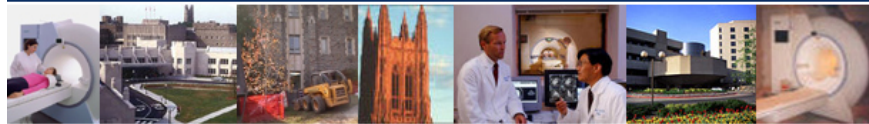


Duke Cardiovascular Magnetic Resonance Center



Patient Care

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Version: 3/01/2016

Investigator Initiated Project Title:

Microvascular Dysfunction in Nonischemic Cardiomyopathy:
Insights From CMR Assessment of Coronary Flow Reserve

Center: Duke Cardiovascular Magnetic Resonance Center,
Division of Cardiology, Duke University Medical Center

NCT03249272

Investigators:

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Study Synopsis

Study Title	Microvascular dysfunction in nonischemic cardiomyopathy: Insights from CMR assessment of coronary flow reserve
Primary Objective	<ul style="list-style-type: none"> To determine the prevalence of microvascular dysfunction (MVD) by a CMR measurement of whole-heart (global) perfusion reserve in subtypes of nonischemic cardiomyopathy
Secondary Objective	<ul style="list-style-type: none"> To compare the compare the CMR measurement of global perfusion reserve with regadenoson and adenosine To ascertain the relationship between microvascular dysfunction and other CMR metrics including regional myocardial scarring and perfusion
Study Design	<ul style="list-style-type: none"> Single-center, observational study
Planned Enrollment	<ul style="list-style-type: none"> 30 patients with hypertrophic cardiomyopathy 30 patients with idiopathic dilated cardiomyopathy 15 controls with atypical chest pain
Subject Selection Criteria	<p>Inclusion Criteria</p> <ul style="list-style-type: none"> Men or women aged 18 years or older Cardiomyopathy patients <ul style="list-style-type: none"> -Patients presenting for CMR with the clinical diagnosis of hypertrophic cardiomyopathy based on left ventricular wall thickness of at least ≥ 15 mm in the absence of any other cardiac or systemic cause of hypertrophy -Patients presenting for CMR with the clinical diagnosis of idiopathic dilated cardiomyopathy based upon left ventricular ejection fraction $\leq 40\%$, LV end-diastolic diameter ≥ 55 mm or left ventricular end-systolic diameter ≤ 45 mm, and the absence of coronary stenoses on angiography. Control patients with atypical chest pain <ul style="list-style-type: none"> -Patients presenting for CMR evaluation of chest pain but without evidence of obstructive coronary artery disease either by coronary angiography or stress testing. <p>Exclusion Criteria</p> <ul style="list-style-type: none"> Decompensated heart failure or hemodynamic instability Prior coronary revascularization (PCI or CABG) or myocardial infarction (as evidenced by previously elevated CPK-MB or troponin levels) Accelerating angina or unstable angina Inability to physically tolerate MRI or implanted objects that are MRI incompatible Inability to provide written informed consent obtained at time of study enrollment. Severe claustrophobia Advanced heart block or sinus node dysfunction Hypersensitivity or allergic reaction to regadenoson or adenosine Hypotension Active bronchospasm or history of hospitalization due to bronchospasm History of seizures Recent cerebrovascular accident Use of dipyridamole within the last 5 days Contraindication to aminophylline Severe renal insufficiency with estimated glomerular filtration rate < 30 ml/min/ 1.73 m² Pregnant or nursing
Length of Study	<p>Projected duration of enrollment: 1-1.5 years</p> <p>Projected duration of analysis and manuscript preparation: 0.5-1 year</p> <p>Total study duration: ~2.5 years</p>
Primary Endpoint	The primary endpoint will be the prevalence of MVD by CMR. The hypothesis is that global perfusion reserve will be significantly lower in patients with hypertrophic cardiomyopathy and idiopathic dilated cardiomyopathy than in controls with atypical chest pain. We also anticipate that differences in prevalence of MVD between the cardiomyopathy groups will be observed.

Secondary Endpoints	The secondary endpoints will include <ul style="list-style-type: none">• A comparison of CMR global perfusion reserve using regadenoson versus adenosine.• A comparison of the correlation coefficients between the extent of MVD and other CMR metrics, including myocardial scar size, extent of regional perfusion defects, and morphological and function characteristics such as LV mass, LVEF, and LV volume.
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Scientific Rationale

Coronary microvascular dysfunction (MVD) has been implicated as an important marker of cardiac risk and has been thought to directly contribute to the pathogenesis of a wide variety of cardiomyopathies.^{1,2} For instance, MVD is believed to cause ischemia (with reduction in coronary flow reserve) in patients with hypertrophic cardiomyopathy (HCM) despite the presence of angiographically normal epicardial coronary arteries. The implication is that MVD in HCM may lead to the ventricular arrhythmias, sudden death, and heart failure. Similarly, patients with idiopathic dilated cardiomyopathy (IDCM) have blunted coronary flow reserve, which appears to be independently associated with poor prognosis.

Several etiologic mechanisms have been proposed to explain the occurrence of MVD, including structural and functional abnormalities¹:

- (a) increased microvascular resistance due to reduced vascular luminal caliber.
- (b) reduced density of microvessels associated with replacement scarring.
- (c) inappropriate vasoconstrictor responses.
- (d) inadequate vasodilator responses.

Unfortunately, these mechanisms are difficult to study in humans since no technique currently allows the direct visualization of the coronary microcirculation *in vivo*. Thus, MVD has been largely studied using non-invasive imaging techniques, such as positron emission tomography (PET) or single photon emitted computed tomography (SPECT).

Although these methods have provided insight into MVD, much remains unknown. For example, even the prevalence of MVD in patients with various types of cardiomyopathy is unclear, with different studies showing widely different rates. In part, this may be due to limitations of these imaging methods. For example, SPECT provides qualitative information regarding relative differences in regional blood flow, but is not able to assess global changes in myocardial blood flow. PET can quantify regional and global absolute blood flow, but these measurements may exhibit substantial variation in individual measurements depending upon the methodology that is employed (e.g. the arterial input function and/or the extraction model used).³ Both techniques can be affected by partial volume effects due to regional variation in LV wall thickness and the limited spatial resolution of imaging. Additionally, both PET and SPECT have difficulty distinguishing between reductions in blood flow due to ischemia versus scar tissue, which appears to be present in variable degrees depending on the type of cardiomyopathy. Thus, the influence of scar tissue on myocardial blood flow in patients with suspected MVD is unknown.

Cardiovascular magnetic resonance (CMR) is increasingly being used in clinical practice to evaluate cardiac disease. CMR employs a multifaceted imaging approach with separate techniques used to acquire separate sets of raw data, providing information on cardiac morphology, function, regional myocardial ischemia, scarring, and global myocardial perfusion reserve. The advantage of this approach is that image artifacts in one set of data will not affect the quality of the other datasets, and the datasets in combination can be used to distinguish separate pathophysiologies that could confound image interpretation. For example, perfusion defects could be due to ischemia or scar tissue, but since we will obtain both perfusion images and scar images, we will be able to resolve the etiology. Additionally, CMR provides high spatial resolution (over 10-fold higher than PET), and hence partial volume effects will be kept to a minimum and variability in measurements will be reduced.

In the proposed study, we will assess microvascular function as determined by a CMR measurement of whole-heart (global) perfusion reserve. Our laboratory has recently made optimizations to this technique (see below; **Figure 1**) which will improve the reproducibility of measurements. Our goal is

to determine the prevalence of MVD in two common forms of non-ischemic cardiomyopathy, hypertrophic cardiomyopathy (HCM) and idiopathic dilated cardiomyopathy (IDCM). We hypothesize that our optimized technique will provide robust detection of MVD and that our multifaceted approach will provide new insights into the pathophysiology of MVD, including the influence of myocardial scarring upon the presence and severity of MVD.

The Duke Cardiovascular Magnetic Resonance Center (DCMRC)

We believe the DCMRC is well positioned to successfully perform the proposed investigation. The DCMRC was the first dedicated CMR clinical service in the United States, and is the largest. We currently perform ~3,500 CMR scans per year, and of these, ~700 are stress perfusion CMR studies. The DCMRC also has an active research program. Faculty members in the DCMRC developed and validated the delayed enhancement technique (DE-CMR), which is now utilized in all CMR centers worldwide to identify scar tissue associated with myocardial infarction and non-ischemic cardiomyopathy.⁴⁻¹⁰ Our group also published the first report describing how to combine stress perfusion CMR with delayed-enhancement CMR (DE-CMR) for differentiating ischemia from scar.^{11,12}

Pilot Data and Anticipated Findings

As noted earlier in this proposal, we will use a multifaceted CMR imaging approach, which includes:

- (a) cine-CMR to assess left ventricular mass, volume, and function
- (b) coronary sinus velocity-flow CMR with and without vasodilator stress to measure global myocardial blood flow and perfusion reserve
- (c) first-pass perfusion CMR with and without vasodilator stress to evaluate regional perfusion defects
- (d) delayed enhancement CMR after contrast administration to provide high spatial resolution images of scar tissue.

With regard to coronary sinus velocity-flow CMR (CS-CMR), our group has recently introduced optimizations to this technique (**Figure 1**). The optimizations are related to [a] sequence design (e.g. importance of water-fat in-phase measurement), [b] sequence settings (need for high bandwidth imaging), and [c] imaging protocol (e.g. precise double oblique plane needed within 1 cm of the origin of the coronary sinus).

We have studied a small group of individuals with hypertrophic cardiomyopathy (n=5), idiopathic dilated cardiomyopathy (n=4), and normal controls (n=3). These pilot data suggest that the optimizations reduce measurement variability and

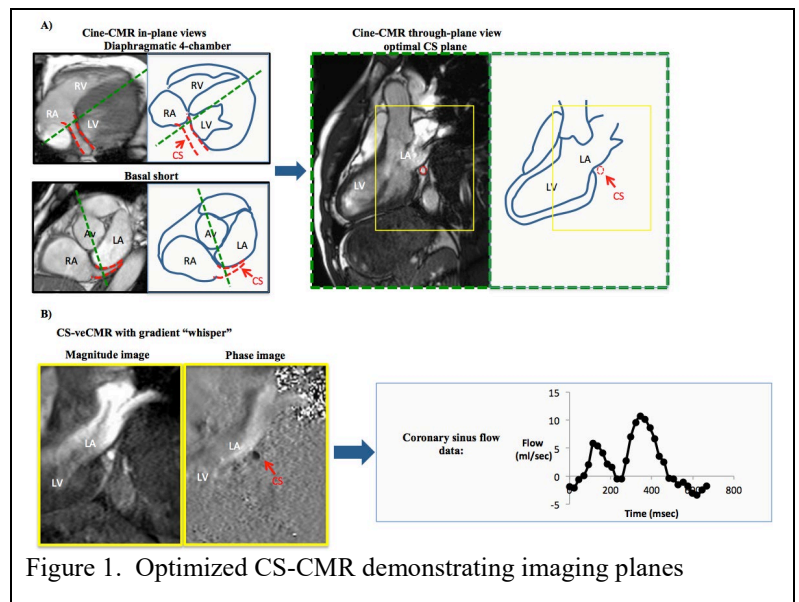


Figure 1. Optimized CS-CMR demonstrating imaging planes

improve reproducibility compared with conventional imaging. Additionally, we have observed several other interesting findings:

(1) Global perfusion reserve by CS-CMR was often reduced in both patients with hypertrophic cardiomyopathy and idiopathic dilated cardiomyopathy (Figure 2, top). However, we also noted variability in the severity of MVD. For instance, in patients with HCM with mild LVH, borderline normal global perfusion reserve by CS-CMR was observed. In patients with IDCM, there seemed to be less dependence on LVH but a stronger relationship between the severity of LV systolic dysfunction and the severity of MVD.

(2) We observed differences in MVD phenotype identified by CMR between patients with hypertrophic cardiomyopathy and idiopathic dilated cardiomyopathy. An example is shown in **Figure 2**. Although global perfusion was reduced to similar degrees in both, differences were clearly present on stress perfusion CMR and delayed enhancement CMR. The patient with hypertrophic cardiomyopathy (Figure 2, left) demonstrated visible, focal perfusion defects on first-pass perfusion CMR and moderate scarring on delayed enhancement CMR. The patient with idiopathic dilated cardiomyopathy (Figure 2, right) did not have any focal perfusion defects and scarring was minimal despite the same level of MVD (global perfusion reserve = 1.2 for both).

One plausible explanation for this finding could be that MVD in hypertrophic cardiomyopathy is a result of abnormalities in arterioles, whereas in idiopathic dilated cardiomyopathy, the abnormalities may lie in the more distal microcirculation (e. g. due to capillary rarefaction).

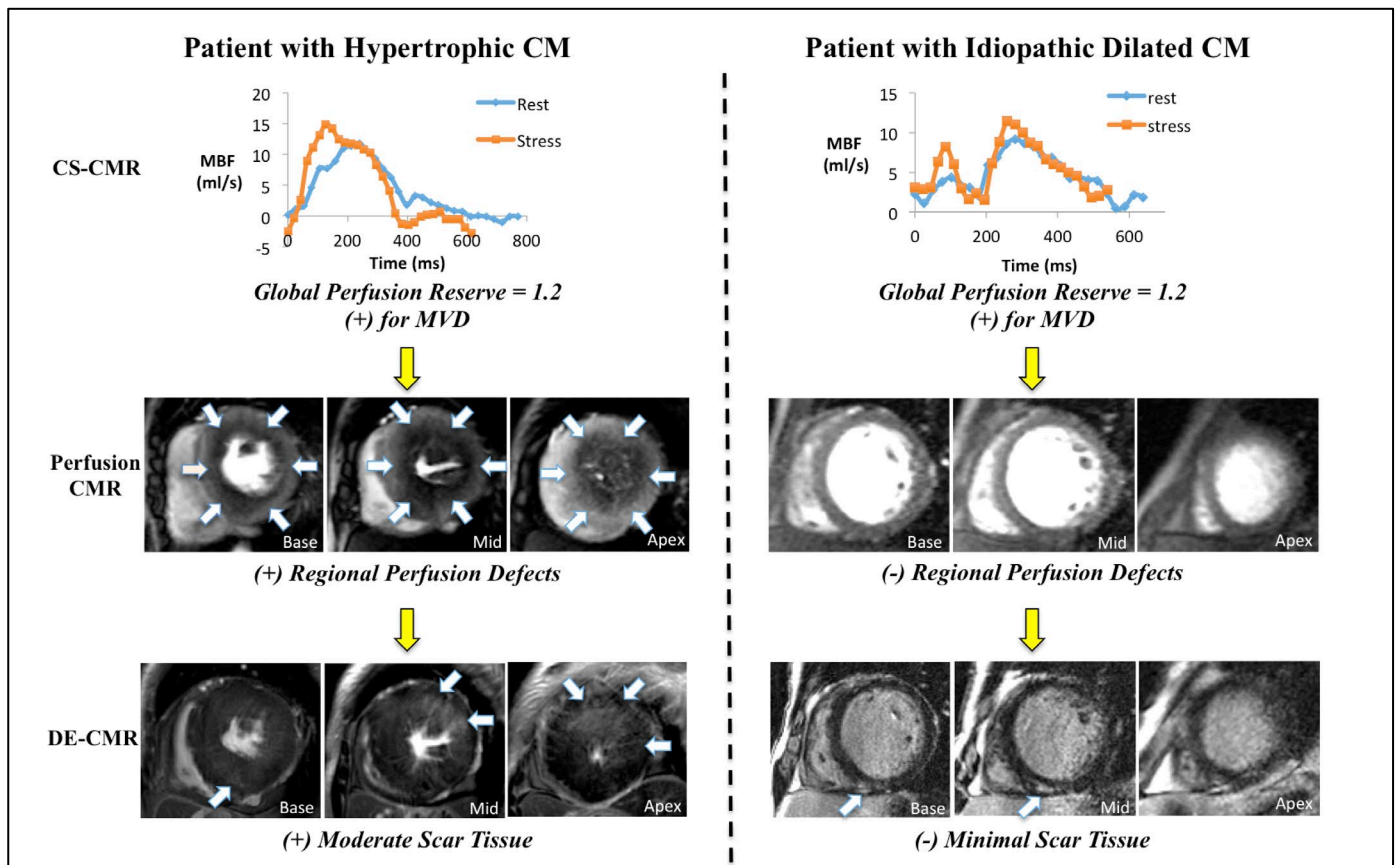


Figure 2. Different MVD phenotype in hypertrophic cardiomyopathy (left) versus idiopathic dilated cardiomyopathy (right) identified by multifaceted CMR. In both examples, there is evidence of severe MVD with markedly reduced global perfusion reserve by CS-CMR. In HCM, regional perfusion defects by perfusion CMR (arrows) and extensive myocardial scarring by DE-CMR, (arrows), are also present. However, in idiopathic dilated cardiomyopathy, no regional perfusion defects are seen with only minimal scar.

Hypothesis and Aims

Primary Objective

We propose to study the prevalence of MVD in patients with hypertrophic cardiomyopathy and idiopathic dilated cardiomyopathy compared to controls with atypical chest pain. MVD will be assessed by CS-CMR and will be defined by presence of global perfusion reserve <2.0 , which is a standard threshold used in prior studies.² Based on our initial observations, we believe our measurement of MVD will clearly distinguish between patients with cardiomyopathy and control patients with atypical chest pain, who have normal coronary arteries and no evidence of ischemia. The prevalence of MVD will be greater than 50% for both HCM and IDCM, but will be different between patients with hypertrophic cardiomyopathy and idiopathic dilated cardiomyopathy.

Secondary Objectives

To demonstrate the generalizability of global perfusion reserve as measure of MVD, we will compare the CS-CMR measurement of global perfusion reserve with regadenoson versus that adenosine to demonstrate that they are similar. Specifically, the global perfusion reserve in all participants receiving regadenoson will be compared to those receiving adenosine using a two sample t-test. We will also explore whether differences within the subgroups are present (e.g. controls receiving regadenoson vs controls receiving adenosine; HCM patients receiving regadenoson vs HCM patients receiving adenosine; IDCM patients receiving regadenoson vs IDCM receiving adenosine). However, given the relatively small numbers, we expect the within subgroup comparisons to be mainly for the generation of hypotheses for subsequent studies.

Additionally, based on our initial observations, we anticipate that the presence and severity of MVD will be dependent on different morphological and functional characteristics in HCM and IDCM. Specifically we will examine:

- (1) the relationship between MVD and scarring
- (2) the relationship between MVD and regional perfusion defects
- (3) the relationship between MVD and LV morphologic characteristics (mass, volume, ejection fraction)

As a tertiary objective, we will also estimate the amount of interstitial fibrosis present in the myocardium of patients HCM and IDCM, compared to controls. Interstitial fibrosis will be estimated using MODified Look-Locker Inversion-recovery sequence (MOLLI) sequence, which is performed prior to and after contrast administration. We will attempt to discern if an association exists between MVD and increases in interstitial fibrosis.

Methods

Patient Selection

We will recruit patients referred to the DCMRC with a documented history of hypertrophic cardiomyopathy or idiopathic dilated cardiomyopathy. Those agreeing to consent will be enrolled and undergo the multifaceted CMR protocol (**Figure 3**). Patients with history of coronary artery disease will be excluded.

To serve as controls, we will recruit patients referred for CMR for atypical chest pain. Only patients with low Framingham Risk (10% or less CHD risk at 10 years) and no history of CAD will be considered for recruitment. Controls will be excluded if they have evidence of obstructive coronary artery disease either by coronary angiography or stress testing.

Other Exclusion Criteria

Patients will also be excluded for the following reasons:

- Decompensated heart failure or hemodynamic instability
- Prior coronary revascularization (PCI or CABG) or myocardial infarction (as evidenced by elevated CPK-MB or troponin levels)
- Accelerating angina or unstable angina
- Inability to physically tolerate MRI or implanted objects that are MRI incompatible
- Inability to provide written informed consent obtained at time of study enrollment.
- Severe claustrophobia
- Advanced heart block or sinus node dysfunction
- Hypersensitivity or allergic reaction to regadenoson or adenosine
- Hypotension
- Active bronchospasm or history of hospitalization due to bronchospasm
- History of seizures
- Recent cerebrovascular accident
- Use of dipyridamole within the last 5 days
- Contraindication to aminophylline
- Hypersensitivity or allergic reaction to gadolinium contrast media
- Severe renal insufficiency with estimated glomerular filtration rate <30 ml/min/ 1.73 m²
- If they are pregnant or nursing

A detailed clinical history will be performed in all patients at the time of CMR. Information including age, gender, symptomatology, blood pressure, weight, height, and cardiac risk factors will be recorded. 12-lead ECGs will be obtained in all. Blood will be drawn for measurement of creatinine (point of care) and hematocrit. In women of childbearing potential, a serum HCG will be drawn.

Study Specific Procedures

Upon agreeing to participate in the study, subjects will be randomized to receive regadenoson or adenosine.

CMR Overview

The CMR methodology and analysis to be used are fully described in the references. The entire scan procedure and scan time should take around 45 minutes. A cardiologist or fellow will supervise the scan, assess/triage patients, and initiate treatment if needed. A schematic of the imaging procedure is shown in **Figure 3**.

1. Standard Scout images of the heart leading to double oblique horizontal long axis view (4-Chamber) of the heart
2. Cine Image of the horizontal long axis view of the heart
3. Prescribed double oblique short axis view of the heart with first slice at the insertion points of the mitral valve in diastole.
4. Cine images of the short axis of the left ventricle from the base to and including the apex for entire LV coverage.

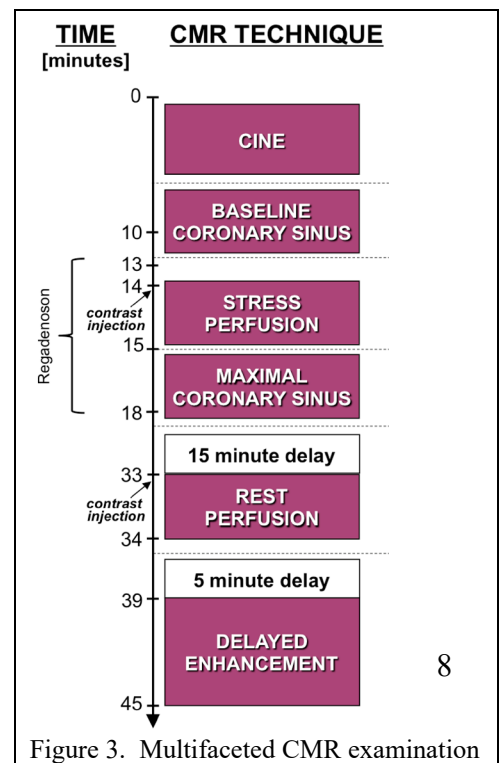


Figure 3. Multifaceted CMR examination

5. Cine images of the long axis views of the heart (2 chamber, 3 chamber, 4 chamber).
6. MOLLI images will be obtained prior contrast administration.
7. Coronary sinus imaging at rest as described in Figure 1.
8. Patients and controls will receive either (a) regadenoson (0.4 mg IV) injection over 10 seconds followed by saline flush or (b) adenosine $140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ x 3 minutes. During the injection or infusion, the participants will be monitored (continuous ECG and blood pressure monitoring) for 3-4 minutes. Half of the participants will receive Regadenoson, while the remainder will receive adenosine. Any serious adverse reactions during or after vasodilator stress will be recorded.
9. Stress perfusion images of 4-5 short axis slices (basal, mid, and apical) per heart beat for 60 heart beats beginning after 1-1.5 minutes after the regadenoson injection.
10. Bolus contrast injection of 0.1 mmol/kg of gadolinium contrast (gadoteridol or gadoteric acid) followed at 30 cc of saline flush beginning beginning after 1-1.5 minutes after regadenoson administration or after 2 minutes of adenosine infusion.
11. Coronary sinus imaging during stress (Figure 1).
12. In those receiving regadenoson, aminophylline (100 mg IV) will be administered over 30-60 seconds after completion of coronary sinus imaging. If bronchospasm is noted, participants will be treated with inhaled albuterol metered dose inhaler.
13. After a 15 minutes wait period, rest perfusion images of 4-5 short axis slices per heart beat for 60 heart beats using the identical imaging location and parameters as the stress perfusion images.
14. Bolus contrast injection of 0.1 mmol/kg of gadolinium contrast (gadoteridol or gadoteric acid) followed at 30 cc of saline flush beginning at the start of rest perfusion imaging.
15. Delayed enhancement images
 - a. (Starting immediately after rest perfusion imaging) True FISP IR single shot non-breath held images (stack of short-axis views from base to apex with normal myocardium nulled). These are performed with the patient instructed to breath quietly with minimal respiratory motion
 - b. (Starting immediately after single shot True FISP IR) breath held, ECG gated, segmented inversion recovery FLASH sequence with inversion time adjusted to null the myocardium. Slice locations should be identical to cine CMR locations
 - c. Repeat step A (single-shot non breath-held stack of delayed enhancement images) with the TI fixed at 600 ms.
16. MOLLI images will be obtained after contrast administration.

CMR details and parameters

(1) Cine:

- SSFP cine sequences are ECG gated and breath held
- Slice Thickness of 6mm with a distance of 8 mm between slices (gap of 2 mm)
- Temporal resolution of less than 45 ms
- Flip angle maximized (>60 degrees)
- After obtaining horizontal long axis of the heart, obtain double oblique short axis at the level of

the insertion points of the mitral valve in diastole. Proceed with short axis cines every 10mm from the base to the apex until the myocardium is not visualized

(2) Coronary sinus at rest

A 3 step process was used to determine the optimal view for en face velocity encoded CMR of the CS. A graphical demonstration of the steps is shown in **Figure 1A-B**.

- Step 1: Acquisition of optimal CS-CMR imaging plane (Figure 1). In addition to the standard long axis cine planes, 2 additional orthogonal views (diaphragmatic 4-chamber, basal short-axis) are obtained to localize the coronary sinus. The diaphragmatic 4-chamber is obtained by adjusting the standard 4-chamber, inferiorly to visualize the CS entering into RA. A basal short axis is obtained based upon the location of the CS on the diaphragmatic 4-chamber. The optimal CS-CMR plane was acquired orthogonal to the CS in both in-plane views, within 1-2 cm of the CS ostium, ensuring the through-plane CS perpendicular to the main direction of the CS motion.
- Step 2: To obtain CS flow data, a velocity encoded CMR sequence to image en-face CS flow. The CS is centered with small field of view such that the CS is at the iso-center of the magnet for the reduction of velocity offset errors (Rolf, JCMR 2011,13:18). The small field of view is adapted to patient body size (**Figure 1A** right panel).
- Step 3: Optimize CS-CMR sequence parameters. The velocity encoded CMR sequence is performed with “whisper” gradients. The maximum gradient amplitude is minimized at < 22 (mT/meter). The slew rate of bipolar gradient is also minimized to < 50 mT/mms in order to reduce eddy currents and maxwell terms. (**Figure 1B, left panel**). CS-CMR images are then acquired at rest using a breath-hold retrospective triggered gradient-echo sequence. Summary of sequence parameters are listed below:
 - a) For 3.0T: TR/TE, 5.2/2.5 ms; temporal resolution: 31.3ms; For 1.5T: TR/TE, 7.4/4.8 ms; temporal resolution: 44.5ms; 3 lines per segment, flip angle, 20°; section thickness, 10 mm; field of view;
 - b) The velocity encoding was set to a maximum velocity of 70-90 cm/sec and set higher if there is aliasing.
 - c) The contour of the cross-sectioned CS was traced base on both anatomic and flow cine images at each time frame. CS blood flow (ml/sec) was calculated by summing the flow per cardiac phase over the cardiac cycle.

(3) Stress Perfusion

- Saturation-recovery non-segmented imaging with GRE readout
- Short-axis view imaging (at least 4 slices / per heart beat)
- Slice thickness 6-8 mm
- Parallel imaging, 2-fold acceleration
- In-plane resolution, ~ 1.8-2.8 x 2.0 mm
- Temporal resolution ~ 100 – 125 ms
- Regadenoson (0.4 mg IV) injection over 10 seconds followed by saline flush under continuous ECG and blood pressure monitoring for 3-4 minutes while patient table is partially outside of bore for easy patient monitoring OR
- Adenosine ($140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) infusion under continuous ECG and blood pressure monitoring for 3-4 minutes while patient table is partially outside of bore for easy patient monitoring
- Patient table is pushed in and then contrast is given (0.075 – 0.1 mmol/kg, 3-5 ml/sec) followed

- by at least 30 ml saline flush (3-5 ml/sec)
 - After imaging for 40-50 heart beats by which time contrast has transited the LV myocardium.
- (4) Coronary sinus during adenosine stress immediately following stress perfusion imaging.
- So that global perfusion reserve can be calculated, imaging will be performed at the identical location and parameters as with CS-CMR at rest.
 - In those receiving regadenoson, aminophylline (100 mg IV) will be administered over 30-60 seconds after completion of coronary sinus imaging.
- (5) Rest Perfusion
- After ~15 minute wait for contrast to washout from stress perfusion imaging, perfusion imaging repeated without pharmacologic stress using same dose of gadolinium contrast
- (6) Delayed Enhancement MRI (single-shot non breathheld):
- SSFP Single Shot ECG-triggered non-breath held inversion recovery sequence (short-axis stack from base to apex) with appropriate inversion time to null the myocardium
 - Flip angle is 45 degrees, matrix size is 192 in frequency encode direction.
 - Data acquisition is centered in mid-diastole
 - Slice Thickness of 6mm with a distance of 8 mm between slices (gap of 2 mm)
 - Images are acquired with triggering set to every third or fourth heart beat to allow recovery of relaxation between slices.
- (7) Delayed Enhancement Images (segmented breathheld):
- ECG-gated breath hold segmented inversion-recovery GRE sequence with inversion time adjusted to null signal in normal myocardium.
 - Temporal resolution should be below 200 ms
 - Flip angle should be 25 degrees
 - TE should be 3-4 msec and TR should be 8-9 msec
 - Slice locations should be identical to cine MRI locations (6-8 short axis, 2- and 4-chamber long axis) with slice thickness of 6 mm. The field of view should be set to the minimum possible without fold-in artifact obscuring the left ventricle. Timing is set to acquire image data in mid-diastole. Typical voxel is 1.9 X 1.4 X 6 cm.
- (8) MOLLI images
- SSFP ECG-gated, breathheld in three short axis location performed prior to contrast and after gadolinium contrast administration
 - Flip angle is 45 degrees.
 - Data acquisition is centered in mid-diastole
 - Pre contrast images will be obtained using 2 inversion recovery pulses followed by 5 and 3 readout heartbeats respectively, separated by 3 wait heartbeats.
 - Post contrast images will be obtained using 3 inversion recovery pulses followed by 4, 3, and 2 readout heartbeats with an extra heartbeat between inversions.

Image Analysis

For the primary aim of the study, the prevalence of MVD will be determined by the measurement of global perfusion reserve. Global preserve reserve is calculated from the following formula:

$$\text{Global Perfusion Reserve} = \frac{\text{coronary sinus blood flow during maximal vasodilation}}{\text{coronary sinus blood flow at rest}}$$

Coronary sinus blood flow will be measured by contouring the coronary sinus at each time point within the cardiac cycle on both the magnitude and phase CS-CMR images at each cardiac frame. Total coronary sinus blood flow is then calculated by summing the flow per cardiac frame over the cardiac cycle. A global perfusion reserve of <2.0 will be regarded as abnormal and consistent with MVD.

Flow measurements will undergo a baseline correction using basal myocardium reference region located closest to the coronary sinus during at late-systole or mid-diastole when the motion is the most static. The baseline correction was performed for each cardiac frame to get the corrected coronary sinus blood flow. Coronary sinus blood flow_{corr}=V_{corr}×A_{CS}, where coronary sinus blood flow_{corr} is baseline corrected. V_{corr} comes from subtracting mean velocity of static region of interest from CS velocities. A_{CS} is area of CS at each time frame.

For the secondary aims, specific analyses will also be performed for each CMR technique to determine myocardial scar size, extent of regional perfusion defects, and morphological and function characteristics such as LV mass, LVEF, and LV volume.

Myocardial scarring will be determined by visual inspection using the American Heart Association 17-segment model. A patient is adjudicated to have myocardial scarring if there is at least more than one quarter of a myocardial segment of DE. This is done to reduce the effect of imaging artifacts, which can occasionally mimic DE. The extent of regional scarring is scored according to the spatial extent of delayed hyperenhancement within each segment (0 = no hyperenhancement (HE); 1 = 1%–25% HE; 2 = 26%–50% HE; 3 = 51%–75% HE; and 4 = 76%–100% HE). Global infarct size will be calculated by contouring of hyperenhanced regions from the stack of short-axis of delayed enhancement CMR images.

Regional myocardial perfusion will be scored by visual inspection using the American Heart Association 17-segment model (minus the apex) for the presence or absence of perfusion abnormalities and the spatial extent of hypoperfusion within each segment. Perfusion defect size will be calculated by contouring of hypoenhanced regions from stress perfusion CMR images.

For calculation of left ventricular mass, volume, and ejection fraction, endocardial borders on the stack of short-axis cine images in end diastole and end systole, and the epicardial borders in end diastole, will be traced using planimetry. End diastolic volume (EDV) and end systolic volume (ESV) are calculated from the product of the area end-diastole and end systole respectively and the thickness of the imaged slice (10 mm). Left ventricular ejection fraction (LVEF) is calculated using the following formula:

$$\text{LVEF} = \frac{\text{end diastolic volume} - \text{end systolic volume}}{\text{end diastolic volume}}$$

Left ventricular mass is calculated by subtracting endocardial from epicardial volumes and multiplying by myocardial density (1.05 g/cm³).

The amount of interstitial fibrosis will be estimated by calculating the extracellular volume fraction from the MOLLI images.

The myocardial extracellular volume fraction (ECF) is calculated from the following formula:

$$ECF_{\text{myo}} = [1 - \text{hematocrit}] * \Delta R1_{\text{myo}} / \Delta R1_{\text{blood}}$$

where $\Delta R1 = (R1_{\text{post-contrast}}) - (R1_{\text{pre-contrast}})$ for myocardium or blood, respectively, and $R1 = 1/T1$. ECF values above that of a normal population are considered abnormal (elevated) and are inferred to represent increased fibrosis.

All image analyses will be performed blinded to patient identity and clinical characteristics.

Statistical Methods

Sample size Determination

The determination of sample size is based upon the primary aim of the study to assess the prevalence of MVD in subtypes of cardiomyopathy. Using a cutoff value of ≥ 2.0 as normal for global perfusion reserve, our pilot data demonstrates that MVD is present in approximately 70% patients with HCM, whereas none of the 3 controls had MVD. Samples sizes of 25 in each group (HCM and IDCM) will provide 71% power to detect a difference of 25% in the presence of MVD at $\alpha=0.05$.

Expected Start Date / Length of Enrollment

Immediate start date once protocol is approved. Given rate of enrollment of pilot data, we anticipate enrollment will be completed in 12-18 months.

Data Handling and Record Keeping

Data will be stored securely under the patient's name. All clinical data, images and procedural data will be de-identified prior to any in-house or public presentation of data or images. Coded data will only be released to those listed as study investigators or support staff. Clinical CMR images will be stored in the clinical PACS system of the Cardiac Magnetic Resonance Imaging Center (WebPAX, Heart Imaging Technologies, LLC www.heartit.com) system. All electronic research data will be stored on Duke School of Medicine servers on a secured shared drive in a database that is only accessible to the research team. The principal investigator, sub-investigators, study research coordinators, regulatory personnel, Duke University Medical Center Institutional Review Board, and any applicable regulatory agencies will have access to the study records. The data and associated identifiers will be kept for up to 6 years in a locked research facility accessible only to designated personnel, at which time the data will be destroyed.

Serious Adverse Events Reporting

A cardiologist or fellow will supervise the scan, assess/triage patients, and initiate treatment if needed. A serious adverse event will be defined as any untoward medical occurrence that:

- (1) Results in death.
- (2) Is life-threatening. Any adverse experience that places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that, had it occurred in a more serious form, might have caused death).

- (3) Requires inpatient hospitalization or prolongation of existing hospitalization.
- (4) Results in persistent or significant disability or incapacity.
- (5) Is a congenital anomaly/birth defect.
- (6) An event that required intervention to prevent permanent impairment or damage or is considered medically important.

All adverse events will be reported to the Duke Institutional Review Board, as follows

- (1) Immediately (within 24 hours) upon learning of an unanticipated study-related death, study personnel will notify the IRB and provide a brief summary of the event. Then, within 1 week (five business days), study personnel will send to the IRB a formal safety event submission.
- (2) For a reportable serious adverse event, study personnel will notify the IRB within five business days of the investigator becoming aware of the event. Study personnel will send a formal safety event submission.
- (3) For any other problem or event requiring prompt reporting to the IRB, within ten business days of the investigator becoming aware of the event, study personnel will send to the IRB a formal safety event submission.

Any serious adverse events will also be reported to the Federal Drug Administration through the MedWatch program at the time that the IRB is notified. A copy will also be provided to Astellas Pharma, Inc.

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