UNIVERSITY OF LEEDS

Heart failure in Patients with Diabetes: cells, crosstalk and consequences

PROTOCOL VERSION NUMBER AND DATE Version 1.0 April 20th 2024

RESEARCH REFERENCE NUMBERS

IRAS number: 343489
Funder's code: BHF RE_23_130040-3
Sponsor's code: 2024-NCT37
CPMS ID: 62251
ClinTrials ID: NCTXXXXXXXX
Edge ID: XXXXXXX

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Chief Investigator:

Signature: ..... Date: 17/04/2024

Name: (please print):

Klaus Witte.....

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LIST OF ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
CRF	Case Report Form
NHS R&D	National Health Service Research & Development
PI	Principal Investigator
PIS	Participant Information Sheet
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SSG	Study Steering Group

KEY STUDY CONTACTS

Chief Investigator	Dr Klaus Witte LIGHT building University of Leeds Woodhouse Lane Leeds LS2 9JT k.k.witte@leeds.ac.uk 00447768254073 00441133926642
Study Co-ordinator	As above
Sponsor	Head of Research Regulatory Compliance, The Secretariat, The University of Leeds, Woodhouse Lane, Leeds, LS2 9JT. Email: governance-ethics@leeds.ac.uk Telephone 0113 3437587
Joint-sponsor(s)/co-sponsor(s)	N/A
Funder(s)	British Heart Foundation
Key Protocol Contributors	Klaus Witte, John Gierula, Scott Bowen, Lee Roberts, Cedric Duval, Khalid Naseem, Mark Kearney.

STUDY SUMMARY

Study Title	Heart failure in Patients with Diabetes: cells, crosstalk and consequences
Internal ref. no. (or short title)	IRAS 343489
Study Design	Observational cohort study
Study Participants	Six groups: people with and without heart failure with and without reduced ejection fraction, and with and without and type II diabetes mellitus.
Planned Size of Sample (if applicable)	600
Follow up duration (if applicable)	5 years of digital contact for vital outcomes
Planned Study Period	5 years of recruitment and 5 years for long term follow-up
Research Question/Aim(s)	1. Conduct a systematic analysis of cardiac muscle, skeletal muscle, endothelial cells, and blood platelets from CHF patients to elucidate the alterations induced by T2DM; 2. Investigate how T2DM influences intercellular communication to promote the progression of CHF; 3. Establish correlations between abnormalities in these cells correspond to heart abnormalities, patient symptoms and clinical outcomes.

FUNDING AND SUPPORT IN KIND

FUNDER(S) (Names and contact details of ALL organisations providing funding and/or support in kind for this study)	**FINANCIAL AND NON FINANCIALSUPPORT GIVEN**
British Heart Foundation	£1,000,000

ROLE OF STUDY SPONSOR AND FUNDER

The University of Leeds will act as Sponsor for this study. As Sponsor, the University of Leeds assumes overall and final responsibility for the initiation and management of this study including study design, conduct, data analysis and interpretation, manuscript writing, and dissemination of results.

The study will be funded through a grant from the British Heart Foundation (BHF). As funder the BHF will have no input on the conduct of the study, the analysis or write-up.

ROLES AND RESPONSIBILITIES OF STUDY MANAGEMENT COMMITEES/GROUPS & INDIVIDUALS

Study Steering Group

The Study Steering Group will consist of the Clinical Co-PIs (Witte and Kearney), and the key collaborators (Professor Kalid Naseem, Dr Cedric Duval, Dr Scott Bowen and Professor Lee Roberts, and Dr John Gierula. The nurse assigned to this study (to be decided) will also form part of this group. The Study Steering Group will meet every three months and will coordinate recruitment and all study-related activities and will also be responsible for reviewing all adverse events and for declaring these within 24 hours to the Sponsor.

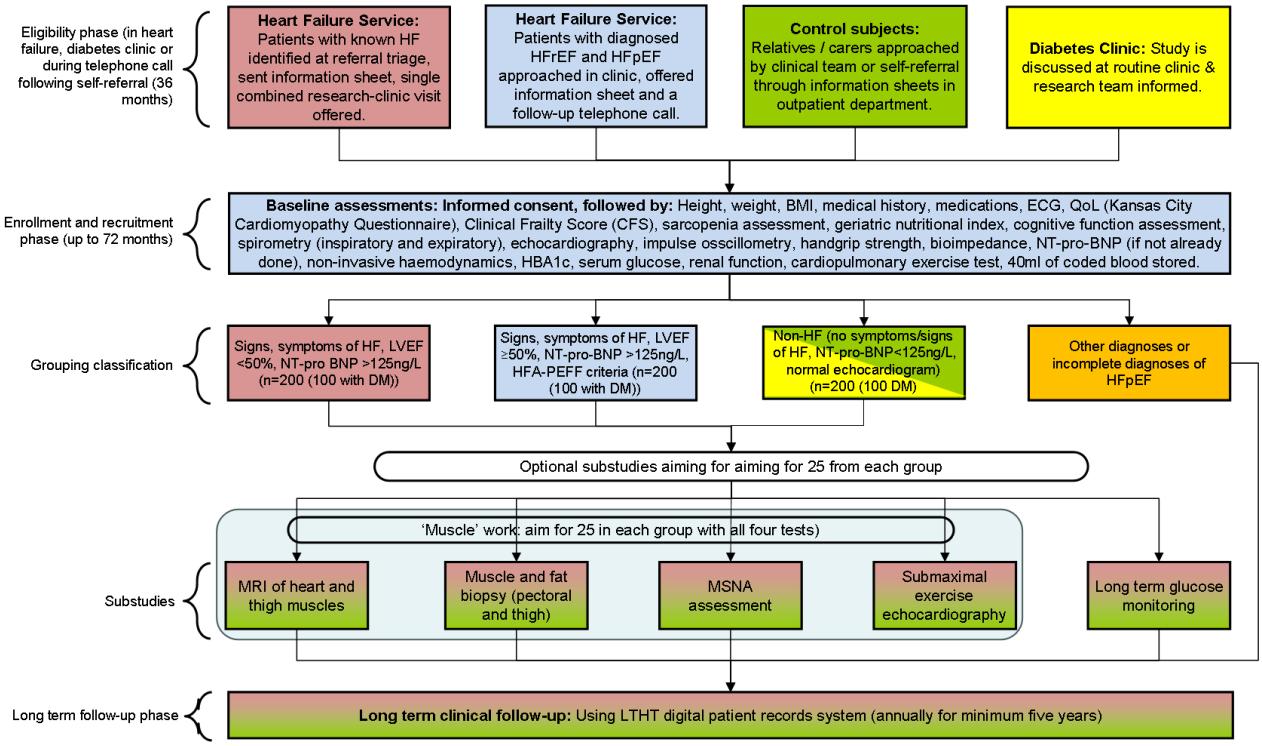
Patient & Public Involvement Group

A PPI Advisory Group consisting of two patients and one carer has been formed to aid study design, set-up, design and writing of the patient-facing documentation. In the preparatory phase, they have helped with patient-facing documents and ethical approval application. During the recruitment phase, they will meet with the Steering Group every six months to review recruitment progress and participate in safety and adverse events monitoring. They will therefore be involved in the 6-monthly decision to continue the study or to cease recruitment.

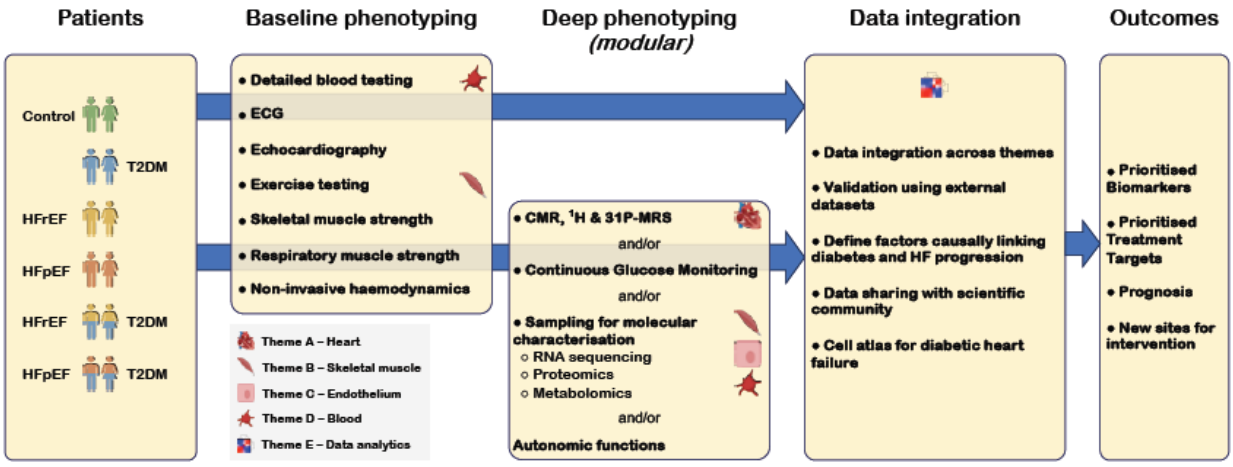
PROTOCOL CONTRIBUTORS Dr Klaus Witte, Dr John Gierula, Dr Cedric Duval, Dr Scott Bowen, Prof. Lee Roberts, Prof M. Kearney, Prof. K. Naseem.

KEY WORDS Chronic heart failure, type II diabetes mellitus, organ crosstalk

STUDY FLOW CHART



STRATEGY OF THE OVERALL PROJECT



SECTION B: STUDY PROTOCOL

TITLE: Heart Failure in Patients with Diabetes Mellitus: cells, crosstalk and consequences.

ABSTRACT: Heart failure is a leading cause of mortality and morbidity worldwide[1],[2] and is characterized by symptoms such as shortness of breath, peripheral oedema and impaired exercise capacity due to structural or functional heart disease. The condition is associated with impaired longevity, high levels of morbidity (poor quality of life) and enormous costs for the healthcare system. In the western world, approximately 1-2% of the population suffer from heart failure, increasing to over 10% of people over the age of 70. The prognosis of patients with severe heart failure is similar to that of patients with cancer.

Diabetes mellitus is reaching pandemic proportions across the globe. A condition previously characterised simply as a relative or absolute deficiency of insulin and diagnosed by elevated sugar levels in the bloodstream, it is now much better understood as a syndrome of impaired glucose tolerance accompanied by a range of metabolic abnormalities that can be observed in all tissues of the body. This metabolic remodelling is accompanied by higher risks of chronic disease particularly cardiovascular disease including atherosclerosis but also, even in the absence of coronary artery disease, a considerable increase in the risk of heart failure.

These two morbid and life-limiting illnesses commonly occur together, each worsening the other in a synergistic relationship that leads to greater symptoms, resistance to therapies and shorter longevity.

Further detail on how these diseases interact is urgently required. However, in addition to understanding the clinical impacts on patients, in order to focus existing therapies and develop new options, we need to understand how diabetes mellitus and heart failure interact at a metabolic level, which cellular-based pathways are affected and how these lead to abnormal cellular function, and thereby to tissue and organ dysfunction. We also need to understand how communication between tissues contributes to the overall pathophysiology.

The present investigation will therefore be a single-centre non-randomised, observational study involving an unselected but highly phenotyped cohort of patients with and without heart failure and with and without type 2 diabetes mellitus and controls without disease. Patients will be recruited from heart failure and diabetes clinics and controls will be identified from these patients' carers and their relatives through direct contact and through advertisements in outpatient departments.

Participants will undergo cardiac ultrasound, exercise testing, assessments of lung function, non-invasive haemodynamics, autonomic function, hand and leg muscle strength, and lung function assessment along with blood testing. At additional optional visits, unselected volunteers will be given the opportunity to participate in optional substudies where they will undergo steady state exercise with simultaneous heart ultrasound, assessments of autonomic function, longer term glucose monitoring, sampling of a small amount of fat and muscle, and MRI scanning of heart and thigh muscles. Participants' direct involvement will end at that point although we will continue to monitor them using their digital records on an annual basis for up to 5 years to gain information on the prognostic value of the metabolic and haemodynamic testing. Tissue and blood samples will be coded and stored in a Human Tissue Authority-approved freezer until analysis following which they will be related to clinical variables with the aim of identifying mechanisms by which metabolic disease (in this case

diabetes mellitus) influences the progression of heart failure with the long term objective of informing the development of more effective treatment strategies.

The present investigation will allow us to advance our understanding of how metabolic disease and heart failure interact with the goal of developing targeted interventions that could open new treatment avenues.

BACKGROUND AND RATIONALE: Heart failure is a disease of heterogeneous aetiology, which is characterized by typical symptoms such as shortness of breath, peripheral oedema and inability to exercise due to structural or functional heart disease.[3] Chronic heart failure (HF) results in high mortality and morbidity in patients as well as enormous costs for the healthcare system. Furthermore, the prognosis of patients with severe heart failure is similar to that of patients with cancer.[4] In the Western world, around 1-2% of the population suffers from heart failure, while the figure rises to over 10% in people more than 70 years of age although recent figures from western Europe are projecting rates of more than 15%.

Heart failure is classified into two main groups based upon the presence or absence of impaired left ventricular function. People with heart failure have reduced ejection fraction (HFrEF) or preserved ejection fraction (HFpEF). Over the past decades, significant advances have been made in managing HFrEF through the development of evidence-based pharmacotherapies, implantable devices, and innovative care models. Until recently the medical management of HFpEF was limited to symptom control using diuretics and rate control of atrial fibrillation.

Modern treatment of HF with reduced ejection fraction (HFrEF): Medical management of HFrEF has expanded to include four classes of agents proven to prolong life and reduce hospitalizations. Guidelines do not advocate any specific drug alone, but rather that optimal therapy for HFrEF should include all four medications.[5] When these agents are titrated to their optimal doses in a coordinated program, these agents together can achieve a considerable gain in life expectancy.[6] However, the reality from observational studies is that whilst a dose response is apparent in patients with co-morbidities,[7] the prognosis remains poorer for these patients, especially those with diabetes mellitus than those without co-morbidities in whom an almost normal life expectancy is possible.[8]

Is the prognosis of heart failure really improving? The challenge facing people with heart failure and their families, carers and healthcare teams, is that although patient-orientated outcome rates in trials seem to be improving, this is not universally reflected in data from registries. Whilst recently published data from Denmark suggests that the mortality from HF is improving in younger patients, this was not seen in older people (figure 1).[9] Recent data from the USA go further, suggesting that gains in prognosis made 20 years ago have reversed (figure 2). It is likely, that these data are partially due to the increasing proportion of those with HF who have HFpEF.[10] These people are generally older, have more co-morbdities, and have a limited response to standard medical therapies.

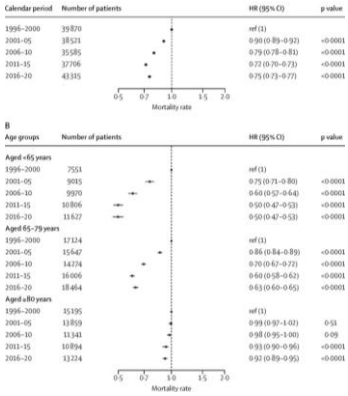


Figure 1: The challenge facing heart failure patients, carers and healthcare teams. Reproduced from 9.

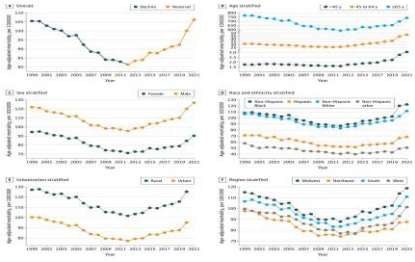


Figure 2: The reversal of gains in prognosis in HF in the USA. Reproduced from 10.

The challenge of HF with preserved ejection fraction (HFpEF): HF has traditionally been viewed as a failure of LV systolic function, with reduced LV ejection fraction (EF) used to define systolic dysfunction, assess prognosis, and select patients for therapeutic interventions.[11] During the last two decades a syndrome that includes symptoms of breathlessness and congestion but in the presence of 'normal' ejection fraction has been increasingly recognised and it is now well established that HF can occur in the presence of LVEF in the normal range. As discussed above HFpEF accounts for a substantial and

increasing proportion of clinical cases of HF.[12,13] These individuals have similar symptoms and impairments in quality of life as those with heart failure with HFrEF.[14]

Moreover, the clear understanding that we have gained about the pathophysiology of HFrEF has contributed to the development of therapies that extend longevity, and clinical trials of drugs targeting activation of the renin angiotensin aldosterone (RAAS) and sympathetic nervous system (SNS) have been shown to reduce the risk of death and hospitalisation due to progressive HF in patients with HFrEF. These agents have not however, shown such favourable results in patients with HFpEF.[15] where (with the possible exception of sodium-glucose co-transporter 2 inhibitors,[16,17]) the benefits of the agents are attenuated for those with higher LVEF, [18,19] and for the overall population with HFpEF no therapies have been shown to improve survival.[20,21] This suggests that mechanisms leading to the development and progression in patients with HFpEF may be different from patients with HFrEF. Fundamentally, perhaps HFpEF is either a condition involving different pathophysiological drivers, or our diagnostic criteria are incomplete and not sufficiently specific.

The impact of metabolic disease: The development and persistence of both phenotypes of heart failure is increasingly appreciated to be contributed to by abnormal metabolism, not only of the cardiomyocytes but also of multiple tissues throughout the body where the heart failure syndrome and metabolic disease independently lead to a variety of structural and functional changes in muscle, fat, and vascular tissue by way of a series of as yet imprecisely identified cross-talk mechanisms. This might explain the adverse relationship between type II diabetes mellitus (T2DM) and heart failure, where in addition to being contributory to the development and persistence of HF,[22] such that 25-40% if people with HF also have T2DM, the presence of T2DM as a co-morbidity worsens considerably the prognosis of HF.[23,24]

Relevance of diabetes mellitus: The pathophysiological causes of how T2DM contributes to the development and poorer prognosis of heart failure are not yet sufficiently understood. However, it is well known that metabolic abnormalities have functional and structural effects on the heart and peripheral muscles,[25] which appear to be at least partially reversible.[26]

The problem of persistent symptoms in HF: The significant advances in the treatment of heart failure in recent years have focused primarily on prognosis and long-term survival. The approval of new treatments is driven almost exclusively by the impact on long-term survival. Symptom-oriented approaches, on the other hand, have received much less attention over the last 30 years, despite the fact that most patients remain symptomatic despite optimal therapy, and that this persistence of symptoms drives the impaired quality of life. In a cohort of 461 patients seen in a heart failure clinic, after one year of optimized treatment, we found that only a minority had improved symptoms.[27] The majority remained symptomatic with ongoing daily limitations, and this was particularly the case with people with diabetes mellitus as a co-morbidity.

Skeletal muscles in HF: Exercise intolerance is the major symptom in HFrEF, but only part of this can be explained by cardiac (central) dysfunction.[28] Peripheral skeletal muscle pathology is considered a key therapeutic target in HFrEF, as it directly exacerbates symptoms and independently predicts survival.[29] Muscle pathology in HFrEF is characterised by fiber atrophy and weakness alongside early fatigue, consequent to impaired energy metabolism, and abnormal fiber type shifts (Type I to II).[28] Underpinning this muscle pathology

are a variety of mechanisms including elevated protein degradation (e.g. *via* MuRF1), pro-inflammatory cytokines (e.g. *via* IL6, TNFα), reactive oxygen species, and mitochondrial dysfunction. Treatments that slow, or even reverse, the progression of skeletal muscle pathology could offer an opportunity to improve clinical outcomes in HFrEF. However, to date, there remains no established pharmacological treatment for skeletal muscle pathology in HFrEF. Many of the adverse changes in skeletal muscles seen in HFrEF are also present in HFpEF,[30] and include abnormal skeletal muscle blood flow, not expected in HFpEF,[31] suggesting that the pathways by which these changes are induced, based upon data from HFrEF models are more complex than originally assumed.[32]

The lungs and diaphragm in HF: It is unsurprising that the function of lungs and diaphragm are related to symptoms in people with HF even in the absence of chronic airways disease. We have previously described that both expiratory function and inspiratory function are abnormal in people with HFrEF[33] and also that the airways are abnormal even during non-forced assessment with impulse osscillometry.[34],[35] HFpEF is associated with both skeletal muscle and diaphragm weakness[36] in the presence of catabolism (breakdown of structural and contractile proteins).[37] The origins of this catabolism are unclear but could stem from skeletal muscle-cardiac and diaphragmatic cross-talk.[38]

Skeletal muscle-cardiac communication in HF: The heart has the highest energy consumption in the body, which ensures continuous contraction. The energy supply required for this is covered by different substrates such as fatty acids, glucose (sugar), lactate (lactic acid), ketone bodies and amino acids. When healthy, the heart is nourished primarily by fatty acids which are used by the mitochondria (the powerhouse of the cell) to generate energy in the form of ATP). In heart failure, cardiac metabolism is perturbed and associated with impaired mitochondrial function. This is accompanied by increased glucose utilization, which supplies mitochondrial-independent energy (ATP) via glycolysis. Furthermore, it is already known that brown and white adipose tissue and muscle,[39] regulate systemic metabolism through a metabolite-inter-organ signalling axis that implies cross-talk between organs, so abnormal skeletal muscle or lipid metabolism could influence cardiac metabolism both in health and disease.[40,41] It is quite possible, based on our previous data, that these changes could have a negative impact on cardiac metabolism.

Where we are: Our group are global leaders in the field of tissue pathology in heart failure and diabetes mellitus as evidenced by our publication record.[41,42,43,44,28,45,46,47,48,49] These data have thrown light on the problem relating skeletal muscle, fat, the endothelium, the diaphragm and cardiac dysfunction in HFrEF. The explosion of HFpEF and the development of metabolic therapies such as the sodium-glucose co-transporter 2 inhibitors have led to an even greater need to understand the disease processes and how they affect cellular energetics and cross-talk. Our previous work has been undertaken exclusively in patients undergoing pacemaker implantation and this limitation has been a consistent feature in the above publications. Amongst other goals, this project will provide validation and stepwise progress in a wide range of people with and without heart failure and importantly, will also provide unique data in a group of people without disease.

RESEARCH QUESTION

How does the combination of HF and DM lead to the adverse prognosis, poorer response to therapies and worse symptoms?

The present project aims to define the relationship between HF and DM in order to provide an answer to the above question and also begin the process of developing interventions that could help improve the treatment of these most vulnerable subgroups.

There are therefore several overarching primary objectives:

1. Conduct a systematic analysis of the function of skeletal muscle, endothelial cells, fat cells and blood platelets from HF patients to elucidate the alterations induced by T2DM.
2. Investigate how T2DM influences intercellular communication between organs (including platelets and coagulation factors) to promote the progression of the syndrome of HF.
3. Establish correlations between abnormalities in these cells and organ-level cardiac, diaphragm, renal, and skeletal muscle function, patient symptoms and clinical outcomes.

Secondary objectives include:

4. What metabolic pathways are abnormal in people with heart failure, diabetes mellitus and both?
5. Can we identify using metabolomics, a substrate or compound that underlies the metabolic abnormalities?
6. Does organ 'cross-talk' have a negative influence on the functions of skeletal muscle, fat and cardiac function?
7. Is peripheral autonomic activation at higher heart rates reflected in higher activation of cardiac sympathetic tone in HFrEF?
8. Do the laboratory data describing muscle and fat metabolomics relate to clinical variables, response to therapy and prognosis?
9. Can we confirm the skeletal muscle findings from pectoralis biopsies in people having pacemakers in groups with and without heart failure not having pacemakers?

STUDY DESIGN AND SETTING

This will be a single-centre UK-based, unblinded, observational, mechanistic study carried out in 600 people with and without the two phenotypes of heart failure, with and without diabetes mellitus and without either.

PARTICIPANT RECRUITMENT AND ELIGIBILITY CRITERIA

Identification, approach and consent: Participants will be identified from the clinical service at Leeds Teaching Hospitals NHS Trust.

Group A - heart failure: Patients will be approached at specific points during their pathway of care at time points designed to make participation as easy as possible. Those newly referred but with a *previous diagnosis* of HF will be approached by the clinical team during the triage process. They will receive a letter and information offering them direct entry to a research-orientated, one-stop clinic which will be followed-up by the clinical team with a telephone call to answer questions and confirm their interest. Only when they have agreed to have their information passed to the research team, will their details be forwarded in order to make the appointment for the baseline visit. The conduction of baseline tests during the routine clinical visit can avoid a second visit.

Patients with signs and symptoms and a raised NT-pro-BNP but with a *possible diagnosis* of heart failure will be approached in the NHS clinic by the clinical team, once the diagnosis has been confirmed. They will be offered an information sheet, the opportunity to ask further questions and, if during a telephone call, they are interested a further appointment at the research clinic.

Patients attending a HF clinic with an *existing diagnosis* will also be approached at the time of their routine visit, offered an information sheet and the opportunity to ask questions. If they are interested, an appointment for the baseline research visit will be made.
The source of Group A patients will be recorded to allow us to differentiate those with *de novo* HF from those with a previous diagnosis. This will also be facilitated by the documentation of the date of diagnosis.

Group B - diabetes mellitus: Patients attending the diabetes service at Leeds Teaching Hospitals NHS Trust will be approached by the clinical team, offered an information sheet and given a week to digest this. Their details will be passed to the research team who will contact them after one week to confirm interest and organise the baseline visit to the research unit where written consent will be taken.

Participation requirements for groups A and B will include the diagnoses (either HF or DM) AND fulfilling the inclusion and exclusion criteria below:

Group C – control subjects: These will be sourced in two ways. The clinical teams will approach carers and relatives of people with heart failure and diabetes attending the clinics. We will also prepare posters to be displayed in outpatient departments making visitors aware of the study, offering the opportunity for self-referral. These posters will describe the study, the tests that would be done, and telephone numbers for direct contact. There will also be a QR code that will link to an online Form hosted by the University of Leeds to make contact easier. Potential participants will only be required to enter their names and telephone numbers to allow the research team to make contact. Upon self-referral, we will contact them, arrange to post out the information sheets, and offer the opportunity to ask questions. We will either make the appointment immediately or offer them another call at one week to answer further questions.

Participation in Group C will require the subject to be older than 50 years, have neither HF nor DM, nor any long term chronically disabling condition as determined by the investigators (for example chronic airways disease or arthritis limiting day-to-day activity) AND fulfil the inclusion and exclusion criteria as follows:

***Inclusion criteria**:*	***Exclusion criteria**:*
• Age >18 years • Ability to provide written informed consent • Persons who are legally competent and mentally able to follow the instructions of the study staff	• Anemia Hb <8 mg/dl • Patients with acute infectious diseases (e.g. pneumonia) • Patients with heart failure due to sepsis • People with acute myocardial ischemia, which is manifested, for example, by angina pectoris or ECG changes under stress • Patients with acute liver or kidney failure or severe COPD (FEV_1<1.0) • Pregnant and breastfeeding women • People who are institutionalized on official or court orders • People who are dependent or employed by the sponsor or investigator • Taking study medication (of an investigational drug) 30 days before the start of the study

Participants will not be paid for participation, although reasonable travel costs will be refunded according to guidelines laid out by INVOLVE for those attending for research purposes only.

STUDY CLINICAL PROCEDURES:

Baseline visit:

At the baseline visit the following tests will be done in the order in which they are listed here:

1) *Demographic variables including address postcode, medical history, co-morbidities, aetiology of heart failure if relevant, and year of diagnosis of HF (and/or DM), current medication, activity assessment, date of diagnosis of HF and HFA-PEFF[50] score will be recorded:*

	Functional	Morphological	Biomarker (SR)	Biomarker (AF)
Major	septal e' < 7 cm/s or lateral e' < 10 cm/s or Average E/e' ≥ 15 or TR velocity > 2.8 m/s (PASP > 35 mmHg)	LAVI > 34 ml/m² or LVMI ≥ 149/122 g/m² (m/w) and RWT > 0,42 #	NT-proBNP > 220 pg/ml or BNP > 80 pg/ml	NT-proBNP > 660 pg/ml or BNP > 240 pg/ml
Minor	Average E/e' 9 -14 or GLS < 16 %	LAVI 29-34 ml/m² or LVMI > 115/95 g/m² (m/w) or RWT > 0,42 or LV wall thickness ≥ 12 mm	NT-proBNP 125-220 pg/ml or BNP 35-80 pg/ml	NT-proBNP 365-660 pg/ml or BNP 105-240 pg/ml
Major Criteria: 2 points	≥ 5 points: HFpEF			
Minor Criteria: 1 point	2-4 points: Diastolic Stress Test or Invasive Haemodynamic Measurements			

Figure 3: The Heart Failure Association preserved ejection fraction score.

2) *Two quality of life questionnaires* (the Kansas City Cardiomyopathy Questionnaire and EQ5D-5L) and one *cognitive function* assessment score.

3) *Frailty assessment:* Frailty is an important risk factor for adverse outcomes in heart failure.[51] One component contributing to frailty is muscle strength. Hence, we will include an assessment of frailty known as the clinical frailty scale (CFS). This is not a questionnaire and requires no input from the patients. We will also however, include a questionnaire specific for sarcopenia to improve the identification muscle loss,[52] and, using the baseline variables of body height, weight and serum albumin (from the blood tests) we will document the geriatric nutritional index tool which is related to prognosis in HF (GNRI = (14.89 × serum albumin [g/dL]) + (41.7 × weight [kg] / ideal body weight [kg]).[53,54] We will also ask the patient to answer 5 questions that form the sarcopenia assessment (SARC-F).[55]

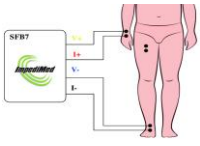
4) *Blood tests for kidney function, glucose levels and HbA1c, platelet and clotting function and metabolic assessment.* We will collect 50mls of venous blood through a single blood draw during the visit which will be divided into tubes for clinical and research purposes. The 40mls required for research purposes will be aliquoted and coded for participant anonymisation and transferred to the University of Leeds for analysis of platelet function, metabolomics and proteomics as described below. The blood sample will be collected through a small cannula through which, after blood sampling, a small wire will be passed through the cannula up the vein in order to collect some of the cells lining the inner surface of the vein.[56][57] This is painless and takes less than one minute. If the participant is to undergo an exercise test (see below), the cannula will be left in to allow us to take a small (2.5ml) blood sample at the immediate cessation of exercise.

Figure 4: The handgrip dynamometer

5) *Handgrip strength:* Lower handgrip strength is a marker of adverse outcome in HF,[58] and we have previously demonstrated a relationship to exercise capacity. As a non-invasive marker of skeletal muscle function we will ask each patient to perform maximal handgrip strength assessment using a standard handgrip dynamometer (Jamar). Maximal strength will be recorded as the best of three attempts on the non-dominant arm. Endurance will be assessed by the duration of the ability to hold a minimum of 80% power (best of three attempts).

6) *Bioimpedance to assess lean mass:* Higher lean muscle mass and higher fat mass are associated with better outcomes in people with heart failure.[59] Body composition assessment may help define and clarify the obesity paradox in heart failure where, despite a higher rate of heart failure in obese people, worse rates of diabetes (an adverse factor in CHF) and lesser exercise tolerance, obesity is associated with better outcomes in cohort studies of people with prevalent heart failure. We will use a portable bioelectrical impedance device (ImpediMedSFB7) to assess the proportion of muscle and fat.

Figure 5: Bioimpedance to assess body composition.



7) *Lung and diaphragmatic function assessment.* All participants will undergo formal spirometry to measure inspiratory and expiratory capacity. We will also use impulse osscillometry to measure non-forced airways resistance.[34,35]

Figure 6: Impulse osccillomtery to assess airways resistance.

8) *Echocardiography:* This has been described previously and is routinely in use in our research projects.**Error! Bookmark not defined.** **Error! Bookmark not defined.**[60] All participants will undergo complete echocardiography with two-dimensional grayscale and tissue Doppler images recorded in two- and four-chamber. The images are stored in the "Echopac" digital imaging system and analyzed offline (GE, Milwaukee, WI, USA). This analysis includes a calculation of LV end-diastolic and end-systolic volumes using the biplane disk method (modified Simpson method). The frame at the R wave is taken as end diastole and the frame with the smallest LV volume is taken as end systole. The LV end-systolic volume (ESV) index (ESVi) is calculated as ESV/body surface area (BSA) in each phase. Each measurement is averaged from three beats. BSA is calculated using the Mosteller equation. Our data to date have confirmed good reproducibility. This method of LV volume calculation is not only widely used and accepted,[61,62] but the key variable is the ESV, which has a particularly high reproducibility.**Error! Bookmark not defined.**

Figure 7: The monitoring system (Finapress) measures cardiac output and peripheral resistance using finger cuffs.

9) *Non-invasive cardiac output measurement:* This will be performed using the Finapres Nova hemodynamic monitor (Finapress Medical Systems, Enschede, Netherlands) during the pacemaker programming protocol described above. The device has been validated using invasive methods of hemodynamic assessment. It continuously measures stroke volume, cardiac output and peripheral resistance using finger plethysmography.

10) *An exercise ECG with metabolic gas analysis (cardiopulmonary exercise test)*. This is a common functional test established in the clinic to assess a patient's performance. The patient will walk continuously on a treadmill until exhaustion. The aim is to evaluate the patient's maximum performance. As part of the examination, blood pressure, pulse and oxygen saturation will be measured at three minute intervals. A stress ECG is usually carried out to assess prognosis, confirm diagnosis and measure response to therapy. It is a routine test in people with heart disease. Immediately upon ceasing exercise, a small blood sample (2.5ml) will be taken from the cannula used for the initial blood test.

Clinical care: All data will be entered into a bespoke eForm on the Leeds Teaching Hospitals NHS trust electronic patient record system (PPM+) and will be available for all clinicians with access to view. Any abnormal findings will be discussed with the participant and passed to the participant's general practitioner or a secondary care appointment will be made with the relevant team. This eForm will allow the input of a unique number that will link to the tissue and blood samples and allow the results of these to be linked with the clinical data.

Substudies:

We will offer participation in six optional substudies and one for patients with a pacemaker). We recognise that not all participants will wish to be involved with these but they will be included in the information sheet and consent forms as optional.

10) *Optional substudy 1 - thigh and pectoralis muscle biopsy:* We have previously undertaken biopsy samples from many hundreds of patients (without and without heart failure) without complication. These have all been collected in people having a pacemaker. Doing this during a pacemaker procedure makes the sampling entirely

pain free and easy to undertake. However, our hypotheses now need confirming in a true control group and also in a wider range of people with the two phenotypes of HF including those that do not need a pacemaker.

We aim to sample tissue from pectoralis muscle and quadriceps muscle in around 25 people in each of the six groups. Previous studies that have assessed skeletal muscle function in HF patients have used muscle biopsies from the *vastus lateralis* muscle of the thigh. This particular muscle is more susceptible to the effects of training and detraining. The pectoralis area provides easy access to fat and, as a muscle not involved in locomotion, the pectoralis muscle gives a better estimation of muscle changes in people irrespective of their lifestyle (sedentary or not). We would like to be able to account for this in our laboratory-based assessments of muscle function.

Pectoralis fat sampling: Under local anaesthetic and using aseptic technique we will make a 5mm incision under the collar bone on the non-dominant side. This reveals the subcutaneous adipose tissue beneath which the pectoralis muscle is easily visible. Three small samples of fat (<3mm in diameter) and three small samples of muscle (<3mm diameter) will be taken and immediately stored as outlined below in liquid nitrogen or other medium depending upon the testing to be done.[47] The incision will be closed with skin adhesive and a dressing applied if required.

Figure 8. The Magnum microbiopsy needle.

Thigh muscle biopsy: The point of incision will be determined by asking the patient to tense their leg so that the correct muscle can be identified. This point will then be marked on them with a marker pen. In each case, the biopsy will be taken from the middle third of the outer part of the thigh. The area surrounding the incision point will be shaved with a disposable razor (if necessary) and covered with iodine. Lidocaine will be injected to anaesthetise the area where the biopsy is to be taken. We have previously used a Bergstrom needle to take samples. Since our early work, where we used the Bergstrom needle, a new technique (microbiopsy) which is more acceptable to patients has been proved to achieve adequate samples allowing metabolic activity to be assessed.[63] This is done with a 16-guage needle (16-gauge Magnum® core disposable biopsy needles (1.65-mm diameter, Magnum® Needle, MN1610; Bard, Covington, GA, USA) and does not require an incision (Figure 8). The clinical tolerance in a study of people with COPD was excellent with pain scores of 0-1 and we expect the pain scores to be similar in people with (and without) heart failure. The total weight of 1–3 microbiopsy samples in these studies ranged from 11–20 mg. These tissue samples will be treated exactly the same as outlined above and below for the *pectoralis major* biopsy. Following the procedure, the biopsy wound will be dressed in several layers, including adhesive skin closure tape, a soft adhesive sterile dressing, a waterproof vapour permeable dressing and a pressure dressing comprising gauze pads and an elastic bandage.

The absolute contra-indications to these biopsy procedures are:

1) Participants with a known bleeding disorder,
2) Participants still taking formal anticoagulation (warfarin or NOACS),

The relative contra-indications to this procedure are:

1) Participants who have demonstrated a tendency to keloid (hypertrophic) scarring,
2) Participants on medications that affect the immune system (e.g. steroids),
3) Participants on aspirin-like drugs (which are known to prolong bleeding),
4) Post-biopsy management: Patients will receive advice on how to look after the dressings in the days following the procedure.

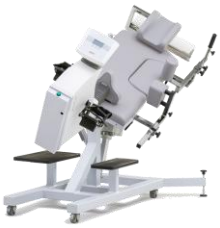


Figure 9. The recumbent echocardiography bench to perform exercise echocardiography.

11) Optional substudy 2 - Submaximal exercise on a stress echocardiography ergometer: We will invite participants for a second visit to allow us to assess how their heart adapts to exercise. Participants will be asked to exercise on a recumbent bicycle at a steady workload of 40-50% of that achieved during the baseline peak exercise test. During this time, we will perform an echocardiogram and the Finapress cardiac output assessment to collect information on contractility (aortic velocity time integral for cardiac output), diastolic function (mitral filling), LV volumes and peripheral vascular resistance[64] at rest and moderate submaximal exercise. This could be particularly important for people with HFpEF who we propose might fall into two groups with and without impaired contractility on exercise.

12) Optional substudy 3 - Sympathetic nervous system activity assessed by microneurography and heart rate variability. The autonomic nervous system is responsible for controlling heart rate, blood pressure and digestion and can be divided into two systems, the sympathetic ('fight or flight') system and the parasympathetic ('rest and digest') system. With advancing age and heart problems especially heart failure, the balance between the sympathetic and parasympathetic branches of the autonomic nervous system alters with increased sympathetic and decreased parasympathetic activity. This change is detrimental for heart function and contributes to decreased quality of life, breathlessness during exercise[65] and shortened life.

The sympathetic nervous system plays a large role in maintenance and progression of heart failure. Treatments that improve heart failure status whilst also lowering sympathetic activation are often associated with improved outcomes, but lowering sympathetic activity also improves heart failure status and outcomes – rather like a chicken and egg scenario that leads to a vicious cycle of deterioration. We want to explore whether heart rate increases lead to increased sympathetic tone, and whether heart rates *above* the critical heart rate augment this. This would give some mechanistic information about the adverse effects of higher heart rates on outcomes.

In order to assess the sympathetic nervous system activation we will invite a subgroup of participants to undergo microneurography for Motor Nerve Sympathetic Activity (MSNA) assessment.

The Leeds Cardiovascular Clinical Research Facility and the PI independently have experience of this technique,[65,66,67,68,69,70] but briefly, microneurography uses small electrodes with a tip diameter of 1-3µm (less than the diameter of a hair), meaning that there is no need for local anaesthetic. The electrode is inserted into a nerve just under the skin of the leg. As the electrode is inserted, participants may report a brief sensation of 'pins and needles' or itching along the course of the nerve when the electrode is inserted, but pain is rare. Fewer than 10% of subjects who underwent microneurography experienced symptoms such as muscle weakness or a sensation of numbness, but these subsided within hours and a maximum of two weeks.[71]

To prevent infection, all electrodes used in microneurography experiments will be sterilised before insertion and used only once. If skin lesions or infections are present on the area to be recorded from, microneurography will not be performed. Recordings will be made for 15 minutes. We plan to invite 100 patients to undergo microneurography at baseline (25 from each group) aiming for 20 complete datasets from each group.

These participants will also be invited to carry a heart rhythm monitor for 24 hours to document the natural rise and fall of their heart rate (heart rate variability) over 24 hours. This is a routine test in cardiac patients.

13) Optional substudy 4 – Force Frequency Relationship assessment (for participants with pacemakers) force frequency relationship (FFR)

The echocardiographic approach will be the same as for the baseline resting echocardiography as outlined above. In those people with pacemakers we will undertake echocardiography at three or four pacemaker-induced heart rates in order to subsequently calculate the FFR. This study requires no patient effort.

PACING PROTOCOL: Echocardiographic images are acquired at rest with intrinsic atrial rhythm or a baseline rate of 40 beats per minute, after which atrial pacing is initiated in DDD mode (or VVI in patients with atrial fibrillation (AF)) for CRT patients. For subjects without CRT, we will program AAI mode (or DDD with long AV delays to avoid RV pacing). We do not exclude patients with single-chamber VVI pacemakers to avoid the deleterious effects of RV pacing. Basic heart rate programming will be the next highest multiple of ten over the intrinsic heart rate. After four minutes, another series of images is recorded and then the stimulation frequency is increased at stepwise intervals of 15 bts/min, with images recorded every four minutes. This is repeated until the maximum predicted heart rate set by Åstrand (200 years old) is reached or the patient develops discomfort (angina or palpitations). This point is considered peak and stimulation is reset to basal settings. For safety reasons, the subjects are asked to stay in the clinic for another 30 minutes.

BLOOD PRESSURE MEASUREMENT: Calculating the end-systolic pressure-volume ratio requires measuring the LV pressure at end-systole . Systolic blood pressure (SBP) is used as a surrogate. Blood pressure is measured according to Riva Rocci.[72]

FORCE-FREQUENCY CALCULATION: Dividing systolic pressure by LVESVi (SP/LVESVi) yields a surrogate of contractility[73,74,75,76,77] which has been validated against invasive methods.[73,77] We will plot heart rate versus contractility for each patient and at those with a parabolic curve define the 'critical HR' as the HR where the relationship between SBP/LVESVi reaches its maximum value. For those where multiple increases in HR induce a decrease in SBP/LVESVi, we will define the resting or baseline HR as “critical HR”.[78]

14) Optional study 5: Cardiac Magnetic Resonance for cardiac contractility and ischaemia assessment

An MRI scan is standard of care for heart failure patients to identify the severity of the left ventricular dysfunction, the involvement of the right heart, the presence of myocardial scar and ischaemia. We have previously used it to describe contractility at higher heart rates.

We will invite all participants in each of the 6 groups to have a cardiac MRI scan unless they have had one of sufficient quality in the preceding 6 months in which case we will ask them if we can use their MRI data for research purposes.

An MRI scan does not involve ionising radiation. Exclusion criteria for a clinical MRI scan at Leeds Teaching Hospitals NHS Trust will be followed. If participants have a pacemaker, standard operating protocols will be followed according to the MRI unit at Leeds Teaching Hospitals NHS Trust to ensure that the device is MRI-conditional and programmed accordingly.

15) *Optional study 6: Continuous ambulatory blood glucose measurement*

Continuous blood glucose monitoring (CGM) is increasingly routine in people with type 1 diabetes mellitus. As described above, it is our hypothesis that impaired glucose tolerance in people with heart failure is a driver of adverse outcomes. However, it is also possible that even in people with heart failure but without diabetes mellitus, impaired glucose tolerance and metabolic changes associated with this contribute to the progression of the condition. We therefore plan to add a period of two weeks of CGM to around 30 people in each of the six groups. The diabetes team in Leeds Teaching Hospitals Trust use the Libre Freestyle device routinely and we plan to use this device in this study. If participants are interested, this sensor can be applied at the baseline visit. The nurses in the diabetes centre and those in the CV-CRF will work together to provide the sensor and the limited education required. The data are stored in the senor up to every minute and can be downloaded to either

a bespoke monitoring device or through a mobile phone application. No participant interaction is required. If the participant wishes not to connect their mobile phone to the sensor, they can simply send us the sensor and we can download the data. On the other hand, we can provide a reader that connects to the sensor or the participant can download the application. In either of these circumstances, we would provide the participant with an NHS email address to which they can connect their reader or their application so that we can safely view and download the information. After two weeks, we will telephone the participant and organise that the sensor and reader are posted to us (in the event of no planned further optional visits), or advise the participant that the sensor can be discarded (in the event that the participant has used the mobile telephone application and no further visits are planned). Should the participant have planned a further optional visit, we would organise al of this at that visit. Complications are rare and limited to cutaneous reactions.[79,80] Since the device will only be used once for two weeks by each participant we do not expect to have any long term skin problems.

Timing and combination of the substudies

It is not expected that all participants would undertake all substudies although it is entirely possible to do these within four visits since some can be combined. The baseline visit is expected to last around 2 hours.

Participants with a pacemaker could have the force frequency assessment at the baseline visit (substudy 4). Participants willing to have the continuous blood glucose assessment (optional substudy 6) could have this placed during the baseline visit. The biopsies are likely to require an additional visit of their own, but the steady-state exercise test and the microneurography and Holter heart rhythm monitor (substudies 2 and 3) can all be accomplished during a single visit of 60 minutes. The MRI scan of the heart and thigh muscles (optional study 5) will require a further separate visit.

STUDY TISSUE ANALYSIS METHODS

Subcutaneous adipose tissue (SAT) analysis: SAT will be subdivided into 5 pieces, with one piece being placed into MACS tissue storage solution, one into 4% PFA (for histology), one into PBS (for studies of angiogenesis and vascular density), one to BIOPS for respiratory assessment, and a final piece flash frozen in liquid nitrogen and stored at -80ºC (for RNA, protein and metabolite/lipid extraction and qPCR and transcriptomics, immunoblotting, metabolomics, lipidomics and proteomics).

Skeletal muscle sampling: Muscle samples will be split into approximately four portions. One portion of muscle will be immediately frozen in liquid nitrogen and stored thereafter at -80ºC for further molecular analyses e.g. for assessing changes in protein content that regulate muscle growth and atrophy, muscle regeneration, and metabolite and lipid content whereas another portion will undergo microscopy imaging to assess muscle structure. A further portion of muscle will be put into a specialised buffer that will allow functional measures of mitochondrial respiration (for energetics) and contractility (forces) to be performed within fresh isolated fibres. A final portion of muscle will also be prepared in another buffer that will allow isolation of other cells such as satellite or endothelial cells, which will allow cell cross talk to be explored.

Endothelial cells from the venous cannula: These will be separated into two batches, one of which will be immediately frozen for RNA analysis and the other placed in medium to allow culture.

Blood sampling: The 50mls of blood will be processed, half will be spun to provide plasma and both whole blood and plasma will initially be stored at -80°C. To investigate cardiac and peripheral metabolism, "metabolomics/lipidomics" analyses will be carried out as a first step. The "metabolome" includes molecules up to 2kDa such as sugars, lipids, amino acids and their derivatives. For further "biomarker" identification, peptides or proteins will be analysed using non-targeted "peptidomics"/"proteomics" as well as specific ELISA analyses, and determination of circulating microRNAs, non-coding RNA sections, which play an important role play in gene regulation, as well as circulating immune cells using FACS analysis.
One aliquot will be used to explore platelet and clotting function in the six participant groups.

It is likely that of the samples taken, analysis will not use all of the tissue available. Any remaining tissue or blood (we estimate 5-10mls) will be stored appropriately at the University of Leeds in a HTA approved environment until the end of the study. If our work has provided new avenues for which we require the tissue to be stored for longer than the current funding (5 years), we will apply for an amendment to allow longer storage and additional testing. We have therefore asked participants to consent for 10 years of storage.

STUDY ENDPOINTS:

Study primary endpoint: Are there differences in skeletal muscle cellular metabolism assessed by mass spectrometry-based metabolomics and lipidomics between the two phenotypes of heart failure compared with controls and what influence does diabetes mellitus have?

Secondary and exploratory objectives:

1. How does T2DM influence intercellular communication to promote the progression of HF.
2. Are there correlations between abnormalities in these cells and cardiac abnormalities, patient symptoms and clinical outcomes?
3. What metabolic pathways are abnormal in people with heart failure, diabetes mellitus and both?
4. Can we identify using metabolomics, a substrate or compound that underlies the metabolic abnormalities?
5. Does organ 'cross-talk' have a negative influence on the functions of skeletal muscle, fat, platelets and clotting function, and cardiac function?
6. Is autonomic activation reflected in metabolic abnormalities in HFrEF or HFpEF?
7. Is lung function, especially that of the diaphragm and smooth muscle of the medium-sized bronchioles reflective of the metabolic abnormalities and how do these relate to the two major HF phenotypes?
8. Do the laboratory data describing muscle and fat metabolomics relate to clinical variables and prognosis?
9. Is cardiac contractility impairment as measured by cardiac magnetic resonance related to the tissue-based metabolic abnormalities and can we identify different subgroups of contractile function within the heart failure and diabetes groups.

STATISTICAL ANALYSIS AND POWER CALCULATION:

Participants will be divided into 6 groups. Patients with HF will be categorised using the European Society of Cardiology 2016 guidelines on the diagnosis of HFrEF or HFpEF.[81] Participants with HFpEF will be classified according to the presence of primary or secondary HFpEF according to the Heart Failure Association classification.[82] The documentation in participants with HFpEF will include whether the condition is secondary (restrictive cardiomyopathy, hypertrophic cardiomyopathy, constrictive pericarditis or valvular heart disease) or

primary with the presumed leading phenotype and contributors highlighted (by a tick box) according to the recent statement from the Heart Failure Association (age, sex, type 2 diabetes mellitus, obesity, sleep apnoea, arterial hypertension, arterial hypotension, pulmonary hypertension, chronic obstructive pulmonary disease, iron deficiency, coronary artery disease, atrial fibrillation, high heart rate, chronotropic incompetence, atrial functional mitral regurgitation, functional tricuspid regurgitation, cachexia and sarcopenia, very high ejection fraction (>65%/>70%), LVEF between 50% and 55%, HFpEF in patients with cancer).

The presence of diabetes will be determined according to European guidelines as a documented history of one of a fasting glucose value of ≥126mg/dL (7.0mmol/L), a 2-hour plasma glucose after a 75g oral glucose tolerance test of ≥200mg/dL (11.1mmol/L), a HbA1c ≥ 6.5% (48mmol/L) or a random blood glucose level of ≥200mg/dL (11.1mmol/L).

Key primary analyses will be the comparison between the groups in terms of skeletal muscle cell metabolism. Laboratory data will first be analysed for its distribution to determine whether parametric or non-parametric testing should be done. Data with a wide distribution might also be log-transformed to facilitate analysis. The data will be mean centred and scaled (either auto-scaled or pareto scaled). We expect the metabolomic data to be normally distributed with similar means and medians. The data will then by analysed using multivariate techniques (principal component analysis, partial least squares – discriminant analysis) and where appropriate the robustness of the models will be validated (permutation testing) as we have performed previously and is best practice for metabolomic data analysis.[83]

Power calculations for metabolomic analyses were made using pilot metabolomic analyses. These data were generated from plasma of age and sex-matched eligible patients with or without HFrEF undergoing routine pacemaker implantation (Leeds Teaching Hospitals NHS Trust). HFrEF patients had stable HFrEF >3 months with LVEF <50%. Controls had pacemaker-requiring bradycardia, without confounding conditions (e.g. CVD, diabetes, dyslipidaemia, exercise intolerance, CHF or LV dysfunction) (Ethical approval: Leeds West Research Ethics Committee 11/YH/0291; Leeds Teaching Hospitals R&D committee CD11/10015). From these data we used a key metabolite, β-aminoisobutyric acid (BAIBA) to calculate power. Plasma BAIBA concentration is lower in age, sex and BMI-matched CHF patients (Figure 10). Based on this data plasma BAIBA concentration in control patients is 1 +/- 0.5 (SD) (Figure 10). To detect a 0.59-fold change (Fig X) in BAIBA concentration with Beta=0.8 and alpha * ; requires n = 23 per group. We have based the power calculations for the muscle biopsy studies on this.

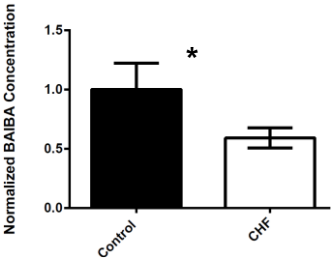


Figure 10: LC-MS analysis of plasma from CHF and control patients. Plasma BAIBA concentration is lower in CHF (n = 10). *, p < 0.05

Appropriate sample sizes for the histology were determined based on our pilot and/or published data. For human experiments, primary outcome measure will be a difference in muscle fibre size. To detect a change of 20% with 80% power at an alpha level of P<0.05, assuming a standard deviation of 28%, requires n=18 patients. To account for a 10% potential attrition rate, a final sample size of a minimum of n=20 patients is required.

LONG TERM PATIENT ORIENTATED OUTCOMES:

Understanding the adverse profile of diabetes mellitus in HF could lead to optimizing treatments for patients with and without diabetes mellitus. This could include metabolism-based targeted therapy to achieve an improvement in metabolism and a personalisation of anti-platelet and anti-coagulation approaches for those with both

conditions. The present investigation will also allow us to expand our understanding of heart-muscle crosstalk with the aim of developing targeted interventions that could open up new avenues of treatment.

RISK ASSESSMENT:

For the patients with HF, this study is additional to standard medical care due to the tissue sampling, the autonomic assessments and the exercise echocardiogram. The other tests are commonly but less rigorously undertaken during the clinical assessment of heart failure. For the group with diabetes mellitus and no HF and the controls, the HF assessments throughout will be research. We will not use additional ionising radiation.

Complications of the clinical procedures: The clinical tests (echocardiography, blood tests and exercise testing) are routine in clinical practice. Exercise testing can leave patients feel a little tired the following day. The questionnaires ask no intrusive questions. and are all validated and in routine use in clinical settings. Any questions that the participants feel uncomfortable with can be left blank.

For the convenience of the participants, the blood tests would occur through a small cannula inserted into a vein. This allows sampling at baseline and a small additional sample after the end of the exercise test. Prior to removing the cannula, a small guide wire can be passed up the cannula around 1cm into the vein. When this is withdrawn it takes some endothelial cells from the inner surface of the vein. This is painless but allows us to explore the function of these cells that form the 'teflon' lining of the veins which is often dysfunctional in people with diabetes and heart failure. This is a routine assessment in our research into the vascular changes in diabetes mellitus. The passing of this wire is painless and is not associated with any additional risk either of bruising or infection.

Risks of the MSNA testing:

This has been done for more than 20 years at Leeds Teaching Hospitals NHS Trust with no long term complications. Some participants can feel a little tingling over the lateral aspect of their calf in the first hours after the test, and very rarely this can last two weeks (but has never lasted longer).

Risks of the pectoralis and thigh muscle biopsy: While this procedure is generally not harmful, some patients may experience some discomfort during the following aspects of the procedure:

- During the injection of local anaesthetic;
- During the extraction of the muscle tissue with the needle, some patients may experience a pushing and pulling sensation within the muscle although this is not painful.
- Very occasionally if the needle catches some of the fascia covering the muscle a sharp pain may be experienced; this is very much like the discomfort of a “dead leg” felt when struck on the leg at sport. It eases after a short period of time and does not interfere with subsequent exercise.

Following the biopsy, patients will not generally experience much discomfort as the area around the biopsy site will have been numbed by the local anaesthetic. Once the local anaesthetic wears off, they may find that the muscle is sore or bruised. After about four hours the muscle can feel rather stiff. However, this will only persist for about 24 hours and then ease off. We would advise against engaging in any contact sports, swimming, or explosive/high-intensity exercises for at least four to five days after the procedure.

The microbiopsy procedure is very low risk with side effects identified as:

- Bleeding from the skin wound.
- Bruising (which can take several days to appear).
- Soreness lasting several days.

Previous research groups using the Bergstrom needle (which we do not plan to use) have reported some of the following which I have listed here simply to point out that firstly the microbiopsy technique is far safer than the technique that we used previously (albeit we had no complications in 25 patients) and also that it is conceivable although unlikely that these events could occur:

- Bleeding from the skin wound.
- Bruising (which can take several days to appear).
- Soreness lasting several days.
- Bleeding within the muscle—can present either as swelling within the muscle or extensive bruising.
- Altered sensation or numbness of an area of skin adjacent to the biopsy scar. This is caused by injury to a small sensory nerve branch and after a few months awareness of this disappears.
- Denervation of a part of the muscle distal to the biopsy. This is caused by damage to a motor nerve branch and will result in atrophy of the affected segment of muscle. This will not be initially obvious but will only become apparent after several months. Although this may be cosmetically visible this complication has never been demonstrated as having any effect on muscle function or performance.
- Superficial wound infection—this is extremely rare and is unlikely to require any treatment. The following are signs/symptoms of infection:
    - Malaise - ususual feelings of triedness/lethagry
    - Fever - feeling atypically warm and/or cold
    - Swelling/hardness of the infected area
    - Pain, tenderness and redness around the infected area
    - Drainage/pus eminating from the infected site
- Deep wound infection—there are no reported cases of this complication with needle muscle biopsies but it can occur in open muscle biopsy in immuno-compromised patients.

These complications have ***not* been reported with the microbiopsy technique, but we will monitor these very closely and provide the ethics committee with an annual update of complications.**

Risks of the CMR scan

Participants can feel claustrophobic in which case the scan can be stopped. The stress part involves the use of an agent to make the heart go faster which can cause the sensation of palpitations and flushing. The contrast used to show blood supply to the heart muscle is routinely used clinically, is not iodine based and rarely causes a contrast reaction. The MRI unit has standard operating protocol for this situation.

Risks of the long term glucose monitoring sensor

These are very uncommon and usually involve a local skin reaction. This resolves within a few days. The sensor (patch) rarely falls off and participants will be taught what to do if this is the case: simply to post it as arranged or bring it to the research unit at their next visit.

Abnormal results:

It is possible that in control participants, or participants without diabetes (or participants without known heart failure) we will find abnormal results. These will be dealt with along usual clinical pathways of care either through their primary care physician or directly to the secondary care service.

PARTICIPANT COMPLIANCE AND SAFETY:

Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant involved in this research with a clinically relevant event not necessarily caused by or related to the research.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Related and Unexpected Serious Adverse Event (RUSAE)	An SAE occurring to a research participant which, in the opinion of the Principle Investigator was: - 'Related' that is, it resulted from the administration of any of the research procedures, and - 'Unexpected' that is, the type of event is not listed in the protocol as an expected occurrence.

All SAEs and RUSAEs will be reported to the Sponsor within one working day of the study team becoming aware of them using the governance-ethics@leeds.ac.uk email. All RUSAEs will also be reported by the CI to the REC within 15 working days.

ROLES AND RESPONSIBILITIES:

Chief Investigator (CI) / delegate or independent clinical reviewer:

1. Clinical oversight of the safety of patients participating in the study, including an ongoing review of the risk / benefit.
2. Using medical judgement in assigning the SAEs seriousness, causality and whether the event was anticipated (in line with the Reference Safety Information) where it has not been possible to obtain local medical assessment.
3. Using medical judgement in assigning whether and event/reaction was anticipated.
4. Review of specific SAEs and RUSAEs in accordance with the trial risk assessment and protocol.

Sponsor:

1. Central data collection and verification of AEs, SAEs, RUSAEs according to the trial protocol onto a database.

Study Steering Group (SSG):

In accordance with the Trial Terms of Reference for the SSG, reviewing recruitment and safety data every three months.

Reporting death

For most studies, we would report all deaths, including deaths deemed unrelated to the study, if they occur earlier than expected to the sponsor. However, given the population involved, including the fact that one of our aims is to assess the prognostic value of the tests being undertaken, it is likely that over 5 years, around 30-40% of patients (and around 10% of controls) will die. Furthermore, the observational nature of the study and the very benign nature of the testing being undertaken, ***we propose to report only deaths deemed by the SSG to be due to the study.***

Pregnancy

This will lead to immediate withdrawal from the study.

DATA MANAGEMENT AND PATIENT CONFIDENTIALITY:

All investigators and study site staff will comply with the requirements of the Data Protection Act 2018 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

Personal information will be collected through direct patient contact during the study visits and through the electronic health records systems of the participating NHS organisation.
Data will be maintained on NHS computers and will only leave this secure site when collated into a single file anonymised (deidentified). This can be done automatically using the Leeds Teaching Hospitals PPM+ system.

Prior to a paper copy of the consent form being given to the participant, it will be scanned and stored in the electronic CRF and the patients electronic health record.

Only the CI, members of the Steering Group, the Sponsor and direct study team members will have access to the password protected folder in the NHS system containing the identifiable dataset.

A unique identifier added at the time of the blood tests and tissue sampling into the PPM+ electronic patient record system will allow the samples to be pseudonomyised prior to transfer to the University of Leeds in order to maintain the connection between clinical information and the blood/tissue analysis.

The Leeds Teaching Hospitals PPM+ system will hold all the records of all patients and participants. Since all data will be collected as part of that system, the clinical testing results will be stored according to NHS criteria. Any electronic dataset produced by collation of these data (for example from the excel product of the eForm will be stored in its usable form for 5 years after the end of the study procedures such that it is possible that some participants' data will be available in that form for up to 10 years.

The data from the continuous blood glucose monitoring system and the heart rhythm monitors in the research participants (including the controls) will be handled in the same way that the clinical data are currently handled.

They can be downloaded into the patient management system of Leeds Teaching Hospitals NHS Trust and will be available to clinicians and researchers.

Dr Witte (the CI) will be the data custodian.

Archiving will be carried out along SOP for the Clinical Research Facility at Leeds General Infirmary and data will be stored for 5 years following the end of the follow-up phase.

WITHDRAWAL CRITERIA:

Participants will be free to withdraw from the study at any time. Anonymised baseline samples and data collected will be kept and analysed as planned. Withdrawal from the study will be communicated to the participant's usual care provider. The reasons for withdrawal will be recorded on the eCRF. We have accounted for a 15% drop out rate in the sample size. Patients withdrawing from the study will be followed through the Leeds electronic patient record system as though they had completed the study for up to 5 years after the date of their withdrawal.

We do not expect the investigation to lead to a deterioration of the clinical condition. Hence we do not plan any early stopping criteria based upon clinical testing or risks. However, should recruitment prove challenging, each year we will review whether the study will reach its goals. It will be the responsibility of the SSG to decide to continue recruitment or to change the protocol to improve same.

STUDY CONDUCT:

Study conduct throughout will be according to the principals of the Declaration of Helsinki. All patients will provide written formal consent prior to enrolment. The study is registered on ClinTrials.gov (NCTXXXXXXX).

Safeguarding implications: The usual processes for which the investigators and the clinical teams receive annual training will be activated should information with safeguarding implications come to light.

Research Ethics Committee (REC) and other Regulatory review & reports: The study protocol and all patient facing material have been submitted for HRA approval. The study will only begin at each site when HRA approval and local R&I approval have been secured. All correspondence with the REC will be retained in the Study Master File.

For NHS REC reviewed research: Substantial amendments that require review by NHS REC and HRA approval will not be implemented until those reviews and approval are in place and other mechanisms are in place to implement them at sites. All correspondence with the REC will be retained. The CI will notify the REC of the end of the study. If the study is terminated prematurely, the CI will notify the REC, including the reasons for the premature termination. Within one year after the end of the study, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC.

Regulatory Review & Compliance: Before any site can enrol patients into the study, the Chief Investigator/Principal Investigator or designee will ensure that appropriate approvals from participating organisations are in place.

For any amendment to the study, the Chief Investigator or designee, in agreement with the sponsor will submit information to the appropriate body in order for them to issue approval for the amendment. The Chief Investigator or designee will work with sites (R&D departments at NHS sites as well as the study delivery team) so they can put the necessary arrangements in place to implement the amendment to confirm their support for the study as amended.

Amendments: All amendments will be submitted by the CI following Steering Group approval to the Sponsor for initial review. It is the sponsor's responsibility to decide whether an amendment is substantial or non-substantial for the purposes of submission to the REC. all amendments both, substantial and non substantial will be submitted to the HRA using the HRA amendment tool following sponsor review. All amendments will also be notified to the local R&D office of participating organisations.

Peer review: This study has undergone several stages of peer review. Initial scientific peer review was carried out by the local research team and then by the funder as part of the decision to support the project.

Patient & Public Involvement: The study has been reviewed in a two-stage process by members of the public and patients. An initial proposal was presented to the Leeds PPI group and subsequently, the final protocol was reviewed and discussed by self-selected members of the PPI group that formed the PPI-advisory group for this study. Patients and carers were involved in shaping the design, methodology of the research. The PPI-AG will be involved in writing up the results for lay members and the dissemination of the findings.

Dissemination: A dissemination plan will be developed in collaboration with the PPI advisors, sponsor and funder. It is likely that this will include formal scientific publication in peer-reviewed speciality medical and translational science journals to coincide with a press release. We will also provide an annual information sheet for participants and a final summary document at the end of the study.

Protocol compliance: The Study Steering Group (SSG) will monitor compliance with the protocol and record all instances of protocol deviations, non-compliances, or breaches.
A serious breach in this setting will be defined as a breach of the protocol or of the conditions or principles of Good Clinical Practice (or equivalent standards) which is likely to affect to a significant degree the safety or physical or mental integrity of the trial subjects, or the scientific value of the research.
All deviations and serious breaches of the protocol will be documented in the patient records and in the electronic CRF and immediately reported to the Chief Investigator and Sponsor (governance-ethics@leeds.ac.uk) within one working day of the team becoming aware of them.
Reports of serious breaches will give details of when the breach occurred, the location, who was involved, the outcome and any information given to participants. An explanation will be given, and the CI will work with the sponsor to prepare this report which will also include what further action the sponsor plans to take.
Serious or recurrent deviations will lead to a ceasing of study recruitment until an investigation has been completed to determine the cause and measures are in place to avoid repetition.

End of the study: The study will end once 600 participants have been recruited and the last patient has completed 5 years of follow-up. However, the steering group together with the patient advisory group can decide to terminate the study early in the event of safety issues.

Continuation of the study: We believe, based upon preliminary data that 200 people in each group will be sufficient to deliver clinically and statistically significant outcomes. However, a comprehensive assessment of this nature including the metabolic and clotting assessments has never been undertaken previously. Should we require greater numbers to achieve more accurate information (or for example, we find that fewer people than expected agree to the tissue sampling) we will approach the ethics committee (with agreement of the Leeds Ethics and Governance team) to increase the sample size for all or specific groups as required.

INDEMNITY:

The University, when acting as Sponsor, has insurance cover in force, which meets claims against it and where those claims arise from the Universities own negligence in its role and activities relating to the study (and which is subject to the terms, conditions and exceptions of the relevant policy). Clinical negligence indemnification will rest with the participating NHS Trust under standard NHS arrangements.

ACCESS TO THE FINAL DATASET:

The final dataset will initially be available to members of the Steering Group and subsequently an anonymised version will be available to other researchers including outside of the University of Leeds in response to a reasonable request and confirmation of appropriate data protection policies and a mutual data sharing agreement.

DISSEMINIATION POLICY AND AUTHORSHIP POLICY:

We will disseminate the results through publications in peer-reviewed journals, at conferences and we will also work with the PPIE group to produce a document aimed at participants. Authorship eligibility will be decided at the end of the study, but it is expected that it will include the Steering Group Members and other team members who have contributed to the data collection, analysis and write up according to the international guidelines.

APPENDICIES:

Appendix 1 – Schedule of Clinical Procedures

	Baseline	Optional visit	Optional visit	Optional visit
Informed consent	X			
Demographics	X			
Medical history	X			
Quality of life questionnaire (KCCQ)	X			
Quality of life questionnaire (EQ5D-5L)	X			
Cognitive function assessment	X			
Clinical Frailty Score	X			
Sarcopenia (SARC-F) questionnaire	X			
Blood tests	X			
Exercise test	X			
Echocardiogram	X			
Pacemaker check (where relevant)	X			
Handgrip strength	X			
Bioimpedance assessment	X			
Lung function, diaphragm and airways resistance assessment	X			
Non-invasive cardiac output assessment (Finapress)	X			
FFR assessment (optional & for people with pacemakers)	X (optional)			
Ambulatory continuous blood glucose assessment (optional)	X (optional)			
Pectoralis fat and muscle biopsy (optional)		X		
Thigh muscle biopsy (optional)		X		
MSNA and 24 hour heart rhythm assessment (optional)			X	
Echocardiogram during submaximal exercise (optional)			X	
Cardiac (and thigh) MRI scan (optional)				X

Appendix 2 – Tissue and blood sample processing and disposition

Thigh muscle sampling:

1) Confirm identification

2) Confirm consent and review planned procedure with participant

3) Ensure appropriate tissue transportation available (for muscle tissue: Eppendorf tube with liquid nitrogen, BIOPS and OCT solutions)

4) Check subject's unique identifier for the samples is prepared and tubes are labelled (also with 'T', 'P' or 'F')

5) Subject is to be made comfortable on the bed.

6) Left knee to be supported by a pillow with a pad below it.

7) Items needed:

- Dressing pack
- Fenestrated drape
- Iodine or similar sterilisation liquid
- 10 mls lignocaine 1%
- Blue needle x 1 and green needle x 1
- Magnum device
- Skin adhesive
- Dressing and bandage
- 11 blade scalpel

8) Identify lower lateral aspect of the quadriceps muscle and mark area to be biopsied with pressure

9) Sterilise the area using swabs in dressing pack

10) Cover with fenestrated disposable drape

11) Inject 5mls lignocaine to the hub of the blue needle and exchange for the green needle injecting a further 2-5mls as necessary

12) Make a small nick in the skin with the scalpel if necessary

13) Pass the Magnum microbiopsy needle as directed in the instructions for this device x 3 passes checking that sufficient muscle has been sampled each time.

14) Place each muscle and fat sample into a cryo, biops, or OCT tube and label with identification number from PPM+ eForm

15) Close the puncture site with skin adhesive if required

16) Dress appropriately and wrap with bandage.

Pectoralis muscle and fat sampling

1) Confirm identification

2) Confirm consent and review planned procedure with participant

3) Ensure appropriate tissue transportation available (for muscle, Eppendorf tubes containing liquid nitrogen, BIOPS and OCT, for adipose tissue, Eppendorf tubes containing 4% PFA, BIOPS, liquid nitrogen, MACS and PBS)

4) Check subject's unique identifier for the samples is prepared and tubes are labelled (also with 'T', 'P' or 'F')

5) Subject is to be made comfortable on the bed.

6) Items needed:

- Dressing pack
- Fenestrated drape
- Iodine or similar sterilisation liquid
- 10 mls lignocaine 1%
- Blue needle x 1 and green needle x 1
- Skin adhesive
- Dressing and bandage
- 11 blade scalpel
- Small self-retaining retractor
- Small needle holder
- Forceps
- Small artery clamp x1

7) Identify location to be incised and mark area to be biopsied with pressure

8) Sterilise the area using swabs in dressing pack

9) Cover with fenestrated disposable drape

10) Inject 10mls lignocaine to the area injecting a further 10mls as necessary

11) Make a small incision (5-6mm) deep enough to allow access to muscle and dilate as standard to visualise pectoralis muscle

12) Sample fat (x 3) and muscle (x3)

13) Place each muscle and fat sample into a cryo, biops, or OCT tube and label with identification number from PPM+ eForm

14) Close the incision site with skin adhesive

15) Dress the wound appropriately.

Following the biopsy procedure(s), subjects are to be asked to rest for a minimum of one hour prior to mobilising. Simple pain relief to be offered (paracetamol).

Sample disposition

- Fat tissue (aiming for four equal pieces) to be labelled with unique identifier and 'F':

MACS tissue storage solution x 1

4% PFA x 1

PBS x 1

BIOPS x 1

Liquid nitrogen x 1

- Muscle tissue (three equal samples of quadriceps and three equal samples of pectoralis) to be labelled with unique identifier and 'P' or 'T':

PBS x 1

BIOPS x 1

Liquid nitrogen x 1

- Blood sampling

50mls of venous blood will be drawn according to quality standards using a single needle stick following aseptic techniques. The blood will be aliquoted following usual procedures for the handling of blood samples as follows:

Order Code	Volume	Cap Colour	Cap Ring Colour	Tube Type	Tests	Special Instructions
Blood samples should be taken in the following order:						
Blood Culture - (aerobic followed by anaerobic) - If insufficient blood for both culture bottles, use aerobic bottle only. Fill with 3-10mls. Higher volume withtn range increases detection rate						DO NOT OVERFILL
KFK 305	4ml	Gold	Gold	Clotting Accelerator and Separation Gel	Routine Biochemistry, Thyroid and Steroid Hormones, Tumour markers and Troponin. Haematinics (B12, Folate, Ferritin)	Not for use ith Renal Unit, CCU, Transplant Units and patients on LV Heparin
KFK 306	4ml	Red	Yellow	Clotting Accelerator and Separation Gel	Immunology (Autoantibodies / Allergy /Specific Proteins)	
KFK 307	6ml	Red	Black	Clotting Accelerator	Bacterial and Viral Serology, Antibiotic Assays	Serology - Please ensure tubes are filled to the line
KFK 122	3.5ml	Blue	Black	Trisodium Citrate	Coagulation Requests	
KFK 387	6ml	Yellow	Black	ACD-B	Transplant Immunology and Tissue Typing	
KFK 320	3ml	Green	Yellow	Li Heparin and Separation Gel	Use instead of Gold tube for units mentioned in Special Instructions. Ammonia	Do not use for Lithium Levels
KFK 028	4ml	Green	Black	Li Heparin	Renin, Aldosterone PLEASE CONTACT LABORATORY BEFORE COLLECTION	Do not use for Lithium Levels
KFK 389	4ml	Lavender	Black	EDTA	Haematology Requests. Cyclosporin, Tacrolimus, HbA1c, Blood Lead, Cadmium, ACTH, PTH TPMT, Lymphocyte Subsets. Bacterial and Viral Nucleic Acid Detection	If requesting HbA1c with either FBC or PTH please provide separate samples for each Please ensure tubes are filled to line. Multiple tests require additional tubes
KFK265	6ml	Pink	Black	EDTA for Crossmatch	HLA Testing and Molecular Tissue Typing	
KFK 330	2ml	Grey	Black	NaF/Ox	Glucose, Lactate, Alcohol	
KFK262	6ml	Royal Blue	Black	Sodium Heparin Trace Elements	Copper, Zinc, Aluminium, Selenium, Chronium, Mercury, Maganese	

Sample	Pathology	Roberts lab	Naseem lab	Ariens lab
Serum	1			
			1	1
Plasma	2	2		
Glucose sample	1			
Micro-nutrients				

One 'gold' (routine biochemistry – renal function, liver function, albumin, haematinics, NT-pro-BNP, thyroid function, troponin), one 'grey' (for glucose) and two 'lavendar' (HbA1c, blood count) tubes will be transferred to the pathology department using the subject's NHS number as ID.

Appendix 3 - Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made

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