PLASMA UROCORTIN 1 IN HUMAN HEART FAILURE

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ABSTRACT

Background: The urocortins are emerging as potentially important contributors to neurohumoral regulation of the circulation with recent reports attributing a powerful array of hemodynamic, renal and neurohumoral effects to the urocortins in cardiac failure. These peptides also appear to have cardioprotective effects in the setting of ischemia-reperfusion. Little is known concerning the plasma concentrations of the urocortins in health and disease. We have investigated plasma urocortin 1 as a potential diagnostic marker of heart failure and documented its relationships to symptoms, measures of cardiac function and concurrent levels of other circulating neurohormones.

Methods and Results: In two hundred and ninety nine patients with recent onset dyspnea and/or peripheral edema presenting to primary care, plasma urocortin 1 and other vasoactive hormones were assayed and echocardiography performed. Heart failure was present in 74 patients (25%) according to predefined diagnostic criteria. Urocortin 1 levels were increased in patients with heart failure and were related to functional class, clinical signs of heart failure, echocardiographic indicators of left ventricular dimensions and function, plasma creatinine, and concurrent circulating levels of plasma natriuretic peptides, adrenomedullin and endothelin 1.

Conclusions: Plasma urocortin 1 is elevated in heart failure (in proportion to the degree of cardiac dysfunction) in concert with the generalized neurohormonal activation seen in this condition. Urocortin levels predict heart failure independent of age, previous history of prior myocardial infarction, diabetes, hypertension, fractional shortening and NTproBNP levels.

Key words (3-5) Urocortin, Heart Failure, Cardiac Natriuretic Peptides, adrenomedullin, endothelin
INTRODUCTION

The urocortins are endogenous vasoactive peptides known to exert powerful beneficial neurohormonal, hemodynamic and renal actions in experimental heart failure (1-5). In stable human heart failure infusions of urocortin 1, at the doses chosen, had little hemodynamic effect whereas urocortin 2 markedly augmented cardiac output whilst reducing systemic vascular resistance (6,7). Urocortins are cardio-protective effects in experimental ischemia/reperfusion (8).

Members of the corticotrophin releasing factor (CRF) family, the urocortins are ancient, with genetic profiling demonstrating their presence through 550 million years of evolution (9). The complementary DNA to urocortin 1 was cloned in 1995 (10) and two related peptide sequences, named urocortin 2 and urocortin 3, were discovered subsequently (11-13). The gene for urocortin 1 is found in chromosome 2p23-2p21 and encodes a 123 amino acid polypeptide precursor (14,15).

Urocortin 1 expression and peptide release are widespread in the body; occurring in the central nervous system, gastrointestinal tract and heart, among other sites (16-19). Plasma levels, metabolism and regulation of the urocortins are still largely undefined. Urocortin expression and peptide release from cultured cardiomyocytes is increased by ischemia (20). Secretion in vitro is also enhanced by cytokines including interleukin(IL) –1 beta, IL-6 and tumor necrosis factor (TNF)-alpha (21); factors elevated in heart failure and acute coronary syndromes. Urocortin expression is increased in hypertrophied rat hearts and in the cardiac ventricles of patients with both dilated and hypertrophic cardiomyopathy (18,22). Circulating levels in humans have been reported at about 8pmol/L (23). In human heart failure, one report indicates levels are elevated but (perhaps unexpectedly) fall with increasing severity of symptoms as well as with reducing
LVEF and increasing age (24). Bioactivity and detectable immunoreactivity may be modulated by a CRF binding protein present in humans but not all mammalian species. This factor may play an inhibitory role in binding to urocortin and preventing receptor activation (25,26).

Urocortins act predominantly through two receptor subtypes, CRF-1 and CRF-2. Receptors possess 7 trans-membrane domains and are G-protein coupled. CRF-2(a) receptors constitute the dominant peripheral CRF-2 receptor form, particularly in the heart and vasculature (15,19). Receptor concentrations are high in the left ventricle and intra-myocardial vessels (19).

In the current study we set out to establish whether or not plasma urocortin 1 levels were systematically elevated in the presence of symptomatic heart failure, and to establish the relationship of plasma concentrations of this peptide with concurrent symptomatic status, echocardiographic measurements of cardiac dimensions and function and with concurrent circulating concentrations of neurohormones known to be activated in the presence of heart failure.

METHODS

Patients: Patients were participants in the Natriuretic Peptides in the Community study, a prospective randomized trial of the diagnostic value of NTproBNP in diagnosis of suspected new onset heart failure in primary care (27). The protocol was approved by an ethics committee of the New Zealand Ministry of Health. All patients provided written informed consent. Patients presenting with breathlessness and/or peripheral edema were referred to the study by their family doctor. Each patient underwent further cardiologic assessment including ECG, chest x-ray and echocardiography (ATL HDI 5000). Blood samples for neurohormone assays were collected from seated patients without prior resting or other special patient preparation. Blood was
collected into EDTA (7.5mg/ml, Vacutainer, Becton Dickinson), centrifuged within 2 hours of venesection (3000 rpm for 10 minutes) with plasma stored at -80°C prior to radioimmunoassay.

The definitive diagnosis of heart failure was provided by a panel of three consultants who reviewed each clinical presentation and all test results (excluding neurohormone levels). The panel decided whether individual patients met the European Society of Cardiology diagnostic criteria for a diagnosis of heart failure (28). This required the presence of appropriate symptoms and signs of pulmonary or peripheral congestion and the presence of objective evidence of cardiac dysfunction. In the case of doubt, response to treatment was considered. This standard was based on that used in other recent studies of the diagnosis of heart failure (29,30).

**Radioimmunoassays:** Blood samples were collected using standard venepuncture technique into tubes containing ethylenediaminetetraacetic acid. Samples were centrifuged and frozen at -70 degrees C prior to immunoassay. Urocortin 1 radioimmunoassay was performed on plasma samples as previously described (31). Sample preparation included steps previously employed to extract total CRF (bound + free) from human plasma. One milliliter of plasma was mixed with 2ml methanol and centrifuged. The supernatant was adjusted to contain 0.003% Triton X-100, dried under an air stream at room temperature, and reconstituted in 0.5ml assay buffer.

For assay, 100μl plasma extract or standard plus 100μl anti serum PBL#5779 diluted 1:165,000 were incubated at 4°C for 24 hours before addition of 100μl radiolabel (¹²⁵I Tyr-Ucn-1) (prepared by the chloramine T method and purified by reverse phase HPLC) containing 10,000cpm. The assay was incubated an additional 24 hours at 4°C after which bound and free label was separated by a solid phase second antibody (donkey anti-rabbit, Sac-Cell, IDS, Bolden, UK). The radioimmunoassay characteristics (mean ± standard deviation) include: zero binding,
35.5 ± 6.3% (n=25); standard curve 50% effective concentration, 414 ± 69pg/ml (88.1 ± 14.6 pmol/L); and a detection limit of 6.6 ± 2.7pg/ml (1.4 ± 0.6pmol/L) in plasma after 2-fold concentration during extraction; n=25). Cross reactivity with urocortins 2 and 3 was less than 0.004%, and less than 0.002% with human CRF, human urotensin 2 or the cardiac natriuretic peptides including ANP, BNP and NTproBNP. Within assay coefficients of variation (CVs) calculated from variance between assay duplicates were 13.4% over 0-52pg/ml (0-11pmol/L), 10.3% over 56-239pg/ml (12-51pmol/L) and 3.3% over 239-1490pg/ml (51-317pmol/L). Inter-assay CV was 17.5% at 63pg/ml (13.5pmol/L; n=23), 16.2% at 111pg/ml (23.7pmol/L; n=20) and 13.3% at 145pg/ml (30.9pmol/L; n=23). Recovery of urocortin 1 added to plasma was 61.2% at 141pg/ml (30pmol/L), 60.9% at 329pg/ml (70pmol/L) and 59.3% at 470pg/ml (100pmol/L).

Urocortin results are given without correction for recovery. The urocortin 1 reference range encompassing 95% of results for healthy subjects (2-16pmol/L) was determined in 98 subjects drawn at random from the Christchurch, New Zealand, electoral roll and matched to the study population for age and gender.

Immoassays for NTproBNP, BNP, ANP, NTproANP, CNP, adrenomedullin and endothelin-1 were measured using our previously validated and published radioimmunoassays (32-37).

**Statistical Methods:** Plasma concentrations of neurohormones are expressed as mean and standard deviation (when normally distributed) or median and inter-quartile range when skewed. Variables with non-normal distributions were compared using the Wilcoxon’s rank test. Other continuous variables are compared by Student t tests. Categorical variables were compared with the Fisher exact test. In univariate analyses, Spearman rank coefficient was used to determine
correlations between variables, presented as R values. Modeling to investigate independent
determinants of urocortin 1 was performed by means of multiple linear regression. Multiple
logistic regression with a forward stepwise selection based on the significance of the Wald Score
if a candidate variable were entered into the regression, was used to test urocortin 1 and other
variables for potential independent association with a diagnosis of heart failure. Urocortin I and
NTproBNP were compared for their ability to discriminate presence or absence of heart failure
by receiver-operator (ROC) analysis (38). The authors had full access to and take full
responsibility for the integrity of the data. All authors have read and agree to the manuscript as
written.

RESULTS

Patient characteristics: Two hundred and ninety nine patients with a median age of 72 years
(range 40-95 years), 65% women, were included in the study (Table 1). Forty-nine percent of
patients presented with dyspnea alone, 12% with edema alone and 39% with both symptoms.
There were 80, 109, 84 and 26 patients in New York Heart Association (NYHA) functional
classes I, II, III and IV respectively. Seventy-four (74) of 299 (25%) were diagnosed as having
heart failure by the panel (8, 22, 27 and 17 in NYHA classes I, II, III and IV respectively).
Underlying etiologies included ischemic heart disease in 27 (36%) and was multifactorial with
contributions from 2 or more of ischemia, diabetes, hypertension and/or atrial fibrillation in 23
(31%). Valvular heart disease was present in 5 patients (7%), dilated cardiomyopathy in 5 (7%),
isolated diastolic heart failure in 11 (15%) with cause unknown in a further 6 patients (8%).
Common non-heart failure conditions leading to presentation among the other 225 patients
included respiratory disease (exacerbation of COPD and malignancies), myocardial ischemia
with exertional dyspnea, and obesity. Other less frequent causes included anxiety with
hyperventilation, thoracic deformity and chronic pulmonary venous thromboembolic disease.

Hypertension was similarly frequent in both heart failure (48%) and non-heart failure (53%) groups. However, diabetes (28 versus 10%; p<0.001), previous myocardial infarction (34 versus 8%; p<0.001) and prescription of both loop diuretics (38 versus 18%; p<0.001) and ACE inhibitors (47 versus 20%; p<0.001) were more frequent in patients with heart failure.

**Plasma Urocortin 1 Concentrations:** Mean plasma urocortin 1 in normal control subjects at 7.2 ± 2.9 pmol/L (n=98) was significantly lower than levels recorded in the 225 non-heart failure symptomatic patients (11.1 ± 3.2 pmol/L) which in turn were lower than in the 74 patients with a final diagnosis of heart failure (13.6 ± 4.1 pmol/L; p<0.001 for all inter-group comparisons).

Levels of urocortin I and NTproBNP in symptomatic subjects with and without a final diagnosis of heart failure are compared in Figure 1. Urocortin levels fell within a much tighter distribution and heart failure induced lesser absolute and proportional increments in urocortin 1 than in NTproBNP.

The significant inter-group differences in plasma urocortin 1 were independent of age and gender. Levels were significantly higher in men than in women in normal subjects and in the study population, whether the study group was considered in totality or separately for those with and without a final diagnosis of heart failure (Table 2). No significant relationship to age was seen in the study group, the normal reference range population or in any sub-group. We also observed the expected increases in other neurohormones in heart failure (Table 1).

Urocortin 1 levels were related to symptoms, exhibiting stepwise increases from 10.6±1.3 to 10.8±1.3; 11.5±1.4 and 13.3±1.5 pmol/L in NYHA functional classes I through IV respectively (p=0.007) for the group overall. A similar trend was seen in the 74 patients with a final diagnosis
of heart failure (11.5±3.5; 12.4±2.5; 14.3±4.3 and 14.6±5.0 pmol/L respectively). Levels from patients in NYHA classes I and II combined were compared with those in NYHA classes III and IV combined. Severe symptoms (NYHA III/IV, n=44) were associated with significantly higher mean plasma urocortin I levels than mild (NYHA I/II, n=30) symptoms (14.4±3.9 versus 12.1±3.9 pmol/L; P=0.017).

Peptide levels were also significantly related to jugular venous pressure (measured by standard clinical technique as the distance of the highest excursion of internal jugular venous waves in centimeters above the sternal angle with torso at 45 degrees and legs horizontal) in those with heart failure (R=0.309, n=72, P=0.008) but not in those without (R=0.05, n=222, P=0.495).

Urocortin was related to left ventricular dimensions and function. Correlations with left ventricular end diastolic dimension, left ventricular end systolic dimension, fractional shortening and LVEF were significant (R=0.190, 0.228, -0.291 and -0.187 respectively with sample number varying between 212 for calculated left ventricular mass to 259 for left ventricular end diastolic dimension and corresponding P values varying between 0.002 and <0.0001). R values tended to strengthen when analyses was confined to the 74 patients with heart failure (R=0.192, 0.266, -0.313 and -0.238 respectively) and lost significance when only the 255 patients without heart failure were considered (P>0.05 for all).

Significant positive relationships were observed between plasma urocortin 1 levels and levels of the other neurohormones measured, including ANP, NTproANP, BNP, NTproBNP, CNP, adrenomedullin and endothelin 1 (R values varying between 0.210 for endothelin 1 and 0.398 for CNP levels, sample size varying between 286 and 298 with P values <0.0001 for all relationships; Figure 2).
An inverse relationship with renal function was observed. Urocortin was directly related to plasma creatinine (R=0.249, n=294, p<0.0001) and inversely related to estimated GFR (R= -0.107, n=286, p=0.072). By multiple linear regression analysis, only gender (P<0.0001), NTproBNP levels (P=0.002) and a diagnosis of heart failure (P=0.034) remained independently predictive of increased plasma urocortin levels (R squared=0.16). Other variables which were significantly associated in univariate analysis but not in this multivariate analysis included plasma creatinine, all neurohormones with the exception of NTproBNP, and the different measures of cardiac function.

With respect to predictors of heart failure, univariate relationships were observed for fractional shortening, creatinine clearance, NTproBNP and plasma urocortin 1.

By multivariate analysis, employing stepwise multiple logistic regression, urocortin remained an independent predictor of heart failure (P=0.005 Table 3) independent of age, history of prior myocardial infarction, diabetes, LVEF, NTproBNP and calculated creatinine clearance. The likelihood of a final diagnosis of heart failure rose from 15 to 20% to 39% in first, second and third tertiles of plasma urocortin 1 respectively. In comparison, for tertiles of plasma NTproBNP the corresponding figures were 5, 12 and 58%. In patients falling within both the lowest tertile of urocortin 1 and the lowest tertile of NTproBNP, 5% had heart failure.

Conversely, in those in the upper tertile plasma concentrations of both peptides, the risk of heart failure was 75% and 49% of all patients with heart failure fell within this sub-group (Figure 3).

Urocortin 1 and NTproBNP were assessed for their ability to predict the presence or absence of heart failure by receiver-operator analysis. Urocortin 1 yielded an area under the curve (AUC) of 0.68 (95% CI, 0.61-0.75, p<0.001). The best-performing value of urocortin 1 (12.3pmol/L) was associated with sensitivity, specificity, positive and negative predictive values of 61, 67, 38 and
84% respectively. This was clearly a weaker performance than that observed for NTproBNP with an AUC of 0.85 (95% CI, 0.80-0.90, p<0.0001), sensitivity of 80%, specificity of 81%, positive predictive value of 58% and negative predictive value of 93%. Area under the ROC was significantly less for urocortin 1 when compared to NTproBNP (P<0.01; Figure 4).

**DISCUSSION**

In patients with a final diagnosis of heart failure, plasma urocortin levels were double those in the normal reference range population. Symptomatic patients without a final diagnosis of heart failure also had elevated urocortin at intermediate levels approximately 50% higher than those observed in the normal range. Plasma urocortin levels were related to symptoms (NYHA class), signs (jugular venous pressure), concurrent levels of other neurohormones known to be activated in heart failure and to echocardiographic measures of cardiac dimensions and function. Urocortin was independently predictive of heart failure. Urocortin 1 is clearly not as powerful a stand-alone indicator of cardiac function or as good a marker of the presence of heart failure as the natriuretic peptides (Figure 4). However, it does add additional and independent information beyond that offered by established predictors as reflected in our multivariable analysis. Intuitively, this is best appreciated from our tertile analysis (Figure 3). When NTproBNP results were considered alone plasma levels in the upper tertile carried a 58% chance of a final diagnosis of heart failure; when both NTproBNP and urocortin levels fell in the upper tertile the likelihood rose to 75% and half of all patients with heart failure exhibited top tertile levels for both peptides.

Even in symptomatic patients without heart failure, urocortin 1 was increased above normal. The reasons for this are uncertain, but it is possible a burden of cardiovascular disease within the non-
heart failure study group produced increased urocortin 1 at an intermediate level between truly healthy subjects and those with symptomatic or overt heart failure. Further, urocortin may also be stimulated by non-cardiovascular illness. The stimulation of urocortin secretion in vitro by IL-1, IL-6 and TNF alpha (21) raises the possibility that an array of inflammatory states, with attendant cytokine activation, may result in some increase in plasma urocortin 1. Notably the R squared value (0.16) from the multivariate model assessing independent determinants of plasma urocortin 1 only reflects 16% of the interindividual variation in plasma urocortin 1 levels. This suggests other factors which influence plasma urocortin levels remain to be found. Further studies in health and disease should establish the spectrum of stimuli which trigger increases in circulating urocortin 1.

Ng et al have also reported elevated levels of urocortin in heart failure. In contrast to this previous report we observed an inverse relationship with left ventricular ejection fraction rather than the positive correlation previously reported (24). In addition, we observed a continuous increase in plasma urocortin levels across NYHA functional classes I-IV in contradistinction to the report from Ng et al in which levels appear to peak in NYHA class I and II and were somewhat less in the NYHA classes III and IV. We observed no relationship with age rather than the inverse relationship reported previously (24).

From the present data set, it is not possible to determine why our results differ from the previous report in such clear-cut fashion. Answers may lie within the differing characteristics of the two immunoassays employed. They are likely to detect differing epitopes and/or partial degradation products of urocortin. The potential effects of corticotrophin binding protein on assay performance may also differ between assays. The assay employed in the current report exhibits no significant cross-reactivity with relevant alternative peptides and has been fully validated.
Notably, in the current report plasma urocortin levels follow the common pattern of neurohormones known to be activated in heart failure. That is, they increase with increasing severity of symptoms and cardiac dysfunction and rise in parallel with other neurohormonal markers including the natriuretic peptides, adrenomedullin and endothelin 1. Clearly further corroborating data are required.

In contrast to the cardiac peptides, plasma urocortin 1 is higher in men than in women. This pattern was apparent in the symptomatic cohort overall and in age and gender matched healthy subjects. The underlying reasons for the gender difference are unknown. Levels of urocortin are also mildly inversely related to renal function by univariate analysis, although in multivariate tests, this relationship to creatinine clearance was not independent of other predictors of elevated urocortin 1.

In summary, plasma urocortin 1 levels are elevated in human heart failure with significant positive relationships to other circulating neurohormones known to be activated in this condition. They are also related to symptoms, signs (i.e. jugular venous pressure) and to echocardiographic measures of cardiac structure and function. In contradistinction to a previous report (24), we found an inverse rather than positive relationship to left ventricular ejection fraction. In addition, the relationship between urocortin 1 and symptomatic status was continuous with levels showing a continuous increment between NYHA class I-IV.

Our findings suggest that plasma urocortin levels are unlikely to assume a role as a diagnostic marker in heart failure. However, they do add to our overall understanding of the pathophysiological response to heart failure. In experimental heart failure, deliberate antagonism
of endogenous urocortins clearly exacerbates hemodynamic, neurohormonal and renal impairment (3). Taken together with previous studies defining the bioactivity of the urocortins in experimental heart failure (1-5), it appears likely that elevated plasma urocortin 1 in human heart failure reflects a beneficial compensatory response to this condition.
Funding Sources

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Disclosures

None.

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REFERENCES


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Figure Legends

Figure 1
Plasma NTproBNP (A) and Urocortin 1 (B) box plots showing median, 25th and 75th percentiles, and smallest and largest values. Identical axes are employed to display both skewed NTproBNP data and normally distributed Urocortin 1 data to facilitate direct comparison. For both peptides levels in heart failure are significantly higher than in patients with symptoms from other causes (P<0.001 for both urocortin 1 and NTproBNP).

Figure 2
Plasma Urocortin 1 (in patients presenting with dyspnea and/or edema) plotted against concurrent levels of NTproBNP, BNP, CNP, Adrenomedullin (ADM) and Endothelin 1 (ET1) levels. R values are given together with 95% confidence intervals.

Figure 3
Distribution of patients with Heart Failure (n=74) according to tertiles (low, mid and high) of plasma NTproBNP and urocortin 1 levels derived from the total study group (n=299). Forty nine percent (36/74) of patients with a final diagnosis of heart failure exhibit upper tertile levels of both peptides.

Figure 4
Receiver-operator curves, Areas Under Curves (AUC (95% Confidence Intervals)) for discrimination of heart failure by Urocortin 1 (AUC 0.68 (0.61-0.75)) and NTproBNP (AUC 0.87 (0.82-0.92)). AUCs differed significantly P<0.001.
<table>
<thead>
<tr>
<th>Clinical, Echo and Hormone Variables</th>
<th>Heart Failure (n=77)</th>
<th>Not Heart Failure (n=228)</th>
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<td>Age, y, mean</td>
<td>74 (11)</td>
<td>72 (11)</td>
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<tr>
<td>Gender, female n (%)</td>
<td>39 (51)</td>
<td>159 (70)</td>
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<td>Atrial fibrillation, n (%)</td>
<td>23 (30)</td>
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<td>Heart rate, bpm, mean (SD)</td>
<td>73 (16)</td>
<td>74 (15)</td>
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<td>Systolic BP, mmHg, mean (SD)</td>
<td>140 (24)</td>
<td>148 (22)</td>
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<td>Diastolic BP, mmHg, mean (SD)</td>
<td>76 (17)</td>
<td>80 (14)</td>
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<td>Serum creatinine, mmol/L</td>
<td>0.114 (0.04)</td>
<td>0.09 (0.03)</td>
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<td>Creatinine clearance (ml/min)</td>
<td>58.6 (28.1)</td>
<td>69.6 (28.6)</td>
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Neurohormones, median (25th and 75th percentiles)

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<tr>
<td>N-CNP, pmol/L</td>
<td>32.4 (25.4, 39.4)</td>
<td>24.7 (21.0, 29.5)</td>
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<td>CNP, pmol/L</td>
<td>1.85 (1.35, 2.15)</td>
<td>1.45 (1.13, 1.67)</td>
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<td>ANP, pmol/L</td>
<td>32 (26, 45)</td>
<td>17 (12, 25)</td>
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<td>N-terminal proANP, nmol/L</td>
<td>1.77 (1.36, 2.44)</td>
<td>0.77 (0.49, 1.17)</td>
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<td>BNP, pmol/L</td>
<td>30 (16, 45)</td>
<td>8 (5, 14)</td>
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<td>N-terminal proBNP, pmol/L</td>
<td>183 (103, 319)</td>
<td>38 (20, 76)</td>
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<td>Endothelin-1, pmol/L</td>
<td>1.7 (1.4, 2.1)</td>
<td>1.0 (2.6, 1.2)</td>
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<td>Adrenomedullin, pmol/L</td>
<td>7.7 (6.5, 9.7)</td>
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Echocardiographic variables

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<td>LVEDD, mm</td>
<td>57.5 (10)</td>
<td>50.2 (5.8)</td>
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<td>44.7 (11.5)</td>
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<td>LV mass, g</td>
<td>229.7 (90.4)</td>
<td>177.6 (56.7)</td>
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<td>Fractional shortening, %</td>
<td>23.9 (9.7)</td>
<td>34.3 (7.7)</td>
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<td>LVEF, %, mean (SD)</td>
<td>44.1 (14.7)</td>
<td>60.7 (9.9)</td>
<td>&lt;0.0001</td>
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<td>LA area, cm², mean (SD)</td>
<td>26.2 (5.9)</td>
<td>20.7 (4.8)</td>
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<td>E-wave velocity, cm/s</td>
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<td>52.0 (17.1)</td>
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<td>58.3 (24.7)</td>
<td>69.1 (17.2)</td>
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<td>Deceleration time, msec</td>
<td>175 (75)</td>
<td>215 (170)</td>
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</tbody>
</table>
TABLE 2. Plasma Urocortin 1 (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td>7.2±2.9</td>
</tr>
<tr>
<td>M</td>
<td>33</td>
<td>9.1±2.7</td>
</tr>
<tr>
<td>F</td>
<td>65</td>
<td>6.4±2.6</td>
</tr>
<tr>
<td><strong>Heart Failure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>13.6±4.1</td>
</tr>
<tr>
<td>M</td>
<td>37</td>
<td>14.6±4.5</td>
</tr>
<tr>
<td>F</td>
<td>37</td>
<td>12.5±2.6</td>
</tr>
<tr>
<td><strong>Non-Heart Failure Illness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>225</td>
<td>11.1±3.2</td>
</tr>
<tr>
<td>M</td>
<td>68</td>
<td>12.1±3.3</td>
</tr>
<tr>
<td>F</td>
<td>157</td>
<td>10.7±3.1</td>
</tr>
</tbody>
</table>

All three groups "Total" values differ (P<0.001 for all pair-wise comparisons).

Mean values in males (M) are greater than in females (F) in all three groups (P<0.001 for all).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>Sig (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urocortin I (median)</td>
<td>2.97</td>
<td>1.39-6.38</td>
<td>0.005</td>
</tr>
<tr>
<td>NTproBNP (median)</td>
<td>7.12</td>
<td>2.71-18.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (per 10 years increment)</td>
<td>1.28</td>
<td>0.78-2.13</td>
<td>0.328</td>
</tr>
<tr>
<td>LVEF (per 1 % fall)</td>
<td>1.08</td>
<td>1.04-1.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prior MI</td>
<td>2.27</td>
<td>0.95-5.92</td>
<td>0.063</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.80</td>
<td>1.39-10.42</td>
<td>0.009</td>
</tr>
<tr>
<td>e GFR (per 40ml/min decline)</td>
<td>1.58</td>
<td>0.71-3.53</td>
<td>0.261</td>
</tr>
</tbody>
</table>
A. NT-proBNP (RIA, pmol/L).  B. Urocortin I (pmol/L) Box plots give median, 25th and 75th percentiles, and the smallest and largest values excluding outliers.  Outliers are defined as any value greater than 1.5x the interquartile range and are indicated by open circles.
\[ \text{Urocortin I (pmol/L)} \]

\[ \begin{array}{ccc}
\text{NT-proBNP (pmol/L)} & \text{BNP (pmol/L)} & \text{CNP (pmol/L)} \\
n=299 & n=294 & n=286 \\
r=0.293 (0.19-0.39) & r=0.311 (0.20-0.41) & r=0.384 (0.28-0.48) \\
p<0.001 & p<0.001 & p<0.001
\end{array} \]

\[ \begin{array}{ccc}
\text{ADM (pmol/L)} & \text{ET (pmol/L)} \\
n=298 & n=292 \\
r=0.331 (0.23-0.43) & r=0.244 (0.13-0.35) \\
p<0.001 & p<0.001
\end{array} \]
NT-proBNP (tertiles)

Heart Failure Patients (%)

Urocortin I (tertiles)

Heart Failure Patients

0 20 40 60 80

Low
n=100

Mid
n=101

High
n=8

Low
Mid
High
1 - Specificity

Sensitivity

Reference Line
NTproBNP
Urocortin 1

Circulation
Heart Failure

Journal of the American Heart Association
Plasma Urocortin 1 In Human Heart Failure
Susan P. Wright, Robert N. Doughty, Chris M. Frampton, Greg D. Gamble, Tim G. Yandle and A. Mark Richards

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