Circulating levels of non-phosphorylated undercarboxylated matrix Gla protein are associated with disease severity in patients with chronic heart failure

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ABSTRACT

We recently demonstrated that circulating MGP [matrix Gla (γ-carboxylated glutamate) protein] levels were associated with left ventricular dysfunction and increased mortality in patients with symptomatic aortic stenosis. We hypothesized that patients with chronic HF (heart failure) would have dysregulated MGP levels. We examined plasma dp-cMGP (non-phosphorylated carboxylated MGP) and dp-ucMGP (non-phosphorylated undercarboxylated MGP) in 179 patients with chronic HF and matched healthy controls as well as the relationship between MGP and cardiac dysfunction as assessed by echocardiographic measurements, inflammation [CRP (C-reactive protein)] and neurohormonal activation [NT-proBNP (N-terminal proB-type natriuretic peptide)] and the prognostic value of MGP levels in relation to mortality in these patients. We found markedly enhanced plasma dp-cMGP and, in particular, of dp-ucMGP in chronic HF with increasing levels with disease severity. Elevated MGP species were associated with ischaemic aetiology, increased CRP and NT-proBNP levels, as well as systolic and diastolic dysfunction. Finally, dp-ucMGP was associated with long-term heart transplant-free survival (n = 48) in univariate, but not in multivariate, analysis. However, plasma dp-ucMGP was markedly higher in patients who died because of progression of HF (n = 12) and gave prognostic information also in multivariate analysis. In conclusion, a dysregulated MGP system could be involved in left ventricular dysfunction in patients with chronic HF.

Key words: all-cause mortality, calcification, chronic heart failure, left ventricular function, matrix γ-carboxylated glutamate protein (MGP).

Abbreviations: AngII, angiotensin II; ACE, angiotensin-converting enzyme; ASA, aspirin; CAD, coronary artery disease; CI, confidence interval; CRP, C-reactive protein; DCM, dilated cardiomyopathy; E/A, early transmitral diastolic filling velocity/late transmitral diastolic filling velocity; ECM, extracellular matrix; eGFR, estimated glomerular filtration rate; HF, heart failure; HR, hazard ratio; IDCM, idiopathic DCM; LV, left ventricular; LVEF, LV ejection fraction; MGP, matrix Gla (γ-carboxylated glutamate) protein; cMGP, carboxylated MGP; dp-cMGP, non-phosphorylated cMGP; MI, myocardial infarction; NYHA, New York Heart Association; NT-proBNP, N-terminal proB-type natriuretic peptide; ucMGP, undercarboxylated MGP; dp-ucMGP, non-phosphorylated ucMGP; VIF, variance inflation factor.

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INTRODUCTION

Chronic HF (heart failure) is an important cause of cardiovascular morbidity and mortality. Five-year mortality is still above 50%, suggesting that important pathogenic mechanisms remain unchallenged by current treatment modalities. The development of HF is characterized by several cellular and molecular processes, including cardiomyocyte hypertrophy, increased ventricular volume due to dilatation of the ventricular cavity and changes in the ECM (extracellular matrix) including fibrosis [1,2]. Both these changes are collectively referred to as ventricular remodelling and accommodate the increased myocardial wall stress. However, over time, myocardial remodelling turns maladaptive, leading to a progressive change in myocardial shape and decrease in myocardial function. In this process, abnormal regulation of the ECM seems to be of major importance [1,2].

MGP [matrix Gla (γ-carboxylated glutamate) protein] is a 10-kDa circulating calcification inhibitor containing five Gla residues and three serine residues, which may be phosphorylated [3]. Both the Gla residues and phosphorylated serine residues have a high affinity for calcium and are formed in a post-translational reaction. MGP has been shown to inhibit vascular calcification at least partly by its ability to bind nascent crystals and direct binding and inhibition of BMP (bone morphogenic protein) [4]. MGP has also, at least indirectly, been implicated in ECM remodelling as a result of its function as a mineral-binding protein as well as its ability to bind to vitronectin [5]. MGP function depends on vitamin-K-dependent γ-carboxylation of glutamate residues, a process inhibited by warfarin [4,6,7]. Thus, although the relationship between warfarin and vascular calcification in humans has not been elucidated, observational studies suggest that inhibition of MGP carboxylation by warfarin results in extensive calcification of arteries in vitro and in vivo due to synthesis of the inactive ucMGP (undercarboxylated MGP) [8].

Several DNA microarray studies have demonstrated increased MGP mRNA expression in the left ventricle during acute and chronic pressure overload in mice and humans [9–11] as well as in experimental HF in rats and mice [12,13]. Recently, Mustonen et al. [14] demonstrated that MGP is rapidly up-regulated in response to cardiac overload, well before the development of LV (left ventricular) hypertrophy and remodelling following MI (myocardial infarction) in rats. However, clinical data on human HF is lacking, and most data in experimental HF are based on mRNA analyses, and even protein analysis of MGP is challenging.

On the basis of its role in ECM calcification and remodelling, we hypothesized a role for MGP in the progression of chronic HF. To explore this hypothesis, we measured plasma levels of the various forms of MGP in patients with chronic HF and healthy controls and related MGP levels to the degree of HF as assessed by clinical, haemodynamic and neurohormonal measurements.

MATERIALS AND METHODS

Study subjects

A total of 179 patients with stable HF for >6 months in NYHA (New York Heart Association) functional class II–IV were consecutively included in the study (Table 1). Patients with acute coronary syndromes during the last 6 months and patients with significant concomitant disease, such as infection, malignancy or autoimmune disease, were excluded. The underlying cause of HF was classified as CAD (coronary artery disease, n = 74), DCM (dilated cardiomyopathy, n = 96) or others (n = 9) on the basis of patient history, cardiac morphology and coronary angiography. Control subjects were 33 sex- and age-matched healthy individuals (mean age 59.8 ± 2.9 years). The study was approved by the regional committee for ethics in medicine and conducted according to the Declaration of Helsinki. Informed consent was obtained from all individuals.

Classification of mortality

Deaths in the study were classified as: 1, death due to progressive HF defined as any of cardiogenic shock, pulmonary oedema, HF symptoms or signs requiring intravenous therapy or oxygen, confinement to bed because of HF symptoms or sudden death during hospital stay for aggravated HF; 2, sudden deaths defined as any of witnessed instantaneous death in the absence of progressive circulatory failure lasting for 60 min or more, unwitnessed death in the absence of pre-existence progressive circulatory failure or other causes of death, or death within 28 days after resuscitation from cardiac arrest in the absence of pre-existing circulatory failure or other causes of death, death during attempted resuscitation or death within 60 min from the onset of new symptoms unless a cause other than cardiac was obvious; and 3, other deaths. All deaths were classified by an independent experienced clinician according to prespecified definitions from medical records and other documents and, if in doubt, confirmed by a telephone call to the relatives of the patients.

Biochemistry and blood sampling

For NT-proBNP (N-terminal pro-B-type natriuretic peptide), CRP (C-reactive protein) and MGP measurements, peripheral venous blood was drawn into pyrogen-free tubes with EDTA as anticoagulant. The tubes were immediately immersed in melting ice and centrifuged within 30 min at 2000 g for 20 min to obtain platelet-poor plasma. All samples were stored at −80°C and thawed less than three times. NT-proBNP and CRP were assayed on a MODULAR platform (Roche Diagnostics).
Levels of circulating dp-ucMGP (non-phosphorylated ucMGP) and dp-cMGP (non-phosphorylated cMGP (carboxylated MGP)) were determined in plasma using sandwich ELISA techniques as described previously [15]. Plasma levels of LDL (low-density lipoprotein)-cholesterol, HDL (high-density lipoprotein)-cholesterol and creatinine were measured enzymatically on a Roche/Hitachi 917 analyser (Roche Diagnostics). eGFR (estimated glomerular filtration rate) was calculated based on the Cockcroft–Gault formula.

### Echocardiography

Echocardiographic imaging of the heart was performed from parasternal and apical views by use of a GE Vivid 7 ultrasonic digital scanner (GE Vingmed). We obtained conventional two-dimensional images, M-mode and colour Doppler as well as pulsed-wave Doppler recordings of blood flow velocities in the cardiac ostias [16]. Doppler echocardiographic calculations of stroke volume and cardiac output were performed on the basis of the aortic annular cross-sectional area and aortic annular flow velocity data. LVEF (LV ejection fraction) was obtained by the modified biplane Simpson method [16]. Peak systolic right ventricular pressure was estimated from the TIP_{max} (maximal tricuspid regurgitative blood flow velocity). Dimensional and velocity parameters were averaged from at least three (five in atrial fibrillation) cardiac cycles. Diastolic dysfunction was defined as: 0, normal; 1, abnormal relaxation pattern; 2, pseudonormal filling dynamics; 3, restrictive filling dynamics. We also used a modified version of the scheme first introduced by Appleton et al. [17] to grade diastolic function (0–3) in patients with sinus rhythm based on E/A (early transmitral diastolic filling velocity/late transmitral diastolic filling velocity) ratios. We used the grading system recommended by the American Society of Echocardiography [18], but owing to the large age span in our population, we used age-specific cut-off values for E/A ratios based on the Norwegian material for normal echocardiographic values reported from the Tromso study [19].

### Statistical analysis

We chose to use parametric statistics on all measures since they ultimately would be included in a multiple regression model. Variables not normally distributed as evaluated by the Kolmogorov–Smirnov test were log-transformed prior to statistical analyses, but are also presented as non-transformed data. Both in the linear regression and survival analysis (see under), continuous
variables expressed per S.D. change (log/SD[log]) were used. Differences between groups were analysed by one-way ANOVA a priori for more than two groups, followed by Student’s t test with Bonferroni correction. Relationships between variables were tested by simple linear (bivariate) regression analysis (Pearson correlation) or by bivariate logistic regression on standardized values (categorical variables; Table 1). Stepwise regression was used to identify predictors of myocardial dysfunction. Kaplan–Meier analysis with log-rank test was performed to compare the number of events per S.D. increase. Cox proportional hazard analysis was performed to estimate HRs (hazard ratios) using forward stepwise conditional method. Only variables with a statistically significant association with MGP (Table 1) or all-cause/anticipated (heart transplantation) mortality respectively were included in the model. Multicolinearity in the regression models was evaluated by examining the tolerance and VIF (variance inflation factor) in linear regression. The tolerance was >0.4 and the VIF <2.5 for all independent variables. P values are two-sided and considered significant when <0.05.

RESULTS

MGP in patients with HF in relation to clinical characteristics

As can be seen (Figure 1), patients with HF were characterized by markedly elevated dp-cMGP and, in particular, dp-ucMGP levels in all NYHA classes compared with age- and sex-matched healthy controls (n = 33), resulting in a molar abundance of dp-ucMGP compared with dp-cMGP (dp-ucMGP/dp-cMGP molar ratio) in HF patients. As for dp-ucMGP and the molar ratio, particularly high levels were found in those with the most advanced clinical disease (i.e. NYHA class IV) (Figure 1). Plasma dp-ucMGP and dp-cMGP, but not the molar ratio between these parameters, were higher in patients with ischaemic compared with dilated cardiomyopathy. Importantly, also patients with DCM had raised plasma levels of plasma MGP compared with healthy controls (Figure 1). Additionally, plasma levels of dp-ucMGP and dp-cMGP were positively correlated with age, creatinine and CRP (Table 1). A strong positive association was found between use of vitamin K antagonists and MGP levels (Table 1). In addition, increased dp-ucMGP was observed in patients using diuretics, whereas lower levels were observed in ASA (aspirin) users. For dp-cMGP, lower levels were observed in patients using ACE (angiotensin-converting enzyme) inhibitors (Table 1).

Associations between MGP and myocardial function

NT-proBNP, which is strongly associated with LV filling pressures, was strongly correlated with both dp-ucMGP (r = 0.44, P < 0.001) and dp-cMGP (r = 0.39, P < 0.001). As noted above, MGP levels were influenced by serum creatinine, age and warfarin use, and adjusting for these still revealed significant associations with NT-proBNP (dp-ucMGP: $r = 0.26, P = 0.001$; dp-cMGP: $r = 0.21, P = 0.006$). Moreover, both dp-ucMGP and dp-cMGP were correlated with the degree of systolic dysfunction as assessed by LVEF (dp-ucMGP: $r = −0.32, P < 0.001$ and dp-cMGP: $r = −0.22, P = 0.005$). Results for dp-ucMGP are shown (Figure 2A). In addition, both MGP species were associated with diastolic dysfunction with the highest levels in grade 3 diastolic function (i.e. restrictive filling pattern) (Figure 2B). Importantly, stepwise linear regression identified dp-ucMGP as a significant predictor of LVEF together with NT-proBNP and eGFR in a model that, in addition, included age, aetiology, NYHA class, CRP, creatinine/eGFR, warfarin, ASA use and dp-cMGP (Figure 2C). Including the same variables in a model for diastolic function revealed NT-proBNP as the sole determinant of diastolic dysfunction.
Matrix γ-carboxylated glutamate protein in heart failure

**Figure 2** Plasma levels of dp-ucMGP and dp-cMGP according to degree of myocardial dysfunction as assessed by echocardiographic measurements

(A) Association with systolic dysfunction as assessed by LVEF. (B) Association with diastolic dysfunction was defined as: 0, normal; 1, abnormal relaxation pattern; 2, pseudonormal filling dynamics; and 3, restrictive filling dynamics. (C and D) Stepwise regression showing predictors of (C) LVEF and (D) diastolic dysfunction.

**dp-ucMGP and mortality in HF patients**

During a mean follow-up of 2.9 (±1.3 (S.D.)) years, 48 patients died or underwent heart transplantation (i.e. anticipated mortality). A Kaplan–Meier plot demonstrating the association between +1 S.D. increases in dp-ucMGP and incidence of all-cause/anticipated mortality is shown (Figure 3). Patients with +2 S.D. dp-ucMGP levels had higher mortality, corresponding to dp-ucMGP levels above the 85th percentile, indicating that only particularly high levels were associated with all-cause/anticipated mortality. No associations between dp-cMGP and transplant-free survival could be demonstrated (results not shown).

Clinical and demographic variables associated with all-cause mortality are shown (Table 2). Including these variables (NYHA class, aetiology, previous MI, creatinine, CRP, NT-proBNP, warfarin and ASA use) and dp-ucMGP, with continuous variables entered log-transformed (per S.D. change) when appropriate, identified high NYHA class together with eGFR as predictors of all-cause/anticipated mortality (NYHA HR, 3.30 (95% CI (confidence interval), 2.06–5.28) ($P < 0.001$); and eGFR HR, 0.67 (95% CI, 0.50–0.90) ($P = 0.008$) (Figure 3B). NYHA remained the predictor for death due to HF progression (Figure 3B). Including potential confounders as mentioned above identified dp-ucMGP [HR, 5.62 (95% CI, 2.05–15.46); $P = 0.001$] and ASA [HR, 0.12 (95% CI, 0.02–0.96); $P = 0.046$] as the only significant predictors of mortality secondary to progression of HF by stepwise Cox regression. Only one patient died due to this cause in non-users of warfarin, while in warfarin users, dp-ucMGP remained the predictor for death due to HF progression [HR, 3.28 (95% CI, 1.27–8.42); $P = 0.014$] (Figure 3B).

**DISCUSSION**

There are several reports of altered circulating levels of MGP in cardiovascular disorders and, in particular, in relation to vascular calcification and atherosclerosis [15,20,21]. Recently, we reported increased levels of dp-cMGP and, in particular, dp-ucMGP, in patients
with symptomatic aortic stenosis [15]. In the present study we report, to the best of our knowledge, for the first time, increased levels of non-phosphorylated MGP with particularly high levels of the non-phosphorylated uncarboxylated MGP form in patients with chronic HF. Additionally, dp-ucMGP was strongly correlated with the degree of myocardial failure as assessed by clinical, neurohormonal and haemodynamic measurements. Moreover, high levels of dp-ucMGP were independently associated with mortality due to progression of HF. Our findings may suggest that dp-ucMGP could be a marker of myocardial failure and that a dysregulated MGP system may be involved in the progression of HF.

In the circulation, many different MGP species, including p-cMGP (phosphorylated cMGP), p-ucMGP (phosphorylated ucMGP), dp-cMGP and dp-ucMGP can be detected [22]. It has been suggested that dp-MGP has a lower affinity for calcium salts, and increased circulating levels may partly reflect the degree of calcification within the vessel wall [21]. Circulating levels of the non-phosphorylated forms of MGP could therefore be markers of the activity in the MGP system. Also, it has been suggested that the dual measurements of dp-ucMGP is particularly sensitive to changes in vascular vitamin K status and vascular calcification status [22]. Previous studies in various cardiovascular disorders have reported decreased circulating levels of the phosphorylated forms of MGPs. However, based on newly developed ELISAs, we were able to detect the circulating levels of the non-phosphorylated forms of MGP. We found increased levels of both non-phosphorylated forms of MGP in chronic HF, and as for dp-ucMGP, a strong association with the degree of myocardial failure was observed. In particular, high levels of dp-ucMGP were associated with mortality due to worsening of HF, but not all-cause/anticipated mortality, suggesting that circulating dp-ucMGP may reflect processes that are involved in the progression of HF.

Although the role of MGP in vascular calcification is firmly established, our results from the present study suggest that the MGP system, indirectly and directly, could be involved in more fundamental processes associated with myocardial remodelling and the development of HF. The precise function of MGP in the heart is unknown,

Figure 3  Plasma MGP and mortality in HF patients
(A) Kaplan–Meier plot for the association between + 1 S.D. increases in dp-ucMGP and incidence of all-cause/anticipated mortality. (B) Stepwise Cox regression analysis showing predictors of all-cause/anticipated mortality and mortality due to progression of HF in all patients and divided according to use of warfarin. The x-axis is log-transformed. (C) Plasma MGP levels according to cause of death in HF patients. (D) Kaplan–Meier plot for the association between + 1 S.D. increases in dp-ucMGP and mortality due to progressive HF (n = 13). HTx, heart transplantation.
but, considering the lack of myocardial calcification in the MGP-deficient mice, point towards a role of MGP in the heart not related to inhibition of calcification [23]. Moreover, MGP has been associated with ECM binding and apoptosis, as well as tissue growth and development, and all these processes may be relevant to myocardial remodelling [4,5,24]. Previously, raised MGP levels have been found as a rapid myocardial response to pressure overload, potentially induced by AngII (angiotensin II), suggesting a link between neurohormonal disturbances and MGP [9,12,14]. This AngII-mediated increase in MGP was seen in both cardiomyocytes and fibroblasts. In the present study, we find a strong correlation between dp-ucMGP and Nt-proBNP, further supporting such a notion. Moreover, MGP has been demonstrated to modulate the activity of several growth factors that are involved in tissue remodelling, such as BMP (bone morphogenic protein), TGFβ (transforming growth factor β) and VEGF (vascular endothelial growth factor). Such interactions could potentially also be operating within the failing myocardium, contributing to the remodelling of ECM. Although further studies are needed, it is tempting to hypothesize that the MGP system could contribute to myocardial remodelling and progression of HF, at least partly through mechanisms that are independent of vascular calcification.

The balance between the various MGP forms is strongly influenced by the vitamin K status, and the antagonist warfarin enhances the synthesis of ucMGP (inactive MGP). In fact, it has been suggested that circulating dp-ucMGP levels may reflect the vascular vitamin K status [22]. In our study population, patients who were using warfarin had markedly raised levels of the inactive MGP forms. However, raised levels of dp-cMGP and dp-ucMGP were found even when warfarinized patients were excluded from the calculations. Moreover, the association between dp-ucMGP and the degree of HF and mortality due to worsening of HF respectively were seen also after correcting for warfarin use as a potential confounder. Thus, although the use of warfarin could influence our findings, the strong association between circulating dp-ucMGP and the degree of HF and mortality due to worsening of HF respectively were seen also after correcting for warfarin use as a potential confounder. This strong association between circulating dp-ucMGP and the severity of HF seems not merely to reflect this potential confounder.

In the present study, NYHA class was the strongest predictor of all-cause/anticipated mortality. Although NYHA class has previously been shown to give prognostic information in HF [25,26], Nt-proBNP levels are almost invariably superior to symptom status. The

Table 2 Characteristics of the study population according to long-term survival

To convert NT-proBNP values from pM into pg/ml, multiply by 8.47. ARB, angiotensin receptor blocker; DM2, Type 2 diabetes mellitus.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Survivors (n = 131)</th>
<th>Non-survivors (n = 48)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55 ± 12</td>
<td>58 ± 12</td>
<td>0.15</td>
</tr>
<tr>
<td>Male (%)</td>
<td>103 (79 %)</td>
<td>37 (77 %)</td>
<td>0.84</td>
</tr>
<tr>
<td>NYHA functional classes II/III/IV (n)</td>
<td>50/59/22</td>
<td>2/20/26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cause of HF (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>47 (36 %)</td>
<td>27 (56 %)</td>
<td>0.032</td>
</tr>
<tr>
<td>IDCM</td>
<td>78 (60 %)</td>
<td>18 (38 %)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>6 (5 %)</td>
<td>3 (6 %)</td>
<td></td>
</tr>
<tr>
<td>Family history (n)</td>
<td>32 (24 %)</td>
<td>9 (19 %)</td>
<td>0.42</td>
</tr>
<tr>
<td>Hypertension (n)</td>
<td>18 (14 %)</td>
<td>8 (17 %)</td>
<td>0.64</td>
</tr>
<tr>
<td>DM2 (n)</td>
<td>15 (12 %)</td>
<td>7 (15 %)</td>
<td>0.58</td>
</tr>
<tr>
<td>Previous MI (n)</td>
<td>39 (30 %)</td>
<td>22 (46 %)</td>
<td>0.048</td>
</tr>
<tr>
<td>LV systolic function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>31 ± 14</td>
<td>31 ± 14</td>
<td>0.74</td>
</tr>
<tr>
<td>Biochemistry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (ml/min per 1.73 m²)</td>
<td>81 ± 26</td>
<td>71 ± 31</td>
<td>0.039</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>8.0 ± 14.7</td>
<td>10.6 ± 15.8</td>
<td>0.023</td>
</tr>
<tr>
<td>NT-proBNP (pM)</td>
<td>371 ± 541</td>
<td>577 ± 661</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medication (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>91 (70 %)</td>
<td>33 (69 %)</td>
<td>0.93</td>
</tr>
<tr>
<td>ARBs</td>
<td>26 (20 %)</td>
<td>11 (23 %)</td>
<td>0.65</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>107 (82 %)</td>
<td>37 (77 %)</td>
<td>0.49</td>
</tr>
<tr>
<td>Diuretics</td>
<td>89 (68 %)</td>
<td>38 (79 %)</td>
<td>0.14</td>
</tr>
<tr>
<td>Statins</td>
<td>51 (39 %)</td>
<td>27 (56 %)</td>
<td>0.038</td>
</tr>
<tr>
<td>Warfarin</td>
<td>51 (39 %)</td>
<td>25 (52 %)</td>
<td>0.12</td>
</tr>
<tr>
<td>ASA</td>
<td>59 (45 %)</td>
<td>18 (38 %)</td>
<td>0.37</td>
</tr>
</tbody>
</table>
reason for this discrepancy is unclear, but there are some issues that may provide some explanation. First, our study is a single-centre study, where there may be larger concurrence with regard to what NYHA group patients belong to, whereas this classification may be subject to more deviation in multicentre studies, where biochemical risk markers may be more objective. Secondly, a limitation in our present study was that a large proportion of the HF patients included were hospitalized for evaluation of heart transplantation. Since the age limit for transplantation in Norway is 65–70 years, most of the patients included were <65 years. Since IDCM (idiopathic DCM) is more frequent in younger patients with HF, as many as 54% of our HF patients had IDCM. Thus our results may not necessarily be representative of an older HF population. It is also important to underscore that the present study was not designed to evaluate the predictive value of NYHA classification. Similar findings in a younger-than-normal HF population with more IDCM were reported by Neuhold et al. (27), who found that NYHA was a stronger predictor than BNP in a study comprising 786 HF patients of NYHA functional classes I–IV, conducted at an experienced tertiary care centre.

We have demonstrated an elevation in plasma levels of dp-ucMGP in patients with chronic HF, significantly associated with disease severity, including mortality due to progression of HF. Our findings suggest that this particular MGP species could reflect processes that are involved in the progression of myocardial failure and also indicate that the MGP system is involved in myocardial remodelling and the development of HF.

AUTHOR CONTRIBUTION

Thor Ueland conceived and designed the research, analysed and interpreted the data, performed the statistical analysis and drafted the manuscript. Christen Dahl acquired the data and made critical revisions of the manuscript. Lars Gullestad conceived and designed the research, analysed and interpreted the data, handled funding and supervision, and made critical revisions of the manuscript. Svend Aakhus analysed, acquired and interpreted the data, and made critical revisions of the manuscript. Kaspar Broch analysed, acquired and interpreted the data, and made critical revisions of the manuscript. Rolf Aukrust conceived and designed the research, interpreted the data, drafted and made critical revisions of the manuscript. Leon Schurgers conceived and designed the research, interpreted the data, drafted and made critical revision of the manuscript. All authors agree with its contents and approved submission of the manuscript.

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