Clinica Chimica Acta 526 (2022) 49–54

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Vasostatin-1 as a potential novel circulating biomarker in patients with chronic systolic heart failure: A pilot study

Giuseppe Pinto [a], Barbara Colombo [b], Adriano Autieri [a], Luca Foppoli [a], Roberto Spoladore [a], Valentina Ardizzone [a], Alberto Margonato [a,c], Angelo Corti [b,c], Gabriele Fragasso [a,*]

[a] Heart Failure Unit, Department of Cardiology, IRCCS San Raffaele Scientific Institute, Milan, Italy
[b] Division of Experimental Oncology, IRCCS San Raffaele Scientific Institute, Milan, Italy
[c] San Raffaele Vita-Salute, University, Milan, Italy

ARTICLE INFO

Keywords:
Vasostatin-1
Chromogranin A
Heart failure
Natriuretic peptides

ABSTRACT

Background and aims: Previous studies have shown that circulating chromogranin A (CgA) increases in patients with chronic systolic heart failure (HF). Aim of the present study is to evaluate the potential role of circulating vasostatin-1 (VS-1), a cardioregulatory fragment of CgA, as prognostic marker in patients with chronic HF.
Materials and methods: The plasma levels of CgA and VS-1 were determined in 80 patients with chronic systolic HF. Patients were followed-up to evaluate the occurrence of cardiovascular (CV) events.
Results: CgA and VS-1 plasma levels were significantly higher in patients with CV events at follow-up. VS-1, but not CgA, was associated to NT-proBNP. No significant association of CgA and VS-1 with left ventricular ejection fraction (LVEF) was observed. CgA, NT-proBNP and age, but not VS-1, were independent predictors of CV events.
Conclusion: In patients with chronic systolic HF those who experienced CV events had higher levels of VS-1 and CgA. Given its established effect on cardiac cells, the association of VS-1 levels with NT-proBNP levels but not with LVEF, suggests that this fragment might provide complementary information to NT-proBNP and CgA in HF patients.

1. Introduction

Different cell types, including neuroendocrine cells and cardiomyocytes, produce chromogranin A (CgA), a polypeptide belonging to the chromogranin-secretogranin family [1–3]. This protein, which is stored in secretory granules and is exocytotically released in the tissue microenvironment and then in circulation, can exert a variety of regulatory functions in several biological pathways. CgA presents multiple proteolytic sites, and most of its actions are mediated by its fragments [4–6]. The discovery that cardiomyocytes produce CgA and co-secrete it with brain natriuretic peptide (BNP), in particular in dilated and hypertrophic cardiomyopathies, has recently increased the interest aimed at investigating the cardiovascular (CV) effects of CgA and its fragments [7]. These studies have shown that full-lenght CgA can exert negative lusitropic and inotropic effects on cardiac muscles and vasodilator effect on coronary arteries, through nitric monoxide (NO) pathway [8]. Similar inhibitory effects have also been observed with two fragments of CgA, called vasostatin-1 (VS-1) and catestatin (Cst) [9]. Additionally, these fragments can exert protective effects against myocardial ischemia, post-ischemic reperfusion injury, and can modulate calcium release from endoplasmic reticulum [10,11]. Finally, CgA, VS-1 and Cst can also regulate endothelial homeostasis [12]. For example, CgA and VS-1 can enhance the endothelial barrier function and exert anti-angiogenic effects, whereas Cst can exert pro-angiogenic effects [13–16]. Moreover, CgA and its fragment VS-1 have been studied as potential marker of atherosclerosis progression in carotid arteries [17]. Some preclinical studies indicate Cst as a reliable indirect marker of sympathetic nervous system activity which, in HF patients, could potentially highlight those with a more advanced disease [18]. On this basis, Cst resulted as a predictor of in-hospital death during an episode of

Abbreviations: ANS, autonomic nervous system; BNP, brain natriuretic peptide; CgA, chromogranin A; Cst, catestatin; ECL, enterochromaffin-like cells; GDMT, guideline-directed medical therapy; HF, heart failure; ICD, implantable cardioverter defibrillator; IQR, interquartile range; LVEF, left ventricular ejection fraction; mAb, monoclonal antibody; NYHA, New York Heart Association; NO, nitric monoxide; PPI, proton pump inhibitors; SNS, sympathetic nervous system; VS-1, vasostatin-1.

* Corresponding author at: Heart Failure Unit, Istituto Scientifico San Raffaele, via Olgettina 60, 20132 Milano, Italy.
E-mail address: gabriele.fragasso@hsr.it (G. Fragasso).

https://doi.org/10.1016/j.cca.2021.12.024
Received 17 November 2021; Received in revised form 20 December 2021; Accepted 26 December 2021
Available online 29 December 2021

acute worsening HF [19].

Another fragment of CgA, corresponding to its C-terminal region (called serpinin) can also exert CV effects. Contrary to VS-1 and Cst, this fragment exerts positive inotropism and lusitropism through activation of β1-adrenergic receptor without long-term heart remodelling. Furthermore, similarly to VS-1 and Cst, serpinin promotes coronary vasodilation through NO-dependent pathway [20].

Circulating levels of CgA in heart failure (HF) patients have been shown to be significantly associated to NYHA (New York Heart Association) classification and morbidity/mortality rates [21–25]. However, in the GISSI-HF study, after correction for confounding factors, the association between CgA and mortality was no longer significant [26]. The aim of this study was to evaluate the potential prognostic role of VS-1 levels in HF patients in relation to clinical and conventional diagnostic and prognostic tools, such as NT-proBNP levels and left ventricular ejection fraction (LVEF).

2. Methods

Eighty consecutive ambulatory patients (17 females, age 73 (63–78) yrs) with chronic systolic HF (LVEF <45%), NYHA class I-III on optimal guideline-directed medical therapy (GDMT) for at least the previous 3 months were recruited. LVEF was determined by echocardiography performed within 30 days before study inclusion. Transthoracic echocardiographic exams (TTE) were performed using the Vivid E95 ultrasound system equipped with a 4Vc-D 4D Matrix Cardiac transducer (GE Vingmed Ultrasound, Horten, Norway). LVEF measurement was assessed by the modified Simpson biplane method. The plasma levels of CgA, VS-1 and NT-proBNP were analyzed. Blood samples were obtained from fasting outpatients in vacutainer tubes containing EDTA in between 9 a.m. and 1 p.m. at the time of recruitment. Timing of sampling was uniformed to limit the potential day-period variations of CgA plasma levels. Afterwards, blood samples were centrifuged at 2500*g*, at 4 °C, for 10 min, and plasma samples sent to the laboratory in Eppendorf safe-lock tubes at room temperature. At last, plasma samples were preserved at −20 °C until sample testing.

2.1. Follow-up

After study entry, patients were seen approximately twice a year, unless otherwise indicated. All subjects who had not attended the clinic at least once after the study entry date, were actively searched by telephone and/or ordinary mail. Survival status was ascertained by telephone interviews with patients, family members, or their general practitioners using standard questionnaires. Death certificates were obtained where applicable. All hospital visits were performed by a cardiologist of the heart failure clinical team. No nurses were involved in the follow-up.

2.2. Definitions

During the follow-up period, patients were managed by cardiologists through the rapid access to specialized outpatient HF clinic. Hospitalizations were identified and classified according to preset criteria as either a planned or unplanned admission. If the admission was unplanned it was sub-classified into either acute cardiac decompensation or one of the following categories (generically indicated as CV causes): worsening of the severity of HF; new medical problem related to the aetiology of HF (e.g. further myocardial infarction in a patient with HF due to previous myocardial infarction); new medical problem directly related to HF (e.g. cellulitis of oedematous lower limbs); cardiac surgery; percutaneous cardiac interventions. Non cardiovascular causes were: new medical problem not related to HF or its aetiology; social reasons; unplanned admission for change in therapy; improved compliance or further investigation; iatrogenic; other (including surgical admissions).

Death was classified as cardiovascular if it was coded as 390 to 459 (9th International Revision of the International Classification of Death - https://www.cdc.gov/nchs/icd/icd9.htm) on death certificates.

All patients signed informed consent, and the study was approved by the local ethics committee.

2.3. CgA and VS-1 determination

Plasma levels of CgA and VS-1 were assessed by using two sandwich ELISAs previously described [27]. The CgA-ELISA was based on the use of the monoclonal antibody (mAb) B4E11 in the capture step (against an epitope located in the N-terminal domain), and an anti-recombinant CgA polyclonal antiserum in the detection step (against epitopes located in the central region). This assay can detect full-length CgA as well as fragment lacking the C-terminal region, but not VS-1. The VS-1-ELISA was based on the use of mAb 5A8 in the capture step (against an epitope located in the N-terminal domain) and rabbit antibodies against the C-terminal residues of VS-1. This assay can detect VS-1 (CgA1-76), but not full-length CgA or larger fragments [27].

2.4. Statistics

Statistical analysis was performed with the Stata software package (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.). Categorical variables are reported as number (percentage) and compared with the Pearson's chi-squared. Continuous variables, given their non-normal distribution (tested by Shapiro-Wilk W test for normality and Kolmogorov-Smirnov tests of the equality of distributions), are reported as median (interquartile range [IQR]) and compared with the Two-sample Wilcoxon rank-sum (Mann-Whitney) test. Plasma levels of total CgA and VS-1 were compared in patients with and without a CV event during follow up.

The relationships between CgA, VS-1, NT-ProBNP and EF were studied with the univariate linear regression. A p-value < 0.05 was considered statistically significant.

Multivariate Cox regression analysis was used to identify independent predictors of CV events (variables tested on univariate and multivariate analysis were all the variables in Table 2)

3. Results

Table 1 shows the clinical characteristics and concomitant pharmacological therapy of study patients. Mean follow-up from study entry was 644 ± 184 days. Twenty-one patients experienced a CV event at 406 ± 228 days during follow-up. Twenty patients were hospitalized for acute decompensated HF, while death for CV cause occurred in only one patient. No patients underwent heart transplantation during follow-up.

CgA and VS-1 plasma levels were significantly higher in patients with events compared to those without (Fig. 1). In particular, CgA values were 6.79 nM (3.70–11.15) vs 2.42 nM (1.32–4.96), $p < 0.001$. VS-1 values were 0.28 nM (0.18–0.37) vs 0.21 nM (0.13–0.26), $p = 0.04$, respectively. Patients who experienced a CV event were older [76 (73–79) vs 70 (62–77) yrs, $p = 0.006$], and had higher baseline plasma levels of NT-proBNP [1799 (1068–5542) vs 798 (350–1662) pg/ml, $p < 0.001$], while ejection fraction was not different [38 (30–43) vs 37 (33–43) %, $p = 0.684$]. Table 2 shows clinical characteristics and laboratory findings stratified by the CV event.

In all patients a positive correlation between levels of VS-1 and NT-ProBNP ($R2 = 0.259$, $p < 0.05$) was observed (Fig. 2). On the contrary, no statistically significant correlation was observed between levels of CgA and NT-proBNP. No significant association of CgA and VS-1 with LVEF was observed.

To evaluate whether the values of CgA and VS-1 were influenced by the intake of proton pump inhibitors (PPI), a class of drugs known to increase the release of CgA from the gastric mucosa, we compared, within all patients group, these variables according to the concomitant use of PPI. The median values of CgA and VS-1 were increased in

Table 1
Baseline clinical characteristics and pharmacological therapy of the whole study population.

CLINICAL CHARACTERISTICS	
Age	73 (63–78)
Gender (male)	63 (79%)
Idiopathic cardiomyopathy	26 (32%)
Ischemic cardiomyopathy	26 (32%)
NYHA class I	15 (19%)
NYHA class II	56 (70%)
NYHA class III	9 (11%)
EF	37% (33–43)
ICD	21 (26%)
Sinus rhythm	58 (72%)
AF	22 (28%)
Diabetes	25 (31%)
Hypertension	52 (65%)
Hypercholesterolemia	37 (46%)
Family history for CAD	16 (20%)
Chronic kidney disease	11 (14%)
Smoking	6 (8%)
Hypothyroidism	10 (12%)
Hyperthyroidism	7 (9%)
PHARMACOLOGICAL THERAPY	
Beta-Blockers	62 (78%)
ACE-inhibitors	40 (50%)
ARBs	19 (24%)
Mineralocorticoid receptor antagonists	40 (50%)
Loop diuretics	62 (78%)
PPI	55 (69%)
Nitrates	20 (25%)
Digoxin	19 (24%)
Dihydropyridine calcium channel blockers	8 (10%)
Anticoagulants	31 (39%)
Antiarrhythmics	14 (17%)

Abbreviations: ACE, angiotensin converting enzyme; AF, atrial fibrillation; ARBs, angiotensin-II receptor blockers; CAD, coronary artery disease; EF, ejection fraction; ICD, implantable cardioverter defibrillator; NYHA, New York Heart Association; PPI, proton pump inhibitors.

patients who were on PPI compared to those not taking PPI (Fig. 3). In particular CgA values were 4.97 nM (2.55–9.01) vs 1.29 nM (0.81–2.35), $p < 0.001$. VS-1 values were 0.24 nM (0.19–0.32) vs 0.16 nM (0.12–0.24), $p = 0.002$, respectively. There were no statistically significant differences as regard NT-proBNP values among the whole patient population stratified by the intake of PPI.

A multivariate regression analysis was used to identify independent predictor of CV events. According to the final multivariate model, among all the analyzed variables, age (CI 1.01–1.16, $p = 0.016$), NT-proBNP (CI 1.01–1.08, $p = 0.049$) and CgA (CI 1.04–1.25, $p = 0.006$) were the only independent predictors of CV events. VS-1 levels were higher in patients experiencing CV events, although this molecule was not retained as a predictor in the multiple regression analysis (Supplementary materials, Tables 1–2).

4. Discussion

Endocrine properties of cardiac cells are an established notion. On this basis research in HF and other CV conditions has been heavily focused on the potential role of new molecules as diagnostic and prognostics markers or even therapeutic targets. These endocrine functions are ensured, among others, by natriuretic peptides [28]. All the endocrine substances produced by the heart, given their endocrine, autocrine and/or paracrine mechanism of action, in concert with neurotransmitters released by the Autonomic Nervous System (ANS), increase the variety of chemical signals that may contribute to cardiac physiological and pathophysiological states [29]. In HF, chronic activation of sympathetic nervous system (SNS) has adverse prognostic significance and may accelerate the pathological processes [30]. In this context CgA and its fragments (VS-1 and Cst) have been found to exert a negative inotropic effect mainly through NO pathway, but perhaps also through a NO independent pathway, interacting with beta-adrenergic receptors, counterbalancing the SNS activation [7–9]. Whether the influence of these molecules on inotropism is beneficial or detrimental may depend on the specific heart condition under study and remains to be established.

The clinical potential of CgA in HF and the possible relationships with other common parameters, such as natriuretic peptides and LVEF, has been investigated without consistent results [21–26]. Our study has evaluated, for the first time, the role of VS-1 (the CgA1-76 N-terminal fragment) in HF patients, and its relationships with well-known

Table 2
Clinical characteristics and laboratory findings stratified by the CV event.

	CV events (21)	Free from CV events (59)	
Gender (male)	18 (85%)	45 (76%)	P = 0.550
Age (yrs)	76 (73–79)	70 (62–77)	*P = 0.006*
NYHA class			P = 0.242
I	2 (9%)	13 (22%)	
II	15 (72%)	41 (69%)	
III	4 (19%)	5 (9%)	
EF (%)	38 (30–43)	37 (33–43)	P = 0.684
NT-proBNP (pg/ml)	1799 (1068–5542)	798 (350–1662)	*P < 0.001*
Total CgA (nM)	6.79 (3.70–11.15)	2.42 (1.32–4.96)	*P < 0.001*
VS-1 (nM)	0.28 (0.18–0.37)	0.21 (0.13–0.26)	*P = 0.04*
PPI	18 (85%)	37 (62%)	P = 0.093

Abbreviations: CgA, chromogranin A; CV, cardiovascular; EF, ejection fraction; NT-proBNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association; PPI, proton pump inhibitors; VS-1, vasostatin-1.

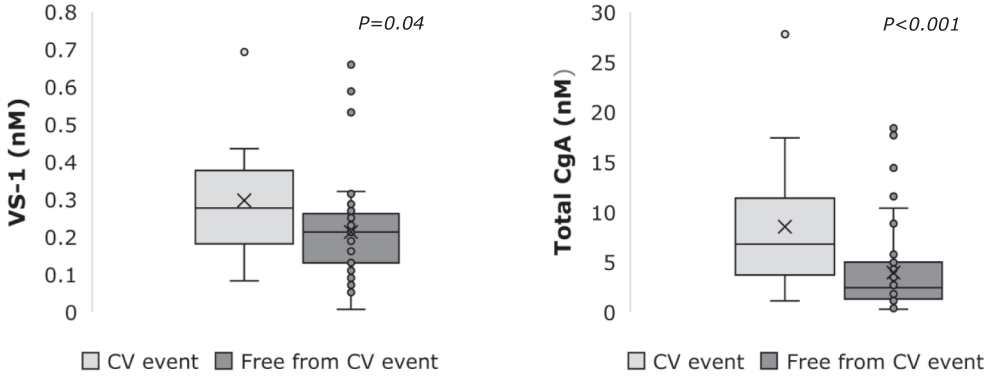

Fig. 1. Box-plots for plasma levels of CgA and VS-1 in patients with chronic systolic heart failure stratified by CV event compared with Mann-Whitney test. Abbreviations: CgA, chromogranin A; VS-1, vasostatin-1.

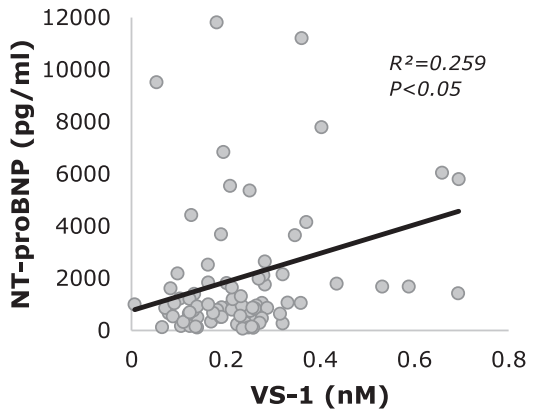

Fig. 2. Scatter plot with linear regression line between VS-1 and NT-ProBNP among all patients. Abbreviations: NT-ProBNP, N-terminal pro brain natriuretic peptide; VS-1, vasostatin-1.

parameters such as NT-proBNP, CgA and LVEF. The results show that VS-1 values are higher in patients experiencing CV events at follow up, mainly determined by increased occurrence of acute decompensated HF. Nevertheless, VS-1 does not appear as an independent predictor of CV events at multivariate analysis. Furthermore, our analysis confirms the potential prognostic role of CgA, as previously reported [21–25]. However, VS-1 positively correlates with NT-proBNP, while CgA does not. The association of VS-1 levels with NT-proBNP levels, but not with LVEF, suggests that this fragment may represent an independent and complementary marker to NT-proBNP and CgA. Despite this association, VS-1 levels resulted not predictive for CV events. A potential explanation for this finding may lie in the relatively small sample size. Moreover, neither of the two molecules correlate with LVEF. Since NT-proBNP is significantly associated with LVEF, the lack of correlation between VS-1, CgA and LVEF suggests that these two molecules may have a clinical meaning different from that of NT-proBNP, possibly indicating a residual neuro-hormonal activation which may be independent by the degree of LV dysfunction, but likely predictable of future HF decompensation. Although only CgA was found as an independent marker of CV events, both the molecules could represent independent markers of disease, not dependent by LVEF and complementary to NT-proBNP, since their release may be related to different neuro-hormonal pathways.

Despite the prognostic role of LVEF is an established asset, it should be acknowledged that LVEF has important limitations as a precise indicator of ventricular contractile performance, due to its load dependency and to the associated high inter- and intra-observer variability of its measurement with different techniques [31]. Additionally, apart from LVEF, patients may differ in terms of clinical characteristics, outcomes, and response to therapy, thereby underscoring the high degree of heterogeneity that exists among chronic HF patients and the need for improved phenotyping of the syndrome [32]. On this ground, it has been proposed to move away from a classification of HF based on LVEF and symptom severity alone [33]. In this context, the development of more precise prognostic biomarkers is warranted. The need of non-natriuretic peptide biomarkers in heart failure with preserved and reduced ejection fraction has recently been emphasized [34]. On the basis of the results of the present study, it appears that VS-1, CgA and NT-proBNP could yield a complementary role in predicting the occurrence of events in HF. The preliminary results of this pilot study should be tested in bigger study populations.

Finally, it should be taken into account the fact that natriuretic peptides determination may not be accurate in presence of different conditions. In fact, independently from coexisting HF, BNP and NT-proBNP levels are higher in older patients [35], women [35], black population [36] in patients with renal dysfunction [37], sepsis [38], atrial fibrillation [39], and hyperthyroidism [40]. On the other hand, natriuretic peptide levels may be disproportionately lower in hypothyroidism [40], in patients with obesity [41] or with advanced end-stage heart failure (the latter probably due to increased fibrosis) [42]. All these frequently encountered conditions in the HF population, definitely diminish the accuracy of natriuretic peptides in a fair proportion of patients.

4.1. PPI and CgA

Plasma CgA levels may increase also in patients with other diseases, such as chronic kidney disease, pancreatitis or systemic inflammatory diseases. Among iatrogenic causes of increased CgA plasma values the intake of PPI drugs stands out. Indeed, PPI are known to induce hyperplasia of enterochromaffin-like cells (ECL) [43] and, consequently, to increase the release of CgA from the gastric mucosa [44]. It has been shown that increased CgA values could be associated to either ECL hyperplasia and hyperfunction [44] and to low-dose PPI intake for short periods [45]. Notably, PPI induce CgA secretion to a greater extent than anti-H2 drugs [46]. On this basis, PPI intake has been considered a confounding factor in CgA dosing.

Interestingly, compared to patients not on PPI, we observed a 4.0-fold increase of the median plasma levels of CgA and only a 1.5-fold increase of VS-1 levels in patients on PPI therapy. These data might suggest, knowing all the limits of this assumption, that VS-1 could be less susceptible than CgA to PPIs-induced changes.

On this ground, the development of ELISAs for other fragments with known cardiovascular activity but not yet evaluated in patients with HF, such as serpinin ($CgA_{411-436}$), and a better knowledge of fragments already detected with ELISAs and studied as potential markers for diagnosis and prognosis in HF, such as Cst ($CgA_{352-372}$) [47], along with detection of VS-1 in HF patients is definitely warranted.

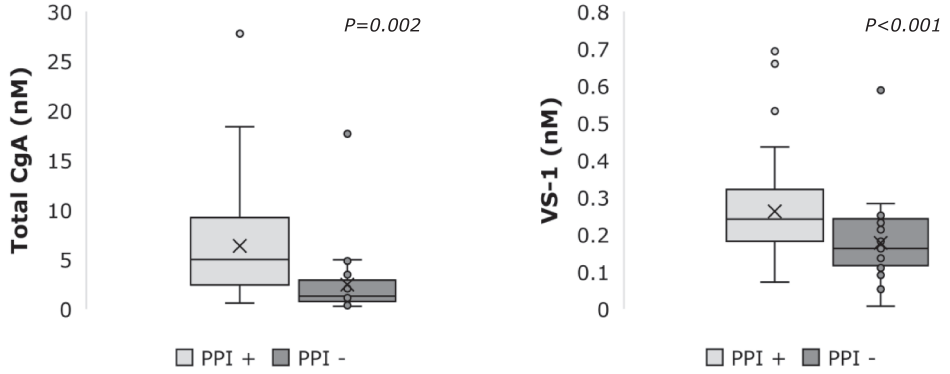

Fig. 3. Box-plots for plasma levels of CgA and VS-1 in patients with chronic systolic heart failure stratified by proton pump inhibitors intake compared with Mann-Whitney test. Abbreviations: CgA, chromogranin A; PPI, proton pump inhibitors; VS-1, vasostatin-1.

4.2. Study limitations

The relatively small sample size represents the main limitation of the present study. Moreover, the generalizability of the results are limited because there might be confounders, other than PPIs consumption, that could affect CgA and VS-1 values.

5. Conclusions

VS-1 and CgA plasma levels appear significantly higher in HF patients experiencing a CV event. Considering the experimental evidence on the mechanisms of release and action of VS-1, these results could suggest a prognostic role for CgA and VS-1 in chronic systolic HF, although only CgA appears as an independent marker of CV events at a multivariate analysis. Plasma levels of VS-1, unlike those of CgA, were associated with NT-proBNP. Given the significant association between NT-proBNP and LVEF, the lack of correlation between VS-1, CgA and LVEF may suggest that the evaluation of these fragments could be as independent and complementary markers to NT-proBNP, especially in those cases where natriuretic peptides elevation may be aspecific. Possibly, CgA and VS-1 release occurs through pathophysiological pathways different from known mechanical mechanisms involved in HF onset and progression. In fact, the increment of both molecules probably indicates a residual neuro-hormonal activation, independent by the degree of LV dysfunction, but likely predictable of future HF decompensation. Finally, VS-1 appears less susceptible than CgA to PPIs-induced changes and, therefore, more easily interpretable in the daily clinical context.

Disclosures

No sources of funding.

All authors have substantially contributed to the manuscript, have read and approved it.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Informed consent was obtained from all patients for being included in the study.

No animal studies were carried out by the authors for this article.

CRediT authorship contribution statement

Giuseppe Pinto: Investigation. **Barbara Colombo:** Methodology. **Adriano Autieri:** Investigation. **Luca Foppoli:** . **Roberto Spoladore:** Visualization. **Valentina Ardizzone:** Investigation. **Alberto Margonato:** Supervision. **Angelo Corti:** Methodology, Data curation. **Gabriele Fragasso:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2021.12.024.

References

[1] K.B. Helle, A. Corti, M.-H. Metz-Boutigue, B. Tota, The endocrine role for chromogranin A: a prohormone for peptides with regulatory properties, Cell Mol. Life Sci. 64 (22) (2007) 2863–2886, https://doi.org/10.1007/s00018-007-7254-0.

[2] A. Corti, Chromogranin A and the tumor microenvironment, Cell Mol. Neurobiol. 30 (8) (2010) 1163–1170, https://doi.org/10.1007/s10571-010-9587-8.

[3] M. Mandalà, M. Stridsberg, K.B. Helle, G. Serck-Hanssen, Endothelial handling of chromogranin A, Adv. Exp. Med. Biol. 482 (2000) 167–178, https://doi.org/10.1007/0-306-46837-9_13.

[4] K.A. Radek, B. Lopez-Garcia, M. Hupe, I.R. Niesman, P.M. Elias, L. Taupenot, S. K. Mahata, D.T. O'Connor, R.L. Gallo, The neuroendocrine peptide catestatin is a cutaneous antimicrobial and induced in the skin after injury, J. Invest. Dermatol. 128 (6) (2008) 1525–1534, https://doi.org/10.1038/sj.jid.5701225.

[5] R.H. Angeletti, S. Aardal, G. Serck-Hanssen, P. Gee, K.B. Helle, Vasoinhibitory activity of synthetic peptides from the amino terminus of chromogranin A, Acta Physiol. Scand. 152 (1994) 11–19, https://doi.org/10.1111/j.1748-1716.1994.tb09780.x.

[6] R. Ramella, O. Boero, G. Alloatti, T. Angelone, R. Levi, M.P. Gallo, Vasostatin 1 activates eNOS in endothelial cells through a proteoglycan-dependent mechanism, J. Cell. Biochem. 110 (2010) 70–79, https://doi.org/10.1002/jcb.22510.

[7] M. Pieroni, A. Corti, B. Tota, F. Curnis, T. Angelone, B. Colombo, M.C. Cerra, F. Bellocci, F. Crea, A. Maseri, Myocardial production of chromogranin A in human heart: a new regulatory peptide of cardiac function, Eur. Heart J. 28 (9) (2007) 1117–1127, https://doi.org/10.1093/eurheartj/ehm022.

[8] T. Pasqua, A. Corti, S. Gentile, L. Pochini, M. Bianco, M.H. Metz-Boutigue, M. C. Cerra, B. Tota, T. Angelone, Full-length human chromogranin-A cardioactivity: myocardial, coronary, and stimulus-induced processing evidence in normotensive and hypertensive male rat hearts, Endocrinology 154 (2013) 3353–3365, https://doi.org/10.1210/en.2012-2210.

[9] S. Fornero, E. Bassino, M.P. Gallo, R. Ramella, R. Levi, G. Alloatti, Endothelium dependent cardiovascular effects of the Chromogranin A-derived peptides Vasostatin-1 and Catestatin, Curr. Med. Chem. 19 (2012) 4059–5067, https://doi.org/10.2174/092986712802429984.

[10] M.C. Cerra, M.P. Gallo, T. Angelone, A.M. Quintieri, E. Pulerà, E. Filice, B. Guérold, P. Shooshtarizadeh, R. Levi, R. Ramella, A. Brero, O. Boero, M.H. Metz-Boutigue, B. Tota, G. Alloatti, The homologous rat chromogranin A1–64 (rCGA1-64) modulates myocardial and coronary function in rat heart to counteract adrenergic stimulation indirectly via endothelium-derived nitric oxide, FASEB J. 22 (11) (2008) 3992–4004, https://doi.org/10.1096/fj.08-110239.

[11] T. Angelone, R. Mazza, M.C. Cerra, Chromogranin-A: a multifaceted cardiovascular role in health and disease, Curr. Med. Chem. 19 (2012) 4042–4050, https://doi.org/10.2174/092986712802430009.

[12] A. Corti, E. Ferrero, Chromogranin A and the endothelial barrier function, Curr. Med. Chem. 19 (2012) 4051–4058, https://doi.org/10.2174/092986712802429975.

[13] L. Crippa, M. Bianco, B. Colombo, A.M. Gasparri, E. Ferrero, Y.P. Loh, F. Curnis, A. Corti, A new chromogranin A-dependent angiogenic switch activated by thrombin, Blood 121 (2013) 392–402, https://doi.org/10.1182/blood-2012-05-430314.

[14] M. Theurl, W. Schgoer, K. Albrecht, J. Jeschke, M. Egger, A.G.E. Beer, D. Vasiljevic, S. Rong, A.M. Wolf, F.H. Bahlmann, J.R. Patsch, D. Wolf, P. Schratzberger, S. K. Mahata, R. Kirchmair, The neuropeptide catestatin acts as a novel angiogenic cytokine via a basic fibroblast growth factor-dependent mechanism, Circ. Res. 107 (11) (2010) 1326–1335, https://doi.org/10.1161/CIRCRESAHA.110.219493.

[15] K.B. Helle, A. Corti, Chromogranin A: a paradoxical player in angiogenesis and vascular biology, Cell. Mol. Life Sci. 72 (2) (2015) 339–348, https://doi.org/10.1007/s00018-014-1750-9.

[16] T. Watanabe, The Emerging Roles of Chromogranins and Derived Polypeptides in Atherosclerosis, Diabetes, and Coronary Heart Disease, Int. J. Mol. Sci. 22 (11) (2021) 6118, https://doi.org/10.3390/ijms22116118.

[17] T. Bachetti, A. Ferrari Bardile, T.L. Aloi, B. Colombo, E. Assi, G. Savino, A. Vercelli, R. Colombo, A. Corti, Plasma levels of vasostatin-1, a chromogranin A fragment, are associated with carotid artery maximum stenosis: A pilot study, Int. J. Cardiol. 236 (2017) 438–443, https://doi.org/10.1016/j.ijcard.2017.02.019.

[18] J. Bozic, M. Kumric, T. Ticinovic Kurir, H. Urlic, D. Martinovic, M. Vilovic, N. Tomasovic Mrcela, J.A. Borovac, Catestatin as a Biomarker of Cardiovascular Diseases: A Clinical Perspective, Biomedicines 9 (12) (2021) 1757, https://doi.org/10.3390/biomedicines9121757.

[19] J.A. Borovac, D. Glavas, Z. Susilovic Grabovac, D. Supe Domic, L. Stanisic, D. D'Amario, C.S. Kwok, J. Bozic, Circulating sST2 and catestatin levels in patients with acute worsening of heart failure: a report from the CATSTAT-HF study, ESC Heart Fail. 7 (5) (2020) 2818–2828, https://doi.org/10.1002/ehf2.12882.

[20] B. Tota, S. Gentile, T. Pasqua, E. Bassino, H. Koshimizu, N.X. Cawley, M.C. Cerra, Y. P. Loh, T. Angelone, The novel chromogranin A-derived serpinin and pyroglutaminated serpinin peptides are positive cardiac β-adrenergic-like inotropes, FASEB J. 26 (7) (2012) 2888–2898, https://doi.org/10.1096/fj.11-201111.

[21] C. Ceconi, R. Ferrari, T. Bachetti, C. Opasich, M. Volterrani, B. Colombo, G. Parrinello, A. Corti, Chromogranin A in heart failure; a novel neurohumoral factor and a predictor for mortality, Eur. Heart J. 23 (2002) 967–974, https://doi.org/10.1053/euhj.2001.2977.

[22] M.E. Estensen, A. Hognestad, U. Syversen, I. Squire, L. Ng, J. Kjekshus, K. Dickstein, T. Omland, Prognostic value of plasma chromogranin A levels in patients with complicated myocardial infarction, Am. Heart J. 152 (5) (2006) 927.e1–927.e6, https://doi.org/10.1016/j.ahj.2006.05.008.

[23] G. Fragasso, R. Spoladore, F. Maranta, A. Corti, G. Lattuada, B. Colombo, M. Locatelli, A. Salerno, G. Calori, L. Briceno, A.B. Alfieri, G. Perseghin, A. Margonato, Increased low-grade inflammation is associated with lack of functional response to carvedilol in patients with systolic heart failure,

J. Cardiovasc. Med. (Hagerstown) 14 (1) (2013) 49–56, https://doi.org/10.2459/JCM.0b013e328345a1f6.
[24] D. Gruson, A.-C. Pouleur, T. Lepoutre, J.-M. Ketelslegers, S.A. Ahn, M.F. Rousseau, Chromogranin A Predicts 6 Years Mortality in Patients With Reduced Ejection Fractions, J. Cardiac. Fail. 18 (8) (2012) S73, https://doi.org/10.1016/j.cardfail.2012.06.471.
[25] B. Dieplinger, A. Gegenhuber, J. Struck, W. Poelz, W. Langsteger, M. Haltmayer, T. Mueller, Chromogranin A and C-terminal endothelin-1 precursor fragment add independent prognostic information to amino-terminal proBNP in patients with acute destabilized heart failure, Clin. Chim. Acta 400 (1–2) (2009) 91–96, https://doi.org/10.1016/j.cca.2008.10.012.
[26] H. Røsjø, S. Masson, R. Latini, A. Flyvbjerg, V. Milani, M.T. La Rovere, M. Revera, A. Mezzani, G. Tognoni, L. Tavazzi, T. Omland, GISSI-HF Investigators, Prognostic value of chromogranin A in chronic heart failure: data from the GISSI-Heart Failure trial, Eur. J. Heart Fail. 12 (2010) 549–556, https://doi.org/10.1093/eurjhf/hfq055.
[27] A. Corsello, L. Di Filippo, S. Massironi, F. Sileo, A. Dolcetta Capuzzo, M. Gemma, C. Carlucci, C. Cusini, B. Colombo, A. Dallatomasina, G.M. Franchi, A. Corti, M. F. Manzoni, S. Gasman, Vasostatin-1: A novel circulating biomarker for ileal and pancreatic neuroendocrine neoplasms, PLoS ONE 13 (5) (2018) e0196858, https://doi.org/10.1371/journal.pone.0196858.
[28] C. Lugnier, A. Meyer, A. Charloux, E. Andrès, B. Gény, S. Talha, The Endocrine Function of the Heart: Physiology and Involvements of Natriuretic Peptides and Cyclic Nucleotide Phosphodiesterases in Heart Failure, J. Clin. Med. 8 (10) (2019) 1746, https://doi.org/10.3390/jcm8101746.
[29] B. Tota, T. Angelone, R. Mazza, M.C. Cerra, The chromogranin A-derived vasostatins: new players in the endocrine heart, Curr. Med. Chem. 15 (14) (2008) 1444–1451, https://doi.org/10.2174/092986708784567662.
[30] J.N. Cohn, T.B. Levine, M.T. Olivari, V. Garberg, D. Lura, G.S. Francis, A.B. Simon, T. Rector, Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure, N. Engl. J. Med. 311 (13) (1984) 819–823, https://doi.org/10.1056/NEJM198409273111303.
[31] P.A. Pellikka, L. She, T.A. Holly, G. Lin, P. Varadarajan, R.G. Pai, R.O. Bonow, G. M. Pohost, J.A. Panza, D.S. Berman, D.L. Prior, F.M. Asch, S. Borges-Neto, P. Grayburn, H.R. Al-Khalidi, K. Miszalski-Jamka, P. Desvigne-Nickens, K.L. Lee, E. J. Velazquez, J.K. Oh, Variability in ejection fraction measured by echocardiography, gated single-photon emission computed tomography, and cardiac magnetic resonance in patients with coronary artery disease and left ventricular dysfunction, JAMA Netw. Open 1 (4) (2018) e181456, https://doi.org/10.1001/jamanetworkopen.2018.1456.
[32] T. Ahmad, M.J. Pencina, P.J. Schulte, E. O'Brien, D.J. Whellan, I.L. Piña, D. W. Kitzman, K.L. Lee, C.M. O'Connor, G.M. Felker, Clinical implications of chronic heart failure phenotypes defined by cluster analysis, J. Am. Coll. Cardiol. 64 (2014) 1765–1774, https://doi.org/10.1016/j.jacc.2014.07.979.
[33] M.A. Konstam, F.M. Abboud, Ejection fraction: misunderstood and overrated (changing the paradigm in categorizing heart failure), Circulation 135 (8) (2017) 717–719, https://doi.org/10.1161/CIRCULATIONAHA.116.025795.
[34] E. Oikonomou, G. Vogiatzi, S. Tsalamandris, K. Mourouzis, G. Siasos, G. Lazaros, G. Skotsimara, G. Marinos, M. Vavuranakis, D. Tousoulis, Non-natriuretic peptide biomarkers in heart failure with preserved and reduced ejection fraction, Biomark Med. 12 (7) (2018) 783–797, https://doi.org/10.2217/bmm-2017-0376.
[35] M.M. Redfield, R.J. Rodeheffer, S.J. Jacobsen, D.W. Mahoney, K.R. Bailey, J. C. Burnett, Plasma brain natriuretic peptide concentration: impact of age and gender, J. Am. Coll. Cardiol. 40 (5) (2002) 976–982.
[36] D.K. Gupta, J.A. de Lemos, C.R. Ayers, J.D. Berry, T.J. Wang, Racial differences in natriuretic peptide levels: the Dallas Heart Study, JACC Heart Fail. 3 (2015) 513–519, https://doi.org/10.1016/j.jchf.2015.02.008.
[37] M.A. Roberts, D.L. Hare, K. Sikaris, F.L. Ierino, Temporal trajectory of B-type natriuretic peptide in patients with CKD stages 3 and 4, Dialysis, and kidney transplant, Clin. J. Am. Soc. Nephrol. 9 (6) (2014) 1024–1032, https://doi.org/10.2215/CJN.08640813.
[38] M. Maeder, T. Fehr, H. Rickli, P. Ammann, Sepsis-associated myocardial dysfunction: diagnostic and prognostic impact of cardiac troponins and natriuretic peptides, Chest 129 (5) (2006) 1349–1366, https://doi.org/10.1378/chest.129.5.1349.
[39] S. van Doorn, G.-J. Geersing, R.F. Kievit, Y. van Mourik, L.C. Bertens, E.E.S. van Riet, L.J. Boonman-de Winter, K.G.M. Moons, A.W. Hoes, F.H. Rutten, Opportunistic screening for heart failure with natriuretic peptides in patients with atrial fibrillation: a meta-analysis of individual participant data of four screening studies, Heart 104 (15) (2018) 1236.1–1237, https://doi.org/10.1136/heartjnl-2017-31278110.1136/heartjnl-2017-312781.supp1.
[40] M. Kohno, K. Murakawa, K. Yasunari, Y. Nishizawa, H. Morii, T. Takeda, Circulating atrial natriuretic peptides in hyperthyroidism and hypothyroidism, Am. J. Med. 83 (1987) 648–652, https://doi.org/10.1016/0002-9343(87)90893-x.
[41] J.V. St Peter, G.G. Hartley, M.M. Murakami, F.S. Apple, B-type natriuretic peptide (BNP) and N-terminal pro-BNP in obese patients without heart failure: relationship to body mass index and gastric bypass surgery, Clin. Chem. 52 (2006) 680–685, https://doi.org/10.1373/clinchem.2005.062562.
[42] W.L. Miller, J.C. Burnett, K.A. Hartman, M.P. Henle, M.F. Burritt, A.S. Jaffe, Lower rather than higher levels of B-type natriuretic peptides (NT-pro-BNP and BNP) predict short-term mortality in end-stage heart failure patients treated with nesiritide, Am. J. Cardiol. 96 (6) (2005) 837–841, https://doi.org/10.1016/j.amjcard.2005.05.032.
[43] A.n. Tran-Duy, B. Spaetgens, A.W. Hoes, N.J. de Wit, C.D.A. Stehouwer, Use of Proton Pump Inhibitors and Risks of Fundic Gland Polyps and Gastric Cancer: Systematic Review and Meta-analysis, Clin. Gastroenterol. Hepatol. 14 (12) (2016) 1706–1719.e5, https://doi.org/10.1016/j.cgh.2016.05.018.
[44] H.L. Waldum, J.S. Arnestad, E. Brenna, I. Eide, U. Syversen, A.K. Sandvik, Marked increase in gastric acid secretory capacity after omeprazole treatment, Gut 39 (5) (1996) 649–653, https://doi.org/10.1136/gut.39.5.649.
[45] S. Sanduleanu, M. Stridsberg, D. Jonkers, W. Hameeteman, I. Biemond, G. Lundqvist, C. Lamers, R.W. Stockbrügger, Serum gastrin and chromogranin A during medium- and long-term acid suppressive therapy: a case-control study, Aliment. Pharmacol. Ther. 13 (1999) 145–153, https://doi.org/10.1046/j.1365-2036.1999.00466.x.
[46] M. Giusti, M. Sidoti, C. Augeri, C. Rabitti, F. Minuto, Effect of short-term treatment with low dosages of the proton-pump inhibitor omeprazole on serum chromogranin A levels in man, Eur. J. Endocrinol. 150 (2004) 299–303, https://doi.org/10.1530/eje.0.1500299.
[47] J.A. Borovac, D. Glavas, Z. Susilovic Grabovac, D. Supe Domic, D. D'Amario, J. Bozic, Catestatin in Acutely Decompensated Heart Failure Patients: Insights from the CATSTAT-HF Study, J. Clin. Med. 8 (8) (2019) 1132, https://doi.org/10.3390/jcm8081132.