Gender and renal function influence plasma levels of copeptin in healthy individuals

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Gender and Renal function influence plasma levels of Copeptin in Healthy Individuals

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ABSTRACT

This study sought to identify confounding factors for the interpretation of copeptin levels in healthy individuals. The natriuretic peptides are recognised as diagnostic and prognostic tools in heart failure (HF). Interpretation of BNP and NTproBNP levels is multifaceted as their secretion is influenced by many variables. A newly identified glycopeptide called copeptin is comparable to the natriuretic peptides in diagnosis and prognosis of HF and as a prognostic biomarker after acute myocardial infarction (AMI). Copeptin, derived from the C-terminal portion of the precursor to arginine vasopressin (AVP), is secreted stoichiometrically with vasopressin, hence can be used as a surrogate marker of the AVP system. 706 healthy volunteers were recruited from a local HF screening study. Participants with a history of cardiovascular disease and those with echocardiographic abnormalities were excluded from the study. Copeptin and NTproBNP levels were assayed using in-house immunoluminometric assays. Median copeptin levels were significantly higher in the male volunteers compared with the females (4.3 [0.4-44.3] vs. 3.2 [1.0-14.8] pmol/L; $P<0.001$). In males, copeptin was correlated with eGFR ($r_s=-0.186$, $P<0.001$). In females, the correlation of copeptin with eGFR was weak ($r_s=-0.097$, $P=0.095$). Deceleration time (DT) and left atrial size correlated with higher copeptin levels ($r_s=0.085$; $P=0.029$, $r_s=0.206$, and $P<0.001$ respectively). Only gender ($P<0.001$), eGFR ($P<0.001$), left atrial size ($P=0.04$) and DT ($P=0.02$) remained independently predictive of plasma copeptin. This study suggests that gender and renal function specific partition values should be used to interpret copeptin values in future studies of this biomarker in HF or ischaemic heart disease.

INTRODUCTION

The morbidity and mortality of heart failure (HF) exerts a huge burden on industrialised societies. The patho-physiological mechanism responsible is the activation of the neuro-hormonal systems [1]. Therapies have been designed to antagonise these hormones and prevent the deleterious progression of the condition. For example, angiotensin converting enzyme inhibitors and β-blockers have transformed the management of HF. Novel peptides like the natriuretic peptide systems have been increasingly recognised as diagnostic and prognostic tools in HF. Although B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NTproBNP) have a high sensitivity and negative predictive value for the detection of HF, specificity remains low and therefore positive predictive value of the test is not high [2]. The diagnostic accuracy of BNP and NTproBNP is influenced by many variables. BNP has been found to increase with age independent of age-related alterations in renal function [3,4]. BNP levels are higher in females inferring a relationship with oestrogen status [5]. High natriuretic peptides levels are independently related with BMI and renal dysfunction [6]. Hence interpretation of BNP and NTproBNP levels is multifaceted.

New peptides are being identified to complement existing tools to improve the diagnostic and prognostic accuracy of clinical disease. A newly identified glycopeptide called copeptin has been shown to be comparable to the natriuretic peptides in diagnosis and prognosis of HF [7]. In a study by Gegenhuber et al. copeptin was found to be comparable with BNP for 1 year all cause mortality in patients with acute destabilized HF. In addition, copeptin has also shown promise as a prognostic biomarker after acute myocardial infarction (AMI), with accuracy equivalent to NTproBNP [8].

Copeptin is a glycoprotein derived from the C-terminal portion of the precursor to arginine vasopressin, which is of unknown physiological function [9]. Arginine vasopressin (AVP) is a hormone synthesised in the para-ventricular nuclei of the hypothalamus and stored in neuro-secretory vesicles in the posterior pituitary gland. It is secreted in response to stimuli to promote water conservation, contributing to volume regulation and cardiovascular homeostasis [10]. Concerns exist regarding the accuracy and reliability of AVP quantification largely in part due to its instability ex vivo, platelet binding and rapid plasma clearance [11,12]. In comparison to AVP, copeptin is stable ex vivo for up to 14 days in EDTA at room temperature [9]. The

stoichiometric generation of copeptin allows it to be used as a surrogate marker for the AVP system.

With the introduction of copeptin as a potential diagnostic and prognostic factor in HF and acute coronary syndromes there is a need to understand variables, which may affect copeptin levels in normal individuals, in order to derive a working normal range for diagnosis or prognosis of heart disease [7,8]. The objectives of this population based study was to identify these confounding factors for the interpretation of plasma copeptin levels, which may lead to improved utility of this marker.

MATERIALS AND METHODS

Study population

Healthy volunteers were derived from a heart failure screening study performed in the local community. From patient records information regarding history of ischaemic heart disease (myocardial infarction (MI) or angina), hypertension, diabetes mellitus, smoking and cardiovascular medication was sought. This study complied with the Declaration of Helsinki and was approved by the local ethics committee. All healthy volunteers gave written informed consent for physical examination, echocardiography and peripheral blood sampling. Participants with a history of ischaemic heart disease, hypertension, diabetes mellitus and those with echocardiographic abnormalities (including segmental wall motion abnormalities, valvular disease, LV hypertrophy [LVH]) and those on cardiovascular medications were excluded from the present study. The estimated glomerular filtration rate (eGFR) of these subjects was derived using the modification of diet in renal disease (MDRD) formula [13].

Blood Sampling

Venesection was performed in recumbent volunteers. Samples for measuring the serum concentrations of the propeptides were collected in pre-chilled tubes containing EDTA and aprotinin. Plasma was stored at –70ºC until assay and all analyses were done in a single batch. Samples for measuring plasma creatinine were collected in tubes containing lithium and heparin.

Echocardiography

Transthoracic echocardiography was performed in patients using a Sonos 5500 instrument (Philips Medical Systems, Reigate, Surrey, UK). A 16-segment left

ventricular wall motion index (LVWMI) based on the American Society of Echocardiography model was derived by scoring each LV segment (1 = normal, 2 = hypokinesis, 3 = akinesis and 4 = dyskinesis (Paradoxical Motion)) and dividing the total by the number of segments scored. Left ventricular ejection fraction (LVEF) was calculated using the biplane method of discs formula [14]. All the normal volunteers in this study had a LVWMI = 1 (i.e. no segmental wall motion abnormalities), and no evidence of valvular disease or LVH. Left ventricular mass was calculated using the Devereux et al. formula [15] and indexed for body surface area to obtain left ventricular mass index. LVH is diagnosed when the LV mass index is greater than 134g/m^2, 110g/m^2 in males and females respectively [16].

The transmitral peak flow during early (E) and the atrial (A) filling phase was determined using pulsed wave Doppler examination at the tips of the mitral valve leaflets. The E/A ratio, left ventricular isovolumetric relaxation time (IVRT) and deceleration time (DT) were calculated from these traces.

NTproBNP assay

Our NTproBNP assay was based on a non-competitive assay [17]. Sheep antibodies were raised to the N-terminal of human NTproBNP and monoclonal mouse antibodies were raised to the C-terminal. The N-terminal IgG was affinity-purified and biotinylated. Samples or NTproBNP standards were incubated in C-terminal IgG-coated wells with the biotinylated antibody for 24 h at 4°C. Detection was with methyl-acridinium ester labelled streptavidin. The lower limit of detection was 0.3 fmol/ml. There was no cross reactivity with atrial natriuretic peptide, BNP, or C-type natriuretic peptide.

Copeptin Assay

The sandwich immunoluminometric assay used to determine copeptin levels has been reported previously [9]. In brief tubes were coated with sheep polyclonal antisera directed against amino acid sequence 132-164 of preprovasopressin as a solid phase antibody. Sheep antibody raised against the amino acid sequence 149-164 of preprovasopressin was used as a tracer. Dilution of peptide representing 132-164 of preprovasopressin in normal horse serum was used as calibrators. The immunoassay was conducted by incubating 50µL of sample/standard and 200µL of tracer in the coated tubes for two hours at room temperature. Test tubes were washed with 1ml of

wash solution and bound chemiluminescence was measured on a LB952T luminometer (Berthold, Germany). The 95% confidence interval (CI) for copeptin was 4.0–4.4 pmol/L [9].

Statistical analysis

Statistical analysis was performed using Statistics Package for Social Sciences version 12.0 (SPSS Inc, Chicago, Illinois). Variables that did not follow a Gaussian distribution were log transformed prior to statistical analysis to satisfy modelling assumptions. Concentrations of copeptin, NTproBNP and plasma creatinine had a non-Gaussian distribution and were log transformed. For continuous variables in two independent groups the Mann Whitney U test was used. Spearman's correlation coefficients were used to investigate the influence of patient characteristics on NTproBNP and copeptin levels in univariate analyses. Scatter diagrams were constructed to illustrate the general trend between the two variables. Boxplots were also constructed consisting of median boxes, which represent the interquartile ranges and the whiskers representing the 2.5^{th} and 97.5^{th} percentiles. To analyse the interaction of multiple independent variables on NTproBNP and copeptin levels, the univariate general linear model was used. A P value below 0.05 was deemed to be statistically significant.

RESULTS

Baseline characteristics of the 706 healthy volunteers stratified according to gender are presented in Table 1. 57.8% of the healthy volunteers were male. The mean age in the male volunteers was significantly lower than in the females (59.7 vs. 64.0 years; P<0.001). The body mass index (BMI) in the male group was comparable to that in the female group. Mean heart rate was higher in the female volunteers compared with the male cohort (74.8 vs. 70.5 beats/min; P<0.001). The mean systolic blood pressure (BP) in the male volunteers was comparable to the females. The diastolic BP was significantly higher in the males than compared with the females (78.1 (12.2) vs.74.4 (11.7) mmHg; P=0.004). Mean eGFR was significantly higher in the males than in the females (79.1 [42.5-113.8] vs. 70.2 [39.9-99.4] ml $min^{-1}1.73\ m^2$; P<0.001). Median copeptin levels were higher in the male volunteers than compared with the females (median 4.3 [range 0.4-44.3] vs. 3.2 [1.0-14.8] pmol/L; P<0.001), figure 1a. In contrast median NTproBNP levels were higher in females compared with the males

(53.3 [5.7-991.9] vs. 12.8 [5.7-932.8] pmol/L; P<0.001), figure 1b. Left atrial size was significantly higher in the male cohort compared with the females (3.4 (0.4) vs. 3.1 (0.5) cm; P<0.001). The E/A ratio was significantly higher in the males compared with the females (0.9 (0.2) vs. 0.8 (0.2); P<0.001). Gender specific definitions of LVH were used because of the significant differences in LV mass index between males and females (88.4 (19.6) vs. 74.5 (22.1) g/m^2; P<0.001 respectively).

Univariate analysis (Clinical parameters)

In univariate analyses copeptin was correlated positively with male gender (r_s=0.341; P<0.001, Table 2). Increasing BMI was significantly related with higher plasma copeptin concentrations (r_s=0.147; P<0.001). MDRD derived eGFR a measure of renal dysfunction was not correlated with copeptin levels in the whole population (r_s=-0.02; P=0.678). No significant relationships were observed between copeptin levels and age, heart rate, systolic BP, diastolic BP or NTproBNP levels.

Due to the very significant differences in copeptin between males and females, we examined these correlations in the 2 genders separately. In females, copeptin remained significantly correlated with BMI (r_s=0.185; P<0.001) and non-significantly with eGFR (r_s=-0.097; P=0.095). In males, the correlation of copeptin with BMI was non-significant (r_s=0.091; P=0.06) but copeptin was very significantly correlated with eGFR (r_s=-0.186; P<0.001), figure 2a. In the whole population, partial correlation analysis (controlling for gender) between copeptin and eGFR was significant (r_s=-0.189; P<0.001), whereas the correlation between BMI and copeptin was non-significant (r_s=0.066; P=0.08).

In accordance with previous reports, NTproBNP was strongly correlated with female gender (r_s=-0.281; P<0.001) and increasing age (r_s=0.371; P<0.001). We observed an inverse relationship between NTproBNP and heart rate (r_s=-0.080; P=0.035). Plasma NTproBNP levels were inversely associated with diastolic BP (r_s=-0.093; P=0.015). NTproBNP was negatively correlated with eGFR (r_s=-0.293; P<0.001), figure 2b.

Multivariate analysis (Clinical Parameters)

In multivariate analysis, male gender (P<0.001) and eGFR (P<0.001) were independent predictors of plasma copeptin levels.

Multivariate analysis, which included age, male gender, heart rate, diastolic BP and eGFR, revealed that age (P<0.001), female gender (P<0.001), heart rate (P<0.001) and eGFR (P<0.04) were independent predictors of NTproBNP levels.

Univariate Analysis (Clinical parameters and Echocardiographic parameters)

Copeptin was positively correlated with LV mass index (r_s=0.091; P=0.033). Variables that may reflect preload such as DT (deceleration time) [18] and left atrial size correlated with higher copeptin levels (r_s=0.085; P=0.029 and r_s=0.206; P<0.001 respectively). Other indices of diastolic dysfunction such as E/A ratio and left ventricular isovolumetric relaxation time were not significantly correlated to copeptin levels.

NTproBNP was inversely correlated with LV mass (r_s=-0.089; P=0.034). NTproBNP failed to correlate with any other echocardiographic variable.

Multivariate Analysis (Clinical parameters and Echocardiographic parameters)

The significant clinical and echocardiographic variables in univariate analyses were used as covariates in multivariate analyses. Gender and eGFR remained strong independent predictors of copeptin levels, see Table 3. Deceleration time and left atrial size retained a weak independent relationship with copeptin levels.

In males, independent predictors of copeptin were eGFR (P<0.001), left atrial size (P<0.03) and deceleration time (P<0.04). In females, none of these variables were independent predictors of copeptin.

Age, female gender and heart rate remained as strong independent predictors of plasma NTproBNP levels, whilst eGFR was a weak predictor.

DISCUSSION

NTproBNP is considered an established diagnostic and prognostic marker for HF. The significance of new prognostic markers is best established by comparing them with existing markers. A study by Gegenhuber et al. showed that the prognostic utility of copeptin was comparable to that conferred by the natriuretic peptides for 1 year all cause mortality in acute destabilized HF patients [7]. Stoiser et al. revealed that copeptin was an excellent predictor of composite endpoints in patients with advanced HF superior to that conferred by BNP [19]. In our study no correlation was observed

between NTproBNP and copeptin inferring that these peptides mediate their effects via different pathways, responsible for the progression of HF [20].

The normal range for copeptin in individuals without cardiovascular disease has not been established. The magnitude of effects of variables such as age and gender and their potential importance in the interpretation of copeptin levels remains unclear.

In our study copeptin levels were found to be higher in male volunteers than compared with females, which corroborates findings by Khan et al. [8]. This contrasts with NTproBNP, which is higher in females. Copeptin was correlated with BMI in univariate analyses. However in the general linear model including both clinical and echocardiographic parameters, BMI was displaced from the model and was not an independent predictor of copeptin levels. The MDRD formula derived eGFR, a measure of renal function was an independent predictor of copeptin levels. This may suggest that copeptin is mainly cleared from the kidneys or that the AVP system is activated in patients with renal impairment. We observed a lack of effect of age on plasma copeptin levels. Our study suggests that gender and renal function have to be taken into consideration when interpreting copeptin levels. In male subjects especially, there is a strong relationship between copeptin and eGFR. This is in contrast to NTproBNP, which is influenced by many factors, such as gender, age, renal function and body mass index. In this study NTproBNP levels were higher in females than males, which corroborates findings by Costello et al. who showed that NTproBNP was associated with higher BNP in females inferring that oestrogen status may be responsible [21].

Plasma copeptin was correlated significantly with left atrial size and deceleration time, but not with other indices that may reflect diastolic dysfunction (E/A ratio, IVRT). The mitral valve E wave deceleration time is dependent on many factors including left ventricular relaxation and pressure, left atrial pressure and preload. It is likely that the correlation with copeptin may reflect preload, as this biomarker is released in stoichiometric amounts with arginine vasopressin, which regulates fluid status. The preload is a known determinant of arginine vasopressin release in normal homeostatic physiology. The relationship with left atrial size and deceleration time was especially noted in male subjects.

Our study confirmed previous research that NTproBNP is strongly related to age. Study by Raymond et al. showed that NTproBNP levels doubled with each decade increase in age [22]. They attributed this association due to an increase in myocardial

mass [23]. In our study eGFR, a marker of renal dysfunction was inversely related with NTproBNP levels. However eGFR was a weak independent predictor of NTproBNP levels. Research by Akiba et al. reported that patients with renal failure had high levels of natriuretic peptides [24], which is consistent with our findings.

Investigating the interaction between the AVP system and clinical/echocardiographic variables in a healthy population as opposed to patients with cardiovascular disease is important in understanding how these neuro-hormonal systems behave in health. This study demonstrates that relationships that exist in health may not follow through into disease. Copeptin in healthy individuals was related with gender and eGFR but not with age. The relationship with eGFR was stronger in the male subjects. The normal range in female subjects was independent of eGFR and echocardiographic measurements. However research by Khan et al. revealed that this was not the case in patients with AMI, as copeptin was correlated with age and eGFR and was higher in the male patients [8]. A burst of copeptin release following an AMI could show a relation with age in disease, which may not show up if the normal range was narrow.

Interest has focussed on copeptin as a prognostic marker. Thorough understanding of the physiology as well as the patho-physiology of this marker in a large population based study is required in order to derive normal reference ranges. Further studies will be needed to confirm these findings and to explore the associated mechanisms. In summary, gender and renal dysfunction were major factors influencing copeptin in normal volunteers, with other factors only minimally contributing. The interpretation of copeptin levels must take into account potential confounding factors such as male gender, renal impairment and the fluid status of the subject. Hence a single reference range for normal copeptin will not be valid, considering the need to adjust for the independent effects of gender and renal function. In summary, the reference range in male subjects has to take renal function into account. In females, the reference range may be independent of age and renal function.

A limitation of the current study is that the cohort consisted entirely of white Caucasians. Therefore these findings cannot be extrapolated to other ethnic groups without further studies. Furthermore, although a relationship of copeptin with creatinine and the MDRD formula derived eGFR was documented, a relationship with renal function may necessitate direct measurement of GFR.

CONCLUSION

In conclusion, this study suggests that gender and renal function specific partition values should be used to interpret copeptin values in future studies of this biomarker in HF or ischaemic heart disease.

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Figure Legends

Figure 1a — Boxplot demonstrating gender differences in Copeptin levels.

Figure 1b — Boxplot demonstrating gender differences in NTproBNP levels.

Figure 2a — Scatter graph displaying Spearman Rho correlation between Copeptin and eGFR, in males (solid circles) and in females (hollow circles), (rs=-0.186; P<0.001 and r_s=-0.097; P=0.095 respectively).

Figure 2b — Scatter graph displaying Spearman Rho correlation between NTproBNP and eGFR, in males (solid circles) and females (hollow circles), (r_s=-0.293; P<0.001).

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Figure 1a

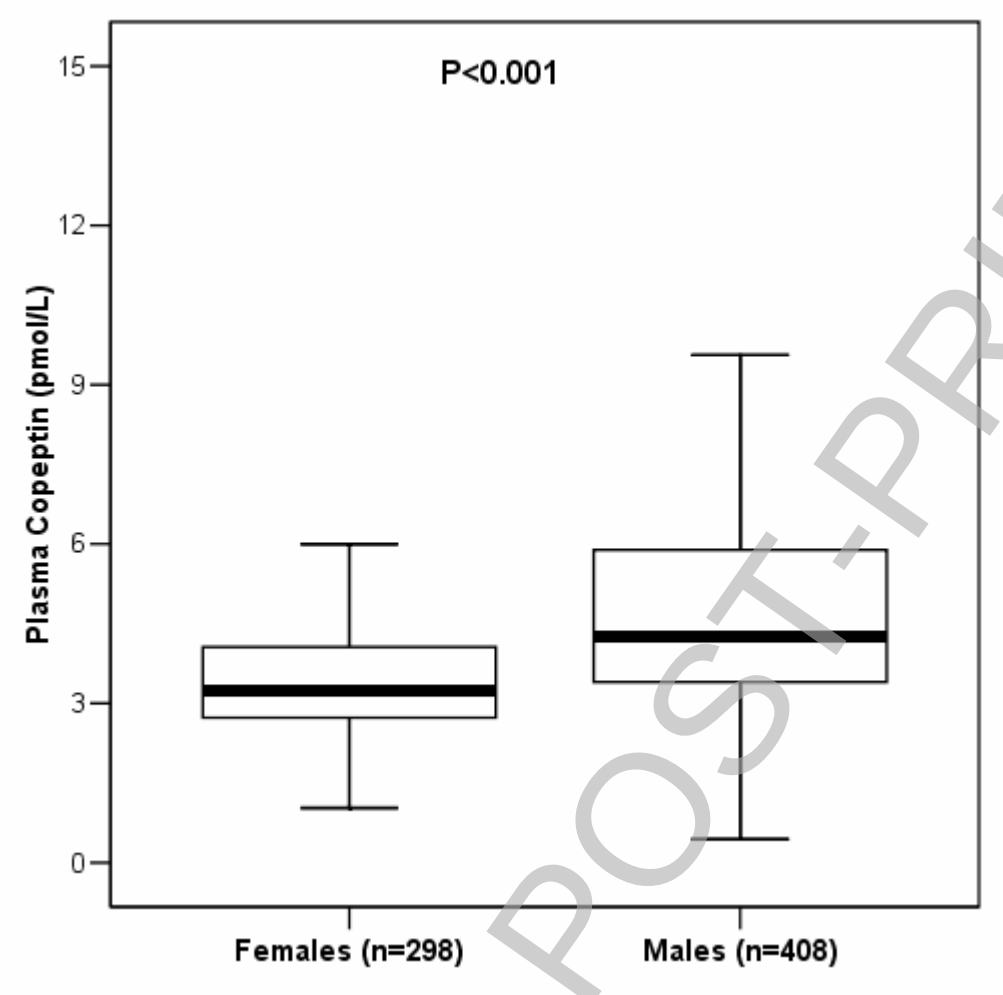

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Figure 1b

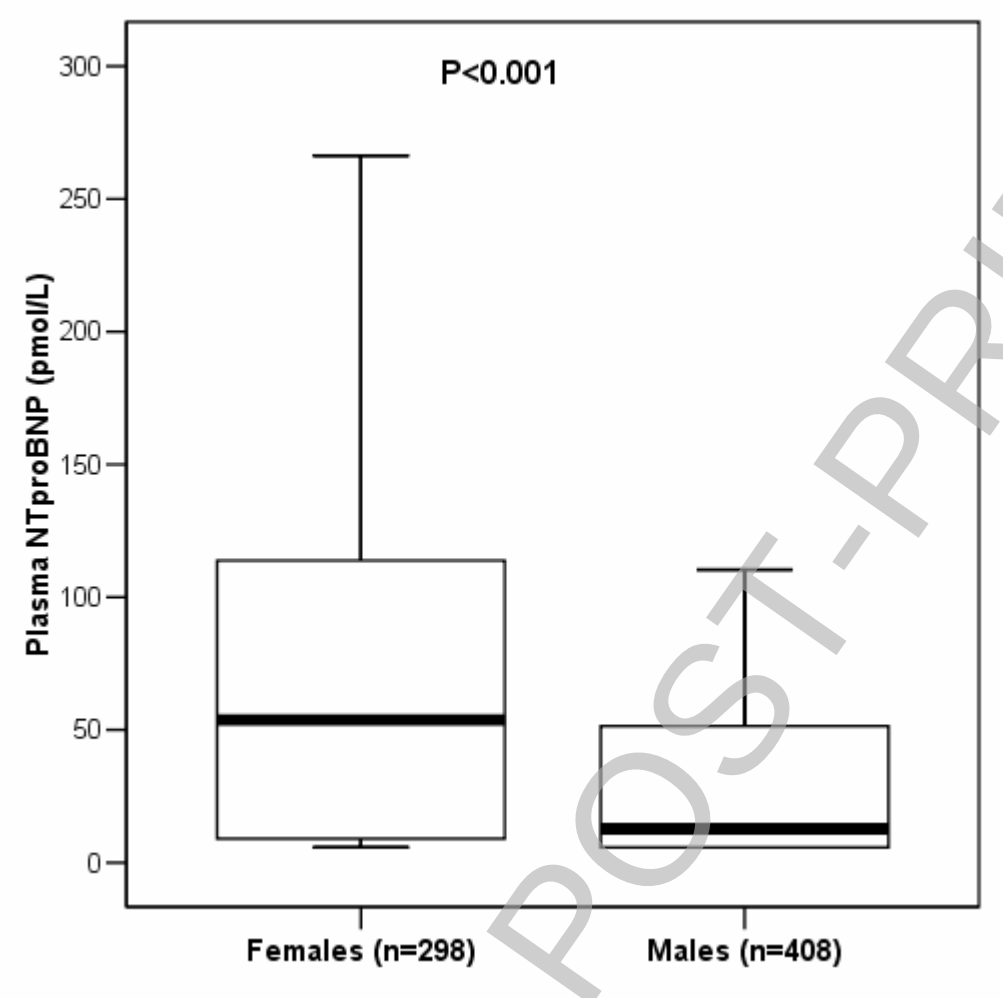

Figure 2a

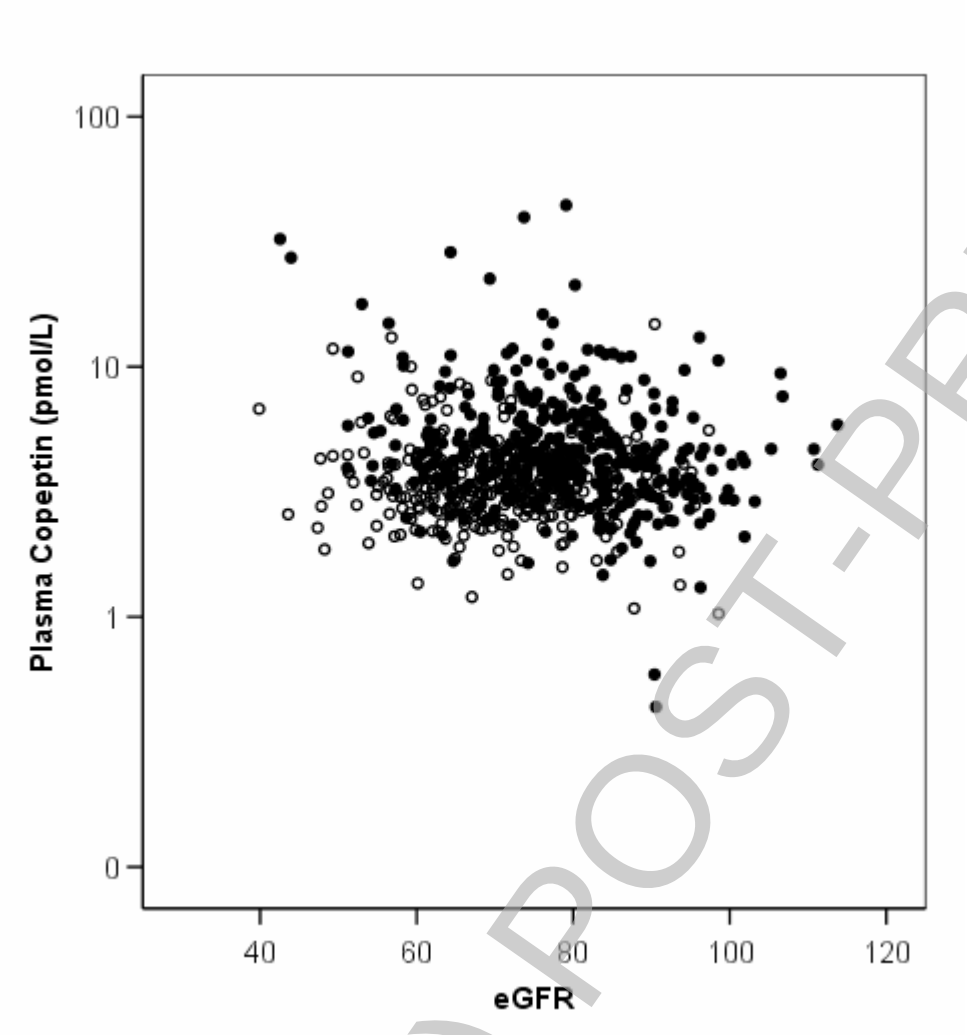

Figure 2b

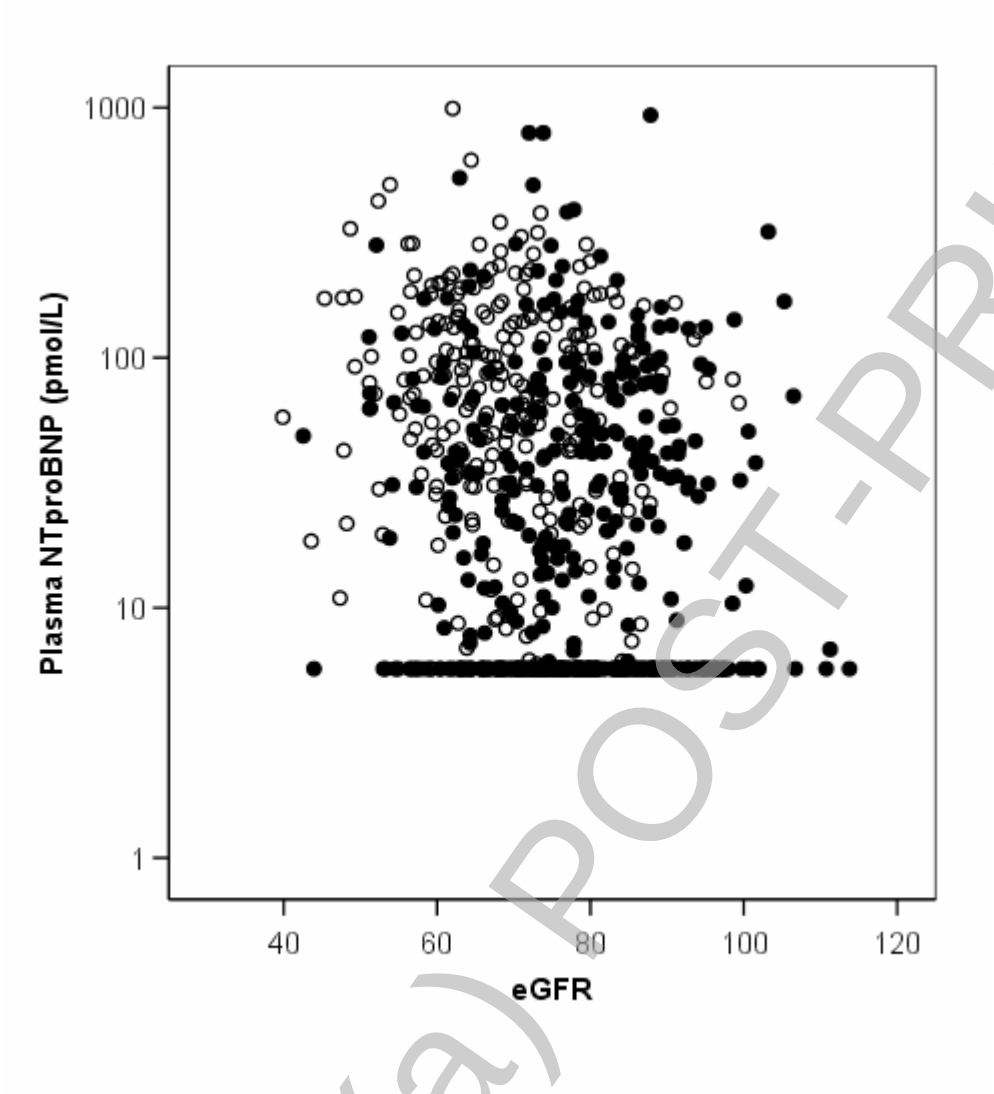

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Table 1. Baseline Characteristics of Study Sample

	Male (n=408)	Female (n=298)	Significance
Clinical Characteristics			
Age (y), mean (range)	59.7 (45.5-80.4	64.0 (55.2-79.4)	P<0.001
eGFR (ml min^{-1} 1.73 m^2 surface area), mean (range)	79.1 [42.5-113.8]	70.2 [39.9-99.4]	P<0.001
BMI (kg/m^2), mean (S.D)	26.2 (3.9)	26.0 (4.3)	P=0.50
Heart rate (min^{-1}), mean (S.D)	70.5 (11.5)	74.8 (11.4)	P<0.001
Systolic BP (mmHg), mean (S.D)	130.5 (17.4)	132.7 (18.1)	P=0.11
Diastolic BP (mmHg), mean (S.D)	78.1 (12.2)	74.4 (11.7)	P=0.004
Biochemical measurements			
Plasma Creatinine (µmol/L), median [range]	91.0 [67.0-156.0]	77.0 [56.0-122.0]	P<0.001
Plasma Copeptin (pmol/L), median [range]	4.3 [0.4-44.3]	3.2 [1.0-14.8]	P<0.001
Plasma NTproBNP (pmol/L), median [range]	12.8 [5.7-932.8]	53.3 [5.7-991.9]	P<0.001
Echocardiographic characteristics			
Left Atrium (cm), mean (S.D)	3.4 (0.4)	3.1 (0.5)	P<0.001
E/A ratio, mean (S.D)	0.9 (0.2)	0.8 (0.2)	P<0.001
DT (msec), mean (S.D)	234.2 (53.8)	236.4 (52.6)	P=0.48
IVRT (msec), mean (S.D)	109.7 (26.3)	113.8 (54.4)	P=0.22
LV mass (g),	164.7 (44.4)	126.9 (45.4)	P<0.001

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mean (S.D)			
LV mass index (g/m^2), mean (SD)	88.4 (19.6)	74.5 (22.1)	P<0.001
LVEF (%), mean (SD)	62.4 (4.9)	62.7 (5.3)	P=0.78

Table 2. Spearman Rho correlations

Clinical parameters	Log Copeptin	Log NTproBNP
Age	r_s=-0.052 P=0.172	r_s=0.371 P<0.001
Male Gender	r_s=0.341 P<0.001	r_s=–0.281 P<0.001
BMI	r_s=0.147 P<0.001	r_s=-0.049 P=0.193
Heart Rate	r_s=0.018 P=0.640	r_s=-0.080 P=0.035
Systolic BP	r_s=0.056 P=0.140	r_s=0.053 P=0.163
Diastolic BP	r_s=0.043 P=0.260	r_s=-0.093 P=0.015
Log Creatinine	r_s=0.310 P<0.001	r_s=-0.085 P=0.024
eGFR	r_s=-0.02 P=0.678	r_s=-0.293 P<0.001
LA size	r_s=0.206 P<0.001	r_s=-0.015 P=0.710
DT	r_s=0.085 P=0.029	r_s= 0.012 P=0.733
E/A ratio	r_s=0.18 P=0.638	r_s=-0.054 P=0.153
IVRT	r_s=0.007 P=0.856	r_s=-0.054 P=0.181
LV Mass	r_s=0.183 P<0.001	r_s=-0.089 P=0.034
LV mass index	r_s=0.091 P=0.033	r_s=-0.009 P=0.831

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Table 3. Independent predictors of plasma Copeptin and NTproBNP

	F	Significance
Independent predictors of plasma Copeptin		
Gender	57.81	P<0.001
BMI	0.06	P=0.79
eGFR	14.50	P<0.001
LA size	4.14	P=0.04
DT	5.67	P=0.02
LV mass index	1.01	P=0.31
Independent predictors of plasma NTproBNP		
Age	45.59	P<0.001
Gender	23.69	P<0.001
Heart Rate	11.16	P<0.001
Diastolic BP	0.49	P=0.48
eGFR	4.65	P=0.03
LV Mass	1.81	P=0.18