FHRWW Stress SPECT Protocol Reduces Radioactive Dosage and Increases Ischemia Detection

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Abstract

BACKGROUND: Radioisotope manufacturers, nuclear camera manufacturers, standard guidelines for myocardial perfusion imaging, all assume rapid isotope uptake followed by an essentially static retention mechanism. Contrariwise, the historic first nuclear cardiology study found measurement of nuclear isotope blood flow kinetics to be diagnostic of heart disease. No clinical investigations appear to have followed that classic finding. We assessed the clinical diagnostic utility of time course myocardial perfusion measurements following single injections.
METHOD: One hundred twenty patients suspected of having heart disease underwent Sestamibi stress images taken at 5 and at 60 minutes following completion of cardiac stress. Quantitative (FRHWW) redistribution differences between the images were assessed. FHRWW results were compared with angiographic findings.
RESULT: Parabolic regression of stenosis on redistribution yielded an effect size of R(CI95%) = 0.72 to 0.95, (P=3.8×10-8). Fifteen percent of individuals with "normal" appearing 60 minute SPECT images were noted to having coronary artery disease using both redistribution data and coronary angiography requiring stent placement. These individuals had "wash in" of Sestamibi (isotope uptake greater at 60 minutes than 5 minutes) and signaled patients at risk of undergoing an acute cardiac event due to vulnerable plaques or tight lesions placing significant amounts of myocardium at risk of infarction. One of the individuals who did not require cardiac catheterization based upon the FHRWW data, underwent coronary angiography based upon rest-stress results AND had a major adverse event as a result of the subsequent cardiac catheterization. There was no detectable CAD, confirming the FHRWW findings and the need for caution in individuals undergoing invasive procedures.
CONCLUSION: Dynamic "uptake /release" models appear to be a superior alternative to the common "uptake/retention" models of technetium-99m isotopes used in nuclear myocardial perfusion imaging. This sequential quantitative diagnostic model, reinforces the work by Blumgart, enabling more accurate diagnosis of coronary artery disease and providing for intervention in individuals requiring stents or bypass, whom would otherwise be missed on rest-stress imaging protocols while avoiding unnecessary coronary angiography among individuals with "normal" Sestamibi redistribution.

Keywords: (MeSH): FHRWW, Sestamibi redistribution, Heart Function Tests; Mathematical Models; Tomography; Emission-Computed, Single-Photon; Vulnerable Plaque; Adverse Cardiac Events.

Introduction

While diagnostic testing and decision making in the treatment of atherosclerotic coronary artery disease (ASCAD/CAD) tends to revolve around evidence of anatomic (cardiac catheterisation,[1] coronary computed tomography, intravascular ultrasound,[2,3] et cetera) disease, it has been well established that this inflammatory[4-6] process may smolder for years and that up to 85% of all myocardial infarctions occur with <30% diameter narrowing. It is; therefore, important for us to look for new ways to uncover this smoldering inflammatory disease process when intervention may be more beneficial to the patient and potentially less costly to the patient and society at a time when health care costs are a major issue.

When teenagers learn to buy their first automobile, they learn an important lesson. Not every car that looks good runs well. This same lesson applies to the diagnosis and treatment of ASCAD. The lesson is to determine how well the car runs, or in this case, how well the heart runs. Hence the importance of physiologic testing; ie. testing to see how well the heart runs. Prior to nuclear imaging of the heart, now called myocardial perfusion[7,8] imaging (MPI), patients ran on a treadmill (exercise stress testing/EST) under the belief that this would precipitate chest pain (angina) and perhaps electrocardiographic

and hemodynamic changes which would indicate problems with coronary blood flow. Until recently, it was believed that this was purely the result of narrowing within the lumen of the artery. It has since been demonstrated that angina is the result of regional blood flow9,10 differences; hence, the importance of quantifying such areas of the heart by analyzing regions of interest (ROI). These differences in regional blood flow work through mechanisms as of yet not completely understood. The ability to precipitate these differences in regional blood flow can be accomplished via the utilization of MPI and pharmacologic or exercise stress testing. A major limitation with utilizing single 60 minute images for interpreting the presence of ischemia on MPI are attenuation artifacts. Such artifacts include anterior attenuation from breast tissue, diaphragmatic attenuation from abdominal obesity, hypertrophic differences in walls of the heart, bundle branch anomalies, et cetera. These attenuation issues have not been significantly corrected with the addition of attenuation correction protocols. While nuclear tracers (isotopes) have improved over the years, many misconceptions[11-17] have plagued the field limiting the accuracy of MPI with sensitivities and specificities remaining at 65-90% in most published investigations.[18]

In 1926 Blumgart[19] first proposed nuclear imaging techniques for the detection of heart disease. His method clearly proposed serial assessment of isotope count activity to determine cardiac function. Decades passed before Love[20] discussed the potential future of Nuclear Cardiology and pointed out that rest images[21] were useless in the detection of coronary artery narrowing. With the advent of technetium-99m- sestamibi, clinicians believed that the isotope was taken up by myocardial tissue within minutes of injection and remained fixed within myocytes. Crane[22] demonstrated this was no so and demonstrated that retention of sestamibi was dependent upon ischemia and cellular viability. This opened the door to the discovery of changes in isotope count over the course of time and reinforced Blumgart's 1926 paper. During our initial[23,24] efforts to better understand the underlying inflammatory component of CAD via MPI, five and sixty minute imaging revealed differences in the qualitative appearances of images seen following pharmacologic stressing of individuals as predicted by Blumgart,[19] Love[20] and Crane.[22] As noted below, others have noticed these differences as well. Furthermore, comparisons of the initial 5-minute imaging with the 60-minute imaging demonstrated different results from the simple "uptake/ retention" rest-stress model. Other investigators have noted similar differences between 5 minute and 60 minute imaging of the heart[25-29] allowing for enhanced detection of congestive heart failure,[26,30-33] cardiomyopathies,[34,35] Prinzmetal's angina[36,37] and underlying coronary artery disease[26,31,38-49] including evidence of washin[46,47] indicative of critical lesions not detected by conventional MPI. In the same way that thrombolysis in myocardial infarction (TIMI) flow is used to look for changes (sometimes subtle, sometimes not) in coronary blood flow in the cath lab, multiple imaging following pharmacologic or EST allows us to take a more advanced look at the physiologic function of the heart, unmasking ischemic heart disease missed by rest-stress imaging.

Given these recent investigations into improving the detection of ischemia by quantifying isotope counts on multiple (sequential) images following pharmacologic and exercise stress, we set out to determine (1) are nuclear cameras used in the "clinical" setting today able to detect differences in radioactive isotope counts over a period of time, which would allow these cameras to detect changes in flow and retention of isotope over time (2) what are the optimal imaging times for the detection of ischemia and (3) how do these changes in isotope counts compare with percent diameter stenosis as seen on coronary angiography?

Methods

Single Photon Emission Computed Tomography (SPECT) Cameras

The Philips Forte Dual Head SPECT camera used general all (GAP) purpose collimators with the heads positioned at 90 degrees per manufacturers specifications. A 15% window was used with a 64 by 64 matrix. In the first part of this study, we additionally looked at differences between 64 x 64 matrix and 128 x 128 matrix. Imaging occurred 5 and 60 minutes post stress with processing of images as described in the literature.[17,23,24] Jet stream software was used per manufacturer's instructions to quantitatively measure regions of interest (ROIs).

The Picker Axis Dual Head SPECT camera used a low energy general all (LEGAR-PAR) purpose collimator with parallel hole positioning. The heads were positioned at 102 degrees per manufacturer specifications. Picker camera software was used for ROI measurements.

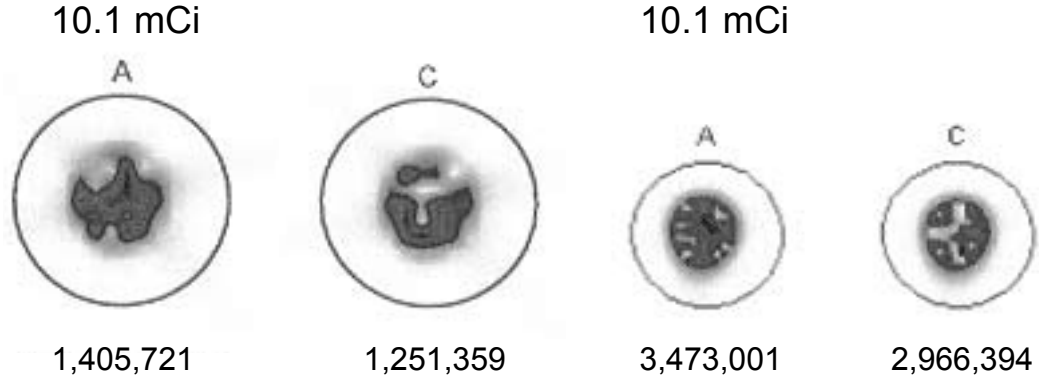

Figure 1: Verification of Technetium-99m-Sestamibi Count Imaging.
A 37.37 MBq syringe of technetium-99m-sestamibi is placed under the camera and counts acquired over 5 minutes. The same syringe is reimaged 55 minutes later for an equal amount of time. When the 64 x 64 matrix was used, the original count was 1,405,721, the second image count was 1,251,359, representing 89% of the original count activity. When the resolution was increased to 128 x 128 matrix, there was an additional 50% loss in data, with a 5-minute image (different syringe sample) count of 3,473,001. The second image count 55 minutes later was 2,966,394, representing a count decrease of 14.6%, 4.6 % more than should have been shown. This reduction is due to data lost as a result of increased grid lines forming the increased number of pixels per image. Subsequently, total information is lost at the gain of localization information (FH Uncertainty principle). Since we are looking for total cell uptake of the tracer, one must use the 64 x 64 matrix as a better index of isotope activity in exchange for resolution.

Determining if Nuclear Cameras used in "Clinical" Practice are able to detect changes in isotope count over time

Establishing technetium-99m decay by radioactive count measurement: Based upon the half-life of technetium-99m, a stable source representing the "uptake/retention" model (viz. a syringe filled with the isotope) has a 10% decay over 55 minutes with a retention of ~90% of the radioactive counts over a 55 minute (the difference between 5 and 60 minute images) span of time. In an effort to establish whether "clinically" used cameras are able to measure this physical decay in technetium-99m, we studied a 37.37 MBq (10.1 mCi) sample of sestamibi at baseline and again 55 minutes later. As shown in figure 1, the resting isotope count was 1,405,721 while the second image obtained 55 minutes later was 1,251,359 representing 89% of the original count activity. Independent of the actual amount of activity which is taken up by a region of myocardium, if the "uptake/retention" model was correct, images taken 55 minutes after the original image would yield counts of 90% of that seen in the first image.

Fleming-Harrington Redistribution Washin Washout (FHRWW)[50-52]: Using Multiple SPECT Camera Images of the Heart to Determine Changes Between 5 and 60-Minute Images.

Optimal Timing to Measure Changes in Radioactive (Sestamibi) Isotope Activity

Measuring regions of interest (ROIs) to quantify changes in technetium-99m isotopes between 5 and 60-minute images to look for changes in isotope "uptake/retention." We began with the knowledge acquired from the first part of this study, that we needed to use the 64 x 64 matrix to accurately measure changes in radioactive decay. We then proceeded to analyze ROIs at 5-minutes (figure 2) and at 60-minutes (figure 3). FHRWW calculations (discussed below) demonstrated that the "uptake/retention" model was clearly wrong as seen by the increase in count activity in the anterior wall (23,600 at 5-minutes and 30,470 at 60 minutes) while the inferior region without ischemia demonstrated a 6% reduction in count activity.

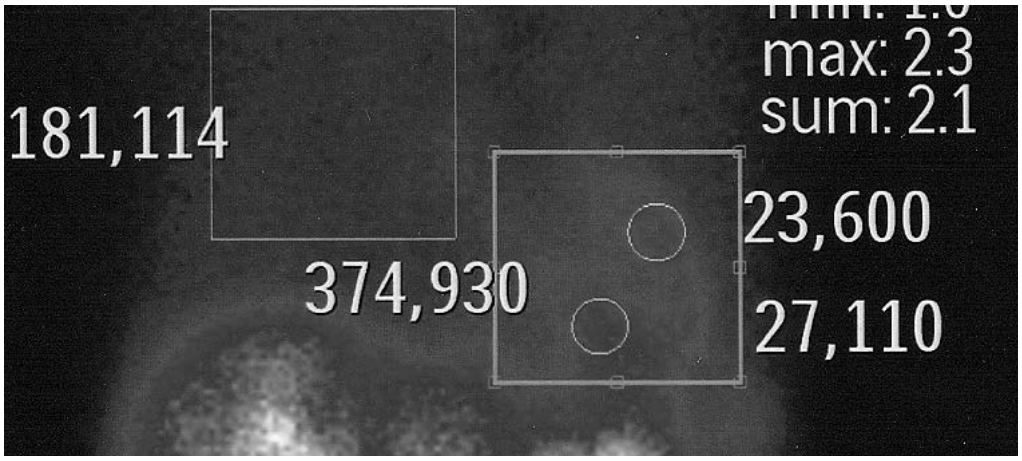

Figure 2: SPECT image acquired 5 minutes post stress with regions of interest including the total heart, anterior and inferior myocardial regions.
Static image acquired at 5 minutes with regions of interest (ROI) showing total heart count of 374,930. The anterior ROI representing the basal anterior region of the perfused by the left anterior descending artery shows 23,600 counts while the inferior ROI shows 27,110 counts.

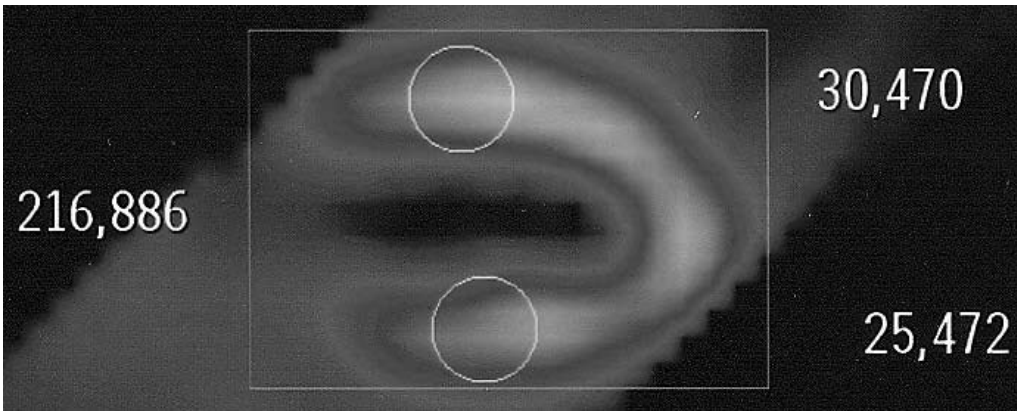

Figure 3: SPECT image acquired 60 minutes post stress with regions of interest including the total heart, anterior and inferior myocardial regions.
Reconstruction of the same myocardial regions noted in figure 2, this time taken from the dynamic image result obtained at 60 minutes post stress, showing a total heart count of 216,886. The anterior ROI shows an increase in count activity (washin) of 30,470 compared with the activity of 23,600 (Figure 2) obtained at 5 minutes. The inferior ROI shows a count of 25,472 compared with the count activity of 27,110 (Figure 2) obtained 5 minutes post stress.

Establishing the optimal time sequence for image acquisition to measure radioactive counts: Using this particular individual (figure 2 and 3), during this second phase of the investigation, we additionally compared the basal anterolateral, mid anterolateral, basal anterior, mid anterior, basal inferior, mid inferior basal inferoseptal and mid inferoseptal ROIs and compared the results with that obtained from coronary angiography. We then measured radioactivity in each of the 8 ROIs over the course of an hour, with the salient findings reported in Figure 4. These results were compared with our previous studies[23,24] (which revealed the ideal timing for detection of inflammatory changes), and based upon the expected 10% decay of technetium-99m over a span of 55 minutes, the remained of our work (FRHWW) focused on ROIs obtained at 5 and 60 minutes post stress. These results confirm that Sestamibi does NOT follow an "uptake/retention" model; but rather an "uptake/release" model consistent with Sestamibi redistribution (FHRWW).

Calculating FHRWW:

By comparing 5 and 60-minute imaging, the expected decay of Tc-99m is 10%. Actual counts from individual ROIs can then be taken and actual redistribution (FHRWW) can be

calculated as follows.

(eq 1). FHRWW (Total Heart) =

$$\frac{\text{ROI counts (total heart) at 5 minutes - ROI counts (total heart) at 60 minutes}}{\text{ROI counts (total heart) at 5 minutes}} \times 100 - 10$$

Where 100 places the answer in percent minus the 10 % expected decay.

(eq 2). FHRWW for a particular myocardial ROI = _ _

$$\frac{\text{ROI counts (region) at 5 minutes -ROI counts (region) at 60 minutes}}{\text{ROI counts (region) at 5 minutes}} \times 100 - 10$$

This can be re-written as:

(eq 3). Let the 5 minute counts = 'x', let the 60 minute counts = 'y', and let % washout = '100*w'.

Then w = (x - y)/x - 0.1 = x/x - y/x - 0.1 = 1.0 - y/x - 0.1 = 0.9 - y/x = .9 – 60 minute counts/5 minute counts.

Comparing washout/washin results with coronary angiographic assessment of percent diameter stenosis

One hundred twenty patients (ages 25 to 82 years) with suspected coronary artery disease underwent myocardial perfusion imaging after signing informed consent per Institutional protocol following adenosine (n=30), lexiscan (n=61), dobutamine[53-57] (n=4) or treadmill1[7,56] (n=25) stress. Following standardized protocols already published[50-52] MPI using both the rest-stress and stress-stress (FHRWW) approach were compared with coronary angiography.[58-59]

Statistical Analysis. Quantification of isotope counts was made using the specific nuclear computer software provided by the nuclear camera companies. These quantified counts were recorded with calculations of FHRWW as noted above. Correlation coefficients, confidence intervals and p-value (significance levels) were determined between %DS and FHRWW using product-moment correlation and least squares regression fitting the hypothesized model of $y=cx^2$. Using the null hypothesis for n = 120 patients, we estimated odds ratios (Ors) for predicting final diagnosis from nuclear procedures. Ors were determined against coronary angiography results for both the rest/stress images and FHRWW images. Statistical analysis and graphics were developed using R-2.6.0 and GGobi software.

Results

To the best of our knowledge verification of the ability of a nuclear camera used for "clinical" purposes to measure sequential radioactive counts confirming differences in technetium-99m-sestamibi resulting from the physical decay of the isotope has not previously been reported in the literature. The first phase of this study was to confirm that cameras used clinically are able to detect differences in radioactivity accurately, assuming they employ the correct matrix to do so. To prove this, we used 37.37 MBq of technetium-99m-sestamibi with both a 64 x 64 matrix and a 128 x 128 matrix. The results of the 64 x 64 and 128 x 128 matrices are shown in figure 1. Over a 55 minute period of time, the expected decay of technetium-99m would be 10%, leaving a residual activity of 90% of the original counts. This represents a *true* "uptake/retention" model since the radioactive compound is retained within the syringe. Using the 64 x 64 matrix, the radioactive counts went from 1,405,721 to 1,251,359 representing an 11% decay with a residual 89% of the original isotope activity. The 128 x 128 matrix reported 3,473,001 at baseline and a count of 2,966,394 55-minutes later for a decay of 14.6% with a residual of 85.4% of the original isotope activity. For this reason, the 64 x 64 matrix more correctly demonstrated the expected decay of the isotope based upon the physical half-life of Tc-99m and was therefore chosen as the standard for performing these studies.

The second question related to the timing of data collection. Prior studies[23,24] demonstrated the ability to detect inflammatory markers on SPECT images at 5 minutes; but, not at later periods of time. Figure 4 displays the results of radioactivity measured using the clinical camera with a 64 x 64 matrix. As shown, the peak detectable radioactivity was measured between 5 and 10 minutes post injection for individuals with "no" coronary artery disease or coronary artery disease without "critical" lesions or "vulnerable" plaques. In individuals with "critical" lesions and/or vulnerable plaques, radioactive counts increase from 5 to 60 minutes (viz. washin)50-52. Combined with the results of instrumentation (figure 1), the ability to detect ischemia based upon blood flow and cellular uptake and release of sestamibi, is best made by comparing counts at 5 minutes and 60 minutes.

The final component of our study compared results of rest-stress imaging and FHRWW with angiographic (%DS) data. There were no differences observed between individuals who were stressed either

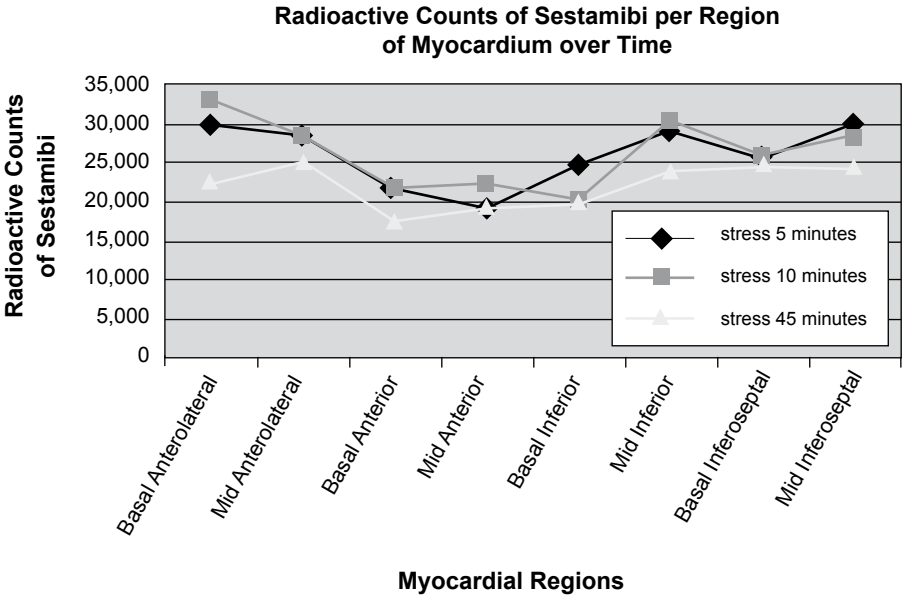

Figure 4: Determination of Optimal Timing of Image Acquisition.
The radioactive counts acquired in eight different myocardial regions vary with time and disease. This graphic demonstrates changes between 5, 10 and 45 minutes post stress. This graph demonstrates that the "uptake/retention" model of technetium-99m-sestamibi is incorrect and that "uptake/release" of technetium-99m-sestamibi occurs throughout the first 45 minutes following stress.

physically or pharmacologically. Outcomes were not changed by the stressor used, as the comparison between rest/stress and FHRWW determination of redistribution was independent of the type of stress. The use of regional wall motion information was used with both the rest/stress protocol and the FHRWW approach, which made it possible to look for wall motion abnormalities and ejection fraction using either approach. Since these observations are identical, regardless of which method was used to report ischemia, we do not report them here.

We analyzed the results of rest/stress image comparisons and FHRWW redistribution images and compared them with findings obtained from coronary angiography.For the rest/stress images, the OR for detecting ischemia was 4.9 with a CI (95%) of 2.3 to 10.3. For FHRWW, the OR was 56.8 with a CI (95%) of 27.5 to 117.2. When the results of these ORs were compared, the t value was greater than 6.6 ($P < .0001$). We obtained results of %DS for each of the epicardial arteries and their branches, and plotted these results against the redistribution results for each of the eight ROIs. This could only be done with the redistribution data, which provided quantitative results, which could be compared with the quantitative results obtained angiographically.

Comparison of the rest/stress image results with the angiographic results showed a sestamibi rest/stress imaging sensitivity of 67%. Of the false negative results (n = 22), 18% (n = 4) had critically narrowed arteries or arteries with vulnerable plaques whose 60-minute images appeared completely normal.[24,25] However, evaluation of the 5-minute images for redistribution (using FHRWW), revealed both qualitative and quantitative decreased uptake, demonstrating a washin effect. The 60-minute post stress images miss this washin phenomena when looked at independent of the 5-minute images, yielding incorrect results.

The standard rest/stress images also yielded a specificity of 88%; of which, only one participant (0.8%) who underwent a coronary angiogram had an adverse outcome requiring cardiopulmonary resuscitation.27

FHRWW calculation was made first by comparing the total radioactive counts of the heart at 5 minutes with the total counts of the heart measured at 60 minutes. The eight regions of interest (ROIs) were then compared using both the 5 and 60-minute image information. Analysis of eight ROIs appears to provide the optimal analysis of specific coronary artery beds (left anterior descending, circumflex and right). These regions include the basal anterior, mid anterior, basal anterolateral, mid anterolateral, basal inferior/posterior, mid inferior, basal inferoseptal and mid inferoseptal. Examples of some of these regions are shown in figures 2, 3, 5 and 6. These multiple examples show how, independent of different color scales and cameras used to qualitatively estimate ischemia; quantitative measurements can be made independently and without interference from hepatic and other tissue uptake. Washout (counts greater at 5 minutes than 60 minutes, excluding the expected isotope decay) and washin (noted counts greater at 60 minutes than 5 minutes) were plotted against the percent diameter stenosis (%DS) determined on coronary angiography. Washin was seen in 15% of the individuals studied and was associated with "normal" appearing 60-minute images. When the results of washout/washin were compared with %DS, a parabolic relationship was demonstrated with $F = 70.4$, $df(1,21)$, $(P=3.8\times10^{-8})$, R(CI95%) = 0.72 to 0.95.

Discussion

The advent of nuclear cardiology by Blumgart[19] in 1925 yielded a new era of medicine with the use of radioactive materials to perform physiologic testing which requires sequential measurements as described by Blumgart to be performed correctly. Unfortunately, decades elapsed[20] before such nuclear materials were made readily available for "clinical" use. The first agent being Thallium-201 (Tl-201) was plagued with limitations including a long half-life (72 hours) resulting in the need to use relatively low doses (6.8-11.1 MBq) of the isotope. Given the limitations57,58 of Compton scatter, tissue attenuation and the emission of mercury x-rays from the isotope, cameras could not acquire adequate counts for imaging until 60 minutes had elapsed. Given the passage of time, much of what Blumgart discussed had been lost to history.[20] ith the advent of Molybdenum-99, Technetium-99m generators, the ability to perform clinical studies using technetium tagged agents (eg. teboroxime, sestamibi, myoview, et cetera) with shorter half-lifes (6 hours) allowing greater doses (up to 1110 MBq) and less Compton scatter and tissue attenuation issues yielded what "appeared to be cleaner images". These images were still plagued with attenuation issues and were reported to be "taken up and retained" within minutes of intravenous injections. Despite the report by DuPont[2]2 and multiple

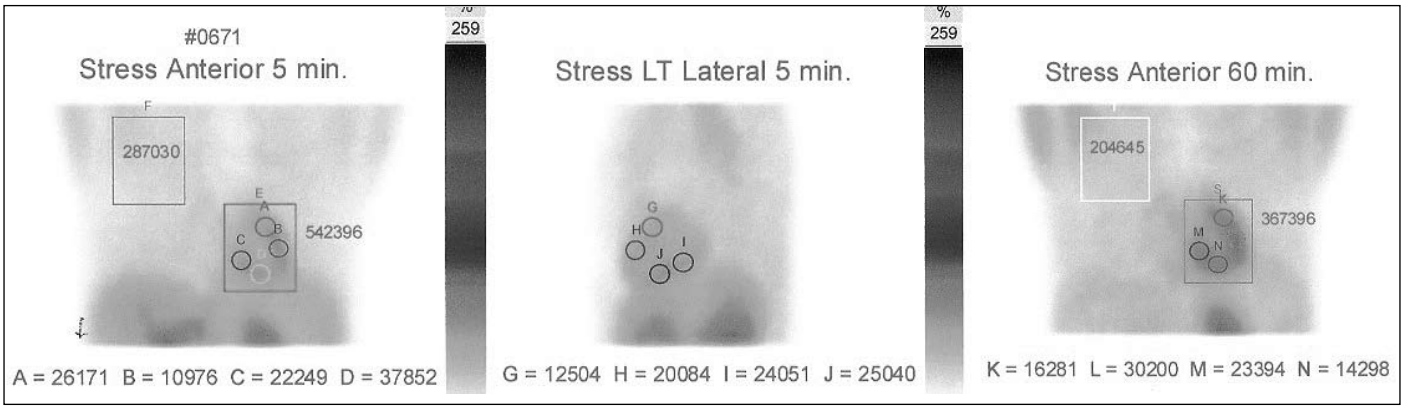

Figure 5: Radioactive Counts Acquired at 5 and 60 Minutes Post Stress.
Anterior images reveal ROI counts at 5 and 60 minutes in the basal anterolateral (A, K), mid anterolateral (B, L), mid inferoseptal (D, N) and basal inferoseptal (C, M) respectively. The counts are shown below the images. The lateral images reveal ROI counts at 5 and 60 minutes in the basal anterior (G, O), mid anterior (H, P), mid inferior (J, R) and basal inferoposterior (I, Q) regions respectively. The total heart and lung counts are not shown or discussed in this paper; however, their ROIs are represented by square boxes of equal size in the anterior images.

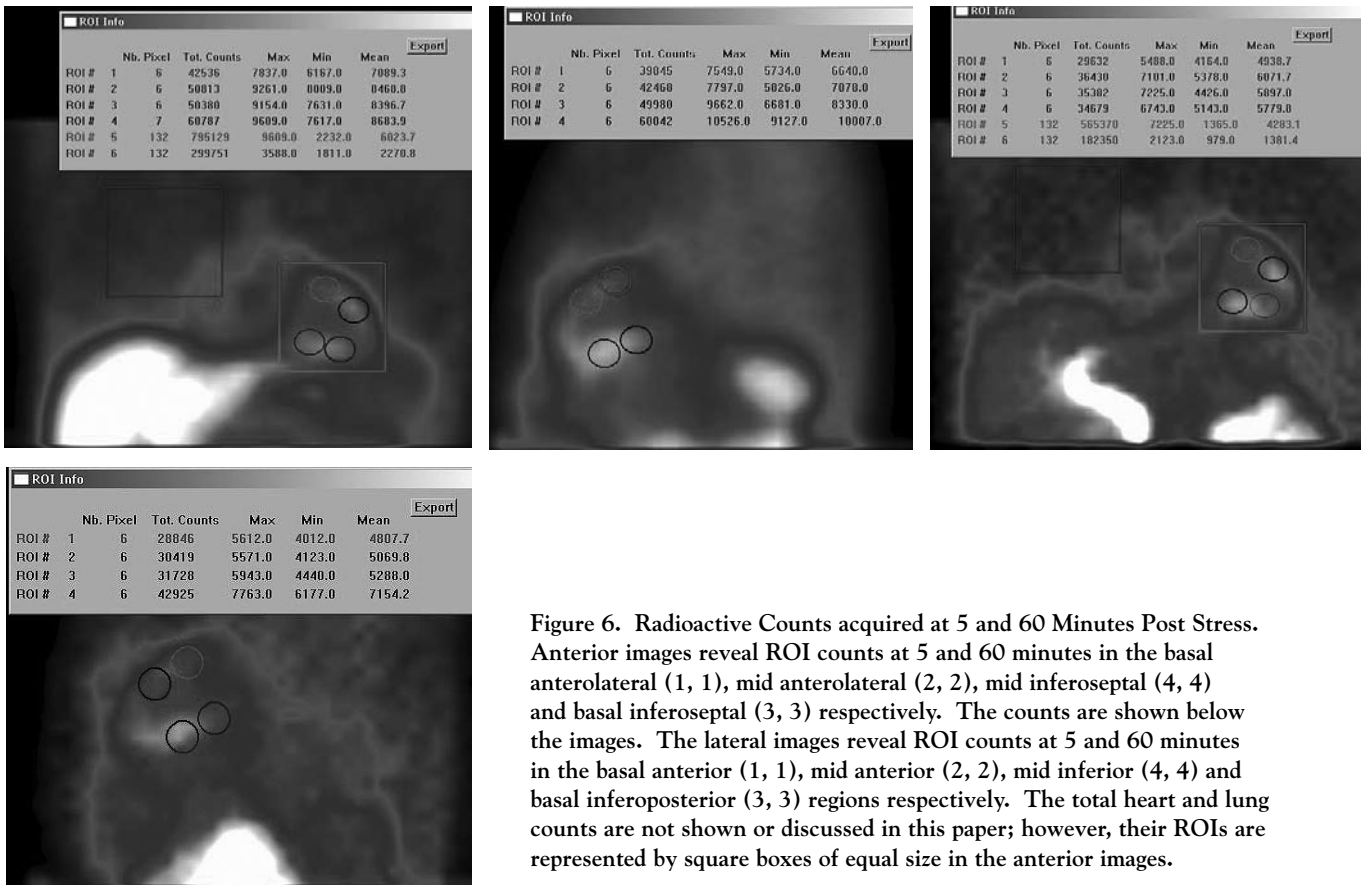

Figure 6. Radioactive Counts acquired at 5 and 60 Minutes Post Stress. Anterior images reveal ROI counts at 5 and 60 minutes in the basal anterolateral (1, 1), mid anterolateral (2, 2), mid inferoseptal (4, 4) and basal inferoseptal (3, 3) respectively. The counts are shown below the images. The lateral images reveal ROI counts at 5 and 60 minutes in the basal anterior (1, 1), mid anterior (2, 2), mid inferior (4, 4) and basal inferoposterior (3, 3) regions respectively. The total heart and lung counts are not shown or discussed in this paper; however, their ROIs are represented by square boxes of equal size in the anterior images.

other research studies[23-49,62] to the contrary, most clinicians have ignored Blumgart's teaching and have limited their investigation to single post-stress imaging despite the teaching of nuclear imaging[60] which notes that "a better indicator of organ function is the change in the activity in a particular organ with time".

Inconsistencies in analysis of cardiac imaging have lead some researchers[63-65] to recommend that these clinical studies be standardized quantitatively; something this study has accomplished. In medical school we were taught that there were two ways to understand the results of a test. The first is to compare it against the population (eg. one obtains an electrocardiogram to look for evidence of ischemia or infarction), the second is to compare it with the results of that test which the patient had previously (eg. poor R-wave progression may actually represent loss of R-waves and evidence of a prior anteroseptal injury/infarction which can be made more confidently in the presence of a change in the electrocardiogram for that patient, opposed to the confidence one has in making such a diagnosis when this is the first electrocardiogram). The same problem applies with looking at a region of the heart on a single 60 minute image post stress. Issues of tissue attenuation including (1) breast artifacts in the anterior wall, (2) diaphragmatic attenuation inferiorly, (3) differences in wall thickness (asymmetric septal hypertrophy) and (4) conduction disturbances influencing timing of systole (left bundle branch morphology) are examples of errors made when one region of myocardium is compared with another region (eg. a single 60 minute image) instead of comparing a region with itself (5 and 60 minute images) and looking for change. Unless there is a change in breast tissue or abdominal obesity between the 5 and 60 minute image, errors resulting from soft tissue attenuation of photon activity are eliminated. The same applies for changes in wall thickness and conduction issues; unless there is a change between the 5 and 60 minute image, the region is being compared with itself and such artifacts are eliminated.

Despite an absence of research to support the ability of "clinical" nuclear cameras to accurately detect changes in radioactive counts which match the expected decay of the nuclear isotope in question clinicians have used these cameras to assess the presence or absence of disease in patients. As Archie Cochrane and history has taught us it is truly dangerous to assume that the way we have been doing something is the correct way to do it in the absence of data to confirm we are right. The first part of this study demonstrated that the cameras we used in this study were able to accurately detect the decay of technetium-99m-sestamibi over a 10% decay. This was best appreciated using the 64 x 64 matrix. Even though manufactures support the use of 128 x 128 matrix as giving greater detail, the use of the 128 x 128 matrix reported a greater decay in the isotope than is physically possible suggesting that information is lost; perhaps as a result of "modulation transfer function" (a concept well known to photographers and imaging engineers).

During the second and third part of our study we looked at the timing sequence, which would yield the most valuable information, not only regarding detecting changes in radioactivity with cardiac tissue, but the ability to detect markers of inflammation[5-7,23,24]. Our previous findings clearly demonstrated the importance of looking for markers of inflammation when detectable, i.e. at 5 minutes post stress. During this study and our most recent work[50-52] we clearly demonstrated that in individuals who had ischemia with non-critical narrowing of arteries, that maximum radioactivity was present during the first 5 to 10 minutes. The inability to maintain the Sestamibi in regions of ischemia has previously been termed "washout" and represents an inability to retain the isotope within the mitochondria in the region. This clearly confirms the work of Crane[22], who demonstrated that

sestamibi redistributes AND is not retained in regions of "ischemia." Our studies also proved this to be the case, independent of which technetium-99m isotope[50] was used or which form of stressor[50-52] was used. Our current study clearly demonstrates this to be true in the "clinical" setting and disproves the "uptake/retention" theory in favor of an continual FHRWW "uptake/release" model which would explain not only the loss of isotope to an ischemic myocardial region over time; but, also accounts for "washin". "Washin" was noted in 15% of the cases where regions of myocardium were supplied by "critically" narrowed arteries and arteries whose "vulnerable plaques" were ready to rupture. In these individuals the combination of severely disturbed flow through critically narrowed and/or unstable coronary lumen passages and relatively large regions of ischemic myocardium with impaired ability to accumulate sestamibi, results in a delay in initial isotope counts. From this perspective, uptake is a function of isotope availability (myocardial blood flow) and cellular health; retention is transient with release of the isotope back into the blood stream or redistribution to other cells, a function of cellular health and re-uptake subject to a cellular refractory period. As a result the delay in isotope delivery is not noted if stress imaging is limited to 60 minute imaging. These individuals, despite "normal" appearing 60 minute images, were at risk of experiencing an acute coronary event with loss of substantial amounts of myocardial tissue.

Conclusion

The standard "uptake/retention" model was observed only in subjects who demonstrated no evidence of coronary ischemia or infarction. In reality, such an "uptake/retention" model is simply an illusion. It is the rapid "uptake/release", "uptake/release", "uptake/release" in non-ischemic, non-infarcted myocardial tissue that makes it appear that the isotope is taken up and retained. In other words, if the amount being released and the amount being taken up are equal, one will not detect a change in isotope count over time, minus the slight decrease occurring from isotope decay. Such individuals would appear to have no change in isotope concentration despite continual turnover of isotope. "Uptake/release" was demonstrated via washout in individuals with coronary ischemia and a delayed "uptake/release" in individuals with washin due to critically narrowed and unstable plaqued regions supplying significant amounts of myocardium. Washin and washout are terms used to convey different types of CHANGE in isotope concentrations which occur over time at different rates to indicate the type of lesions present in the individual being studied. The results of these changes can be compared with changes found on cardiac catheterization as shown in figure 7. As we continue to expand the number of individuals being studied with this method, it is clear that specific regional information can be determined from the parabolic equations used here and graphically displayed in figure 7. The utilization of this knowledge will allow us to better determine which patients will benefit most from coronary angiography and has now been confirmed "qualitatively" by investigators at UCLA and Harvard Universities[66]. With the advent of nuclear cameras capable of fasting image acquisition, we expect that FHRWW will replace rest-stress imaging as the protocol of choice allowing (1) a further reduction in the amount of radioactive material required for ischemic imaging, (2) fasting imaging times (3) with FHRWW the ability to calculate %DS directly from nuclear imaging and (4) when coupled with previously published flow reserve equations, the ability to calculate stenosis[67] flow reserve directly from FHRWW.

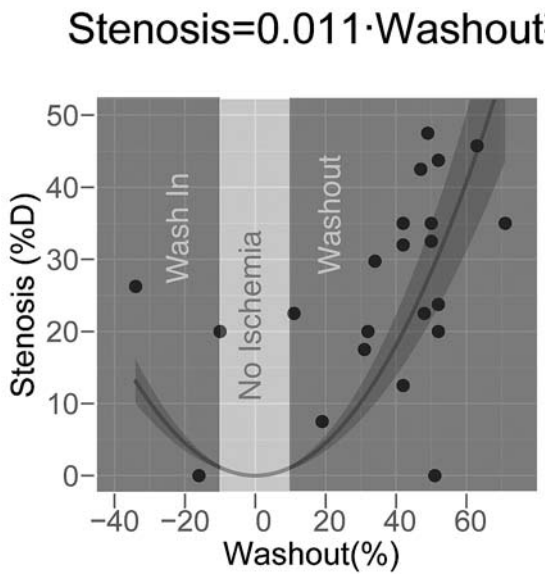

Figure 7. Parabolic Regression of Washout on Percent Diameter Stenosis.
Percent diameter stenosis noted on coronary angiography was plotted against the results of washout. Stenosis = 0.011×Washout2, F = 70.4, df(1,21), (P=3.8×10-8), R(CI95%) = 0.72 to 0.95, Graphic bands show the standard error of the fit. The region of "No Ischemia" is marked in green. "Wash in" is displayed in red to the left of the "No Ischemia" region, while "Washout" is to the right of the "No Ischemia" zone.

References

1. Fleming RM, Kirkeeide RL, Smalling RW, Gould KL. Patterns in visual interpretation of coronary arteriograms as detected by quantitative coronary arteriography. *JACC* 1991;18:945-51.
2. Taki J, Fujino S, Nakajima K, et al. ^{99m}Tc-Sestamibi retention characteristics during pharmacologic hyperemia in human myocardium: Comparison with coronary flow reserve measured by Doppler flowire. *J Nucl Med* 2001;42:1457-63.
3. St. Goar FG, Pinto FJ, Alderman EL, Fitzgerald PJ, Stadius ML, Popp RL. Intravascular ultrasound imaging of angiographically normal coronary arteries: An in vivo comparison with quantitative angiography. *JACC* 1991;18:952-8.
4. Fleming RM, Boyd L, Forster M. Unified Theory Approach Reduces Heart Disease and Recovers Viable Myocardium. 42nd Annual World Congress - International College of Angiology, San Diego, California, USA, June 29, 2000.
5. Fleming RM. The Fleming Unified Theory of Vascular Disease: A Link Between Atherosclerosis, Inflammation, and Bacterially Aggravated Atherosclerosis (BAA). *Angiol 2000*; 51: 87-89.
6. Fleming RM.: Chapter 64. The Pathogenesis of Vascular Disease. Textbook of Angiology. John C. Chang Editor, Springer-Verlag New York, NY. 1999, pp. 787-798.
7. Kern MJ, Lerman A, Bech JW, et al. Physiological assessment of coronary artery disease in the cardiac catheterization laboratory: A scientific statement from the American Heart Association committee on diagnostic and interventional cardiac catheterization, council on clinical cardiology. *Circulation* 2006;114:1321-41.
8. Maddahi J. Myocardial perfusion tracers in the detection and evaluation of coronary artery disease. 48th annual meeting of the

society of nuclear medicine 2001.
9. Fleming RM. Angina and coronary Ischemia are the result of coronary regional Blood Flow Differences. *J Amer Coll Angiol* 2003;1:127-42.
10. Fleming RM., Boyd L., Forster M. Angina is Caused by Regional Blood Flow Differences - Proof of a Physiologic (Not Anatomic) Narrowing, Joint Session of the European Society of Cardiology and the American College of Cardiology, Annual American College of Cardiology Scientific Sessions, Anaheim, California, USA, 12 March 2000, 49th (Placed on internet www.prous.com for physician training and CME credit, April 2000.)
11. Glover DK, Tuiz M, Edwards NC, et al. Comparison between 201Tl and 99mTc Sestamibi uptake during adenosine-induced vasodilation as a function of coronary stenosis severity. *Circulation* 1995;91:813-20.
12. Sinusas AJ, Bergin JD, Edwards NC, et al. Redistribution of 99m-Tc-sestamibi and 201-Tl in the presence of a severe coronary artery stenosis. *Circulation*. 1994;89:2332-41.
13. ahlberg ST, Leppo JA. Physiologic properties of myocardial perfusion tracers. *Cardiol Clin* 1994;12:169-85.
14. Beller GA, Sinusas AJ. Experimental studies of the physiologic properties of technetium-99m isonitriles. *Am J Cardiol* 1990;16:5E-8E.
15. Hurwitz GA, Blais M, Powe JE, Champagne CL. Stress/injection protocols for myocardial scintigraphy with sestamibi and thallium-201: implications of early post-stress kinetics. *Nucl Med Commun* 1996;17:400-9.
16. Husain SS. Myocardial perfusion imaging protocols: Is there an ideal protocol. *J Nucl Med Tech* 2007;35:3-9.
17. Fleming RM., Kirkeeide RL, Taegtmeyer H, Adyanthaya A, Cassidy DB, Goldstein RA. A Comparison of Technetium 99-m Teboroxime Tomography to Automated Quantitative Coronary Arteriography and Thallium - 201 SPECT. *J Am Coll. Cardiol.* 1991;17:1297-1302.
18. Parameswaran RV, Purantharan N, Prasad RD. Comparison of nitrate augmented 99mTc-Sestamibi infusion with nitrate augmented ^{99m}Tc-Sestamibi bolus injection for the detection of myocardial viability. *IJNM* 2006;21:36-40.
19. Blumgart HL, Yens OC. Studies on the velocity of blood flow: I. The method utilized. *Journal of Clinical Investigation*. 1927;4:1-13.
20. Love WD. Isotope Technics in Clinical Cardiology. *Circulation*. 1965;32:309-15.
21. Gorlin R, Brachfeld N, MacLeod C, Bopp P. Effect of nitroglycerin on the coronary circulation in patients with coronary artery disease or increased left ventricular work. *Circulation*. 1959;19:705-18.
22. Crane P, Laliberte R, Heminway S, Thoolen M, Orlandi C. Effect of mitochondrial viability and metabolism on technetium-99m-sestamibi myocardial retention. *Eur J Nucl Med*. 1993;20:20-5.
23. Fleming RM. Using C-Reactive Protein as a Marker of Bacterially Aggravated Atherosclerosis in Acute Coronary Syndromes. *J Amer Coll Angiol* 2003;1:165-71.
24. Fleming RM, Harrington GM. What is the relationship between myocardial perfusion imaging and coronary artery disease risk factors and markers of inflammation? *Angiology* 2008;59:16-25.
25. Pace L, Catalano L, Del Vecchio S, et al. Washout of [99mTc] sestamibi in predicting response to chemotherapy in patients with multiple myeloma. Q J Nucl Med Mol Imaging 2005;49:281-5.
26. Hurwitz GA, Ghali SK, Husni M, et al. Pulmonary uptake of Technetium-99m-Sestamibi induced by dipyridamole-based stress or exercise. *J Nucl Med* 1998;39:339-45.
27. Hurwitz GA, Fox SP, Driedger AA, Willems C, Powe JE. Pulmonary uptake of sestamibi on early post-stress images: angiographic relationships, incidence and kinetics. *Nucl Med Commun* 1993;14;15-22. .
28. Saha M, Forrest TF, Brown KA. Lung uptake of technetium-99m-sestamibi: relation to clinical, exercise, hemodynamic, and left ventricular function variables. *J Nucl Cardiol* 1994;1:52-6.
29. Giubbini R, Bampini R, Milan E, et al. Evaluation of technetium-99m-sestamibi lung uptake: correlation with left ventricular function. *J Nucl Med* 1995;36:58-63.
30. Sugiura T, Takase H, Toriyama T, et al. Usefulness of Tc-99m methoxyisobutylisonitrile scintigraphy for evaluating congestive heart failure. *J Nucl Cardiol* 2006;13:64-8.
31. Kumita S, Seino Y, Cho K, et al. Assessment of myocardial washout of Tc-99m-sestamibi in patients with chronic heart failure: comparison with normal control. *Ann Nucl Med* 2002;16:237-42.
32. Matsuo S, Nakae I, Tsutamoto T, Okamoto N, Horie M. A novel clinical indicator using Tc-99m sestamibi for evaluating cardiac mitochondrial function in patients with cardiomyopathies. *J Nucl Cardiol* 2007;14:215-20.
33. Sugiura T, Takase H, Toriyama T, Goto T, Ueda R, Dohi Y. Usefulness of Tc-99m methoxyisobutylisonitrile scintigraphy for evaluating congestive heart failure. *J Nucl Cardiol* 2006;13:64-8.
34. Ikawa M, Kawai Y, Arakawa K, et al. Evaluation of respiratory chain failure in mitochondrial cardiomyopathy by assessments of 99mTc-MIBI washout and 123I-BMIPP/99mTc-MIBI mismatch. *Mitochondrion* 2007;7:164-70.
35. Meissner K, Sperker B, Karsten C, et al. Expression and localization of P-glycoprotein in Human Heart: Effects of Cardiomyopathy. *J Histochem Cytochem* 2002;50:1351-6.
36. Ono S, Yamaguchi H, Takayama S, Kurabe A, Heito T. Rest delayed images on ^{99m}Tc-MIBI myocardial SPECT as a noninvasive screen for the diagnosis of vasospastic angina pectoris. *Kaku Igaku* 2002;39:117-24.
37. Ono S, Yakeishi Y, Yamaguchi H, et al. Enhanced regional washout of technetium-99m-sestamibi in patients with coronary spastic angina. *Ann Nucl Med* 2003;17:393-8.
38. Fukushima K, Momose M, Kondo C, et al. Myocardial kinetics of (201) Thallium, (99m) Tc-tetrofosmin, and (99m) Tc-sestamibi in an acute ischemia-reperfusion model using isolated rat heart. *Ann Nucl Med* 2007;21:267-73.
39. VanBrocklin HF, Hanrahan SM, Enas JD, et al. Mitochondrial avid radioprobes. Preparation and evaluation of 7'(Z)-[125I] iodorotenone and 7'(Z)-[125I]iodorotenol. *Nucl Med Biol* 2007;34:109-16.
40. Tanaka R, Nakamura T, Chiba S, et al. Clinical implication of reverse redistribution on 99mTc-sestamibi images for evaluating ischemic heart disease. *Ann Nucl Med* 2006;20:349-56.
41. Liu Z, Johnson G 3rd, Beju D, Okada RD. Detection of myocardial viability in ischemic-reperfused rat hearts by Tc-99m sestamibi kinetics. *J Nucl Cardiol* 2001;8:677-86.
42. Shin WJ, Miller K, Stipp V, Mazour S. Reverse redistribution on dynamic exercise and dipyridamole stress technetium-99m-MIBI myocardial SPECT. *J Nucl Med* 1995;36:2053-5
43. Takeishi Y, Sukekawa H, Fujiwara S, et al. Reverse redistribution of technetium-99m-sestamibi following direct PTCA in acute myocardial infarction. *J Nucl Med* 1996;37:1289-94.
44. Fujiwara S, Takeishi Y, Atsumi H, et al. Prediction of functional recovery in acute myocardial infarction: comparison between sestamibi reverse redistribution and sestamibi/BMIPP mismatch. *J Nucl Cardiol* 1998;5:119-27.
45. Ayalew A, Marie PY, Menu P, et al. A comparison of the overall

first-pass kinetics of thallium-201 and technetium-99m MIBI in normoxic and low-flow ischaemic myocardium. *Eur J Nucl Med* 2000;27:1632-40
46. Richter WS, Cordes M, Calder D, Eichstaedt H, Felix R. Washout and redistribution between immediate and two-hour myocardial images using technetium-99m sestamibi. *Eur J Nucl Med* 1995;22:49-55.
47. Meerdink DJ, Leppo JA. Myocardial transport of hexakis(2-methosyisobutyl isonitrile) and thallium before and after coronary reperfusion. *Circulation Research* 1990;66:1738-46.
48. Ayalew A, Marie PY, Menu P, et al. 201 Tl and 99m Tc-MIBI retention in an isolated heart model of low-flow ischemia and stunning: Evidence of negligible impact of myocyte metabolism on tracer kinetics. *J Nucl Med* 2002;43:566-74.
49. akahashi N, Reinhardt CP, Marcel R, Leppo JA. Myocardial uptake of 99m Tc-tetrofosmin, Sestamibi, and 201 Tl in a model of acute coronary reperfusion. *Circulation* 1996;94:2605-13.
50. Fleming RM, Harrington GM, Baqir R. Heart Disease in Men. Chapter 3. Using Multiple Images Post-Stress to Enhance diagnostic Accuracy of Myocardial Perfusion Imaging: The Clinical Importance of Determining Washin and Washout Indicates a Parabolic Function between Coronary Perfusion (Blood Flow) and Cellular ("Uptake/Release") Function. Alice B. Todd and Margo H. Mosley Editors, Nova Publishers, 2009, pp. 75-100. (https://www.novapublishers.com/catalog/product_info.php?products _id=8409)
51. Fleming RM, Harrington GM, Baqir R, Jay S, Sridevi Challapalli, Avery K, Jim Green. The Evolution of Nuclear Cardiology takes Us Back to the Beginning to Develop Today's "New Standard of Care" for Cardiac Imaging: How Quantifying Regional Radioactive Counts at 5 and 60 Minutes Post-Stress Unmasks Hidden Ischemia. *Methodist DeBakey Cardiovascular Journal* (MDCVJ) 2009;5(3):42-48.
52. Fleming RM, Harrington GM, Baqir R, Jay S, Challapalli S, Avery K, Green J. Renewed Application of an Old Method Improves Detection of Coronary Ischemia. A Higher Standard of Care. *Federal Practitioner* 2010;27:22-31.
53. Matsunari I, Haas F, Nguyen NTB, et al. Comparison of Sestamibi, Tetrofosmin, and Q12 Retention in Porcine Myocardium. *J Nucl Med* 2001;42:818-23.
54. Calnon DA, Glover DK, Beller GA, et al. Effects of dobutamine stress on myocardial blood flow, ^{99m}-Tc sestamibi uptake, and systolic wall thickening in the presence of coronary artery stenoses: implications for dobutamine stress testing. *Circulation* 1997;96:2353-60.
55. Calnon DA, Ruiz M, Vanzetto G, et al. Myocardial uptake of ^{99m}Tc-N-NOET and 201 Tl during dobutamine infusion. Circulation 1999;100:1653-9.
56. *Fleming* RM.: Chapter 31. Nuclear Cardiology: Its Role in the Detection and Management of Coronary Artery Disease Textbook of Angiology. John C. Chang Editor, Springer-Verlag New York, NY 1999, pp. 397-406.
57. Fleming RM, Rose CH, Feldmann KM. Comparing a High Dose Dipyridamole SPECT Imaging Protocol with Dobutamine and Exercise Stress Testing Protocols. *Angiology* 1995;46(7):547-556
58. Fleming RM, Gibbs HR, Swafford J. Using Quantitative Coronary Arteriography to Redefine SPECT Sensitivity and Specificity. *Am J Physiol. Imag.* 1992;7:59-65.
59. Fleming RM, Detecting Coronary Artery Disease Using SPECT Imaging: A Comparison of Thallium-201 and Teboroxime. *Am J Physiol Imag* 1992;7(1):20-23.
60. Aodah B. "Nuclear Medicine and Gamma Camera" Instructional Course from the King Saud University College of Applied Medical Science, Biomedical Technology Department. 2007.
61. Everett DB, Fleming JS, Todd RW, Nightingale JM. Gamma-radiation imaging system based on the Compton effect. *Proceedings of the IEEE* 1977;124:995-1000.
62. Fallahi B, Beiki D, Gholamrezanezhad A, et al. Single Tc99m Sestamibi injection, double acquisition gated SPECT after stress and during low-dose dobutamine infusion: a new suggested protocol for evaluation of myocardial perfusion. *Int J Cardiovasc Imaging* 2008. Jun 18. [Epub ahead of print]
63. Researchers make case for standardized analysis of cardiac imaging. Oregon Heart & Vascular Institute, University of Oregon and L.A.'s Cedar-Sinai. March 13, 2008. http://pmr.uoregon.edu
64. Fleming RM. A Tate-en-Tate Comparison of Ejection Fraction and Regional Wall Motion Abnormalities as Measured by Echocardiography and Gated Sestamibi SPECT. *Angiology* 2002;53:313-21.
65. Toriyama T, Takase H, Sugiura T, et al. ^{99m}Tc-sestamibi scintigraphy in the prediction of cardiac events in patients with congestive heart failure. 7th International conference of Nuclear Cardiology-European Society of Cardiology 2005.
66. Sheikine Y, Berman DS, Di Carli MF. Technetium-99m-sestamibi redistribution after exercise stress test identified by a novel cardiac gamma camera: Two case reports. *Clin Cardiol.* 2010;33:E39-E45.
67. Fleming RM, Harrington GM. Quantitative Coronary Arteriography and its Assessment of Atherosclerosis. Part 2. Calculating Stenosis Flow Reserve Directly from Percent Diameter Stenosis. *Angiology* 1994;45(10):835-840.