**Inter-organ cross talk in heart failure: A multisite biomarker sampling study**

**Short title:**

Multisite biomarker sampling in heart failure

Alfred HREC Number: 633/21

**Principal Investigator:** Prof David Kaye

**Associate Investigators:** Assoc Prof Justin Mariani, Dr Shane Nanayakkara, Dr Hitesh Patel, Dr Jason Bloom, Ms Donna Vizi, Ms Liz Dewar, Ms Jia Tang, Mr Nik Hemsley, Dr Waled Shihata, Assoc Prof Bing Wang, Mr Duncan Horlock

**Version Number: v.1**

**October 2021**

# 1 EXECUTIVE SUMMARY

|  |  |
| --- | --- |
| **Executive Summary:** | This is a single centre, investigator-led of the regional origins of biomarkers in patients with heart failure (heart failure with reduced ejection fraction: HFrEF, and heart failure with preserved ejection fraction: HFpEF) and in healthy control subjects, and of the relationship between biomarkers in cardiovascular function. |
| **Objectives:** | To measure the plasma levels of biomarkers (small molecules, proteins and miRNAs in samples of arterial, coronary sinus, renal venous and hepatic venous blood during regional catheterisation.  To cross-correlate regional biomarker levels with   * biomarker release in other organs (e.g. cardio-renal signalling) * cardiac performance (Swan Ganz catheter, echocardiography) * functional capacity (6-minute walk test) * quality of life (KCCQ, EQ-5D) * renal function (eGFR) * medications |
| **Sample size:** | We aim to recruit 50 HFrEF, 50 HFpEF and 25 healthy controls. |

**2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE**

**BACKGROUND**: Over the past 5 years, there have been several significant advances in the management of heart failure patients with the demonstration of favorable effects of the selective sodium–glucose cotransporter 2 inhibitors (SGLT2i) (e.g. the DAPA-HF trial 1) and the angiotensin receptor-neprolysin inhibitor sacubitril/valsartan 2 in HFrEF. More recently, important trials have also been conducted on the effects of both classes of drug in patients with HFpEF including the PARAGON Trial 3 and the recent EMPEROR study 4.

The pathophysiological mechanisms responsible for the beneficial outcomes of these drugs remains somewhat speculative, however in post-hoc cross-sectional analyses of these recent studies the impact of the agents on biomarkers such as NTproBNP and troponin I has been of interest, together with their prognostic impact. Similarly, inflammatory cytokines such as galectin-3 have also been associated with outcome. In more detailed studies, network analysis of the relationships between many biomarkers have been investigated, such as the work of Tromp et al 5 below.

**Chart, bubble chart

Description automatically generated**

**Network analysis of biomarker relationships in HFrEF (left) and HFpEF (right)**

A major limitation of these studies is that biomarker levels are measured in peripheral blood only. The level of any circulating biomarker represents a balance of regional and global secretion and excretion. By performing regional blood sampling from key organs (heart, liver, kidney, lungs) we have conducted numerous studies to better understand the pathophysiology of heart failure. The majority of these studies assessed the levels of individual biomarkers (e.g. noradrenaline). With the advent of multi-marker assay platforms for a range of biomarker classes (e.g. proteins, miRNA, small molecule metabolites) we aim to comprehensively study the regional sources of biomarkers in HF patients and to analyze their relationship to disease pathophysiology with appropriate statistical methods (e.g. principal component analysis). Recently, we showed that whilst a difference in the distribution of inflammatory biomarkers was evident between healthy people and HFpEF patients, this was not explained by cardiac release.

|  |  |
| --- | --- |
|  |  |

**Principal component analysis of inflammatory cytokine levels in arterial blood (left) and the trans-cardiac concentration gradient (right) in controls and HFpEF patients.**

# 3 AIMS

To investigate the inter-relationship between local biomarker release and (1) haemodynamics and (2) the activity of other local biomarker pathways as assessed by the trans-organ concentration gradients of small molecules (i.e. metabolomics), proteins (proteomics) and miRNAs (miRNA array).

# 4 STUDY DESIGN

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Screening Visit**  **(Visit 1)** | **Randomisation (Visit 2)** | **Follow up**  **(Phone call)** |
| Day of study & visit window | **Day -7 (±7)** | **Day 0** | **Day 7** |
| Informed Consent | **X** |  |  |
| Inclusion/exclusion criteria check | **X** |  |  |
| Physical examination | **X** |  |  |
| Electrocardiogram (ECG) | **X** |  |  |
| Medication Review | **X** |  |  |
| Vital Signs | **X** |  |  |
| Peripheral blood test | **X** |  |  |
| Questionnaires (KCCQ & EQ-5D) | **X** |  |  |
| 6-minute walk test | **X** |  |  |
| Trans-thoracic echocardiogram (TTE)  (if not one available within 3 months) |  | **X** |  |
| Catheterisation & multisite sampling |  | **X** |  |
| Adverse Event report |  | **X** | **X** |
| Study review and close |  |  | **X** |

**Healthy Control Subjects (n=25)** will be recruited from the general community via advertising in print and radio and via website postings. Subjects will undergo a detailed medical history check and clinical examination, together with the investigations outlined below.

**Heart Failure Patients (n=100):** Patients will be recruited from the Cardiology Department outpatient clinics and from those patients undergoing clinically indicated right heart catheterisation for the evaluation and management of heart failure.

**Inclusion Criteria**:

Heart failure either HFpEF (n=50) or HFrEF (n=50)

NYHA II-IV.

Ischaemic or non-Ischaemic aetiology.

Stable heart failure therapy for 1 month (a <50% adjustment to diuretics is permissible)

**Exclusion Criteria:**

Prior heart transplantation

Complex congenital heart disease

Unstable heart failure requiring high dose inotropes (milrinone >15ug/min, dobutamine >5 ug/kg/min or adrenaline > 2ug/min) or mechanical circulatory support.

**Sample Size Estimate**: We estimate that 50 HFrEF, 50 HFpEF and 25 controls will be required to conduct the network and PCA analyses.

# 5 STUDY PROCEDURES

* **Medical history and concomitant illness:** will be obtained by interview to review eligibility.
* **Medication history:** will include medications currently taken and prescription medications taken up to 12 weeks before Consent/Screening. Assessment of eligibility will include a review of permitted and prohibited medications.
* **Vital signs**, **anthropometric measurements** and **physical examination:** will be conducted at the times detailed in the study schedule. NYHA class will be determined.
* **12 lead electrocardiogram (ECG):** will conducted by the study team at the scheduled visits. Any new, clinically significant abnormalities will be reported to the patient's medical practitioner.
* **Peripheral blood tests:** baseline bloods will be taken on screening of subjects. Tests include UEC, LFTs, FBE & TFTs, NTproBNP, as well as a bHCG for women of childbearing potential.
* **Quality of Life:** The Kansas City Cardiomyopathy Questionnaire and EQ-5D Questionnaire will be administered.
* **6-minute walk test (6MWT):** This will measure the distance you can walk for six minutes on a hard, flat surface. You will be asked questions to help decide what is the primary factor limiting your walking activity
* **Trans-thoracic echocardiogram** will be performed (if one is not available from the 3 months prior or if the clinical situation has changed) by trained echocardiography staff following standard American Society of Echocardiography measurements. The measurements will include M-mode, 2D, colour, Doppler and tissue Doppler assessments which will give information about left ventricular (LV) mass, LV volumes, LV ejection fraction and LV global longitudinal strain (GLS).
* **Swan-Ganz catheterisation and arterial cannulation**: A 7 Fr venous introducer sheath will be placed in an appropriate cubital vein or the right internal jugular vein under local anaesthesia, using ultrasound guidance. A 3 Fr Arterial cannula will be placed in right radial or brachial artery under local anaesthesia. A Swan Ganz catheter will be advanced to the pulmonary artery under fluoroscopy for measurement of central haemodynamics. Arterial and PA blood gas samples (1.5mL each) will be drawn.
* **Regional blood sampling.** A blood sample (30mL) will be drawn from the pulmonary artery via the Swan Ganz catheter. A 6 Fr multipurpose catheter will then be advanced to the renal vein, hepatic vein and coronary sinus under fluoroscopy via the venous sheath for the collection of blood samples (30mL each) and together with a blood gas sample from the coronary sinus (1.5 mL). This catheterization procedure has been performed by Prof Kaye and colleagues for >25 years at The Alfred Hospital.
* **Sample storage and analysis**. Samples will be stored in a deidentified manner at -80oC in freezers located in Prof Kaye's lab at the Baker Heart and Diabetes Institute. Proteomic analysis will be conducted by contract when appropriate by a commercial vendor (OLINK) using aptamer-based methods. As appropriate individual ELISA or Luminex based assays will be conducted in Prof Kaye and A/Prof Wang's laboratories. miRNA assays will be conducted by A/Prof Wang using miRNA arrays. Metabolomic analysis will be conducted as appropriate at Baker Institute, Monash University or Harry Perkins Centre (NSW) core metabolomic facilities.
* **Statistical analysis**. Between group analysis will be conducted using appropriate testing depending on the comparison (unpaired t-test, Wilcoxon, ANOVA or Kruskal walls etc). Correction for multiple comparison will be applied using Hochberg adjustment. Network analyses and PCA will be performed in R.

# 6 REFERENCES

1. McMurray JJV, Solomon SD, Inzucchi SE, Kober L, Kosiborod MN, Martinez FA, Ponikowski P, Sabatine MS, Anand IS, Belohlavek J, Bohm M, Chiang CE, Chopra VK, de Boer RA, Desai AS, Diez M, Drozdz J, Dukat A, Ge J, Howlett JG, Katova T, Kitakaze M, Ljungman CEA, Merkely B, Nicolau JC, O'Meara E, Petrie MC, Vinh PN, Schou M, Tereshchenko S, Verma S, Held C, DeMets DL, Docherty KF, Jhund PS, Bengtsson O, Sjostrand M, Langkilde AM, Committees D-HT and Investigators. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. *N Engl J Med*. 2019;381:1995-2008.

2. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile MR, Investigators P-H and Committees. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med*. 2014;371:993-1004.

3. Solomon SD, McMurray JJV, Anand IS, Ge J, Lam CSP, Maggioni AP, Martinez F, Packer M, Pfeffer MA, Pieske B, Redfield MM, Rouleau JL, van Veldhuisen DJ, Zannad F, Zile MR, Desai AS, Claggett B, Jhund PS, Boytsov SA, Comin-Colet J, Cleland J, Dungen HD, Goncalvesova E, Katova T, Kerr Saraiva JF, Lelonek M, Merkely B, Senni M, Shah SJ, Zhou J, Rizkala AR, Gong J, Shi VC, Lefkowitz MP, Investigators P-H and Committees. Angiotensin-Neprilysin Inhibition in Heart Failure with Preserved Ejection Fraction. *N Engl J Med*. 2019;381:1609-1620.

4. Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Bohm M, Brunner-La Rocca HP, Choi DJ, Chopra V, Chuquiure-Valenzuela E, Giannetti N, Gomez-Mesa JE, Janssens S, Januzzi JL, Gonzalez-Juanatey JR, Merkely B, Nicholls SJ, Perrone SV, Pina IL, Ponikowski P, Senni M, Sim D, Spinar J, Squire I, Taddei S, Tsutsui H, Verma S, Vinereanu D, Zhang J, Carson P, Lam CSP, Marx N, Zeller C, Sattar N, Jamal W, Schnaidt S, Schnee JM, Brueckmann M, Pocock SJ, Zannad F, Packer M and Investigators EM-PT. Empagliflozin in Heart Failure with a Preserved Ejection Fraction. *N Engl J Med*. 2021.

5. Tromp J, Khan MA, Klip IT, Meyer S, de Boer RA, Jaarsma T, Hillege H, van Veldhuisen DJ, van der Meer P and Voors AA. Biomarker Profiles in Heart Failure Patients With Preserved and Reduced Ejection Fraction. *Journal of the American Heart Association*. 2017;6.