Breast Enhanced Scintigraphy Test (B.E.S.T.) Imaging Utilizes Vascularity/Angiogenesis and Mitochondrial Activity to Distinguish Between Normal Breast Tissue, Inflammation and Breast Cancer.

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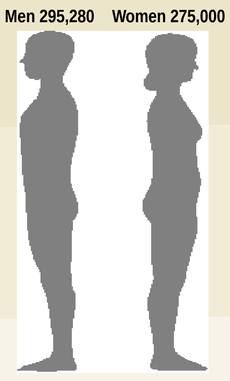
Background: Breast cancer remains the number one cause of cancer deaths among women in the US. Current testing modalities utilize anatomic approaches to detect changes in breast tissue. Nuclear imaging utilizes isotopes to detect physiologic changes in tissue. One of these isotopes, Sestamibi, is actively taken up by mammalian and plant cells alike. In mammalian tissue it is actively taken up by the mitochondria while in plant cells it is taken up by chloroplasts. The mitochondria and chloroplasts are the energy organelles of living tissue. These organelles are present in varying concentrations in normal, inflammatory (wbc's, fibroblasts) and tumor cells. Additionally, the delivery of an isotope to a given tissue is dependent upon a transport mechanism. Within the body, this system is primarily the blood stream. Regions of greater vascularity (eg. Inflammation and/or cancers) deliver greater concentrations of isotope for tissue detection. We have previously shown that the utilization of dipyridamole augments the delivery of isotopes to tissue through enhancement of blood flow. This study was designed to determine if the simultaneous enhancement of blood flow using dipyridamole and measurement of isotope activity using specialized software could distinguish between normal breast tissue, inflammation and breast cancer.
Methods: Three hundred and twenty-seven non-pregnant women between 30 and 60 years of age were studied following referral from their primary care physician. Women enrolled in the study either had a family history of breast cancer, an abnormality on physical exam, mammogram or other concerns (eg. Tumor markers). A history of hormone therapy use was also recorded to look for differences in imaging results. Biopsies of suspicious lesions were taken following nuclear imaging. B.E.S.T. imaging was performed in a fasting state. An intravenous catheter was placed followed by a four-minute infusion of dipyridamole. Two minutes later, Sestamibi was given and imaging began 5-10 minutes later. Subjects were placed in a prone position for lateral views of the breast. Each image took 10 minutes to acquire. Images were then quantified using a specialized software package to analyze the regions of interest (ROI). ROIs were quantified for maximal count activity (MCA) and average count activity (ACA) along with standard deviation of counts.
Results: Differences in MCA and ACA were significant between "normal" and "fibrocystic disease" ($p<0.001$), "fibrocystic disease" and "atypia" ($p<0.001$) and "atypia" and "cancer" ($p<0.05$). Differences between tissue types were confirmed by pathology data. Differences within groups existed for women who were taking hormone therapy and those who did not take hormone therapy ($p<0.05$).

2005 Estimated US Cancer Deaths*

Lung and bronchus	31%
Prostate	10%
Colon and rectum	10%
Pancreas	5%
Leukemia	4%
Esophagus	4%
Liver and intrahepatic bile duct	3%
Non-Hodgkin Lymphoma	3%
Urinary bladder	3%
Kidney	3%
All other sites	24%

Men 295,280 Women 275,000



- 27% Lung and bronchus
- 15% Breast
- 10% Colon and rectum
- 6% Ovary
- 6% Pancreas
- 4% Leukemia
- 3% NoHodgkin lymphoma
- 3% Uterine corpus
- 2% Multiple myeloma
- 2% Brain/ONS
- 22% All other sites

ONS=Other nervous system.
Source: American Cancer Society, 2005

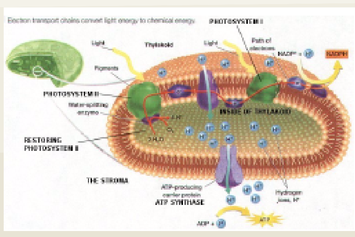
Detection of Breast Cancer Utilizes

•Anatomic Testing
-Mammography
-Ultrasound
-MRI
•Physiologic Testing
-PET (in addition to CT/MRI)
-SPECT imaging

Physiologic Testing

- Uses properties of cellular function to detect the presence or absence of normal and abnormal tissue.
- Depends upon delivery of the testing agent (in this case a nuclear isotope) to the area of interest, and
- A method for detecting the testing agent (in this case a nuclear SPECT camera).

Sestamibi Uptake Within Cells is Dependent Upon Electrochemical Gradients.



- Isotope delivery is dependent upon blood supply to the area or region of (ROI) interest.
  - The greater the blood supply, the greater the delivery of the isotope for detection
- Sestamibi uptake within cells is dependent upon the organelles which produce the energy for those cells. These organelles produce electrochemical gradients as a property of their energy production.
  - In plants sestamibi is taken up by chloroplasts, while in mammalian tissue sestamibi is taken up by mitochondria.

Original Concept

- Sestamibi was FDA approved for the detection of breast cancer in the mid 1990s after earlier work with heart disease revealed breast and lung cancers.
- The imaging study was performed using a resting approach where three sets of images were taken by a SPECT camera 10 minutes after the intravenous injection of
  - 925-1110 mBq (25-30 mCi) of Sestamibi
- This approach was named MIRALUMA.

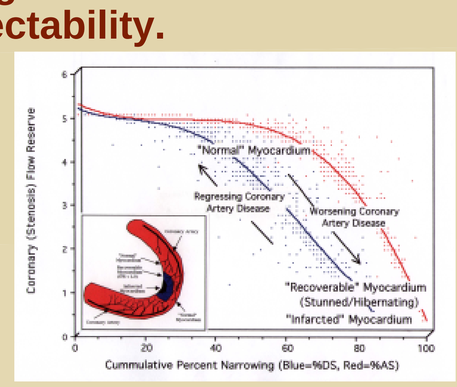
- Three sets of images are obtained 10 minutes after the isotope had circulated throughout the body.
- These images are obtained (10 minutes each) with the patient lying prone on a 6 inch mattress with the breasts in a dependent position.
- The quality of these black and white images lead to concerns about false positive findings.

Using the Properties of Cancers to Enhance Their Detection.

- Cancers have greater metabolic activity than normal tissue does. This greater metabolic activity is associated with a greater number of mitochondrial to carry out energy production.
- Similarly, inflammatory tissue (leukocytes, etc.) are more metabolically active than normal tissue, yet less metabolically active than cancers.
- Cancers require increased blood supply to maintain their greater metabolic activity. To obtain this greater blood supply (nutritional support), they produce new blood vessels (angiogenesis).

Using the Angiogenesis of a Cancer to Increase its Detectability.

- Detection of ischemic coronary artery disease requires "stress" imaging to detect regional differences in coronary flow (CFR) reserve.
- This change in CFR can be brought about either by
  - Exercise or
  - Pharmacologic stress
- This same approach can be used to augment blood flow elsewhere in the body

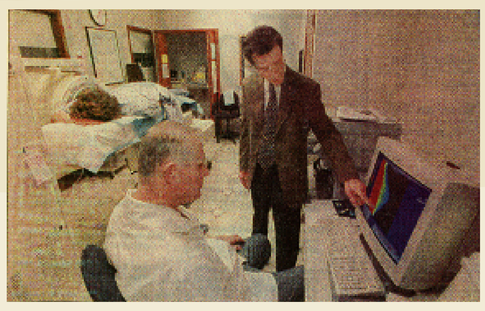


Study Proposal

- If cancers have greater metabolic activity than inflammatory tissue and inflammatory tissue has greater metabolic activity than normal tissue, and
- If cancers have a greater blood supply than normal tissue, then
- Enhancing the delivery of the isotope (sestamibi) to a cancer using the same technique employed for myocardial perfusion imaging (eg. Dipyridamole), should increase the delivery and uptake of sestamibi within a cancer.
  - This difference should be detectable after measuring the amount of radioactivity within a ROI of normal tissue, inflammatory breast tissue (eg. Fibrocystic disease) and breast cancer.

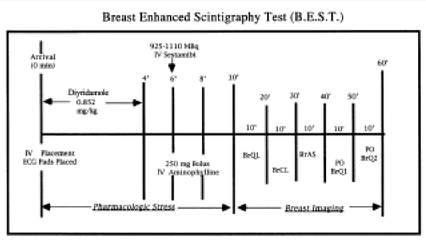
Initial Comparison of Miraluma & Enhanced Approach.

- 10 participants were studied on two separate days.
- On the first day a resting (Miraluma) approach was used.
- On the second day, the enhanced (Breast Enhanced Scintigraphy Test) approach was used.



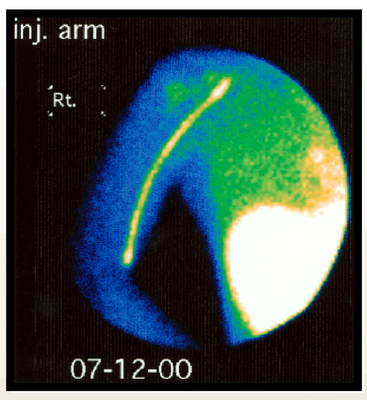
Breast Enhanced Scintigraphy Test.

- Subjects arrive in the morning in a fasting state.
- A catheter is placed in the right antebrachium unless there is an abnormality within the right breast and then the catheter is placed in the left antebrachium.
- Through the IV catheter, both the dipyridamole (to enhance blood flow) and the isotope (sestamibi) is delivered, after which breast images are taken as shown below.

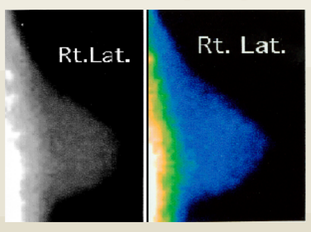


Sequence of Events.

- Once the isotope is injected, it can be traced going through the brachial veins into the subclavian vein (right).
- Following image acquisition and reconstruction, using a 75 PMT SPECT camera with a LEHR collimator and intrinsic resolution of 3.4 mm, images are then displayed.



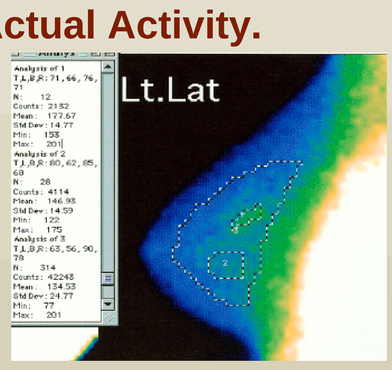
Initial Display & Conversion

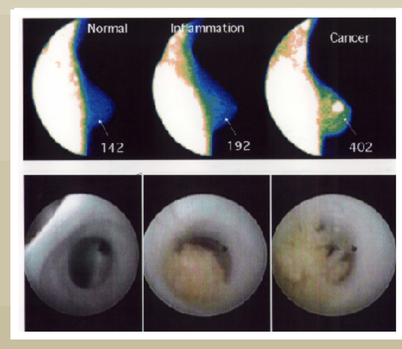


- These images are initially displayed using a black & white format.
- They are then converted to a green-blue image to enhance visual detection of abnormal tissue.

Quantification of Actual Activity.

- Regions of interest (ROIs) are then quantified to determine differences in sestamibi uptake within breast tissue.
- ROI-1 had an average count activity (ACA) of 178 +/- 15.
- ROI-2 had an ACA of 147 +/-15.
- ROI-3 had an ACA of 134 +/- 25.

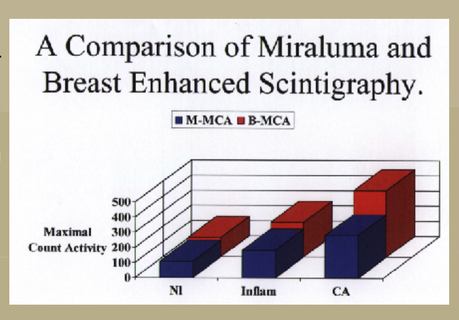




BEST & Miraluma Imaging Results Were Then Compared With Biopsy Information.

The Maximal Count Activity (MCA) Reveals the Greatest Accumulation of Sestamibi Within the Breast and Provides the Best Distinction Between Normal, Fibrocystic (Inflammation) Disease and Breast Cancer.

- The difference in isotope activity between Miraluma (M-MCA) & BEST (B-MCA)was negligible in normal breast tissue.
- This difference increased with breast inflammation.
- The difference between resting and enhanced approaches was statistically ($P<0.05$) significant for breast cancer.

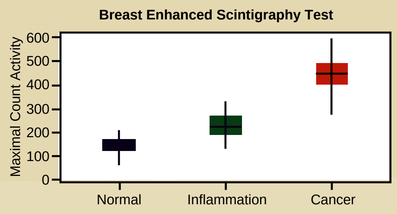


The Next Phase of Reasearch

- The second phase of this research looked at 100 women with a breast lump, family history of breast cancer or abnormal mammogram.
- There was a 20% incidence of breast cancer in this group.
  - Mammography and BEST agreed 76% of the time.
  - Mammography sensitivity was 69% and specificity was 84%.
  - BEST sensitivity was 95% and specificity was 100%.

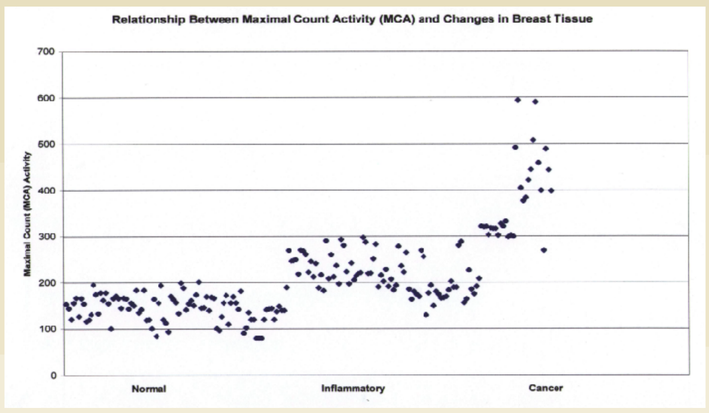
BEST Imaging MCAs

- For normal breast tissue, the MCA was 144 +/- 30 (95% CI:138-150)
- For inflammatory changes of the breast, the MCA was 229 +/- 50 (95%CI:218-240)
- For breast cancer the MCA was 446+/-80 (95%CI:403-489).



P<0.05 between normal and inflammation
P<0.05 between inflammation and cancer

There is a Curvilinear Relationship Between MCA and Changes in Breast Tissue Indicating Changes in Both Mitochondrial Content/Activity and Differences in Blood Flow.



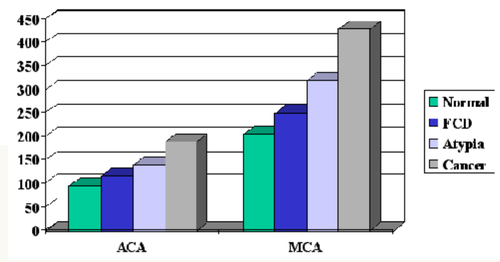
Third Stage of Research

- 327 women, including 139 taking hormone replacement therapy and 188 not taking hormone therapy were studied, looking at ACA, MCA and the variability of count activity within the breast tissue.
- Fibrocystic disease (FCD) had a statistically ($p<0.01$) greater count activity than normal tissue.

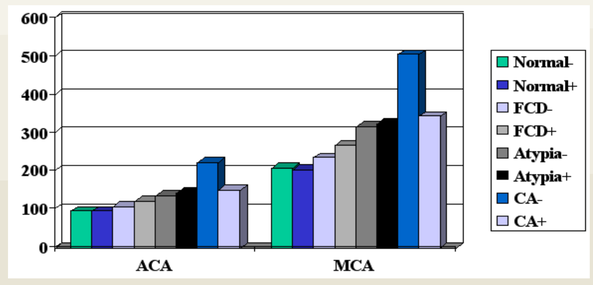
Further Differentiation Based Upon "Atypia" and Count Variability.

- Further efforts were made to subcategorize women with "atypia" who had a statistically ($p<0.01$) greater count activity than women with FCD.
- Differences between "atypia" and breast cancer were statistically ($p<0.01$) higher for women with breast cancer.
- There was a statistically ($p<0.05$ to 0.01) greater variability of breast tissue uptake of sestamibi in women who took hormone replacement therapy.

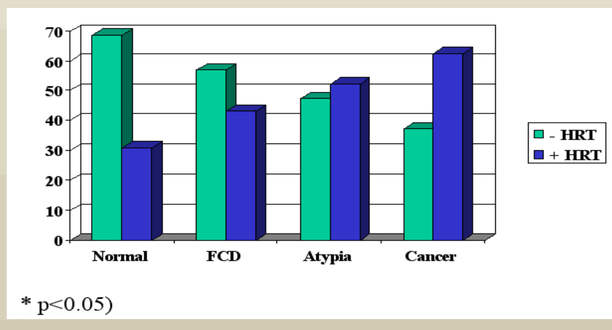
Differences in ACA & MCA.



Differences Between ACA & MCA Among Women Who Were (+) and Were Not (-) Taking HRT.



There Was a Statistically* Greater Incidence of Breast Cancer Among Women Taking HRT.

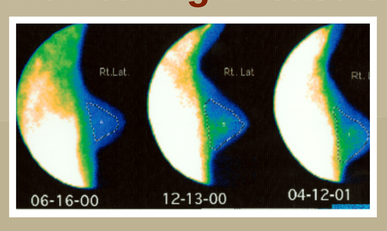


* $p<0.05$)

BEST Imaging

- Enhancement of isotope delivery using dipyridamole results in increased delivery of the isotope to the breast in states of inflammation, atypia and cancer compared with the resting approach (Miraluma).
- This enhanced delivery results in greater mitochondrial uptake as quantitatively measured and displayed as average (ACA) and maximal (MCA) count activity.
- There is increased breast tissue variability in women who are taking hormone replacement therapy.
- These same women have a greater incidence of breast cancer when compared with women who do not take HRT.

Future Directions for BEST Include Earlier Detection of Breast Cancer & Monitoring Breast Changes



- Changes can be tracked over time.
  - 6-16-00 MCA was 142
  - 12-13-00 MCA was 165
  - 4-12-01 MCA was 240
  - Biopsy on 6-20-01 showed DCIS

And Treatment Response

- This patient has had recurrent breast cancer.
- Following radiation therapy, the cancer shows metabolic death with no evidence of mitochondrial activity