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2022 SCREENING SURVEY / TREND STUDIES OF DENSE BREAST CASES

A Clinical Research Collaborative Report

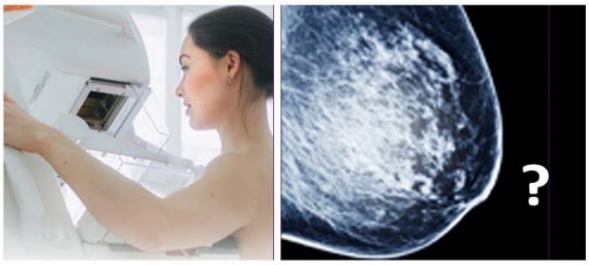
CONTRIBUTORS:

N. Cutter, PhD	-	Co-Principal Investigator / Field Imaging / Bio-Science Officer (Molloy Univ. Research Lab)
R. Bard, MD	-	Co-Principal Investigator / Sr. Medical Diagnostic imaging Dir. (Bard Diagnostics Lab, NYC)
R. Kline, MD	-	Clinical/Scientific Advisor / Epigenetics Analyst & Educational Reporter
L. Goetze, EdD	-	Research Data Coordinator / Medical Communications dir.
N. Novack, PhD	-	Community Outreach Support / Strategic Advisor (NancysList.org)
A. Fiederlein	-	Recruiter & Community Outreach support (ICRS/Integrative Cancer Resource Soc.)
J. Cappello	-	Recruiter & Community Outreach support (Are You Dense? Foundation)
C. R-Dewitt	-	Report editor / Publishing liaison
L. V-Montoya	-	Recruiter & Community Outreach support (ICRS/Integrative Cancer Resource Soc.)
M. Nielsen	-	Community Outreach Support / Strategic Advisor (Women's Health Digest)

ACADEMIC PREFLIGHT STUDY OF ULTRASOUND SCREENING APPLICATIONS FOR DENSE BREAST CASES

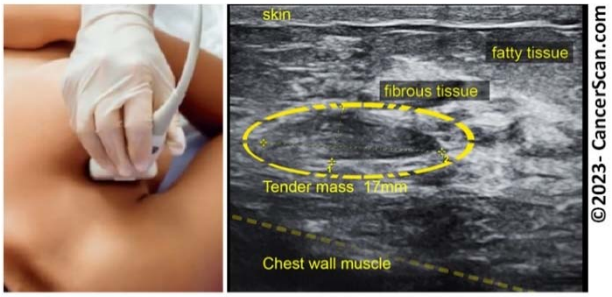
Introduction

Major cases and reports of dense breast tissue nationwide have been historically aligned with the higher risk of developing breast cancer. For 30+ years, criticism over the performance of mammography scans of dense breast tissue (appearing as a white film) tends to mask/cloak over small cancerous tumors which also appears white on a mammogram. This problem often leads to a FALSE POSITIVE REPORT- contributing to undetected cancer cases and potential mortality rates. To date, mammography remains as the national standard screening test for early detection of breast cancer including for the 35-40% of the population of women who have mostly dense breasts.



Inconclusive Mammo readings through tissue density

For women with any level of breast density, the reported rate of false positives potentially aligning with late stage cancers missed by a mammogram has proven to be a national health crisis. Also, a second concern about dense breasts having a naturally higher risk of breast cancer (than women with fatty breasts) is added alarm. (This increased risk is separate from the effect of dense breasts on the ability to read a mammogram.)



Ultrasound tumor detection through tissue density

Scientific Abstract

Breast density is associated with breast cancer risk in women; however, much work is still needed to understand the connection of breast density and increased lifetime risk of breast cancer. Physical activity is considered a significant modifiable factor in breast cancer risk, and since exercise reduces fatty tissue and BMI, it has been thought to increase breast density. However, studies into the relationship between physical activity and breast density have been inconclusive. Currently, New York State has one of the highest incidences of breast cancer especially in pre-menopausal women. Our aim is to compare the association between breast density and risk of invasive breast cancer among pre-menopausal women with low BMI. Our prospective cohort study will use currently available data from the Breast Cancer Surveillance Consortium from pre-menopausal women in NYS as well as exploratory studies in the recruitment of new patients who fit these criteria. Furthermore, we plan to use tissue expression array analysis to help delineate the molecular pathways involved in the development of invasive breast cancer by identifying biomarkers associated with dense breast tissue. Our findings will add potential value for the early detection of poor-outcome breast cancer as well as understand the link with breast density and increased risk of breast cancer in pre-menopausal women.

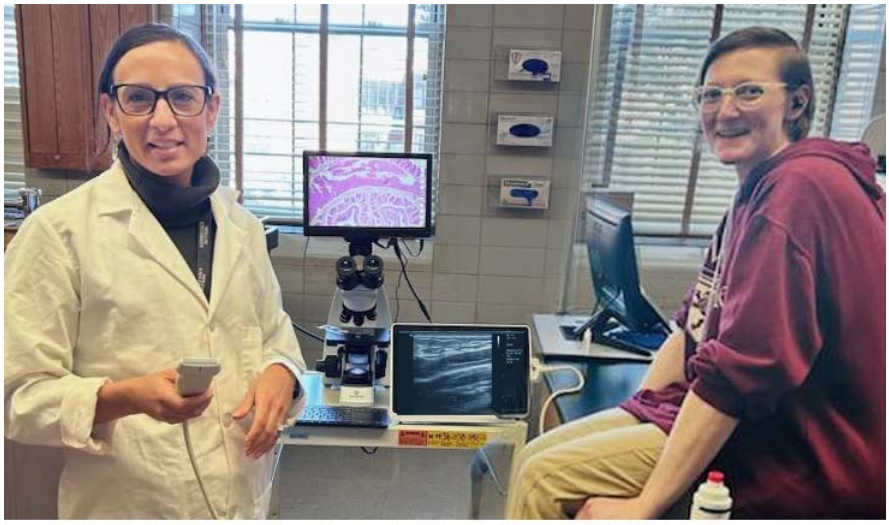
Background of Executable Concepts:

Major cases and reports of dense breast tissue nationwide have been historically aligned with the higher risk of developing breast cancer. For 30+ years, criticism over the performance of mammography scans of dense breast tissue (appearing as a white film) tends to mask/cloak over small cancerous tumors which also appears white on a mammogram. This problem often leads to a FALSE POSITIVE REPORT- contributing to undetected cancer cases and potential mortality rates. To date, mammography remains as the national standard screening test for early detection of breast cancer including for the 35-40% of the population of women who have mostly dense breasts.

Part 1: FIELD REPORT FROM SCANNING TEAM 1

By: Noelle Cutter, Ph.D.

We started working with the Terason in the summer of 2022. The goal of the project was to be able to scan a limited number of younger premenopausal women under the age of 40 who are more likely to have dense breasts based on their status as being endurance based athletes. We have been exclusively using the Terason 3200 based on the recommendation of our lead radiologist Dr. Robert Bard. The Terason became our lab scanner of choice to conduct the screening of 10 individuals in the lab on a voluntary basis to get a readout for their breast density. Endurance athletes are more prone to dense breast tissue, especially athletes under the age of 40. Mammographic density is the strongest risk factor for non-familial breast cancer among women, especially those under the age of 40. Metastasis, tumor relapse, and resistance to therapy remain the principal causes of death for breast cancer patients and the lack of effective therapies calls for an improved understanding of the molecular mechanisms driving breast cancer progression. Early identification of mammographic density is an essential step in preventative health.



Dr. Noelle Cutter (L) co-authored the 2021 Dense Breast Cohort Study. Remote scanning stations include the Molloy University Lab and Bard Diagnostic Imaging center in NYC.

SELECTED CASES

We assembled 10 volunteers for our initial review. These subjects were all selected from the triathlete community who recently either participated in the full distance 140.6 Ironman or the half distance 70.3 Ironman in 2022. From Molloy University (base 1 of our research), I recruited from our student population who also worked in the lab- as well as from my professional contacts in the endurance world.

To note, out of our select volunteers, three have been clinically diagnosed with breast cancer within the last five years. I would say that out of the two women who had cancer, both had dense breasts, and both were Ironmann athletes under the age of 40 when they were diagnosed. They're both over the age of 40 now. So, they knew that already going into our scanning. I am glad to also report that both cases remain in remission. Noting these cases, we did not consider using them as a control by any means, only that this is part of the data that we are collecting as additional considerations that relate to our survey for tissue density, as a means of comparison.

The other eight subjects were young women all under the age of 35- and none of them had any idea about breast density. They've never had any breast screenings in their life besides self-breast examinations- hence, they had no idea about density. Furthermore, they had no idea of how mammographic density is really one of the strongest risk factors for non-familial breast cancer among women and especially among younger women. Undergoing our 'simple' screening session gave them the beginnings of a sense of personal ownership and advocacy about their health. Perhaps this experience (for them) could lead to proactive steps that they need to take- the next time they visit with either their OBGYN or their general physician. They now have an idea about the types of tests to request to be proactive about their health. They also have some clarity about mammographic density in their breasts.

PORTABLE, HOSPITAL GRADE AND FIELD FRIENDLY

My technicians and I found the Terason to be "super portable" and upon scanning our first few subjects, we immediately understood why Dr. Bard suggested this brand and model. The picture quality and imaging system is very clear and the user interface is very easy to read. For someone who is not experienced or well trained in using an ultrasound, with one or two training sessions I was able to easily use the machine to work with our volunteer subjects.

An underlying test is the feasibility of using the Terason itself in actual field work. The portability is astounding (to me). This means we can pack it up. I don't even need any sort of a car. I could throw it on my back and get on a bicycle and bring it to a, uh, a destination. As far as its' performance, my group and I did our own homework about its accuracy and reliability and what we often found was the common statement about it being "battle-tested"- in the EMS world). Targeting the triathlete community, all these features are critical advantages to roadside screening! It's easy to use and even easier to train other research assistants in the lab to use it. And with Dr. Bard (and his expert interpretation) on the receiving end of our scans, that is the ultimate peace of mind and a COMPLETE tele-health scanning paradigm.

To note, our research concept focused on using the ultrasound because it is completely safe and effective at scanning for (something like) image breast density. Particularly for our research, it's portable in the field, so we can bring it to the race locations. I can bring it into the lab, I can bring it into my office. I can really package it up in a small briefcase sized bag, and it can come with me and travel with me anywhere I need to go.

Prof. Noelle Cutter receives a complete ultrasound instructional tour from Terason's remote/WIFI interactive trainer.

TRAINER

Working with the Terason virtual trainer (Mike) made learning the ultrasound device surprisingly easy, and he thoroughly guided me over the phone and via the device itself which is also a computer. He gave us such a comprehensive tour on all of the different probes but of course, we were focused on the one (L20) probe that was suited for of screening of breast density. We found the device itself to cover the most common demands of a hospital-grade or field-grade ultrasound including the ability to capture measurements of critical data like blood flow and density. Recalling my initial training session with Dr. Bard (at his office) aligned with everything because as a field/remote ultrasound tech, I am literally capturing all images for him (our in-house radiologist). This Terason model truly enables this remote scanning paradigm between the field screening tech in communication with the central radiologist- making for a truly ideal and real time professional collaboration!

A VITAL RESEARCH PROJECT TO PREVENTION AND EARLY DETECTION

I believe that because our focus was on women with dense breasts and the fact that this issue is now showing escalating rates, the urgency for this project also aligns with an increased likelihood of developing cancer within their lifetime. We also know that women, especially pre-menopausal women, are often not sent for imaging unless they have a family history. But we know only about five to 10% of breast cancers occur because of an inherited or genetic variant. So the likelihood that we're missing a lot of women (especially young women) who may have an early stage of breast cancer is the big concern.

This project was developed out of the need for a safe, effective, and affordable screening alternative. We wanted to explore and possibly attest to the higher benefits of this modern (and field-friendly) alternative vs. the existing "gold standards" which are far more inaccessible, expensive and potentially invasive- as far contrast or radiation. I appreciate my understanding of ultrasound as a fully safe and effective imaging modality - and one that we can use and easily can be accessed in any physician's office or anywhere else. After our dedicated training course, the roll-out to being a stage 1 ultrasound technician was very comfortable and truly supportive of our goals about ultrasound as the path to the future and "the better way".

As far as goals, our main objectives are (of course) advocacy and to identify women younger using a more safe and effective screening method. Another goal that we found of great interest in enabling ACCESS to women have dense breasts; we wanted to explore ways that we can get them screened sooner. We can have them checked out to make sure that there are no early cancers that are being missed through other tools and techniques that we can use. In addition to this, we can start looking at how density correlates to cancer. Our early studies indicate that many of the pathways enriched in patients with higher mammographic density are targetable, raising the possibility of developing prevention strategies for mitigating density-associated breast cancer risk. We hope to further elucidate those molecular changes and those genetic signatures that may be common in women with dense breasts. Our overall goal is to increase survivability and offer targetable outcomes for the patient.

Part 2: EPIGENETICS and BREAST DENSITY

By: Roberta Kline, MD / Gene Expression Specialist for Women's Health

Increasing amount of studies align epigenetics with what causes higher breast density on a genetic and molecular level. In a recent epigenomic wide association study, hypermethylation of a number of regions (called Differentially Methylated Regions, or DMRs) were found to be associated with higher breast density. These same regions overlapped significantly with ones associated with breast cancer, highlighting potential common pathways and mechanisms mediated through epigenetics.

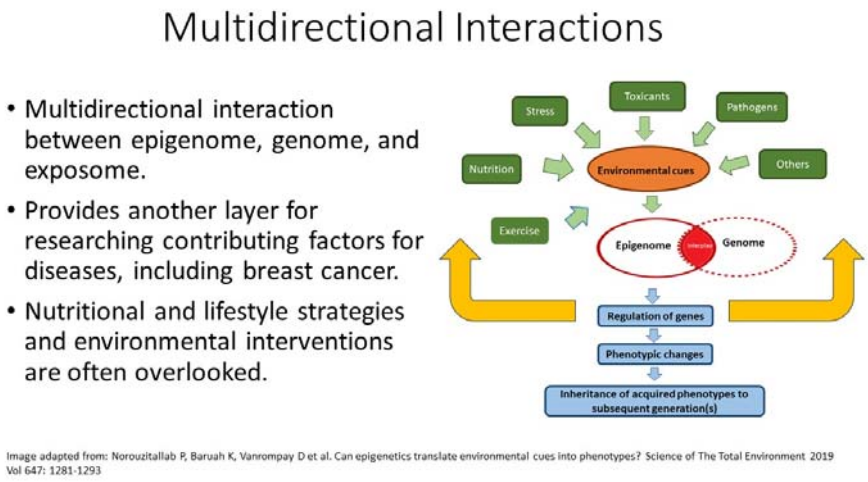


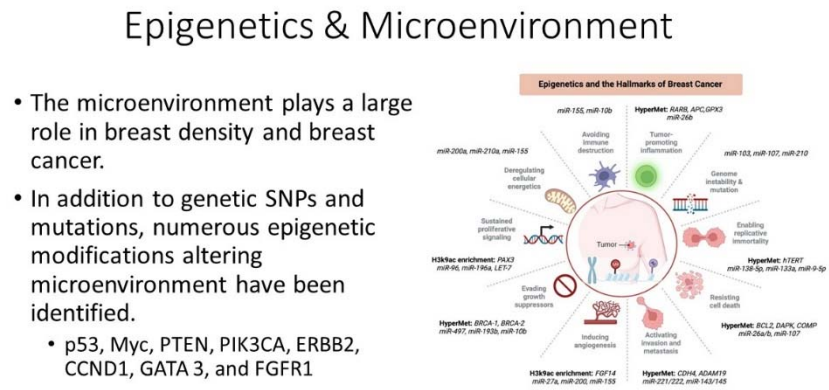
Image adapted from: Norouzitallab P, Baruah K, Vanrompay D et al. Can epigenetics translate environmental cues into phenotypes? Science of The Total Environment 2019 Vol 647: 1281-1293

Epigenetics is emerging as a critical aspect of gene expression, with lots of potential to advance clinical care. Gene expression is the final result of many systems interacting with each other. While alterations in DNA such as genetic SNPs and mutations are the best studied, epigenetic changes are emerging as important regulators, and both of these interact with the exposome- or the sum total of environmental exposures over a person's lifetime.

Currently epigenetic markers are being explored to further refine existing gene expression profiling of breast cancer tumors to assess prognosis and treatment options. Soon we'll be able to go beyond hormone receptor and HER2 status to provide even more personalized strategies. There are multiple clinical trials ongoing in various phases looking at intervention with epigenetic agents. Most of these are focusing on the two main enzymes – the methyltransferases, which we covered, and another key epigenetic mechanism that involves enzymes called histone deacetylases.

Unlike DNA, these epigenetic changes are reversible, offering a powerful opportunity to intervene to potentially prevent as well as treat many health issues including cancer. These interactions are multidirectional: the exposome influences epigenetic changes, DNA affects how we respond to the exposome as well as our ability to create epigenetic changes, and then the resulting genetic expression provides another feedback loop to influence all of these systems as yet another layer.

There are 3 main ways in which this occurs: methylation of DNA, modification of histones – which are the structures around which DNA is coiled, and various types of noncoding RNA – or RNA that doesn't code for active proteins. Depending on the type and location of the modification, these can activate or inhibit gene expression. So they can turn genes on and turn genes off. We'll now focus on DNA methylation, as this is the one most studied and available in clinical practice.



Sarvari, P., Sarvari, P., Mahjoubi, F., & Rubio, K. (2022). Advances of Epigenetic Biomarkers and Epigenome Editing for Early Diagnosis in Breast Cancer. International Journal of Molecular Sciences, 23(17), 9521. https://doi.org/10.3390/ijms23179521

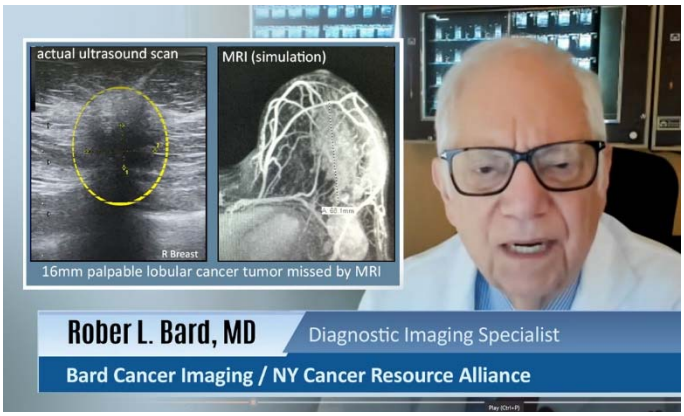
In addition to specific functional pathways involving oncogenes and tumor suppressor genes, there is evidence that epigenetic alterations in genes linked to the microenvironment itself also play a role in both breast density and breast cancer. (FIG-1) Epigenetic changes are seen in genes across multiple biological systems that are involved in the maintenance of the microenvironment – and disruptions are associated with increased breast density and with the development and progression of breast cancer.

Part 3: REMOTE IMAGING INTERPRETATION REPORT / BREAST TISSUE ANALYSIS

By: Dr. Robert L. Bard

My role in this dense breast cohort study is to review all field imaging scans and to generate diagnostic radiology reports to identify or confirm breast density and breast health of all the test subjects acquired remotely. Where Dr. Noelle Cutter scans patients out on the field (in her Molloy University campus and in athletic races), our partnership shows the flexibility of ultrasound screening of breast health, density and disease. Our focus is in women under 39, and women with dense breasts, which is more common in athletic women and women under 40 who are below the age of screening. We are also able to prove the ease of use and the safety factor of ultrasound as a non-radiation utility since it captures images in real-time with quantitative accuracy in distinguishing cancers from benign cysts in dense or lumpy breast tissue.

Dr. Cutter approached this project as more of a SURVEY, collecting data in various underserved communities- seeking out breast density in low BMI subjects as well as specific cultural groups. Her objectives aim to detect breast cancers in women under 39, or women with dense breasts that may not (yet) have the means or the capacity to access routine mammography or even clinical palpation by a physician.



SCANNER OF CHOICE: THE TERASON 3200

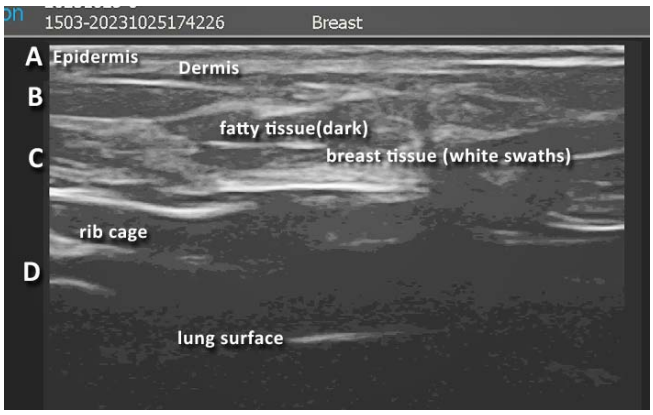
Upon review of a wide range of portable ultrasound devices, we selected the Terason 3200 due to its proven quality, user-friendly interface and its rugged design. Its capacity offers high resolution imaging and high quality probes that can capture anatomical scans comparable to hospital grade appliances. Hence, we are able to see not only the breast tissue, but also the skin and the lymph nodes on the chest wall. This means that if there's a breast cancer that's infiltrating the skin from below (which a clinician may not be able to see and oftentimes cannot feel) this will show that the cancer is a stage two because it's already broken through the bottom layer of the skin or metastasized to the glands.

HUMAN RE-INTERVENTION IN IMAGING

While everybody's talking about artificial intelligence, the cancer imaging society continues to rely on seasoned experience of a seasoned diagnostic over-reader. Specifically, if there's a benign tumor that's calcified, it has the same look and effect as a breast cancer that's not calcified because calcium and cancer absorbs the ultrasound frequency the same way- whereby the area behind them is basically black, not white. So it takes an experienced eye to determine if something suspicious is really serious. As of now, artificial intelligence has not yet reached that level of sophistication in detective work.

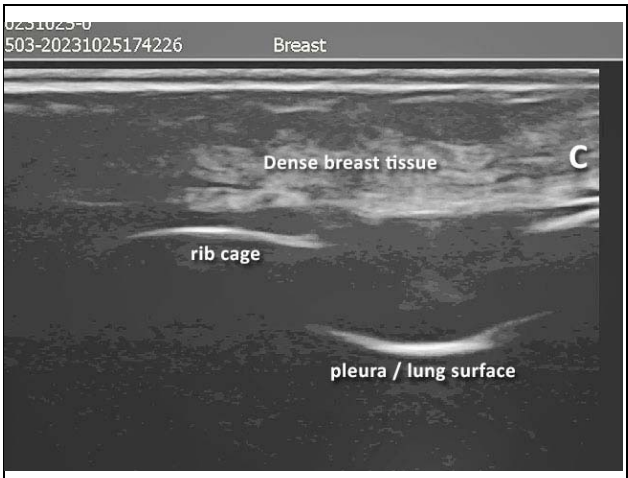
WIFI SUPPORTS THE MEDICAL COLLABORATION PARADIGM

Today's entire emergency response system is well underway the use of WIFI connected devices in their emergency vehicles. The portable rescue services are completely reliant on WIFI connections, allowing for field responders to receive medical guidance and diagnostic reading support from 'central command'. Having access of the supervision of a senior medical professional on the field can save lives where precious seconds count- way before the patient arrives in the emergency room. The ability to have wifi allows the responder and the surgical team to confirm the pathology while receiving expert guidance from anywhere in the world within seconds.



PATIENT #1: STUDY OF BREAST LAYERS

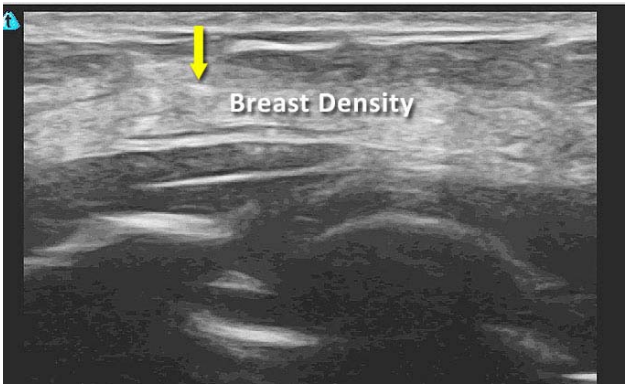
This scan (Patent #1) shows that directly under the white line (A) is a black area that is the epidermis of the skin. The horizontal white band below is the dermis. The resolution of the Terason ultrasound device allows us to see less than half millimeter lesions. Below this is an area (B) that's mixed white and dark. It's got horizontal bands of white and broad swaths of dark fatty tissue, white is breast tissue. The scan shows normal breast tissue, which is gray.



PATIENT #2: ULTRASOUND DEPTH CAPACITY

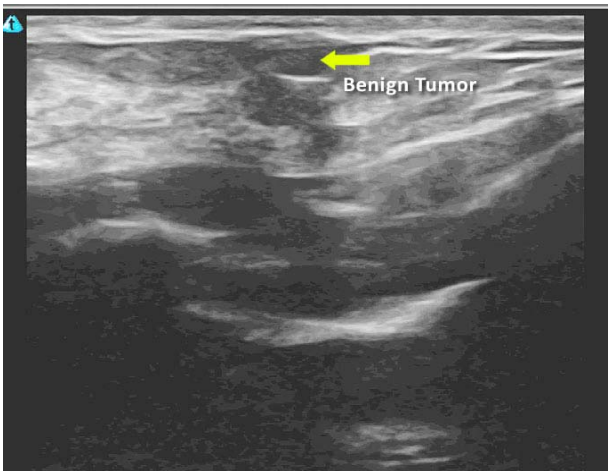
To contrast from patient 1, we have band of white tissue (C), which is normal fatty breast tissue- appearing as white. Below C is the semi-circular bands represent the rib cage and more importantly, the surface of the lung. This is useful because should a cancer of the breast metastasize to the lymph nodes or to the lungs, this is where we might see evidence of this during this simple screening examination.

The dense breast tissue is the band of white above the semi-circular rib cage echoes. And in the middle of the band of whites, you could see tiny black wormy lines, which represent dilated ducts, which are a part of breast disease or cystic breast disease.



PATIENT #6: CATEGORY 4 DENSITY

Once again, the skin is on the top, with the curvilinear ribs in the middle of the scan and the dense tissue as a broad white path in between. In the middle of this white density are tiny, bright white areas representing microcalcium inside the breast. The fibrous tissue is an inflammatory process which heals with scarring and calcification, which can also block the ducts and dilate the ducts. So here we have an example of a very white dense breast with areas of micro calcification, which are part of the healing process. This would be a CATEGORY 4 breast density on mammography because the tissue is homogeneously white.

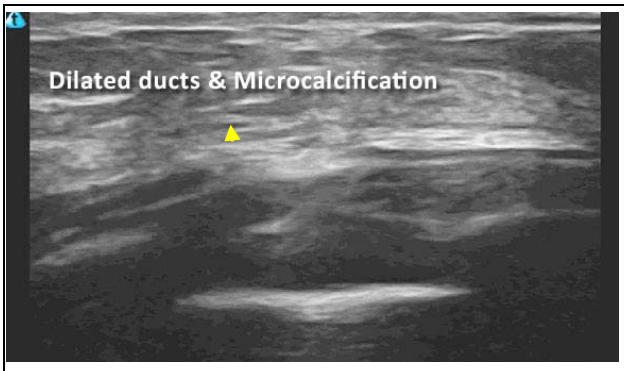


PATIENT #7: BENIGN TUMOR

Here's an example of a benign tumor within the breast as a black area within the white carpet of tissue and sharply demarcated. There is a white trail behind the black area showing that it's a degenerated cystic area or a solid benign tumor that's breaking down into cystic regions. So this is an area that we follow every six months with ultrasound scans. This would show up as a BI-RADS 2 or as a class four density and probably be missed on mammograms. In a prior research project, we did a study showing that the benign tumors like this in dense breasts are missed about 90% of the time by mammography. Also, since the breast tissue is lumpy, they're often hard to palpate clinically.

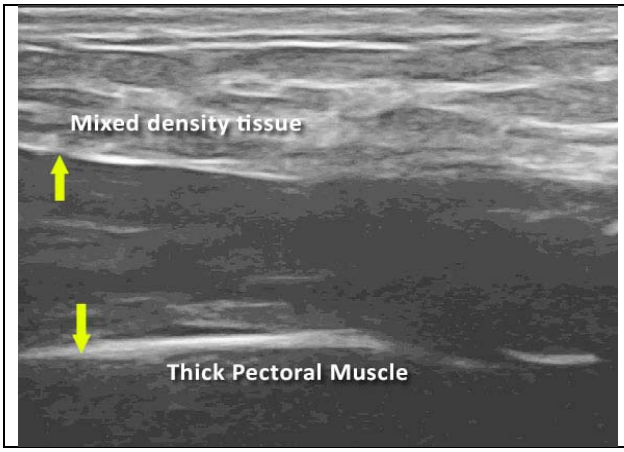
PATIENT #8: CATEGORY 1 DENSITY

This is an example of a class one density mammogram of breast tissue, because this is almost uniformly dark or fatty tissue within the breast. The linear white lines are prominent because the bulk of the tissue is predominantly fatty.



PATIENT #9: CATEGORY 3 WITH MICROCALCIFICATION

This is an example of a class three where we see the white tissue interrupted by horizontal areas of dark and white indicating dilated ducts, some of which have micro calcification within them. Once again, we see the chest wall below that. So if there is a cancer, we can see if it's invading not only the skin, but the muscle tissue below that. With this technology, we find cancers and stage them within minutes.

	PATIENT #10: This is another example of mixed density tissue because you have alternating bands of gray and darker gray. Below that is a very thick pectoral muscle below, which is the white band of the rib cage. So we can see muscle disease as well as the pleural disease (on the lining of the lung). We can also measure the thickness of a muscle. This may be useful in treating muscle wasting diseases interfering with breathing.

FINAL STATEMENT

Breast density is a key factor in early detection because mammograms routinely miss breast cancer in dense breasts, especially in younger and more athletic women. Ultrasound offers a supplemental scan and peace of mind because it finds the pathology and almost instantaneously distinguishes from a benign cyst from a possible cancer - or a definite cancer. Ultrasound even goes further because if there is a definite cancer or something highly suspicious of a cancer look at the other organs such as the glands under the arm to see if it's spread into the lining of the lungs to see if it's invading the pleura or the lymph nodes in the abdomen or the brain. Since we are now armed with expert diagnostic over-readers and remote collaborative imaging options, I urge our gynecologic and obstetric community to take advantage of this affordable non-invasive screening solution.

SPECIAL THANKS

Our clinical research team and our affiliates wish to express our deep appreciation to Alice Chang (CEO) and Terason Ultrasound for their generous support in providing us their time, insight, technological resources for the execution of this review. We also give very special thanks to Dr. Robert Bard of Bard Cancer Diagnostic Imaging and Molloy University for their clinical resources, support staff and institutional facilities that enabled the execution of patient reviews. Additional thanks to the clinical research expertise goes to Dr. Roberta Kline in association with the advocacy and sponsorship efforts of Mr. Joe Cappello, Founder of “Are You Dense?” Foundation- all of whose generous contribution and support aided in the making of this clinical study.