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INTEGRATED DUAL EXERCISE AND LEXISCAN PET: IDEAL PET

Version Date: September 12, 2016

Study Type: Single Center Study

Sponsor: Investigator initiated study, PI, Sharmila Dorbala

Study Enrollment Period: June 2010 to June 2017

Principal Investigator: Sharmila Dorbala, MD

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ABBREVIATIONS USED:

Myocardial Blood Flow: MBF

Positron Emission Tomography: PET

Computed Tomography: CT

Myocardial perfusion imaging: MPI

Coronary Flow reserve: CFR

Coronary artery disease: CAD

Lexiscan + Exercise: Lexercise

SYNOPSIS

Title of Study: Integrated Dual Exercise And Lexiscan PET: IDEAL PET

Planned Study Period: From 4Q2010 - 2Q2017

Study Objective(s)

Aim 1: To *assess the tolerability and safety* of combined symptom limited exercise stress test with Lexiscan (Lexercise PET) compared to Lexiscan alone (Lexiscan PET).

Aim 2: To *assess the image quality* of Lexercise compared to Lexiscan PET.

Aim 3: To compare *relative and absolute myocardial perfusion imaging* with Lexercise compared to Lexiscan PET to identify CAD.

Planned Total Number of Study Centers and Location

Single study center, Brigham and Women's Hospital, Boston, MA, United States

Design and Methodology

This is a single-center, prospective cohort study of subjects undergoing clinically indicated PET/CT MPI, with documented coronary artery disease or an intermediate to high pretest likelihood of coronary artery disease. Following the clinical study, the research study is explained to the subjects. Consenting subjects will return on another day (next calendar day or later) for a repeat stress study with Lexercise or a repeat Lexiscan stress PET. Approximately 50 subjects (not more than 16 patients with normal Rubidium-82 or N-13 ammonia PETMPI, 10 healthy volunteers and 24 patients with reversible perfusion defects) will be enrolled in this study. After successful screening and enrollment, 13 random subjects (8 normal and 5 ischemic) will return for repeat Lexiscan PET, and 37 subjects will return for a Lexercise PET/CT MPI. Lexiscan PET will be performed per standard protocol. For Lexercise, a symptom limited exercise treadmill study with Lexiscan injection at peak exercise is performed. Rubidium-82 or N-13 ammonia will be injected and PET/CT images will be obtained in a list mode acquisition. Images will then be unlisted and myocardial blood flow computed.

Number of Subjects Planned

Approximately 50 subjects will be enrolled into the study.

Main Selection Criteria

Subjects will be eligible if they meet all of the following inclusion criteria and none of the exclusion criteria:

Inclusion criteria:

- Age > 18 years
- Clinically indicated Rubidium-82 or N-13 ammonia PET study
- Ten healthy volunteers recruited using web based advertisements.
- Known coronary artery disease (prior percutaneous coronary intervention, prior coronary artery bypass surgery or Q wave MI on ECG) or intermediate to high pretest likelihood of CAD
- Able to exercise on a treadmill
- Able and willing to provide informed consent to participate in the study

Exclusion criteria:

- Contraindications to exercise stress testing such as
  - unstable angina
  - known severe left main coronary artery stenosis
  - severe or uncontrolled heart failure
  - uncontrolled atrial or ventricular arrhythmias
  - symptomatic hypotension or severe hypertension (systolic blood pressure < 90 or > 200 mmHg, respectively)
  - > 1st degree atrioventricular block in the absence of a functioning pacemaker.
- Contraindications to Lexiscan use such
  - Allergy or intolerance to aminophylline or regadenoson
  - Known severe or oxygen dependent bronchoconstrictive or bronchospastic lung disease [e.g., asthma, wheezing, chronic obstructive pulmonary disease (COPD), etc.].
  - Sick sinus syndrome
  - History of heart transplantation.
  - Dialysis
  - Subject requires emergent cardiac medical intervention or catheterization after the clinical study.
  - Documented myocardial infarction (MI) ≤ 30 days prior to enrollment.
  - History of percutaneous coronary intervention (PCI) ≤ 4weeks prior to enrollment.

- History of coronary artery bypass graft (CABG) ≤ 8 weeks prior to enrollment.
- Severe LV dysfunction, with ejection fraction of < 30%
- Serious non-cardiac medical illness (e.g., disseminated malignancy, severe neurological dysfunction at time of baseline PET study) or a social situation which will preclude research study participation
- History of seizure disorder

Discontinuation criteria:

Subjects should be discontinued for the following discontinuation criteria

- Subject does not fulfill inclusion or exclusion criteria
- Subject experiences a serious or intolerable adverse event
- In the Investigator's opinion, the subject is non-compliant with the protocol requirements (protocol violation)
- Subject's health would be jeopardized by continued participation
- Subject wishes to withdraw consent

Lexiscan will not be administered to patients with clinical or EKG evidence on ischemia during exercise treadmill testing. Clinical or EKG abnormalities during treadmill testing that would preclude administration of Lexiscan are listed below:

a) Clinical
  a. Severe chest pain
  b. Severe lightheadedness or dizziness,
  c. Severe shortness of breath
  d. Exercise induced hypotension (> 10mm Hg decrease in systolic blood pressure)
  e. Exercise induced hypertension (systolic blood pressure >210 mm Hg)
b) ECG changes
  a. Significant ST segment depression (≥2 mm)
  b. Significant ST segment elevation (≥1 mm)
  c. Sustained ventricular or atrial tachycardia,
  d. Exercise induced heart block

Statistical Plan:

The overall aim of this pilot study of 50 patients is to assess the tolerability, safety, diagnostic accuracy and image quality between Lexiscan with symptom limited exercise compared to Lexiscan alone.

Aim 1. For this aim, we will compare the reported side effect profile for Lexercise to Lexiscan PET, using a Pearson Chi square test. A P-value of<0.05 will be considered significant.

Aim 2: For this aim, we will compare objective measures of image quality including signal to noise ratio using dynamic Rubidium-82 or N-13 ammonia images, the frequency of misregistration between PET and CT, the frequency of presumed motion artifact and bowel radiotracer activity. These measures will be compared using a student's t-test or a Pearson Chi square test, as appropriate. A P-value of<0.05 will be considered significant.

Aim 3: For this aim, we seek to compare measures of semi quantitative relative perfusion, absolute perfusion and LVEF using the Lexiscan alone compared to Lexercise PET. Firstly, we will compare semi-quantitative defect size, severity and reversibility between the Lexercise and Lexiscan PET studies. Based on the results of one prior study using N-13 ammonia and comparing dipyridamole versus treadmill exercise[2], a sample size of 24 subjects with reversible defects should be adequate to identify if significant differences exist between Lexercise and Lexiscan PET protocols. Next, we will compare the differences in regional rest and peak stress myocardial blood flow and coronary flow reserve in the defect territory compared to normal territory between the Lexercise and Lexiscan protocols. Finally, we will study the changes in LVEF from rest to peak stress in Lexercise versus Lexiscan PET studies in the normal and ischemic subjects.

I. BACKGROUND:

Myocardial perfusion imaging (MPI) with positron emission tomography (PET) is more accurate than single photon emission computed tomography (SPECT)[3], enables non-invasive quantitation of absolute myocardial blood flow (MBF), and is particularly helpful in the obese individuals and those that are unable to exercise.[3] Presently PETMPI is indicated only for subjects that are unable to exercise[4],

primarily because exercise stress is not easy with PET. However, due to superior image characteristics, faster pace, and radiation dosimetry (compared to Thallium-201), exercise PET with Rubidium-82 is being viewed as an excellent alternative to exercise SPECT. This has become more important lately with the recent shortage of Technetium labeled radiotracers. Although exercise PET is feasible with Rubidium-82,[2] the workflow is challenging due to its short-half-life (75 seconds). Exercise PET with N-13 ammonia has been studied, but this is only possible in sites with an onsite cyclotron and the production and delivery of N-13 ammonia have to be coordinated with the PET scanner due to its 10 minute half-life.[2] Finally, in high volume nuclear cardiology laboratories, routine clinical use of exercise PET poses the concern of high radiation exposure to staff. [5] This is primarily related to the fact that, after the PET radiotracer is injected on the treadmill, the staff is directly and closely exposed to the radioactive patient (while walking and positioning the subject to the scanner immediately after radiotracer injection).

Effects of exercise and vasodilator stress on myocardial blood flow:

Exercise is the most important physiological stimulus that increases myocardial oxygen demand. Exercise results in vasodilation of the normal epicardial coronary arteries (Figure, Top panel).[6] The resultant increase in coronary blood flow and shear stress lead to endothelial release of nitric oxide and several other vasoactive mediators that cause further vasodilation of the downstream microvasculature and increased MBF. In contrast, diseased epicardial coronary arteries vasoconstrict in response to exercise resulting (Figure, bottom panel) in an increased flow gradient with reduced blood flow to the downstream microvasculature during exercise as compared to normal territories.
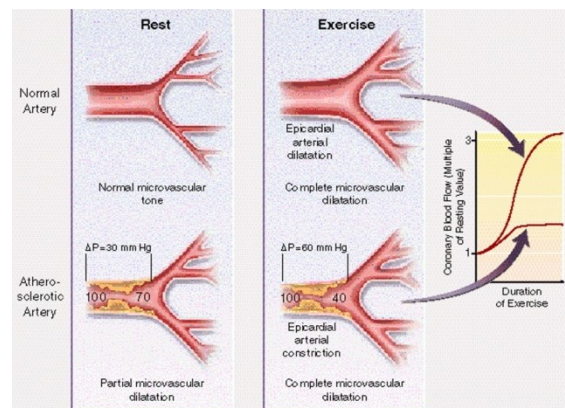
Wilson R. N Engl J Med 1996;334:1735-1737

Vasodilator stress agents do not typically result in vasoconstriction of diseased coronary arteries. They produce differential vasodilation of both normal and diseased coronary arteries by predominantly by an endothelium independent mechanism resulting in estimation of primarily endothelium independent changes in MBF, and to a lesser extent endothelium independent changes in MBF.[7]

Combined standard symptom limited exercise stress testing with injection of Lexiscan at peak exercise (Lexercise PET) may be a novel protocol to optimally image maximal endothelium dependent (exercise) and endothelium independent (Lexiscan) alterations in MBF, using relative and absolute myocardial perfusion PET. Symptom limited exercise produces endothelial activation and vasodilation of normal and vasoconstriction of diseased coronary arteries. Addition of vasodilator stress at maximal exercise results in further (maximal) vasodilation of the normal coronary arteries; but minimal or no additional

vasodilation of the vasoconstricted diseased coronary arteries. This may result in improved defect contrast compared to the normal coronary arteries and better detection of CAD. Indeed a prior study demonstrated that defect size is larger with treadmill exercise stress compared to vasodilator stress with dipyridamole.[8] Also, the precise reasons for larger perfusion defects with exercise compared to dipyridamole PET were not studied; although, true ischemia from exercise was proposed as a possible mechanistic link for larger perfusion defects with exercise. If endothelial dysfunction and true vasoconstriction are imaged with exercise PET, then, we can expect to metabolic abnormalities (switch from fatty acid to glucose metabolism) in the corresponding myocardial segments that may be imaged using tracers of fatty acid metabolism such as β Methyl-*p*-[123I]-iodophenyl-pentadecanoic acid (BMIPP) or C-11 Palmitate. Lastly, quantitative MBF is estimated predominantly with vasodilator stress and occasionally with bicycle exercise stress.[9] Quantitative PET with Lexercise PET may be a novel method to image combined alterations in MBF related to both endothelium dependent and independent mechanisms of MBF.

II. SPECIFIC AIMS:

The overall purpose of the proposed research study is to examine in detail, using relative and quantitative Rubidium-82 or N-13 ammonia PET, the feasibility, safety, utility and the mechanistic basis of combined symptom limited exercise treadmill test with vasodilator stress compared to vasodilator stress alone in the evaluation of known or suspected coronary artery disease. The following specific aims are proposed:

Aim 1: To *assess the tolerability and safety* of the combined symptom limited exercise and Lexercise protocol (Lexercise PET) compared to Lexiscan alone (Lexiscan PET).
Hypothesis 1: Lexiscan will cause further/maximal vasodilation in a subject that is already vasodilated from exercise; therefore, the safety of this protocol needs to be ascertained. Lexiscan is expected to be well tolerated when injected during maximal exercise stress without any increase in major adverse events.

Aim 2: To **assess the image quality** of Lexercise PET compared to Lexiscan.

Hypothesis 2: Image quality as assessed by myocardial count density and heart to liver ratio will be superior with Lexercise compared to Lexiscan. However, misregistration artifacts from upward diaphragmatic creep will be higher with Lexercise compared to Lexiscan.

Aim 3: To compare **relative and absolute PET** with Lexercise compared to Lexiscan PET in identifying CAD.

Hypothesis 3: Lexercise PET offers the advantage of imaging reduced myocardial blood flow from vasoconstriction in diseased territories and maximal vasodilation in normal territories. Therefore, in patients with ischemic scans, the defect contrast on relative will be better with Lexercise compared to Lexiscan PET. We hypothesize that the differences in regional CFR between normal and abnormal territories will be greater with Lexercise compared to Lexiscan.

Significance of the mechanistic questions of this proposal:

The overall goal of this proposal is to evaluate the feasibility, utility and mechanistic underpinnings of a novel protocol incorporating combined maximal symptom limited exercise treadmill test with Lexiscan compared to Lexiscan alone. The combined standard symptom limited exercise stress testing with Lexiscan has several novel clinical and research applications:

1. This protocol provides exercise data (hemodynamic, electrocardiographic and clinical data) combined with high quality PET imaging that is important in patient management.
2. A PET MPI study can be performed with physiological exercise stress using any of the available flow tracers (Rubidium-82, N-13 ammonia, or the new compound BMS747158, an F-18 labeled agent) with minimal radiation exposure to the staff.
3. This protocol makes it feasible to assess both endothelium dependent (exercise) and endothelium independent (Lexiscan) mechanisms affecting MBF.
4. This combined assessment may prove to have a superior diagnostic accuracy compared to Lexiscan alone in diagnosing milder degrees of stenoses and balanced ischemia due to ECG changes, symptoms, hemodynamic changes, and improved defect contrast on relative and absolute PET.[8]
5. Also, using Rubidium-82 or N-13 ammonia and exercise PET, peak stress left ventricular ejection fraction can be computed.
6. Finally, the results of this study could be extrapolated to stress imaging with cardiac magnetic resonance imaging, making exercise CMR feasible.

b) Previous studies leading up to or supporting the proposed research

A major strength of this project is that it represents an outgrowth of techniques pioneered at BWH. Within our existing Division of Nuclear Medicine/PET and Cardiovascular Imaging, this project is a natural extension of our work to date. We have extensive clinical and research experience with Rubidium-82 and N-13 ammonia PET, and have performed over 6000 clinical studies since the initiation of our PET program in the fall of 2003. We have been using Lexiscan with Rubidium-82 PET since January 2009 and with N-13 ammonia PET since July 2011. Also, quantitative myocardial blood flow estimates have been routinely performed using a home grown software program (soon to be commercially available).

Rationale for specific aim 1:

Study of both endothelium dependent and independent mechanisms of myocardial blood flow is clinically relevant and important.[9] [10] Sub maximal / low-level exercise with vasodilator stress has been studied and used clinically to improve image quality and patient tolerance of side effects.[11] The combined protocol of Lexercise proposes to use a maximal symptom limited exercise stress combined with maximal coronary vasodilation with Lexiscan. The safety of this "dual full stress" protocol needs to be established before widespread clinical application. At the BWH, we have been using combined symptom limited exercise with Lexiscan and SPECT imaging since January 2009 in approximately 5 cases per week. The results have not been formally analyzed yet, but, in general the protocol is well tolerated without any major side-effects. There has been one instance of a side-effect in a subject who exercised ~9 minutes and had sub-maximal heart rate response, received Lexiscan and vomited during recovery. This was associated with high grade heart block that resolved spontaneously. But, this combined symptom limited exercise and Lexiscan protocol has not been used in conjunction with PET imaging.

Rationale for specific aim 2:

Due to increased blood flow to the extremities and reduced splanchnic flow during maximal exercise treadmill testing, the quality of myocardial perfusion images as assessed by a heart to liver ratio, and bowel uptake will be superior with Lexercise compared to

Table 2. Dipyridamole and Exercise Treadmill Stress Image Data

	Dipyridamole	Exercise	p Value
Rest ^{82}Rb dose (MBq)	786 ± 206	770 ± 171	NS
Stress ^{82}Rb dose (MBq)	789 ± 185	757 ± 188	NS
SRS	2.2 ± 3.7	2.1 ± 4.1	NS
SSS	7.2 ± 8.3	6.6 ± 7.7	NS
SDS	5.0 ± 6.0	4.5 ± 5.3	NS
Rest defect (% LV)	4.6 ± 7.6	4.3 ± 7.9	NS
Stress defect (% LV)	15.2 ± 16.2	14.4 ± 16.0	NS
Rest myocardial uptake (Bq/cc)	11,293 ± 3,668	12,192 ± 4,597	NS
Stress myocardial uptake (Bq/cc)	14,476 ± 4,029	22,405 ± 10,747	<0.001
Myocardium/lung rest	10.9 ± 4.8	12.4 ± 14.5	NS
Myocardium/lung stress	12.3 ± 4.4	14.8 ± 9.3	NS
Myocardium/gut rest	1.2 ± 0.7	1.1 ± 0.4	NS
Myocardium/gut stress	1.8 ± 1.5	5.1 ± 4.0	<0.0001
Myocardium/liver rest	3.8 ± 3.6	2.8 ± 0.9	NS
Myocardium/liver stress	3.4 ± 1.4	26.9 ± 66.8	0.02
Myocardium/blood pool rest	2.5 ± 0.8	2.5 ± 0.8	NS
Myocardium/blood pool stress	3.0 ± 0.8	3.8 ± 1.1	<0.001

LV = left ventricle; SDS = summed difference score; SRS = summed rest score; SSS = summed stress score.

Lexiscan PET. However, with PET MPI, imaging is started soon after stress while the subject is still breathing heavy and hence the effects on image quality, both relative PET and dynamic PET images need to be determined. A prior study[2] from the Ottawa heart institute, evaluated treadmill exercise PET with Rubidium-82. Myocardial uptake of Rubidium-82 was significantly greater with exercise compared with

dipyridamole stress (See Table reproduced from reference [2]). Higher ratios of myocardial uptake to gut, blood pool, and higher stress myocardial uptake were observed with exercise stress compared with dipyridamole stress. No significant differences in inferior wall defects due to misregistration were noted between exercise and dipyridamole stress studies using a dedicated PET scanner.

Rationale for specific aim 3:

A prior study,[2] from the Ottawa heart institute, evaluated treadmill exercise PET with Rubidium-82. This study included 50 patients (74% with known CAD and 26% with intermediate to high pretest likelihood of CAD) that underwent both exercise and dipyridamole Rubidium-82 PET within a median of 6 days of each other. More patients preferred exercise stress over dipyridamole. The summed stress (A), rest (B) and difference scores (C) were highly correlated (Figure below reproduced from reference[2]). Bland-Altman analysis revealed no significant differences in defect size between the treadmill and dipyridamole studies. Also, the agreement between treadmill and dipyridamole studies was similar to the agreement between repeat dipyridamole studies. The authors concluded that treadmill exercise 82Rb PET is feasible and provides imaging results of similar diagnostic content and better quality compared with dipyridamole stress.
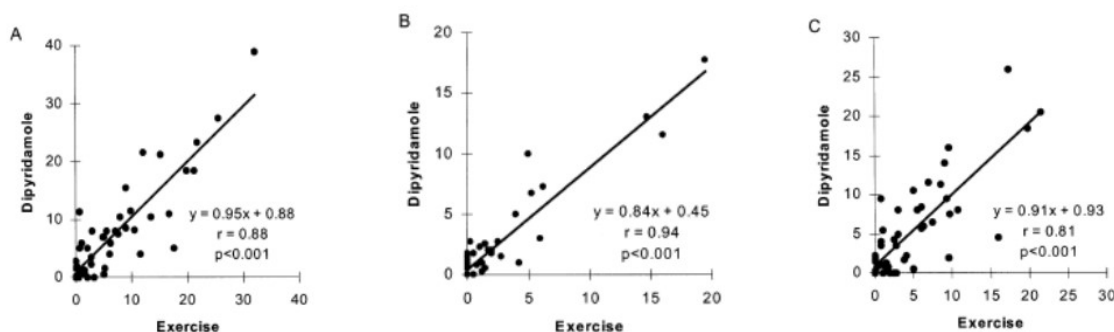
In a separate study from the same group, treadmill exercise PET with N-13 ammonia was compared to dipyridamole PET.[8] This study included 26 patients (41% with prior MI, 35% with prior PCI and 15% with prior CABG) that underwent both exercise and dipyridamole N-13 ammonia PET within a median of 6 days of each other. The summed stress, rest and difference scores were well correlated between the treadmill and dipyridamole studies. The defect size and severity was larger with treadmill exercise compared to dipyridamole PET (See Table below reproduced from reference[8]).

Table 3. Semi-Quantitative and Quantitative Analysis of N-13 PET Myocardial Perfusion Images

	Exercise Stress	Dipyridamole Stress	p Value	Correlation	Significance of Correlation
Summed stress score	9.1 ± 5.7	6.9 ± 5.9	<0.01	0.80	<0.001
Summed rest score	3.4 ± 3.3	3.2 ± 2.8	NS	0.79	<0.001
Summed difference score	5.8 ± 4.7	3.7 ± 4.6	<0.02	0.61	0.001
LV defect size (stress) (%)	19.3 ± 11.5	13.8 ± 13.6	<0.02	0.62	0.001
LV defect size (rest) (%)	8.1 ± 9.4	6.4 ± 6.5	NS	0.57	0.003

Values are mean ± SD.
LV = left ventricle; PET = positron emission tomography.

Myocardial count density of N-13 ammonia was significantly greater with dipyridamole compared to exercise stress. Successful performance of exercise ammonia required coordination between the cyclotron team and the imaging team. The authors concluded that treadmill exercise produces larger and more severe perfusion defects than dipyridamole and may reflect true ischemia.

III. STUDY METHODS

This is a single-center, prospective cohort study of subjects undergoing clinically indicated PET/CT MPI, with documented coronary artery disease or an intermediate to high pretest likelihood of coronary artery disease based on age gender and symptoms as described in the ACC/AHA/ASNC appropriate use criteria for cardiac radionuclide imaging. [12] Following completion of the clinical study, the research study is explained to the subjects. Consenting subjects will return on another day for a repeat stress study with Lexercise or a repeat Lexiscan stress PET. Approximately 50 subjects (not more than 16 patients with normal Rubidium-82 PETMPI and 34 with reversible perfusion defects) will be enrolled in this study. After successful screening and enrollment, 13 random subjects (8 normal and 5 ischemic) will return for repeat Lexiscan PET, and 34 subjects will return for a Lexercise PET/CT MPI, on the next calendar day or within 2 weeks of the clinical Rubidium-82 or N-13 ammonia PET study.

SUBJECT SELECTION

Main Selection Criteria
Subjects will be eligible if they meet all of the following inclusion criteria and none of the exclusion criteria:

    Inclusion criteria:

- Age > 18 years
- Clinically indicated Rubidium-82 or N-13 ammonia PET study
- Ten healthy volunteers recruited using web based advertisements.

- Known coronary artery disease (prior percutaneous coronary intervention, prior coronary artery bypass surgery or Q wave MI on ECG) or intermediate to high pretest likelihood of CAD
- Able to exercise on a treadmill
- Able and willing to provide informed consent to participate in the study

Exclusion criteria:
- Contraindications to exercise stress testing such as
    - unstable angina
    - known severe left main coronary artery stenosis
    - severe or uncontrolled heart failure
    - uncontrolled atrial or ventricular arrhythmias
    - symptomatic hypotension or severe hypertension (systolic blood pressure < 90 or > 200 mmHg, respectively)
    - > 1st degree atrioventricular block in the absence of a functioning pacemaker.
- Contraindications to Lexiscan use such
    - Allergy or intolerance to aminophylline or regadenoson
    - Known severe or oxygen dependent bronchoconstrictive or bronchospastic lung disease [e.g., asthma, wheezing, chronic obstructive pulmonary disease (COPD), etc.].
    - Sick sinus syndrome
- History of heart transplantation.
- Dialysis
- Subject requires emergent cardiac medical intervention or catheterization after the clinical study.
- Documented myocardial infarction (MI) ≤ 30 days prior to enrollment.
- History of percutaneous coronary intervention (PCI) ≤ 4weeks prior to enrollment.
- History of coronary artery bypass graft (CABG) ≤ 8 weeks prior to enrollment.
- Severe LV dysfunction, with ejection fraction of < 30%
- Serious non-cardiac medical illness (e.g., disseminated malignancy, severe neurological dysfunction at time of baseline PET study) or a social situation which will preclude research study participation
- History of seizure disorder

Discontinuation criteria:

Subjects should be discontinued for the following discontinuation criteria
- Subject does not fulfill inclusion or exclusion criteria

- Subject experiences a serious or intolerable adverse event
- In the Investigator's opinion, the subject is non-compliant with the protocol requirements (protocol violation)
- Subject's health would be jeopardized by continued participation
- Subject wishes to withdraw consent

Lexiscan will not be administered to patients with clinical or EKG evidence on ischemia during exercise treadmill testing. Clinical or EKG abnormalities during treadmill testing that would preclude administration of Lexiscan are listed below:

c) Clinical
   a. Severe chest pain
   b. severe lightheadedness or dizziness,
   c. severe shortness of breath
   d. exercise induced hypotension (> 10mm Hg decrease in systolic blood pressure)
   e. exercise induced hypertension (systolic blood pressure >210 mm Hg)
d) ECG changes
   a. Significant ST segment depression (≥2 mm)
   b. Significant ST segment elevation (≥1 mm)
   c. Sustained ventricular or atrial tachycardia,
   d. Exercise induced heart block

a) **Source of subjects and recruitment methods**

Patients will be recruited from the nuclear cardiology laboratory at the Brigham and Women's Hospital. Among them, stable and eligible patients meeting all inclusion and exclusion criteria will be invited to participate in this study and those who provide informed consent will be enrolled. Referring physicians will be informed about the study and asked to discuss the study with their patients being referred for PET myocardial perfusion imaging. Ten healthy volunteers will be recruited for this study using web based advertisements.

IV. SUBJECT ENROLLMENT

a) **Methods of enrollment including procedures for patient registration and or randomization**

The following recruitment methods will be used:
- This is a pilot study of subjects referred for a clinically indicated myocardial perfusion PET study. Majority of the subjects will be recruited from the clinical practices of the Brigham and

Women's hospital. The patients' referring physician will introduce the study to the subjects prior to the clinical PET study and those subjects that are willing to consider the study will be given the written informed consent for the study for review. Subjects' questions will be answered by Dr. Sharmila Dorbala. There will be no time restriction to sign the consent form or participate in this study. Subjects that express an interest will be evaluated and provided they meet all the inclusion and exclusion criteria, they will be considered for this study.

- On occasion, we may identify subjects with reversible perfusion defects from our clinical Rubidium-82 or N-13 ammonia PET studies. In those instances, physicians requesting myocardial perfusion imaging studies (primary or specialist health care provider) will be contacted personally explaining the purpose of the study. The physicians of those subjects will be asked if they felt comfortable introducing the study to their subjects either verbally during the office visit or through a recruitment letter. If the physician introduces the study verbally, the subject will be asked if they would like to speak with a study physicians after their appointment. If they would like to speak with someone, Dr. Dorbala will be notified and she will explain the study to the subject and go over the inclusion and exclusion criteria for the study. If they are interested in participating in the study an appointment will be made for their first visit.

- Hospital in-patients with request for pharmacologic stress: Attending physicians will be asked if they felt comfortable introducing the study to their subjects that they schedule for in-patient pharmacologic stress myocardial perfusion study verbally during their visit. If the physician introduces the study verbally, the subject will be asked if they would like to speak with a coordinator after the visit. If they would like to speak with someone, the coordinator will be notified and they will explain the study to the subject and go over the inclusion and exclusion criteria for the study. If they are interested in participating in the study an appointment will be made for their first visit.

- Special attention will be paid to enroll women and minorities in the study.

- Ten healthy volunteers will be recruited for this study using web based advertisements. They will all undergo Lexiscan (regadenoson) followed by Lexercise protocol (exercise + regadenoson) on a separate day.

b) **Procedures for obtaining informed consent (including timing of the consent process)**

For subjects interested in the speaking to a study staff, Study PI, will go over the inclusion and exclusion criteria and will describe the study and procedures to the potential subject over the phone or in person. If the subject qualifies and is interested he/she is encouraged to discuss the study with his/her primary care physician or family members. Upon arrival for the study, the subject will be given a copy of the consent form to read. After the subject has finished reading the consent form, one of the physician investigators will describe the study in detail and answer any questions or concerns

the subject might have. If the subject agrees to participate in the study they will be asked to provide his/her written informed consent. Subjects will be given a copy of the signed consent form for their records. The investigator will inform the subject that they have the alternative not to participate in the study and can withdraw their consent at any point during the study.

The subjects will be given as much as 2 weeks to consider participation in the trial. Pregnant women and minors are excluded from this trial. Subjects that are determined by the physician to be unable to consent due to their physical or mental condition will not be enrolled in this trial.

V. STUDY PROCEDURES

The patient will have 1-2 study visits and healthy volunteers will have 2-3 study visits. Study visit 1 is the same for patients and healthy volunteers.

a. **Study visit 1**

a. The subjects that are interested in the study will meet with Dr. Dorbala and go over the consent form and details of study participation.

b. This screening visit will take about 1 hour. The research study team will ask the subject questions about medical history, medications, and prior hospitalizations to make sure that the subject qualifies for this study. Blood pressure, heart and breathing rates, temperature, and height and weight will be measured. Pregnancy is not allowed due to the unknown risks radioactivity may have on the mother or the unborn baby from the beginning of the study period through the end of the study. A blood pregnancy test will be performed on the day of the PET scan in women with child bearing potential.

Patients:

a. **Study visit 2**

The study visits 1 and 2 can be conducted on the same day. Study visit 2 is for the performance of the Lexercise stress Rubidium-82 or N-13 ammonia PET study. This will be scheduled at least the next calendar day or within 2 weeks of the clinical Rubidium-82 or N-13 ammonia PET study. While we aim to conduct the research scan within 2 weeks of the clinical scan, if there is a scanner malfunction or patient schedule conflict, we would like to extend the time frame for enrollment to 4 weeks. Approximately 20 cc of blood will be drawn following completion of the Rubidium-82 or N-13 ammonia imaging study and evaluated for biochemical markers of ischemia.

b. **Study Visit 3**

Study visit 3 is for the performance of the Lexercise stress Rubidium-82 or N-13 ammonia PET study. This will be scheduled at least the next calendar day or within 2 weeks of the clinical Rubidium-82 or N-13 ammonia PET study. Approximately 20 cc of blood will be drawn following completion of the Rubidium-82 or N-13 ammonia imaging study and evaluated for biochemical markers of ischemia. A part of this blood sample (10 cc) will be sent to the laboratory of Dr. XX for the isolation of microRNAs involved in angiogenesis and vascular inflammation. This blood sample will be stored for future analysis.

Healthy volunteers:

a. Study visit 2

The study visits 1 and 2 can be conducted on the same day. Study visit 2 is for the performance of the rest and regadenoson stress Rubidium-82 or N-13 ammonia PET study. No blood drawing will be performed during this visit.

b. Study visit 3

Study visit 3 is for the performance of the Lexercise stress Rubidium-82 or N-13 ammonia PET study. This will be scheduled at least the next calendar day or within 2 weeks of the Visit 2 rest/stress scans. While we aim to conduct the Visit 3 "Lexercise stress" scan within two weeks of the 2 rest/stress scans, if there is a scanner malfunction or patient schedule conflict, we would like to extend the time frame for enrollment to 4 weeks. Approximately 5 cc of blood will be drawn following completion of the Rubidium-82 or N-13 ammonia imaging study for troponin levels. Blood samples will not be drawn for micro RNA measurements in the healthy volunteer cohort.

Detailed Description of PET MPI Methods: The proposed research will utilize the resources of the Cardiovascular Imaging center at Brigham and Women's Hospital, Boston MA. Specifically, PET imaging will be performed on a state-of-the-art 64 slice cardiac PET/CT unit.

Before the test: All patients scheduled for a rest- vasodilator stress Rubidium-82 PET or N-13 ammonia will receive written instructions to fast overnight, avoid caffeine containing beverages and medications for 24 hours prior to the test and bring all their medications (including prescription, over the counter, dietary supplements, herbal and vitamins) with them. Upon arrival for the test, all patients are given a written questionnaire about allergies and possibility of pregnancy (women of child bearing age will undergo a blood pregnancy test prior to the rest Rubidium-82 or N-13 ammonia PET study), as per laboratory protocol. Patients without objection will be detailed about the study and related procedures and

consenting patients will be recruited into the study. An experienced exercise physiologist/imaging fellow obtains a detailed clinical history from each patient and confirms it by a review of electronic medical records. Written informed consent for the study is checked and IV line inserted, ECG module connected and the patient brought into the PET/CT room for imaging. A detailed datasheet will be used to record information on demographics, history on coronary risk factors, reason for the test, nature of symptoms (chest pain type, shortness of breath description), resting ECG interpretation (as approved by a cardiologist), list of medications, resting and stress hemodynamics, dose of Lexiscan, dose of Rubidium-82 or N-13 ammonia injected, reason for test termination, symptoms and ECG changes during stress test.

Rubidium-82 or N-13 ammonia PET image acquisition:

Following skin preparation and ECG lead placement, patient undergoes a symptom limited standard Bruce exercise treadmill test. At peak exercise (when patient is unable to exercise for more than 1 minute), the treadmill is stopped and patient is immediately positioned on the imaging table. Image acquisition is started with a CT scout image for positioning the heart (10 mA, AP scout, 9 seconds). Then 0.4 mg of regadenoson is given by intravenous push over 10 seconds followed by 10 ml of normal saline flush. Right after the saline flush, 40-60 mCi of Rubidium-82 or 20 mCi of N-13 ammonia is injected and emission imaging started in a list mode fashion started at the time of injection of radiotracer. Following completion of the emission imaging, a CT transmission scan (20 mA, 120 KV, non-gated, free tidal breathing) is obtained for correction of photon attenuation by soft tissues. .

Rest and Lexiscan stress images from the clinical study and the Lexercise stress emission images will be unlisted for analysis into a static file, a gated file and dynamic image frames (36 frames of 5 seconds each, 8 frames of 15 seconds each, and 6 frames of 30 seconds each for stress and 2 groups for rest of 24 frames at 5 seconds each and 8 frames at 30 seconds each). In subjects with serious or bothersome adverse effects, aminophylline will be administered intravenously (1 mg/kg, to a maximum of 150 mg) to alleviate the symptoms.

Myocardial blood flow quantification:

Myocardial blood flow (in ml/gm/min) at rest and after vasodilator stress will be quantified using a software based on GFADS and a 2 compartment model, developed and validated at the Brigham and Women's Hospital.[13] Rest and peak stress myocardial blood flow will be computed in ml/gm/min and global and regional (vascular territories assigned as described below) coronary flow reserve computed by dividing stress myocardial blood flow by rest myocardial blood flow. Coronary vascular resistance at rest and stress will be computed by dividing mean aortic pressure [derived as 2 (diastolic blood pressure) + systolic blood pressure/3] by myocardial blood flow. In addition to calculating the myocardial blood flow for the left ventricle as a whole, myocardial blood flow will be determined for each of three vascular beds

(left anterior descending [LAD], left circumflex [LCx], and right coronary artery [RCA]). Subendocardial and subepicardial myocardial blood flow will be assessed globally.

Interpretation of relative myocardial perfusion images:

The semi-quantitative clinical interpretation of Rubidium-82 or N-13 ammonia images will be performed using a standard 17 segment model and a 0-4 scale (0=normal, 1= mild defect, 2=moderate defect, 3=severe defect, 4= absent radiotracer uptake). The sum of the scores in the 17 segments at stress and rest will be computed as the summed stress score (SSS) and summed rest score (SRS) respectively. The summed difference scores (SDS) will be derived as SSS – SRS. The SSS, SRS and SDS will be converted into percentages to compute total percent myocardium abnormal, scarred or ischemic determined as (100/68)*SSS, (100/68)*SRS or (100/68)*SDS, respectively. Perfusion defects on relative PET will be assigned vascular distributions as described previously.[14] LV volumes and EF will be estimated using FDA approved software (EcTb), with automatic edge detection and manual refinement when needed.

Interpretation of absolute myocardial perfusion images:

Myocardial blood flow at rest, stress and CFR are calculated for the left ventricle as a whole, as well as for each of three vascular beds (left anterior descending [LAD], left circumflex [LCx], and right coronary artery [RCA]). Polar plots of CFR will be generated and defect extent and severity computed in relation to a quantitative CFR normal limits database (developed at BWH).

Study Endpoints Assessed:

- Diagnostic value:
    - Defect size, severity, extent and contrast with Lexiscan alone compared to exercise Lexiscan protocol
    - Left ventricular ejection fraction at rest and immediately post stress will be compared in the Lexiscan alone and the exercise Lexiscan protocol.
    - Myocardial blood flow estimates and coronary flow reserve will be compared between the Lexiscan alone and Lexercise protocols.
    - Comparison to coronary angiography results when available.
- Safety and tolerability:
    - Side effects, ECG changes, blood pressure changes, will be recorded.
    - Safety blood work will be drawn after the Lexiscan and the Lexercise PET study to measure serum troponin T levels.
    - Radiation dose to the staff will be measured using personal dosimeters after the Lexiscan as well as the Lexercise PET study.
- Image quality:

- o   Motion artifacts
- o   Image registration
- o   Sub diaphragmatic activity (liver heart ratios)
- o   Defect contrast
- o   Signal to noise ratio and
- o   Dynamic image evaluability will be assessed

VI. BIOSTATISTICAL ANALYSIS

The overall aim of this pilot study of 50 patients is to assess the tolerability, safety, diagnostic accuracy and image quality between Lexiscan with symptom limited exercise compared to Lexiscan alone.

Aim 1. For this aim, we will compare the reported side effect profile for Lexercise to Lexiscan PET, using a Pearson Chi square test. A P-value of<0.05 will be considered significant.

Aim 2: For this aim, we will compare objective measures of image quality including signal to noise ratio using dynamic Rubidium-82 images or N-13 ammonia, the frequency of misregistration between PET and CT, the frequency of presumed motion artifact and bowel radiotracer activity. These measures will be compared using a student's t-test or a Pearson Chi square test, as appropriate. A P-value of<0.05 will be considered significant.

Aim 3: For this aim, we seek to compare measures of semi quantitative relative perfusion, absolute perfusion and LVEF using the Lexiscan alone compared to Lexercise PET. Firstly, we will compare semi-quantitative defect size, severity and reversibility between the Lexercise and Lexiscan PET studies. Based on the results of one prior study using N-13 ammonia and comparing dipyridamole versus treadmill exercise[2], a sample size of 35 subjects with reversible defects should be adequate to identify if significant differences exist between Lexercise and Lexiscan PET protocols. Next, we will study the changes in LVEF from rest to peak stress in Lexercise versus Lexiscan PET studies in the normal and ischemic subjects. Finally, we will compare regional rest and peak stress myocardial blood flow and coronary flow reserve between Lexercise and Lexiscan PET studies.

VII. RISKS AND DISCOMFORT

Time: Participation in this trial entails an exercise vasodilator stress Rubidium-82 PET or N-13 ammonia scan. The total time spent for the study procedures is approximately 1 hour.

Intravenous line insertion: Possible side effects intravenous line insertion are pain, bruising, or infection where the needle entered the skin.   Some people faint or get dizzy during insertion of the intravenous line.

Radioactive imaging agents may have the following side effects: The average dose from the research radiation is approximately 2.7 mSv, which is approximately 6% of the amount allowed per year for a person who routinely work with radiation sources. For healthy volunteers, the average dose from the research radiation is approximately 6.9 mSv, which is approximately 13.8% of the amount allowed per year for a person who routinely work with radiation sources. Other side effects include: metallic taste in the mouth, burning at the injection site, facial swelling, numbness of the hand and arm, low blood pressure, nausea, angina and chest tightness. Radiation exposure associated with both rest and stress imaging is well within acceptable safety standards as set forth by the FDA. Radiation may cause physical or genetic damage to a fetus, so subjects that are pregnant or breast feeding will be excluded. A pregnancy test will be performed prior to enrolling patients with childbearing potential.

Lexiscan: In previous studies where vasodilator stress agents were given to patients with heart disease at the FDA approved doses, they have been reported to be associated with the following side effects including dyspnea-shortness of breath (28%), headache (26%), flushing (16%), chest discomfort (13%), angina pectoris-chest pain caused by myocardial ischemia (12%), dizziness (8%), chest pain (7%) and nausea (6%), flushing, feeling of heat, hypoesthesia (decreased sensitivity), paresthesia (tingling of the arms and legs), sensation of heaviness, abdominal pain, palpitations, unpleasant taste in the mouth and feeling hot (each 5%). Increases in heart rate and mild decreases in blood pressure have also been associated with administration of vasodilator stress agents.

In addition to the risks or discomforts listed above, the study drug and procedures may have unknown side effects. There is always the possibility that subjects will have a reaction that is currently not known or not expected. All drugs can cause an allergic reaction that, if not treated promptly, could be life-threatening. Symptoms of such a reaction are: throat tightness, itching, hives ,wheals ,vomiting ,difficulty breathing or turning blue.

Risks of withholding medications: Certain medications such as beta-blockers, nitrates, diuretics, calcium channel blockers and bronchodilators may have to be held for 12-24 hours prior to the study. Withholding these medications may result in chest pain or angina, high or uncontrolled blood pressure, high blood glucose, or in very rare instances a heart attack. For these reasons we will only enroll subjects that are clinically stable without uncontrolled angina, hypertension or are acutely ill for any reason. The medications that will be withheld and the length of the time withheld are shown below. To minimize the

risk, the prescribing physician will be consulted to give permission for his patient to withhold the specified medication for the specified length of time.

Hold on the morning of the test

> Diabetic agents: Insulin Half of Normal Morning Dose can be taken.
> Hold oral Hypoglycemics till after the test completion: Actos, Amaryl, Avandamet, Avandia, Avandaryl, Byetta, Diabeta, Diabinese, Fortamet, Glipizide, Glucophage, Glucovance, Glucotrol, Glyburide, Metformin, Micronase, Prandin

Hold 4-6 hours prior to testing

> Nitroglycerine, Deponit, Nitro-Bid, Nitro-Disc, Nitro-Dur, Nitro-Gard, Nitrol, Nitrostat, TransdermNitro, Trasdermal-NTG, Nitro paste

Hold 12 hours prior to testing

> Nitrates, Isosorbide Dinitrate Isordil, Dilatrate, Sorbitrate, Isosorbide Mononitrate, Imdur, Ismo, Monoket

Hold 24 hours prior to testing

> **Bronchodilators** Aerolate, Aminophylline, Aquaphyllin, Asmalix, Choledyl, Elizophylline Slo-bib, Slo-Phyllin, Thoe-Dur, Theovent
>
> **Calcium Channel Blockers**
>
> Amlodipine Cauduet, Lotrel, Norvasc, Diltiazem Cardizem CD, Cardizem SR,Cartia XT, Dilacor XR, Diltia XT, Tiazac, Taztia XT, Felodipine Plendil, Isradipine DynaCirc, Nicardipine, Hydrochloride, Cardene, Cardene SR, Nifedipine Adalat, Adalat CC, Procardia, Procardia XL
>
> Nimodipine Nimotop, Nisoldipine Sular, Verapamil, Hydrochloride, Calan, Calan SR, Covera, Isoptin, Isoptin SR, Verelan
>
> **Miscellaneous**
>
> Cafergot, Cialis, Levitra, Ranexa, Ranolazine, Trental, Pletal, Viagra.

Hold 48 hours prior to testing

> Dipyridamole, Persantine, Aggrenox

Aminophylline: Side effects of aminophylline may include nausea, headache, restlessness, convulsions, rapid breathing, a rapid heart rate, and allergic reactions such as rash.

VIII. POTENTIAL BENEFITS

No direct benefit is anticipated to individual subjects. The results of this study may benefit society through evaluation of the diagnostic utility of novel protocol utilizing combined symptom limited exercise vasodilator stress test to image endothelium dependent and endothelium independent factors affecting myocardial blood flow.

IX. MONITORING AND QUALITY ASSURANCE

Subject safety will be ensured by strict adherence to inclusion and exclusion criteria and close monitoring. Subjects that experienced serious adverse events during the study will be removed from the study and followed up, but no other study procedures will be performed. We will monitor the subjects closely for seizures and ischemic symptoms. History of seizures is an exclusion criteria for the study. Individuals who experience seizures for the first time during the initial regadenoson study will be excluded from the second regadenoson with exercise study. Intravenous aminophylline, a competitive agonist, will be administered for ischemic symptoms. Subjects with ischemic symptoms will be monitored until complete resolution (current clinical practice). As listed in page 22, study endpoints, safety blood work will be drawn after the Lexiscan and the Lexercise PET study to measure serum troponin T levels. This study does not involve treatment and hence a data safety monitoring board.

This is a pilot clinical study of about 50 subjects. The study PI, Sharmila Dorbala, MBBS, will be responsible for monitoring the study procedures after every subject is enrolled, including the accuracy and completeness of the case report form entries, source documents and informed consent after every enrollment. These documents will be appropriately maintained on submission.

Specification of Source Documents

The following information will be included in the source medical records:

• Demographic data (age, sex, and race)

• Inclusion and exclusion criteria details

• Participation in study and signed and dated informed consent forms

• Visit dates

• Medical history and physical examination details

• Key efficacy and safety data

• Adverse events and concomitant medication

• Results of relevant examinations

• Laboratory printouts

• Reason for premature discontinuation

Ethical Conduct of the Study

The investigator(s) and all parties involved in this study will conduct the study in adherence to GCP, ICH Guidelines and the applicable laws and regulations.

Subject Confidentiality

The principal investigator will be responsible for oversight on maintaining subject confidentiality. All study staff are trained in Partners HIPAA procedures as well as in ethical conduct of research. Documents relating to the study that contain personal data that may disclose the identity of the subject will remain with the investigator and kept confidential in a locked cabinet accessible only to study staff. Subject confidentiality will be maintained by utilizing subject identification code numbers to correspond to treatment data in the study computer files. All study center personnel will comply with privacy rules of this institution, the ICH guideline for good clinical practice, HIPAA and applicable state law. Subject confidentiality will be maintained by limiting access to data collected to only co-investigators, study staff. Data and specimens will not be stored at Partners or non-Partners sites for future uses not described in the protocol.

X. REFERENCES:

1. Dilsizian V, Bateman TM, Bergmann SR, et al. Metabolic imaging with beta-methyl-p-[(123)I]-iodophenyl-pentadecanoic acid identifies ischemic memory after demand ischemia. *Circulation.* Oct 4 2005;112(14):2169-2174.

2. Chow BJ, Ananthasubramaniam K, dekemp RA, et al. Comparison of treadmill exercise versus dipyridamole stress with myocardial perfusion imaging using rubidium-82 positron emission tomography. *J Am Coll Cardiol.* Apr 19 2005;45(8):1227-1234.

3. Di Carli MF, Hachamovitch R. New technology for noninvasive evaluation of coronary artery disease. *Circulation.* Mar 20 2007;115(11):1464-1480.

4. Klocke FJ, Baird MG, Lorell BH, et al. ACC/AHA/ASNC guidelines for the clinical use of cardiac radionuclide imaging--executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/ASNC Committee to Revise the 1995 Guidelines for the Clinical Use of Cardiac Radionuclide Imaging). *Circulation.* Sep 16 2003;108(11):1404-1418.

5. Schleipman AR, Castronovo FP, Jr., Di Carli MF, et al. Occupational radiation dose associated with Rb-82 myocardial perfusion positron emission tomography imaging. *J Nucl Cardiol.* May-Jun 2006;13(3):378-384.

6. Wilson RF. Assessing the severity of coronary-artery stenoses. *N Engl J Med.* Jun 27 1996;334(26):1735-1737.

7. Bache RJ. Vasodilator reserve: a functional assessment of coronary health. *Circulation.* Sep 29 1998;98(13):1257-1260.

8. Chow BJ, Beanlands RS, Lee A, et al. Treadmill exercise produces larger perfusion defects than dipyridamole stress N-13 ammonia positron emission tomography. *J Am Coll Cardiol.* Jan 17 2006;47(2):411-416.

9. Camici PG, Crea F. Coronary microvascular dysfunction. *N Engl J Med.* Feb 22 2007;356(8):830-840.

10. Camici PG, Rimoldi OE. The clinical value of myocardial blood flow measurement. *J Nucl Med.* Jul 2009;50(7):1076-1087.

11. Holly TA, Satran A, Bromet DS, et al. The impact of adjunctive adenosine infusion during exercise myocardial perfusion imaging: Results of the Both Exercise and Adenosine Stress Test (BEAST) trial. *J Nucl Cardiol.* May-Jun 2003;10(3):291-296.

12. Hendel RC, Berman DS, Di Carli MF, et al. ACCF/ASNC/ACR/AHA/ASE/SCCT/SCMR/SNM 2009 appropriate use criteria for cardiac radionuclide imaging: a report of the American College of Cardiology Foundation Appropriate Use Criteria Task Force, the American Society of Nuclear Cardiology, the American College of Radiology, the American Heart Association, the American

Society of Echocardiography, the Society of Cardiovascular Computed Tomography, the Society for Cardiovascular Magnetic Resonance, and the Society of Nuclear Medicine: endorsed by the American College of Emergency Physicians. *Circulation.* Jun 9 2009;119(22):e561-587.

13. El Fakhri G, Sitek A, Guerin B, et al. Quantitative dynamic cardiac 82Rb PET using generalized factor and compartment analyses. *J Nucl Med.* Aug 2005;46(8):1264-1271.

14. Dorbala S, Vangala D, Sampson U, et al. Value of vasodilator left ventricular ejection fraction reserve in evaluating the magnitude of myocardium at risk and the extent of angiographic coronary artery disease: a 82Rb PET/CT study. *J Nucl Med.* Mar 2007;48(3):349-358.