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Pratik M. Pimple Date

Is Mental Stress Induced Myocardial Ischemia Associated With Coronary Artery Disease Burden?

By

Pratik M. Pimple, MBBS

Master of Public Health

Department: Epidemiology

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Viola Vaccarino, MD, PhD

Faculty Thesis Advisor

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By

Pratik M. Pimple

Bachelor of Medicine Bachelor of Surgery (MBBS)

Maharashtra University of Health Sciences

2010

Faculty Thesis Advisor: Viola Vaccarino, MD, PhD

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Abstract

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By: Pratik M. Pimple

Objective: Mental stress-induced myocardial ischemia (MSMI) is thought not to be related to severity of coronary artery disease (CAD), in contrast to physical stress-induced myocardial ischemia (PSMI), but previous studies are conflicting and did not consider potential confounders or interactions.

Methods: We used single-photon emission tomography (SPECT) myocardial perfusion imaging in conjunction with a standardized psychological stressor and, on a separate day, with physical stress primarily via exercise treadmill testing, in 98 subjects with a history of myocardial infarction in the previous 6 months. We calculated a summed stress score (SSS) of perfusion defects with mental stress (MSSS) and physical stress (PSSS) using both a reader-independent, software-based method, and physician-based readings. Using linear regression analysis, we examined the association between the MSSS and two quantitative measures of CAD severity, the Gensini score and the Duke CAD Severity Index (DCSI). For comparison, we performed a similar analysis with the PSSS. We adjusted for all Framingham risk factors and depression status and assessed interaction effects with age.

Results: Forty-nine subjects were ≤ 50 years of age, 49 were female and 39 subjects were whites. For MSMI, there was no association between software-assessed MSSS and either the Gensini score or the DCSI, even after adjustment for Framingham risk factors, depression and medication status. For PSMI, we found a significant interaction effect between age and software-assessed PSSS for both Gensini score and DCSI. In subjects of age ≤ 50 years, PSSS was associated with both Gensini score (regression coefficient: 0.050, 95% CI: 0.014 to 0.086, p value= 0.006) and DCSI (regression coefficient: .067, 95% CI: 0.011 to 0.122, p value = 0.02). PSSS was not found to be associated with either Gensini score or DCSI in subjects older than 50 years. Similar results were found using physician-assessed outcome variables.

Conclusion: Mental stress-induces myocardial ischemia is not associated with CAD severity. In contrast, physical stress myocardial ischemia is positively associated with CAD, although this association is only observed in younger MI patients. Mechanisms other than plaque burden may underlie ischemia triggered by mental stress.

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THESIS CHAPTER I

Background

Coronary heart disease (CHD) is a major cause of death in developed countries like the USA, and a growing problem in developing world as well. CHD accounts for about 7.2 million deaths in the world per year, which is about 12% of total worldwide deaths(1). It has been 65 years since the Framingham Heart Study started in Framingham, Massachusetts(2). Since its inception, we have gained considerable knowledge through extensive research in cardiovascular disease (CVD) epidemiology. We have conclusively identified age, male gender, race, hypertension, dyslipidemia, smoking and diabetes mellitus as important risk factors for CVD(2) and there are effective preventive and therapeutic measures in place for some of the risk factors that are modifiable. These measures have significantly reduced morbidity associated with CVD(3, 4) but CVD remains a major health challenge worldwide(5). It has been observed that CVD events can occur with few of the established Framingham risk factors or none at all(6, 7). Approximately 20% of 30-year predicted risk for CVD is not explained by Framingham risk factors (C statistics = 0.8)(8). Hence, we need to continue finding novel risk factors for CVD prevention and treatment purposes. One of the important emerging risk factors for CVD is psychological stress.

Psychological stress has been long suspected to be a risk factor for CVD, but only recently it has been given due attention, to better understand the role of stressful conditions in acute coronary events and to find the mechanism of action of mental stress(9-12). Jain et al state that, "Despite the identification of emotional stress as a trigger in the first description of angina pectoris by Heberden, as well as the well-publicized death of John Hunter, a renowned British surgeon, in 1793, after a heated argument with his colleagues, the role of mental stress and

behavioral and psychological factors in cardiovascular medicine has remained inadequately understood."(13, 14). Many researchers have observed that depression is highly prevalent in cardiac patients with 31% to 45% of total CVD patients suffering from clinically significant depressive symptoms and 15% to 20% patients fulfill the criteria for major depressive disorders(15). Eller et al found in their systematic review, that work-related stress leading to high psychological demands, low control, and job strain in combination with lack of social support, are moderate risk factors for future CVD events in many studies conducted in the US and Europe(10). Steptoe et al in their most recent meta-analysis found that social isolation and work place stress increase the risk of CVD by 50% and 30% respectively(16). Acute emotional distress has also been observed to trigger acute coronary syndromes (ACS) in subjects with no apparent cardiovascular conditions or coronary artery blockade(17). Steptoe et al found the relative risk for ACS being preceded by acute emotional distress like anger, or depressed mood, to be 2.5(16). The prevalence of depression is on the rise in both developed and developing countries, with current data showing that estimated one in ten adults in the US report to be suffering from major depressive disorder(18) and psychological illnesses like depression are found to be the leading cause of disability worldwide.(1) Hence, continued research on the effects of psychological stress on CVD and on the mechanisms of action behind this association is needed

Mental Stress-Induced Myocardial Ischemia (MSMI):

Mental stress-induced myocardial ischemia (MSMI) can be defined as transient myocardial ischemic response to intense mental stress (12). MSMI can be elicited in laboratory settings using standardized mental stressors and assessment of cardiac function through different imaging techniques(12). MSMI is analogous to the ischemia induced by exercise (e.g., by walking on a treadmill) or pharmacological agents (e.g., by administration of adenosine), during

standard cardiac testing (referred to here as “physical-stress induced myocardial ischemia,” or PSMI), except that the stressor used is psychological instead of physical(12).

Two components are used in assessment of MSMI in a laboratory setting: The first component involves use of validated mental/emotional stressor for eliciting cardiac response to mental stress. Commonly used mental stressors in laboratory are 1) mental arithmetic, 2) verbal recall of recent events which angered the subject, 3) color-word cognitive processing conflict task (commonly known as Stroop test), and 4) public speaking task involving personal distressful attributes or simulated events(12, 19). The second component involves assessment of myocardial ischemia during mental stress(12, 19) using a variety of non-invasive assessments and criteria of diagnosing MSMI. The methods and criteria used are as follows: 1) Electrocardiographic assessment with ST segment depression during mental stress as the criteria of diagnosis of MSMI, 2) Radionuclide ventriculography or echocardiography with three criteria for diagnosis of MSMI, the presence of new or worsening wall motion abnormalities, a fall in ejection fraction of $\geq 5\%$, and a fall in ejection fraction of $\geq 8\%$, 3) Single photon computed emission tomography (SPECT) or positron emission tomography (PET) for myocardial perfusion imaging (MPI) with criteria of diagnosis being perfusion defect score ≥ 3(12, 19). As shown above, considerable variation of mental stressors and diagnostic criteria have been used by researchers in laboratory settings.

Both Strike et al(12) and Burg et al(19) in their review recommend that for assessment of MSMI, MPI using PET or SPECT should be used because : 1) Flow changes during mental stress may be mild, variable, and may be actually insufficient to generate functional signs of ischemia such as regional wall motion abnormalities on echocardiography(20, 21). 2) Worsening of left ventricular (LV) systolic function (ejection fraction) can also be observed as a result of an increase in systemic vascular resistance(22) and it has been observed in healthy volunteers in response to mental stress, in absence of ischemia(23). 3) Use of electrocardiographic assessment

underestimates the prevalence of MSMI, as compared to other criteria of assessment(12, 24). 4) MSMI diagnosis with SPECT scan has shown 75% reproducibility(25).

Both review articles also found that public speaking and anger recall task are more potent in inducing mental stress than mental arithmetic and Stroop test. Also, anger recall test has been found to be more consistent in reproducibility than arithmetic and Stroop testing(12, 19, 26).

The incidence of MSMI have been found to be variable, ranging from 20% to 70% in subjects with pre-existing coronary artery disease (CAD)(12). Some of this variation can be explained by the various methods of MSMI assessment as explained above. A recent study by Jiang et al found the incidence of MSMI to be 43.5% in their study population with patients with stable coronary artery disease(27). They also found that incidence of MSMI is associated with gender and marital status, with female gender and single status having higher risk of MSMI(27).The incidence of MSMI has also been found to be associated with co-existing LV dysfunction, with Akinboboye et al(28) finding that incidence of MSMI was about five times more in patients who had severe LV dysfunction than in those who had normal LV function. Hasan et al(29) found this incidence of MSMI to be about 1.5 times in subjects with LV dysfunction as compared to normal LV function, but their results were non-significant.

MSMI is associated with worse prognosis in subjects with pre-existing, stable CAD. Sheps et al(30) found in their Psychophysiological Investigations of Myocardial Ischemia (PIMI) study that new or worsened wall motion abnormalities during the speech test at baseline observation significantly predicted death at average 3.5 to 5 year follow up with a rate ratio of 3.0. Krantz et al(31), Jiang et al(32) and Jain et al(33) found similar results in their study, with two and three fold increase in subsequent cardiac events, when comparing subjects having MSMI as compared to subjects not having MSMI. A recent study by Babyak et al(34) found that MSMI, as diagnosed by fall in ejection fraction $\geq$ 5% was associated with two fold increase in subsequent clinical events or death.

MSMI, PSMI and Coronary Artery Disease Burden:

Even though MSMI is analogous to PSMI, it has many different characteristics. MSMI is observed to be typically painless and without any overt symptoms and it occurs at lower levels of oxygen demand than exercise-induced myocardial ischemia(35-40). MSMI has been observed to happen despite previous revascularization procedures(41, 42). MSMI is thought not to be related to severity of coronary artery disease (CAD) as compared to PSMI, which is directly related with CAD burden(12). There have been few studies where the association between MSMI and CAD burden has been evaluated, but the results present in the literature are conflicting and this lack of association between MSMI and CAD burden has not been conclusively proven yet.

Researchers have found no association between MSMI and indicators of obstructive CAD, such as history of myocardial infarction, history of revascularization by percutaneous transluminal coronary angioplasty (PTCA) or stenting, or coronary artery bypass surgery (CABG)(27, 30, 34, 41-43). The findings of these studies suggest that there is no relationship between CAD burden and MSMI. A major criticism of these studies is that these results were unadjusted descriptive comparisons between the two populations (MSMI positive and MSMI negative). More importantly, these studies compared parameters which are indirectly associated with CAD burden, but did not evaluate the actual difference in underlying CAD burden among MSMI positive and negative subjects.

Among the studies where the number of major coronary vessels involvement in CAD (one, two or three vessel disease) was compared between MSMI positive and negative patients, results are conflicting. Brodov et al found that subjects with MI preceded by potential trigger activity (PTA) (emotional stress) had half the odds of having three vessel disease and disease ofleft anterior descending artery (LAD) as compared to MI not preceded by PTA(44). Krantz et al observed similar distribution of subjects with one, two or three vessel disease in MSMI positive

and negative subjects, but they did not contrast these results with PSMI assessment(45). Hassan et al found that MSMI positive subjects were more likely to have single vessel CAD (86%) as compared to PSMI positive subjects (54%), indicating that CAD burden is less associated with MSMI than PSMI(46). In contrast to these results indicating that MSMI is not a strong correlate of CAD burden, Legault et al found that subjects with speech induced ischemia had a significantly higher jeopardy score and a greater mean number of diseased vessels (2.1 vs. 1.2) as compared to subjects without speech induced ischemia(47). A recent study by Stepanovic et al found similar results, with MSMI positive subjects having a higher frequency of two or more diseased coronary vessels as compared to MSMI negative subjects(48). Both these studies lack comparison of their results of MSMI assessment with PSMI assessment(47, 48). Contrasting the association between MSMI and CAD burden with the association between PSMI and CAD in the same population is useful. A strong association between CAD burden and PSMI is already established through scientific research. A study where MSMI is not associated with CAD while PSMI is associated with CAD, in the same population, can make a better case that that the lack of association between MSMI and CAD is not due to confounding factors or low power. Such a study can also make the argument that the pathophysiology between these two types of ischemia differs. Another contrasting results found in literature are two studies by Specchia G et al, reporting that the prevalence of having two or more diseased vessels was significantly higher in subjects with MSMI, as compared to MSMI negative subjects(49, 50). However, the results of these two studies need to be interpreted cautiously, as all subjects who were MSMI positive, were also PSMI positive(49, 50).

One hypothesis which supports the lack of association between MSMI and CAD burden is the observation that in subjects with CAD, coronary resistance falls during mental stress in regions with significant stenosis(21, 51-53). In regions without significant stenosis, there is lower resistance at baseline as compared to regions with stenosis, but these regions show increased

resistance during mental stress, indicating that mental stress has different, possibly opposite, effects on the vasomotor tone of coronary arteries as compared to exercise stress(21, 51-53).

The main methodological limitations of the studies mentioned above are: 1) Lack of adjustment for potential confounders and assessment of interactions; 2) Lack of comparison of the association between MSMI and CAD burden and PSMI and CAD burden; 3) Lack of use of quantitative measures of CAD burden, such as CAD scoring systems (54, 55); 4) Use of ischemia assessments that are not the state-of-the art. Only two studies used myocardial perfusion imaging using SPECT for diagnosis of MSMI(42, 46).

Conclusion:

Methodological limitations and conflicting results present in the existing literature suggest a need for further research in the association between MSMI and CAD burden. Confirmation of non-association between MSMI and CAD burden and comparison of these results with association between PSMI and CAD burden will add significant scientific evidence to our current hypothesis that PSMI and MSMI have different underlying pathophysiological mechanisms. If it is proven that MSMI is not affected by underlying severity of CAD, this conclusion will mean that the pathophysiological mechanisms underlying MSMI are significantly different from those of PSMI. This finding has substantial implications for risk assessment, risk prediction, prevention and treatment strategies. It will also mean that vulnerable subjects with pre-existing CHD can have an adverse prognosis due to emotional stress, irrespective of the severity of their underlying disease and of treatment strategies aimed at reducing CAD blockage, such as revascularization procedures.

In our analysis, we will overcome the limitations of the previous studies by 1) Use of regression analysis with adjustment for potential covariates like all Framingham risk factors (age, gender, race, hypertension, dyslipidemia, smoking and diabetes mellitus), income status,

depression and medications. 2) Comparison of the association between MSMI and CAD burden with the association between PSMI and CAD burden in the same population, 3) Use of validated, state-of-the art techniques for assessment of MSMI and PSMI, including myocardial perfusion imaging with SPECT, and a standardized public speaking task or standardized exercise stress test (56) or pharmacological stress, and 4) Use of composite, validated measures of CAD burden, the Modified Gensini score(54, 55), and the Duke CAD Severity Index(55, 57).

THESIS CHAPTER II

Thesis Manuscript

Is Mental Stress Induced Myocardial Ischemia Associated With Coronary Artery Disease Burden?

Authors list: **Pratik M Pimple, Viola Vaccarino.**

Abstract

Objective: Mental stress-induced myocardial ischemia is thought not to be related to severity of coronary artery disease (CAD), in contrast to physical stress-induced myocardial ischemia, but previous studies are conflicting and did not consider potential confounders or interactions.

Methods: We used single-photon emission tomography (SPECT) myocardial perfusion imaging in conjunction with a standardized psychological stressor and, on a separate day, with physical stress primarily via exercise treadmill testing, in 98 subjects with a history of myocardial infarction in the previous 6 months. We calculated a summed stress score (SSS) of perfusion defects with mental stress (MSSS) and physical stress (PSSS) using both a reader-independent, software-based method, and physician-based readings. Using linear regression analysis, we examined the association between the MSSS and two quantitative measures of CAD severity, the Gensini score and the Duke CAD Severity Index (DCSI). For comparison, we performed a similar analysis with the PSSS. We adjusted for all Framingham risk factors and depression status and assessed interaction effects with age.

Results: Forty-nine subjects were ≤ 50 years of age, 49 were female and 39 subjects were whites. For MSMI, there was no association between software-assessed MSSS and either the Gensini score or the DCSI, even after adjustment for Framingham risk factors, depression and medication status. For PSMI, we found a significant interaction effect between age and software-assessed

PSSS for both Gensini score and DCSI. In subjects of age ≤ 50 years, PSSS was associated with both Gensini score (regression coefficient: 0.050, 95% CI: 0.014 to 0.086, p value= 0.006) and DCSI (regression coefficient: .067, 95% CI: 0.011 to 0.122, p value = 0.02). PSSS was not found to be associated with either Gensini score or DCSI in subjects older than 50 years. Similar results were found using physician-assessed outcome variables.

Conclusion: Mental stress-induces myocardial ischemia is not associated with CAD severity. In contrast, physical stress myocardial ischemia is positively associated with CAD, although this association is only observed in younger MI patients. Mechanisms other than plaque burden may underlie ischemia triggered by mental stress.

Important Abbreviations list:

Cardiovascular disease (CVD)

Mental Stress induced Myocardial Ischemia (MSMI)

Physical Stress induced Myocardial Ischemia (PSMI)

Coronary Artery Disease (CAD)

Myocardial Infarction (MI)

Mental Summed Stress Score (MSSS)

Physical Summed Stress Score (PSSS)

Mental Summed Difference Score (MSDS)

Physical Summed Difference Score (PSDS)

Summed Rest Score (SRS)

Duke CAD Severity Index (DCSI)

Introduction

Psychological stress has been long suspected to be a risk factor for CVD, but only recently it has been given due attention, to better understand the role of stressful conditions in acute coronary events and to find the mechanism of action of mental stress(9-12). Mental stress-induced myocardial ischemia (MSMI) can be defined as transient myocardial ischemic response to intense mental stress (12). It is an important method of assessing the effects of stress and emotion on cardiac function. MSMI can be elicited in laboratory settings using standardized mental stressors and assessment of cardiac function through different imaging techniques(12). MSMI is analogous to the ischemia induced by exercise (e.g., by walking on a treadmill) or pharmacological agents (e.g., by administration of adenosine), during standard cardiac testing (referred to here as "physical-stress induced myocardial ischemia," or PSMI), except that the stressor used is psychological instead of physical(12).

MSMI is observed to be typically painless and without any overt symptoms and it occurs at lower levels of oxygen demand than exercise-induced myocardial ischemia(35-40). MSMI has been observed to happen despite previous revascularization procedures(41, 42). MSMI is thought not to be related to severity of coronary artery disease (CAD) as compared to PSMI, which is directly related with CAD burden(12), but there have been very few studies where association between MSMI and CAD burden has been evaluated, and the results present in the literature are conflicting. Studies comparing indirect measure of obstructive CAD like history of myocardial infarction, history of revascularization by percutaneous transluminal coronary angioplasty (PTCA) or stenting, and history of coronary artery bypass surgery (CABG) among MSMI positive and negative subjects did not find any statistically significant differences among these parameters, indicating no association between MSMI and CAD burden(27, 30, 34, 41-43). Among the studies where number of major coronary vessels involvement in CAD (one, two or three major vessel disease) and MSMI status was compared, three studies found results indicating

no association between CAD burden and MSMI(44-46), but four studies found the prevalence of CAD burden (presence of two or more major vessels involvement) to be significantly greater in MSMI positive subjects, as compared to MSMI negative subjects(47-50).

The methodological limitations of all these studies mentioned above are: 1) all these studies compared CAD burden (indirectly or directly) with MSMI status only in descriptive table with chi square statistics. They did not involve any adjustment for potential confounders and assessment of interactions, 2) Lack of comparison of the association between MSMI and CAD burden and PSMI and CAD burden; 3) Lack of use of quantitative measures of CAD burden, such as CAD scoring systems (54, 55).

Hence, we will be evaluating the association of CAD burden with MSMI in our study, with following analytical strengths, in order to overcome the limitations mentioned above, 1) use of regression analysis with adjustment for potential covariates like all Framingham risk factors (age, gender, race, hypertension, dyslipidemia, smoking and diabetes mellitus), income status, depression and medications, 2) Comparison of the association between MSMI and CAD burden with the association between PSMI and CAD burden in the same population, and 3) Use of quantitative, validated measures of CAD burden (Modified Gensini score(54, 55), Duke CAD Severity Index(55, 57)). We performed analysis on 98 subjects, recruited from the cross-sectional study "Sex Differences in Myocardial Ischemia Triggered by Emotional Factors after Myocardial Infarction (MI)" (MIMS). The main aim of MIMS study is to evaluate gender differences in stress-induced ischemia after an MI.

In this analysis, the primary hypothesis is that in this population of middle-aged men and women with stable coronary artery disease, MSMI is not associated with CAD burden, while PSMI is associated with it, even after controlling for Framingham risk factors, income, and depression status.

Methods

Null Hypothesis:

1) There is no association between mental stress induced myocardial ischemia (MSMI) and coronary artery disease (CAD) burden in our sample of middle aged men and women with stable CAD.

2) There is no association between physical stress induced myocardial ischemia (PSMI) and coronary artery disease (CAD) burden in our sample of middle aged men and women with stable CAD.

Characteristics of Study Design:

Our study is cross-sectional, but used an experimental design through which ischemia was induced in the laboratory. Both our exposure (composite measures of CAD burden) and outcomes (MSMI and PSMI), were measured as continuous variables.

Characteristics of Study Population, Inclusion/ Exclusion criteria:

Sex Differences in Myocardial Ischemia Triggered by Emotional Factors after Myocardial Infarction (MI) (MIMS) study enrolled 98 subjects between year 2009 and year 2012. All subjects were between 18 and 59 years of age and had history of documented myocardial infarction (MI) within the previous 6 months. Men and women were frequency-matched for age (± 2 years), type of MI (STEMI or NSTEMI) and months since the MI (± 2 months). Anti-ischemic medications, including beta-blockers, ace-inhibitors, calcium channel blockers and nitrates, were withheld for 24 hours before testing, according to standard protocol for exercise stress imaging studies(58).

Subjects with the following characteristics were excluded from the study: 1) history of unstable angina, myocardial infarction, or decompensated heart failure in the past week of enrollment; 2) deemed to be unsafe to hold anti-ischemic medications for the 24 hours prior to the

testing; 3) systolic blood pressure >160 mm Hg or diastolic blood pressure >100 mm Hg on the day of the test; 4) physical limitations with inability to exercise on a treadmill (Duke activity status index(62) (DASI) < 5 METs); 5) history of current alcohol or substance abuse or dependence within the past year of enrollment; or history of severe psychiatric disorder other than major depression, such as schizophrenia; 6) history of serious medical disorder other than cardiovascular disease that may interfere with the study results, e.g. cancer or renal failure; 7) use of exogenous estrogens or progesterone within past 3 months of enrollment; 8) current psychotropic medication treatment within past month of enrollment except treatment for depression; 9) current pregnancy or breast feeding; 10) severe aortic stenosis. Along with assessment of MSMI and PSMI, extensive data were collected evaluating demographic, behavioral, psychological and other related data at baseline, and inflammatory, autonomic, hemodynamic, endothelial function changes during both mental and physical stress. The MIMS study was approved by the Emory Institutional Review Board, and all subjects signed an informed consent.

Measurement of Outcome Variables:

The primary outcome of our analysis was mental stress induced myocardial ischemia (MSMI), and the secondary outcome for comparison was physical stress induced myocardial ischemia (PSMI). There are two components for the assessment of MSMI and PSMI: 1) Use of a validated stressor for mental and physical stress, and 2) Assessment of myocardial ischemia during stress and at rest.

Mental and physical stressors used:

In our study, we used public speaking task as a mental stressor. A scenario involving a real-life stressful situation that could happen to anyone was read to each subject. They were then asked to make up a realistic story around this situation. They were given two minutes to plan the

story and prepare a statement and then three minutes to present it in front of an audience and a video camera.

For physical/exercise stress, subjects underwent the Bruce protocol for exercise stress, consisting of walking on a treadmill(56). Exercise target was set at 85% of predicted heart rate according to the patient's sex and age, as it is standard for stress imaging studies(56). For subjects who were unable to reach the heart rate target by walking on the treadmill, we did a pharmacological stress test with a 4-minute infusion of adenosine(56).

Method of assessment of myocardial ischemia:

We assessed myocardial ischemia during stress and rest using 99m-Tc-sestamibi gated single-photon emission computed tomography (SPECT) myocardial perfusion imaging. Three scans were done on each subject, one during baseline (rest period), one during mental stress and (on a separate day) one during exercise or adenosine stress.

SPECT scan protocol:

For assessment of myocardial perfusion at rest, subjects were injected 8-15 mCi Tc-99m according to body weight and, after about 45 minutes, resting myocardial blood flow images were acquired for 15 minutes.

For mental stress, three times the rest dose of Tc-99m was given at 1 minute after the beginning of the speaking task, and was followed by SPECT scan imaging. For physical stress, subject were injected with 30-45 mCi Tc-99m by bolus intravenous injection according to body weight, at peak exercise stress. Subjects underwent SPECT scanning a minimum of 45 minutes after injection of Tc-99m.

SPECT scan image analysis:

We performed gated SPECT imaging using conventional methodology(63). Data were acquired after injection of Technetium 99M Sestamibi according to an established protocol for

nuclear medicine studies. The SPECT images were analyzed in two ways: 1) Software assessment with Emory cardiac SPECT toolbox(64), and 2) Physician assessment of images.

Software assessed outcome: Gated SPECT scan images were processed and analyzed using the Emory cardiac toolbox software version 3.1 (ECTb)(64). For each phase (rest, mental stress and physical stress), the SPECT images were re-oriented along the short axis using GE Xeleris software(65). These short axis images were then transferred to the Emory Cardiac Toolbox for interpretation. Each stress scan was processed alone. The rest scans were also processed alone, but the rest scan was processed with the same rest scan, renamed as stress scan, in order to produce unbiased normalization of rest images. The myocardial center, base and apex positions were kept the same between the stress only and rest only processing to maintain consistency. In each region, the system quantified defect severity on a 4-point scale from normal to absent perfusion. The regional severity scoring was then summed up across the 17 segments yielding a total score. Quantitative parameters (summed stress score (SSS), summed rest score (SRS)) were directly outputted from ECTb software, and described the extent and the severity of the perfusion defects across 17 segments of the myocardium. (See Appendix, figure 1) All this process was done by same technologist, who was blinded to subject's condition of the stress (mental or exercise).

Physician assessed outcome: Gated SPECT scan images were also scored separately by one investigator (PR) blinded to condition of the stress (mental or exercise). Images from both baseline, exercise and mental stress were reconstructed in short axis, vertical long axis and horizontal long axis views. The scoring of perfusion is similar to the automatic scoring described above, except that it was based on visual interpretation(66). In each region, the defect severity was quantified on a 4-point scale from normal to absent perfusion. The regional severity scoring was then summed up across the 17 segments yielding a total score. Separate total scores were obtained for the rest and stress conditions, with the rest score assessing fixed or irreversible

defects. A reversible defect (or ischemia) score were obtained by subtracting the rest score from the stress score (i.e., summed difference score: SDS).

Definition of outcome variables:

Our main outcome variables were the mental/physical summed difference scores (MSDS and PSDS), describing the presence and severity of reversible myocardial perfusion defects (indicators of ischemia) during mental stress and physical stress, respectively. We also examined the summed rest score (SRS), describing fixed (irreversible) myocardial perfusion defects, recorded at the baseline rest condition, and the mental/physical summed stress scores (MSSS and PSSS), describing the total perfusion abnormalities (both fixed and reversible), recorded during mental and physical stress, respectively. These indices are mathematically related as follows:

MSDS = MSSS - SRS

PSDS = PSSS - SRS

Measurement of Predictor Variables:

CAD burden was assessed by calculating the Gensini score(54) and the Duke CAD severity index(57). All 98 subjects underwent coronary angiography at Emory University, and the CAD lesions were systematically assessed using a 17-segment modified American Heart Association model(67). A single investigator abstracted the coronary angiography data of all the subjects from the Emory University medical database, blinded to the SPECT data. Only the most recent coronary angiography, coinciding with the date of myocardial infarction, was used for the abstraction of coronary artery angiographic data. Patients with prior percutaneous coronary intervention or coronary artery bypass grafting were scored based on post-procedural disease burden.

The CAD scoring systems were calculated based on published algorithms(54, 57). The Gensini scoring system takes into account the geometrical severity of coronary artery lesions, the

cumulative effects of multiple obstructions, and the significance of jeopardized myocardium(54, 55). The Duke CAD severity index gives higher prognostic weight to both number of involved vessels and increasing severity of Left Anterior Descending (LAD) artery stenosis, with more proximal disease weighted higher(55, 57).

Measurement of Covariates:

Age was calculated by subtracting date of birth from date of enrollment in the study and rounded off to two decimal places. Sociodemographic, lifestyle and medical history data were assessed using standardized questionnaires by trained personnel. Height and weight were measured and BMI was calculated by dividing square of height (in meters) from weight (in Kilograms). Depression status was assessed by use of Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-NP), a semi-structured interview for making the diagnoses of major depressive disorders and other mental disorders(68).

Statistical Analysis:

Results were expressed as mean ± standard deviation for continuous variables and percentages for categorical variables. Mental stress summed score (MSSS) and physical stress summed score (PSSS) were compared according to demographic characteristics and medical history in our population. These scores were chosen for this descriptive analysis, instead of the difference scores, due to their normal distribution, as explained below. Statistical differences in the MSSS and PSSS between groups were determined by use of the Student *t* test for dichotomous variables and by analysis of variance (ANOVA) method for categorical variable with more than 2 categories.

Normality assumptions for the outcome variables were evaluated using criteria of skewness (absolute value <1), kurtosis (absolute value <1) and Kolmogorov-Smirnov (KS) test (p value of the test > 0.05). By using these criteria, we found that MSSS and PSSS were normally

distributed, while mental/physical summed difference score (MSDS and PSDS) were not normally distributed. Hence, to examine the association between mental stress myocardial ischemia and CAD burden and to contrast this relationship with physical stress myocardial ischemia, we performed two linear regression models with MSSS and PSSS as outcome variables, measure of CAD burden (Gensini score/ Duke CAD Severity Index) as primary exposure variables, and adjusting for rest myocardial perfusion defect score (SRS). As proved in the appendix section, this modeling strategy will give us exactly the same results for the regression coefficients of our primary exposure variable, as a linear regression models where the dependent variables are MSDS and PSDS. Using this strategy we will obtain non-biased standard errors and correct p values for our models, as the outcome variables MSSS and PSSS are normally distributed, while MSDS and PSDS are not.

We assessed for confounding from a pre-specified set of potential confounding factors, including traditional CAD risk factors, such as age, gender, race, smoking status, hypertension, hyperlipidemia, and diabetes, by adding all of these covariates in the above models. Based on previous literature (12, 69), and clinical considerations, we also considered depression, income, obesity, and use of beta-blockers as covariates in the model. These potential confounders were added one at a time in the above model, and the difference in regression coefficients was assessed. If adjusted regression coefficients changed by more than 10% in comparison to the regression coefficients adjusted for traditional CAD risk factors only, these factors were retained in the final model. We assessed interaction effects of all these covariates by adding interaction terms with exposure variable in the model, and then performing chunk test to assess the statistical significance of these interaction terms. If this chunk test was found to be significant, we dropped one interaction term at a time, to end up with a final model with significant confounders and interaction terms. Because we had so many covariates and interaction terms in our model, we also

assessed for collinearity among our independent (exposure) variables, using the criterion of variance inflation factor (VIF) > 10.

All statistical analyses were conducted using SAS version 9.3 for Windows (SAS, Cary North Carolina). A p value of less than 0.05 was considered statistically significant.

Results

Group and subject characteristics:

Five subjects did not have data on myocardial perfusion for mental stress or physical stress or both, and thus they were dropped from the analysis, yielding a final sample size of 93 subjects. As shown in Table 1, of the 98 total subjects, 49 (50%) were female, 49 (50%) subjects were 50 years of age or younger, 39 (40%) subjects were whites, 54 (55%) subjects were African-Americans and the remaining 5 (6%) subjects were of other race. A large proportion (50%) had income less than $35,000. In general, CAD risk factors were prevalent, including smoking (28% for current smokers, 34% past smokers), hypertension (69%), hyperlipidemia (73%), and obesity (46%). The mean ±SD for the Gensini score was 17.44 ± 32.15, range: 0 to 192. Corresponding statistics for the Duke CAD Severity Index were 14.72 ± 18.04, range: 0 to 74.

For **software assessed** outcomes, the mean for the mental summed stress score (MSSS) was 9.81 ± 6.06 (SD) with a range 0 to 27. The mean for the physical summed stress score (PSSS) was 10.27 ± 6.18 (SD) and range 0 to 32. There was no statistically significant difference in software assessed MSSS and PSSS between different categories of age, gender, ethnicity, and smoking status (Table 1). Among three categories of race, 'other race' subjects had significantly higher MSSS and PSSS as compared to Whites and African-Americans (p value = 0.04 for MSSS and 0.003 for PSSS), but this result can be spurious due to the fact that the “other race” category had only 5 subjects. Hence, for regression analysis, we decided to collapse this category and use

'Whites vs. non-Whites' as dichotomous variable. Subjects with more than $75,000 had significantly less MSSS and PSSS as compared to other income categories (P value = 0.07 for M_SSS and 0.02 for P_SSS). Also, subjects with hypertension, hyperlipidemia, diabetes, obesity and depression had higher MSSS and PSSS scores, and subjects on regular beta blocker medication had lower MSSS and PSSS scores as compared to those without it, but these differences were not statistically significant. In general, PSSS tended to be higher than MSSS (Table 1). Overall, results were similar when **physician assessed** MSSS and PSSS were examined (Table 2).

Results of Linear Regression:

Our outcomes included both physician and software derived summed stress scores, adjusted for the rest summed scores to yield coefficients and p values for ischemia. The exposure included both the Duke CAD Severity Index and the Gensini score. The results below are presented in a way that we can compare mental summed stress scores and physical summed stress scores using each of the different combinations of measured outcomes and exposures.

Outcome Assessment of MSSS and PSSS using Software:

Exposure measured by Gensini score:

The unadjusted (only adjusted for the SRS as discussed in methods and appendix section) regression coefficient for the association between PSSS and Gensini score tended to be higher than the coefficient for the association between MSSS and Gensini score in our whole sample. However, these results were not significant (p value=0.48 and 0.91 respectively). Even after adjusting for age , gender, race, smoking status, hypertension, hyperlipidemia, diabetes, depression status, beta blocker medication status, and rest sum scores (income level and obesity status were not found to be significant confounders, nor did they interact with other exposure),

these finding did not change for either PSSS or MSSS (adjusted p value = 0.41 and 0.83 respectively) (Table 3.1).

With all confounders and interaction terms in the model, we assessed for collinearity and did not find any significant collinearity problem in our model. We assessed for interaction in our study sample, and found borderline significant interaction effect of age category (age ≤ 50 years, as compared to age > 50 years) for the association between PSSS and Gensini score (p value = 0.058). On age stratified analysis, we found that in subjects with age ≤ 50 years, with **every 1 point increase** in Gensini score, PSSS score increased by 0.050 (95% CI: 0.014 to 0.086, p value= 0.006); while the association between PSSS and Gensini score in subjects with age > 50 years was non-significant (regression coefficient: -0.014 (95% CI: -0.043 to 0.015), p= 0.33) (Table 3.2).

For comparison, we performed similar analysis for the MSSS~ gensini score association, and found that age interaction was not present in this association. The Gensini score was not associated with MSSS in either patients of age ≤ 50 years or those of age >50 years (Table 3.2). No other confounder was found to have interaction effects with either MSSS or PSSS.

Exposure assessed by Duke CAD Severity Index:

Similar results were found when examining the association between software assessed MSSS and PSSS scores and Duke CAD Severity Index (DCSI), with adjustment done in the models with the same set of confounders as mentioned in the above section (Table 4.1 and 4.2). No significant unadjusted or adjusted associations between MSSS~ DCSI and PSSS~ DCSI were found in our whole sample (Table 4.1), but in subjects with age ≤ 50 years, PSSS was significantly associated with DCSI (regression coefficient: 0.067 (95% CI: 0.011 to 0.122), p value = 0.02). MSSS was borderline associated with DCSI (regression coefficient: 0.041 (95% CI: -0.001 to 0.183), p value = 0.06) in subjects with age ≤ 50 years. For subjects with age >50 years, DCSI was not found to be associated with PSSS, while it was found to be negatively

associated with MSSS (regression coefficient: -0.059 (95% CI: -0.104 to -0.015), p= 0.01). (Table 4.2)

Outcome Assessment of MSSS and PSSS by physician:

The results for physician-assessed MSSS and PSSS as outcomes and Gensini score and DCSI as primary exposures were similar to what we obtained for software-assessed MSSS and PSSS as outcomes, with adjustment done in the models with the same set of confounders.

Exposure measured by Gensini score:

For analysis with Gensini score as primary exposure, both unadjusted and adjusted associations with MSSS and PSSS were non-significant, even though regression coefficients were consistently greater with PSSS as outcome, compared to MSSS. (Table 5.1) However, again we found a significant age interaction for both the MSSS ~ Gensini score association and the PSSS~ Gensini score association (both p values < 0.05). In subjects with age ≤ 50 years, **every 1 point increase** in Gensini score was associated with 0.072 points increase in PSSS (95% CI: 0.021 to 0.123, p value = 0.005); and 0.057 points increase in MSSS (95% CI: 0.010 to 0.105, p value = 0.02). In subjects with age > 50 years, both of these associations were non-significant. (Table 5.2)

Exposure assessed by Duke CAD Severity Index:

Results of the analysis examining the association between MSSS (physician assessed) and the DCSI, as well as the association between PSSS (physician assessed) and the DCSI showed similar results. The unadjusted and adjusted associations were no-significant in the entire sample (Table 6.1). However, as seen in the above analyses, we found significant age interactions, such that in subjects ≤ 50 years of age, both PSSS and MSSS were positively related to DCSI, with an association that was statistically significant for PSSS (regression coefficient: 0.103 (95% CI: 0.024 to 0.183), p value = 0.01). In contrast, for subjects >50 years of age, DCSI

was not found to be associated with either P_SSS or M_SSS, with a negative coefficient for both associations (Table 6.2).

Discussion

In this study of subjects with previous myocardial infarction and stable coronary artery disease (CAD), we found that in younger patients (age ≤ 50 years), physical stress myocardial ischemia (PSMI) is positively and consistently associated with measures of CAD burden assessment, while mental stress myocardial ischemia (MSMI) is generally not associated with CAD burden. To the best of our knowledge, this is the first study where association between MSMI, PSMI and CAD burden is extensively studied, with due consideration to confounding and interaction assessment. Neither type of ischemia is associated with CAD burden among patients older than 50 years of age.

We assessed these associations through use of two types of outcome variables (software and physician assessment) and two types of composite measures of CAD burden (Gensini score and Duke CAD severity Index) to validate our results. We consistently found PSMI to be associated with CAD burden, while the association between MSMI and CAD burden was much weaker and generally non-significant except for the association between physician assessed MSMI and the Gensini score. Hence, we can reasonably conclude that PSMI is associated with CAD burden, while MSMI is not.

The physician-assessed MSMI may be more prone to bias than the software assessment. Thus, the operator-independent readings obtained with the ECTb software may potentially be more informative from a research perspective. Software assessed outcomes are measured with minimum human interference. In addition, software-based assessments may have lower random error and greater power to detect myocardial perfusion changes during stress, as software can catch even small changes in perfusion, which are not visible to the naked eye. Even with these

strengths, we do acknowledge the shortcomings of giving more weightage to software assessed outcome as it is an evolving field, and the process of software assessment of myocardial perfusion imaging (MPI) requires more validation studies and greater use in research settings.

A reason why we found significant associations between PSMI and CAD burden only in the younger population can be due to the fact that subjects who have MI at younger age are a more vulnerable population, with significantly higher burden of cardiovascular risk factors and CAD burden, as compared to older subjects(70). Even in our study, though insignificant, we found higher CAD burden in younger population (age ≤ 50 years) than older population (age > 50 years).

A finding in literature which supports our results of association between PSMI and CAD burden and more importantly, the non-association between MSMI and CAD burden, is the observation that in subjects with CAD, coronary resistance falls during mental stress in regions with significant stenosis(21, 51-53). In regions without significant stenosis, there is lower resistance at baseline as compared to regions with stenosis, but these regions show increased resistance during mental stress, indicating that mental stress has different, possibly opposite, effects on the vasomotor tone of coronary arteries as compared to exercise stress(21, 51-53).

Strengths and limitations:

Strengths of our study are as follows:

1) Use of validated techniques for assessment of MSMI and PSMI: Myocardial perfusion imaging with SPECT scan analysis (considered better than other procedures of MSMI detection as i) flow changes during mental stress may be mild, variable, and may be actually insufficient to generate functional signs of ischemia such as regional wall motion abnormalities on echocardiography(20, 21), ii) Worsening of LV systolic function (ejection fraction) can also be observed as a result of an increase in systemic vascular resistance (22) and it has been observed in healthy volunteers in

response to mental stress on some occasions too(23), iii) Use of electrocardiographic assessment underestimates the prevalence of MSMI, as compared to other criteria of assessment(12, 24), iv) MSMI diagnosis with SPECT scan has shown 75% reproducibility(25).) Use of public speaking task as mental stressor is another advantage, as it is considered more potent in inducing mental stress than mental arithmetic and Stroop test(12, 19). For physical stress, we used the established Bruce protocol(56) using walking on treadmill or pharmacological stress testing with Adenosine infusion.

2) Use of composite measure of CAD burden like Gensini score(54) and Duke CAD Severity Index(57), with proper weightage given to blockade in major arteries as compared to minor arteries(55), instead of broad criteria of CAD burden like number of vessels involved(44-50).

3) Robust regression analysis with due consideration given to assumptions of linear regression (assessment of normality of outcome variables), confounding and interaction assessment.

4) To the best of our knowledge, ours is the first study assessing MSMI prevalence in an MI population, where 50% of the subject population are females and 50% are 50 years old or younger.

Even with these strengths, our study is not without limitations. The most important limitation is its relatively small sample size. Also, residual confounding due to unmeasured/unknown confounders cannot be discounted. Another limitation of our study is its cross sectional design, which prevents us from conclusively proving or disproving a causal relationship between MSMI and CAD burden. Finally, our results are based on a young MI sample and may not be generalizable to a more broadly defined population of stable CAD patients.

Conclusion

In a sample of subjects with previous myocardial infarction and stable coronary artery disease (CAD), we found that physical stress myocardial ischemia (PSMI) is positively associated with measures of CAD burden assessment, while mental stress myocardial ischemia (MSMI) appears not to be associated with CAD burden. The association between PSMI and CAD was present in younger patients ≤ 50 years old, but not in the older patients. These findings are consistent with the hypothesis that PSMI and MSMI have different underlying pathophysiological mechanisms. For physical stress myocardial ischemia, existing CAD burden holds importance in predicting PSMI and in turn, future cardiac events. In contrast, mental stress induced myocardial ischemia is not dependent on coronary artery disease burden and there must be other pathophysiological mechanisms responsible for this phenomenon. Our results need to be replicated in larger samples. The interaction with age we found also needs to be replicated in larger samples and its underlying mechanisms need to be clarified.

THESIS CHAPTER III

Public Health Implications and Future Directions

Summary

This thesis analysis examines the association between mental stress induced myocardial ischemia (MSMI) and coronary artery disease (CAD) burden, and contrasts these results with the association between physical stress induced myocardial ischemia (PSMI) and CAD burden. We found that PSMI is positively associated with measures of CAD burden, while MSMI appears not to be associated with CAD burden. We found that this association was present in the younger population with age ≤ 50 years, while it was not present in the older population. This analysis supports the notion that MSMI is a distinct phenomenon from PSMI, whose relationship with CAD is well established.

Public Health Implications

Proving no association between MSMI and CAD burden, in contrast to PSMI, has important public health implication. It confirms the long standing knowledge of differences in pathophysiology between PSMI and MSMI in subjects with stable CAD. Assessing CAD burden is a common practice in clinical setting for a subject with myocardial infarction. These results point to the fact that, in contrast to ischemia due to physical exertion, severity of CAD cannot predict ischemia due to mental stress, for example future myocardial infarctions triggered by emotional factors. Thus, new methods need to be developed to predict and prevent MSMI. This result and extrapolation holds particular importance for female population, as it is generally observed that women with MI have smaller infarcts and less coronary narrowing as compared to men, but have more severe presentation and higher rates of complications and mortality which in many reports persist even after accounting for age(71).

Another reason why these findings are important, is that MSMI has been found to be often silent(12). It has been observed that subjects with MSMI have more incidence of ambulatory myocardial ischemia than subjects without it(41, 72) and subjects with MSMI have worse prognosis as compared to subjects without it(30). Thus, MSMI can easily go unrecognized, while placing patients at a higher risk. Based on these findings and our results, we can extrapolate that in the general population, standard clinical tests for CAD detection and risk stratification may not be helpful for the relatively large portion of patients suffering from MSMI, despite the fact that MSMI, as PSMI, carries an adverse prognosis.

Future directions

Future studies with larger sample size and follow up for cardiac events are needed to clarify the associations we observed and examine in more detail predictors of MSMI and its prognostic implications. We also need to study the pathophysiological causes of MSMI and its mechanism of action on the heart and vessels. Finally, novel methods of assessment of MSMI need to be developed, for example noninvasive, low cost techniques that can be easily implemented in regular cardiology clinical practice.

TABLES, FIGURES AND DERIVATION

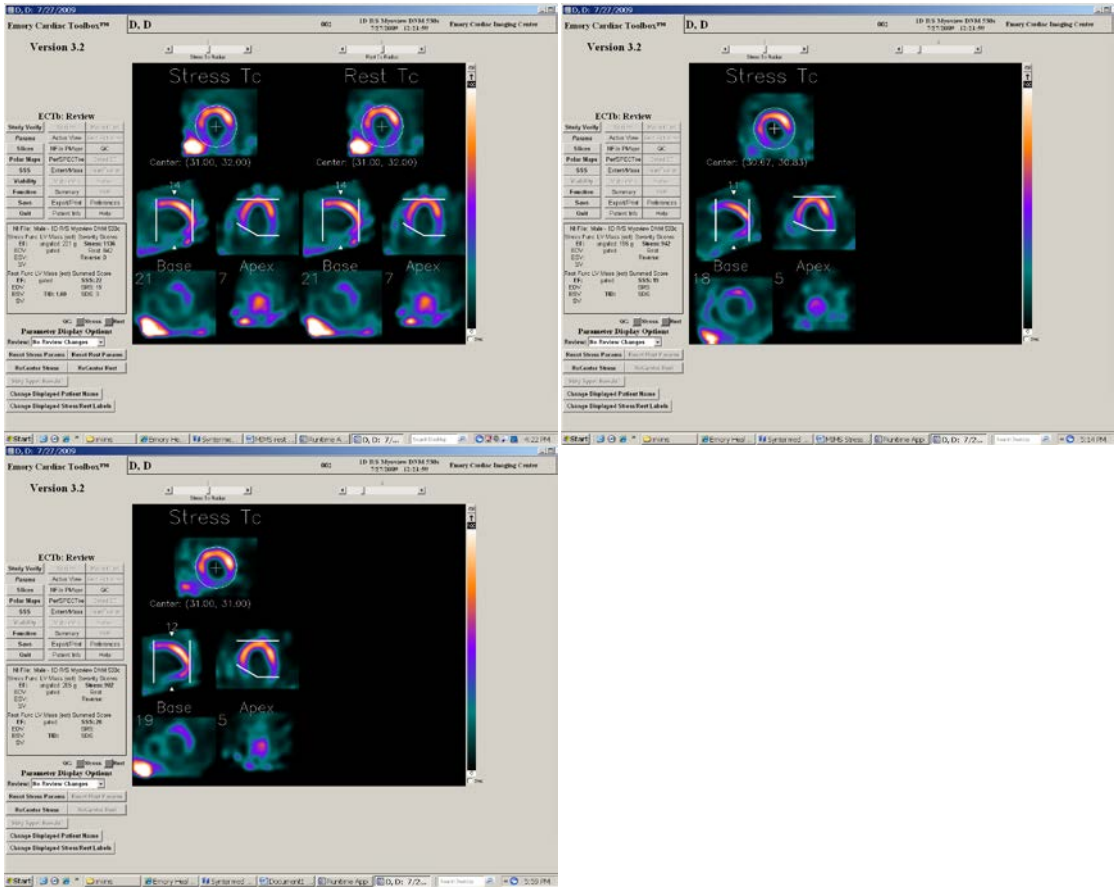

Figure 1: Analysis of SPECT images of a subject using the Emory Cardiac SPECT Toolbox software. The image on the top left shows the result of analyses of two rest images (one renamed as stress for normalization purposes). The image on the top right shows the result of analysis of stress A and image on the bottom left shows result of analysis of stress B (the technician was blinded to the type of stress).

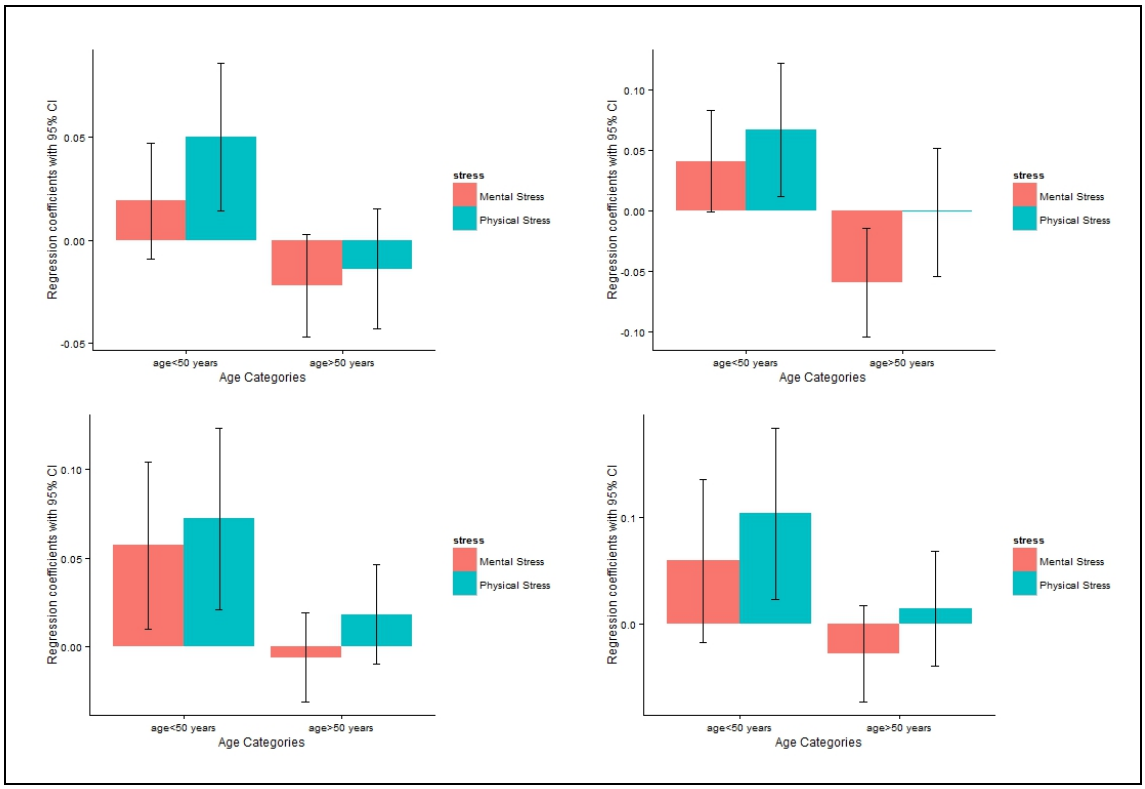

Figure 2: Regression coefficients with 95% CI for association of MSSS and PSSS with CAD burden, stratified by age (age≤ 50 years and age > 50 years): 1) Top left: Software assessed outcomes Vs. Gensini score, 2) Top right: Software assessed outcomes Vs. DCSI, 3) Bottom left: Physician assessed outcomes Vs. Gensini score, 4) Bottom right: Physician assessed outcome Vs. DCSI.

Derivation proof (See Method section, statistical analysis for reference)

Variable and regression coefficients explanation:

Y_1 = Mental Summed Stress Score (MSSS)

Y_2 = Mental Summed Difference Score (MSDS)

X_1 = Primary exposure variable (Gensini score/Duke CAD Severity Score)

X_2 = Summed Rest Score (SRS)

β_0 and β_0' = Intercept for model 1 and 2 respectively

β_1 and β_1' = Regression coefficient of X_1 for model 1 and 2 respectively

β_2 and β_2' = Regression coefficient of X_2 for model 1 and 2 respectively

To prove:

For model 1 ($\mathbf{Y_1 = \beta_0 + \beta_1 * X_1 + \beta_2 * X_2}$) and model 2 ($\mathbf{Y_2 = \beta_0' + \beta_1' * X_1 + \beta_2' * X_2}$), $\mathbf{\beta_1 = \beta_1'}$; that is, irrespective of outcome variable (MSSS or MSDS), the regression coefficient for primary exposure remains the same.

Proof:

Let's start with model 1:

$Y_1 = \beta_0 + \beta_1 * X_1 + \beta_2 * X_2$

Subtracting X_2 from both sides, we get:

$(Y_1 - X_2) = \beta_0 + \beta_1 * X_1 + (\beta_2 - 1) * X_2$

But, as given in methods section (Relationship between different outcome variables),

$(Y_1 - X_2) = Y_2$

Therefore, $Y_2 = \beta_0 + \beta_1 * X_1 + (\beta_2 - 1) * X_2$ (Model 3).

Now, comparing model 3 with model 2, we can see that

$\boldsymbol{\beta_1 = \beta_1'}$

Conclusion: Hence, we have proved that irrespective of outcome variable (MSSS or MSDS), the regression coefficient for primary exposure remains the same. Similar derivation will apply to model relationship between Physical Summed Stress Score (PSSS) and Physical Summed Difference Score (PSDS). Also, this derivation will not change even if we add other variables to our model for confounding and interaction assessment.

Table 1: Demographic and clinical characteristics of the subjects, with corresponding **software assessed** Mental Summed Stress Score (MSSS) and Physical Summed Stress Score (PSSS)

Characteristics	Frequency (n=98)[#]	MSSS*	PSSS*
Demographics			
Age			
Age less than 50 years	49 (50%)	9.85 (5.82)	10.20 (6.61)
Age more than 50 years	49 (50%)	9.76 (6.37)	10.34 (5.80)
Gender			
Male	49 (50%)	10.63 (6.21)	11.59 (6.41)
Female	49 (50%)	9.00 (5.87)	8.98 (5.72)
Race			
White	39 (40%)	8.76 (5.84)	9.05 (4.62)
Black	54 (55%)	9.96 (5.93)	10.34 (6.58)
Other races	05 (05%)	16.00 (6.40)	18.80 (6.78)
Income status[+]			
Less than $35,000	48 (50%)	10.00 (5.18)[%]	9.93 (5.78)[%]
$35,000 - $75,000	29 (30%)	11.38 (8.16)[%]	12.37 (7.69)[%]
More than $75,000	19 (20%)	7.21 (3.98)[%]	7.94 (3.64)[%]
Smoking status			
Non smokers	37 (38%)	9.79 (6.15)	10.11 (6.38)
Past smokers	33 (34%)	10.03 (6.42)	10.39 (6.12)
Current smokers	28 (28%)	9.54 (5.71)	10.32 (6.23)
Medical history			
Hypertension[$]			
Absent	30 (31%)	9.32 (6.63)	10.14 (6.79)
Present	67 (69%)	10.02 (5.85)	10.32 (5.95)
Hyperlipidemia[$]			
Absent	26 (27%)	8.60 (4.62)	10.00 (4.39)
Present	71 (73%)	10.25 (6.49)	10.37 (6.75)
Diabetes[$]			
Absent	77 (79%)	9.49 (5.53)	9.88 (5.77)
Present	20 (21%)	11.05 (7.87)	11.89 (7.64)
Obese (BMI ≥ 30 kg/m^2)[$]			
Absent	53 (54%)	9.38 (5.76)	9.86 (5.99)
Present	45 (46%)	10.30 (6.42)	10.74 (6.43)
Lifetime h/o depression[+]			
Absent	60 (62%)	9.50 (6.15)	10.06 (5.91)
Present	36 (38%)	10.38 (6.06)	10.68 (6.77)
Beta blocker[$]			
Absent	12 (12%)	10.45 (7.87)	10.91 (6.92)
Present	85 (88%)	9.72 (5.84)	10.17 (6.10)

* : Data given as mean (Standard deviation); [#] : data given as frequency (percentage); [%] : P value significant for bivariate comparison; [$]: 1 observation missing; [+]: 2 observations missing

Table 2: Demographic and clinical characteristics of the subjects, with corresponding **physician assessed** Mental Summed Stress Score (MSSS) and Physical Summed Stress Score (PSSS)

Characteristics	Frequency (n=98)[#]	MSSS*	PSSS*
Demographics			
Age			
Age less than 50 years	49 (50%)	4.45 (5.80)	5.09 (6.16)
Age more than 50 years	49 (50%)	5.71 (6.68)	6.62 (7.01)
Gender			
Male	49 (50%)	5.21 (6.34)	6.19 (6.85)
Female	49 (50%)	4.96 (6.24)	5.52 (6.41)
Race			
White	39 (40%)	4.45 (5.62)	4.97 (5.57)
Black	54 (55%)	5.21 (6.40)	6.00 (7.04)
Other races	05 (05%)	8.60 (9.32)	11.0 (8.12)
Income status[+]			
Less than $35,000	48 (50%)	5.15 (5.95)[%]	5.78 (6.07)[%]
$35,000 - $75,000	29 (30%)	7.00 (7.77)[%]	8.26 (8.30)[%]
More than $75,000	19 (20%)	2.26 (2.98)[%]	2.95 (3.49)[%]
Smoking status			
Non smokers	37 (38%)	4.77 (6.02)	5.29 (6.49)
Past smokers	33 (34%)	4.39 (6.41)	5.67 (6.70)
Current smokers	28 (28%)	6.33 (6.43)	6.85 (6.80)
Medical history			
Hypertension[$]			
Absent	30 (31%)	5.59 (7.17)	5.62 (6.33)
Present	67 (69%)	4.86 (5.86)	5.97 (6.78)
Hyperlipidemia[$]			
Absent	26 (27%)	4.84 (5.15)	5.36 (5.05)
Present	71 (73%)	5.17 (6.64)	6.04 (7.12)
Diabetes[$]			
Absent	77 (79%)	5.04 (6.14)	5.93 (6.88)
Present	20 (21%)	5.25 (6.85)	5.58 (5.60)
Obese (BMI ≥ 30 kg/m^2)[$]			
Absent	53 (54%)	5.65 (6.59)	6.25 (6.59)
Present	45 (46%)	4.43 (5.86)	5.38 (6.68)
Lifetime h/o depression[+]			
Absent	60 (62%)	5.32 (6.53)	6.20 (6.43)
Present	36 (38%)	4.83 (5.89)	5.36 (7.05)
Beta blocker[$]			
Absent	12 (12%)	6.58 (8.73)	7.91 (9.18)
Present	85 (88%)	4.87 (5.86)	5.58 (6.21)

* : Data given as mean (Standard deviation); [#] : data given as frequency (percentage); [%] : P value significant for bivariate comparison; [$] : 1 observation missing; [+] : 2 observations missing

Table 3.1: Unadjusted and adjusted regression coefficients for the analysis of association between **software assessed** Summed Stress Scores [Mental (MSSS) and Physical (PSSS)] with Gensini score

Outcome	Unadjusted regression coefficient	Unadjusted P value	Adjusted regression coefficient*	Adjusted P value
MSSS	-0.001(-0.019 to 0.017)	0.91	-0.002(-0.021 to 0.017)	0.83
PSSS	0.008(-0.014 to 0.029)	0.48	0.009(-0.013 to 0.032)	0.41

*: Adjustment done for age, gender, race, smoking status, hypertension, hyperlipidemia, diabetes mellitus, beta blocker medication and depression status.

Table 3.2: Analysis of association between **software assessed** Summed Stress Scores [Mental (MSSS) and Physical (PSSS)] with Gensini score, stratified by age ≤ 50 years and age > 50 years.

Outcome	Unadjusted regression coefficient	Unadjusted P value	Adjusted regression coefficient*	Adjusted P value	P value for interaction
Age less than or equal to 50 years					
MSSS	0.015(-0.012 to 0.041)	0.28	0.019(-0.009 to 0.048)	0.18	0.41
PSSS	0.019(-0.014 to 0.053)	0.26	0.050(0.014 to 0.086)	0.006	0.06
Age more than 50 years					
MSSS	-0.009(-0.034 to 0.016)	0.46	-0.022(-0.047 to 0.004)	0.10	0.41
PSSS	-0.003(-0.031 to 0.025)	0.83	-0.014(-0.043 to 0.015)	0.33	0.06

*: Adjustment done for age, gender, race, smoking status, hypertension, hyperlipidemia, diabetes mellitus, beta blocker medication and depression status.

Table 4.1: Unadjusted and adjusted regression coefficients for the analysis of association between **software assessed** Summed Stress Scores [Mental (MSSS) and Physical (PSSS)] with Duke CAD Severity Index

Outcome	Unadjusted regression coefficient	Unadjusted P value	Adjusted regression coefficient*	Adjusted P value
MSSS	-0.002(-0.033 to 0.029)	0.88	-0.007(-0.039 to 0.026)	0.69
PSSS	0.024(-0.013 to 0.061)	0.20	0.029(-0.009 to 0.067)	0.13

*: Adjustment done for age, gender, race, smoking status, hypertension, hyperlipidemia, diabetes mellitus, beta blocker medication and depression status.

Table 4.2: Analysis of association between **software assessed** Summed Stress Scores [Mental (MSSS) and Physical (PSSS)] with Duke CAD Severity Index, stratified by age ≤ 50 years and age > 50 years.

Outcome	Unadjusted regression coefficient	Unadjusted P value	Adjusted regression coefficient*	Adjusted P value	P value for interaction
Age less than or equal to 50 years					
MSSS	0.040(-0.002 to 0.081)	0.07	0.041(-0.001 to 0.083)	0.06	0.05
PSSS	0.040(-0.015 to 0.095)	0.16	0.067(0.011 to 0.122)	0.02	0.17
Age more than 50 years					
MSSS	-0.032(-0.076 to 0.013)	0.16	-0.059(-0.104 to -0.015)	0.009	0.05
PSSS	-0.006(-0.045 to 0.056)	0.83	-0.001(-0.053 to 0.053)	0.99	0.17

*: Adjustment done for age, gender, race, smoking status, hypertension, hyperlipidemia, diabetes mellitus, beta blocker medication and depression status.

Table 5.1: Unadjusted and adjusted regression coefficients for the analysis of association between **physician assessed** Summed Stress Scores [Mental (MSSS) and Physical (PSSS)] with Gensini score

Outcome	Unadjusted regression coefficient	Unadjusted P value	Adjusted regression coefficient*	Adjusted P value
MSSS	0.015 (-0.009 to 0.040)	0.23	0.010 (-0.015 to 0.035)	0.43
PSSS	0.025 (-0.003 to 0.053)	0.08	0.023 (-0.005 to 0.050)	0.11

*: Adjustment done for age, gender, race, smoking status, hypertension, hyperlipidemia, diabetes mellitus, beta blocker medication and depression status.

Table 5.2: Analysis of association between **physician assessed** Summed Stress Scores [Mental (MSSS) and Physical (PSSS)] with Gensini score, stratified by age ≤ 50 years and age > 50 years.

Outcome	Unadjusted regression coefficient	Unadjusted P value	Adjusted regression coefficient*	Adjusted P value	P value for interaction
Age less than or equal to 50 years					
MSSS	0.057 (0.007 to 0.107)	0.03	0.057(0.010 to 0.105)	0.02	
PSSS	0.073 (0.019 to 0.127)	0.008	0.072(0.021 to 0.123)	0.005	
					<0.05
Age more than 50 years					
MSSS	-0.006 (-0.030 to 0.018)	0.61	-0.006(-0.030 to 0.019)	0.63	
PSSS	0.004 (-0.026 to 0.033)	0.81	0.018(-0.010 to 0.046)	0.21	

*: Adjustment done for age, gender, race, smoking status, hypertension, hyperlipidemia, diabetes mellitus, beta blocker medication and depression status.

Table 6.1: Unadjusted and adjusted regression coefficients for the analysis of association between **physician assessed** Summed Stress Scores [Mental (MSSS) and Physical (PSSS)] with Duke CAD Severity Index

Outcome	Unadjusted regression coefficient	Unadjusted P value	Adjusted regression coefficient*	Adjusted P value
MSSS	0.012 (-0.033 to 0.057)	0.59	0.010 (-0.035 to 0.054)	0.67
PSSS	0.036 (-0.015 to 0.087)	0.16	0.039 (-0.011 to 0.088)	0.12

*: Adjustment done for age, gender, race, smoking status, hypertension, hyperlipidemia, diabetes mellitus, beta blocker medication and depression status.

Table 6.2: Analysis of association between **physician assessed** Summed Stress Scores [Mental (MSSS) and Physical (PSSS)] with Duke CAD Severity Index, stratified by age ≤ 50 years and age > 50 years.

Outcome	Unadjusted regression coefficient	Unadjusted P value	Adjusted regression coefficient*	Adjusted P value	P value for interaction
Age less than or equal to 50 years					
MSSS	0.063 (-0.020 to 0.146)	0.13	0.059(-0.017 to 0.135)	0.13	
PSSS	0.116 (0.030 to 0.203)	0.009	0.103(0.024 to 0.183)	0.01	
					<0.05
Age more than 50 years					
MSSS	-0.029 (-0.073 to 0.015)	0.20	-0.028(-0.073 to 0.017)	0.22	
PSSS	-0.022 (-0.077 to 0.033)	0.43	0.014(-0.039 to 0.068)	0.60	

*: Adjustment done for age, gender, race, smoking status, hypertension, hyperlipidemia, diabetes mellitus, beta blocker medication and depression status.

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EMORY UNIVERSITY | Institutional Review Board

TO: Laura Vaccarino, MD
Principal Investigator

DATE: May 7, 2012

RE: **Continuing Review Expedited Approval**
CR4_IRB00009248

IRB00009248
Sex Differences in Myocardial Ischemia Triggered by Emotional Factors after MI

Dear Dr. Vaccarino,

Thank you for submitting a renewal application for this protocol. The Emory IRB reviewed it by the expedited process on 04/30/2012, per 45 CFR 46.110, the Federal Register expeditable category F[8c], and/or 21 CFR 56.110. This re-approval is effective from **04/30/2012** through **04/29/2013**. Thereafter, continuation of human subjects research activities requires the submission of another renewal application, which must be reviewed and approved by the IRB prior to the expiration date noted above.

Any reportable events (e.g., unanticipated problems involving risk to subjects or others, noncompliance, breaches of confidentiality, HIPAA violations, protocol deviations) must be reported to the IRB according to our Policies & Procedures at www.irb.emory.edu, immediately, promptly, or periodically. Be sure to check the reporting guidance and contact us if you have questions. Terms and conditions of sponsors, if any, also apply to reporting.

Before implementing any change to this protocol (including but not limited to sample size, informed consent, study design, you must submit an amendment request and secure IRB approval.

In future correspondence about this matter, please refer to the IRB file ID, name of the Principal Investigator, and study title. Thank you.

Sincerely,

Steven J. Anzalone, M.S.
IRB Analyst Assistant
This letter has been digitally signed

CC: Ibeanu Ijeoma Epidemiology

Parrott	Janice	MedCardio
Shallenberger	Lucy	MedCardio
Bremner	James	Psychiatry - Main
Esteves	Fabio	Radiology - Main
Garcia	Ernest	Radiology - Main
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Veledar	Emir	MedCardio

Emory University
1599 Clifton Road, 5th Floor - Atlanta, Georgia 30322
Tel: 404.712.0720 - Fax: 404.727.1358 - Email: irb@emory.edu - Web: http://www.irb.emory.edu/

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Study Identification Information

1.0 * Enter the Full title of the study (include any version dates from the sponsor)

Sex Differences in Myocardial Ischemia Triggered by Emotional Factors after MI

2.0 * Enter a SHORT identifying title for tracking purposes:

MIMS

3.0 What is the estimated start date of this study:

01-Apr-09

4.0 What is the estimated completion date of this study:

31-Mar-11

5.0 * Name of Principal Investigator. Limit is one person; Emory affiliation is required. If name does not appear in menu, the person probably does not yet have an eIRB account. For more information about obtaining an eIRB account, click here.

Laura Vaccarino **Dept**:MedCardio

6.0 Names of Emory Co-Investigators. May include Emory personnel and non-Emory persons with sponsored eIRB accounts. If name does not appear in menu, the person probably does not yet have an eIRB account. For more information about obtaining an eIRB account, click here.

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Garcia	Ernest	Radiology - Main
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Quyyumi	Arshed	MedCardio
Raggi	Paolo	MedCardio
Veledar	Emir	MedCardio

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Last	First	Dept
Ibeanu	Ijeoma	Epidemiology
Parrott	Janice	MedCardio

Shallenberger	Lucy	MedCardio

8.0 **Names of other Emory Study Staff not listed above. If name does appear in menu, the person probably does not yet have an eIRB account. For more information about obtaining an eIRB account, click here.**

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View	Uphoff	Irina	MedCardio	Other
View	Vasudevan	Sowmya	MedCardio	Other
View	Wenger	Nanette	MedCardio	Other

9.0 **Enter information on Non-Emory Study Staff:** (this is for non-Emory personnel who will not be logging into eIRB)

	Name	Affiliation	Type
View	Amit Shah	Emory University - new fellow beginning July 1, 2009	Research Fellow
View	Patricia Engel	Emory RSPH student	Other study personnel type
View	Pratik Manohar Pimple	RSPH student	Other study personnel type