Oxygen-Sensitive Magnetic Resonance Imaging in Women with Chest Pain and Non-Obstructive Coronary Artery Disease: A Mechanistic Insight into the Pathophysiology of Microvascular Disease

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A thesis is submitted to McGill University in partial fulfillment of the requirements of the degree of a Masters of Science in Experimental Medicine

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Abstract

Background: Among women with chest pain and non-obstructive coronary arteries (NOCAD), approximately 50% have evidence of coronary microvascular dysfunction (CMD), which carries a heightened risk for long-term cardiovascular events. Current diagnostic tools used in the assessment of women with chest pain and NOCAD are invasive and have provided conflicting results. Oxygen sensitive magnetic resonance imaging (OS-CMR) using hyperventilation/breath-hold manoeuvres is an emerging technique that allows for a non-invasive assessment of myocardial oxygenation. We aimed to study the coronary vascular response to breathing manoeuvres as monitored by OS-CMR in women with chest pain and NOCAD.

Methods: An interim analysis was performed for a study that will eventually incorporate 25 women with chest pain and NOCAD and 25 healthy volunteers. Fourteen women with chest pain and angiographically verified non-obstructive coronary artery disease (<50% stenosis) and sixteen healthy volunteers were recruited. All patients were scanned using a 3 Tesla magnetic resonance imaging (MRI) scanner with a standardized cardiac MRI protocol inclusive of cine imaging, T2-weighted imaging, native T1 mapping, rest and stress Blood Oxygen Level Dependent (BOLD) CMR, effectively providing OS-CMR images. During OS-CMR, patients performed guided hyperventilation for 60s followed by a 30s breath hold, acting as a vasoactive stimulus. The coronary vascular response was assessed by measuring myocardial signal intensity changes in OS-CMR images during the breath-hold and during hyperventilation. Regional heterogeneity in myocardial oxygenation was assessed through calculation of radial, circumferential, and combined absolute differences in signal intensity between myocardial segments in the endocardium and epicardium during the breath-hold. In women with chest pain and NOCAD, coronary vascular function was also assessed during adenosine-induced hyperemia using first-pass CMR perfusion imaging.

Results: There was no significant difference in age, body mass index, myocardial mass or cardiac output between women with chest pain and NOCAD compared to healthy volunteers. By adenosine first-pass perfusion imaging, the mean MPR index was significantly lower than previously reported normal reference values at 1.32±0.23, consistent with impaired microvascular function. Women with chest pain and NOCAD had a higher mid-T2 on edema imaging compared to healthy volunteers (42.0 ms (SD: 1.9) vs. 38.8 ms (SD: 2.2), p=0.001). Although there was no significant difference in global myocardial oxygenation response during a breath-hold (7.3 ± 6.6% vs. 3.9 ±3.4%, p=0.07), women with chest pain and NOCAD had higher regional heterogeneity in myocardial oxygenation (combined absolute difference: 15.8 ± 4.7% vs. 10.1 ± 2.6%, p =0.009). Intramyocardial heterogeneity in women with chest pain and NOCAD was correlated with a lower myocardial perfusion reserve (r=-0.67, p =0.07).

Conclusion: In our interim analysis, women with chest pain and NOCAD with a reduced myocardial perfusion reserve demonstrated an increase in regional heterogeneity in oxygenation, suggesting the possibility of microvascular maldistribution as a pathophysiological response in CMD. Breathing manoeuvres in combination with OS-CMR provides important mechanistic insights into the pathophysiology of women with chest pain and NOCAD.

Résumé

Contexte: Parmi les femmes souffrant de douleurs thoraciques et d'artères coronaires non obstructives (NOCAD), environ 50% présentent des signes de dysfonction microvasculaire coronaire (DMC), qui entraîne un risque accru d'événements cardiovasculaires à long terme. Les outils de diagnostic présentement utilisés dans l'évaluation des femmes souffrant de douleur thoracique et de NOCAD sont invasifs et ont donné des résultats contradictoires. L'imagerie par résonance magnétique sensible à l'oxygène (OS-CMR) utilisant l'hyperventilation /maintient du souffle est une technique émergente qui permet une évaluation non invasive de l'oxygénation du myocarde. Notre objectif était d'étudier la réponse vasculaire coronaire aux manœuvres respiratoires sous surveillance OS-CMR chez les femmes souffrant de douleurs thoraciques et de NOCAD.

Méthodes: Une analyse préliminaire a été réalisée pour une étude qui éventuellement inclura 25 femmes souffrant de douleurs thoraciques et de NOCAD et 25 volontaires en bonne santé. Quatorze femmes souffrant de douleurs thoraciques et de coronaropathie non obstructive vérifiée par angiographie (sténose>50%) et seize volontaires en bonne santé ont été recrutées. Toutes les patientes ont été scannées à l'aide d'un scanner Tesla 3 d'imagerie par résonance magnétique (IRM) avec un protocole d'IRM cardiaque standardisé incluant l'imagerie ciné, l'imagerie pondérée en T2, le mapping T1 native, le CMR dépendant du taux d'oxygène dans le sang au repos et au stress (BOLD), fournissant ainsi des images OS-CMR. Au cours de l'OS-CMR, les patients ont pratiqué une hyperventilation guidée pendant 60 secondes suivie d'une pause de 30 secondes, agissant comme un stimulus vasoactif. La réponse vasculaire coronaire a été évaluée enmesurant les changements d'intensité du signal myocardique dans les images OS-CMR pendant la garde du souffle et pendant l'hyperventilation. L'hétérogénéité régionale de l'oxygénation du myocarde a été évaluée par le calcul du radial, circonférentiel, et la différence absolues de l'intensité du signal entre les segments du myocarde de l'endocarde et de l'épicarde lors de la tenue de la respiration. Chez les femmes souffrant de douleur thoracique et de NOCAD, la fonction vasculaire coronaire a également été évaluée au cours d'une hyperhémie induite par l'adénosine, à l'aide de l'imagerie de perfusion CMR de premier passage.

Résultats: Il n'y avait pas de différence significative d'âge, d'indice de masse corporelle, de masse myocardique ou de débit cardiaque entre les femmes souffrant de douleur thoracique et NOCAD par rapport aux volontaires en bonne santé. Les femmes souffrant de douleurs thoraciques et de présentaient une imagerie d'œdème plus élevée au milieu de T2 par rapport aux volontaires en bonne santé (42,0 ms (SD: 1,9) par rapport à 38,8 ms (SD: 2,2), p 0,05). Bien qu'il n'y ait pas eu de différence significative entre la réponse à l'oxygénation globale du myocarde lors d'une halte respiratoire (7,3 ± 6,6% et 3,9 ± 3,4%, p = 0,07), les femmes souffrant de douleur thoracique et de NOCAD présentaient une plus grande hétérogénéité régionale dans l'oxygénation du myocarde (différence absolue combinée: 15,8 ± 4,7% vs 10,1 ± 2,6%, p = 0,009). L'hétérogénéité intramyocardique était corrélée à une réserve de perfusion myocardique inférieure (r = -0,67, p = 0,07).

Conclusion: Dans notre analyse préliminaire, les femmes souffrant de douleur thoracique et de NOCAD avec une réserve de perfusion myocardique réduite ont montré une augmentation de

l'hétérogénéité régionale dans l'oxygénation, suggérant la possibilité d'une mauvaise distribution microvasculaire en tant que réponse physiopathologique dans la CMD. Les manœuvres respiratoires en combinaison avec OS-CMR fournissent des informations mécanistiques importantes sur la physiopathologie des femmes souffrant de douleurs thoraciques et de NOCAD.

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This thesis would not have been possible without the contribution of several of my mentors and the endless support and encouragement from my friends and family.

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I would like to acknowledge the support of my family. To my siblings Ahmed, Mouad, and Mubrouka, thank you for all the encouragement and support during my MSc year. To my dearest mother Faeza, I would like to thank you for your patience and endless love, and always celebrating each and every step along this long educational journey. You have instilled within me a life-long understanding that in times of challenge comes growth. Thank you to my biggest mentor, my father Mohamed, for your wisdom, motivation, and endless support throughout my educational career. You have always instilled within me a desire to pursue more, learn more, and achieve more than I could have ever imagined.

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Finally, to our newest addition to the family. You have been the greatest motivation of all. I hope in this thesis, you find an inspiration and drive to be forever curious, patient and enduring, and to always strive in the endless pursuit of knowledge.

List of Abbreviations and Acronyms

BOLD: Blood oxygen level dependent

CTH: Capillary transit time heterogeneity

CSX: Cardiac syndrome X

CMRI: Cardiovascular magnetic resonance imaging

CAD: Coronary artery disease

CMD: Coronary microvascular dysfunction

CRT: Coronary reactivity testing

HADS: Hospital anxiety and depression score

MRI: Magnetic resonance imaging

MPR: Myocardial perfusion reserve

NOCAD: Non-obstructive coronary artery disease

OS-CMR: Oxygen sensitive magnetic resonance imaging

OSA: Obstructive sleep apnea

RF: Radiofrequency

SI: Signal intensity

SSFP: Steady state free precession

Preface and Statement of Originality

All the writing in this thesis is the sole work of the main author, Malik Elharram (ME). In addition, all the tables and figures were designed and generated by ME. To ensure reproducibility and accuracy of the results, an additional comparative analysis for the oxygen-sensitive cardiac magnetic resonance (OS-CMR) images was completed by Elizabeth Hillier (EH). Development of a novel OS-CMR technique used to assess regional heterogeneity (Section 3.5) was performed by EH and evaluated in this dataset by ME.

The thesis was made possible through the contribution from the expertise of my supervisors. Dr. Louise Pilote brings her expertise in epidemiology and the study of women and vascular health. Her contribution to the thesis was with the design of the study, statistical analysis of the data, and recruitment of patients in Montreal. Dr Pilote obtained funding for the study. Dr. Matthias Friedrich brings his expertise in cardiovascular magnetic resonance imaging and contributed his knowledge and teaching on the analysis of oxygen sensitive MRI (OS-CMR). Dr. Todd Anderson brings his expertise on the assessment and evaluation of women with non-obstructive coronary artery disease, and he contributed to the design of the study and recruitment of patients in Calgary.

1.0 Introduction

1.1 Introduction to Magnetic Resonance Imaging

The signal for magnetic resonance imaging (MRI) is generated from the nuclei of hydrogen protons in human tissues. In the absence of a magnetic field, hydrogen atoms magnetic moments, or spins, are randomly oriented. When placed in a large static magnetic field (B_0), the spin of the hydrogen atoms align in parallel or anti-parallel with the direction of the magnetic field, creating a net magnetization vector (B_0) in the z-direction as shown in **Figure 1.**[1]Hydrogen atoms undergo precession or rotation at a speed that is proportional to the strength of the applied magnetic field, represented by the Larmor equation ($\omega = \gamma \cdot B_0$, where ω is the Larmor frequency, γ is the gyromagnetic ratio constant, and B_0 is the main magnetic field strength). Therefore, the Larmor frequency of precession is proportional to the applied magnetic field. In a 1.5T MRI, the precession frequency of hydrogen atoms is 64MHz, while in a 3T MRI it is 128 MHz.[1-3]

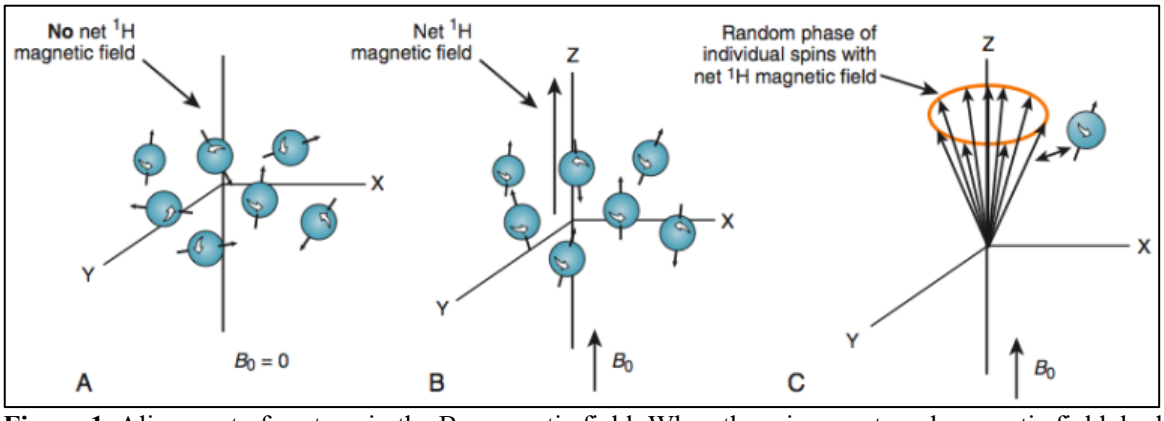

Figure 1. Alignment of protons in the B_0 magnetic field. When there is no external magnetic field, hydrogen protons are randomly oriented **(A)**. When the protons are placed in a strong magnetic field (B_0), a net magnetization will be produced parallel to the main magnetic field **(B & C).**[1]

The magnetic system can introduce energy by applying a radiofrequency pulse (RF) of the same frequency as the Larmor frequency. When the energy is applied to the hydrogen atoms,

the spins will absorb the energy from the RF pulse and the main magnetic field strength will be tilted away from the +z direction at an angle, known as the flip angle (α) (**Figure 2)**.[2,3]

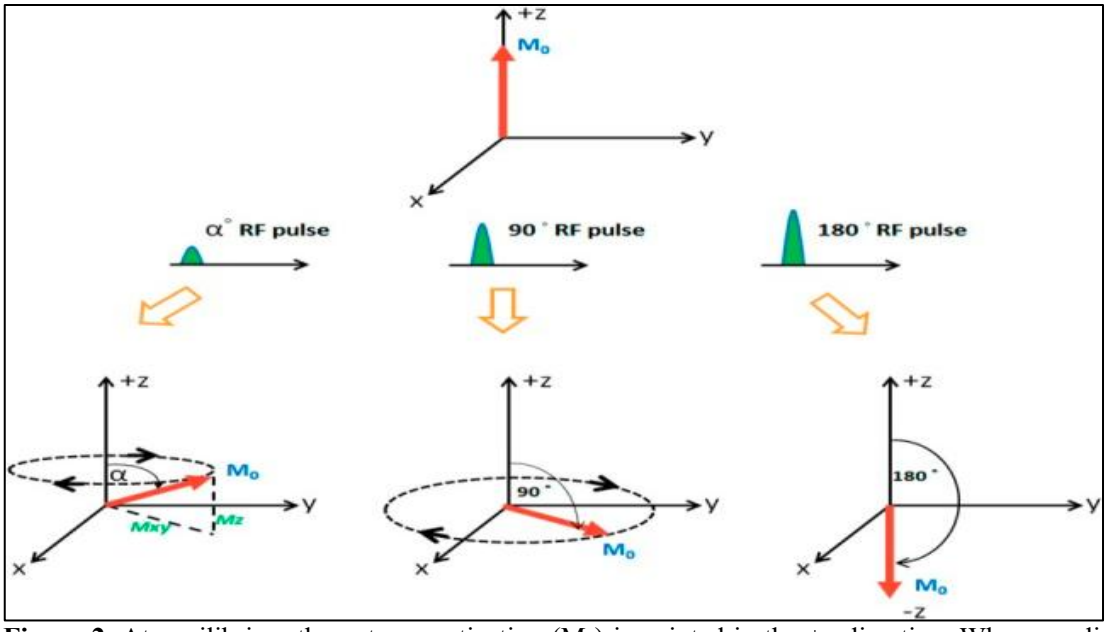

Figure 2. At equilibrium the net magnetization (M_0) is pointed in the +z direction. When a radiofrequency pulse is applied, the net magnetization vector absorbs the energy from the RF pulse and rotates away from the +z direction and makes an angle with respect to the +z direction (flip angle, α). The degree of the angle depends on the magnitude and duration of the RF pulse.[1]

When the RF pulse is turned off, the magnetization returns to the equilibrium state in a process known as relaxation, which emits a signal that is read by the MRI. Magnetization can be decomposed into two types of relaxation: longitudinal spin-lattice magnetization or T1 relaxation (relaxation component in line with the +z direction) and the transverse spin-spin magnetization or T2 relaxation (relaxation component in line with the x-y plane).T1 relaxation is defined as the time it takes to regain 63% of its equilibrium value following the termination of the RF pulse, while T2 relaxation is the time required to regain 37% of its initial equilibrium value. Both T1 and T2 relaxation occur simultaneously **(Figure 3).**[1-4]

Values of T1 are related to energy exchanged between proton spin and the surrounding molecules (spin-lattice interaction), whereas the value of T2 is related to energy exchanges between any two-proton spins (spin-spin interaction).[2] Additionally, the transverse phase (T2) is

also affected by static inhomogeneities in the local magnetic field, which can be caused by inhomogeneities in the applied local magnetic field or by local differences in the magnetization of tissues (i.e. paramagnetic contrast agents or iron deposition).[1] Effective transverse relaxation time, or T2*, describes the exponential decay in signal that results from the combination of spin-spin relaxation and static field inhomogeneities. Values of T1, T2 and T2* can be used to exploit differences in tissue characteristics and form the basis of MRI sequences used. [1-3]

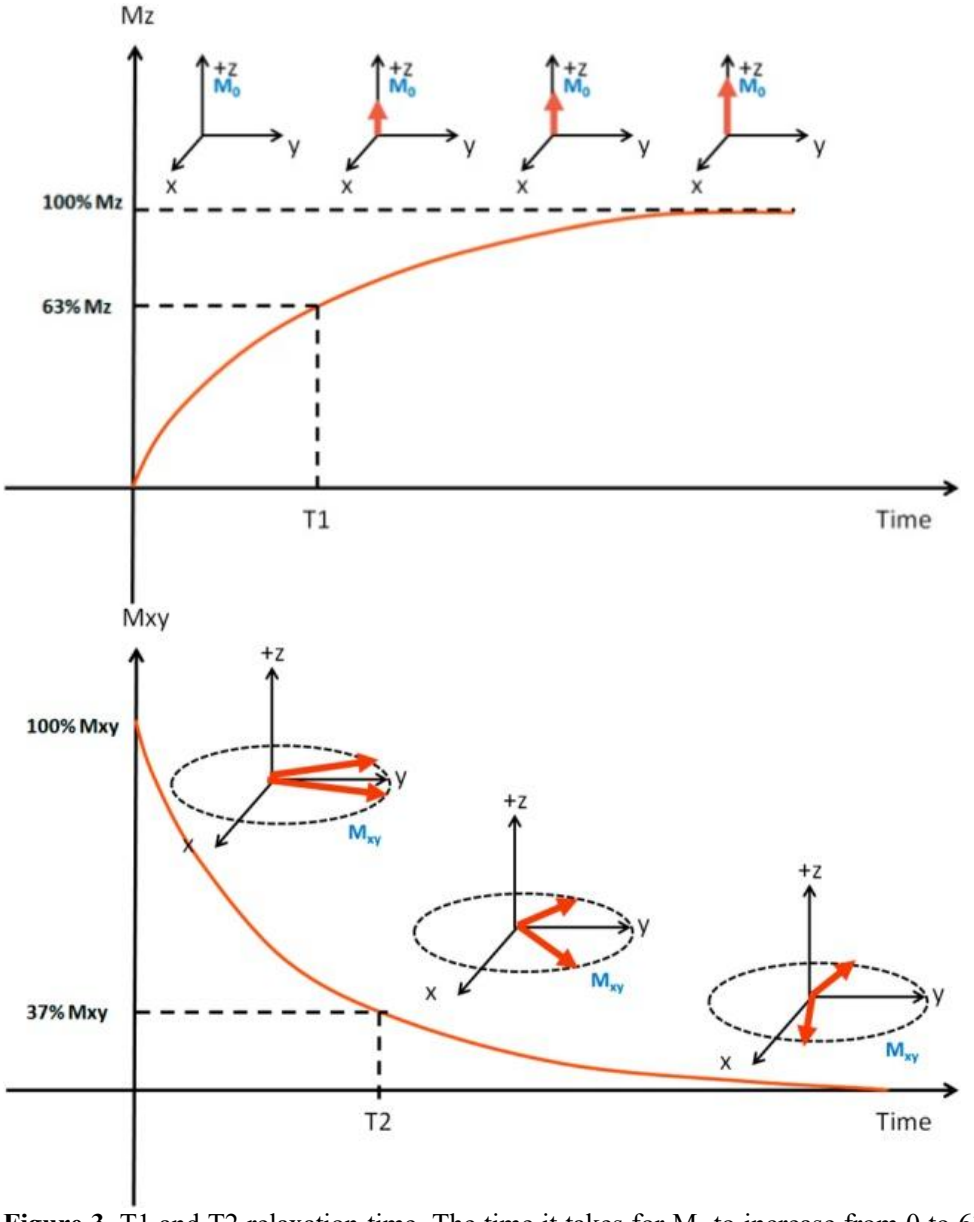

Figure 3. T1 and T2 relaxation time. The time it takes for M_z to increase from 0 to 63% of M_0 is known as T1. The time it takes for M_{xy} to decrease to 37% of M_{xy} is known as T2.[1]

1.2 Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance imaging (CMR) provides an accurate and reproducible measure of cardiac morphology, flow, volume, ventricular function, perfusion, and tissue characteristics of all four cardiac chambers, without the use of ionizing radiation.[3,4] We will briefly describe a number of CMR parameters that will be examined in this thesis.

Cardiac Function Imaging

An advantage of CMR is the ability to obtain dynamic cine images of the heart at multiple phases throughout the cardiac cycle. This dynamic series of images provides an opportunity to depict regional wall and valvular motion in addition to the quantification of cardiac volume and function.[4] As a result of an excellent contrast between myocardium and blood, changes of the cardiac chamber and ventricular wall can be readily visualized, allowing one to quantify the ventricular function with high accuracy and reproducibility.[5] Global function of the right and left ventricle can be acquired in multiple short axis views, including parameters of ventricular volumes, stroke volume, myocardial mass, and ejection fraction.[5,6]

Myocardial T1 and T2 mapping

T1-weighted imaging can be useful to identify normal anatomic structures, including the pericardium and pathological features including myocardial fibrosis. Increased pre-contrast myocardial T1 time has been reported in cardiomyopathies such as myocarditis, amyloidosis and hypertrophic cardiomyopathy. In contrast, patients with iron overload or fatty infiltration such as in Anderson-Fabry's disease show a decreased pre-contrast T1myocardial time.[7]

T2 weighted imaging is generally used to look for evidence of myocardial edema in the acute setting. Protons bound to free water have a longer T2 relaxation time, leading to areas of high signal intensity with an increased content of free water. Accumulation of water in the

myocardium is associated with different types of typically acute pathology including acute myocardial infarction, severe ischemia, myocarditis, stress-induced cardiomyopathy, or graft rejection. [2,8]

First-pass myocardial perfusion imaging

CMR first-pass myocardial perfusion imaging is increasingly being used for the diagnosis and risk stratification of patients with coronary artery disease.[9,10]Qualitative analysis of first-pass myocardial perfusion imaging relies on visual assessment of perfusion defects on first-pass imaging and is a subjective method of analysis as it is highly dependent on the expertise of the reader. Quantitative perfusion imaging can be performed by tracking the signal intensity change in images acquired during the first pass of an intravenously administered gadolinium-based contrast agent.[11]The slope and extent of the signal intensity increase in T1-weighted images correlates with myocardial perfusion.[9] Typically, coronary vascular function is assessed by comparing a first-pass obtained at baseline with one acquired during the presence of a pharmacological vasodilator (commonly adenosine). Areas with an impaired coronary vascular response, e.g. caused by coronary artery stenosis, show a delayed and diminished signal intensity change **(Figure 4).**

The signal intensity curves used to evaluate myocardial blood flow can be analyzed through semi-quantitative or quantitative methods. Semi-quantitative analysis of stress CMR perfusion is based on the calculation of the maximal upslope of the tissue attenuation curve, whereas a quantitative method is based on a model dependent deconvolution using the SI-curves.[9] In a quantitative analysis, a variety of tracer kinetic models can be used to provide quantification of absolute myocardial blood flow. Although marked heterogeneity exists across

CMR acquisition protocols and reference standards, semi-quantitative and quantitative perfusion analysis have demonstrated similar diagnostic accuracy for the assessment of significant CAD.[11]

Both semi-quantitative and quantitative parameters can be used to analyze a myocardial perfusion reserve (MPR), a quantitative marker for the relative difference between baseline and vasodilator images. An MPR quantifies the maximal observed increase in myocardial blood flow (above baseline) in response to exercise or pharmacological stimulation and is expressed as the ratio between myocardial signal intensity at peak stress vs. rest.[10,12]

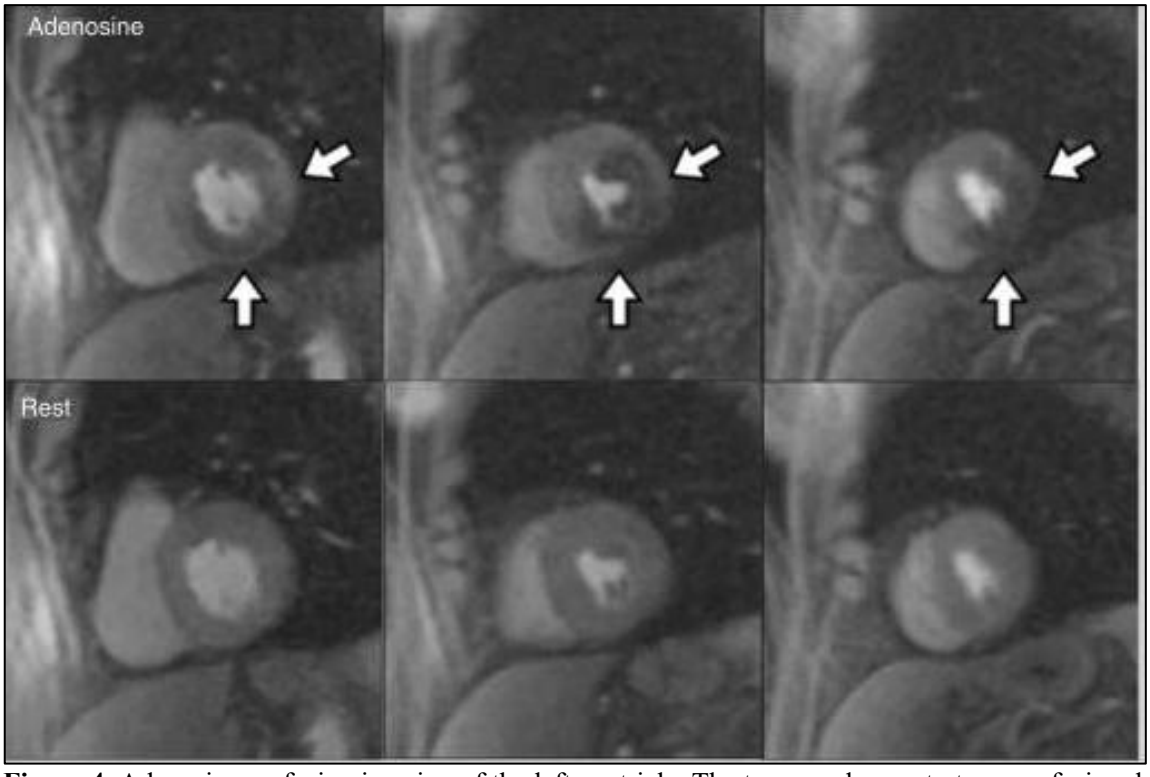

Figure 4. Adenosine perfusion imaging of the left ventricle. The top row demonstrates a perfusion defect in the anterolateral, inferolateral, and inferoseptal wall.[10]

1.3 Oxygen-Sensitive CMR (OS-CMR)

Magnetic susceptibility of red blood cells is strongly dependent on the blood oxygen saturation, whereby binding of oxygen to the heme molecules in red blood cells results in a detectable susceptibility difference between plasma and red blood cells.[3]While oxygenated hemoglobin is diamagnetic exhibiting weak stabilization of the magnetic field surrounding the molecule, deoxygenated hemoglobin is paramagnetic, resulting in pronounced spin-spin interaction and a loss of magnetic field homogeneity. In myocardial tissue these in-homogeneities are primarily caused by the magnetic properties of the ferrous iron component of hemoglobin.[13]Differences in local magnetic field inhomogeneities from deoxygenated hemoglobin result in local frequency variations that lead to changes in T2*, which are the basis of oxygen-sensitive images and the assessment of myocardial oxygenation changes.[3] In the myocardium, nearly 90% of blood volume is within capillaries, and changes in blood oxygenation in the capillary bed leads to changes in magnetic field variations between red blood cells and plasma.[13]After an excitation pulse, during the recovery of the transverse magnetization, these field variations cause hydrogen spins to lose coherence, leading to a reduction of the observed signal intensity in OS-CMR images. This phenomenon is referred to as the myocardial blood oxygenation level dependent (BOLD)effect.[3]

A decrease of the oxygenation of arterial blood, or a decrease of blood flow, or an increased myocardial oxygen utilization will result in a relative increase in the concentration of deoxyhemoglobin, which will increase the amount of paramagnetic material present (local magnetic field inhomogeneity), thereby leading to a reduction in signal intensity detected on OS-CMR. In contrast, increased blood flow, in the absence of an increased utilization, will decrease

the concentration of deoxyhemoglobin, decreasing the paramagnetic material present, thereby leading to a signal intensity increase in OS-CMR images.[13]

1.4 Women with Chest Pain and Non-Obstructive Coronary Artery Disease

Chest pain syndromes with non-obstructive coronary artery disease (NOCAD) comprise a large proportion of poorly diagnosed and treated patients**.** In most women, coronary artery disease (CAD) is characterized by atypical clinical presentation, later onset of disease, and a higher mortality, leading to diagnostic and therapeutic challenges for physicians.[14,15]Although more women than men die annually of cardiovascular disease, women presenting with symptoms and signs of myocardial ischemia are more likely to have NOCAD on coronary angiography.[14]As many as 50% of women presenting with symptoms of angina have normal or minimal CAD on coronary angiography, compared to 7-17% of men.[15]Historically, patients with NOCAD were given the diagnosis of Cardiac Syndrome X (CSX), and initial studies had suggested that these patients had a relatively benign prognosis.[16]More recently however, evidence has shown that a subpopulation of women with coronary microvascular dysfunction (CMD) are at a heightened risk for future cardiovascular events.[17-19] Data from the Women's Ischemia Syndrome Evaluation (WISE) study have shown that nearly 50% of women who present with chest pain and NOCAD have evidence of coronary microvascular dysfunction on coronary reactivity testing (CRT), with reportedly higher rates of cardiovascular events including hospitalization for heart failure, sudden cardiac death and myocardial infarction.[20]In the original WISE study, women with coronary microvascular dysfunction (defined as a reduced coronary flow reserve on invasive coronary reactivity testing) had an increased risk for cardiovascular death, myocardial infarction, stroke and heart failure, and an increased risk of mortality after adjusting

for baseline cardiovascular risk factors (**Figure 5).** [21]Furthermore, longer term follow up from the WISE study have demonstrated a worse prognosis in women with NOCAD, where the 10-year all cause death and cardiac death rates were 17% and 11% in women with non-obstructive CAD, and 10% and 6% in women with normal coronary arteries.[22]Additionally, in a recent Veterans Administration Database, patients with non-obstructive CAD in three coronary arteries, had a similar annual risk for myocardial infarction and death as patients with single-vessel obstructive CAD. [23] Despite this, chest pain syndromes with NOCAD remain poorly understood and the true prevalence of CMD in women is unknown and likely underestimated. Moreover, no published guidelines in the diagnosis and management of CMD currently exist. With a lack of clear diagnostic criteria, the development and responses to appropriate treatment of CMD cannot accurately be assessed. Patients with chest pain syndromes in the setting of NOCAD therefore represent a large heterogeneous population of poorly diagnosed and treated patients.

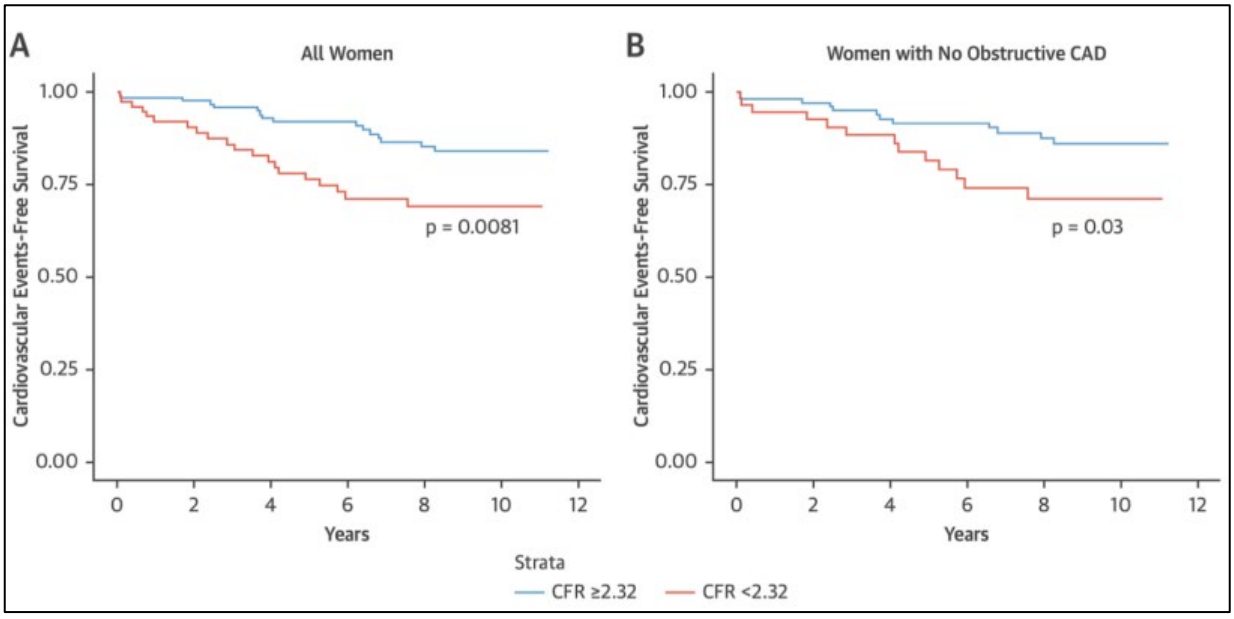

Figure 5. Kaplan-Meier analysis showing percentage of women surviving free from major adverse cardiovascular events (MACE) during long term follow up stratified by coronary flow reserve (CFR) in all women (**A)**, and women with chest pain and NOCAD (**B).** Women with a reduce coronary flow reserve (coronary microvascular dysfunction) had a worse prognosis independent of cardiovascular risk factors.[21]

1.5 Syndrome of Chest Pain and NOCAD

NOCAD is an all-encompassing term for chest pain syndromes without hemodynamically significant obstructive coronary artery disease, and defined as either less than 50% narrowing, less than 20% narrowing or unimpaired coronary blood flow, depending on the study.[24]Studies involving patients with chest pain syndromes and NOCAD have revealed a number of underlying ischemic and non-ischemic etiologies, with various overlapping nomenclature (**Figure 6)**.[25,26]CSX is a narrower definition of NOCAD, that requires proof of ischemic changes on non-invasive testing, and excludes patients with occlusive focal vasospastic abnormalities (i.e. Prinzmetal's angina).[27] Microvascular angina is a term used to describe a cardiac ischemic mechanism for angina in patients with normal coronary arteries and involves structural and functional abnormalities of the coronary microcirculation. Coronary microvascular dysfunction occurs in the setting of microvascular angina and is defined as an abnormal coronary flow reserve in the response to stress stimulus.[26]CMD is believed to comprise a large proportion of patients with chest pain syndromes and NOCAD and carries a worse prognosis.[19-22]

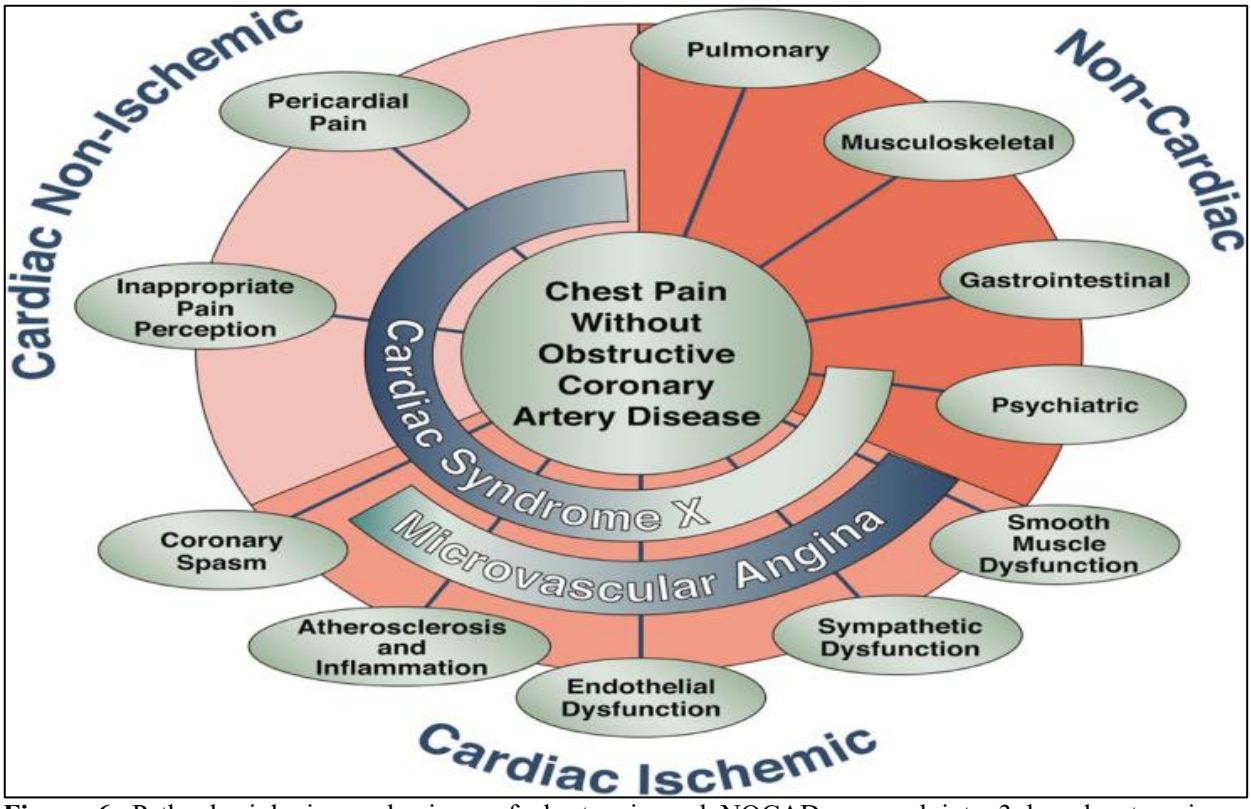

Figure 6. Pathophysiologic mechanisms of chest pain and NOCAD grouped into 3 broad categories: noncardiac, cardiac ischemic, and cardiac nonischemic. Microvascular angina overlaps partially with cardiac syndrome X, but it has a narrower cardiac ischemic pathophysiological basis.[25]

1.6 *Coronary Microvascular Dysfunction*

The coronary microcirculation represents a continuous network of functionally distinct vessel segments of decreasing size, ranging from large epicardial coronary arteries (>400μm) to small pre-arterioles and coronary capillary bed (<10μm).[26] The pre-arterioles and arterioles make up most of the resistance circuit of the heart and control the regulation and distribution of blood flow through changes in vessel resistance and tone. This adaptive mechanism helps to prevent myocardial ischemia during the development and progression of epicardial stenosis **(Figure 7)**.[26]

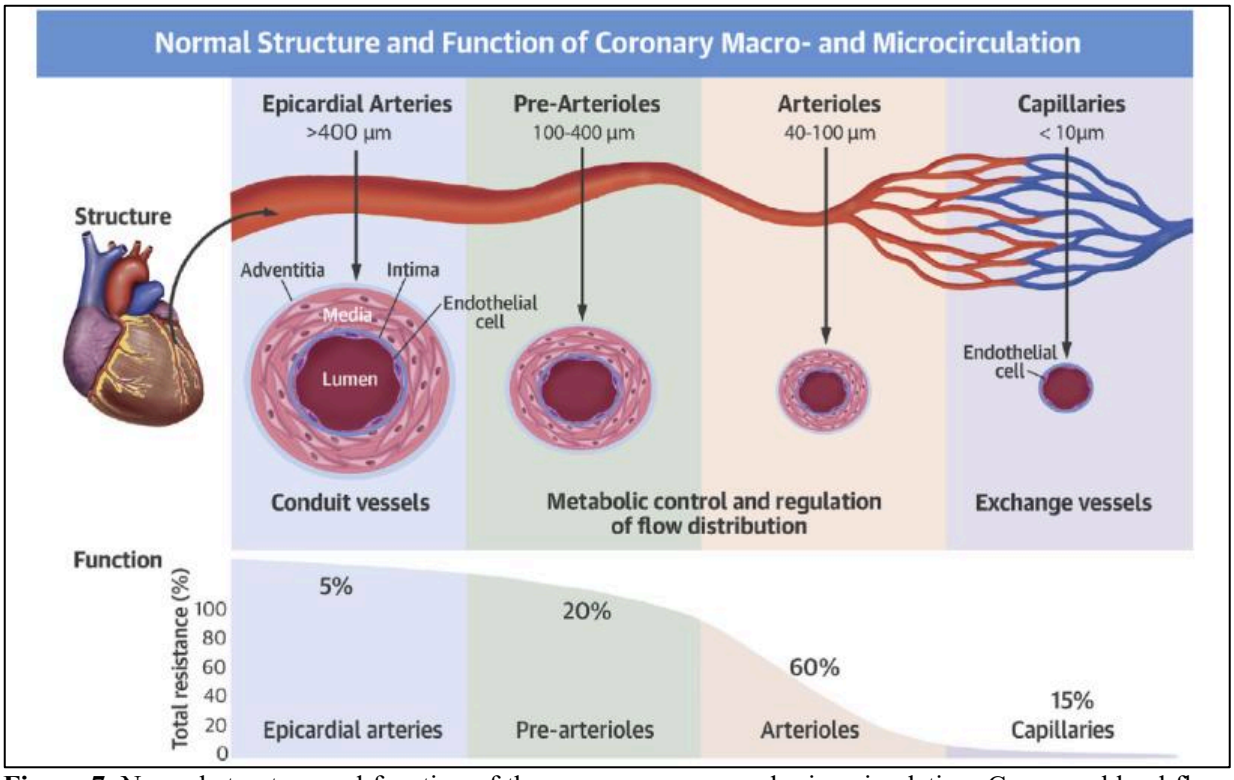

Figure 7. Normal structure and function of the coronary macro and microcirculation. Coronary blood flow remains constant over a wide range of perfusion pressure through changes in resistance in the microcirculation.[26]

CMD results from structural and functional impairment in the vasodilatation of the coronary microvasculature, leading to inadequate coronary blood flow response to an increase in physiologic demand (**Figure 8)**.[28]Structural abnormalities of the microvasculature include decreased luminal size from microvascular atherosclerosis, intimal smooth muscle thickening, and capillary rarefaction. Functional abnormalities in the coronary microcirculation are related to endothelial independent and dependent dysfunction.[26]Endothelium-independent dysfunction is thought to be related to impairment of smooth muscle relaxation due to structural abnormalities from microvascular hypertrophy or surrounding myocardial irregularities.[29,30]Attenuated response to vascular smooth muscle relaxation by vasodilators substances including adenosine, have been shown in patients with coronary microvascular dysfunction. Endothelium-dependent dysfunction is caused by a pathologic constriction of a

vessel or vascular bed due to an imbalance in the homeostatic regulation of the vascular endothelium.[28] In the presence of normal endothelial function, an increased physiological demand triggers vasodilation of the microcirculation by releasing vasoactive substances including nitric oxide. In the setting of endothelial dysfunction however, the vascular endothelium is injured, and vasodilator response is attenuated, resulting in blunted coronary blood flow augmentation.[26]

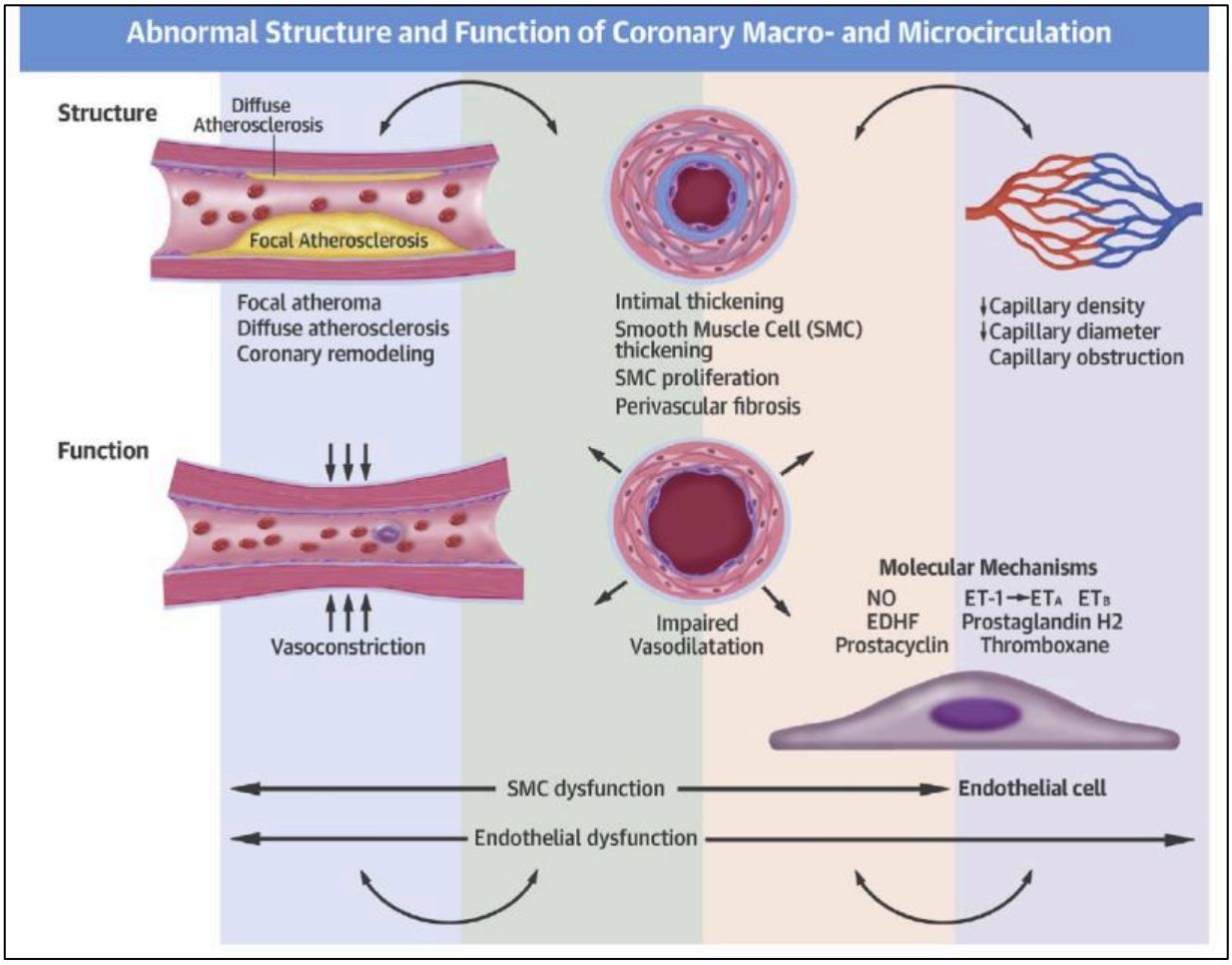

Figure 8. Pathogenesis of coronary microvascular dysfunction. Structural and functional abnormalities result in reduced flow mediated response to increased physiologic demand.[26]

Although there exists no technique to directly visualize the coronary microcirculation, varying diagnostic techniques have been used to distinguish between the various etiologies of

NOCAD.[28] Coronary reactivity testing (CRT) involves the measurement of micro and macrovascular responses of the coronary vasculature to vasoactive substances including acetylcholine, nitroglycerine, and adenosine and serves as the gold standard in the assessment of patients with CMD.[28,31-33]Responses of coronary vasculature can be measured by quantifying changes in epicardial coronary diameter, and by measurements of blood flow velocity using Doppler flow wires.[24]Although CRT is recommended by some cardiovascular guidelines in patients undergoing angiography for suspicion of NOCAD,[34-36] few centers are actually performing this test mainly due to the lack of a standardized protocol and concerns over catheterization laboratory time.[20] Additionally, the administration of vasoactive substances during CRT is often poorly tolerated in these patients.[37]Moreover, the use of quantitative arteriography has recognized limitations in the accurate measurements of the severity of atherosclerotic lesions, and the presence and extent of microvascular disease can have a significant effect on the measurement of flow reserve independent of the level of stenosis severity.[39,40]Therefore, while CRT remains the currently proposed procedure in the evaluation of patients with chest pain syndromes and NOCAD, it is invasive and has limited its use in the diagnosis of patients with CMD.

Several non-invasive techniques have been studied for the diagnoses of CMD, relying mainly on the measurement of a coronary flow reserve, calculated as the ratio of hyperemic to rest absolute myocardial blood flow. Positron emission tomography (PET) is the most validated non-invasive approach for quantitative assessment of coronary flow reserve, and the reproducibility of this technique has been well established in several experimental animal and human studies.[41] Doppler echocardiography of the left anterior descending coronary artery can also be used to quantify coronary blood flow velocity at rest and during vasodilator stress to

determine a coronary flow velocity reserve.[42] CMR is an emerging technique that can also be used to quantity myocardial perfusion reserve in a similar manner as PET, although with a high spatial resolution, lack of ionizing radiation, and the ability to perform a comprehensive assessment of cardiovascular structure and function.[25,40]

1.7 The Use of Cardiovascular MRI in patients with Chest Pain and NOCAD

CMR first-pass perfusion imaging can be used to calculate the indexed ratio of the perfusion time intensity curve upslope in response to vasodilator stress and expressed as the myocardial perfusion reserve index (MPRI).[43]Panting et al. were the first to examine MPRI using adenosine stress CMRI in patients with CSX. The study found subendocardial MPRI did not increase significantly with vasodilatation using adenosine in patients with CSX compared to controls, indicating that chest pain in these patients might be caused by underlying microvascular ischemia.[44] Further work to confirm the original findings of this study, however, have been controversial. In a study by Vermeltfoort *et al.* to assess subendocardial and subepicardial perfusion using CMRI at rest and under stress with adenosine infusion, patients with CSX were found to have significant perfusion increases to adenosine, without evidence of subendocardial hypoperfusion.[45] In contrast however, in a study by the WISE group examining 118 women with suspected CMD as determined by CRT and 21 asymptomatic reference controls, the group found that women with an abnormal CRT had reduced MPRI in both the subepicardium and subendocardium compared to controls. It was found in the study that an MPRI threshold of 1.84 predicted coronary reactivity testing abnormality with a sensitivity of 73% and specificity of 74%.[43] In a more recent paper, Liu et al assessed adenosine perfusion CMRI for diagnosing microvascular angina in patients with chest pain and NOCAD and correlated the findings to the

index of microcirculatory resistance (IMR), an invasive thermodilution-based marker of microvascular function. The authors found that an MPRI <1.4 accurately detected impaired perfusion related to CMD (IMR >25 U; Fractional flow reserve >0.8).[46]

The role of CMR first-pass perfusion imaging in patients with chest pain syndromes and NOCAD remains uncertain, and further studies are needed. While stress perfusion is considered a non-invasive diagnostic tool, it still utilizes pharmacological agents, which are often poorly tolerated and can have serious side effects in patients including chest pain, dyspnea, and arrhythmia.[37]Furthermore, recent data have shown that the length of a breath-hold is a significant confounder and therefore, the variable length of breath-holds may lead to a significant biological variability.[47]In summary, while CMR first-pass perfusion imaging has shown promise in detecting CMD, current data do not support its use for diagnosing patients with chest pain and NOCAD.

1.8 *Oxygenation-Sensitive MRI in women with Chest Pain and NOCAD*

OS-CMR using the BOLD effect is an alternative to first pass perfusion that allows for a direct determination of myocardial oxygenation without the application of exogenous contrast agents as a surrogate marker of myocardial blood flow.[13] This eliminates the need for accurate timing of dynamic perfusion imaging during bolus arrival and provides the opportunity of sequence repetition if necessary.[48]Moreover, OS-CMR eliminates the need for contrast agents such as gadolinium, which is problematic in patients with renal failure, and can lead to nephrogenic systematic fibrosis and more recently, has been shown to deposit in deep neuronal structures.[49,50]Wacker *et al.* completed one of the first studies examining the role of OS-CMR in a cohort of patients with known single-vessel CAD (>70% stenosis on coronary angiogram)

compared to healthy controls. Myocardial measurements were obtained at rest and during dipyridamole stress. The results demonstrated that T2* increased significantly in healthy volunteers, but was reduced at rest, with a modest response to vasodilation in myocardial segments supplied by a stenotic coronary artery.[51] The utility of OS-CMR in CAD patients was additionally examined by Friedrich *et al*. who demonstrated a significant comparison between stress-induced angina OS-CMR with the results generated by thallium single photon emission computed tomography (SPECT).[52]While prior studies did not distinguish between the impact of blood flow, perfusion, and blood volume on signal intensity on BOLD images, Voehringer et al. showed in an animal model using intracoronary injection of vasoactive substances, that signal intensity observed in oxygenation sensitive images reflected myocardial blood oxygenation rather than blood flow.[53]Since, OS-CMR demonstrated its ability to directly measure tissue oxygenation, it emerged as a promising diagnostic tool for CAD.

Manka *et al*. extended these conclusions by conducting a study using 3T MRI to assess the ability of OS-CMR to detect stress inducible myocardial ischemia. The study was composed of forty-six patients with suspected or known CAD who underwent CMRI prior to clinically indicated coronary angiography. 3T OS-CMR was found to accurately differentiate between normal, ischemic and non-ischemic myocardial segments, demonstrating the ability of OS-CMR to non-invasively identify patients with significant CAD.[54]OS-CMR has been shown to correlate well with quantitative coronary angiography and conventional CMRI perfusion imaging in the detection of CAD, validating its use in the measurement of perfusion and microcirculatory oxygenation.[55]With the ability to directly examine myocardial oxygenation without gadolinium, OS-CMR serves as a valuable alternative to first pass perfusion for cardiac pathology.

While the role of OS-CMR has been well studied in CAD, its clinical utility in the diagnostic workup of patients with chest pain and NOCAD has yet to be fully elucidated.In studies performed on patients with hypertension and hypertrophic cardiomyopathy, [56,57] OS-CMR was able to detect microcirculatory changes, suggesting it may be a useful surrogate marker for microvascular disease. Furthermore, OS-CMR has the potential of providing mechanistic insights into the pathophysiology of diseases affecting the microvasculature. This has been demonstrated in patients with 1 or 2 vessel CAD, where oxygenation signal intensity during hyperemia in 'remote to ischemia' segments were intermediate compared to segments of stenosed and normal coronary arteries, reflecting its ability to identify microvascular disease.[13]Furthermore, microvascular disease has been associated with diffuse fibrosis and more recently, edema. Both, fibrosis and edema can be readily identified by a combined application of native T1 mapping and T2 mapping using OS-CMR. Increased T2 indicates edema, while an abnormally high T1 in the absence of edema is a marker for fibrosis or infiltration. In focal abnormalities, the regional distribution adds further diagnostic information.[58]

Despite the evidence supporting its use in patients with NOCAD, only one study in the literature has examined OS-CMR in a cohort of patients with CSX. In all, 18 patients with CSX, were examined compared with 14 controls who underwent CMRI scanning at 3T using a BOLD protocol. Absolute myocardial blood flow was measured during adenosine stress and oxygenation was measured using a T2-prepared BOLD sequence. No significant differences were found in myocardial blood flow at stress, BOLD signal change, and coronary flow reserve measurements in patients with CSX and controls respectively.[59]The aforementioned study comprised a small cohort of patients and might not have been powered to detect any significant difference. Furthermore, the study examined transmural perfusion, and not subendocardial or

subepicardial blood flow, which have previously been associated with microvascular disease.[44,45] Additionally, patients in the cohort were on average 62 years of age. Hyperemic myocardial blood flow response to vasodilator stress has been shown to be reduced in a healthy older population in comparison to a younger population.[60] Despite these limitations, this remains the only existing study of OS-CMR in patients with chest pain syndromes and NOCAD, and further research is needed to validate these findings.

1.9 Breathing Manoeuvres in OS-CMR

Carbon dioxide is a potent vasodilator in the cerebral and coronary vascular beds, and recent studies have shown that breathing maneuvers can significantly affect coronary perfusion.[61,62] During patient-induced hyperventilation, the concentration of arterial carbon dioxide decreases, resulting in a coronary vasoconstrictive response, which can be detected as a negative change in signal intensity on oxygen-sensitive imaging. Furthermore, during a breath-hold maneuver, arterial concentration of carbon dioxide increases, resulting in coronary vasodilatation, which can be detected as a positive change in signal intensity.[62] In a study by Fischer et al. using OS-CMR with hyperventilation/breath hold manoeuvres, a combined manoeuvre had an even greater effect on myocardial oxygenation than an intravenous adenosine administration (signal intensity increase 14.8 ± 6.6% vs. 3.9 ± 6.5%)**(Figure 9)**.[63] Additionally, Roubille et al. examined the clinical utility of OS-CMR with hyperventilation/breath-hold manoeuvre in a cohort of patients with obstructive sleep apnea (OSA).The study found that myocardial vascular response in combined breathing manoeuvres of hyperventilation followed by voluntary apnea was blunted in patients with OSA compared to controls, likely due to underlining coronary microvascular changes involved in the pathogenesis of OSA.[64] The study

suggests a clinical utility of this non-invasive diagnostic tool for detecting microvascular changes involved in CMD. The utilization of breathing manoeuvres offers a safer alternative to pharmacological agents, which often have undesirable side-effects including bradycardia, arrhythmia, chest pain, and bronchospasm.[37]Additionally, the use of pharmacological agents requires an intravenous access and injection of costly vasodilator agents, as well as a longer overall scan time. Given that breathing manoeuvres can affect coronary perfusion quickly and induce a greater myocardial oxygenation response without the added cost of adenosine, OS-CMR could prove to be a non-invasive and cost-effective diagnostic tool for the assessment of CMD in patients with chest pain syndromes and NOCAD.

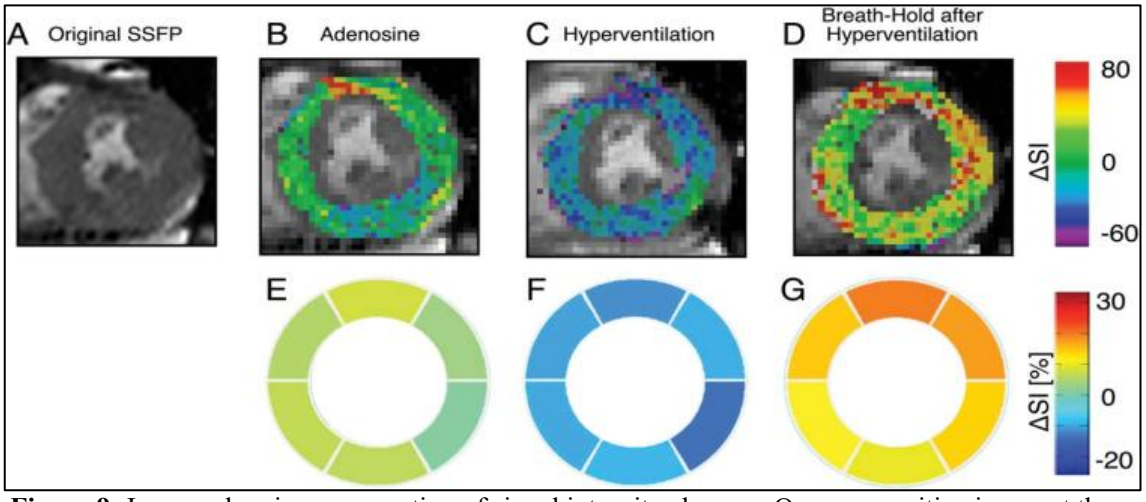

Figure 9: Images showing oxygenation of signal intensity changes. Oxygen sensitive image at the end of systole in **Image A.** Subtraction image displays the absolute difference in signal intensity (SI) from baseline for adenosine, hyperventilation, and maximum breath-hold following hyperventilation. During hyperventilation, a decrease in the concentration of carbon dioxide causes a coronary vasoconstrictive response **(Image C)**, which results in a negative change in SI from baseline. In contrast, a breath-hold maneuver results in an increase in arterial concentration of carbon dioxide and corresponding coronary vasodilatation, which results in a greater change in signal intensity **(Image D).** [64]

2.0 **Objectives**

The overall goal of this study is to evaluate the use of a novel non-invasive diagnostic test, the OS-CMR using a hyperventilation/breath-hold manoeuvre, to assess patients with chest pain and NOCAD.

Primary Aim 1: Determine relative changes in the myocardial oxygenation response, as measured by changes of signal intensity in OS-CMR during a combined hyperventilation/breath-hold manoeuvre, in women with chest pain and NOCAD compared to healthy volunteers.

Primary Aim 2: Assess the relationship between the myocardial oxygenation response observed by OS-CMR during a hyperventilation/breath-hold manoeuvre and CMR first-pass perfusion images using adenosine-induced hyperaemia in women with chest pain and NOCAD.

Hypotheses

1) In comparison to healthy subjects, women with chest pain and NOCAD have an attenuated myocardial oxygenation response to a standardized hyperventilation/breath-hold manoeuvre, as measured by changes of signal intensity in OS-CMR images.

2) In women with chest pain and NOCAD, the myocardial oxygenation response measured by OS-CMR during a hyperventilation/breath-hold maneuver correlates with results obtained by CMR first-pass perfusion imaging using adenosine-induced hyperemia.

3.0 Methodology

3.1 Cohort Selection

Women with chest pain and NOCAD: We recruited a cohort of women aged 40-65 with persistent retrosternal chest pain who underwent cardiac catheterization and were found to have non-obstructive coronary artery disease on angiography (defined as less than 50% luminal diameter stenosis in an epicardial coronary artery). These patients have a clinical diagnosis of exercise-inducible chest pain that is responsive to nitroglycerin, with non-ischemic causes of chest pain excluded on clinical history and physical exam. Documented evidence of ischemia on non-invasive imaging was not a requirement for inclusion within our study. Women were excluded from the study if they had: obstructive coronary artery disease with ≥50% luminal diameter stenosis in any epicardial coronary artery, acute coronary syndrome, Prinzmetal's angina, primary valvular heart disease, cardiogenic shock, prior non-cardiac illness with estimated life expectancy <4 years, chest pain with known non-ischemic etiology (e.g. pericarditis, pneumonia, esophageal spasm), contraindications to MRI (pacemaker, other electronic device, severe claustrophobia), severe asthma (vasodilator stress contraindicated), and renal impairment with an eGFR<45 ml/min/1.73m^2 (gadolinium contrast contraindicated).

Healthy controls: A cohort of women aged 40-65 without a history of cardiovascular disease were recruited as healthy volunteers using public notifications. Women were excluded if they had a known clinical history of vascular disease (i.e. myocardial infarction, revascularization procedure, cerebrovascular event), had been diagnosed with hypertension or were taking antihypertensive medications, had diabetes, cancer or other end-stage disease that may compromise life expectancy, or contraindications to MRI (pacemaker, other electronic device, severe claustrophobia).

All study procedures were approved by a local ethics committee, and all subjects provided written informed consent.

3.2 *Sample Size Calculation:*

Primary Aim 1: We anticipate a sample size of 25 women per group. In our pilot data of 10 healthy women who underwent OS-CMR with a breath holding protocol, the mean change in signal intensity identified was 6.58% with a standard deviation of 2.17%. Given this information, we should be able to detect a statistically significant difference in signal intensity as small as 1.72% with a power of 80%. Based on a previous study with a sample of 29 patients with OSA and 36 controls, the % change in signal intensity between patients with obstructive sleep apnea and controls was 2.6% and 6.7% respectively.[64] Therefore, we anticipate that we should have sufficient power to detect any significant difference in change in signal intensity between our CMD patients and our healthy controls if one does exist.

Primary Aim 2: To compare OS-CMR myocardial oxygenation response using a breath holding manoeuvre to adenosine-based assessments of OS-CMR and first-pass perfusion imaging in CMD patients, we will determine the Pearson correlation coefficient for respective value changes from at rest to following their respective intervention (eg: breath-hold or adenosine). No prior studies have looked at this direct comparison, however in a study by Fischer et al., the response of myocardial oxygenation to breathing manoeuvres was found to have had an impact on myocardial oxygenation almost equally as strong as intravenous administration of a standard dose of adenosine.[63] Given this strong correlation between the two protocols, with our cohort of 25 women per group, we should be able to detect a correlation coefficient as small as 0.5, with a power of 80%.

Interim analysis: The thesis will include an interim analysis for a larger study that will eventually incorporate 25 women with chest pain and NoCAD and 25 healthy controls, as determined by our sample size calculation.

3.3 Baseline Clinical Characteristics

Baseline clinical characteristics were obtained from each patient at the time of enrollment including previous medical comorbidities, medication use, cardiovascular risk factors, and a health status assessment using the Seattle Angina Questionnaire and the Hospital Anxiety and Depression Questionnaire.

The Seattle Angina Questionnaire is a 19-item questionnaire validated for patients with coronary artery disease, which measures five relevant clinical domains including angina frequency, physical function, angina stability, treatment satisfaction, and quality of life. The score for each clinical domain used in the analysis ranges from 0 to 100, with higher scores indicating fewer symptoms and improved clinical functioning.[65]

To evaluate underlying psychiatric comorbidities of women, we used the Hospital Anxiety and Depression Scale (HADS). The HADS aims to measure symptoms of anxiety and depression in a general medical population and consists of 14 items, with seven items for the anxiety subscale, and seven items for the depression subscale. Each item is scored on a response scale with four alternatives ranging between 0 and 3. All response items are summed to obtain two scales (range from 0-15). Recommended cut-offs for anxiety and depression are 8-10 for doubtful cases, and ≥11 for definite cases.[66]

3.4 MRI Protocol and Image Analysis

Protocol for OS-CMR with Breathing Manoeuvres: All patients were scanned using a 3 Tesla MRI scanner (Prisma or Skyra; Siemens Medical Systems, Germany) with a standardized CMR imaging protocol inclusive of cine imaging, T2-weighted imaging, native T1 mapping, rest and stress Blood Oxygen Level Dependent (BOLD). Care was taken to ensure euvolemia prior to the imaging protocol by way of oral hydration and blood sampled immediately prior to imaging to obtain hematocrit. Localizer images were obtained. Cine imaging were performed using a standard steady-state free precession (SSFP)-based pulse sequence in sequential short-axis slices from the atrio-ventricular annulus to apex at 8 mm intervals, 3 long axis views, and in the 4, 3 and 2-chamber orientations (slice thickness 6 mm, gap 2mm, matrix 256 x 205, TE 1.5 msec, temporal resolution 35-40 msec). Native T1 mapping was performed using a short-inversion recovery modified Look-Locker (shMOLLI) pulse sequence in the basal, mid and apical short axis views. OS-CMR imaging was performed during a prolonged end-expiratory breath-hold of 30-60 seconds executed following a 60 second period of hyperventilation (patients receive pre-test instruction and training). Imaging was continuously acquired during the breath hold period using an SSFP-based pulse sequences as previously by described Guensch DP, et al at a 10 mm slice thickness in identical basal, mid and apical views.[62]The acquired images were obtained from onset (baseline eupnea), during hyperventilation, and through to maximum duration of breath-hold (time point closest to 30s) where peak changes in myocardial oxygenation are expected to be observed (**Figure 10).**

Image Analysis: Standard function parameters were calculated including ejection fraction,

stroke volume, diastolic volume, cardiac output, and myocardial mass indexed to the body surface area. T1 and T2 images were analyzed using manually defined epicardial and endocardial contours. Segments were entirely excluded if more than 33% of the segment area was removed during analysis due to artifact.

Analysis of the changes in the signal intensity response to the breathing manoeuvres were assessed in three separate analysis: breath-hold, hyperventilation, and an additional sensitivity analysis **(Figure 10).**

Breath-hold Analysis:

Myocardial oxygenation changes were assessed by measurement of global signal intensity changes in OS-CMR images from the initiation of the breath-hold (after hyperventilation) to maximum breath-hold (time point closes to 30s). The mean myocardial signal intensity (SI) change on OS-CMR images was automatically calculated after manual tracing of endocardial and epicardial contours and further segmented automatically according to the American Heart Association definition.[67]SI change was expressed as a % (change in SI [%]) using the first image of the breath-hold as the baseline and measured at a time point closest to thirty seconds. SI change was reported as an individual value for each segment in addition to a global value for each slice.

Hyperventilation Analysis:

Myocardial oxygenation changes during hyperventilation was assessed by measurement of global signal intensity change in OS-CMR images from the end of hyperventilation (maximal vasoconstriction) to a point at rest (resting coronary artery tone). SI changes were reported as an

individual value for each AHA segment, in addition to a global value for each slice.

Sensitivity Analysis:

To address the potential confounding impact of differences in coronary vascular response to hyperventilation between the two groups, an additional sensitivity analysis was performed assessing myocardial oxygenation changes during the breath hold compared to a point at rest. For this analysis, myocardial oxygenation changes were assessed by measurement of global signal intensity change in OS-CMR images from the end of hyperventilation (maximal coronary vasoconstriction) to a point at rest (normal coronary artery tone). SI changes were reported as an individual value for each AHA segment, in addition to a global value for each slice.

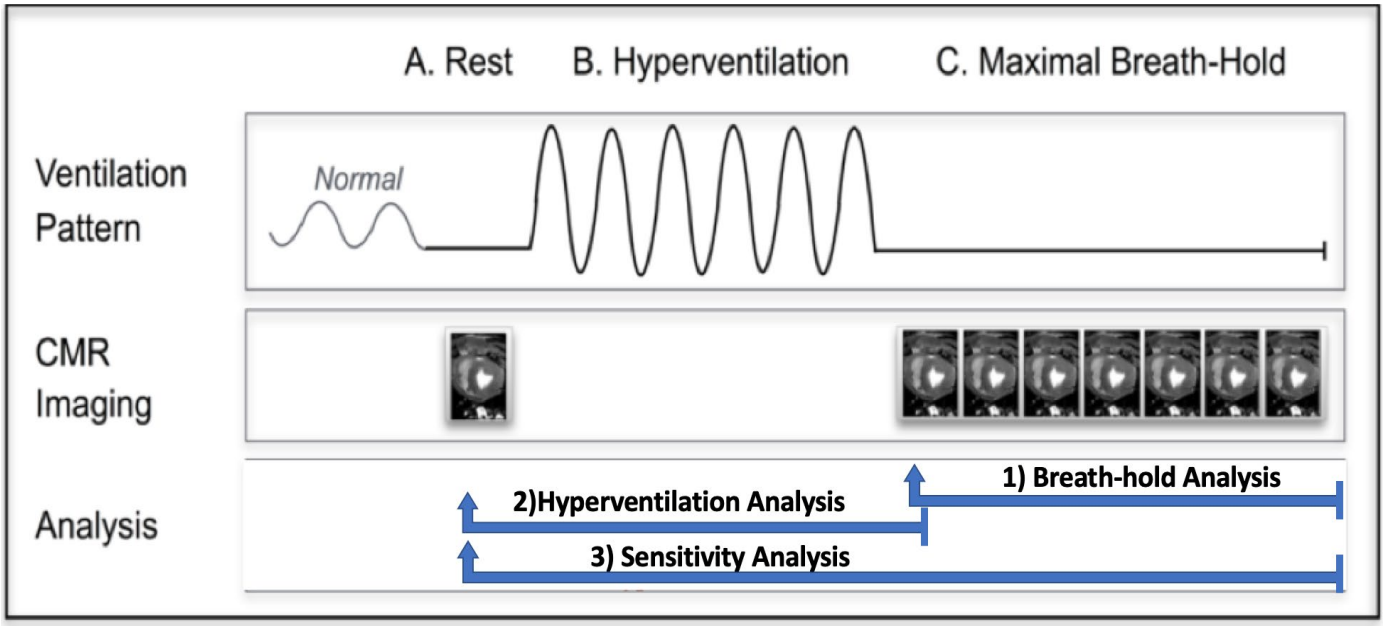

Figure 10. Myocardial signal intensity changes on OS-CMR during**1)** Breath-hold nearest to 30 seconds to the initiation of the breath-hold **2)** End of hyperventilation to a point at rest **3)** Breath-hold nearest to 30 seconds to a point at rest.[64]

Protocol of CMR during Adenosine Stress: In the women with chest pain and NOCAD, incremental imaging was performed during adenosine infusion, providing reference image

datasets for both OS-CMR and first-pass perfusion with contrast administration

OS-CMR imaging was repeated following adenosine infusion at a rate of 140 μg/kg/min for 3-minutes. Adenosine infusion was continued at the same rate for immediate performance of first pass perfusion imaging during dual bolus infusion of 0.0075 followed by 0.075 mmol/kg gadolinium contrast (Gadovist®, Bayer Inc. Canada) at a rate of 3.5 ml/sec (followed by a 30 ml saline flush at same rate). The latter was performed using a saturation-recovery turbo-FLASH pulse sequence. Rest perfusion imaging was then performed 10 minutes later using the same bolus profile. For first pass stress perfusion imaging time signal intensity curves were generated for each myocardial segment using semi-automated tracking of the endocardial and epicardial borders. Corresponding time signal intensity curves were derived for each myocardial segment and the maximal slope of these curves reported for each of the 16-myocardial segments. This analysis was repeated for rest perfusion imaging and a myocardial perfusion reserve was calculated (MPR calculated for each segment, where; MPR = stress max slope / rest max slope).

3.5 Regional Variability in Myocardial Oxygenation

In order to gain an understanding of regional differences in myocardial oxygenation that could underlie microvascular dysfunction, we devised an analysis to represent intersegmental differences in myocardial oxygenation between myocardial segments. Regional variability in myocardial oxygenation was assessed by first analyzing the global change in signal intensity during a breath-hold for both the endocardial and epicardial layers of the myocardium independently for radial, circumferential, and combined differences (**Figure 11)**. The endocardium and epicardium were each subdivided into six myocardial segments for a total of 12 segments per slice. Radial differences were defined as the sum of the absolute differences

between the endo and epicardium in each of the myocardial segments, circumferential differences were the sum of the absolute differences between myocardial segments within the endocardium and epicardium, and combined differences were the sum of the absolute radial and circumferential differences.

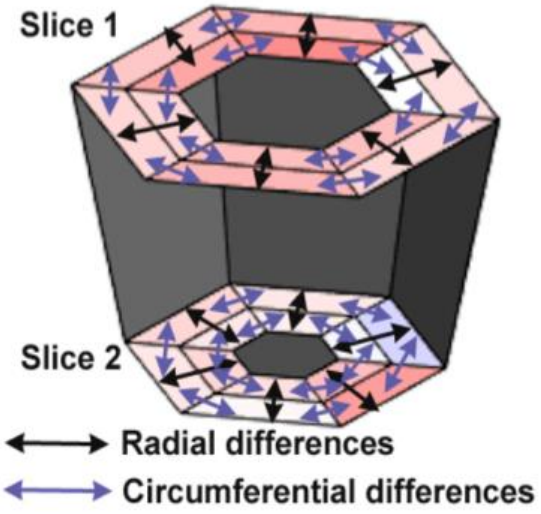

Figure 11. Schematic demonstrating the determination of regional variability in signal intensity using radial and circumferential differences. Radial differences were defined as the sum of the absolute differences between the endo and epicardium in each of the myocardial segments, whereas circumferential differences were the sum of the absolute differences between myocardial segments within the endocardium and epicardium.

3.6 Statistical Analysis

CMR Data Analysis: All CMR analyses were performed in a blinded fashion at the McGill University Health Center Research Institute CMR Core Lab and at the Stephenson Cardiovascular MR Center using software certified for CMR image analysis (cvi[42], Circle Cardiovascular Imaging Inc., Calgary, AB, Canada). All OS-CMR images were anonymized to group status and analyzed by two individual readers trained in OS-CMR analysis.

Statistical Analyses: Descriptive analyses compared baseline clinical and MRI values between patients with chest pain and NOCAD and healthy volunteers using mean and standard deviation for continuous variables, and frequency distributions for categorical variables. An independent t-test or Mann Whitney U test was used to compare data between groups for continuous variables, and chi-square statistic for categorical variables. Associations between the change in SI, regional variability in SI, T2, and myocardial perfusion reserve, were assessed with a Pearson correlation coefficient. To estimate the probability of being in the chest pain and NOCAD group, univariate logistic regression models were developed to include age, body mass index, cardiac output, myocardial mass, end diastolic volume, end systolic volume, Mid T1, Mid T2, Global % change in signal intensity, radial, circumferential, and combined differences. All readings were replicated by a second independent reader and inter-observer reliability was assessed using a two-way intraclass correlation test.

All statistical analysis was performed using SPSS version 23 (SPSS IBM, New York, USA). Results were considered statistically significant at a $p < 0.05$.

4.0 Results

4.1 Cohort Selection

Thirty-eight patients met the inclusion criteria for our study and were recruited from July 2017 to December 2018. Twenty women had chest pain and NOCAD and 18 were healthy volunteers (**Figure 11)**. Two patients with chest pain and NOCAD were lost to follow up or did not complete an MRI scan and were excluded from our study. Thirty-six patients underwent OS-CMR using a breath-holding maneuver. Six patients were excluded from the OS-CMR analysis as a result of poor image quality and breathing artifacts. Of the fourteen patients with chest pain and NOCAD, ten patients underwent adenosine perfusion MRI. Two patients did not complete the adenosine perfusion MRI as a result of an intolerance to adenosine and two patients were excluded from the analysis due to significant image artifact.

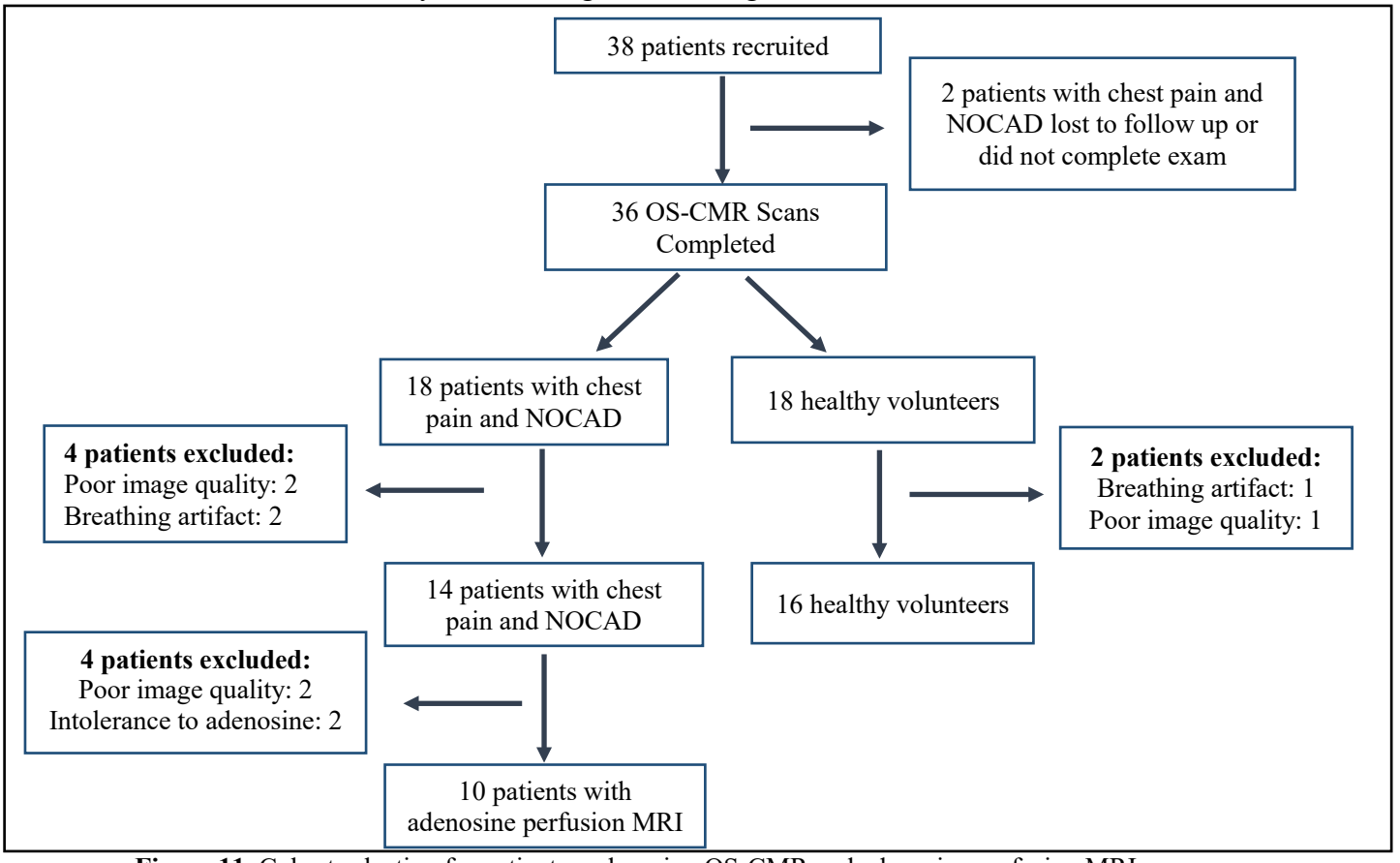

Figure 11. Cohort selection for patients undergoing OS-CMR and adenosine perfusion MRI

4.2 Baseline Clinical Characteristics

Clinical characteristics of our cohort are described in **Table 1.** The mean age of our cohort was 53±5years. There was no significant difference in age and body mass index between women with chest pain and NOCAD and healthy volunteers. Nearly 80% of women with chest pain and NOCAD had at least one traditional cardiovascular risk factor, with dyslipidemia in 9 (60%), hypertension in 7(50%) and diabetes mellitus type II in 2 patients (14%). Women with chest pain and NOCAD had a higher mean hospital anxiety score compared with healthy volunteers (mean hospital anxiety score: 10.1 vs. 3.2, p=.001). At enrollment, cardioprotective medications were present in 13 women with chest pain and NOCAD (93%), including cholesterol lowering medications (70%), aspirin (60%), calcium channel blocker (50%), and nitrates (53%).

Table 1. Baseline clinical characteristics between women with chest pain and NOCAD and healthy volunteers

	Women with chest pain and NOCAD (n=14)	Healthy volunteers (n=16)
Mean age (SD)	53.7 (6.6)	52.5 (3.3)
Mean body mass index (SD)	27.9 (6.8)	27.3 (5.2)
Cardiac risk factors		
Dyslipidemia (%)	9 (64)	0
Hypertension (%)	7 (50)	0
Diabetes Mellitus Type II (%)	2 (14)	0
Obesity (%)	3 (21)	4 (25)
Active smoker (%)	0	0
Depression/Anxiety (%)	7 (50)	2 (33)
Hospital anxiety and depression score		
Mean Anxiety Score (SD)	10.1 (3.0) *	3.2 (3.1) *
Mean Depression (SD)	5.4 (2.9)	3.3 (2.3)
Cardiac Medications		
Cholesterol lowering medications (%)	10 (71)	0
Aspirin (%)	9 (60)	1 (6)
Calcium channel blockers (%)	7 (50)	0
Nitrates (%)	8 (53)	0
Beta blockers (%)	4 (29)	0
ACEi/ARB (%)	1 (7)	0
Diuretics (%)	2 (14)	1 (6)

Standard deviation (SD), Angiotensin converting enzyme inhibitor (ACEi), Angiotensin receptor blocker (ARB), *$p<0.05$

Table 2 describes the clinical and angiographic characteristics of women with chest pain and NOCAD. While the predominant symptom in women with NOCAD was chest pain, dyspnea was also noted in four patients (29%). The majority of patients had normal coronary arteries on coronary angiography, with one patient presenting with minimal coronary artery disease (less than 50% stenosis). Acetylcholine provocation was performed in five patients, all of whom displayed abnormal coronary vasoconstriction. A wide range of health status were reported by women with chest pain and NOCAD through the Seattle Angina Questionnaire. At baseline, women were less likely to describe any improvement in their anginal symptoms (median angina stability score: 50 (25-100)) and reported that their anginal symptoms had a significant impact on their quality of life (median disease perception/quality of life score: 64 (31-100)).

Table 2. Angiographic and clinical characteristics of women with chest pain and NOCAD

Characteristic	Women with Chest pain and NOCAD (n= 14)
Predominant Symptom	
Chest pain (%)	14 (100)
Dyspnea (%)	4 (29)
Coronary Angiography	
Normal coronary arteries (%)	11 (91)
Minimal coronary artery disease (<50%) (%)	1 (8)
Positive acetylcholine reactivity test (%)	5 (36)
Seattle Angina Questionnaire	
Physical limitation, median (Range)	92 (50-100)
Angina Stability, median (Range)	50 (25-100)
Angina Frequency, median (Range)	80 (50-100)
Treatment Satisfaction, median (Range)	75 (63-100)
Disease Perception/Quality of Life, median (Range)	64 (31-100)

4.3. Cardiac Function and T1 and T2 Mapping

Table 3 compares cardiac function and T1 and T2 mapping between healthy volunteers and women with chest pain and NOCAD. There were no differences in left ventricular end diastolic or systolic volume, stroke volume, cardiac output, cardiac index or myocardial mass between women with chest pain and NOCAD and healthy volunteers. Healthy volunteers had a lower left ventricular ejection fraction compared to women with chest pain and NOCAD, but well within the normal range (LVEF: 73% vs. 77%, $p = 0.04$). Women with chest pain and NOCAD had a lower native T1 relaxation time compared to healthy volunteers (1201 ms vs. 1225 ms, $p= 0.05$), and a higher mid T2 relaxation time (42 ms vs. 38.2 ms, $p =0.001$).

Table 3. Functional and T1 and T2 Mapping in women with chest pain and NOCAD and healthy volunteers

	Women with chest pain and NOCAD (n=14)	**Healthy volunteers(n=16)**
Left ventricular end diastolic volume, mean (SD), mL/m2	57.7 (10.7)	60.0 (14.7)
Left ventricular end systolic volume, mean (SD), mL/m2	14.9 (5.1)	19.1 (10.5)
Stroke volume, mean (SD), ml/m2	42.8 (7.9)	46.2 (5.4)
Cardiac output, mean (SD), L/min	5.5 (1.2)	5.5 (1.0)
Cardiac index, mean (SD), l/min/m2	3.2 (0.8)	3.2 (0.6)
Myocardial mass systole, mean (SD), grams/m2	65.2 (13.4)	62.8 (6.9)
Left ventricular ejection fraction (%), mean (SD)	77 (7.2)	73 (4.7) *
MRI Mapping		
Native Mid T1 (ms), mean (SD)	1201 (66)	1224 (67.8) *
Mid T2 (ms), mean (SD)	42 (1.9)	38.8 (2.2) *

* $p<0.05$

4.4 Adenosine perfusion MRI

Results of the adenosine perfusion MRI in women with chest pain and NOCAD are illustrated in **Table 4.** The global mean myocardial perfusion reserve was 2.05 (SD: 0.44), with a mean myocardial perfusion reserve index of 1.32 (SD: 0.24), consistent with impaired microvascular function (**Figure 12).** Myocardial perfusion reserve correlated negatively with T2 relaxation time (r = -0.67, p= 0.069) as shown in **Figure 13.**

Table 4. Results of myocardial perfusion reserve in women with chest pain and NOCAD

MRI Parameter	**Women with chest pain and NOCAD(n=10)**
Mean MPR Basal Slice (SD)	2.22 (0.62)
Mean MPR Mid Slice (SD)	2.09 (0.50)
Mean MPR Apical Slice (SD)	1.63 (0.5)
Mean MPR Global (SD)	**2.05 (0.44)**
Mean Global MPRI (SD)	**1.32 (0.24)**

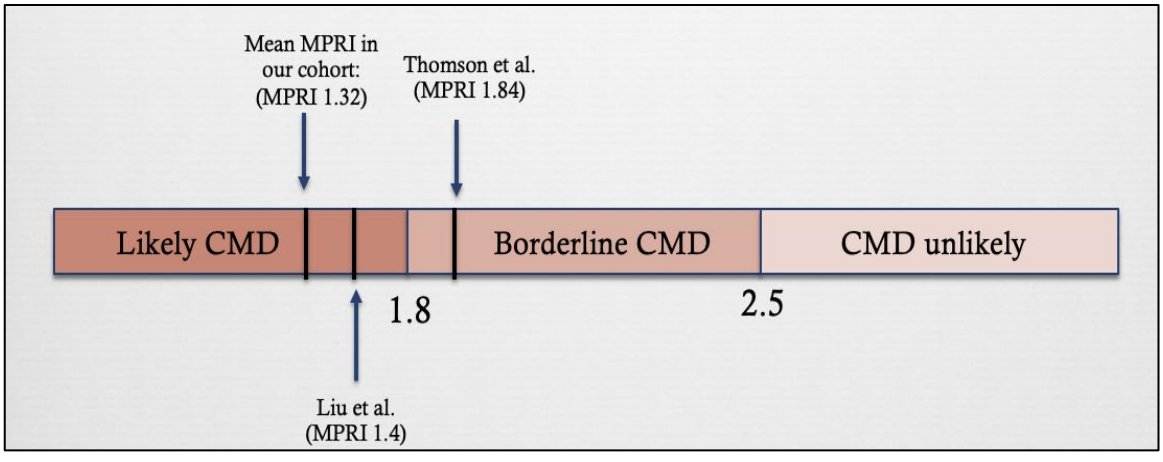

Figure 12. Myocardial perfusion reserve index cut-offs that have been used in studies in women with coronary microvascular dysfunction on invasive coronary angiography.[43,46]

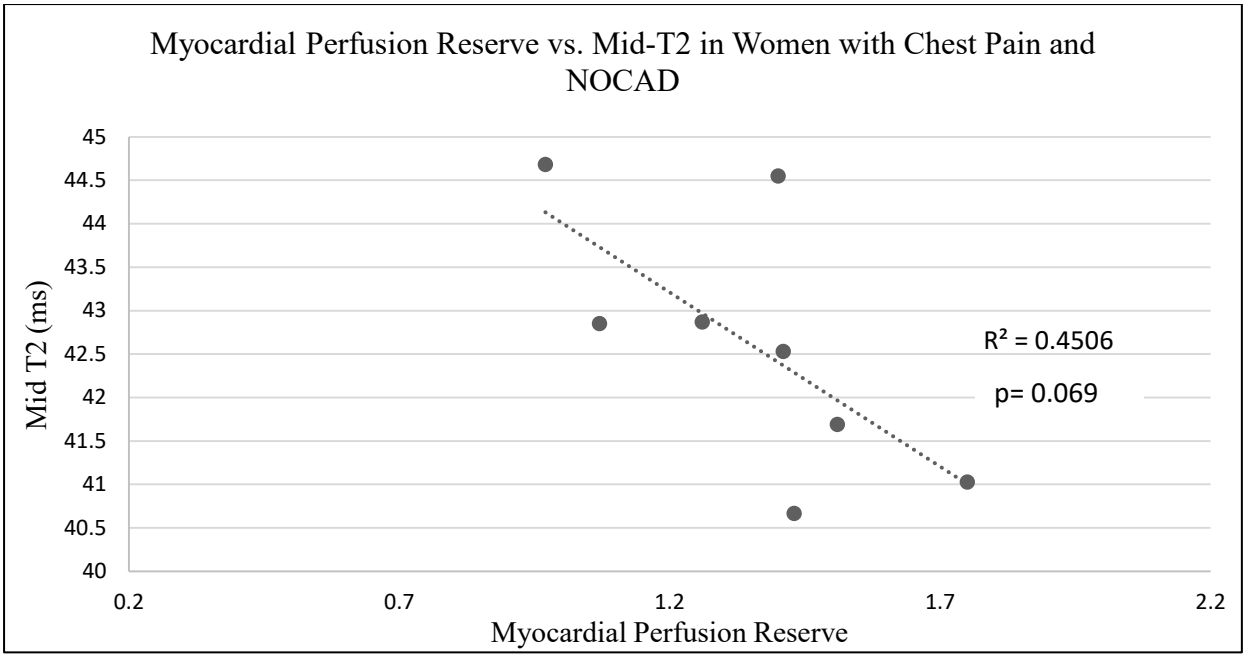

Figure 13. Myocardial perfusion reserve vs. mid T2 relaxation time in women with chest pain and NOCAD. Women with chest pain and NOCAD with reduced myocardial perfusion reserve had a higher mid T2.

4.5 Oxygenation-Sensitive MRI with Breathing Manoeuvres

All patients within our cohort undergoing OS-CMR were able to complete the breathing maneuvers. During image analysis, 7.2% of all myocardial segments had to be excluded, due to susceptibility artifacts or a thinning myocardium. Inter-observer reliability was 0.92, signifying a high degree of consistency in the image analysis between the two readers.

Response to Breath-Hold

During the thirty second breath-hold manoeuvre, women with chest pain and NOCAD showed a trend towards a greater global increase of myocardial oxygenation compared to controls, albeit without reaching statistical significance (7.4± 6.6% vs. 3.9 ±3.4%, p=.07) **(Table 5)**. Values of myocardial oxygenation during the breath-hold in women with chest pain and NOCAD and our control group were within the range of normal values for age referenced controls (**Table 6)**. Upon segmentation of the myocardium into an endocardial and epicardial layer, there remained a trend towards a greater increase in myocardial oxygenation in women with chest pain and NOCAD (endocardium: 7.1 ± 7.0% vs. 4.1 ± 3.0%, p = 0.15; epicardium: 6.9 ± 6.6% vs. 4.0 ± 3.8%, p= 0.14). Global mean values in myocardial oxygenation during a breath-hold manoeuvre correlated negatively with values of reduced myocardial perfusion reserve (r= -0.64, p =0.046) **(Figure 14).**

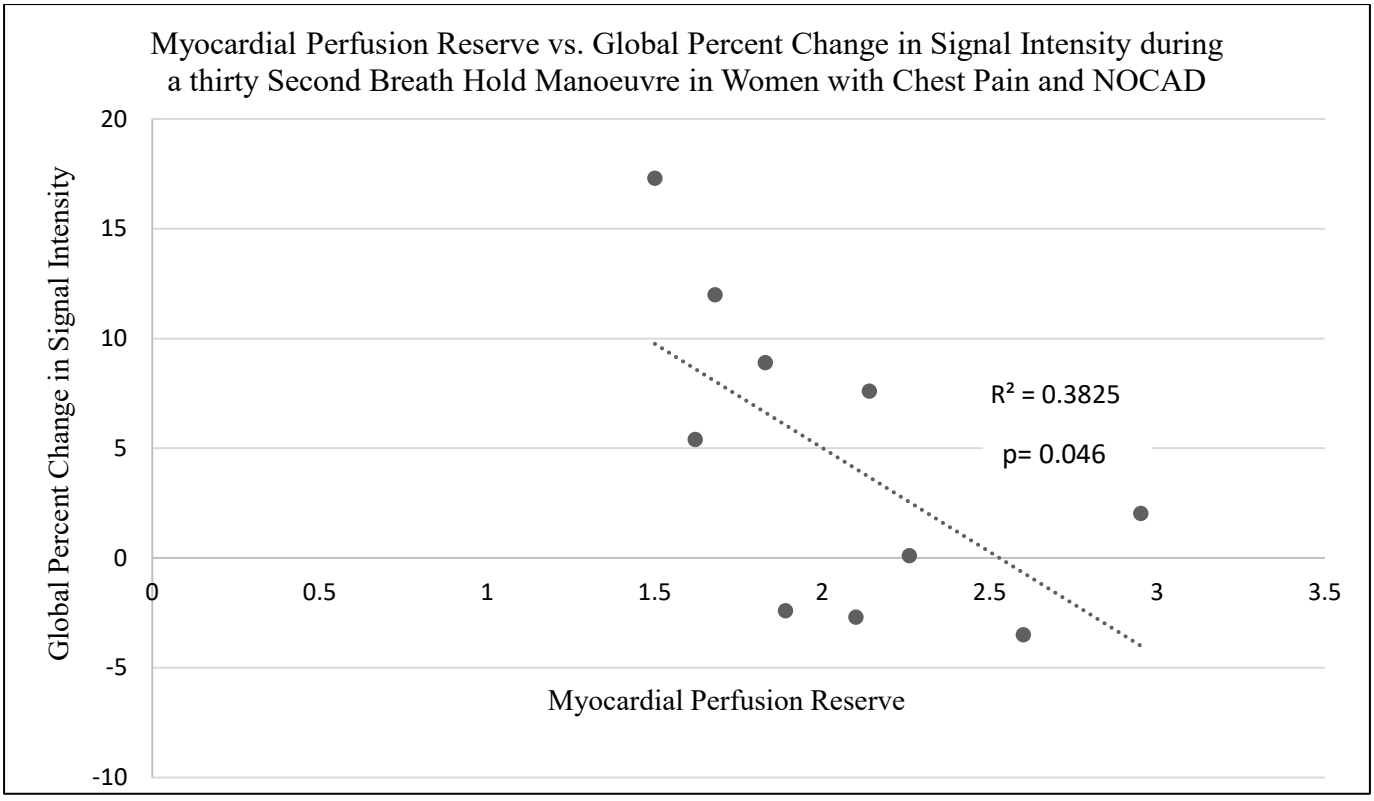

Figure 14: Myocardial perfusion reserve vs. global change in signal intensity during a thirty second breath-hold manoeuvre in women with chest pain and NOCAD

Response to Hyperventilation

During hyperventilation (end of hyperventilation to baseline image), women with chest pain and NOCAD had a trend towards a greater reduction in myocardial oxygenation compared to healthy volunteers (-8.4 ± 7.4% vs. -3.6± 3.4%, p= 0.08) (**Table 5)**, and below the normal values for age referenced controls **(Table 6).** In women with a history of anxiety or depression, there was a trend towards a lower myocardial oxygenation during hyperventilation compared to women without a history of anxiety or depression (-9.5 ± 8.0% vs. -4.3 ± 6.4%, p=.07). A negative correlation existed between a reduction in myocardial oxygenation during hyperventilation and hospital anxiety score (r= -0.35, p=0.10) as demonstrated in **Figure 15.**

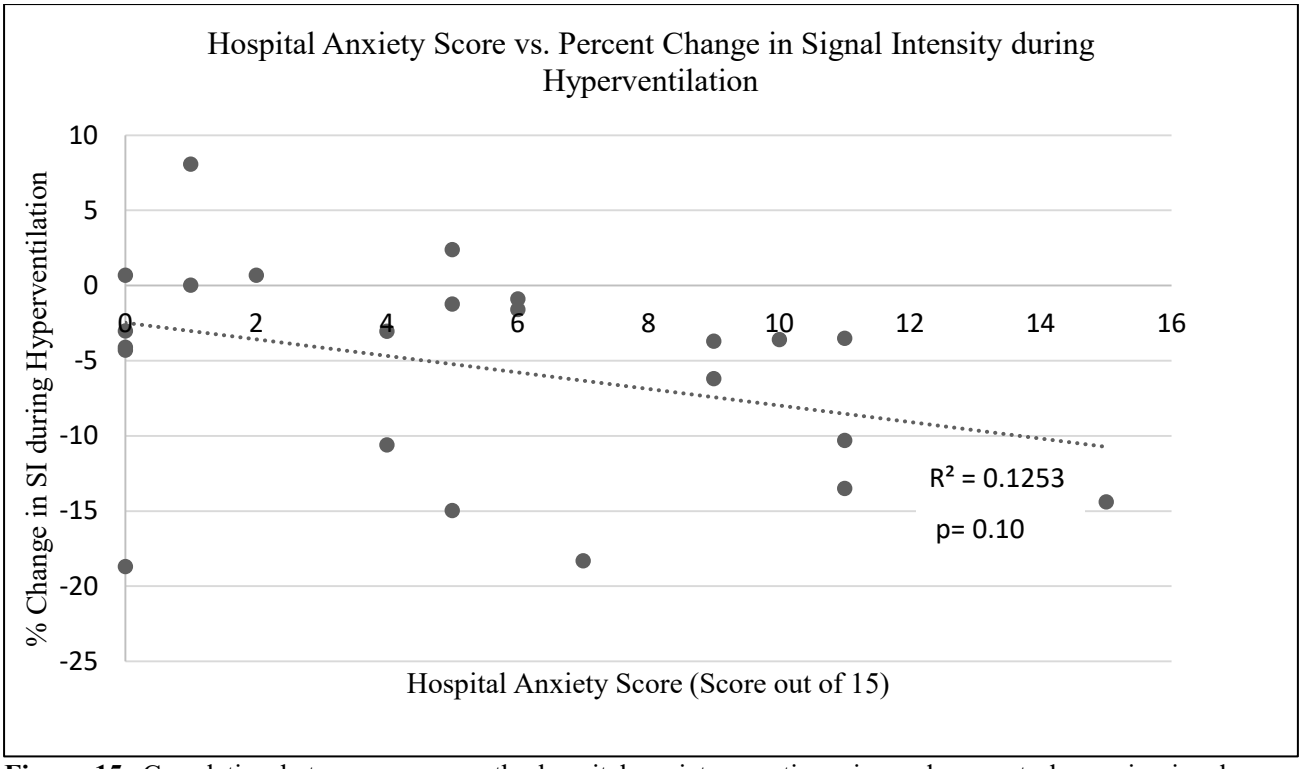

Figure 15: Correlation between score on the hospital anxiety questionnaire and percent change in signal intensity during hyperventilation. Women with a higher hospital anxiety score demonstrated a lower myocardial oxygenation response during hyperventilation

Sensitivity Analysis

During the thirty second breath-hold, when using rest (eupnea) as a baseline, there remained no significant difference in myocardial oxygenation, although there was a trend towards a more blunted global response in myocardial oxygenation during a breath-hold manoeuvre in women with chest pain and NOCAD compared to healthy volunteers (-1.3 ± 7.4% vs. -0.25 ± 7.9%)**(Table 5).**

Table 5: Changes in myocardial signal intensity on OS-CMR in women with chest pain and NOCAD compared to healthy volunteers

	Women with chest pain and NOCAD(n=14)	Healthy Volunteers(n=16)
30s Breath Hold (% Δ in SI)		
Basal Slice	8.4 (9.0)	4.3(4.6)
Apical Slice	6.6 (6.9)	3.6 (3.6)
Global mean (%Δ in SI)	7.4 (6.6)	3.9(3.4)
Subendocardium (% Δ in SI)		
Basal Slice	7.2 (9.2)	5.0 (6.2)
Apical Slice	7.7 (7.3) *	3.3 (2.6) *
Global	7.1 (7.0)	4.1 (3.0)
Subepicardium (% Δ in SI)		
Basal Slice	7.7 (8.8)	4.7 (5.7)
Apical Slice	6.2 (6.7)	3.3 (2.8)
Global	6.9 (6.6)	4.0 (3.8)
Hyperventilation (% Δ in SI)	-8.4 (7.4)	-3.6 (7.0)
Sensitivity Analysis: 30s Breath Hold with comparison to rest (%Δ in SI)	-1.3 (7.4)	-0.25 (7.9)

* $p<.05$

Table 6. Changes in myocardial oxygen reserve in healthy volunteers across age ranges

Age Group	Hyperventilation (%ΔSI)	Breath-Hold (%ΔSI)
18-34	-8.58±8.15	6.10±5.93
35-55	-5.02±6.99	5.05±3.65
56-70	-7.72±6.92	5.24±6.71

4.6 Regional Variability in Myocardial Oxygenation

Upon segmentation of myocardial oxygenation according to the 6 AHA segments in basal and apical slices, women with chest pain and NOCAD had a significantly higher range and standard deviation of segmental values compared to healthy volunteers (mean range of segmental values: 35.9 vs. 15.7, p = 0.002, mean standard deviation of segmental values: 7.6 vs. 4.4, p=0.001). Variability in AHA segmental values are demonstrated by two reference cases in **Figure 16.**

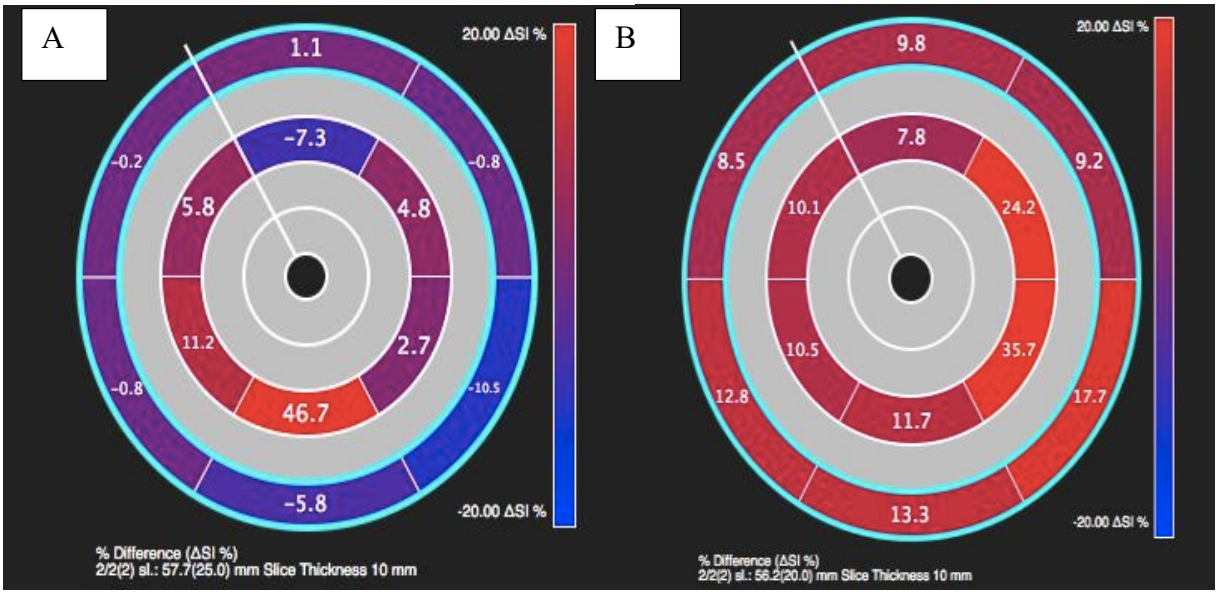

Figure 16: Myocardial oxygenation response during a breath-hold manoeuvre distributed across AHA segments in **A)** 56 year old female with a history diabetes mellitus type 2 and hypertension with chest pain and NOCAD and **B)** 53 year old women without a history of cardiovascular disease. Marked intersegmental variability can be noticed in the apical and basal slices in women with chest pain and NOCAD.

Compared to healthy volunteers, women with chest pain and NOCAD had larger radial and circumferential differences in their myocardial oxygenation response during a breath-hold manoeuvre (mean radial difference: 3.9± 1.2% vs. 2.6± 0.90%, p= .005, mean circumferential

difference: 7.7 ± 3.2% vs. 4.8 ± 1.2%, p=.004). The mean combined difference was also greater in women with chest pain compared to healthy volunteers (15.8± 4.7% vs. 10.1± 2.6%, p =.009) as demonstrated in **Figure 17**. Myocardial perfusion reserve correlated negatively with increased variability in myocardial oxygenation (r= -0.62, p= 0.07) as shown in **Figure 18.**

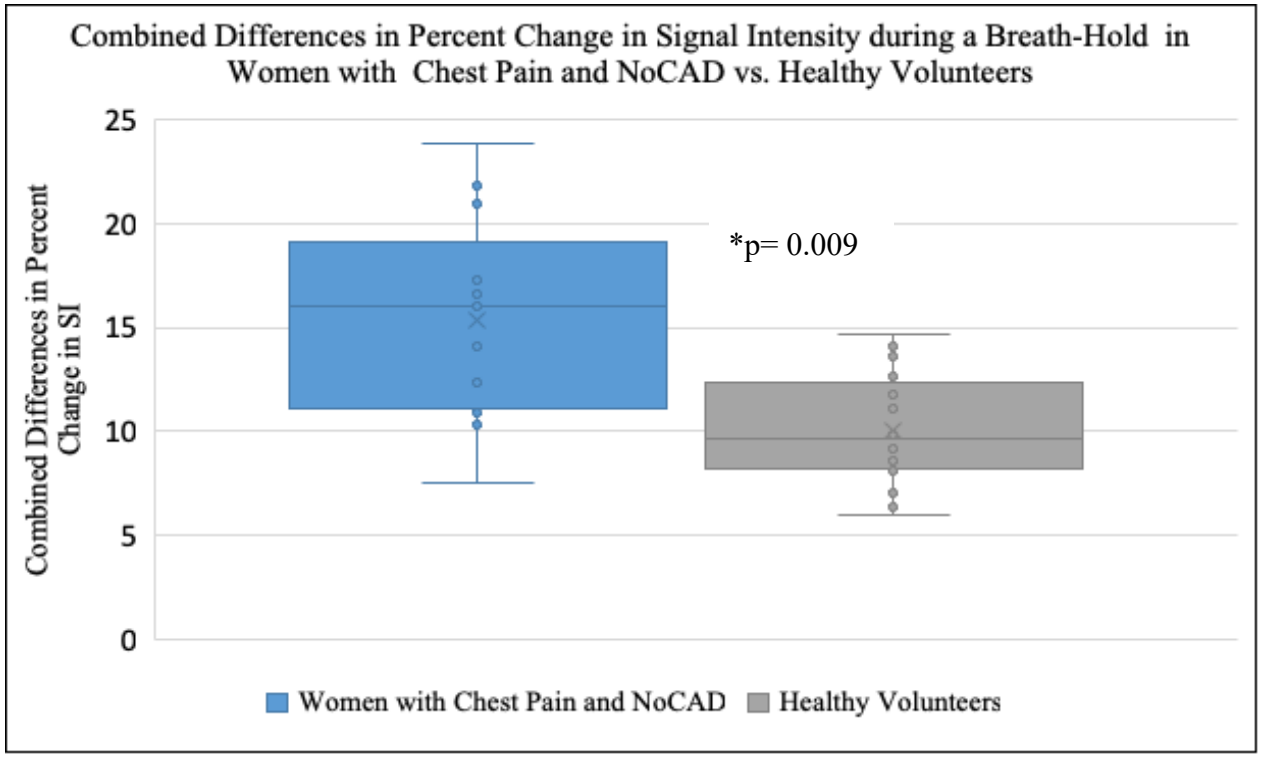

Figure 17. Combined differences in percent change in signal intensity during a breath-hold manoeuvre in women with chest pain and NOCAD compared to healthy volunteers. Women with chest pain and NOCAD had greater regional differences in myocardial oxygenation during a breath-holding manoeuvre compared to healthy volunteers.

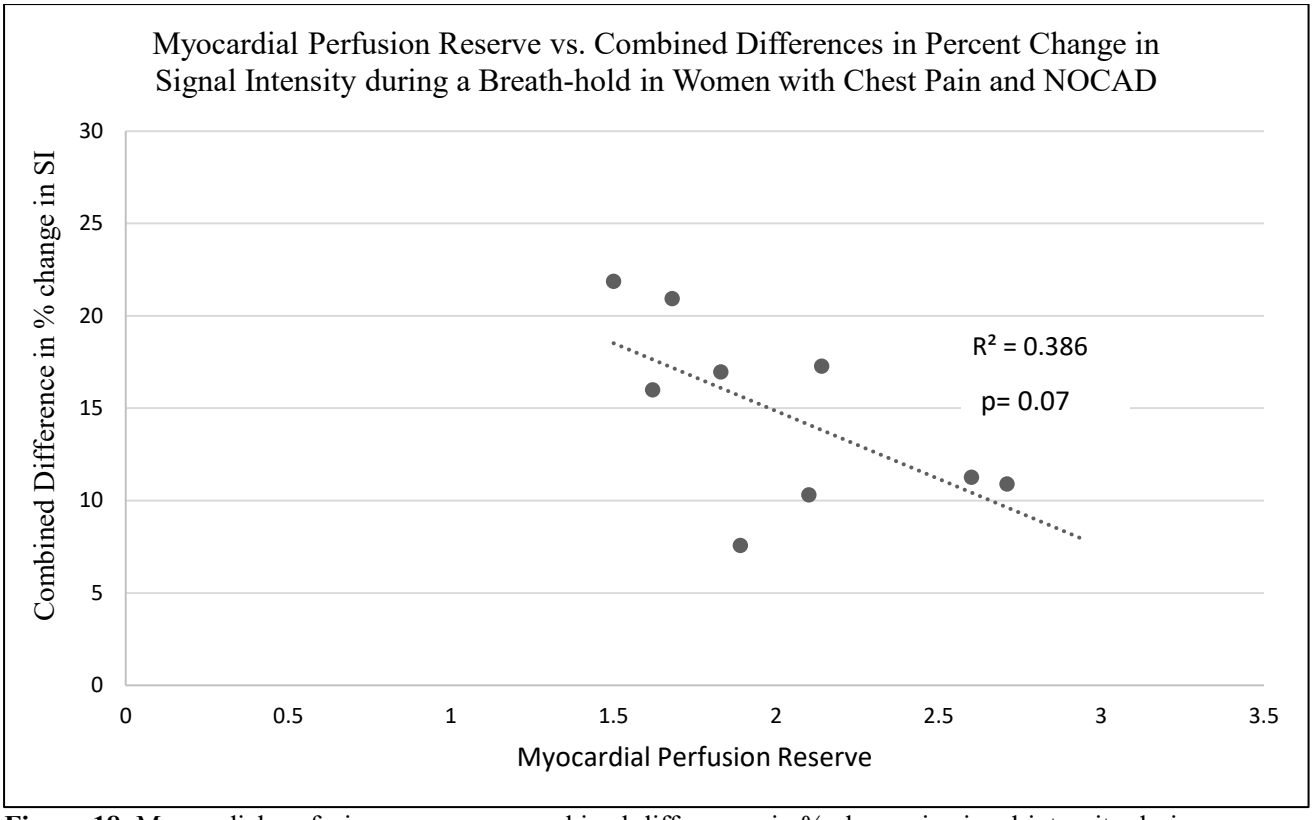

Figure 18. Myocardial perfusion reserve vs. combined differences in % change in signal intensity during a breath-hold manoeuvre in women with chest pain and NOCAD. Women with reduced myocardial perfusion reserve demonstrated higher regional variability in myocardial oxygenation.

4.7 Predictors of Women with Chest Pain and NOCAD

To estimate the association between chest pain and clinical and MRI characteristics in women aged 40-65, univariate logistic regression models were developed to include age, body mass index, cardiac output, myocardial mass, end diastolic volume, end systolic volume, mid T1, mid T2, global % change in signal intensity, and radial, circumferential, and combined differences in % change in signal intensity. In the univariate analysis (**Table 7)** mid T2 (OR: 2.0, 95% CI: 1.23-3.22), radial differences in SI (OR: 2.42, 95% CI:1.08-5.4), circumferential differences in SI (OR: 3.02, 95% CI: 1.2-7.6) , and combined differences in SI (OR: 1.5, 95% CI: 1.1-2.0) were all significantly associated with chest pain ($p<.05$ for all). A multivariable regression analysis was not performed due to the small sample size.

Table 7. Univariate logistic regression analysis on the predictors of chest pain in women aged 40-65

	β (95% CI)	SE β	Wald's χ2	p-value	Odds Ratio estimate (95% CI)	C-statistic
Age	0.050 (-0.97-0.20)	0.075	0.44	0.50	1.05 (0.91-1.21)	0.59
BMI	0.018 (-0.10-.014)	0.06	0.08	0.77	0.02 (-0.10-0.14)	0.50
Cardiac Output	0.0288 (-0.65-0.72)	0.35	0.007	0.93	1.02 (0.52-2.04)	0.47
Myocardial mass	0.02 (-0.05-0.10)	0.037	0.42	0.52	1.02 (0.95-1.10)	0.51
EDV	-0.015(-0.07-0.043)	0.030	0.25	0.62	0.99 (0.93-1.04)	0.67
ESV	-0.1 (-0.26-0.06)	0.08	1.4	0.23	0.91 (0.77-1.07)	0.66
Mid T1	-0.02 (-0.05-0.005)	0.01	2.44	0.12	0.98 (0.96-1.005)	0.69
Mid T2	0.69 (0.20- 1.17)	0.25	7.74	0.005	2.0 (1.23-3.22)	0.88
Global % Change in SI	0.14 (-0.02-0.30)	0.08	3.006	.08	1.15 (0.98-1.36)	0.70
Radial Differences in % Change in SI	0.88 (0.08-1.7)	0.41	4.6	0.03	2.42 (1.08-5.4)	0.83
Circumferential differences in % change in SI	1.1 (0.19-2.02)	0.47	5.6	0.018	3.02 (1.2-7.6)	0.81
Combined difference in % change in SI	0.39 (0.09-0.68)	0.15	6.6	0.013	1.5 (1.1-2.0)	0.69

5.0 Discussion

In our interim analysis of thirty patients undergoing OS-CMR using a combined breathing manoeuvre, there was no significant difference in the global myocardial oxygenation reserve between women with chest pain and NOCAD compared to healthy volunteers. In women with chest pain and NOCAD, there was a negative correlation between the adenosine-induced hyperemic response (MPR) and global myocardial oxygenation reserve. While our preliminary findings dispute our initial hypothesis of a blunted myocardial response in women with NOCAD, our results provide valuable mechanistic insights into the pathophysiology of microvascular dysfunction, which may serve as a stepping stone for future research and treatment.

5.1 Myocardial oxygenation reserve in women with chest pain and NOCAD

While we found no significant difference in global myocardial oxygenation reserve in women with chest pain and NOCAD compared to healthy volunteers, there was a trend towards an increased myocardial oxygenation response in women with chest pain and NOCAD. Furthermore, in women with chest pain and NOCAD undergoing adenosine perfusion MRI, a lower myocardial perfusion reserve (indicating worsening microvascular dysfunction) was correlated with a higher global myocardial oxygenation response during a breath-hold.

Only one other study has evaluated the role of OS-CMR in women with chest pain and NOCAD. The study examined 18 patients with CSX compared to 14 controls who underwent CMRI at 3T using a BOLD protocol with adenosine induced hyperemia. The study similarly found no significant difference in BOLD signal change during a hyperemic stimulus in patients with CSX compared to controls.[59]

One explanation for an increased global oxygenation reserve in women with chest pain and NOCAD could in part be explained by a vasospastic response of coronary vessels during hyperventilation. Our results suggest that women with chest pain and NOCAD showed a stronger response to hyperventilation, apparent through a more pronounced reduction in myocardial oxygenation. Given that our measure of signal intensity is based on the change in signal intensity from baseline (end of hyperventilation or maximal vasoconstriction), women with chest pain and NOCAD could have a higher change in signal intensity as they vasoconstrict more than healthy volunteers, and therefore exhibit a more significant change from baseline (end of hyperventilation). Indeed, when we analyzed the extent of the breath hold response with a eupneic baseline during our sensitivity analysis (excluding the hyperventilation manoeuvre), we found a trend towards a lower oxygenation response in women with chest pain and NOCAD compared to healthy volunteers. Our findings of a trend towards an increase in myocardial oxygenation in women with chest pain and NOCAD, therefore, could possibly be explained through underlying vasomotor abnormalities mediated through enhanced vasoconstriction in hyperventilation.

A further explanation of our finding of no significant difference in myocardial oxygenation response during a hyperemic stimulus in women with chest pain and NOCAD could be explained through a reduction of myocardial oxygen extraction as a result of alterations in capillary morphology. At rest, the normal myocardium is heterogeneously perfused, and oxygen extraction efficacy is improved through homogenizing capillary flow patterns as myocardial blood flow increases. Capillary transit time heterogeneity (CTH) is a parameter that quantifies capillary heterogeneity and is defined as the standard deviation of capillary transit time.[68] Homogenization of capillary flow during hyperemia is an intrinsic property of microvascular

networks, and critical in limiting functional shunting of blood, thereby increasing the efficiency of oxygen extraction.[69] Hypercapnia has an important impact on CTH. In a study examining the impact of hypercapnia on red blood cell kinetics in anesthetized mice, the authors found hypercapnic induced vasodilation resulted in a decrease in CTH and therefore more efficient oxygen extraction.[69] Furthermore, studies evaluating the impact of CTH on the BOLD signal have shown that processes that reduced CTH results in a subsequent decrease in BOLD oxygenation signal due to improved oxygen extraction.[69] **(Figure 20).**

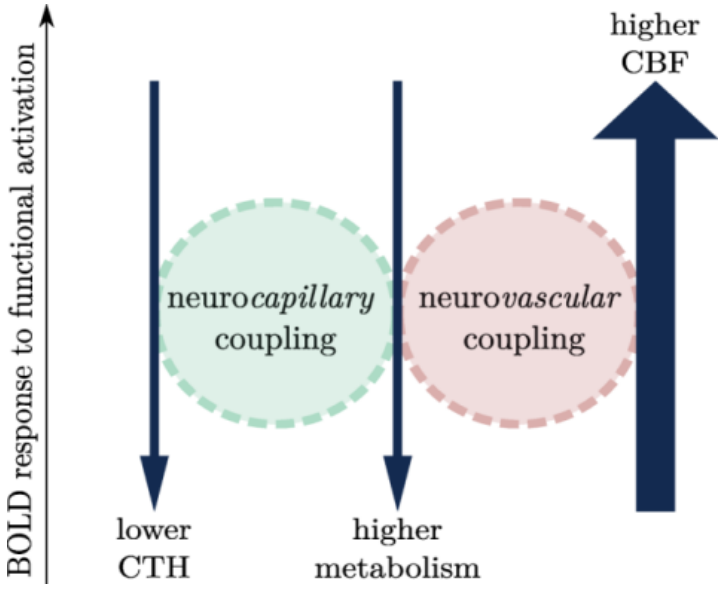

Figure 20. Contributions of coronary blood flow, capillary transit time heterogeneity, and metabolism to the BOLD signal in the brain during functional activation. While increases in coronary blood flow increase BOLD signal, reduction in CTH (through homogenization of blood flow) serve to decrease BOLD signal through an increase in oxygen extraction.[69] CBF: Coronary blood flow CTH: Capillary transit time heterogeneity

CMD is associated with changes in capillary wall morphology, basement membrane thickening, and endothelial dysfunction.[26]Studies on myocardial biopsies from patients with CMD have shown evidence of sclerosis of small arteries, and arterioles with perivascular fibrosis, extramural compression, and capillary rarefraction.[70]Microvascular disease could result in an increase in CTH through disruption in capillary morphology, altering capillaries' ability to

homogenize blood flow during hyperemia. Such disruption could subsequently result in a parallel reduction in oxygen extraction and an increase in oxygenation concentration in the capillary bed.[68] BOLD response to myocardial oxygenation is highly dependent on changes in deoxygenated hemoglobin in the capillary, where nearly 90% of blood volume is located.[13]Therefore, alterations in capillary morphology in women with chest pain and NOCAD could subsequently affect the ability to homogenize capillary blood flow during a breath-hold response, resulting in a decreased capacity to extract oxygen, and therefore a lower fraction of deoxygenated hemoglobin, leading to a higher BOLD signal. Indeed, studies evaluating the myocardium of rats with streptozotocin-induced diabetes, have shown that damage to capillary morphology give rise to less efficient oxygen extraction.[71,72]Our findings of an increased global oxygenation reserve in women with chest pain and NOCAD and its correlation with worsening microvascular dysfunction could be an important explanation of the alterations in capillary morphology that function to reduce myocardial oxygenation extraction in patients with CMD.

A third explanation to explain the lack of significant difference in myocardial oxygenation reserve between our groups could be explained through the underlying heterogeneity that exists in patients with chest pain and NOCAD (**Figure 6).** From prior studies, approximately 50% of patients with chest pain and NOCAD have evidence of CMD. Given the small sample size (only 10 women undergoing adenosine perfusion MRI), the differences between the groups may not be evident yet in our interim analysis.

5.2 Regional heterogeneity of myocardial oxygenation

In women with chest pain and NOCAD, we observed an increase in regional heterogeneity in myocardial oxygenation compared to healthy volunteers. Furthermore, we

noted, that regional heterogeneity of myocardial oxygenation correlated well with measures of worsening microvascular dysfunction (worsening myocardial perfusion reserve). Our results are the first to describe regional variations in myocardial oxygenation in patients with chest pain and NOCAD, which potentially offers an insight into the underlying pathophysiology of microvascular dysfunction.

The coronary microcirculation has commonly been described as a "black box", owing in part to our limitation to image the microvasculature in vivo, as current coronary angiography visualizes only 5% of the coronary tree.[73] Historically, evaluating patients with chest pain and NOCAD has been primarily functional rather than structural, through examination of a coronary flow reserve or an index of microcirculatory resistance on angiography or advanced imaging.[28] By directly examining myocardial oxygenation in vivo, OS-CMR provides an important understanding of structural alterations in myocardial oxygenation that occur in the coronary microvasculature during a hyperemic stimulus.

A hallmark of microvascular networks is heterogeneity, whereby microvascular networks supply surrounding tissue regions through stochastic angiogenetic processes.[73]Vessel arrangement and morphology respond to changes in physiologic demand through vascular adaptation, altering regional blood flow distribution through intravascular signalling and local shunting of blood.[73] Dysfunction in microvascular networks could lead to disruption in this delicate homeostatic process, leading to alterations in myocardial blood flow and eventually oxygenation.

In obstructive coronary artery disease, flow-limiting stenosis causes a large global or regional mismatch of supply and demand in the myocardium, resulting in an increased oxygen extraction and arteriovenous difference of oxygen content downstream of the corresponding

coronary territory. In contrast however, in microvascular dysfunction, microvascular networks are altered through damage to small arterioles and capillaries, resulting in functional shunting, local hypoxia, and a reduced mean arteriovenous difference of oxygenation. Rather than a large perfusion deficit and reduced myocardial oxygenation distal to a stenosed vessel, microvascular disturbances that occur in CMD may result in an increased heterogeneity of flow and distribution through functional shunting of blood, whereby under-perfused regions mix with over-perfused regions.[73] Therefore, a bulk reduction in myocardial oxygenation might not be typical for microvascular dysfunction but rather a mosaic of high and low blood supply **(Figure 20).**[73]

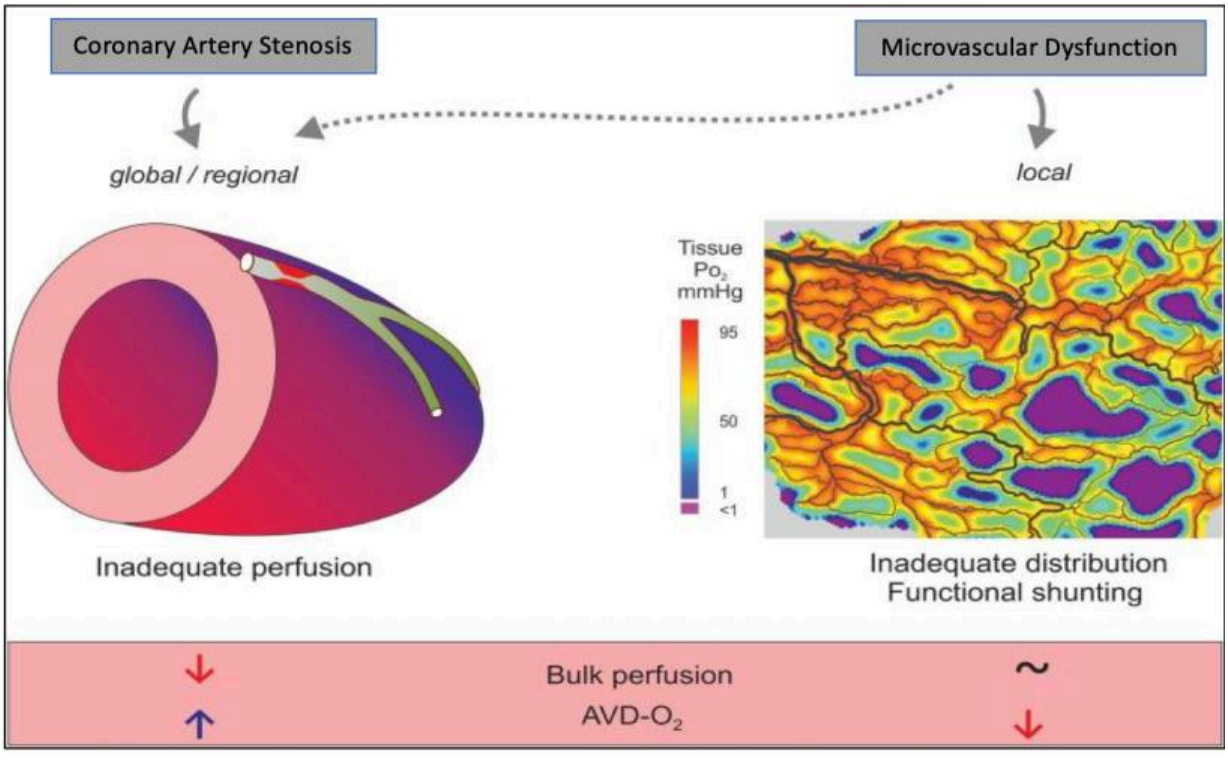

Figure 20. Flow limiting stenosis in an epicardial coronary artery (left) will cause a regional or global myocardial supply to demand mismatch, with increased 0_2 extraction and arteriovenous difference in oxygen content. Microvascular dysfunction (right) causes heterogeneity of flow and oxygen as a result of inadequate distribution and functional shunting from impaired microvasculature resulting in regions with low flow and high extraction that border regions with high flow and low extraction.[73]

Past studies assessing patients with microvascular dysfunction have done so through reliance on global measures of perfusion or oxygenation, which fails to account for important

regional differences in the microvasculature. In a recent paper, Gould et al. argues the importance of a more comprehensive assessment of regional heterogeneity in coronary flow reserve in patients with microvascular angina. The authors provide a clinical example of a patient **(Figure 21)** with a normal global CFR of 3.0, however with a profound regional deficit inferiorly, that is unaccounted for as a result of a surrounding high CFR in the left ventricle.[74]

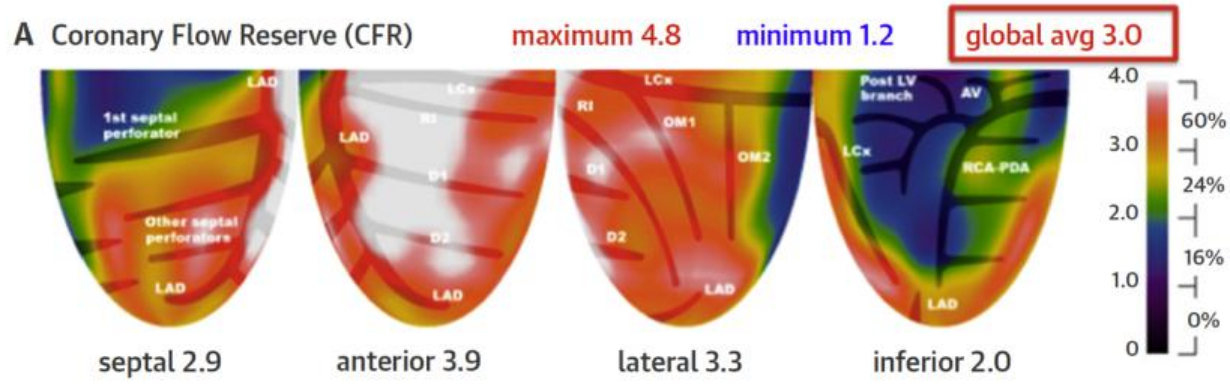

Figure 21. Limitations of global coronary flow reserve. Although a global high CFR of 3.0 reflects increased myocardial oxygenation in the left ventricle, it fails to account for regional perfusion deficits inferiorly.[74]

The simplicity of a global value to describe myocardial perfusion and oxygenation limits our assessment of common regional heterogeneity and complex interactions that exist in microvascular dysfunction. While our findings suggested that there was no significant difference in global myocardial oxygenation, a regional examination of the myocardium revealed important heterogeneity and regional differences in myocardial oxygenation, which could help explain the complex dysregulation of underlying coronary microvascular networks that occur in CMD.

5.3 Myocardial oxygenation response to hyperventilation

In women with chest pain and NOCAD, we noticed a trend towards a greater reduction in signal intensity (decreased oxygenation through vasoconstriction) during hyperventilation compared to healthy volunteers. Furthermore, amongst all women, a trend existed towards a greater reduction in oxygenation during hyperventilation in women with a higher hospital anxiety score.

Our findings that women with chest pain and NOCAD having a greater reduction in oxygenation during hyperventilation could be an important sign of underlying vasomotor abnormalities in these patients. Coronary vasospasm is part of the spectrum of vasomotor abnormalities seen in patients with microvascular dysfunction and affects both the microvasculature and large coronary vessels.[28] In normal endothelial function, acetylcholine and other physiological stimuli (e.g. exercise) produce vasodilation of the coronary arteries through endothelium-dependent release of nitric oxide and subsequent vasodilation of the coronary vessels. When the coronary endothelium is damaged however, nitric oxide release is deficient, and there is paradoxical smooth muscle vasoconstriction due to direct muscarinic receptor activation.[73]In the original WISE cohort, there was a high prevalence of abnormal endothelial dependent response to acetylcholine, which were associated with an increased risk of adverse cardiovascular events in follow up.[21]

Hyperventilation-induced angina has been well described in the literature, and earlier studies identified hyperventilation as a provocative test for coronary artery vasospasm in patients with suspected vasospastic angina.[75,76] In a study by Nakao et al., 206 patients in whom coronary spasm was documented by angiography using acetylcholine and 183 patients without angina, underwent vigorous hyperventilation for 6 minutes. Of the spasm group, 127 showed positive

responses to hyperventilation including ST elevation (n=111), ST depression (n=15). None of the patients in the non-spasm group showed any ischemic changes.[77] The findings suggested that vigorous hyperventilation over longer periods could be a highly specific test for the diagnosis of coronary artery spasm.

Hyperventilation-induced vasospasm is thought to occur as a result of increases in intracellular calcium concentration through a reduction in hydrogen protons (via respiratory alkalosis), which competes with calcium via an active transmembrane protein.[78] It remains uncertain as to why patients with endothelial dysfunction are more susceptible to an intravascular alkalotic state. Microvascular rarefaction and damage to intercellular gap junction proteins in endothelial dysfunction could play an important role in facilitating abnormal increases in intracellular calcium. Our preliminary findings suggest the potential for OS-CMR, using short-term hyperventilation as a stimulus, to potentially serve as a valuable technique in assessing patients with vasomotor abnormalities.

Interestingly, we also noticed a trend towards a higher hospital anxiety score in women with a decreased myocardial oxygenation response (increased vasoconstriction) to hyperventilation. Anxiety disorders have been associated with adverse cardiovascular outcomes in several epidemiological studies,[79]yet the pathophysiological mechanism remains unexplored. Hyperventilation is a common clinical presentation in anxiety and panic disorders.[80] Our findings of a trend towards an increased coronary vasoconstrictive response to hyperventilation could point to vasomotor abnormalities as a possible underlying link. Frequent episodes of hyperventilation in anxiety disorders could induce ischemia through recurrent coronary vasoconstriction and may also be a trigger of chronic endothelial dysfunction and cardiovascular remodelling. Additionally, elevated sympathetic activation in anxiety disorders could augment

α-adrenoceptor activation during hyperventilation resulting in reduced cardiac perfusion by microvascular constriction. Women with CMD could be experiencing an exaggerated response to hyperventilation related to anxiety states.

5.4 Role of T2 mapping in women with chest pain and NOCAD

In women with chest pain and NOCAD, we observed a higher mid-T2 relaxation time compared to healthy volunteers. Furthermore, in a subset of patients undergoing adenosine perfusion MRI, we found that mid-T2 correlated well with worsening microvascular function (reduced myocardial perfusion reserve). T2 mapping has emerged as a valuable tool in CMR for the assessment of myocardial edema, which has demonstrated important diagnostic value in several acute and chronic cardiac diseases such as acute myocardial infarction and myocarditis.[81]In patients with biopsy proven myocarditis, T2 mapping was able to detect corresponding myocardial regions with underlying inflammation.[82]The value of T2 mapping in patients with NOCAD however, has yet to be fully elucidated. Our novel findings of a higher mid-T2 relaxation time in women with chest pain and NOCAD, could suggest a potential diagnostic role for myocardial mapping in patients with microvascular dysfunction. Furthermore, higher values of T2 could suggest the possibility of an underlying inflammatory process in patients with chest pain and NOCAD. Endothelial dysfunction involves an active inflammatory state, with chemokine and adhesion molecule expression, and leukocyte recruitment. In patients with CSX, elevated inflammatory markers, including C-reactive protein have been observed when compared to healthy controls.[83]CMD has been known to occur more frequently in patients with underlying inflammatory disorders including rheumatoid arthritis and connective tissue diseases.[84]Furthermore, in patients with stress induced cardiomyopathy, strong

evidence exists that enhanced sympathetic activity, induces a transient myocardial dysfunction, potentially through abnormal vasoreactivity, endothelial and microvascular dysfunction. The use of CMR in patients with stress induced cardiomyopathy has identified transient areas of myocardial edema and markers of myocardial inflammation (increase in T2), in the absence of significant necrosis or fibrosis.[85]Our findings of an increase in mid-T2 in women with chest pain and NOCAD could potentially be explained through recurrent underlying stress-induced microvascular injury as a result of a heightened sympathetic response, potentially explained through underlying psychological comorbidities in these women.

Higher mid-T2 relaxation time could support microvascular dysfunction as an active inflammatory disease of the microvasculature, offering an important insight into the pathogenesis of this disease. Furthermore, our results suggest that T2 weighted imaging could serve as an additional tool in the comprehensive assessment of women with chest pain and NOCAD using OS-CMR.

5.5 Potential limitations

Our study has several important limitations to note. Firstly, as this is an interim analysis, our findings are based on a limited sample size that is not powered to detect a significant difference between our groups. We anticipate a complete analysis of our findings once our recruitment process for our study has been finished (December 2020). Secondly, our selection of patients from different sites (Calgary and Montreal) could provide the potential for a systemic bias given the possible differences in MRI technicians and scanners. To ensure that no differences exist between sites, we have ensured appropriate quality control measures in our study to standardize the MRI protocol for both sites to ensure consistency in the data collection.

Furthermore, as patients in Calgary are located at a higher altitude, acclimatization due to altitude could theoretically impact oxygen affinity at a lower partial pressure of oxygen, which could impact the BOLD signal. Prior studies however, examining patients from Calgary, Montreal, and Switzerland have demonstrated the same clinical response to hyperventilation/breath-holding manoeuvres irrespective of altitude.[62,86]Furthermore, we are beginning the process of recruiting patients with chest pain and NOCAD in Montreal, and healthy volunteers in Calgary, and plan to examine whether differences in patient location impact our findings. Additionally, given the exploratory nature of this thesis, we tested multiple hypothesis in our cohort. As a result, the significance of some of our findings could be due to chance from multiple statistical comparisons. We intend to perform analytical adjustments for multiple testing upon analysis of our completed cohort. Finally, in our analysis of regional differences, the myocardium was segmented into endocardial and epicardial layers, where circumferential and radial differences were calculated. Separation of the myocardium into endo and epicardium layers was often challenging in a thin myocardium (typically inferolateral wall), leading to an SI result representing a very small voxel area that might not be generalizable for that particular region. To ensure the consistency of our SI results, for each myocardial region, we used two independent readers for all OS-CMR scans, and segments were excluded based on a collective consensus when the myocardium was considered too thin or if a susceptibility artifact comprised a significant portion of the region (>33% of the individual segment area).

5.6 Future Directions

Our preliminary findings on the use of OS-CMR in the assessment of women with chest pain and NOCAD, provides a mechanistic insight into the pathophysiology of microvascular disease, and offers an important direction for future research.

Our preliminary results suggest that there exists no difference in the global myocardial oxygenation response to a hyperemic stimulus (breath-hold) in women with chest pain and NOCAD. However, this finding must be tempered by the hyperventilation heightened vasoconstrictive response. Indeed, we observed that women with chest pain and NOCAD had a trend towards an increased reduction in myocardial oxygenation during hyperventilation, potentially mediated through abnormal vasoconstriction. Furthermore, we observed an increase in regional heterogeneity in myocardial oxygenation in women with chest pain and NOCAD. These novel findings could point to microvascular dysfunction being a heterogeneous and patchy phenomenon, with microvascular maldistribution as an important driver of disease, rather than a global diffuse reduction in myocardial oxygenation reserve. Future research should further investigate regional differences in myocardial oxygenation and perfusion and examine the impact of regional heterogeneity on long-term cardiovascular outcomes. Future research should also attempt to validate our preliminary findings, investigating the role of hyperventilation using OS-CMR in patients with documented acetylcholine-inducible coronary vasospasm on angiography. Finally, our observation of an increase in mid-T2 relaxation time in women with chest pain and NOCAD could point to a potential role of inflammation in these patients.

6.0 Conclusion

In summary, in our preliminary analysis, women with chest pain and NOCAD did not display any significant changes in myocardial oxygenation during a breath-hold manoeuvre compared to healthy volunteers. Our findings, however, suggest that women with chest pain and NOCAD had higher regional variability in myocardial oxygenation and mid-T2 compared to healthy volunteers, and these values correlated well with worsened myocardial perfusion reserve. Furthermore, in women with chest pain and NOCAD, there existed a trend towards a more pronounced vasoconstriction during hyperventilation. OS-CMR provides important mechanistic insights into the pathogenesis of coronary microvascular dysfunction.

7.0 References

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