Urotensin II receptor expression in human right atrium and aorta: effects of ischaemic heart disease

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Background. Urotensin II (UII) and its receptor UT are involved in control of the cardiovascular system and are implicated in heart failure. We measured UT expression by quantitative PCR (Q-PCR) in atrial and aortic tissue, and plasma UII while simultaneously assessing cardiac function in 40 patients undergoing coronary artery bypass surgery.

Methods. RNA extracted from atrial and aortic samples was probed with specific Q-PCR UT and housekeeper (glyceraldehyde-3-phosphate dehydrogenase, GAPDH) TaqMan® primers. Plasma UII was measured using radioimmunoassay. Left ventricular ejection fraction (LVEF) was measured using preoperative trans-thoracic echocardiography and ventriculography, and intraoperatively using transoesophageal echocardiography. Q-PCR data are expressed as difference in cycle threshold ($\Delta C_t = C_{UT} - C_{GAPDH}$; high number indicates low expression).

Results. There was no difference in $\Delta C_t$ in either atrium or aorta between patients with normal (LVEF >50%) or those with impaired (LVEF <50%) preoperative systolic function. There was a weak negative correlation ($r^2 = 0.245$, $P = 0.031$) between intraoperative LVEF and $\Delta C_t$ in 19 patients possibly indicating down-regulation of UT with worsening LVEF. Atria expressed significantly more UT than aorta ($P = 0.011$). In the absence of non-diseased controls, plasma UII was higher than a historical control group.

Conclusions. This is the first study to simultaneously measure UT (mRNA), UII, and cardiovascular function. Collectively, these pilot data may suggest a down-regulation of UT within the right atrium of patients with heart failure.

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Urotensin II (UII) and its receptor UT are widely distributed in central and peripheral tissues. In general, activation of UT on vascular smooth muscle causes vasoconstriction whereas activation of endothelial UT produces vasodilation. Exogenous UII has positive inotropic effects.¹⁻³ However, the vascular effects of UII are highly variable with marked inconsistency between species in response to exogenous UII and notable divergence in the response of different vascular beds and different regions of the same vessel.⁴⁻⁵ The observation that basal haemodynamics and general vasopressor responsiveness are unaltered in UT knockout mice supports the suggestion that the receptor is functionally silent under basal conditions, but this may change in cardiovascular disease.⁶

In man, iontophoresed topical UII caused vasodilation in healthy controls but vasoconstriction in subjects with chronic heart failure⁷ and hypertension.⁸ Moreover, UII infusions have very few cardiovascular effects in healthy control subjects.⁹¹⁰ The current consensus is that increased plasma UII concentrations are a potentially useful biomarker of congestive heart failure (CHF) and unlike N-terminal brain natriuretic peptide (N-BNP) appear to be elevated in systolic dysfunction irrespective of severity.¹¹ Moreover, plasma UII concentrations do not appear to be
affected by age or gender. Immunohistochemical and in situ hybridization analysis of tissue bank and autopsy specimens have demonstrated increased expression of UII peptide and mRNA in cardiac tissue from patients with heart failure. This increased UII expression was most pronounced in areas of injury, particularly the subendocardium and infarction border zones of the left ventricle. Increased UT expression was also noted in these areas.

In the present study, we have addressed the following questions: (1) Is there a relationship between myocardial or aortic UT and plasma UII and left ventricular systolic function? (2) Is there any relationship between atrial and aortic UT expression? (3) Is there a relationship between myocardial or aortic UT and plasma UII and symptoms according to preoperative New York Heart Association Grade of Dyspnoea (NYHA) or Canadian Cardiovascular Society Angina Severity (CCS) scores in patients with ischaemic heart disease and different degrees of heart failure?

Methods

Patients, anaesthesia, and tissue collection

With local research ethics committee approval and written informed consent, 40 patients (three females) presenting for elective coronary artery bypass grafting were recruited. Patients with diabetes mellitus, chronic kidney disease (creatinine >177 mmol litre\(^{-1}\)), and aortic valvular disease were excluded. All patients received 2 mg of lorazepam p.o. 2 h before theatre. Anaesthetic technique was standardized and consisted of etomidate and fentanyl for induction of anaesthesia at a dose sufficient to abolish eye-lash reflex while maintaining haemodynamic stability. Pancuronium (0.1 mg kg\(^{-1}\)) was administered followed by 1–2% isoflurane in oxygen/air mixture. Heparin was administered pre-bypass (300–400 u kg\(^{-1}\)). All patients had left ventricular function assessed before operation via either a ventriculogram or trans-thoracic echocardiogram, which was then classified as either good [left ventricular ejection fraction (LVEF) >50%] or impaired [moderate (LVEF 30–50%) and poor (LVEF <30%)]. Right atrial and thoracic aortic punch biopsy samples were collected from patients during surgery at the same stage for each patient and transferred into RNAlater\(^{\text{TM}}\) (Ambion) as a temporary transport medium. Atrial tissue was obtained as the right atrium was cannulated with the venous drainage tube immediately pre-bypass. Aortic tissue was obtained towards the end of the bypass period in the form of punch biopsies to facilitate proximal vein graft anastomosis. Five millilitres of blood were collected from each patient post-induction of anaesthesia and post-tracheal intubation into EDTA Monovette containers to which aprotinin (3 TIU in 100 ml) was added. Blood samples were maintained on ice before centrifugation at 3000g for 20 min at 4°C, after which plasma was removed and stored at −70°C.

Measurement of systolic function intraoperatively

Because of the limitations of preoperative assessment of systolic function, a 5.5 MHz multiplane transoesophageal echocardiography (TOE) probe (Hewlett-Packard Omniplane II, 21369A) (Philips, Bothell, WA, USA) was used to perform intraoperative scans where possible. This was attached to a Hewlett-Packard Sonos 5500\(^{R}\) Echocardiography system (Hewlett-Packard Inc., Andover, MA, USA). Scans were analysed using AGFA Heartlab Cardiovascular software. Scans were performed by one of the three personnel with detailed analysis of images at a later stage by a consultant cardiac anaesthetist (J.S.). Ejection fraction was calculated using Simpson’s biplane method.

Cell culture

In assay validation experiments [simplex compared with duplex quantitative PCR (Q-PCR)], human embryonic kidney cells stably expressing the human UT (HEK\(_{\text{hUT}}\)) were used as a control high expressing tissue. HEK\(_{\text{hUT}}\) cells were maintained in minimal essential media supplemented with 10% fetal bovine serum (FBS), 100 IU ml\(^{-1}\) penicillin, 100 μg ml\(^{-1}\) streptomycin, and 2.5 μg ml\(^{-1}\) fungizone. Stock cultures were additionally supplemented with geneticin (G418) 800 μg ml\(^{-1}\) to maintain expression. Cultures were grown at 37°C with 5% CO\(_2\) humidified air. Confluent cultures were washed twice in phosphate buffered saline and total RNA extracted using a Tri-reagent\(^{R}\) preparation.

Determination of hUT mRNA by Q-PCR

Atrial and aortic samples were submerged in liquid nitrogen and crushed using a frozen pestle and mortar. The subsequent tissue powder was then processed with either 4 ml (right atrium) or 2 ml (thoracic aorta) of Tri-reagent\(^{R}\) and 1 ml aliquots stored at −70°C. For atrial samples, 1 ml aliquots were processed individually. On the basis of initial data from atria, both 1 ml aliquots of thoracic aorta were combined. Total RNA was extracted and final RNA pellets (patient and cultured cells) were resuspended in PCR grade water and the mass of RNA determined using an Eppendorf Biophotometer. RNA purity was assessed from the 260/280 nm ratio which was, in all but three aorta samples, >1.7. In three initial atrial samples RNA integrity was further assessed using the RNA 6000 Nano chip in an Agilent 2100 Bioanalyzer.\(^{13}\) Mean (range) RNA integrity numbers (RIN) of 6.5 (6.3–6.8) (RIN=1 complete degradation, RIN=5–10 adequate integrity, and RIN=10 no degradation) were obtained further indicating adequate integrity.\(^{14}\)

Total RNA was subjected to DNase digestion using a proprietary kit, Turbo DNA-free\(^{R}\), according to the manufacturer’s instructions. In an attempt to facilitate standardization of further procedures, 5 μg of RNA was added to a
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50 μl reaction. However, due to low yields from thoracic aortic samples, a smaller 25 μl reaction was needed and the mass of RNA included could not be standardized. ‘Cleaned’ samples were reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and the resulting cDNA was stored at −20°C. UT mRNA quantity was assessed by Q-PCR using commercially available TaqMan® gene expression assays from Applied Biosystems for the human UT receptor (Hs00255820_s1) and the housekeeper glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The thermal profile for Q-PCR reactions in the StepOne instrument (Applied Biosystems) was 2 min at 50°C, 10 min at 95°C, 50 cycles of 15 s at 95°C, and 1 min at 60°C. Non-template controls were included for all samples.

Assay validation

In order to conserve patient samples, we decided to assess hUT and GAPDH mRNA in a duplex assay so constructed standard curves (consisting of serial 10-fold dilutions of stock mRNA extracted from HEK₂₉₃UT cells) using hUT and GAPDH primers separately (Simplex) and simultaneously (Duplex). As starting mRNA was serially diluted, cycle threshold increased for UT and GAPDH in both single and duplex assays. Reaction efficiencies [calculated using the formula; $\text{Eff} = 10^{-\frac{1}{\text{slope}}}-1 \times 100$] were 91.0 and 91.4 for GAPDH in simplex and duplex and 86.2 and 87.1 for UT in simplex and duplex, respectively. In all subsequent experiments, a duplex assay format was used.

Extraction and assay of plasma UII

UII was extracted from plasma samples as previously described.¹⁵ ¹⁶ Plasma samples (2 ml) were acidified by the addition of an equal volume of trifluoroacetic acid (TFA, 1%) and left for 30 min, after which up to 4 ml of sample was loaded onto Strata C18-E solid phase extraction cartridges previously equilibrated with 2 ml of 60% acetonitrile in 1% TFA followed by 9 ml of 1% TFA. After sample addition, columns were washed with 6 ml of 1% TFA and UII peptide finally eluted by the addition of 2.5 ml of 50% acetonitrile/1% TFA (v:v). Samples were dried at room temperature, under vacuum using a centrifugal evaporator. UII peptide was determined using a commercially available radioimmunoassay kit (Phoenix Pharmaceuticals, CA, USA) with a range of 1–128 pg per tube and intra-assay coefficient of variation of 7.5%. There is no cross-reactivity with endothelin-1, angiotensin II, or urotensin-related peptide with this kit.

Statistical analysis

On the basis of previous studies,¹⁵ ¹⁷ we calculated that 18 patients per group would be required to demonstrate a 15% difference in plasma UII concentrations between patients with normal LVEF and those with impaired LVEF (moderate or poor LV function), with $\alpha = 0.05$ and $\beta = 0.1$. Relative quantities of UT receptor mRNA in all patient samples were assessed as comparative cycle thresholds ($\Delta C_t = C_{UT} - C_{GAPDH}$). UII RIA standard curves were analysed using GraphPad Prism V5.5 (La Jolla, CA, USA). The majority of data were not-normally distributed and in some subgroup analyses the numbers were small, so non-parametric testing was applied to the whole data set. UT mRNA expression and plasma UII concentrations were analysed using one-way ANOVA (Kruskal–Wallis) with Dunn’s multiple comparison test, Mann–Whitney (non-paired), and the Wilcoxon signed-rank test as appropriate using GraphPad Prism software. The relationship between right atrial UT and ejection fraction was analysed using the Pearson correlation coefficient. Incidence of myocardial infarction data was analysed using Fisher’s exact test. In all cases, $P \leq 0.05$ was considered statistically significant.

Results

Basic patient data and concurrent medication are shown in Table 1. Data are expressed as median and range unless stated otherwise. In addition, all patients were taking aspirin and either glyceryl trinitrate or isosorbide mononitrate and 38 patients were receiving HMG CoA reductase inhibitors. All patients had documented multi-vessel coronary artery disease. Thirty-seven patients underwent only coronary artery bypass grafting, but additional procedures were performed in three patients (mitral valve repair in two and carotid endarterectomy in one). On the basis of preoperative assessment of LV function, 20 patients had good and 20 impaired LV function. In the impaired group, 15 were moderately and five were severely impaired.

Quantitative PCR assessment

Right atrium

Right atrial samples were available from 38 of 40 patients and TaqMan Q-PCR was performed on all 38. (One sample was used for method development with preliminary primers and in one case emergency bypass was initiated which prevented sample retrieval.) These samples yielded a mean (range) total RNA quantity of 55.5 (8.4–103.2) μg. There were no differences in $C_t$ values for the housekeeper gene when comparing good and impaired LV function groups [median (range): 21.5 (18.4–31.5), 20.7 (18.2–28.6), $P > 0.05$] or when performing subgroup analysis [21.5 (18.4–31.5), 20.9 (18.2–28.6), and 20.5 (18.4–27.5) for good, moderate, and poor, respectively; $P > 0.05$]. These data confirm that GAPDH expression is not affected by LV function and is therefore an appropriate housekeeper.

There were no differences in $\Delta C_t$ values between patients with good and those with impaired LV function (Fig. 1a). Further subgrouping to good, moderate, and poor LV function also failed to reveal any differences in
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Table 1  Patient details, basic clinical data, and medication data. Data are presented as mean (range) for age and BMI or numbers (with % in parentheses).

<table>
<thead>
<tr>
<th></th>
<th>Good (&gt;50%)</th>
<th>Impaired (≤50%)</th>
<th>Moderate (30–50%)</th>
<th>Poor (&lt;30%)</th>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>68 (43–84)</td>
<td>67 (47–80)</td>
<td>68 (58–80)</td>
<td>64 (47–78)</td>
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<td>BMI (kg m⁻²)</td>
<td>28.5 (21.2–36.2)</td>
<td>28.4 (24.0–36.2)</td>
<td>28.9 (24.0–36.2)</td>
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<td>Ex-smoker</td>
<td>15 (75%)</td>
<td>14 (70%)</td>
<td>10 (67%)</td>
<td>4 (80%)</td>
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<tr>
<td>Hypertension</td>
<td>15 (75%)</td>
<td>14 (70%)</td>
<td>11 (73%)</td>
<td>3 (60%)</td>
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<tr>
<td>Previous PCI*</td>
<td>3 (15%)</td>
<td>2 (10%)</td>
<td>1 (6.7%)</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Sinus rhythm</td>
<td>16 (80%)</td>
<td>18 (90%)</td>
<td>14 (93%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Previous MI†</td>
<td>6 (30%)</td>
<td>13 (65%)</td>
<td>7 (46%)</td>
<td>5 (100%)</td>
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<td>Unstable†</td>
<td>4 (20%)</td>
<td>7 (35%)</td>
<td>5 (33%)</td>
<td>2 (40%)</td>
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<tr>
<td>Critical LMS</td>
<td>6 (30%)</td>
<td>5 (25%)</td>
<td>4 (26%)</td>
<td>1 (20%)</td>
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<tr>
<td>ACEI/ARB§</td>
<td>14 (70%)</td>
<td>17 (85%)</td>
<td>13 (86%)</td>
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<td>Diltiazem</td>
<td>7 (35%)</td>
<td>6 (30%)</td>
<td>4 (26%)</td>
<td>2 (40%)</td>
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<tr>
<td>Nicorandil</td>
<td>7 (35%)</td>
<td>5 (25%)</td>
<td>4 (26%)</td>
<td>1 (20%)</td>
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<td>β-blockers</td>
<td>15 (75%)</td>
<td>15 (75%)</td>
<td>11 (73%)</td>
<td>4 (80%)</td>
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<td>Ca²⁺ antagonists</td>
<td>11 (55%)</td>
<td>10 (50%)</td>
<td>9 (60%)</td>
<td>1 (20%)</td>
</tr>
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<td>Clopidogrel</td>
<td>3 (15%)</td>
<td>5 (25%)</td>
<td>5 (33%)</td>
<td>0 (0%)</td>
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\[ \Delta C_t \] (Table 2). With patients rated according to NYHA classification (Fig. 2A), there were also no differences in \( \Delta C_t \) values. However, when comparing \( \Delta C_t \) with CCS (Fig. 2n) angina scale, there was a small but statistically significant difference between grades 2 and 3. When \( \Delta C_t \) was plotted against two-chamber mid-oesophageal TOE ejection fraction, there was a weak negative correlation \( (r^2=0.245, P=0.031) \) (Fig. 3).

**Thoracic aorta**

Thoracic aortic samples were available from 28 patients and TaqMan Q-PCR was performed on all 28. These samples yielded a mean (range) total RNA quantity of 1.5 (0.2–4.5) μg. For two patients, \( \Delta C_t \) could not be calculated as the \( C_t \) for UT was below the limit of detection (undetectable after 50 cycles of PCR); therefore, \( \Delta C_t \) values were obtained for 26 patients. There were no differences in \( C_t \) values for the housekeeper gene when comparing good and impaired LV function groups \( [21.9 \ (20.2–31.1), 26.5 \ (15.6–35.3), P>0.05] \) or when performing subgroup analysis \( [21.9 \ (20.2–31.1), 26.6 \ (18.6–35.3), \text{and} \ 26.3 \ (21.6–27.9) \text{for good, moderate, and poor respectively;} \ P>0.05] \). Again these data confirm the utility of GAPDH as an appropriate housekeeper.

There were no differences in \( \Delta C_t \) values between patients with good and those with impaired LV function (Fig. 1b). \( \Delta C_t \) values in atrium were significantly \( (P=0.011) \) lower than in aorta (Fig. 1c), indicating higher UT expression. Although the numbers were small, further subgrouping to normal \( (n=12) \), moderate \( (n=11) \), and poor \( (n=3) \) LV function also failed to reveal any differences in \( \Delta C_t \) (Table 2). With patients rated according to NYHA (Fig. 2c) classification or CCS (Fig. 2n) angina scale, there were also no differences in \( \Delta C_t \) values.

\[ \text{Plasma UII concentrations} \]

Plasma samples were obtained from all 40 patients. There were no differences between patients with good and those with impaired LV function (Fig. 4a). A historical control is included for comparison. Further subgrouping to normal, moderate, and poor LV function also failed to reveal any differences (Table 2). With patients rated according to NYHA (Fig. 4n) classification or CCS (Fig. 4c) angina scale, there were also no differences in plasma UII concentrations.

**Discussion**

We have shown that atrial tissue expresses greater levels of UT mRNA than matched aortic samples, but that there are no major changes in the expression of this receptor as a consequence of heart disease (we have no disease-free control group data). However, based on the ejection fraction measured with the mid-oesophageal two-chamber view, there was a weak but significant down-regulation of UT as a consequence of increased myocardial dysfunction (higher \( \Delta C_t \) and lower ejection fraction). As there are limited data available regarding UT expression, our right atrial and thoracic aortic results should be viewed as a pilot study. However, there are no published studies which have assessed myocardial and thoracic aortic UT while simultaneously performing a detailed intraoperative TOE and collecting plasma for UII measurement; the simultaneous sampling and measurement of UT and UII as described here is a significant advance as we are able to detail cardiac physiology and molecular biology in live human subjects. There was no control group in this study, but plasma UII concentrations were increased compared...
UII receptor expression in human right atrium and aorta

Fig 1 UT expression measured by quantitative real-time PCR as a function of good (>50%) and impaired (≤50%) LV function in atrium (A) and thoracic aorta (B). There were no differences between the two groups. Data are presented as median, 25th and 75th centiles, and range for (n) samples. In (c), the entire data set for both tissues is compared. ΔCt values in thoracic aorta were significantly higher than in the atrium indicating lower expression. In 25 cases, there were paired samples; when these were analysed using a Wilcoxon signed rank test, the significant difference remained (P=0.033).

with historical data from healthy subjects determined previously in our laboratory by identical methods.16

There have been several studies examining UII/UT system in heart failure and despite some early contradictory results, the current consensus is that plasma UII is elevated.11 18–21 At apparent variance with our data, a difference has been shown in plasma UII between moderate and severe congestive heart failure.22 We feel the difference is due to the patients in our study being sicker, as patients with moderate and severe CHF had mean LVEF measurements of 24% and 18% respectively. This compares with mean LVEF of 56% and 45% for patients with moderate and poor LV function in our study. Different methods of determining LVEF were used and mean LVEF may have been higher in our study because of the increased preload seen with open chest conditions. In addition, an elegant iontophoresis study has shown that UII dilates skin microvasculature in normal control subjects but produces a constriction in patients with heart failure.7 If this were a global phenomenon, then increased afterload might be anticipated, underscoring a need to develop clinically useful UT antagonists.23 24 Only one study to date has assessed myocardial UII and UT in humans,12 and it correlated tissue bank (n=19, diseased tissue) and unused donor heart/autopsy specimens (n=8, healthy controls) with 6-month-old haemodynamic indices. Mean (SD) age in the ischaemic heart disease group was 49 yr (6.6) compared with 64.8 yr (13.5) in our study. This younger age group may represent a more aggressive form of ischaemic heart disease relative to our older cohort. Plasma UII was not measured and diabetic patients were included. The main finding of this important study (on which the current project is based) was the increased expression of both UII and UT (protein and mRNA) in diseased hearts. These changes were most pronounced in the cardiomyocytes surrounding scar tissue in the left ventricle. A study using a rat model of congestive heart failure recently examined the expression of UII-related peptide which was up-regulated in both atrial and ventricular tissue.25 Interestingly, the authors also assessed UT and found an up-regulation in atrial but not ventricular tissue.25 This apparent up-regulation may be a result of an experimentally induced severe disease presentation; the procedure resulted in ~30% mortality.

In the present study, we have concentrated on systolic function and ejection fraction as markers of severity of heart failure. Abnormalities of left ventricular relaxation or diastolic heart failure are increasingly recognized in anaesthesia and critical care. Diastolic heart failure may represent up to 33% of heart failure cases in the 50–70 age group.26 Intraoperative TOE examinations were conducted during surgery and therefore time constraints precluded a detailed assessment of both systolic and diastolic functions. All TOE examinations in our study were performed under general anaesthesia. The effects of anaesthesia on cardiac function are often inconsistent and depend on several factors including drug used, circulating volume, cardiovascular function, and positive pressure ventilation. In NYHA class III–IV patients undergoing cardiac surgery who were anaesthetized using an opioid-based technique, cardiac output was unchanged compared with awake conditions because of the balance between reduced systolic (dP/dtMAX) and improved diastolic function.27 In contrast, a study of patients with normal left ventricular
function (EF >0.5) exposed to both volatile anaesthesia and opioids, increased cardiac filling and left ventricular end-diastolic area were demonstrated in the open chest condition. The absolute numbers from our TOE-derived estimation of ejection fraction were higher than anticipated. This may be explained by the increased preload seen in the open chest condition.28 We detected a weak negative correlation between right atrial UT and ejection fraction as measured in mid-oesophageal two-chamber views.

Plasma UII concentrations were increased relative to a historical cohort from our laboratory. 16 However, within the current study (where blood was collected from patients under general anaesthesia), plasma UII concentrations were not significantly different in patients with normal or impaired LV function. Does general anaesthesia per se affect biomarkers of heart failure? Data on the effect of anaesthesia on BNP concentrations are limited. Studies assessing the effect of anaesthesia on atrial natriuretic peptide have not demonstrated any significant changes in plasma concentrations.29 The effects of anaesthesia on plasma UII concentrations are unknown, but there is some evidence30 for an interaction between GABA A and UII signalling (at least in astrocytes). An assessment of the effects of anaesthesia on plasma UII is currently the subject of ongoing research within our department.

<table>
<thead>
<tr>
<th>Preoperative LV function</th>
<th>Good (LVEF &gt;50%)</th>
<th>Moderate (LVEF 30–50%)</th>
<th>Poor (LVEF &lt;30%)</th>
</tr>
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<tbody>
<tr>
<td>Ejection fraction*</td>
<td>67 (40–75) (10)</td>
<td>56 (35–75) (8)</td>
<td>45 (33–48) (3)</td>
</tr>
<tr>
<td>Plasma UII (pg ml⁻¹)</td>
<td>4.43 (1.65–6.72) (20)</td>
<td>3.68 (2.41–4.95) (15)</td>
<td>4.40 (3.82–4.62) (5)</td>
</tr>
</tbody>
</table>

Fig 2 UT expression is not affected by preoperative assessment of cardiac dysfunction using NYHA classification of heart failure or the CCS classification system. Atrial and aortic data are presented in (A) and (B), and (C) and (D), respectively. Data are presented as median, 25th and 75th centiles, and range for (n) samples. NYHA grade 1, no limitation of physical activity; grade 2, mild limitation of physical activity; grade 3, marked limitation of physical activity but comfortable at rest; and grade 4, unable to perform any physical activity without discomfort, symptoms at rest also. CCS class 0, asymptomatic; class 1, angina with strenuous exercise; class 2, angina with moderate exertion; class 3, angina with mild exertion; and class 4, angina with any level of physical exertion. There were no differences between the groups except in the atria where CCS classification system data (n) was significant by Kruskal–Wallis with a post hoc difference between class 2 and class 3 (Dunn’s test).
Of the 20 patients with impaired LV function, 15 had moderate impairment and only five had severely impaired LV function. We have used the right atrium as a surrogate marker for the left ventricle. Clearly, this has potential limitations, but this model provided a practical solution to obtaining adequate numbers of clinical specimens within the ethical constraints of obtaining cardiac tissue samples from living patients. The right atrium undergoes structural changes secondary to both congestive heart failure and myocardial infarction. In an experimental dog heart failure model, right atrial fibrosis and remodelling occurred which was subsequently attenuated by angiotensin-converting enzyme inhibitors (ACEI).31 Proliferation of atrial fibroblasts and increased susceptibility to stretch may both contribute to post-myocardial infarction bradyarhythmias.32 33 In patients with symptomatic congestive heart failure and an estimated EF of 25% who were subjected to electrophysiological testing, atrial remodelling was noted. This was characterized by both structural and mechano-electric changes.34 Increased UII/UT expression in atrial tissues, although not as pronounced as ventricular changes, has been described.12 Collectively, the above studies might suggest that the right atrium is a reasonable surrogate marker of the disease processes of the left ventricle. Right atrial tissue provided a homogenous sample of cardiac muscle. Conversely, thoracic aortic punch biopsies were a more heterogeneous tissue consisting of endothelium, smooth muscle, and atheroma. One major weakness inherent to all mRNA measurement-based approaches is that it is assumed that message equals expressed receptor protein. Indeed, we have assumed that increased UT mRNA will translate into increased UT protein that is correctly incorporated into the tissues. At best, we can say alterations in mRNA can only provide an approximation of variations in protein.

In conclusion, our data suggest that increased myocardial UT expression previously described may be a ventricular scar tissue phenomenon as UT is not globally up-regulated and may even be down-regulated within the right atrium. Further studies are required.


