.001**
Norepinephrine (ng/L)	440 (304–612)	430 (259–624)	517 (343–844)	**<0.001**	**<0.001**	0.213	**<0.001**
Epinephrine (ng/L)	22 (10–49)	25 (10–44)	31 (10–63)	0.138	0.293	0.952	0.319

Glomerular filtration rate (GFR) was estimated from plasma creatinine based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. ACEi/ARB, angiotensin converting enzyme inhibitors/angiotensin receptor blockers; BMI, body mass index; BP, blood pressure; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; ICD, implanted cardiac defibrillator; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; LVMI, left ventricular mass index; MRA, mineralocorticoid receptor antagonists; NT-proBNP, N-terminal fraction of pro-B-type natriuretic peptide; PRA, plasma renin activity.

NYHA class, and HF therapy (beta-blocker, ACEi/ARB, MRA, and furosemide) ($p = 0.002$).

4. Discussion

In the present study we assessed for the first time neurohormonal function across all EF categories, measuring 5 biomarkers reflecting the compensatory natriuretic response (NT-proBNP), RAAS activation (PRA, aldosterone), and sympathetic outflow (norepinephrine, epinephrine). Except for epinephrine, circulating levels of all these neurohormones were similar between HFpEF and HFmrEF, and higher in HFrEF than both categories above, beyond the contribution of therapies impacting on RAAS and SNS axes. Significant proportions of patients with either HFpEF or HFmrEF displayed elevated neurohormone levels: in detail, more than half of patients in both categories had at least 2 biomarkers elevated, and a subgroup (7% in both HFmrEF and HFpEF) displayed evidence of activated RAAS, SNS, and natriuretic peptide system. Finally, patient survival was poorer in parallel with the number of elevated biomarkers independently from EF categories.

More than 3 decades ago, an analysis of neurohormone levels in chronic systolic HF led to a change in paradigm in the treatment of this condition. In 1984, Cohn et al. characterized 106 patients with systolic HF, reporting that plasma norepinephrine was elevated, and independently predicted mortality [18]. This ground-breaking paper prompted a reappraisal of HF pathophysiology, allowing to understand how sustained SNS and RAAS activation yields detrimental effects on the cardiovascular system and contributes to HF progression [19–21]. For example, increased sympathetic outflow acts as a compensatory response to the hemodynamic derangement induced by heart damage, but becomes maladaptive over time, inducing salt and water retention, peripheral tissue hypoperfusion, arrhythmic burden, and adverse cardiac remodeling [18–20]. This conceptual background ultimately led to clinical trials that demonstrated the strong survival benefit deriving from therapies for neurohormonal antagonism, resulting in a significant improvement in patient prognosis [21].

In the following years, only a few studies have assessed neurohormones in HF, all of them focusing on systolic HF. Two studies were *post-hoc* analyses of the Valsartan in Heart Failure (Val-HeFT) trial, in which 93% of patients received ACEi/ARB, though only 37% were on beta-blockers, and 5% on MRA [22–24]. Natriuretic peptides (either BNP or NT-proBNP) emerged as the strongest predictors of outcome, further confirming their established role in risk stratification of HF patients, and norepinephrine and PRA had similar prognostic value [23,24]. Of note, the subgroup of Val-HeFT patients receiving both an ACEi and a beta-blocker had lower aldosterone (94 ng/L) and norepinephrine (394 ng/L) circulating concentration than in our HFrEF population. Still, Val-HeFT enrolled younger patients (mean age 62 years) and excluded subjects with severe impairment of renal function, both affecting neurohormonal levels.

The prognostic role of PRA emerged also in other 2 studies from our group, confirming its independent prognostic value either in the whole HFrEF population or in HFrEF patients with chronic

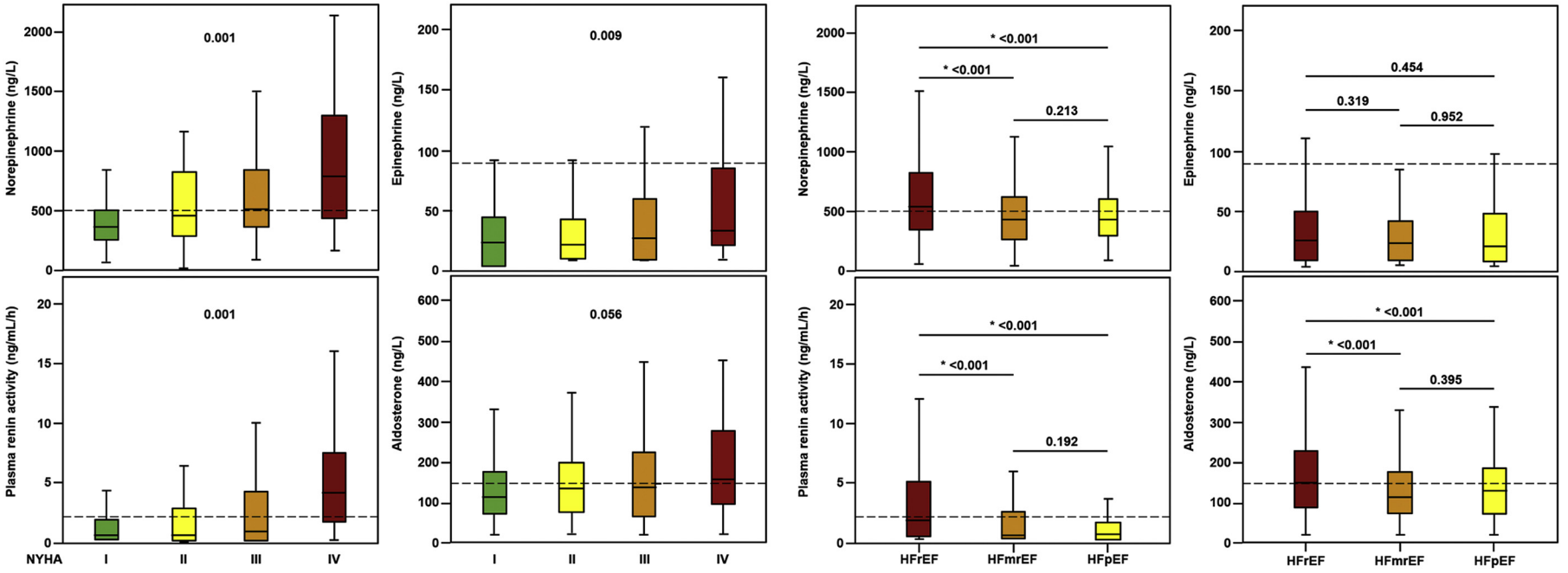

Fig. 1. Neurohormonal function across New York Heart Association (NYHA) functional class (*left*) and left ventricular ejection fraction categories (*right*).
Dashed lines indicate upper reference levels (plasma renin activity, PRA: 2.2 ng/mL/h; aldosterone, 150 ng/dL; norepinephrine, 500 ng/L; epinephrine, 90 ng/L). Normal values of these biomarkers, as established in healthy subjects aged >50 years, are: PRA, 0.5 ± 0.4 ng/mL/h; aldosterone, 96 ± 47 ng/dL; norepinephrine, 342 ± 134 ng/L; epinephrine, 52 ± 20 ng/L [12]. HFmrEF, HF with mid-range ejection fraction; HFpEF, HF with preserved ejection fraction; HFrEF, heart failure with reduced EF.

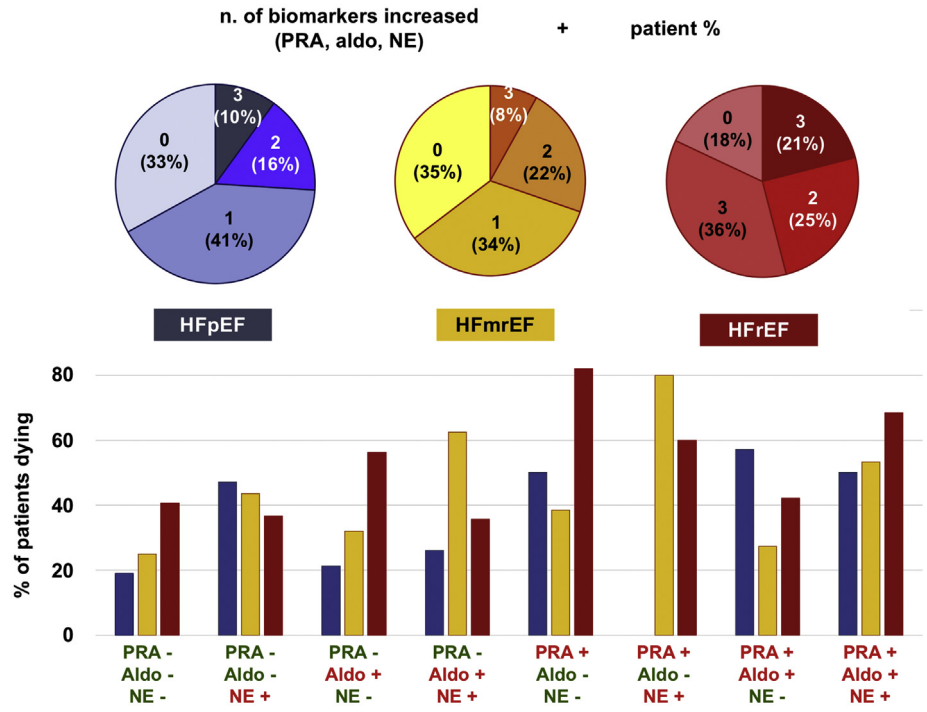

Fig. 2. Sympathetic and renin-angiotensin-aldosterone system activation across ejection fraction categories and its prognostic impact. *Upper panel:* The percentages of patients with 0, 1, 2 or 3 neurohormones increased are reported in pie charts. HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; NE, norepinephrine NT-proBNP, N-terminal fraction of pro-B-type natriuretic peptide PRA, plasma renin activity. *Lower panel:* For the three EF categories, the percentages of patients dying during follow-up are reported as a function of the number of neurohormones elevated.

kidney disease [25,26], despite optimal pharmacological and non-pharmacological treatment. Finally, a *post-hoc* analysis of the AdreView Myocardial Imaging for Risk Evaluation in Heart Failure (ADMIRE-HF) trial, reported that norepinephrine was an independent predictor of outcome in a model including baseline heart rate, carvedilol equivalent dose, and the heart-to-mediastinum ratio at cardiac iodine-123 metaiodobenzylguanidine scintigraphy [27]. These results somehow corroborate the prognostic relevance of norepinephrine even in patients receiving beta-blockers (92% in that cohort). With regard to the other neurohormones, to our knowledge epinephrine has never been analyzed, and aldosterone appeared a weak predictor of outcome in the Val-HeFT cohort [23].

In this study, we assess for the first time the extent of neurohormonal activation in patients without severe impairment of left ventricular systolic function, reporting similar circulating concentrations of norepinephrine, PRA, and aldosterone (as well as NT-

Table 2
Heart failure with preserved ejection fraction: characteristics of patients with or without neurohormonal activation.

HFpEF					
	3 biomarkers elevated (n = 18, 10%)	2 biomarkers elevated (n = 31, 16%)	1 biomarker elevated (n = 77, 41%)	No biomarkers elevated (n = 63, 33%)	*p* for trend
Age (years)	70 (50–77)	73 (66–79)	71 (58–79)	65 (55–78)	0.214
Men (n, %)	9 (50)	17 (55)	43 (56)	36 (57)	0.960
BMI (kg/m^2)	25.9 (22.4–30.1)	29.5 (27.2–34.4)	28.0 (24.5–31.0)	27.5 (24.2–31.2)	0.112
LVEF (n, %)	50 (50–52)	50 (50–55)	50 (50–56)	50 (50–55)	0.735
NYHA I–II/III–IV, n (%)	6/11 (33/61)	17/12 (55/39)	52/18 (68/23)	40/15 (64/24)	**0.011**
Ischemic etiology (n, %)	2 (11)	13 (42)	13 (17)	16 (25)	**0.024**
eGFR (mL/min/1.73 m^2)	82 (52–93)	58 (36–68)	64 (47–75)	78 (59–95)	**0.001**
ACEi/ARB (n, %)	9 (50)	22 (71)	50 (65)	39 (62)	0.306
Beta-blockers (n, %)	6 (33)	15 (49)	41 (53)	29 (46)	0.435
MRA (n, %)	14 (78)	11 (36)	14 (18)	8 (13)	**<0.001**
Diuretics (n, %)	15 (83)	18 (58)	38 (49)	36 (57)	**0.043**
Digoxin (n, %)	7 (39)	6 (19)	12 (16)	12 (19)	0.144
ICD (n, %)	0 (0)	2 (7)	1 (1)	1 (2)	0.519
CRT (n, %)	0 (0)	2 (7)	2 (3)	0 (0)	0.363
PRA (ng/mL/h)	5.2 (3.2–10.2)	0.9 (0.2–2.7)	0.6 (0.2–1.3)	0.3 (0.2–0.9)	**<0.001**
Aldosterone (ng/L)	230 (172–293)	207 (170–377)	138 (76–183)	75 (49–107)	**<0.001**
Norepinephrine (ng/L)	991 (758–1364)	592 (495–878)	476 (302–602)	347 (242–409)	**<0.001**
Epinephrine (ng/L)	31 (10–54)	40 (22–67)	17 (10–38)	22 (10–60)	**0.022**
NT-proBNP (ng/L)	850 (277–2064)	860 (273–1998)	645 (263–1593)	522 (201–1411)	0.406

ACEi/ARB, angiotensin converting enzyme inhibitors/angiotensin receptor blockers; BMI, body mass index; CRT, cardiac resynchronization therapy; ICD, implanted cardiac defibrillator; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonists; NT-proBNP, N-terminal fraction of pro-B-type natriuretic peptide; NYHA, New York Heart Association; PRA, plasma renin activity.

Table 3
Heart failure with mid-range ejection fraction: characteristics of patients with or without neurohormonal activation.

HFmrEF	3 biomarkers increased (n = 15, 8%)	2 biomarkers increased (n = 42, 22%)	1 biomarker increased (n = 65, 34%)	No biomarkers increased (n = 67, 35%)	*p* for trend
Age (years)	68 (60–76)	70 (60–76)	69 (59–75)	64 (58–73)	0.320
Men (n, %)	10 (67)	29 (69)	47 (72)	52 (78)	0.711
BMI (kg/m^2)	27.3 (25.0–32.4)	25.7 (23.7–28.7)	28.0 (25.9–30.4)	26.6 (24.7–28.9)	0.129
LVEF (n, %)	43 (40–46)	43 (40–46)	45 (40–45)	45 (41–46)	0.783
NYHA (I–II vs. III–IV)	7/8 (47/53)	32/10 (76/24)	49/16 (75/25)	51/16 (76/24)	0.109
Ischemic etiology (n, %)	2 (13)	19 (45)	28 (43)	28 (42)	0.158
eGFR (mL/min/1.73 m^2)	53 (37–87)	58 (40–81)	71 (55–92)	82 (66–92)	**<0.001**
ACEi/ARB (n, %)	12 (80)	37 (88)	46 (71)	52 (78)	0.214
BB (n, %)	7 (47)	28 (67)	46 (71)	43 (64)	0.357
MRA (n, %)	7 (47)	15 (36)	16 (25)	11 (16)	**0.034**
Diuretics (n, %)	9 (60)	31 (74)	40 (72)	27 (40)	**0.005**
Digoxin (n, %)	5 (33)	9 (21)	9 (14)	11 (16)	0.308
ICD (n, %)	0 (0)	1 (2)	1 (2)	3 (5)	0.636
CRT (n, %)	0 (0)	6 (14)	4 (6)	0 (0)	**0.004**
PRA (ng/mL/h)	8.2 (4.1–11.9)	3.7 (0.5–5.9)	0.7 (0.2–1.6)	0.2 (0.2–0.5)	**<0.001**
Aldosterone (ng/L)	246 (178–459)	170 (94–250)	131 (73–177)	81 (46–112)	**<0.001**
Norepinephrine (ng/L)	690 (565–967)	569 (451–817)	418 (294–658)	252 (161–409)	**<0.001**
Epinephrine (ng/L)	35 (10–48)	31 (11–65)	27 (10–55)	19 (10–36)	0.238
NT-proBNP (ng/L)	1197 (538–2695)	646 (220–3118)	604 (189–1983)	431 (117–891)	**0.025**

ACEi/ARB, angiotensin converting enzyme inhibitors/angiotensin receptor blockers; BMI, body mass index; CRT, cardiac resynchronization therapy; ICD, implanted cardiac defibrillator; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonists; NT-proBNP, N-terminal fraction of pro-B-type natriuretic peptide; NYHA, New York Heart Association; PRA, plasma renin activity.

proBNP) in patients with HFpEF and HFmrEF, although much lower than those observed in HFrEF. These findings could not be attributed merely to heterogeneity in the use of medications impacting on neurohormonal function, as demonstrated through a dedicated analysis. Notably, contemporary cohorts of patients with HFrEF and HFmrEF are unlikely to be naïve from drugs acting on the RAAS and SNS axes. Therefore, the assessment of neurohormonal activation, as well as its clinical and prognostic values, must be necessarily performed with background pharmacological therapy.

Since neurohormonal activation is less prominent when EF is ≥40%, a lower prognostic benefit from therapies counteracting the activation of SNS and RAAS may be expected. This may explain the systematic failure of clinical trials on drugs for neurohormonal antagonism in HFpEF, which have often included patients with EF values now included in the HFmrEF range [28]. Furthermore, the low degree of neurohormonal activation is in agreement with our current understanding of HFpEF pathophysiology, in which comorbidities such as hypertension, obesity, anemia, kidney dysfunction and diabetes seem to play a crucial role [29]. On the other hand, NT-proBNP was increased in the large majority of patients, and 10% of patients with HFpEF and 8% with HFmrEF displayed an elevation of 3 neurohormones (PRA, aldosterone, norepinephrine), with a progressive worsening of prognosis in parallel with the number of neurohormones elevated.

Our findings suggest that neurohormonal activation be present in a significant proportion of HF patients across the whole EF spectrum, even on guideline-recommended treatment. As a pathophysiological background, peripheral resistance to their biologic action is a powerful stimulus to the production and secretion of natriuretic peptides [30], whereas clinical severity and cardiac dysfunction are major drives to sympathetic activation and renin secretion [25,26], and aldosterone breakthrough often occurs in presence of administration of ACEi/ARB [31]. The persistence of neurohormonal activation on conventional treatment predicts worse prognosis and likely identifies a patient subset needing an enhanced therapeutic effort in HFrEF, and possibly also patients with HFmrEF and HFpEF who may benefit from neurohormonal antagonism [6]. Therefore, the biohumoral panel we propose may represent an additional tool for patient characterization in chronic HF, as well as being potentially relevant for the selection of patients for trials on neurohormonal modulation.

Some limitations of this preliminary, hypothesis-generating study must be acknowledged. First, we assessed relatively small patient sets (n = 189 each) to compare groups of equal size, matched for year of baseline assessment. Our findings should then be verified in larger cohorts, especially with regard to the prognostic impact of neurohormonal activation. Second, the HFmrEF and HFpEF categories may include patients who had a favorable response to therapy (recovered EF). Nevertheless, the relevance of this phenomenon was likely modest, given the low number of device carriers in the HFpEF and HFmrEF subgroups; further, the evolution of EF over time is not considered in the definitions of HFpEF and HFmrEF [4]. Third, while we report that greater neurohormonal activation portends a worse prognosis, our analysis does not allow to establish whether high neurohormone levels are just a reflection of HF severity or have themselves a negative impact on the natural history of the disease. Fourth, predicting response to HF medications based on neurohormonal profiling remains largely speculative; in other words, high levels of markers of RAAS activation does not necessarily mean that neurohormonal drugs will improve prognosis. With regards to the assessment of prognostic significance of neurohormone profiling, we also acknowledge that variables for adjustment in multivariable analysis were arbitrarily selected, although being established outcome predictors in HF. Fifth, neurohormone testing requires dedicated facilities and expertise, and the feasibility and cost-effectiveness of neurohormonal phenotyping for risk prediction and/or therapy optimization requires dedicated studies. Sixth, serial neurohormone evaluations may be more informative on patient prognosis than a single testing, which nonetheless seemed to hold some prognostic significance. On the other hand, an in-depth assessment of the prognostic relevance of neurohormonal activation on patient outcome was beyond the scopes of this study, whose main goal was to assess neurohormonal activation across EF categories. Finally, as in the majority of outcome studies, changes in functional status or therapy during follow-up were not considered, although for example changes in functional capacity could have been informative, and changing HF therapy regiments over time could impact on biomarkers and thus outcome.

In conclusion, NT-proBNP and circulating biomarkers of RAAS and SNS activation (PRA, aldosterone, norepinephrine) are elevated in a substantial proportion of patients with HFpEF and HFmrEF, beyond the effects of therapies impacting on neurohormonal function. Characterizing the neurohormonal profile of individual patients may refine prognostic stratification and deserves consideration in the design of future clinical trials.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.08.040.

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