Chromogranin A in heart failure

A novel neurohumoral factor and a predictor for mortality

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Background In chronic heart failure, several hormonal systems are activated with diagnostic and prognostic implications. We tested the hypotheses that serum Chromogranin-A (CgA) — a 49 kDa acid protein present in the secretory granules of neuroendocrine cells — is increased in chronic heart failure and that CgA levels are a predictive factor for mortality.

Methods and Results In 160 patients with chronic heart failure, we measured serum CgA and other neuroendocrine hormones. The results showed that CgA is increased in chronic heart failure and the increase is related to the clinical severity of the syndrome: CgA levels in New York Heart Failure (NYHA) class II (median 146·9 ng . ml−1, inter-quartiles 108·3–265·5) were significantly higher (P<0·05) than in class I (median 109·7 ng . ml−1, inter-quartiles 96·7–137·6), and significantly lower (P<0·05) than in class III (median 279·0 ng . ml−1, inter-quartiles 203·6–516·1). Class IV patients showed the highest serum levels of CgA (median 545·0 ng . ml−1, inter-quartiles 231·8–1068·3), being statistically significantly different from class III patients (P<0·001). The association between survival and some recognized variables of prognostic significance, including CgA was also studied. The results showed that ejection fraction, noradrenaline, atrial natriuretic peptide, NYHA class and CgA were significant univariate prognosticators; however, in the multivariate analysis by the Cox proportional-hazard model, CgA and NYHA class were the only independent predictive factors for mortality (P<0·005, RR=1·22, 95% CI=1·06–1·41 and P=0·04, RR=1·58, 95% CI=1·02–2·46, respectively).

Conclusions CgA is a pro-hormone, precursor of several active fragments likely to exert biological effects in chronic heart failure. CgA serum levels are increased in patients with chronic heart failure and are a predictive factor for mortality.


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serum concentration of CgA increases after cardiac arrest, strenuous exercise and hypoglycaemia[15–17]. CgA increase has been proposed as a diagnostic marker of several neuroendocrine tumours, such as pheochromocytoma, parathyroid adenoma, carcinoid, pancreatic islet-cell and aortic body tumours[18–20]. Although there are no experimental data on CgA in chronic heart failure, based on the observation that CgA is widely distributed in the neuroendocrine system and is co-secreted with hormones such as catecholamines and natriuretic peptides[12–14], we tested the hypotheses that, in patients with chronic heart failure, serum CgA is increased and its levels are a predictive factor for mortality.

Methods

One-hundred and sixty consecutive elective patients with chronic heart failure, who were managed in a common programme for assessment and treatment at the Fondazione Salvatore Maugeri, Medical Centres of Gussago and Montescano, Italy, were evaluated between 1993 and 1997. Ethical approval was requested and obtained by the participating Institutions. Patients hospitalized for a possible cardiac transplant were also enrolled. Table 1 reports the clinical characteristics of the patients by New York Heart Association (NYHA) class. Mean age of the chronic heart failure population was 56 ± 10 years, males being predominant (83·1%). The most frequent aetiology was coronary artery disease (61·9%), followed by idiopathic dilated cardiomyopathy (25·6%) and valvular disease (6·3%). Up to 94% of the patients were taking diuretics, 80·6% ACE-inhibitors, 72% digitalis and 63·2% nitrates. Only 13·2% were on beta-blockers, while no patients were treated with intravenous inotropic agents or with mechanical–support assistance. Left ventricular volumes and ejection fraction were calculated by conventional two-dimensional echocardiography using a standard area–length algorithm for end-diastolic and end-systolic volume determination[21]. The studied population resembles the population of chronic heart failure patients followed by Hospital Cardiological Units in Italy[22], in terms of clinical characteristics and therapeutic regimens (including the use of ACE-inhibitors and beta-blockers). Serum CgA was also measured in 103 healthy subjects, age- and sex-matched with patients constituting the normal control group: none of them was admitted to hospital, nor had acute or chronic illness, or reported any symptoms related to the cardiovascular system.

Neurohormonal measurements

The techniques used for the assays are described in previous papers[9,23–25]. In summary, venous blood was sampled after 30 min supine rest, centrifuged within 1 h, and stored at −80°C until the assay. Plasma noradrenaline and adrenaline levels were measured by high-performance liquid chromatography with electrochemical detection. Levels of plasma renin activity, aldosterone and atrial natriuretic peptide were measured by radioimmunoassay.
Table 2 Neuroendocrine characteristics of patients with chronic heart failure by NYHA class

<table>
<thead>
<tr>
<th>NYHA Class</th>
<th>n</th>
<th>Noradrenaline pg.mL⁻¹</th>
<th>Adrenaline pg.mL⁻¹</th>
<th>Aldosterone pg.mL⁻¹</th>
<th>PRA ng.mL⁻¹.h⁻¹</th>
<th>ANP pg.mL⁻¹</th>
<th>CgA ng.mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYHA I</td>
<td>9</td>
<td>351 (205–720)</td>
<td>65 (45–110)</td>
<td>4.2 (1.7–6.3)</td>
<td>161 (117–379)</td>
<td>160 (113–192)</td>
<td>110 (97–138)</td>
</tr>
<tr>
<td>NYHA II</td>
<td>63</td>
<td>367 (260–491)</td>
<td>44 (24–75)</td>
<td>118 (2.1–19.3)</td>
<td>138 (79–258)</td>
<td>162 (116–251)</td>
<td>147 (108–266)</td>
</tr>
<tr>
<td>NYHA III</td>
<td>57</td>
<td>471 (307–671)</td>
<td>49 (28–92)</td>
<td>140 (4.9–34.9)</td>
<td>180 (107–369)</td>
<td>244 (177–355)</td>
<td>279 (204–516)</td>
</tr>
<tr>
<td>NYHA IV</td>
<td>31</td>
<td>489 (315–815)</td>
<td>50 (26–86)</td>
<td>29.3 (19.0–41.2)</td>
<td>238 (111–566)</td>
<td>326 (236–500)</td>
<td>545 (232–1068)</td>
</tr>
</tbody>
</table>

NYHA = New York Heart Association; PRA = plasma renin activity; ANP = atrial natriuretic peptide; CgA = Chromogranin-A. Values are expressed as median (interquartiles).

CgA measurement

CgA has a measurable plasma half-life of 18.4 min\(^{[18,26]}\), fitting a two-compartment model, with a rapid half-life of 16 min followed by a longer half-life of 520 min. The model shows a compartment ratio of 23.8:1 (extra-/intra-vascular), thus suggesting circulating CgA binds to tissues\(^{[27]}\). CgA is highly stable in both plasma and serum even after repeated freeze/thaw cycles and overnight at 4°C. The correlation among CgA, different clinical variables and therapy was also measured. Given the marked correlation between systolic and diastolic volumes (r=0.98), only left ventricular end-systolic volume was used.

The ‘Cox proportional-hazard model’ was used to test the association between the studied variables and survival. Transplantation was considered a time-dependent variable. In order to evaluate the differences between transplanted and non-transplanted patients, a multivariate linear model was used: patients undergoing transplantation differed from the non-transplanted patients only for left ventricular end-systolic volume and CgA (P<0.05). In our analysis, patients who received emergency transplantation (n=4), defined according to the Criteria of the United Network for Organ Sharing (UNOS) as Status I\(^{[30]}\), were considered as survivors; however, the outcome of the different predictors was similar by treating the patients who received emergency transplantation as deaths. A univariate analysis based on the Cox model was performed on the single variables and the Akaike Information Criterion\(^{[31]}\) and was used to select variables for the Cox proportional-hazard model.

Results

CgA levels and clinical severity

Table 2 reports data on the neuroendocrine characteristics of patients with chronic heart failure. Due to the non-normal distribution of values, these are expressed as medians (inter-quartiles). Although the limited sample size does not allow a precise stratification of the neuroendocrine response by NYHA class, patients in NYHA classes III and IV exhibit higher neuroendocrine activation, especially for plasma renin activity and atrial natriuretic peptide.
Figure 1 shows the serum CgA concentrations by NYHA class and ejection fraction. CgA levels were markedly elevated in patients with chronic heart failure, if compared to healthy subjects. Serum CgA levels in class II are significantly higher than in class I and significantly lower than in class III. Class IV patients show the highest serum levels of CgA.

CgA levels and mortality

Of the 160 patients with chronic heart failure who were followed-up (mean 629 days, SD 312; median 727), there were 54 deaths (33.8%) and 106 survivors (time range for deaths was 3 to 964 days; mean 259 days, SD 231; median 210). The mean follow-up period of the survivors was 818 days (SD 119; range 656–1055; median 798).

The cumulative survival of all patients was 84%, 77%, 70% and 68% at 6, 12, 18 and 24 months, respectively. Twenty-five patients underwent transplantation during the follow-up period; among the transplanted patients, four underwent emergency transplantation, one of whom died 3 days after transplantation. Of the 54 patients who died, 14 were in NYHA class II, 19 in class III and 21 in class IV. All deaths could be attributed to cardiovascular causes, except for three patients who died following digestive and cerebral haemorrhage, and lung cancer, respectively.

Table 3 and Fig. 2 show the results for the predictors in the univariate and multivariate analyses according to the Cox model, respectively.

The univariate analysis has identified ejection fraction, noradrenaline, atrial natriuretic peptide, NYHA class, and CgA as significant predictors for mortality.

Table 3 Univariate analysis: predictors for mortality in 160 patients with chronic heart failure (Cox proportional-hazard model)

<table>
<thead>
<tr>
<th>Variable</th>
<th>P (likelihood ratio test)</th>
<th>Risk ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF</td>
<td>0.012</td>
<td>0.61 (0.37–0.89)</td>
</tr>
<tr>
<td>NYHA</td>
<td>&lt;0.0001</td>
<td>2.40 (1.68–3.42)</td>
</tr>
<tr>
<td>LVE SV</td>
<td>0.27</td>
<td>1.13 (0.92–1.38)</td>
</tr>
<tr>
<td>Transplant</td>
<td>0.09</td>
<td>0.95 (0.37–2.48)</td>
</tr>
<tr>
<td>PRA</td>
<td>0.11</td>
<td>1.73 (0.96–3.12)</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>0.16</td>
<td>1.02 (0.98–1.07)</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>0.013</td>
<td>1.04 (1.01–1.09)</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>0.82</td>
<td>1.02 (0.84–1.24)</td>
</tr>
<tr>
<td>ANP</td>
<td>0.0007</td>
<td>1.13 (1.06–1.21)</td>
</tr>
<tr>
<td>CgA</td>
<td>&lt;0.0001</td>
<td>1.07 (1.05–1.09)</td>
</tr>
</tbody>
</table>

EF=ejection fraction; NYHA=New York Heart Association; LVE SV=left ventricular end systolic volume; PRA=plasma renin activity; ANP=atrial natriuretic peptide; CgA=Chromogranin-A; CI=confidence intervals.
mortality ($P=0.012$; $P=0.013$; $P<0.0001$; $P<0.0001$, respectively).

The multivariate analysis showed that CgA was an independent predictive factor for mortality ($P=0.005$) with a Risk Ratio of 1.22 (95% CI=1.06–1.41) calculated using the interquartile range as significant change. NYHA class was also a significant predictor ($P=0.04$, RR=1.58, 95% CI=1.02–2.46 for a class change), but the inclusion in the survival analysis of a subjective evaluation of clinical severity, such as NYHA class, does not affect the value of CgA levels as a predictor factor for mortality.

Kaplan–Meier survival plots were used to show the impact of serum CgA levels and NYHA class on survival (Fig. 3): the analysis combines NYHA classes I and II since a low rate of events occurs in patients with mild chronic heart failure. The survival probability of patients decreases with the increase of serum CgA levels. In patients with chronic heart failure with serum CgA levels below the median, the survival probability at 2 years was 90%, 79% and 75% for patients in NYHA class I and II, NYHA class III and NYHA class IV, respectively. The survival at 2 years of patients with CgA values above the median was 66%, 58% and 22% for patients in NYHA class I and II, NYHA class III and NYHA class IV, respectively.

**Discussion**

The results of our study represent the first finding of an increase of serum CgA levels in patients with chronic heart failure, also being a predictive factor for mortality. We have observed that CgA levels are related to the clinical severity of the syndrome: NYHA class II patients have significantly higher levels of CgA than class I patients or healthy subjects, suggesting that the increase in CgA occurs in the early chronic heart failure stages. Moreover, we have found in NYHA class IV patients, CgA levels as high as those of patients with pheochromocytoma and other neuroendocrine tumours.$^{18–20}$
CgA is the major soluble protein present in the secretory vesicles throughout the neuroendocrine system: it is released exocytotically with the many different co-stored hormones. Therefore, its increase in circulating blood could be linked to neuroendocrine activation. A similar paradigm has been applied for instance to neuropeptide Y, a 36-amino acid peptide co-stored with noradrenaline: its increase is considered an activation of the sympathetic outflow\[^7\]. Unlike neuropeptide Y, CgA has multiple locations and is co-stored not only with noradrenaline, but also with other hormones activated in chronic heart failure, such as natriuretic peptides. Furthermore, CgA is preferentially secreted into the lymph and has a measurable plasma half-life\[^18,26\]; therefore, it is a suitable index with which to detect a ‘tonic’ neuroendocrine activation rather than single ‘phasic’ responses to stress.

CgA has been considered for years as a protein involved in hormone storage and granulogenesis\[^12,13,17,20\]. However, recent findings have provided evidence that suggests that CgA can also function as a pro-hormone precursor. In fact, the biological fate of CgA is to undergo rapid processing, by means of pro-hormone convertases and other proteases\[^32,33\] in a tissue-specific manner\[^34,35\]. Among the CgA derived peptides functionally characterized, residues 248–293 correspond to pancreastatin, a pancreatic peptide able to inhibit insulin secretion\[^36\] and the fragment 347–419, known as parastatin, inhibits parathormone secretion in the parathyroid gland\[^37\].

Furthermore, the specific biological effect of some newly characterized systems controlled by the CgA network, namely catestatin and vasostatins, could become of specific importance in the pathophysiology of chronic heart failure. Catestatin corresponds to the fragment 344–364 within CgA and is a potent non-competitive inhibitor of nicotinic cholinergic receptor mediated catecholamine release\[^38\]. Thus, the secretion of CgA, followed by its appropriate proteolytic processing, results in a autocrine/paracrine negative feedback control on local catecholamine release. For this effect, catestatin IC\(_{50}\) is \(~0.2–0.4 \mu\text{M}\)^[^38], thus well within the range of CgA that we have found in our chronic heart failure patients. It has recently been proposed that the major fibrinolytic enzyme, i.e. plasmin, binds specifically and with high affinity CgA generating peptides exerting catestatin activity\[^39\]. Since plasminogen and tissue plasminogen activator — that is synthesized and secreted also by catecholaminergic cells — bind to cell surface leading to local plasmin activation, this represents a mechanism of local CgA processing. In view of the involvement in the inflammatory responses in chronic heart failure, the interrelations between CgA and the fibrinolytic system may have important implications in the control of local sympathetic activity\[^39\].

Vasostatins comprise a family of different molecules generated from the aminoterminal portion of CgA. The most characterized ones are the fragments comprising CgA peptides 1–76 and CgA 1–113, known as vasostatin I and II\[^40–43\], which bind to specific receptors on the smooth muscle cells of the resistance vessels, causing dilatation.

Vasostatin antagonizes endothelin- and noradrenaline-induced increase of vascular tone, both in resistance and conduit vessels by an endothelium-independent mechanism\[^41–43\]. Recently, we have also shown that vasostatins can modulate the adhesion of fibroblasts and smooth muscle cells to extracellular matrix proteins suggesting a a role in the interstitial remodelling processes\[^44,45\].

In view of the above considerations, it can be hypothesized that CgA plays a role in the pathophysiology of chronic heart failure by exerting favourable actions including antiadrenergic, vasodilating, possibly anti-remodelling effects and counteracting the widespread neuroendocrine activation of this syndrome. These responses, mediated by autocrine/paracrine mechanisms induced by local production of CgA, may represent an extreme attempt of the organism to counteract the detrimental effects of excessive neuroendocrine activation. Therefore, accumulation of circulating CgA immunoreactivity also implies that the neuroendocrine system is strenuously stimulated as in the advanced stages of the disease or in the cases with the worst prognosis.

In accordance with the hypothesis, the results of our study indicate that CgA measurement is an independent prognosticator for mortality in patients with chronic heart failure. Interestingly, the predictive power of CgA was maintained irrespective of the clinical status (Fig. 3) even in these selected ‘high risk’ patients. The survival probability of patients with CgA above the median was about threefold lower than that of patients with CgA levels below the median, irrespective of the NYHA class.

CgA, due to its easy measurement in serum, can be considered an additional marker of prognosis that was the most sensitive prognosticator in our model, when compared to other recognized prognostic factors, such as ejection fraction, noradrenaline and atrial natriuretic peptide.

**Limitations of the study**

As stated in the Methods section, the results of our study are based on a population of patients with chronic heart failure hospitalized for assessment and therapy. This population well represents the patients enrolled in similar studies or hospitalized in cardiological units\[^22\]. However, the population of our study differs from a general population of patients with asymptomatic left ventricular dysfunction by characteristics such as age or disease severity. In particular, there are few poorly symptomatic patients in our population. Therefore, CgA levels as a prognostic factor in a population of asymptomatic patients cannot be addressed in our study.

A specific study conducted in a general population with asymptomatic left ventricular dysfunction is, indeed, needed to address this issue.
Moreover, the series of neurohormones evaluated in this study is limited to those available or included in the panel of assays performed in our centres at the beginning of our investigation; thereafter, new knowledge in this field was reached, in particular on natriuretic peptides and on endothelins[5,46–49] which unfortunately could not be retrospectively assayed in an adequate subset of patients. Therefore, further studies are necessary to validate the prognostic value of CgA in comparison with brain natriuretic peptide or endothelins.

**Conclusions and implications**

Our results demonstrate the importance of CgA level as an indicator linked to neuroendocrine activation and an independent predictor of mortality in patients with chronic heart failure, in respect to other recognized predictive factors, such as ejection fraction, noradrenaline, and atrial natriuretic peptide. The hypothesis that CgA levels could be also important for the assignment of specific treatment regimens and for the follow-up of the therapeutic effect in patients with chronic heart failure is stimulating and needs to be addressed in further studies.

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**References**


