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Regional circulatory distribution of novel cardiac bio-markers and their relationships with haemodynamic measurements

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ABSTRACT

Background: Regional sampling may identify sites of production or removal of novel biomarkers in the circulation; their relationship to haemodynamic measurements may clarify their association with the pathophysiology of heart failure.
Methods: Samples were obtained from up to eight circulatory sites from 22 patients with left ventricular dysfunction undergoing elective cardiac catheterisation. The plasma concentrations (PC) of six biomarkers [mid-regional pro-atrial natriuretic peptide (MR-proANP), C-terminal pro-endothelin-1 (CT-proET-1), mid-regional pro-adreno-medullin (MR-proADM), high sensitivity pro-calcitonin (hsPCT), copeptin and galectin-3 (Gal-3)] were measured.
Results: Plasma concentrations of MR-proANP were highest in the pulmonary artery (PA) and left ventricle, suggesting myocardial production. Lower concentrations of copeptin, CT-proET-1, MR-proADM and hsPCT were found in the supra-renal inferior vena cava (SRIVC) sample suggesting renal extraction. Plasma concentrations of Galectin-3 varied little by sampling site. Plasma concentrations of MR-proANP ($R = 0.69$, $P = 0.002$), MR-proADM ($R = 0.51$, $P = 0.03$), CT-proET-1 ($R = 0.60$, $P = 0.009$) and Copeptin ($R = 0.47$, $P < 0.05$) measured from PA samples correlated with PA systolic pressure. There was no relation between any measured marker and cardiac index.
Conclusions: Regional sampling shows variation in the plasma concentration of various novel peptides that provides clues to sites of net production and removal. Plasma concentrations of several biomarkers were positively correlated with pulmonary artery pressure.

1. Introduction

Cardiac biomarkers, such as natriuretic peptides, have an established role in the diagnosis and risk stratification of heart failure (HF) [1–5]. Other biomarkers may reflect the diverse pathological processes, in the cardiovascular and other systems, which contribute to the development and progression of HF and are potential targets for novel treatments. Plasma concentrations of biomarkers reflect the net balance between production and clearance but cannot distinguish between these two. Little is known about sites of production or disposal of many biomarkers or their relationships with haemodynamics, which could help explain their associations with adverse outcome [6–10].

We sought to evaluate regional differences of six novel biomarkers sampled across multiple sites in the arterial and venous circulation to explore likely sites of production and disposal. We also studied their association with haemodynamic measures of left and right heart function. The biomarkers were chosen to reflect different pathways potentially involved in heart failure: mid-regional pro-atrial natriuretic peptide (MR-proANP), a stable fragment of the ANP prohormone which reflects atrial pressure or transmural stress [11–12]; mid-regional pro-adreno-medullin (MR-proADM), a vasodilator peptide that may also offer cytoprotection [13–15]; C-terminal pro-endothelin-1 (CT-proET-1), which mirrors the production of endothelin, a potent vasoconstrictor reflecting endothelial dysfunction [16–17];C-terminal pro-arginine vasopressin (CT-proAVP or copeptin), a measure of AVP production [18]; ultrasensitive procalcitonin (hsPCT), a marker of infection [19–22]; and galectin-3, a measure of tissue injury and fibrosis [23–24]. All of these markers are elevated in heart failure and, in some way, reflect the severity of disease and prognosis [6–9].

2. Methods

2.1. Study population

Twenty-two patients with left ventricular dysfunction (either a reduced left ventricular ejection fraction [LVEF <50%] or an increased left atrial diameter [LAD ≥4.0 cm] on two-dimensional echocardiography or a raised left ventricular end-diastolic pressure [LVEDP >16 mm Hg] invasively measured) undergoing elective left and

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right heart catheterisation were enrolled (seven patients had significant valvular disease, nine had ischemic heart disease (IHD), one had both severe mitral regurgitation and IHD, two had an atrial septal defect and one had complete heart block with superior vena cava obstruction). The mean age was 65 (±12) years (ranging from 30 to 83), thirteen (59%) were men, seven (32%) had atrial fibrillation, one had chronic lung disease, 19 had reduced functional capacity due to breathlessness, five had a left ventricular ejection fraction <40% and 62% were treated with loop diuretics (Table 1).

The investigation conformed to the principles outlined in the Declaration of Helsinki and all subjects gave their written informed consent.

2.2. Cardiac catheterization

Cardiac catheterisation was performed in the afternoon with patients in a fasting state. Catheters were inserted via the femoral artery and vein to measure: pulmonary artery (PA), pulmonary capillary wedge (PCWP), right atrial, systemic arterial and LV end-diastolic pressures. Cardiac output was measured by the direct Fick method. Venous samples were collected from up to five circulatory sites: the low inferior vena cava (LIVC), supra-renal inferior vena cava (SRIVC), high inferior vena cava (at the level of the hepatic vein; HIVC), superior vena cava (SVC) and pulmonary artery (PA). Arterial samples were collected from the left ventricle (LV) and aortic root. In five cases, samples were also obtained from the coronary sinus. After samples were collected, all patients underwent coronary arteriography and left ventriculography.

Table 1
Patient characteristics. The data shown is mean and standard deviation if the variable is normally distributed or median and inter-quartile range if not. List of abbreviations used: ACE – Angiotensin-converitng Enzyme; ARB – Angiotensin Receptor Blocker; IHD — Ischaemic Heart Disease; COPD — Chronic Obstructive Pulmonary Disease; BMI — Body Mass Index; eGFR — estimated Glomerular Filtration Rate; LVEDD — Left Ventricle End-Diastolic Diameter; LVEF — Left Ventricular Ejection Fraction; LAD — Left Atrial Diameter; NHYA – New York Heart Association.

Patient characteristics	
Demographic	
Age — years	65 (12)
Male — no. (%)	13 (59)
IHD — no. (%)	10 (45)
Diabetes — no. (%)	2 (9)
Hypertension — no. (%)	4 (18)
COPD — no. (%)	1 (4)
Atrial fibrillation — no. (%)	7 (32)
NYHA class — no. (%)	
I	3 (14)
II	18 (82)
III	1 (4)
BMI — kg/m^2	25 (7)
Heart rate — bpm	75 (15)
Blood results	
Haemoglobin — g/dL	13.6 (1.8)
Creatinine — umol/L	89 (19)
Urea — mmol/L	6.1 (4.1–7.5)
eGFR — mL/min/1.73 m^2	77 (18)
Echocardiographic data	
LVEF — %	55 (41–60)
LVEF < 40% — no. (%)	5 (23)
LAD — mm	45 (10)
Moderate or severe mitral regurgitation	5 (23)
Moderate or severe tricuspid regurgitation	8 (36)
Medications — no. (%)	
Beta-blocker	15 (67)
ACE inhibitor or ARB	11 (50)
Loop diuretics	14 (63)
Aldosterone antagonists	7 (32)

Plasma concentrations were compared using aortic samples as reference. In addition, an estimate of net cardiac production or removal was made by comparing biomarker concentrations in the PA with calculated mixed venous blood using the formula (mixed venous concentration = ((3 × SVC) + HIVC) / 4. Further comparisons were made to explore net renal (LIVC to SRIVC), hepatic (SRIVC to HIVC) and lung (PA to LV) production or removal.

Samples were collected into ethylene-diamine-tetra-acetic acid (EDTA) vacutainers. The vacutainers were centrifuged immediately after collection at 3000 rpm for 15 min at 4 °C. The plasma was removed from each and stored in a cryotube at −80 °C prior to batch analysis. The plasma concentrations (PC) of MR-proANP, CT-proET-1, MR-proADM, hsPCT and copeptin were measured using a kryptor analyser (B.R.A.H.M.S. AG, Henningsdorf, Germany), a fully automated system based on time-resolved amplified cryptate emissions (TRACE) technology. Galectin-3 was measured using a manual enzyme-linked immuno-sorbent assay (BG Medicine, Waltham MA, USA).

All samples were measured in duplicate and the average value of the two measurements was used.

The lower limits of detection for these assays were 2.1 pmol/L for MR-proANP, 0.05 nmol/L for MR-proADM, 3.0 pmol/L for CT-proET-1, 4.8 pmol/L for copeptin and 7 ng/L for hsPCT. The functional assay sensitivities, defined as the concentration at which the inter-assay coefficient of variation (CoV) was 20%, were 10 pmol/L for MR-proANP, 0.25 nmol/L for MR-proADM, 10 pmol/L for CT-proET-1, 12 pmol/L for copeptin and 30 ng/L for hsPCT.

3. Statistical methods

Regional differences were expressed as the mean and percentage change. The plasma concentrations of biomarkers were log transformed if they were not normally distributed. Where samples were missing, comparisons were made only where there was a sample in both regions. The differences in the mean assay values between each of the circulatory sites were compared using the paired t-test for related samples. Formal adjustments for multiple comparisons were not made as the study was exploratory to generate rather than prove a hypothesis. The correlations between plasma concentrations of different biomarkers and haemodynamic measurements were examined using Pearson correlation coefficient and scatter plots for samples obtained in the aorta and in the pulmonary artery. Due to the exploratory nature of this study, statistical significance was set at a P-value <0.1.

4. Results

4.1. Patient characteristics

Demographic and echocardiographic characteristics, medications and blood results for the study population are reported in Table 1.

4.2. Differences in regional circulatory distribution and relationship with haemodynamic measurements (Figs. 1 to 6 and Table 2).

4.2.1. MR-proANP

Plasma concentrations of MR-proANP fell between aorta and SRIVC, suggesting renal extraction, and increased again in the pulmonary artery suggesting a contribution from the coronary sinus and/or right heart (Fig. 1). The drop in concentration between the aorta and the SRIVC was close to 10% (P = 0.0004) and there was a significant relationship between eGFR and this difference, again suggesting renal clearance (Fig. 7). MR-proANP measured from PA samples correlated with age [R = 0.59; P = 0.010] and systolic PA [R = 0.69; P = 0.002] and mean PA [R = 0.78; P < 0.001] and end diastolic left ventricular filling [R = 0.55; P = 0.02] pressures.