Myocardial Mechanisms in Heart Failure with Preserved Ejection Fraction (MM-HFpEF)
A Heart Share Clinical Study

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EXECUTIVE SUMMARY

Heart failure (HF) with preserved ejection fraction (HFpEF) affects up to 32 million people worldwide. Risk factors for HFpEF include older age, hypertension, diabetes, dyslipidemia, obesity and lung disease.[1,2] There are few effective therapies for HFpEF.[3,4] To address this public health need, the National Institute of Health (NIH) established the Heart Share initiative to investigate pathophysiologic mechanisms and diversity in HFpEF. Heart Share includes several observational clinical research studies. The Myocardial Mechanisms in HFpEF Study (MM-HFpEF) is a prospective, multicenter, mechanistic, observational Heart Share study. The goal of MM-HFpEF is to identify altered biological pathways regulating cardiac structure and function in patients with HFpEF. To address this goal, right ventricular (RV) septal endomyocardial tissue will be procured via three transvenous endomyocardial biopsies (EMB) obtained for research purposes in patients with HFpEF referred for clinically indicated or research right heart catheterization (RHC). A blood sample will be obtained at the time of the EMB and biobanked for future correlative assays. Data from the patient's clinical evaluation or from research tests performed as part of the Heart Share Deep Phenotyping Protocol (if the patient is participating in both) will be recorded to characterize participants. The EMB samples will be used for digital histopathology and for omics analyses performed at Core Facilities designated by the MM-HFpEF Core Laboratory (Johns Hopkins University) and the Heart Share MM-HFpEF Working Group. The first broad hypothesis is that discovery multi-omics including non-destructive quantitative digital histopathology, transcriptomics, proteomics, spatial transcriptomic profiling, and spatial proteomic profiling with appropriate bioinformatic approaches will identify novel mechanisms mediating cardiac dysfunction in HFpEF as compared to non-failing (NF) Controls and HF with reduced (HFrEF). The second broad hypothesis is that among patients with HFpEF, there is myocardial pathophysiology diversity which can be identified by clinically defined or machine learning (ML) based clustering of clinical and omics data. Analytic techniques will compare findings among patients with HFpEF with different clinical and/or myocardial histopathologic phenotypic profiles. Comparator group tissues are available from hearts harvested for organ donation but ultimately not used for transplantation (NF Controls) and at explant of failing heart in patients with HFrEF undergoing cardiac transplantation. These samples will be provided to the MM-HFpEF Core Laboratory via an established collaboration with the University of Pennsylvania Cardiac Tissue Repository. Up to 300 HFpEF patients will be enrolled over a five-year period at up to 10 sites participating in the MM-HFpEF study.

1. BACKGROUND

Heart failure (HF) with preserved ejection fraction (HFpEF) affects approximately 3.0 million people in the US and up to 32 million people worldwide. As there is an incomplete understanding of HFpEF pathophysiology, there are few effective therapies for HFpEF.[3,4] Initially, HFpEF was viewed as a sequela of hypertensive heart disease as treatment of hypertension prevents HF.[5,6] Subsequently, a paradigm proposed that pro-inflammatory medical conditions frequently associated with HFpEF caused coronary microvascular endothelial cell inflammation, oxidative stress and deranged nitric oxide (NO) signaling with microvascular and myocardial diastolic dysfunction.[7] Later, this paradigm was expanded to include the potential for deranged NO signalling to lead to nitrosylation and dysfunction of key unfolded protein response (UPR) effectors causing impaired clearance of dysfunctional proteins.[8,9] To date, this paradigm has not been translated into effective therapy for HFpEF. Most recently, the potential for pathophysiologic diversity has been proposed with unique pathophysiologic HFpEF phenotypes requiring specific therapies.[10] Indeed, specific therapy for the "obese HFpEF phenotype" now exists.[11,12] However, the number of HFpEF phenotypes with specific systemic or myocardial pathophysiologic changes and unique therapeutic needs is unclear.[13]

Most evidence supporting current pathophysiologic paradigms in HFpEF comes from cardiac imaging/hemodynamic studies in humans or studies of cardiac tissue from animal models of HFpEF. Findings in animal models may or may not predict human myocardial pathophysiology.[14,15] In contrast to HF with reduced EF (HFrEF), patients with HFpEF do not undergo cardiac transplantation where the failing heart is explanted and can be used for pathophysiologic research. Cardiac biopsies can be obtained at cardiac surgery for severe aortic stenosis (AS) or coronary artery disease (CAD).[16-38] However, studies with myocardial samples from EMB in patients with typical HFpEF and from surgical samples in patients with AS found very different findings.[39,40] Studies most relevant to understanding HFpEF pathophysiology are those where EMB was performed to rule out specific etiologies (i.e. inflammatory or infiltrative disease) in patients presenting with a HFpEF-like syndrome with some tissue used for research. The need for EMB to rule out infiltrative or inflammatory cardiomyopathies has now been largely eliminated with advances in cardiac magnetic resonance imaging, scintigraphy and positron emission tomography.[4] Thus, studies of HFpEF myocardial pathophysiology now use biopsies done for research purposes.[8,29,39-66] Most previous HFpEF EMB studies were hypothesis-driven studies of hypertrophy, fibrosis, systolic or diastolic function or single biochemical pathways. Only recently have discovery approaches using transcriptomics or metabolomics been performed in human HFpEF myocardium, both from a single center.[49,50] There is an urgent need to confirm and extend these exciting initial findings in a multicenter study inclusive of higher numbers of HFpEF patients and (potentially) more diverse HFpEF phenotypes with expanded omics approaches.

Heart Share is a National Institute of Health (NIH) funded initiative investigating pathophysiologic diversity in HFpEF. Heart Share includes studies with novel analyses of clinical data and images from previous NIH studies of HFpEF and NF Control subjects, retrospective and prospective HFpEF registries using electronic medical record (EMR) data and patient surveys and a prospective deep phenotyping (DP) clinical study including comprehensive imaging and physiologic studies with biopsies of fat and skeletal muscle in HFpEF and NF Controls. The Heart Share MM-HFpEF study will investigate myocardial mechanisms mediating cardiac dysfunction in HFpEF.

2. STUDY HYPOTHESES AND AIMS

The long-term goal of this study is to identify novel and therapeutically relevant biological pathways regulating perturbations in cardiac structure and function broadly (vs HFrEF or NF Controls) and within different HFpEF phenotypes. The first broad hypothesis is that discovery omics approaches coupled with appropriate bioinformatics will identify novel mechanisms mediating cardiac dysfunction in HFpEF. The second broad hypothesis is that there is pathophysiologic diversity at the myocardial level in HFpEF. Such diversity may track clinical characteristics (clinical phenotyping) or require computed approaches incorporating omics data. The following Specific Aims will address these hypotheses.

Aim 1. Procure three right ventricular (RV) septal endomyocardial tissue samples via transvenous EMB in patients with HFpEF referred for clinically indicated or research right heart catheterization (RHC).

Aim 2. Characterize the clinical profile of patients with HFpEF undergoing EMB.

Aim 3. Phase 1 Myocardial Tissue Multiomics: In the first approximately 100 HFpEF subjects undergoing research RV septal EMB and in simulated RV septal EMB samples from explanted heart samples from 30 NF control (unused donor) and 30 HFrEF (transplant recipients) subjects, perform quantitative, non-destructive, digital histopathology (FFPE sample), bulkRNAseq (frozen sample 1), liquid chromatography tandem mass spectrometry proteomics using data independent analysis (DIA) (frozen sample 2) and digital spatial transcriptomic and proteomic (subset) analyses (remaining FFPE sample).

Appropriate statistical approaches will determine differentially expressed (DE) genes from the bulk RNAseq data and DE proteins between HFpEF and the two comparator groups and within clinical or ML computed HFpEF phenogroups. Bioinformatic analyses will assess biological pathways suggested by the DE genes and proteins and examine any discordant information based on RNA versus protein analysis. To address the “abundant RNA/protein” limitation to whole myocardium sampling wherein the large volume of myocyte RNA/proteins may prevent detection of RNA/protein changes in other cells or the interstitium, spatial transcriptomics and in a subset, spatial proteomics will be performed in the FFPE samples. Given the rapidly evolving technology and potential for new publications relevant to this field, these analytic approaches will be reassessed by the MM-HFpEF Core Laboratory and the MM-HFpEF Tissue Working Group prior to proceeding on the first 100 EMB.

Aim 4. Phase 2 Myocardial Tissue Multiomics: In the second 200 HFpEF subjects undergoing research EMB, sample processing techniques, appropriate controls and biologic analyses will be designed by the MM-HFpEF Core Laboratory and the Heart Share MM-HFpEF Working Group to build on findings in Aim 3, evolution in omics technology and other advances in knowledge. Phase 2 analyses will include but not be limited to, snRNA-seq, targeted metabolomics, myofilament functional assessments, further spatial transcriptomic or proteomic analyses, further HFpEF phenogroup characterization and more in-depth study of specific pathways suggested to mediate HFpEF pathophysiology based on Aim 3 findings.

Aim 5. Venous blood samples (5 ml) will be taken from the superior vena cava at the time of RHC and processed for serum (red top tubes) to undergo proteomics analysis to investigate potential novel peripheral biomarkers of unique HFpEF phenogroups defined by myocardial analyses in Aims 3 and 4.

For Aims 3-5, tissue and serum analyses will be performed at Facilities chosen by the MM-HFpEF Core Laboratory and the Heart Share MM-HFpEF Working Group.

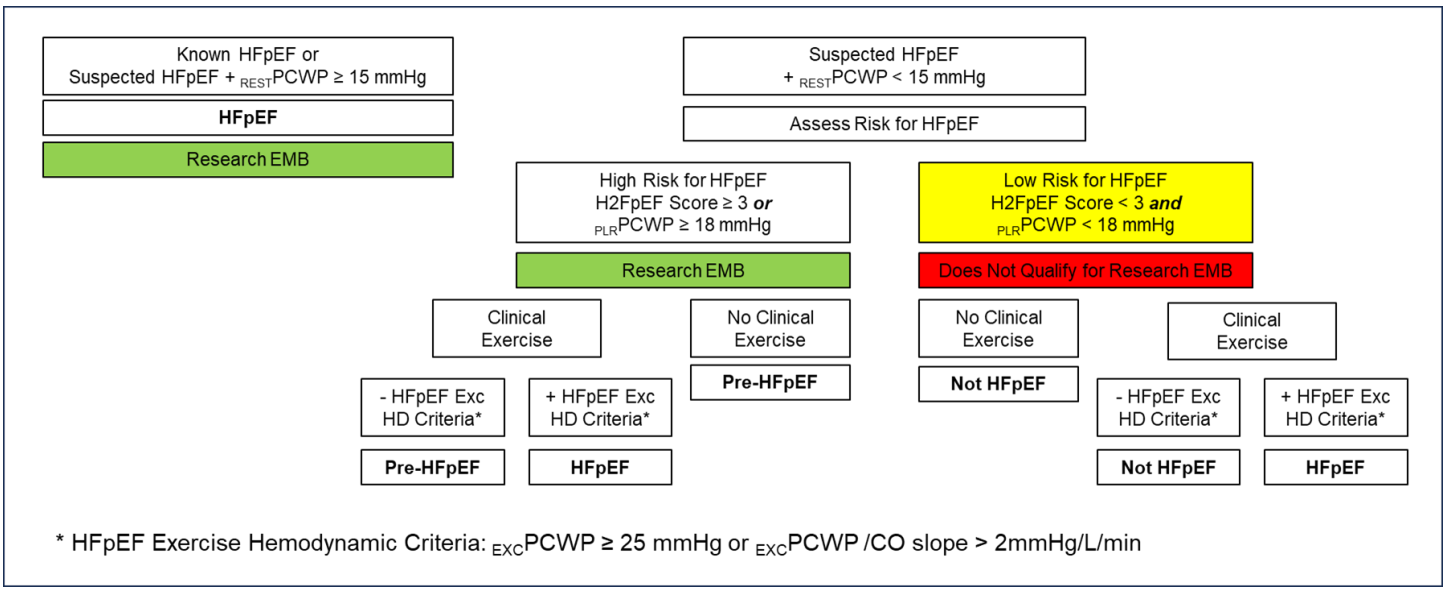
The NF Control and HFrEF comparator groups are not included in this protocol. Ex-vivo RV EMB samples are available from hearts harvested for organ donation but ultimately not used for transplantation (NF Controls) and at explant of failing heart in patients with HFrEF undergoing cardiac transplantation. Ex-vivo RV septal biopsy samples and corresponding clinical data will be provided to MM-HFpEF Core Laboratory by the Gift-of-Life organ donor program and the University of Pennsylvania Cardiac Tissue Repository through established, NIH vetted agreements.

3. STUDY DESIGN

3.1 General Description:

Patients will be consented for research RHC (if not planned for clinical purposes; 4.1 Study Procedures - RHC) and/or research EMB. Within six months of participation, patients will have had a clinically indicated transthoracic echocardiogram, NT-proBNP level, complete blood count, basic metabolic panel and electrocardiogram and/or will have these as research studies as part of this study or the Heart Share Deep Phenotyping Protocol (section 3.4).

Patients will initially be classified as “known HFpEF” or “suspected HFpEF” per criteria below (3.2 Study Population). If the patient has known HFpEF or if the patient meets resting hemodynamic criteria ($_{REST}$PCWP ≥15 mmHg) for HFpEF, the patient will have confirmed HFpEF and the research EMB will be performed (Figure).



If the patient has suspected HFpEF and does not meet resting hemodynamic criteria for HFpEF, the risk of HFpEF will be assessed (Figure).

If the risk of HFpEF is high as evidenced by a H2FpEF score ≥ 3 or a passive leg raise (PLR) PCWP ≥ 18 mmHg, the patient will undergo research EMB. As clinical studies indicate that these patients have impaired functional capacity and increased risk of future HF,[67,68] they will be classified as pre-HFpEF unless clinically indicated exercise hemodynamic assessment meets criteria for HFpEF (Figure).

If the patient has low risk of HFpEF based on the H2FpEF score or the $_{PLR}$PCWP, the patient does not qualify for research EMB and is classified as “not HFpEF” unless clinically indicated exercise hemodynamic assessment meets criteria for HFpEF (rare).

The research EMB (4.2 Study Procedures – EMB) will be performed using fluoroscopic or echocardiographic guidance per operator preference. If an EMB is indicated clinically, the three research EMB will be performed after the clinical EMB (usually 3-5 biopsies, per clinician specifications) have been obtained. Before and immediately after the research EMB, transthoracic echocardiographic assessment of tricuspid valve structure and regurgitation and pericardial fluid will be performed and findings recorded, along with blood pressure and heart rate.

After the research EMB and post-EMB imaging, other clinically indicated procedures (i.e. exercise hemodynamics, drug studies for pulmonary vascular function) to further evaluate HFpEF will be performed. Patients may participate in other research protocols as long as the studies do not interfere with the study entry algorithm (figure) and the ability to perform the EMB.

After the research EMB and any other clinically indicated procedures, patients will be observed for clinical stability in the catheterization laboratory post procedural area and then discharged according to routine post-procedural practice at the participating site. At discharge and at 30 days post procedure, the clinical record will be reviewed, and a case report form completed documenting any procedural related adverse events due to research RHC or EMB.

Up to 300 patients will be enrolled over a five-year period at up to 10 sites participating in the MM-HFpEF study.

3.2 Study Population

3.2.a: Inclusion criteria: Patients with known or suspected HFpEF will be identified by electronic medical record searches, referrals from clinicians, focused screening in the clinical practice including patients scheduled for RHC to diagnose or assess severity of HFpEF, responses to posting of research opportunities or review of patient lists from previous HFpEF studies.

HFpEF criteria

1. Age ≥30 years.
2. Left ventricular ejection fraction ≥50% measured by echocardiography, CMR or MUGA (measured within one year + clinical stability)
3. Definition of HFpEF: signs and/or symptoms of HF, NYHA functional class II-IV, and <u>at least one</u> of the following:
    a. Elevated BNP (≥75 pg/ml in sinus rhythm or ≥225 pg/ml in atrial fibrillation/flutter) or NTproBNP (≥225 pg/ml in sinus rhythm or ≥675 in atrial fibrillation/flutter) at baseline. Choice of BNP or NTproBNP is based on availability at each clinical center.
    b. Prior HF hospitalization (primary reason for the hospitalization is HF with elevated natriuretic peptide levels [using the thresholds listed above], requiring IV diuresis for HF, or pulmonary edema or pulmonary vascular congestion on chest radiography).
    c. Previously documented elevated pulmonary capillary wedge pressure (PCWP) at rest (≥15 mmHg) or during exercise (≥25 mmHg for supine exercise or PCWP/cardiac output ratio ≥2 mmHg/L/min for upright exercise).
    d. Elevated H_2FPEF score[69] (≥5) or HFA-PEFF[70] score (≥5).

Suspected HFpEF criteria

1. Age ≥30 years.
2. Left ventricular ejection fraction ≥50% measured by echocardiography, CMR or MUGA (measured within one year + clinical stability)
3. Signs and/or symptoms of HF, NYHA functional class II-IV and <u>all of the following</u>:
    a. Does not meet BNP or NT-proBNP criteria for HFpEF (above)
    b. No prior HF hospitalization meeting HF criteria (above)
    c. No previous hemodynamic catheterization documentation of HF (as above)

3.2.b: Exclusion criteria:

According to current guidelines,[71] patients deemed at increased risk of right ventricular (RV) septal EMB complications as evidenced by any of the below will be excluded.

1. Inadequate echo or fluoroscopic images.
2. Neck anatomy unfavorable for jugular venous cannulation
3. Therapy with direct oral anticoagulants without cessation for a period (age, renal function, and agent specific) deemed adequate to normalize coagulation according to local clinical guidelines.
4. Previous or ongoing therapy with warfarin with INR ≥ 1.6 measured day before or of EMB
5. Platelet count < 50,000/ml
6. Active bleeding or coagulation disorder
7. Infection or fever
8. Endocarditis
9. Pregnancy
10. Intracardiac thrombus
11. RV Aneurysm
12. Clinically significant tricuspid, pulmonary or aortic valve stenosis
13. Tricuspid or pulmonary mechanical valve prosthesis
14. Left bundle branch block

A known contrast media hypersensitivity is not a contraindication as contrast is not routinely used but the presence of contrast media hypersensitivity should be noted to preclude use. For patients with an indication for anticoagulation (ie atrial fibrillation), who are treated with warfarin, bridging according to local protocols if deemed advisable by the patient's clinician.

Patients with hemodynamically significant left valve disease, pericardial disease, known hypertrophic cardiomyopathy, severe non-revascularized coronary disease, or known inflammatory (i.e. myocarditis or sarcoidosis) or infiltrative (i.e. amyloidosis) cardiac diseases or known non-group 2 pulmonary hypertension are excluded.

3.3 Duration of participation

For patients undergoing research RHC and EMB, the duration of the procedure consumes 4-8 hours (registration, check in, pre-procedural preparation, procedure (30-90 minutes), post-procedure monitoring and dismissal). For patients undergoing clinically indicated RHC without clinically indicated EMB, the portion of the study that involves research is expected to add an additional 5 to 15 minutes to the RHC procedure (for the three EMB). Pre-EMB echocardiography will be performed during access and the EMB procedure itself, so will not add significantly to procedural time. Post-EMB images will be obtained in the interval between EMB and any other RHC measurements (5-10 min). For patients undergoing clinically indicated RHC with clinically indicated EMB, the portion of the study that involves research is expected to add an additional 5 to 10 minutes to the RHC procedure (for three research EMB).

The study will not require any further participation by patients other than that stated above, but participants will be encouraged to participate in the main HeartShare Deep Phenotyping Protocol to maximize phenotypic characterization. Subsequent annual clinical chart review will be performed for up to five years after enrolling to allow analysis of any relationships between study variables (i.e. histopathology, transcriptomic, proteomic or metabolomic findings) and outcomes. The study will end when the tissue samples are analyzed, within approximately 1- 5 years of EMB procedure.

3.4 Primary Study Data Collected from Review of Clinical Records and Study Procedures

3.4.a: Medical History and Physical Examination: age, sex, history of hypertension, diabetes, hyperlipidemia, coronary disease, atrial fibrillation, pacemaker, myocardial infarction, chronic kidney disease, obesity (BMI ≥ 30), collagen vascular disease, lung disease (obstructive, restrictive), previous thoracic radiation or valve disease and blood pressure, heart rate, height, weight.

3.4.b: Laboratory values: creatinine, blood urea nitrogen, NT-proBNP, Hgb, Hct, platelets, white blood cells, and INR day of or day before procedure (only if on warfarin and holding for EMB).

3.4.c: Electrocardiogram: rate, rhythm (sinus, atrial flutter or AF, ventricular pacing), QRS duration, LBBB pattern (yes/no)

3.4.d: Echocardiogram: Left atrial dimension and/or volume (method), LV end diastolic dimension and/or volume (method), LV septal and posterior wall thickness, LV ejection fraction (value/method), right ventricular size (semiquantitative) and function (semiquantitative), aortic, mitral, tricuspid and pulmonary valve function (stenosis or regurgitation grade)

3.4.e: Resting hemodynamics: HR, systemic blood pressure, RA (a,v,mean), RV (systolic, end-diastolic), PA (systolic, diastolic, mean), PCWP (a,v, mean), cardiac output method (Fick indirect, Fick direct, thermodilution), cardiac output, cardiac index, PVR, PA compliance.

3.4.f: Passive Leg Raise hemodynamics: PCWP (a,v, mean)

3.4.g: Clinically indicated exercise hemodynamics (if performed): Exercise method (arm, bike upright, bike supine, other), peak level (watts, other), and same hemodynamic measures as in 3.4.e (as available).

3.5 Primary Safety Data Collected

3.5.a: Pre-EMB echo: Echocardiographic tricuspid regurgitation grade (semi-quantitative) and pericardial effusion (window, dimension)

3.5.b: Immediate post-EMB echo: Echocardiographic tricuspid regurgitation grade (semi-quantitative) and pericardial effusion (window, dimension), tamponade physiology (yes/no))

3.5.c: Research RHC complications: death, chest pain, hemoptysis, pneumothorax, access site bleeding or hematoma exceeding usual expected severity, new atrial fibrillation/flutter, other clinically significant arrhythmias (ventricular tachycardia requiring therapy, heart block requiring therapy), pulmonary artery perforation, new or worsening tricuspid regurgitation.

3.5.d: Research EMB complications: death, chest pain, hemoptysis, pneumothorax, access site bleeding or hematoma exceeding usual expected severity, new atrial fibrillation/flutter, other clinically significant arrhythmias (ventricular tachycardia requiring therapy, heart block requiring therapy), new or worsening tricuspid regurgitation, new pericardial effusion requiring admission or serial echocardiography without intervention, tamponade requiring percutaneous pericardiocentesis, tamponade requiring surgical pericardiocentesis with or without myocardial repair.

4.0 STUDY PROCEDURES

4.1 Right Heart Catheterization (RHC)

Right heart catheterization will be performed as previously described.[72] Prior to cardiac catheterization, an IV cannula will be inserted in a forearm vein for fluid and medication administration. Vascular access is accomplished using ultrasound imaging and local anesthesia. A 9 Fr sheath is deployed under local anesthesia in the right internal jugular vein, and an optional 4 Fr cannula is placed in the radial artery to sample arterial blood and measure blood pressure (if performing Fick cardiac outputs). If left heart catheterization is to be performed for clinical purposes (requiring a larger arterial sheath), EMB will be completed prior to arterial puncture owing to the need to administer heparin following arterial access. A flow guided balloon PA catheter is placed via the jugular venous sheath with or without a micromanometer tipped catheter wire for measurement of fluid filled and/or high-fidelity pressure waveforms in the RA, RV, PA and PCWP positions, all following EMB.

Hemodynamics and cardiac output at rest and during exercise (if indicated) will be measured using gold standard invasive techniques in patients with HFpEF.[72] Resting oxygen consumption and mixed venous and arterial blood samples will be obtained at rest and during exercise (if performed) for Fick calculated cardiac output (if performed).

Passive Leg Raise: As previously described[73], during the passive leg raise maneuver, the legs of the patients should be raised by staff lifting the patients feet so that the legs form an angle of ≈ 50 degrees to the cath lab table. During this maneuver, the patient should be explicitly instructed to relax his/her legs and not to help so as to avoid a Valsalva maneuver. If the patient is to undergo supine bicycle exercise, the feet can be placed in the supine bike pedals which will maintain the ≈ 50 degree angle. The patient should again be encouraged to relax leg muscles and breath normally. After two minutes of elevation by holding legs or putting feet on the bike pedals, the PCWP should be measured as the average of at least 3 beats.

4.2 Endomyocardial biopsy (EMB)

After resting hemodynamics and baseline echocardiographic imaging for tricuspid valve structure and regurgitation and pericardial effusion is performed, a bioptome will be passed via the venous sheath. Three EMB will be performed under fluoroscopic or echocardiographic guidance according to recommendations of the joint European Society of Cardiology (ESC) Heart Failure Association, Heart Failure Society of America and Japanese Heart Failure Society Position Statement on EMB (2021).[71] To minimize risk, operators will need to have averaged at least 20 to 50 RV EMB per year[71] for at least the preceding four years (exclusive of COVID impact in 2020). Continuous electrocardiographic and pulse oximetry monitoring and intermittent blood pressure (if no arterial line used) will be monitored. Echocardiographic assessment for tricuspid valve structure and regurgitation and for pericardial effusion is briefly assessed during each biopsy and at the end of the procedure. After the research EMB, patients are observed for clinical stability in the catheterization laboratory post procedural unit for at least 2 hours prior to discharge. Repeat echo imaging of tricuspid valve structure and regurgitation and for pericardial effusion will be performed in the post-procedural unit prior to discharge.

4.3 Tissue and Data Processing and Storage

4.3.a: Phase 1: First 100 Biopsies: All samples will be placed in sterile, iced saline on the catheterization laboratory table. Immediately after the last biopsy is obtained, the research biopsies will be given to the MM-HFpEF study staff who will place samples on a sterile dry towel to remove saline. One sample will be placed in formalin and will be subsequently embedded in paraffin (FFPE) at the MM-HFpEF Core Laboratory. The other two samples will be flash frozen in individual containers of liquid nitrogen.

4.3.b: Phase 2: Second 200 Biopsies: Samples will be processed as decided by the MM-HFpEF Core Laboratory and the HFpEF Tissue Working Group as Phase 1 enrollment nears completion.

For both phases, venous blood samples (5 ml) will be taken from the superior vena cava, placed in centrifuge tubes (red top), processed in the bio-accession and processing laboratory (BAP) with serum aliquoted into 0.5 ml samples, frozen and shipped with the biopsy specimens to the MM-HFpEF Core laboratory. The samples will be shipped to the MM-HFpEF Core Laboratory where the formalin fixed samples will be embedded in paraffin and frozen samples will be stored at -80 degrees. Deidentified (MM-HFpEF Study #) corresponding baseline, procedural and post-procedural clinical data will be provided within 90 days to the Heart Share Data Translation Center. Follow up data (HF hospitalization, death, presumed cause of death) will be provided yearly for five years after the biopsy.

The data for this trial will be collected in trial-specific electronic data collection forms from source documents by the research staff during the study visit. The study coordinators will be responsible for transmitting the participant unique non-PHI identifiers using standardized data collection instruments to the Heart Share Data Translation Center at Northwestern University. The research coordinators and investigators will perform manual data entry from source documents.

4.4 Risks associated with Study Procedures

Right Heart Catheterization (RHC)

Most RHC performed in this study will be clinically indicated. Few studies have reported the risk of RHC.[74] The RV EMB procedure includes sheath placement in the jugular vein which is part of the RHC procedure as well. No studies have reported the risk of only passing a pulmonary artery catheter through the right heart and pulmonary artery for hemodynamic measurements. Thus, complications cited for RHC are not necessarily additive to RV EMB. Complications in patients undergoing RHC (n=5556) from 2002 to 2013 for any indication at Mayo Clinic in Rochester, MN were recently reported.[74] Overall, complication rates were low (0.216% for RHC) with no deaths attributed to the procedure. For RHC alone, there were no pneumothoraces, 0.036% with ventricular tachycardia, no hemoptysis, 1.3% with worsening tricuspid regurgitation, and no cardiac tamponade events.

Right Ventricular (RV) Endomyocardial Biopsy (EMB)

A recent joint position paper on EMB from the European, Japanese and American HF Societies[71] concluded that EMB is associated with a low rates of major and minor (Table).[71] Some studies reported complications separately for RV and LV EMB while others did not categorize by biopsy site. Patient characteristics including clinical stability and underlying cardiac problem, EMB site (right vs left ventricle), procedural volume and operator expertise were the most important determinants of EMB risk. For example, risk is higher in patients hospitalized with cardiogenic shock due to fulminant myocarditis vs in stable outpatients with NYHA class 2 HFpEF symptoms undergoing EMB to rule out infiltrative vs hypertensive heart disease. It can be difficult to determine whether observed complications are due to the underlying disease process or the EMB itself (i.e. higher rates of reported arrhythmia in a series confined to patients undergoing biopsy for myocarditis (table)). One paper had higher rates of complications than other studies (table, red text). Other papers reported that EMB performed in heart transplant patients had lower complication rates than in native hearts.[75] As transplant patients do not have a fully intact parietal pericardium, they should have lower rates of tamponade. The discharge diagnosis of the hospitalizations where the EMB was performed in non-transplant patients included HF (58%), cardiogenic shock (19%) and myocarditis (4%). The report included non-teaching hospitals where rates were 6x higher and accounted for 25% of the complications (with only 6% of the procedures). In teaching hospitals, the rates were 0.5% vs 0.2% in non-tx vs tx. The rate of complications at the MM-HFpEF Core Laboratory in 3,459 patients was 0.17% for sustained arrhythmia events (requiring pharmacological or electric cardioversion) and 0.06% for pericardial effusion that required pericardiocentesis.

Management of cardiac perforation during EMB includes immediate pericardiocentesis and autotransfusion from the pericardium, close monitoring, and consultation with a cardiac surgical service. Urgent surgical repair of the perforation site may be required in patients with ongoing bleeding or instability related to the perforation.

For RV EMB, few studies have systematically evaluated damage to the tricuspid valve with worsening tricuspid regurgitation as most institutions have not routinely used echocardiographic guidance for EMB or performed routine assessment of tricuspid regurgitation before and after EMB.[74] Since the publication of the HF society position paper, complications in patients undergoing EMB (n=3846) for any indication at Mayo Clinic in Rochester, MN (2002 to 2013) were reported.[74] Overall, complication rates were low (0.208% for EMB) with no deaths attributed to the procedure and individual complication rates similar to those quoted in the position paper (Table). Echo was used in a minority of EMB and this may result in over (indication bias in complex cases) or under (no systematic assessment) estimation of rates of worsening tricuspid regurgitation. In the Mayo study, worsening TR occurred in 5.1% of EMB cases (most asymptomatic and most in cardiac transplantation patients who have many repeated EMB).

Importantly, in large (> 3000 patients) contemporary series from two of the teaching hospitals who will be involved in this study, the risk of death associated with EMB was 0% and the risk of serious complications (tamponade or ventricular arrhythmias) was each less than 2 per 1000 procedures.[74,76]

Major Complications (cited for RV or LV EMB)[71]	Reported Risk range[71]	Risk reported in each study with at least 500 RV (if reported separately) EMB (Table S1 in[71])
Death	0% to 0.07%	One study 0.07% - all others 0%
Cardiac hemopericardium or tamponade	0% to 6.9%	6.9%,0.05%, 0.16%, 1.5%, 0.8%, 0.45%, 0.81%, 0.1%, 0.4%, 0.3%
Pneumothorax/air embolism	0% to 0.8%	0.8%, 0%, 0.07%, 0%
Thromboembolism	0% to 0.32%	0%, 0%, 0%, 0%
Valvular trauma (Tricuspid Regurgitation)	0.02% to 1.1%	1.1%, 0.02%, 0.6%
Severe arrhythmia or atrioventricular block	0% to 11%	11%, 0.13%, 17.5% (all myocarditis pts), 0.8%, 0.19%, 0.61%,0%
Minor Complications		
Transient chest pain	0% to 1.8%	1.8%, 0.1%, 0.19%, 0.61%, 0%,
Deep Vein Thrombosis	0.23% to 3.8%	3.8%, 0.23%,
Vascular access site hematoma/nerve palsy	0% to 0.64%	0.4%, 0%, 0.1%, 0.19%, 0%, 0.64%
Hypotension/vaso-vagal syncope	0% to 4.3%	0.06%, 0.78%, 4.3%
Arterial trauma/vascular damage	0.32% to 2.8%	NA for RV EMB
		Single study with higher rates for all complications

4.5 Strategies to mitigate Risk to Participants

To minimize risk, operators will need to have averaged at least 20 to 50 RV EMB per year[71] for at least the preceding four years (exclusive of COVID impact in 2020). Continuous electrocardiographic and pulse oximetry monitoring and intermittent blood pressure (if no arterial line used) will be monitored. Echocardiographic assessment for tricuspid valve structure and regurgitation and for pericardial effusion is assessed during each biopsy and at the end of the procedure. After the research EMB, patients are observed for clinical stability in the catheterization laboratory post procedural area as per local clinical protocols and as deemed necessary by the EMB operator. Repeat echo imaging of tricuspid valve structure and regurgitation and for pericardial effusion is performed immediately after the procedure in the catheterization laboratory. Only RV EMB will be performed (lower risk of stroke and arterial bleeding). Only clinically stable patients without high-risk features (see **3.2.b: Exclusion criteria)** will be included.

4.5 Tissue Analysis

4.5.a. Background: The MM-HFpEF study seeks to use discovery multi-omics to determine if there are novel, therapeutically relevant and varied (myocardial pathophysiologic heterogeneity) mechanisms mediating myocardial dysfunction in HFpEF. Most studies of human HFpEF tissue have been hypothesis driven analyses of candidate pathways. Unbiased assessment of differences in gene expression (transcriptomics) and/or protein abundance (proteomics) coupled with bioinformatic analyses to determine the pathways indicated by gene or protein changes ("omics" analysis) or integrated gene and protein changes (multi-omics) can be used to discover novel transcriptional or translational changes mediating myocardial pathology in HFpEF.

Transcriptional changes may mediate cardiac pathology in cardiomyopathies[77] and HFpEF.[19,49] However, in endstage cardiomyopathies, dramatic differences in phenotype were unaccompanied by differences in gene expression.[78] Further, transcriptional changes did not correlate tightly with phenotype or protein changes before and after myocardial recovery in HFrEF[79] or in hypertrophic cardiomyopathy (HCM) versus controls.[80] Recent work suggests that several myocardial pathophysiologic changes maybe be predominately or jointly controlled at the translational level[81,82] and thus, study of the proteome may reveal novel or complementary pathophysiologic information beyond changes in gene expression.

Two studies performed transcriptomics on myocardial tissue (bulkRNAseq) using LV or RV samples from HFpEF as compared to HFrEF and/or NF Controls. Both identified 13,000 to 14,000 genes but in one study (LV surgical samples from patients undergoing surgical revascularization for coronary disease with or without evidence of HFpEF), only 743 (5%) genes were differentially expressed (DE) in HFpEF versus NF controls.[19] Most (719) DE genes were down regulated. In the other study with a larger sample size, 67% of genes were DE in HFpEF (RV EMB in patients with rigorously documented HFpEF) versus NF controls and 52% of genes were DE in HFrEF vs NF Controls.[49] Up-regulated (but not down-regulated) genes in HFpEF were no longer significant after adjustment for HFpEF risk factors. Both the HFpEF transcriptomic studies used bioinformatic pathway analyses to interpret the DE gene data and demonstrate alterations consistent with various theories for the pathogenesis of HFpEF.

Gene expression changes in single cells (scRNAseq) or in nuclei isolated from tissue (snRNAseq) may provide greater pathophysiologic insight. For both technical and biologic reasons, fewer genes are usually detected with sc- or snRNAseq (characterized as detecting 10-40% of the transcriptome[83]). To date, scRNAseq or snRNAseq studies in HFrEF and/or NF Controls[77,84-86] or in endstage cardiomyopathies[78] have been performed. The small size of EMBs make snRNAseq analyses challenging but the MM-HFpEF Core Laboratory has used novel methods to perform snRNAseq in preliminary studies in HFpEF EMB.

Proteomics studies in HF and cardiomyopathies have been more limited. In cryopreserved LV tissue from autopsy subjects with or without diastolic dysfunction, mass spectrometry (MS) with isobaric tags to characterize the myocardial proteome revealed 1976 proteins with 57 DE proteins.[87] Network analysis indicated endoplasmic reticulum (ER) stress was significantly altered in the diastolic dysfunction group with down regulation of key proteins involved in the unfolded protein response as reported by others in experimental HFpEF.[8] Another study of LV tissue from diverse HFpEF and NF Control cohorts used newer proteomic techniques but identified only 1043 proteins of which 12 were DE.[26] A comprehensive MS study of the normal human cardiac proteome[88]

identified approximately 8000 proteins were identified in the LV and RV. The MM-HFpEF Core Laboratory has evaluated several proteomic approaches in extensive preliminary studies in HFpEF.

A limited number of studies have examined alterations in metabolism in HFpEF, with the majority examining a single aspect of metabolism such as mitochondrial and oxidative phosphorylation integrity,[18,36] nicotinamide adenine dinucleotide (NAD+) integrity[59,89], somatotropic axis homeostasis,[20] osteopontin mediated disruption of 2-Oxoglutarate Dehydrogenase-Like (OGDHL) mitochondrial signaling.[63] The MM-HFpEF Core Laboratory performed targeted metabolomics and select transcriptomics in RV myocardium from HFpEF, NF Controls and HFrEF showed evidence of decreased fatty acid oxidation in HFpEF relative to NF Controls but no evidence of compensatory increases in ketone utilization, glycolysis or branched chain amino acid metabolism, suggesting insufficient regeneration of citric acid cycle (TCA) intermediates (reduced anaplerosis) and fuel inflexibility. Despite much higher rates of obesity in HFpEF, FA oxidation appeared lower in HFpEF than HFrEF. Tissue metabolomic findings did not corelate with plasma metabolomic findings and methylhistidine, a post-translationally modified amino acid was increased in myocardium and plasma, suggesting stress induced myofibrillar turnover. [50]

4.5.b. Approach: As outlined above, the MM-HFpEF Core Laboratory has performed bulk RNAseq transcriptomics and metabolomic studies in HFpEF with findings implicating unique pathways in HFpEF or HFpEF subtypes.[49,50] The Core laboratory has also performed preliminary (data unpublished) snRNAseq studies and proteomics studies in HFpEF. The larger MM – HFpEF study seeks to build on these preliminary findings and to confirm and extend these “first in HFpEF” discovery omics studies.

The Phase 1 approach in the first 100 patients will be to collect extensive clinical data and perform digital histology and spatial transcriptomics (FFPE samples) with spatial proteomics in a subset. In frozen samples, bulkRNAseq (one frozen biopsy specimen) and (unlabeled) LC-MS MS proteomics (data independent analysis, other frozen biopsy specimen) will be performed in the first 100 patients. This multiomics (histology, transcriptomics, proteomics) analysis will allow unique insight into transcriptional and translational regulation of myocardial structure and function.

In the second 200 HFpEF subjects undergoing research EMB (Phase 2), sample processing techniques, appropriate controls and biologic analyses will be designed by the MM-HFpEF Core Laboratory and the Heart Share MM-HFpEF Working Group to build on findings in Phase 1, evolution in omics technology and other advances in knowledge. Phase 2 analyses will include but not be limited to, snRNA-seq, targeted metabolomics, myofilament functional assessments, further spatial transcriptomic or proteomic analyses, further HFpEF phenogroup characterization and more in-depth study of specific pathways suggested to mediate HFpEF pathophysiology based on Phase I findings.

5.0 INFORMED CONSENT AND STUDY MONITORING

The MM-HFpEF study will report to the Heart Share Observational Study Monitoring Board (OSMB). The Heart Share OSMB was established by the NHLBI in accordance with NIH policies and is responsible for monitoring of patient safety and review of study performance. The OSMB consists of a chair, clinicians or scientists with expertise in heart failure, bioethics, and biostatistics. An NHLBI scientist other than the NHLBI’s Project Scientist serves as the Executive Secretary to the OSMB. The OSMB meets at regular intervals (at least twice per year) and at other times as necessary, as described in the HeartShare OSMB charter. The purpose of monitoring is to (1) verify that the rights and well-being of human participants are protected; (2) ensure that the reported study data are accurate, complete, and verifiable from source documents; and (3) ensure that the conduct of the study is in compliance with the currently approved protocol/amendment, with Good Clinical Practices, and with applicable regulatory requirements.

Informed Consent

The site investigator, or a person designated by the site investigator, will fully inform the participant of all pertinent aspects of the study including the review of the IRB-approved informed consent form. All study procedures and

potential risks will be discussed in detail with each participant. The Informed Consent Form (Consent) will be signed and personally dated by the participant prior to the commencement of any study procedures. All participants will receive a copy of the informed consent form. Electronic copies of the signed Consent forms will be retained at each study site.

Adverse Events
All adverse events occurring within the MM-HFpEF study will be recorded and reported, starting at the beginning of the RHC/EMB procedure and continuing for a 30 day period after the RHC/EMB has been completed.

Adverse events are defined as:

- **Adverse event:** An adverse event (AE) shall be considered any detrimental change in the patient's condition.
- **Anticipated adverse event:** Anticipated adverse events are defined for each of the protocol procedures in the sections specifically written for those procedures.
- **Unanticipated adverse event:** Any adverse event that results in risk or harm to the participant or others that differs from the known, predicted possible effects of the research protocol. An unanticipated adverse event is one that varies in nature, intensity, or frequency from information in the informed consent document.
- **Serious adverse event:** Any event that results in death, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability or incapacity. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

5.0 ETHICAL CONSIDERATIONS

Potential benefits**:** There are no anticipated direct benefits to the subjects as a result of their participation in this study nor will this be implied when obtaining consent. Understanding the molecular mechanism and pathways leading to HFpEF may provide new strategies for treatment.

Risk/benefit ratio: The results of this study may ultimately lead to an effective treatment for HF, which is urgently needed to help millions of patients. Since there is low risk and potential benefits to medical knowledge and society, the risk / benefit ratio is acceptable.

6. STUDY FINANCES

6.1 Funding Source

Heart Share as well as the MM-HFpEF Heart Share study is funded by a collaborative initiative between the NIH and the Accelerating Medicines Partnership® (AMP®). Launched in 2014, the AMP® program is a public-private partnership between the National Institutes of Health (NIH), the U.S. Food and Drug Administration (FDA), multiple biopharmaceutical and life science companies, non-profit and other organizations to transform the current model for developing new diagnostics and treatments. Current AMP projects include:

- Alzheimer's disease (AD 1.0 Biomarkers in Clinical Trials and AD 2.0)
- Autoimmune and Immune-Mediated Diseases
- Autoimmune disorders of rheumatoid arthritis and systemic lupus erythematosus (lupus) (RA/Lupus)
- Bespoke Gene Therapy Consortium (BGTC)
- Common Metabolic Diseases (CMD)
- Heart Failure
- Parkinson's disease (PD)
- Schizophrenia (SCZ)

AMP partners share a common goal of increasing the number of new diagnostics and therapies for patients and

reduce the time and cost of developing them. The AMP program aims to improve understanding of therapeutically relevant biological pathways and validate information that could be relevant for the development of multiple therapeutics.

Through this cross-sector partnership, managed through the Foundation for the NIH (FNIH), NIH and AMP partners are sharing expertise and resources — over $830 million to date, which includes in kind contributions — in an integrated governance structure that enables the best-informed contributions to science from all participants.

6.2 Conflict of Interest

This is a discovery study seeking a signal for pathways involved in HFpEF myocardial pathophysiology using histopathology, transcriptomics, proteomics and metabolomic data. All studies will be published in peer-reviewed scientific journals with access to all data provided to the scientific community at large.

6.3 Subject Renumeration

For subjects undergoing research RHC and EMB, up to $1000.00 will be reimbursed to cover travel, hotel and meals after submission of appropriate receipts and subjects will receive $700.00 renumeration for participation in the research EMB protocol.

For subjects undergoing clinically indicated RHC and/or EMB, with research EMB added on, no travel expenses will be reimbursed and subjects will receive $400.00 renumeration for participation in the research EMB protocol.

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