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Turkish Federation of Breast Diseases Societies

European Journal of Breast Health is the official journal of the **Turkish Federation of Breast Diseases Societies**

The Senologic International Society (SIS) and the National Consortium of Breast Centers (NCBC) are the official supporters of the journal.

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European Journal of Breast Health

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European Journal of Breast Health

European Journal of Breast Health

Aims and Scope

The European Journal of Breast Health (Eur J Breast Health) is an international, scientific, open access periodical published by independent, unbiased, and double-blinded peer-review principles journal. It is the official publication of the Turkish Federation of Breast Diseases Societies, and the Senologic International Society (SIS) is the official supporter of the journal.

The European Journal of Breast Health is published quarterly in January, April, July, and October. The publication language of the journal is English.

EJBH aims to be a comprehensive, multidisciplinary source and contribute to the literature by publishing manuscripts with the highest scientific level in the fields of research, diagnosis, and treatment of all breast diseases; scientific, biologic, social and psychological considerations, news and technologies concerning the breast, breast care and breast diseases.

The journal publishes original research articlesreviews, letters to the editor, brief correspondences, meeting reports, editorial summaries, observations, novel ideas, basic and translational research studies, clinical and epidemiological studies, treatment guidelines, expert opinions, commentaries, clinical trials and outcome studies on breast health, biology and all kinds of breast diseases, and very original case reports that are prepared and presented according to the ethical guidelines.

TOPICS within the SCOPE of EJBH concerning breast health, breast biology and all kinds of breast diseases:

Epidemiology, Risk Factors, Prevention, Early Detection, Diagnosis and Therapy, Psychological Evaluation, Quality of Life, Screening, Imaging Management, Image-guided Procedures, Immunotherapy, molecular Classification, Mechanism-based Therapies, Carcinogenesis, Hereditary Susceptibility, Survivorship, Treatment Toxicities, and Secondary Neoplasms, Biophysics, Mechanisms of Metastasis, Microenvironment, Basic and Translational Research, Integrated Treatment Strategies, Cellular Research and Biomarkers, Stem Cells, Drug Delivery Systems, Clinical Use of Anti-therapeutic Agents, Radiotherapy, Chemotherapy, Surgery, Surgical Procedures and Techniques, Palliative Care, Patient Adherence, Cosmesis, Satisfaction and Health Economic Evaluations.

The target audience of the journal includes specialists and medical professionals in surgery, oncology, breast health and breast diseases.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal conforms with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

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European Journal of Breast Health

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Case Report	1000	200	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media
Current Opinion	300	No abstract	5	No tables	No media

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Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

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Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktiviteleri ve Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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European Journal of Breast Health

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Editorial

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Artificial Intelligence in Senology - Where Do We Stand and What Are the Future Horizons?

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ABSTRACT

Artificial Intelligence (AI) is defined as the simulation of human intelligence by a digital computer or robotic system and has become a hype in current conversations. A subcategory of AI is deep learning, which is based on complex artificial neural networks that mimic the principles of human synaptic plasticity and layered brain architectures, and uses large-scale data processing. AI-based image analysis in breast screening programmes has shown non-inferior sensitivity, reduces workload by up to 70% by pre-selecting normal cases, and reduces recall by 25% compared to human double reading. Natural language programs such as ChatGPT (OpenAI) achieve 80% and higher accuracy in advising and decision making compared to the gold standard: human judgement. This does not yet meet the necessary requirements for medical products in terms of patient safety. The main advantage of AI is that it can perform routine but complex tasks much faster and with fewer errors than humans. The main concerns in healthcare are the stability of AI systems, cybersecurity, liability and transparency. More widespread use of AI could affect human jobs in healthcare and increase technological dependency. AI in senology is just beginning to evolve towards better forms with improved properties. Responsible training of AI systems with meaningful raw data and scientific studies to analyse their performance in the real world are necessary to keep AI on track. To mitigate significant risks, it will be necessary to balance active promotion and development of quality-assured AI systems with careful regulation. AI regulation has only recently included in transnational legal frameworks, as the European Union's AI Act was the first comprehensive legal framework to be published, in December 2023. Unacceptable AI systems will be banned if they are deemed to pose a clear threat to people's fundamental rights. Using AI and combining it with human wisdom, empathy and affection will be the method of choice for further, fruitful development of tomorrow's senology.

Keywords: Artificial Intelligence; breast cancer; breast cancer screening; breast disease; breast imaging; MRI; senology; ultrasound

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Key Points

- Artificial Intelligence (AI) is defined as the simulation of human intelligence by a digital computer or robotic system and has become a hype in current conversations.
- A subcategory of AI is deep learning, which is based on complex artificial neural networks that mimic the principles of human synaptic plasticity and layered brain architectures, and uses large-scale data processing.
- AI-based image analysis in breast screening programmes has shown non-inferior sensitivity, reduces workload by up to 70% by pre-selecting normal cases, and reduces recall by 25% compared to human double reading.

Introduction

Artificial Intelligence (AI) has recently come to the fore in news reports, daily newspapers, periodical magazines and even scientific journals (1, 2). Touching the surface of this topic and ending up with humanised views and expectations is the usual mode of operation. This is more than understandable. One of the pioneers of AI, Turing (3), said that only computers can understand computers. Inputs, embeddings, vectors, matrices, weighted scores, probability distributions, and outputs are pure mathematics. Emotions and feelings are reserved for humans and are the product of a long evolutionary process. Using AI and combining it with human wisdom, empathy and affection will be the recommendation of this editorial, which will focus on AI in senology and its future horizons.

Definition

AI is defined as the simulation of human intelligence by a digital computer or robot system. The term is often used for developed

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systems that are designed to be equipped with the intellectual process characteristic of humans, such as the ability to reason, discover meaning or learn from past experiences (4).

However, the science fiction concept of an AI singularity refers to a super AI singularity that has evolved to a level of intelligence far beyond human performance (5, 6). The implications of such a super AI for the continued existence of mankind are being debated, and you may be familiar with fictional negative outcomes ending in a dictatorship of singularity AI from films, such as the Terminator series or the Matrix trilogy.

Today AI is an umbrella term, and several subfields of AI can be used - although none of them are the super-AI described in Science Fiction. Weak AI can perform certain limited tasks, such as speech recognition. Strong AI may be able to perform any intellectual task that a human brain can perform, if it is strong enough.

Groundhog Day - Fundamentals of AI Training

Maybe some readers are familiar with the American comedy film "Groundhog Day" starring Bill Murray (7). He is playing a raw, narcissistic and cynical television weatherman who falls in love with his co-star Andie MacDowell on a particular February 2nd. He becomes trapped in a time loop and re-lives February 2nd repeatedly. However, each day is different, and he is testing multiple new behaviours ranging from auto-destruction to philanthropy without aging. Further, he acquires new abilities and properties, and gradually changes into a positive, altruistic, and caring person who meets the needs of his beloved co-star that he has courted every day. Finally, she also falls in love with him, and both escape the time loop.

The makers of the film have unknowingly created a simplified and humanised model of AI for us. Bill Murray must re-live this specific date and with each new repetition, explore different actions and find the optimal policy until the desired result (in Bill Murray's case: break out of the time loop with his beloved) is achieved. This corresponds to how AI is trained by humans.

Like Murray does with his February 2nd, artificial neuronal networks repeat several training cycles until all parameters and connectors, like synapses, are finetuned to meet the required output results that human developers define (8, 9), although the scale of AI learning is very different from the film as AI training cycles can consist of millions of repetitions.

Parallel and Sequential Training

Murray is trained in a sequential time loop, one day after another. In comparison, AI training loops can run parallel and sequentially, forward and backwards, at high speed. The rapid improvement of calculating abilities of computers thus allows training of AI and development of solutions with AI in short time frames. Murray took years to solve his problem. A modern AI probably would have taken not even a day - but the process definitely would have seemed less heart-warming and inspiring to us than Murray's story.

The question arises, at what phase of the "Groundhog Day" time loop would "AI in senology" be located today? Is AI in senology still in its beginnings or already advanced along its way, developing into a better form with improved properties? Does AI already change the live trajectories of breast cancer patients, or their caring health professionals? The answers to these questions are complex and full of ambivalence. AI supporters have high hopes and ambitions when it comes to predicting the possible transformation of all areas of society, including medicine and, more specifically, senology, through AI (10, 11).

Bionic Design: How the Structure of AI is Inspired by the Human Brain

AI systems are not only able to mimic the functions of the human brain, but their structure is also heavily influenced by the design of the human brain. Artificial neural networks simulate the principles of human synaptic plasticity and layered brain architecture.

How the Brain Works

Synaptic plasticity refers to the ability of synapses (connections between neurons) to change and adapt in strength and structure over time. Thus, our brain constantly changes structurally with use. A fundamental principle of synaptic plasticity is that "what fires together, wires together". This means that when neurons repeatedly fire at the same time, the synapses between them strengthen, forming a more robust connection and thus reinforce the neural circuitry associated with that particular firing pattern. Conversely, transmission can be weakened by desynchronization of neuronal activity or inhibitory neurons (12).

The cerebral multilayer architecture allows processing of input patterns that improve economy and speed of cerebral function. For example, the visual information of the retina (input) up streams through the neurons of the brain and is perceived at different levels as edges, lines, colours, (intermediate layers) and finally as a complex object. As a result, the brain recognizes the faces or body of, for example, Albert Einstein (output) (13).

How AI Works

The transformation of visual input from simple features to increasingly complex features and then to object recognition in AI is represented by the mathematical approach of convolutional neural networks (14). Between the input layer (corresponding to the retina) and the output layer (corresponding to the object-recognising cortical layer of the brain), intermediate layers, called hidden layers, learn from the raw data and error-correction algorithms. However, what precisely happens in the hidden layers between the input and the output? Each attempt by the network to achieve the output goal is registered and corrected by the network itself. Failed attempts lead to the weakening of unsuccessful data in intermediate layers, analogous to the process of synaptic weighting in the human brain. Due to the high complexity of this process, human observers are ultimately unable to understand how exactly an output is generated from an input in a self-learning network. This is called the "black box" problem, because we humans do not know what the black box of an algorithm looks like and how it works inside (15).

Types of AI

The umbrella term AI covers various methods, such as machine learning (ML), deep learning (DL) or natural language processing (NLP). ML is still the preferred technology in medical systems because of its stable performance, for example in image analysis, diagnosis classification or survival prediction. ML is based on algorithms that learn from data and improve their performance over time (16).

A subcategory of ML is DL, which relies on complex neural networks, that mimic the workings of the human brain and the processing of large amounts of data.

NLP is an artificial neural system of generative intelligence that enables computers to understand, interpret and generate human language. Modern NLPs, such as ChatGPT (OpenAI), Bard (Google) or Galactica (Meta, formerly Facebook) can summarise and simplify documents, answer questions in conversations, translate or autocorrect, or recognize the sentiment of text (17, 18). However, the result of generative AI can sometimes be wrong; the system "hallucinates" and offers information that seems believable but is incorrect (19). A notorious case of this is ChatGPT's generation of confabulated references or wrong biographies for real people, which ChatGPT embellished with various false information, such as fake university degrees or awards.

A similar example of incorrect content creation by AI can be seen in text-image generators, which create artificial images. Midjourney, Dall-E 2 or Stable Diffusion are the world's leading AI-based image generators. While AI-generated images are becoming more advanced and produce impressive results from written prompts, they struggle with highly specific content or small details. For example, when trying to generate images that include people, the number or position of fingers is often incorrect. This is because the models are trained on sometimes blurry and small internet images. Further training of generative AI systems with better and more focused reference images is likely to make such obvious mistakes a thing of the past and compensate for the lack of understanding of human anatomy (20).

More problematic are AI biases based on real, human biases regarding religion, gender, race, or other factors that are represented in the training data. For this reason, the latest version of ChatGPT, as a result of rigorous training, suppresses most responses that do not represent currently accepted ethical, socio-political or legal standards.

Basic Applications of AI With a Focus on Medicine and Senology

The main applications of AI worldwide are in the fields of business/finance and war/defence. In medicine, the following main applications have developed (1, 10, 17, 21, 22).

(a) Medical imaging, aiming at shorter examination times, less contrast media, updated AI-assisted detection and diagnosis, and improved diagnostic accuracy.

(b) Drug discovery to predict molecular interactions and potential new drugs.

(c) Genomics to analyse large genomic datasets for new insights into genetic diseases.

(d) Electronic health records to extract insights and trends for decision making.

(e) Precision medicine to tailor individual treatment plans.

ChatGPT in Senology

Current applications of NLP, such as ChatGPT, in medicine and senology include text generation (e.g., responses, manuscripts, coding), content summarisation (e.g., abstract paraphrasing, meeting notes summarisation), translation (e.g., between languages, text-to-code), classification (e.g., diagnostic classification, patient sentiment analysis), and chatbots (e.g., question and answer, virtual assistants) (18).

Have These Possibilities Already Touched the Field of Breast Healthcare?

A recent PubMed search by the authors in December 2023, focusing on the query "ChatGPT and Senology", yielded only a single result. An alternative search with the prompts "ChatGPT and breast cancer" or "ChatGPT and breast health care" yielded 16 results after removing duplicates. The publications were of low scientific quality and included feasibility or proof of concept studies, case series and expert opinions (LoE 4, 5, GRADE D). Nevertheless, these early publications provide interesting insights. For example, ChatGPT's treatment recommendations were 80% or more in line with human judgement for several tasks, such as providing radiology screening or tumour board recommendations, breast augmentation advice, or top breast cancer-related search queries. In addition, ChatGPT performed significantly better than most human candidates on board exams in radiology, paediatrics and other areas (23-27).

Interestingly, ChatGPT 4 provided 10% to 20% more correct results than ChatGPT 3.5 for well-trained topics such as "screening", but was less successful for "breast pain" or complex clinical cases. Chatbots, particularly context-aware chatbots, resulted in significant time and cost savings compared to radiologists' imaging recommendations.

One may conclude that the future of medical writing will rely heavily on AI and chatbots. However, a major concern was that ChatGPT created non-existent references, cited the wrong journal and date, and lacked depth. These drawbacks provide a significant caveat to the use of ChatGPT and similar large language programs in academia without critical review. Obviously, supervision by professionals is mandatory to ensure accuracy (25, 28-30).

AI in Breast Cancer Screening

Older approaches to screening using computer-aided detection (CAD) systems have been disappointing. While CAD did not improve the diagnostic accuracy of mammography, the insurers paid unnecessary costs for CAD in ultrasound (US) with no proven benefit for women. Today, the application of AI to breast cancer screening seems ready to change old strategies (31-36). Current applications of AI in screening focus on differentiating between benign and malignant tissue and localising of suspicious lesions within breast tissue (37). The newest methods used allow for transfer learning and the use of bilateral and prior images to detect subtle asymmetries and lesion growth. Recently, the authors of the first prospective randomised screening study comparing AI-assisted reading of digital mammography screens with conventional double reading (MASAI study) concluded that AI-assisted reading was safe (38). AI-assisted reading detected more invasive cancers (184 *vs.* 165 invasive cancers) and more *in situ* cancers (60 *vs.* 38 *in situ* cancers) than state-of-the-art double reading. AI also reduced the screen reading workload by 44.3% (38).

Four different applications of AI are currently being studied (39-45):

(a) AI-assisted reading of digital mammography;

(b) AI as a stand-alone decision support system;

(c) AI pre-selection of normal cases;

(d) AI-assisted prediction of breast cancer risk.

The current results for these applications in digital mammography are encouraging.

AI will become a useful tool in the near future and, most importantly, it will help reduce costs and may compensate the initial shortage of specialised radiologists by up to 50% (46). Rule-out and rule-in triage workflows can improve and refine the efficiency and effectiveness of mammography breast cancer screening (47, 48). Complicated cases are sent to two human readers, less suspicious cases to one human reader. Normal cases are directed to one human reader or are analysed only by the stand-alone AI. Thus, the AI can either exclude low-risk cases from double reading, replace the second reader or replace all human readers. Scores are used to express the AI-based stratification of lesion risk assessment.

In addition, the classification of breast density and matrix heterogeneity opens up the possibility of predicting the current and tailored predictive overall risk of breast cancer for each individual woman (49). Recent publications suggest that this type of prediction outperforms clinical risk scores. In addition, the combined assessment of an AI-based lesion detection system and breast density measurements enabled the identification of a greater proportion of women who would develop interval cancer compared than either method alone (50, 51).

AI in Other Breast Imaging Modalities

Digital Breast Tomosynthesis

The lessons learned from digital mammography can also be applied to digital tomosynthesis, as shown by several publications focusing on digital breast tomosynthesis (DBT). The sensitivity of stand-alone AI systems in DBT shows a non-inferior sensitivity, reduces workload by up to 70% due to pre-selection of normal cases, and reduces recall by 25% (52, 53). In contrast, another retrospective analysis of stand-alone AI performance in DBT found a 2% increase in recall rate (54). An AI support system could make advanced and more reliable imaging techniques more accessible and enable more cost-effective breast screening programmes with DBT (55).

Ultrasound

With regard to US, a large multi-vendor, multi-centre study from China found that a DL model could help novice readers in particular, to improve their US reading in terms of accuracy and interobserver agreement for breast cancer diagnosis (56). Adding an AI system to breast US was able to reduce unnecessary lesional biopsies (57). AI support also helped radiologists reduce false-positive findings in breast US interpretation by 37.3%, while maintaining the same level of sensitivity (58). A very recent study by Guldogan et al. (59) evaluated the performance of a commercial AI system for the retrospective BI-RADS category assessment in 715 breast masses detected on breast US. The accuracy of AI was inferior to that of experienced radiologists. However, all lesions categorized as BI-RADS 2 by AI proved subsequently to be benign. The authors stated that considering AI-assigned BI-RADS 2 as safe, this would have avoided 11% (18 out of 163) of benign lesion biopsies and 46.2% (110 out of 238) of follow-up examinations (59).

Breast Magnetic Resonance Imaging

Breast magnetic resonance imaging (MRI) can benefit from AI-assisted k-space sampling, resulting in denoising, improved resolution, reduced artefacts, and up to a 10% reduction in gadolinium dose (60, 61). In addition to clinical indications such as preoperative staging or follow-up under neoadjuvant therapy, this is particularly interesting for the field of personalized breast cancer screening, as these benefits could alleviate concerns about gadolinium uptake, which may deter some patients from undergoing regular MRI in high-risk women or those with dense breast. The DENSE trial has already shown a significant reduction of the interval cancer rate in the supplemental MRI screening group compared with the digital mammography-alone screening group from 5.0% to 2.5% (62). Further simulation models suggested that even an MRI-only screening strategy with a 4-year interval would be cost-effective (63). In addition, Comstock et al. (64) reported a significant difference (7%) of abbreviated MRI in the cancer detection rates between MRI- and DBT-based screening groups.

Contrast-Enhanced Mammography

In terms of contrast-enhanced mammography, DL algorithms for the detection of single mass lesions on CEM outperformed radiologists in terms of classification efficiency in a recent prospective Chinese multicentre study (61).

Nuclear Medicine

AI-assisted positron emission tomography or single photon emission computed tomography studies also promise the same big major benefits: (a) shorter examination times; (b) less radioactivity; and (c) better diagnostic accuracy compared to the old-school approach (65, 66).

Impact of AI on Early Diagnosis and Further Clinical Pathway

At the time of breast cancer diagnosis, the most successful AI systems in recent mammography trials have shown non-inferior sensitivity to expert double reading and a tendency to reduce recalls, avoiding unnecessary anxiety for women and biopsies (39-41, 45, 46, 53).

The average size and stage of breast cancers detected by screening are similar, whether or not AI is added. In the future, AI would only be of significant benefit to patients if it could detect significantly smaller breast cancers at an earlier stage, leading to improved patient survival.

Discussion and Conclusion

In the future, robust studies will be needed to address unresolved issues including: the direct comparison of different AI systems; the effect of different mammography systems on the accuracy of AI systems; the effect of different screening programmes on AI cancer detection or on how the AI system might work within specific breast screening IT systems; and the effect of providing additional breast density and composition information to AI systems for decision making (63). In addition, from a global perspective, AI algorithms trained on image analysis of Western breast composition need to be adapted to the predominantly dense breasts of women in Asia, Africa or Latin America (67). The onset of breast cancer in these women occurs earlier than in Western Europe, Scandinavian countries and the USA. On the positive side, there is evidence that AI systems predicting the presence of breast cancer can be generalized across data from Western countries, although the data are representative of different screening populations and practices (68).

The question remains: can AI in screening bring benefits to breast cancer patients further along the clinical pathway? This pathway includes diagnostic procedures, such as biopsy, imaging for pre-operative staging, treatment and follow-up after screening or clinical detection of breast cancer. In their recent systematic review of AI image analysis, Freeman et al. (69) concluded that there is insufficient evidence to support the introduction of AI into the screening pathway for clinical impact. This is easy to understand. AI implementation and outcomes must meet the gold standard of human expertise. Currently,

many AI tools for these later stages of the clinical pathway do not meet the quality requirements for medical devices that some applications in other fields, such as neuroradiology, cardiology or robotics, do. Eighty percent or more correct AI-generated answers in a tumour conference on the most appropriate therapy are not sufficient, nor are chatbot recommendations on medical procedures in a similar range. Nevertheless, AI tools such as ChatGPT or other NLP's can assist professionals and are particularly valuable for beginners and less trained professionals (17). However, we must remain realistic. The *status quo* can change quickly. A game changer in senology would be the development of new multimodal AI systems that not only detect and characterise cancer better and earlier than humans, but also have global medical knowledge at their disposal. In addition, it would be constantly on the lookout for complex patterns that were previously hidden from humans. Humans would relinquish the gold standard of their expert judgement to AI, and would likely lose the ability to make responsible decisions.

Promises

AI in senology and general AI share an intersection of advantages and disadvantages (70). Undoubtedly, as mentioned above, the main advantage of AI is that it can perform routine and complex tasks much faster and with fewer errors than humans. AI will be able to work cost-effectively 365 days a year, 24 hours a day, without a break. Therefore, future economic decisions will also favour AI in the long term (71). Genomics and research into new breast cancer drugs are likely to benefit most from the speed and efficiency of AI algorithms in processing large data sets (16).

AI's ability to identify subtle and complex patterns that human readers or clinicians might miss will improve data-driven optimisation of diagnosis, patient care, administration, public health and cost-effectiveness, and thereby provide the opportunity to transform breast healthcare. In particular, NLP systems can already provide significant support for academic publishing, translation, medical report summarisation and administrative billing of patient care (18, 30). AI chat bots can help raise awareness and educate women about breast cancer symptoms and lifestyle changes. Emotional support for patients is no longer the privilege of human doctors and nurses. AI can analyse a patient's basic feelings and emotions and respond appropriately (72).

Most importantly, the range of support provided by AI has the potential to evolve into new roles in the future. In just a decade, Western countries will have passed the golden age of the baby boomers and will face a shortage of well-trained breast specialists. AI promises to alleviate such future problems in senology.

Risks

The seven main risks of AI in healthcare have been identified by a recent EU study. These comprise: patient harm due to AI errors; misuse of medical AI tools; bias in AI and the perpetuation of existing inequities; lack of transparency; privacy and security issues; gaps in accountability; and barriers to implementation (22). However, among future general AI applications, the persistent theme of AI misuse could dramatically change our future lives. The sources of misuse are either man-made, such as misuse by authoritarian political systems or corporations, or arise from a super-intelligent AI singularity itself. Elon Musk recently estimated that there is an 80% chance that AI will be a blessing and a 20% chance of a hard landing. (73). Earlier, in May 2023, other prominent leaders from OpenAI, Google DeepMind, Anthropic and other AI labs had also warned that future AI systems could be as deadly as pandemics and nuclear weapons (74, 75).

But what about the present? Our biggest personal concerns relate to the long-term instability of AI models and poor cybersecurity. Most modern NLP training involves continuous learning by scraping information of all kinds from the internet. However, as online information becomes more and more content generated by AIs themselves, one consequence will be the "model collapse" of NLPs. Within a few generations, an AI model will begin to forget improbable events, leading to a degenerated model that no longer reflects the real world (76). This could resemble a schizophrenic human worldview. In addition, the AI model forgets previous examples when learning new information, a "catastrophic forgetting" that resembles human amnesia. Furthermore, contaminated websites can infiltrate AI models with malicious data that is inserted during training to degrade the model's performance (77). This so-called "data poisoning" calls for strong protection by cybersecurity systems.

Implications and Future Impact on Socio-Economic Healthcare System

There are several important conclusions regarding the future impact of AI on the socio-economic health care system (71, 78, 79). The main advantage of AI is probably the potential workflow optimisation by eliminating non-suspicious cases from double reading. Therefore, radiologists with high workloads will initially love these AI systems, as long as they are easy to use. This initial enthusiasm could be reversed if radiologists become increasingly exhausted by having to read more complicated cases per hour than before.

In terms of cost-effectiveness, the system will save significant time and money by reducing the number of cases that need to be double-read. It is expected that between 50–70% of cases will not require further double reading (54). The transition to AI could also negatively affect human jobs in the field of healthcare. Professionals who know how to make use of AI will displace those who do not.

Technological dependency will inevitably increase, especially the role of the human programmer in controlling the AI to prevent the stable system from falling prey to entropy. The examples given of model degeneration and data poisoning underline the need for a strict human interaction and control of AI.

Human neurons and muscles degenerate when they are no longer used. The younger generation in an AI era may become lazy, forget previous skills, and run the risk of trusting AI too much. The transition to AI will also take a lot of time and money. Some developing societies will not be able to afford these costs. As a result, global inequalities are likely to increase.

AI intuition, including its generative approaches, is different from human creativity and lacks the human touch, and has problems thinking outside the box. Combining both human creativity and AI will open new horizons.

Ethics demand transparency, accountability and informed consent in every medical decision. Legal laws and regulations clarify the medical liability. Accordingly, individuals and organisations will be held accountable for the future actions of AI systems, especially if medical AI systems make mistakes or contribute to incorrect diagnoses or treatments.

Progress Under Regulation

According to Sam Altmann, CEO of Open AI, the risks of advanced AI systems were serious enough to warrant government intervention and regulation of AI due to the potential risk of mankind's extinction (75). Released in December 2023, the European Union's AI Act was the first ever, comprehensive legal framework for AI in healthcare worldwide that had been agreed on in order to advance the European approach to trustworthy AI. AI systems identified as high-risk must meet strict requirements, including risk-mitigation systems, high quality datasets, logging of activities, detailed documentation, clear user information, human oversight, and high levels of robustness, accuracy and cybersecurity. Examples of such high-risk AI systems include certain critical infrastructure, medical devices and biometric identification, and categorisation and emotion recognition systems. Unacceptable AI systems will be banned when considered a clear threat to the fundamental rights of people. Companies not complying with the rules will be fined. Fines for breaching the banned AI applications will be €35 million or 7% of global annual turnover, whichever is higher (80).

Future Horizons, Recommendations

Today, modern AI is still in its infancy. Nevertheless, AI will boost digital medicine in all fields, including senology. However, there is still a long way to go before the majority of patients will benefit.

The transition to AI will be disruptive. The authors recommend that healthcare decision-makers and stakeholders in senology will be prepared and open to new AI developments and the necessary regulatory framework.

We suggest that physicians should invest in new AI systems mainly with optimism, with an added dash of caution, and call for strict quality control of the double-edged sword of AI. In the real world, this corresponds to a qualified curriculum of AI training, meaningful systemic controlled randomised trials, but also individual checks of logical plausibility in decision making, for example in AI-assisted tumour board recommendations.

We would like to mention that there is no room for false modesty. Human intellectual abilities have achieved marvellous results in medicine and elsewhere. Most importantly, if mistakes are made in the management of AI, humans can correct them, regulate and manage the risks involved.

We propose that the use of AI, combined with human wisdom, empathy and affection, will be the method of choice for the further fruitful development of tomorrow's senology.

Authorship Contributions

Concept: A.M.; Design: A.M.; Data Collection and/or Processing: A.M.; Analysis and/or Interpretation: A.M., C.M.; Literature Search: A.M., C.M.; Writing: A.M., C.M.

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Dedication

Dedicated to the 30th anniversary of the Osnabrueck Breast Centre at Franziskus-Hospital Harderberg.

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Systematic Review

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Does Post-Mastectomy Radiotherapy Confer Survival Benefits on Patients With 1-3 Clinically Positive Lymph Nodes Rendered Pathologically Negative After Neoadjuvant Systemic Chemotherapy: Consensus from A Pooled Analysis?

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ABSTRACT

The advent of taxane-based chemotherapy has revolutionized breast cancer care. This advance has helped improve the response to downstaging tumors that might otherwise be inoperable. It has also helped in rendering clinically (cN+) positive lymph nodes (LNs) pathologically negative (ypN0). The standard of care for cN+ patients included post-mastectomy radiotherapy (PMRT), regardless of the response to neoadjuvant chemotherapy. However, PMRT in patients with 1–3 positive LNs still lacks definitive guidelines. Numerous retrospective results have been inconclusive about the benefit of PMRT on survival in patients with 1–3 positive LNs. This pooled analysis attempts to reach a consensus. The PubMed database was searched through October 2023. The search yielded 27 papers, of which 11 satisfied the inclusion criteria. The locoregional recurrence-free survival (LRRFS), disease-free survival (DFS), and overall survival (OS) for each study were tabulated when given, and two groups were created, the PMRT and NO PMRT, respectively. The results were then pooled for analysis. The total number of patients was 8340, 4136 in the PMRT group, and 4204 in the NO PMRT group, respectively. The LRRFS, DFS, and OS were 96.9%, 82.1%, and 87.3% for the PMRT group and 93.2%, 79.6%, and 84.8% for the NO PMRT group, respectively. There was no statistical significance in LRRFS, DFS, or OS between the two groups (p = 0.61, p = 0.61, and p = 0.38, respectively). PMRT does not seem to confer survival benefits in patients with pN1 rendered ypN0 for stages T1-3. This pooled analysis's findings should be confirmed prospectively with a longer period of follow-up.

Keywords: Post-mastectomy radiotherapy; neoadjuvant chemotherapy; regional nodal irradiation; clinically positive lymph nodes; pathological complete response

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Key Points

- Taxane-based neoadjuvant chemotherapy has improved response to downstaging and pathological complete response.
- The benefits on survival of post-mastectomy radiotherapy (PMRT) in breast cancer patients with T1-3 and 1-3 positive lymph nodes rendered pathologically negative post-neoadjuvant chemotherapy is not yet established.
- PMRT does not seem to confer survival benefits on breast cancer patients with T1-3 and 1-3 positive lymph nodes rendered pathologically negative post-neoadjuvant chemotherapy.
- Long-term follow-up of patients for 10 years or more is essential to determine the effect of forgoing PMRT on locoregional recurrence.
- Clinicopathological factors such as age, lymphovascular invasion, and tumor size have to be taken into consideration before forgoing PMRT.
- Ongoing prospective studies will determine the basis of radiotherapy administration in these specific groups.

Introduction

The role of post-mastectomy radiotherapy (PMRT) in patients with more than four positive lymph nodes (LNs) has been shown to improve survival. These trials have also shown improvement regardless of tumor size or the number of positive LNs (1). However, the benefit to low-tumor burden LNs (1–3 positive LNs) was debated due to these trials being based on the pre-taxane and human epidermal growth factor receptor 2 (HER2) targeted therapy eras. In addition, some studies

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indicated that these patients demonstrated a low rate of LRR (2, 3). In a series from the Cleveland Clinic, a 10% locoregional recurrence (LRR) rate was reported among patients with 1–3 positive LNs treated with mastectomy and chemotherapy without radiation (4). Other studies placed the LRR rate in the range of 4–10% (5, 6). However, in patients less than forty years of age with lymphovascular invasion (LVI), the five-year LRR rate was 24.3% (7). It is imperative that long-term follow-up be implemented, as 95% of LRRs occur within 10 years after surgical intervention (8).

The conflicting results and lack of evidence led the National Comprehensive Cancer Network to recommend that PMRT be "strongly considered" in patients with 1–3 positive LNs while also taking into account other clinical characteristics, such as life expectancy, age, comorbidities, tumor size, and LVI (9). Furthermore, a joint panel comprised of the American Societies of Clinical, Radiation, and Surgical Oncology recommended PMRT in patients with 1–3 positive LNs and T1-2 as the benefits outweigh the potential toxicities (10).

The advent of taxane-based chemotherapy has revolutionized breast cancer (BC) management. This advance has become a first-line treatment for responders, achieving a higher percentage of pathological complete response (pCR) in both the breast and axilla. Moreover, the addition of anti-HER2 therapy became standard due to its survival benefits (11, 12). The de-escalation in the management of the axilla both surgically and medically is made possible in such patients.

PMRT can lead to numerous side effects, both early and late after treatment. Early side effects, which occur weeks to months apart, can include skin thickening, pleural effusion, and radiation-induced pneumonia. The intermediate to late period, which can take months to years, includes breast fibrosis, pulmonary fibrosis, and fracture of overlying bone, among others (13).

The role of PMRT in the setting of adjuvant therapy has been shown to provide survival benefits for BC patients with positive LNs (1). However, the role of NAC on survival in patients with cN+ is yet to be determined. A prospective trial that is ongoing, namely the NSABP B51/RTOG 1304 (14), has recently presented the five-year results at the San Antonio Breast Cancer Symposium in December 2023 (SABCS) (15). This randomized clinical trial investigated if regional nodal irradiation (RNI) post mastectomy or the addition of regional nodal radiotherapy to whole breast radiotherapy post breast-conserving surgery (BCS) reduced invasive BC recurrence-free interval as a primary endpoint in patients with pathologically positive axillary nodes who are ypN0 after neoadjuvant chemotherapy. The secondary endpoints included LRR-free interval, distant recurrence-free interval, disease-free survival (DFS), and overall survival (OS). The current pooled analysis attempts to answer the question of the survival benefits of PMRT in patients with 1-3 positive LNs and clinical stage T1-3 rendered ypN0 post-NAC.

Materials and Methods

The PubMed database was searched through October 2023. The terminologies used were PMRT, cN+, ypN0, and NAC. The study cohort was required to encompass both cN+ and ypN0. The inclusion criteria were studies that looked at patients who had clinically positive LNs (1-3) and were rendered ypN0 post-neoadjuvant systemic chemotherapy with rates given for either the locoregional recurrence-free survival (LRRFS), DFS, or OS. Exclusion criteria encompassed studies that dealt solely with BCS, whole breast radiotherapy, RNI, and tumor stage T1-4. The data was collected, and patients were then divided into two groups, each exclusively made up of cN+ and ypN0 post-NAC: PMRT and NO PMRT. When given, the number of patients who had RNI was recorded as part of the PMRT group. The number of patients for LRRFS, DFS, and OS rates was calculated for each study when given. The results were then pooled for analysis. A chi-square test with Yates's correction was applied. Confidence intervals (CI) were determined based on a non-central chi-square distribution for Q (a common effect measure). The pooled mean follow-up period was calculated. Subgroup pooled analysis of LRRFS, DFS, and OS was carried out for T1-2 and T2-3 studies, respectively, and the *p*-values were tabulated.

Results

The PubMed search yielded 27 studies in total. Ten were eliminated due to dealing with stage T1-4, BCS, comparison of ypN0 to ypN1, whole breast radiotherapy, survival by subtype, and survival data for regional LN irradiation only. Seventeen were initially found to satisfy the criteria. Five more were eliminated due to incomplete data (Figure 1). The studies included were (16-26). The total number of patients was 8340, with a pooled mean follow-up period of 6.3 years and there were 4136 patients in the PMRT (RNI) group and 4204 patients in the NO PMRT group (Table 1). RNI was included as part of the PMRT in all of the studies. Only five studies gave a breakdown (18, 19, 21, 22, 25). The LRRFS, DFS, and OS were 96.7% (95% CI: 96.5–96.9), 82.1% (95% CI: 81.0–83.2), and 87.3% (95% CI: 86.9–87.7) for the PMRT group, and 93.9% (95% CI: 93.6–94.2), 79.6% (95% CI: 78.7–80.5), and 84.8% (95% CI: 84.3–85.3) for the NO PMRT group, respectively. Some studies did not report figures for LRRFS (17, 19, 23-25) and DFS (22-24). There was no significant

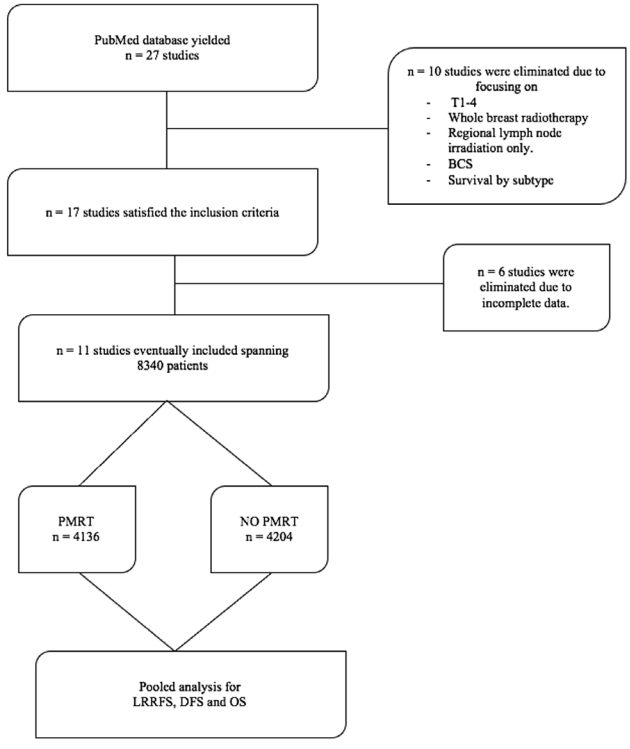

Figure 1. CONSORT flow diagram showing study distribution

PMRT: Post-mastectomy radiotherapy; LRRFS: Locoregional recurrence-free survival; DFS: Disease-free survival; OS: Overall survival; BCS: Breast-conserving surgery

difference between the two groups for LRRFS, DFS and OS at p = 0.61, p = 0.61, and p = 0.38, respectively. The subgroup analysis of the T-stage (Tables 2, 3) also showed no significant differences in LRRFS, DFS, or OS for T1-2 and T2-3 for both groups (Table 4).

Discussion and Conclusion

This pooled analysis included 8340 patients with a pooled mean follow-up period of 6.3 years. The results for survival are in agreement with most of the literature. However, a longer follow-up period of 10 years or more is essential to validate these results, as 95% of LRRs occur within 10 years after surgical intervention (8). Furthermore, LRRFS, DFS, and OS subgroup analysis was performed for T1-2 and T2-3, which also showed no statistical significance. The LRR for the PMRT group in this study was 3.3% and 6.1% for the NO PMRT group, respectively, which are within the reported rates. Only two studies (20, 21) gave 10-year LRR rates, which were 4% *vs.* 7% (study 19) and 2.5% *vs.* 6.5% (study 20) for PMRT *vs.* NO PMRT, respectively. The administration of PMRT in patients who are cN+ and convert to ypN0 post-modern-era NAC remains challenging. Recommendations for PMRT are based on clinical stage and LN status (9, 27). These guidelines are based on the outcomes of randomized trials and patterns of failures (28-30). Patients who achieve pCR in the breast and axilla have a significantly decreased risk of LRR (31). Therefore, patients who are rendered ypN0 are effectively down-staged and might not benefit from PMRT (32). Modern-era NAC has been proven to improve LRR rates in the adjuvant role. McBride et al. (33) looked at patients in two eras and retrospectively analyzed the LRR rates in 1027 patients with T1-2 BC with 1-3 positive LNs treated with mastectomy and adjuvant chemotherapy with or without PMRT during an early era (1978–1997) and a later era (2000–2007). These eras were selected because they represented periods before and after the routine use of sentinel LN surgery, taxane chemotherapy, and aromatase inhibitors. 19% of the 505 patients treated in the early era and 25% of the 522 patients in the later era received PMRT. Patients who received PMRT had significantly higher-risk disease features. PMRT reduced the rate of LRR in the early-era cohort, with 5-year rates of 9.5% without PMRT and 3.4% with PMRT, and 15-year rates of 14.5% versus 6.1%, respectively. However, PMRT did not appear to benefit patients treated in the later cohort, with 5-year LRR rates of 2.8% without PMRT and 4.2% with PMRT. They stated that the risk of LRR for patients with T1-2 BC with 1-3 positive LNs treated with mastectomy and systemic treatment is highly dependent on the era of treatment. Modern treatment advances and the selected use of PMRT for those with high-risk features have allowed for the identification of a cohort at very low risk for LRR without PMRT. Miyashita et al. (34) enrolled patients who received NAC and mastectomy for cT1–4 cN0–2 M0 BC. They evaluated the association between radiotherapy and outcomes of LRR, distant DFS, and OS based on ypN status by multivariable analysis of 3326 patients. Multivariable analysis demonstrated that use of radiotherapy was independently associated with improved LRR for ypN2–3 patients only. The association between radiotherapy and OS was not statistically significant among ypN0 (p = 0.22) and ypN1 patients (p = 0.51). The results from this Japanese nationwide database study did not show significant associations between PMRT and improved survival among ypN0 and ypN1 patients and concluded that radiotherapy may be beneficial only for ypN2–3 BC patients who receive NAC and mastectomy in the modern era. However, another study carried out retrospectively concurred with the lack of benefit of PMRT for patients who achieve ypN0 but disagrees on its possible omission in patients with ypN1 (35). The current pooled analysis is for patients who achieve ypN0, and the results are in agreement for this group of patients.

It was not possible to carry out subgroup analysis for the different molecular subtypes in this pooled analysis due to inadequate data presentation. There are, however, conflicting findings in relation to the benefits of PMRT on subtypes. Cho et al. (18) looked at the benefit of PMRT in ypN0 patients after NAC according to molecular subtypes. They concluded that in patients who achieve ypN0 following NAC and mastectomy, PMRT shows no additional survival benefits for any molecular subtype. However, in another study, it was suggested that among ypN0 patients, only triple-negative breast cancer (TNBC) patients might benefit from PMRT (36). Furthermore, Miyashita et al. (34) suggested that radiotherapy significantly improved the LRR rate only for patients with HER2+ disease in their analysis, and patients with TNBC exhibited a higher LRR rate after NAC and mastectomy regardless of the presence or absence of PMRT. They also observed favorable LRR rates for HR+ patients in both groups. Although they indicated that high-risk subgroups for recurrence, such as those with TNBC and large tumors, are recommended for radiotherapy, their assessment needs further confirmation due to the small sample size. Factors that have also been found to influence LRR in those not receiving PMRT were positive margins, extracapsular extension, age less than forty, and LVI (19, 31, 37, 38). In further analysis, Muhsen et al. (19) examined the relationship between age, LVI presence, and LRR in patients who did not receive PMRT. They found that at 10 years, LRR rates for patients with no LVI and age >40 years were 2% (95% CI, 0.7–3.8), compared with 28% (95% CI, 11.0–22.1) in patients with LVI and age <40 years (p<0.0001). Tumor size has also been implicated as a LRR determinant, with higher rates of LRR seen in tumors ≥2 cm (39). Furthermore, PMRT in patients treated with taxane-based chemotherapy showed no benefit in LRRFS, DFS, or OS (21). Therefore, these LRR factors have to also be taken into account in the context of the type of chemotherapy used.

The addition of RNI to PMRT appears not to influence the LRRFS. Tam et al. (22) included patients treated with chest wall (CW) irradiation alone and CW with RNI. There was no benefit identified with RNI versus CW irradiation alone. Similarly, for BCS, Schlafstein et al. (40) compared the survival of whole breast (WB) radiotherapy alone with WB+ RNI. They found that the 10-year survival for WB alone versus WB + RNI was 83.6% and 79.5%, respectively (p = 0.14) and concluded that for women with cN1 BC who convert to ypN0 following NAC and BCS with SLNB alone, more extensive RNI may not provide a long-term survival benefit. Other trials have demonstrated benefits to DFS and decreased cancer mortality with extensive radiation in the modern era NAC (41, 42). A recently published meta-analysis by the Early Breast Cancer Trialists' Collaborative Group supports this notion. The meta-analysis was carried out on individual patient data from 14324 patients in 16 trials looking at radiotherapy to regional nodes in early BC. They reported that in the newer trials (12167 patients), which started during 1989-2008, RNI significantly reduced distant recurrence and BC mortality with no significant effect on non-BC mortality. However, in the older trials (2157 patients) during 1961–1978 RNI did not have a significant impact on recurrence. They concluded that these contrasting findings could reflect radiotherapy improvements since the 1980s (43).

Table 1. Pooled analysis of survival for all studies

	Author (year)	cN+ rendered ypN0 post NAC patients *n* =	Stage	ypN0 PMRT+(RNI) patients *n* = LRRFS/DFS/OS (%) patients *n*ˆ =	ypN0 NO PMRT patients *n* = LRRFS/DFS/OS % patients *n*ˆ =
Retrospective	Dai et al. (16) (2023)	116	1-2	31 -/90.2/96.7 *n* = 0/28/30 (5 year)	85 -/93.7/97.3 *n* = 0/80/83 (5 year)
Retrospective	Wang et al. (17) (2020)	142	1-2	110 94.5/88.7/96.1 *n* =104/98/106 (5 year)	32 90.1/72.4/95.0 *n* = 29/23/30 (5 year)
Retrospective	Cho et al. (18)* (2019)	189	1-3	111 (98) -/76.9/89.6 *n* = 0/85/99 (5 year)	78 -/77.5/88.9 *n* = 0/60/69 (5 year)
Retrospective	Muhsen et al. (19) (2018)	1087	1-2	163 (150) 96/75/81 *n* = 156/122/132 (10 year)	924 93/73/80 *n* = 859/675/739 (10 year)
Retrospective	Zeidan et al. (20) (2018)	684	1-2	337 97.5/77.3/81.7 *n* = 329/261/275 (10 year)	347 93.5/75.9/78.3 *n* = 324/263/272 (10 year)
Retrospective	Kim et al. (21) (2017)	714	1-2	130 (All) 97/94/98 *n* =126/122/127 (5 year)	584 96/90/96 *n* = 561/526/561 (5 year)
Retrospective	Tam et al. (22) (2017)	523	1-3	206 (146) -/-/86 *n* = 0/0/177 (10 year)	317 -/-/84 *n* = 0/0/266 (10 year)
Retrospective	Rusthoven et al. (23) (2016)	3040	1-3	1962 -/-/88.3 *n* = 0/0/1732 (5 year)	1078 (no PMRT) -/-/84.8 *n* = 0/0/914 (5 year)
Retrospective	Liu et al. (24) (2016)	1560	2-3	903 -/-/84.6 *n* = 0/0/764 (5 year)	657 -/-/81.7 *n* = 0/0/537 (5 year)
Retrospective	Shim et al. (25) (2014)	151	2-3	105 (All) 98.1/91.2/93.3 *n* = 103/96/98 (5 year)	46 92.3/83.0/89.9 *n* = 42/38/41 (5 year)
Retrospective	Le Scodan et al. (26) (2012)	134	2-3	78 96.2/79.2/88.3 *n* = 75/62/69 (5 year)	56 92.5/85.2/94.3 *n* = 52/48/53 (5 year)
Total		8340		4136 *n* = 893*/874#/3609 % (96.7/82.1/87.3)	4204 *n* = 1867*/1713#/3565 % (93.9/79.6/84.8)

PMRT: Post-mastectomy radiotherapy; LRRFS: Locoregional recurrence-free survival; DFS: Disease-free survival; OS: Overall survival; NAC: Neoadjuvant chemotherapy; *: Number of patients for Studies with no LRRFS were deducted for PMRT 4136-3213 = 923 and for NO PMRT 4204 - 2215 = 1989; #: Number of patients for studies with no DFS were deducted for PMRT 4136 – 3071 = 1065 for NO PMRT 4204 – 2052 = 2152; ˆn: The number of patients corresponds to the percentage for each survival outcome (calculated by multiplying the % with the total number of patients for each study)

Table 2. Subgroup analysis for stage 1-2. Pooled analysis for studies that included stage 1-2

	Author (year)	cN+ rendered ypN0 post NAC patients *n* =	Stage	ypN0 PMRT+(RNI) patients *n* = LRRFS/DFS/OS (%) patients *n*^ =	ypN0 NO PMRT patients *n* = LRRFS/DFS/OS (%) patients *n*^ =
Retrospective	Dai et al. (16) (2023)	116	1-2	31 -/90.2/96.7 *n* = 0/28/30 (5 year)	85 -/93.7/97.3 *n* = 0/80/83 (5 year)
Retrospective	Wang et al. (17) (2020)	142	1-2	110 94.5/88.7/96.1 *n* = 104/98/106 (5 year)	32 90.1/72.4/95.0 *n* = 29/23/30 (5 year)
Retrospective	Muhsen et al. (19) (2018)	1087	1-2	163 (150) 96/75/81 *n* = 156/122/132 (10 year)	924 93/73/80 *n* = 859/675/739 (10 year)
Retrospective	Zeidan et al. (20) (2018)	684	1-2	337 97.5/77.3/81.7 *n* = 329/261/275 (10 year)	347 93.5/75.9/78.3 *n* = 324/263/272 (10 year)
Retrospective	Kim et al. (21) (2017)	714	1-2	130 (All) 97/94/98 *n* = 126/122/127 (5 year)	584 96/90/96 *n* = 561/526/561 (5 year)
Total		2743		771 *n* = 715*/631/670 % (96.6/81.8/86.9	1972 *n* = 1773*/1567/1685 % (94.0/79.5/85.4)

PMRT: Post-mastectomy radiotherapy; LRRFS: Locoregional recurrence-free survival; DFS: Disease-free survival; OS: Overall survival; NAC: Neoadjuvant chemotherapy; *: Number of patients for studies with no LRRFS were deducted for PMRT 771 = 740 and for NO PMRT 1972 - 85 = 1887; ^: The number of patients corresponds to the percentage for each survival outcome (calculated by multiplying the % with the total number of patients for each study)

The primary 5-year results of the NSABP B-51 trial recently presented in the SABCS (Dec. 2023) shed light on the role of RNI (15). This prospective trial's protocol specified the final analysis would take place after 172 events, or 10 years after study initiation. It looked at the benefit of RNI on survival in patients who were cN+ and converted to ypN0 after NAC. The number of patients recruited for disease-related end points was 1556. Patients were randomly assigned, with half of them receiving CW irradiation plus RNI after mastectomy or WB irradiation plus RNI after BCS. The other half received no RNI, instead undergoing observation after mastectomy or WB irradiation after BCS. The 5-year estimated LRRFS result for the NO RNI *vs.* RNI was 98.4% *vs.* 99.3% (HR = 0.37; 95% CI, 0.12–1.16), for DFS was 88.5% *vs.* 88.3 (HR = 1.06; 95% CI, 0.79–1.44), and for OS was 94 *vs.* 93.6 (HR = 1.12; 95% CI, 0.75–1.68), respectively. They concluded that the addition of RNI to PMRT or WB did not improve survival outcomes when compared to NO RNI or NO PMRT. Follow-up of patients for long-term outcomes continues. In a retrospective study, Cho et al. (44) found that in patients who achieved ypN0 after NAC and BCS, RNI did not improve LRC or survival, regardless of the subtype or primary tumor response, which is in agreement with the aforementioned trial. In addition to NSABP B-51, the SUPREMO prospective trial, an international trial with most patients contributing from the UK, Europe, and countries such as China, Japan, and Canada, among others, specifically looks at radiotherapy benefits in patients who underwent mastectomy with 1-3 positive LNs. These trials will contribute to the basis of radiotherapy administration in this specific group of patients once the final results are published.

The current pooled analysis is limited by the retrospective nature of the studies included, which contributes to selection bias. Most of the studies are T1-2, which might have influenced the outcome. The analysis for T2-3 is limited by the small number of patients analyzed. However, the inclusion of T1-3 studies in this analysis encompasses the relevant T-stages.

PMRT does not seem to confer survival benefits on patients with T1-3 tumors and 1-3 positive LNs. However, a concrete statement cannot be made in this regard for stage T3 patients due to the small number of patients analyzed. Clinicopathological factors that influence LRR, such as age less than forty, LVI, and tumor size, have to be taken into account before patients can forgo PMRT. Prospective studies with long-term follow-up are required to confirm these findings. These studies also have to take into account the aforementioned prognostic factors. Furthermore, the role of PMRT in the different BC subtypes requires further assessment. The ongoing phase 3 clinical prospective trials' results are essential in guiding the de-escalation of radiotherapy.

Table 3. Pooled analysis for studies that included stage 2-3

	Author (year)	cN+ rendered ypN0 post NST patients *n*=	Stage	ypN0 PMRT+(RNI) patients *n*^ = LRRFS/DFS/OS (%) patients *n* =	ypN0 NO PMRT patients *n*^ = LRRFS/DFS/OS (%) patients *n* =
Retrospective	Liu et al. (24) (2016)	1560	2-3	903 -/-/84.6 *n* = 0/0/764 (5 year)	657 -/-/81.7 *n* = 0/0/537 (5 year)
Retrospective	Shim et al. (25) (2014)	151	2-3	105 (All) 98.1/91.2/93.3 *n* = 103/96/98 (5 year)	46 92.3/83.0/89.9 *n* = 42/38/41 (5 year)
Retrospective	Le Scodan et al. (26) (2012)	134	2-3	78 96.2/79.2/88.3 *n* = 75/62/69 (5 year)	56 92.5/85.2/94.3 *n* = 52/48/53 (5 year)
Total		1845		1086 178*/158#/931 % (97.3/86.3/85.7)	759 94*/86#/631 % (92.2/84.3/83.1)

PMRT: Post-mastectomy radiotherapy; LRRFS: Locoregional recurrence-free survival; DFS: Disease-free survival; OS: Overall survival; NAC: Neoadjuvant chemotherapy; *: Number of patients for studies with no LRRFS were deducted for PMRT 1086-903 = 183 and for NO PMRT 759 - 657 = 102; #: Number of patients for studies with no DFS were deducted for PMRT 1086 – 903 = 183 for PMRT NO PMRT 759 – 657 = 102; ^: The number of patients corresponds to the percentage for each survival outcome (calculated by multiplying the % with the total number of patients for each study)

Table 4. *P*-value for T1-2/ T2-3

Stage	LRRFS *p*-value	DFS *p*-value	OS *p*-value
T1-2	0.67	0.66	0.81
T2-3	0.83	0.97	0.69

LRRFS: Locoregional recurrence-free survival; DFS: Disease-free survival; OS: Overall survival

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Review

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Is STARD3 A New Biomarker for Breast Cancer?

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ABSTRACT

Despite advances in diagnosis and treatment, breast cancer is still one of the three most common cancers in the world and a significant cause of morbidity and mortality. Lipids play a role in many basic physiological pathways in cells, from regulating cell homeostasis to energy expenditure. As in many types of cancer, changes in lipid metabolism and their relationship have been reported in breast cancer. The *STARD3* gene encodes a member of the subfamily of lipid trafficking proteins. It is a sterol-binding protein that mediates the transport of cholesterol from the endoplasmic reticulum to endosomes. It has been shown that STARD3 is correlated with human epidermal growth factor receptor 2 (HER2) amplification since it has the same localization as HER2 in the chromosome. In this review, we aimed to emphasize that investigating lipid metabolism together with the STARD3 biomarker has great potential not only for subtype-specific strategies but also for patient-specific strategies.

Keywords: Breast; cancer; lipid; STARD3

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Key Points

- Breast
- Cancer
- Lipid
- STARD3

Introduction

Breast cancer is the most common malignancy in woman. According to the Global Cancer Observatory (GLOBOCAN) 2020 datas there are 2.26 million new cases and more than 680.000 deaths due to breast cancer globally, which is very concerning (1). Breast cancer can be classified into 4 molecular subtypes; Luminal A [estrogen receptor (ER) and progesterone receptor (PR) positive, human epidermal growth factor receptor 2 (HER2) negative], Luminal B (ER and HER2 positive, PR negative), Basal-Like (ER, PR, HER2 negative) and HER2-enriched (ER and PR negative, HER2 positive) (2). However, some researchers classify breast cancer into 5 subtypes by adding antijen Kiel 67 (Ki67) proliferative markers to the classification. These subtypes; Luminal A (ER and/or PR positive/HER2 negative/low Ki67), Luminal B (ER and/or PR positive/HER2 negative/high Ki67), HER2 positive Luminal B (ER and/or PR positive/HER2 overexpression/any Ki67), non-Luminal HER2 positive (ER and PR amplified/HER2 overexpression), Triple Negative (ER/PR/HER2 negative) (3). Molecular classification is vital for predicting prognosis and clinical outcome, as well as designing treatment strategy based on patients' condition.

According to histological classification, invasive ductal carcinoma accounts for approximately 85% of invasive breast cancers. Breast carcinomas originate from the same part of the terminal duct lobular unit. Invasive ductal carcinoma is the most common type of invasive breast cancer. It accounts for 55% of the incidence of breast cancer at diagnosis (4). Invasive breast carcinomas subtypes and histological variants are known well. In general, breast neoplasias can be classified as carcinoma *in situ* (CIS) and invasive carcinoma. Ductal CIS is a noninvasive and potentially malignant intraductal proliferation of epithelial cells confined to ducts and lobules. Invasive or infiltrative carcinoma is the malignant abnormal proliferation of neoplastic cells that penetrate the stroma trough the duct walls in the breast tissue. Invasive carcinoma and CIS are classified as ductal and lobular depending on the site of tumor origin. Cancers arising from the ducts are called ductal carcinoma, arising from the lobules are called lobular carcinoma. However, it has now been found that such tumor growth variation doesn't correlate with the site or cell of origin but with whether the tumor cells express E-cadherin (5).

HER2 overexpression is present in approximately 15% to 20% of breast cancers. It has generally been associated with an increased risk of the development of systemic metastases and poor survival intratumoral heterogeneity of HER2 expression has been described in 16–36% of HER2-positive BC patients and it is defined as the presence of varying degrees of HER2 overexpression in different areas of the same

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tumor (6). In the HER2-positive group, targeted therapies have been studied mostly in metastatic cases. It has been shown that HER2 overexpression is associated with short disease-free survival and overall survival in node-positive cases. Although the biological consequences of HER2 overexpression on the prognosis of HER2-positive breast cancer and its predictive significance for anti-HER2 therapy are widely understood, the evidence regarding HER2-low breast cancers has been relatively ambiguous, particularly regarding whether it constitutes a separate biological or clinical subtype (7).

Despite the large amount of funding and personnel devoted to breast cancer research over many years, the ethology of breast cancer is not yet fully understood. Treatment resistance and recurrence in breast cancer patients remain unresolved. Because it is a disease with high heterogeneity, the treatment and prognosis of patients are quite different.

Lipid Metabolism-Breast Cancer Correlation

Lipids are in the class of water-insoluble metabolites. Lipid types vary from 10.000 to millions depending on molecular classification (8). Despite this heterogeneity, most lipid molecules consist of fatty acids and cholesterol. The numerous studies to date identifying proteins and genes expressed in cancer cells have almost always identified lipid metabolism as one of the main processes affected. One of the most important characteristics of this condition is that cancer cells depend on the source of fatty acids and cholesterol. This requirement is linked to the increasing need for membranes that support cell growth and division and provide energy to fuel cellular processes such as metastasis (9).

Early studies on lipid metabolism and cancer revealed increasing cholesterol levels and changes on phospholipids in tumor tissues. Studies with radioactive substrates in the early 1960s has shown that cancer cells exhibit a dynamic lipid metabolism and actively synthesize and uptake lipids (10, 11). It's known that lipid metabolism is reprogrammed in tumors. Lipids are can be used on hormonal therapy in the future due to this reprogrammed cholesterol metabolism is association with migration, invasion and cell proliferation on endocrine related cancers (12, 13).

Recent studies have shown characteristic changes in lipid parameters between patients with invasive breast cancer and patients with benign breast tumors. Moreover, these changes were also seen in patients with different molecular types of breast cancer. In addition, postoperative chemotherapy has been found to cause abnormal plasma lipid metabolism changes in breast cancer patients (14). Similarly, it has been thought lipid transduction may be necessary for carcinogenesis and survival in breast cancer (15, 16). However, although many studies have shown the effect of lipid metabolism on carcinogenesis, data on its effects on breast cancer recurrence and survival are limited (17-19).

Some studies have shown that low-density lipoprotein cholesterol (LDL-c) is not associated with breast cancer risk, but serum LDL-c levels may be marker of breast cancer progression (20-22). However, it was demonstrated that significant up-regulation of LDL receptor increased LDL uptake in cancer cells because of the demand of rapid proliferation (23, 24). One recent meta-analysis, based mostly on case-control studies, concluded that high triglyceride levels increase the risk of breast cancer by 8% and low high-density lipoprotein cholesterol (HDL-c) levels increase the risk of breast cancer by 38% (25). Bhat et al. (26) in their study, they found that there was no significant change in HDL-c levels when they compared breast cancer patients with the control group. Borrelli et al. (27) have found that revers relation between HDL-c and breast cancer risk and argued that HDL-c is a biochemical marker that may be associated with an increased risk of breast cancer. In another study, it was observed that oxidized LDL-c levels were also high in breast cancer patients with high blood LDL-c levels, and it was concluded that oxidized LDL-c was associated with the risk of breast cancer. However, it has been suggested that HDL-c is less sensitive to peroxidation due to its lipid and Apo protein content, and therefore acts as an anti-oxidant because it cannot produce reactive oxygen species (28).

STARD3

STARD3 (StAR Related Lipid Transfer Domain Containing 3), also known as metastatic lymph node protein 64 (MLN64), is a sterol-binding protein that forms endoplasmic reticulum-endosome contact areas. The *STARD3* gene located in the q12-q21 zone on chromosome 17 and encodes a protein containing two separate domains. The N-terminus of this protein has the feature of a potential trans membrane region, C-terminus has the same homology to a protein involved in steroid hormone synthesis. Some studies have suggested that the second domain is present in proteins involved in various cell functions and has 37% similarity to STAR (29-31). *STARD3* gene encodes a cholesterol-binding membrane protein (31, 32) and two different cell culture studies conducted in 2005 and 2010 it was shown that this protein may be involved in the actin-dependent movement of late endosytic organelles and cholesterol transfer between this organelles and other membrane-dependent organelles such as mitochondria (33, 34). Due to its activity in lipid metabolism, it has been a matter of curiosity whether STARD3 has any activity in cancer types that may be related to lipid-based hormones. Based on this idea, when its relationship with androgen-dependent breast cancer was investigated, it was seen that STARD3 was closely related to HER2 and it was suggested that STARD3 contributed to proliferation in HER2 cell lines (35). In a study conducted using the quantitative polymerase chain reaction method with DNA samples obtained from frozen tumor samples, the amplification of genes co-localized with HER2 [mediator complex subunit 1 (MED1), STARD3, HER2, growth factor receptor bound protein 7 (GRB7), thyroid hormone receptor alpha (THRA), retinoic acid receptor alpha (RARA), DNA topoisomerase II alpha (TOP2A), insulin like growth factor binding protein 4 (IGFBP4), C-C motif chemokine receptor 7 (CCR7), keratin 20 (KRT20), keratin 19 (KRT19) and gastrin (GAS)] in HER2+ cell lines was evaluated. As a result of the study, it was shown that HER2 amplification and STARD3 were correlated (36). To determine the functional interactions of the STARD3 protein in cellular processes, STRING network analysis was applied. Protein-protein interactions with the top 5 proteins in the shell [MOSPD2 (motile sperm domain-containing protein 2), PGAP3 (post-GPI attachment to proteins factor 3;), STARD3NL (STARD3 N-terminal-like protein), VAPA (vesicle-associated membrane protein-associated protein A), VAPB (vesicle-associated membrane protein-associated protein B/C)] fell within a homology score range of 0.990-0.940 and were statistically highly significant (p<0.05) (Figure 1). Various mechanisms have been proposed regarding STARD3's ability to increase plasma membrane cholesterol. One of these mechanisms involves the newly synthesized STARD3 protein moving to late endocytic organelles via the plasma membrane, thereby increasing cholesterol accumulation in the organelles. The increased cholesterol in late endocytic organelles containing STARD3 can become transportable

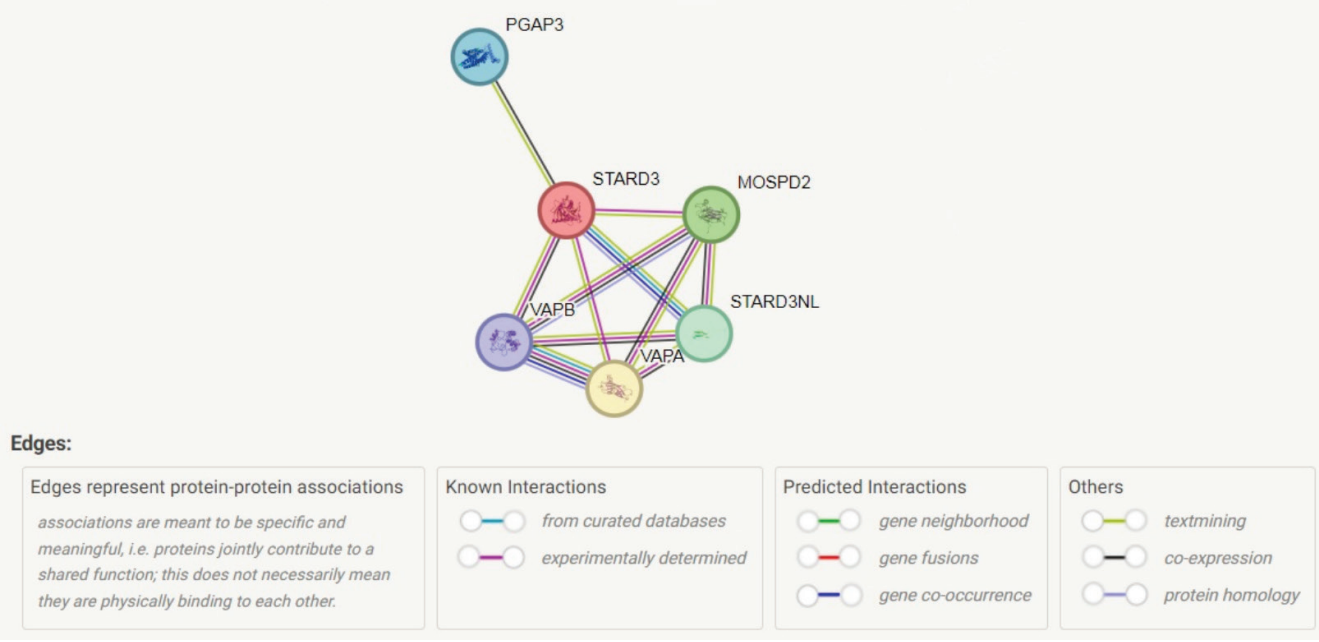

Figure 1. The schematic representation of predicted protein-protein interactions of STARD3 in the STRING database

STARD3: StAR Related Lipid Transfer Domain Containing 3

to the plasma membrane. Finally, the accumulated cholesterol inside the cell is transferred from the endoplasmic reticulum to the plasma membrane (37). In this way, the increased biosynthetic activity of cholesterol observed in cells overexpressing STARD3 also facilitates enrichment of plasma membrane cholesterol. In cells overexpressing STARD3, an increase in mRNA levels encoding HMGR, the rate-limiting cholesterol biosynthesis enzyme, has been observed. Increased cholesterol biosynthesis, especially under low nutrient conditions where membrane biogenesis is limited, allows cells to continue survival and division (38). STARD3 has been determined to play a role in the development of various types of cancer, such as colorectal, prostate and stomach cancers (39). However, STARD3 was found to have the highest expression levels in breast cancer tissues compared to other types of cancer such as prostate and liver cancers (40).

STARD3- Breast Cancer Correlation

In recent years, considering the effect of STARD3 on cells, it has been a matter of curiosity whether it is effective in the lipid metabolism changes seen in breast cancer patients.

Vassilev et al. (38) produced polyclonal rabbit antibodies against the START domain of human STARD3 for the role of the STARD3 protein in breast cancer cells and tissues. These antibodies have been used to suppress STARD3 expression in breast cancer patients and have shown reduced survival of tumor cells in patients given the antibody. In the same study, to gain insight into how STARD3 may support cell survival independent of HER2 amplification generated MCF-7 (HER2-negative) breast cancer cells stably overexpressing STARD3-green fluorescent protein (GFP) or soluble GFP as a control. Remarkably, the overall morphological features of STARD3-GFP high-expressing cells appeared to be strikingly different from those of control GFP cells. STARD3-GFP cell clusters appeared to have increased filipin density, especially at the plasma membrane, compared with control cells. This raises the possibility that STARD3-overexpressing cells may have high cholesterol content. However, based on biochemical cholesterol determination, the total amount of cellular free cholesterol in STARD3-GFP cells was not increased but rather slightly decreased compared with control cells. Based on this, it was concluded that overexpression of STARD3 causes changes in cellular cholesterol distribution and homeostatic control, increasing plasma membrane cholesterol but decreasing ER cholesterol (38).

In 2006, Kao and Pollack (41) investigated the impact of targeted disruption of various genes associated with HER2/neu on cell function. They demonstrated a significant correlation between the inactivation of STARD3 and GRB7 with decreased cell proliferation and progression of the cell cycle, suggesting that the amplification of these genes and the overexpression of their encoded proteins could play a role in cellular tumorigenesis. An immunohistochemical study in breast cancer patients revealed higher expression of STARD3 in malignant breast tissue compared to normal breast tissue, which was associated with tumor size and histological grade. The study concluded that STARD3 could be a potential marker in HER2+ breast cancer patients (42). Silencing STARD3 by siRNA in HER2+ breast cancer cell lines induced apoptosis, suggesting that STARD3 may be necessary for the growth and survival of these cells (43). Similarly, a study by Li et al. (44) found that patients with high STARD3 expression had a lower survival rate compared to those with low expression. The study also demonstrated that inhibiting STARD3 expression reduced PI3K/AKT/mTOR pathway activity and induced apoptosis in MCF-7 cell lines.

Using traditional qualitative PCR and various bioinformatics websites such as Oncomine, GEPIA (gene expression profiling interactive analysis), and Expression Atlas, the expression of STARD3 at mRNA and protein levels in breast cancer was examined. The impact of STARD3 as a prognostic and diagnostic biomarker was assessed. The study revealed that the mRNA expression of STARD3 was significantly higher in HER2+ cell lines compared to ER+ normal cell lines. Based on this, STARD3 was suggested to be a potential diagnostic and prognostic biomarker for HER2+ breast cancer (40). Lodi et al. (45)

investigated the relationship between STARD3 expression and breast cancer-specific survival, comparing it with other relevant patient and tumor characteristics in HER2+ series. In this study, STARD3 DNA copy number showed a strong positive correlation with HER2 DNA copy number. Both STARD3 DNA copy number and RNA expression were found to be strongly associated with HER2. Vinatzer et al. (46) based on their measurements using quantitative RT-PCR, concluded that the overexpression of STARD3 enhances the prognostic power of HER2 overexpression for disease-free survival in breast cancer patients. It has been shown that *MLN64* and *HER2* genes share common transcriptional controls along with a physical connection on chromosome 17q. Based on this, they hypothesized that, in addition to the oncogenic potential of HER2 overexpression, the unbalanced effect of MLN64 contributes to poor clinical outcomes in breast tumors carrying this amplified region (47). HER2 amplification is present in 13–15% of breast cancer cases and biologically leads to a more aggressive malignancy by increasing sensitivity to chemotherapy in cells (48). Furthermore, the evaluation of response to anti-HER2 agents used in chemotherapy is of great importance in treatment monitoring (49). In a study examining STARD3 expression in HER2+ breast cancer patients, a strong correlation was observed between STARD3 and HER2 DNA amplification and RNA expression. Based on these findings, it has been suggested that STARD3 could be evaluated as a subgroup for HER2+ breast cancer, potentially used in treatment planning and patient prognosis monitoring (45). In a similar study, it was observed that STARD3 expression is higher in HER2+ patients compared to HER2-. It has been suggested that STARD3 may have an impact on overall survival, recurrence-free survival, and non-metastatic survival (40). The STARD3 inhibitor has recently been developed and tested in various breast and colon cancer cell lines (50). The study results are promising, but it is a fact that further *in vitro* and *in vivo* research is needed.

Studies conducted in recent years support that STARD3 may be a potential biomarker in the diagnosis of breast cancer, especially since it originates from the same gene region as HER2. However, the limitations of the data we have and the limited number of studies and patient population should also be taken into consideration. Elucidating the molecular mechanism of STARD3 function will provide new insights into its mechanism of action. Future studies will provide evidence on how to regulate the molecule's lipid transfer activities and its role in breast cancer treatment.

Authorship Contributions

Concept: A.N.K., N.I.; Design: A.N.K., N.I.; Data Collection or Processing: A.N.K., N.I.; Analysis or Interpretation: A.N.K., N.I.; Literature Search: A.N.K., N.I.; Writing: A.N.K., N.I.

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Original Article

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Sentinel Lymph Node Biopsy After Neoadjuvant Chemotherapy in cN0 Breast Cancer: Impact of HER2-Positive Status on Survival

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ABSTRACT

Objective: High rates of negative sentinel lymph node biopsy (SLNB) in clinically node-negative (cN0) breast cancer (BC) after neoadjuvant chemotherapy (NAC) have been described. These results are associated with triple-negative (TNBC) and human epidermal growth factor receptor 2 (HER2+) subtypes achieving pathologic complete response (pCR). This study evaluates predictive variables and survival in order to assess the possible omission of SLNB after NAC.

Materials and Methods: Prospective study of women with cN0 BC treated with NAC and subsequent surgery, between April 2010 and May 2021. SLNB technique included, performing axillary lymphadenectomy in the absence of detection or SLNB-positivity. Multivariable logistic regression was used for analysis of NAC-response and SLNB-results in molecular subtypes: HR-/HER2+, TNBC, HR+/HER2- and HR+/HER2+. Kaplan-Meyer and log-rank were used for survival analysis.

Results: A total of 179 patients (50.5±10.1 years) were included. Of these, 39.7% achieved pCR (ypT0/Tis). HR-negative subtypes had higher pCR rates (HR-/HER2+: 59.4%; TNBC: 53.4%), with no cases of SLNB-positive. With residual disease, HR-/HER2+ and TNBC showed low rates of SLNB-positivity (6.7% and 10.3%) versus HR+ (HR+/HER2+: 20%; HR+/HER2-: 44%; $p<0.001$). Multivariable analysis identified independent predictors of SLNB-negativity ($p<0.0001$) to be: HR- [odds ratio (OR)=0.15; 95% confidence interval (CI): 0.06–0.37; $p = 0.0001$], HER2+ (OR=0.34; 95% CI: 0.14–0.81; $p = 0.015$) and high-grade Nottingham (OR=0.42; 95% CI: 0.18–0.99; $p = 0.048$). Disease-free survival showed worse outcomes with SLNB-positivity ($p<0.0001$), HR+/HER2- ($p = 0.0277$), larger tumor size ($p = 0.002$) and residual disease after NAC ($p<0.0001$).

Conclusion: Patient selection based on NAC response, molecular subtype, and survival outcomes is a priority for establishing individualized therapeutic strategies after NAC. Molecular subtypes with higher pCR rates and lower rates of SLNB-positivity could benefit from non-invasive strategies that include omission of SLNB.

Keywords: HER-2/neu; neoadjuvant chemotherapy; sentinel lymph node biopsy; survival; triple negative breast cancer

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Key Points

- SLNB after NAC safe and effective treatment for cN0.
- Molecular subtype tumor size predictors pCR.
- NAC response strongest prognosis predictor.
- SLNB-negative pCR achieved better prognosis.
- HER2+ benefit omission SLNB technique.

Introduction

Sentinel lymph node biopsy (SLNB) in breast cancer (BC) is a validated tool for axillary staging after neoadjuvant chemotherapy (NAC) in patients with clinically negative nodes (cN0) (1). Tumor size and BC molecular subtype are important predictors of NAC-response (2). cN0 patients with triple-negative (TNBC) and human epidermal growth factor receptor 2 (HER2+) BC show high rates of SLNB-negativity (ypN0) (3-5). Patients with a pathological complete response (pCR) show higher disease-free survival (DFS) and overall survival (OS) (6). SLNB after NAC allows better assessment of response to NAC (7-9). Molecular subtypes are important for

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predicting SLNB-negativity with high probability of pCR. There is no standard that recommends omitting axillary surgery in cN0 patients undergoing NAC (10). There are currently several ongoing trials (11), including two prospective trials that aim to assess axillary recurrence-free survival (ARFS) when omitting SLNB after NAC in patients initially diagnosed as cN0 (12, 13). This study presents the survival outcome of a cohort of patients who received NAC, with the aim of providing data for the omission of axillary surgery in selected cases.

Materials and Methods

Between April 2010 and May 2021, 179 women were retrospectively and consecutively included in the study. All patients and their associated data originate from a single healthcare institution: the 'Hospital Clínico Virgen de la Victoria' in the city of Malaga, Spain. It is a first-level hospital, a reference center in BC treatment that provides care to a population of 500,000 inhabitants. The following inclusion criteria were established: Age between 18 and 80 years, newly diagnosed invasive breast carcinoma, clinically negative axilla and/or confirmed through Fine-Needle Aspiration Biopsy (FNAB), undergoing complete SLNB technique with a dual tracer, receiving NAC consisting of Anthracyclines + Taxanes or Cyclophosphamide, receiving adjuvant chemotherapy after surgery, receiving local and axillary radiotherapy after surgery, and receiving Trastuzumab and/or Pertuzumab in HER2-positive patients, as well as hormonal therapy in hormone receptor-positive patients.

Exclusion criteria comprised; age >80 years, as international guidelines did not clarify the use of SLNB in this age group at the beginning of the study; history of previous neoplasia, either BC or any other origin; development of a new neoplasia of a different origin than breast; positive metastasis in the biopsy of a suspicious lymph node by FNAB; any other chemotherapy regimen not mentioned in the inclusion criteria; absence of radiotherapy treatment; absence of hormonal treatment if required; and absence of anti-HER2 treatment if required (Figure 1).

The initial anatomopathological diagnosis of the tumor was performed on samples obtained by core needle biopsy. The material was immediately fixed in buffered neutral formalin and embedded in paraffin. Three-millimeter sections were stained with hematoxalin and eosin (H&E) and macroscopically analyzed for tumor type and histological grade, which adhered to the Nottingham (Scarff-Bloom-Richardson) system. Subsequently, an immunohistochemical analysis was performed to define the molecular subtype.

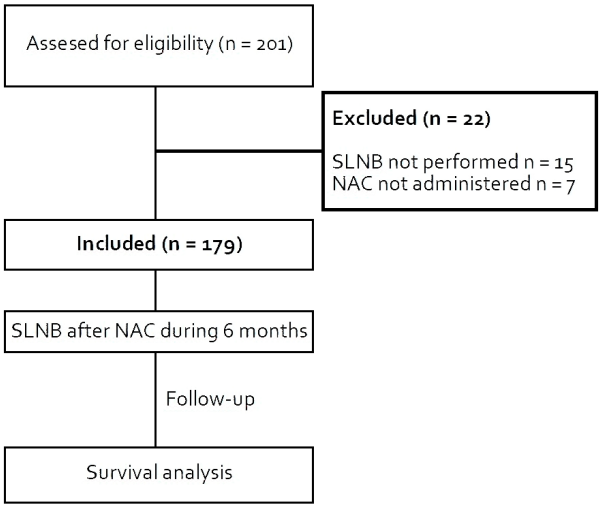

Figure 1. CONSORT flow diagram

SLNB: Sentinel lymph node biposy; NAC: Neoadjuvant chemotherapy

The criteria for NAC indication in cN0 BC patients have been based on the presence of HER2+ or TNBC subtypes and/or the accepted indication for reducing tumor volume to enable more conservative surgery. These NAC indications have been determined by a multidisciplinary team and have been crucial in evaluating the chemotherapy response in these specific cases, thus contributing to establishing a well-defined patient cohort.

SLNB technique was performed by intradermal periareolar injection with 37 MBq of 99mTc-nanocolloid of human serum albumin (Nanocoll®) for lymphoscintigraphy. Intraoperative localization of SLN was performed with gamma probe by an experienced Nuclear Medicine specialist.

During the intraoperative examination, both the tumor and sentinel lymph nodes (SLNs) were promptly sent to the Pathology Department for further analysis. A skilled pathologist conducted a macroscopic evaluation of the lymph node and subsequently sectioned it longitudinally/vertically based on its morphology, creating sections that were 2 mm thick. The most suspicious section, identified macroscopically, was frozen at -20 °C and later cut into 5–10 micrometer-thick sections, which were stained with H&E to assess malignancy. This procedure took approximately 15–25 minutes.

Following the intraoperative assessment of the SLNs, the definitive histopathological study of the tumor and SLN was performed. The tumor was processed with 3-millimeter sections in blocks, and an immunohistochemical study was conducted in separate blocks. Each lymph node was individually fixed in formalin and embedded in separate paraffin blocks. From each block, two 3-micrometer sections were obtained, with an interval between them of 3–5 micrometers, and subsequently stained with H&E. Tumor and lymph node involvement were defined according to the American Joint Committee on Cancer (AJCC - 8th edition) Breast Cancer Staging standard (14) and the Residual Cancer Burden (RCB; MD Anderson Cancer Center, Houston, Texas, USA) (15). This comprehensive approach allowed for accurate assessment and characterization of the NAC response, contributing to the robustness of the study's findings. Isolated tumor cells, micrometastases, and macrometastases were considered as tumor presence at the lymph node level. The cases from our series evaluated through the Miller and Payne system, before the development of Symmans' RCB system, were reevaluated and assigned an RCB index and class and *yp* stage for a correct evaluation of the series. Axillary lymph node dissection (ALD) was performed with intraoperative SLNB-positive and with definitive positive results.

Clinical follow-up after surgery was scheduled every 6–12 months for a period of at least five years.

Statistical Analyses

Clinical variables were prospectively recorded and evaluated with parametrical or non-parametrical test according to appropriateness. Our hypotheses included assessing survival outcomes (DFS, OS, and ARFS) after NAC and identifying predictive factors for negative SLNB results in patients achieving a pCR. The primary outcome was OS, with secondary outcomes including DFS and ARFS. The study's variables encompassed patients' demographics, clinical characteristics,

tumor subtypes, NAC response, and corresponding outcomes, which were analyzed. For OS and DFS Kaplan-Meier analysis and the log-rank test were used. For all analyses, SPSS, version 22 for Windows was used (SPSS, Inc., Chicago, IL, USA).

Results

The clinical characteristics of the patients are shown in Table 1. Most frequent NAC protocol was anthracyclines and taxanes (n = 156; 87.2%), including docetaxel and cyclophosphamide/carboplatin,

Table 1. Patient and tumour characteristics

Variable	No. of patients*
Total number of patients	179 (100.0)
Age, in years [SD; range]	50.5‡/49.9† (±10.1; 29–77)
Body mass index, kg/m²	
<18.5	2 (1.1)
18.5-24.9	74 (41.3)
25-29.9	55 (30.7)
≥30	42 (23.5)
NA	6 (3.4)
Menopausal status	
Premenopausal	88 (49.2)
Perimenopausal	16 (8.9)
Postmenopausal	75 (41.9)
Tumor size, in mm [± SD; range]	33.2‡/30† [±13.7; 10–100]
cT stage	
T1	24 (13.4)
T2	135 (75.4)
T3	14 (7.8)
T4	6 (3.4)
c-Stage	
I	24 (13.4)
IIA	113 (75.4)
IIB	14 (7.8)
III	6 (3.4)
Hystological type	
Ductal invasive NST	165 (92.2)
Lobular invasive	6 (3.4)
Metaplasic invasive	4 (2.2)
Mucious invasive	2 (1.1)
Apocrin invasive	2 (1.1)
Laterality	
Right breast	98 (54.7)
Left breast	81 (45.3)
Location	
External	83 (46.4)
Internal	33 (18.4)
Center	37 (20.7)
Multifocal	26 (14.5)
Nottingham grade	
1	8 (4.7)
2	61 (35.9)
3	101 (59.4)
Surgical procedure	
Lumpectomy	154 (86)
Mastectomy	25 (14)
Hormone receptor (HR)	
Positive	89 (49.7)
Negative	90 (50.3)
HER2-neu receptor (HER2)	
Positive	77 (43)
Negative	102 (57)
Molecular subtypes	
HR-/HER2+	32 (17.9)
HR+/HER2+	45 (24.6)
HR+/HER2+	44 (25.1)
TNBC	58 (32.4)
Pathological response (RCB symmans)	
pCR	69 (38.5)
RCB-I	17 (9.5)
RCB-II	79 (44.1)
RCB-III	14 (7.8)
ypT category after NAC	
ypT0	52 (29.1)
ypTis	19 (10.6)
ypTmi	2 (1.1)
ypT1	57 (31.8)
ypT1a	3
ypT1b	14
ypT1c	40
ypT2	44 (24.6)
ypT3	5 (2.8)
ypN category after NAC	
ypN0	140 (78.2)
ypN0(i+)	6 (3.4)
YpN1mi	5 (2.8)
YpN1a	18 (10.2)
ypN2	6 (3.4)
ypN3	1 (0.6)
Pathology of SLNs	
Tumour-negative	140 (79.5)
Tumour-positive	36 (20.5)
Macrometastasis	25 (14.2)
Micrometastasis	5 (2.8)
ITCs	6 (3.4)
Follow-up, in months [SD; range]	50.9‡ / 45.3† [± 29.3; 12–124]
Progression during NAC	1 (0.6)
Global recurrence	21 (11.7)
Locoregional recurrence	11 (6.1)
Distant recurrence	17 (9.5)
Decreased	10 (5.6)

*With percentages in parentheses unless indicated otherwise; values are ‡mean and †median with [± SD, range]. NST: No special type; HR: Hormone receptor; HER2: Human epidermal growth factor receptor 2; TNBC: Triple negative breast cancer; NAC: Neoadjuvant chemotherapy; pCR: Pathological complete response; SLN: Sentinel lymph node; ITC: Isolated tumour cell; SD: Standard deviation

palbociclib or T-DM1. HER2 therapy and hormone therapy were used, if indicated. Breast surgery was performed six months after NAC. Median (range) time between diagnosis and NAC was 36 (14–67) days and mean NAC was 5.9±1 months. SLN-negatives (n = 140) not submitted to ALD were followed from diagnosis for a mean of 51±29 months with no case of axillary involvement. There were 36 cases which were SLNB-positive [HR+: 28 (77.8%), TNBC: 6 (16.7%) and HER2+/HR-: 2 (5.5%)] and in three cases ALD was performed due to SLNB non-detection.

In total 71 patients (38.5%) had breast pCR (Table 2) and higher rates was obtained in HER2+ (p = 0.046) and HR- (p<0.0001). HR+/HER2- was associated with breast pCR in 6.8%, compared to 59.4% in HR-/HER2+ and 53.4% TNBC patients (p<0.001). Significant predictors of pCR were HR- (p<0.0001), Nottingham score (p = 0.0013), HER2+ (p = 0.05), and cT/tumour size (p = 0.04/p = 0.0018). HR- (p = 0.0006) and HER2+ (p = 0.0087) were independent predictors of pCR (Table 3).

The most frequent molecular subtype in the 36 patients with *yp*N+ status (20.5%) was HR+ (77.8%). Breast pCR was a significant predictor of SLNB-negativity (97.2%; p<0.001). The strongest predictors of *yp*N0 before surgery were molecular subtype (p<0.001), tumour size (p = 0.005), and Nottingham score (p = 0.003) (Table 4).

Disease progression occurred in 21 (11.7%), subdivided into local recurrence (n = 11; 6.15%), and disseminated disease (n = 16; 8.93%). Mean time from surgery to local recurrence was 25±17 months,

Table 2. Pathological response of breast to primary systemic therapy

NAC response (RCB)		**n**	**(%)**
pCR - Complete response (ypT0/Tis)		69	(38.5)
pCR with axillary involvement			
	ypN0(i+)	2	(1.1)
Partial response or no response		108	(60.3)
	ypT1	57	(31.8)
	ypT2	44	(24.6)
	ypT3	5	(2.8)

NAC: Neoadjuvant chemotherapy; pCR: Pathological complete response; RCB: Residual Cancer Burden

Table 3. Univariable and multivariable analysis of predictors of pathologic complete response with their pathologic complete response rates

	% pCR	**Univ. (p-value)**	**Multiv. (p-value)**	**Multiv. OR**	**95% CI for OR**	
					Lower	**Upper**
Tumor size						
≤30	49.46	0.0018	0.0102	1.8136	0.8889	3.7002
>30	26.74					
ki-67						
Value >20	45.86	0.0008	0.1239	2.2066	0.8051	6.0480
Value ≤20	17.78					
Grade						
3	47.52	0.0013	0.0834	2.0246	0.9111	4.4989
1-2	23.19					
HR						
Negative	54.44	0.00001	0.0006	3.8019	1.7784	8.1281
Positive	22.47					
HER2						
Positive	46.75	0.046	0.0087	2.7446	1.2913	5.8333
Negative	32.35					
Multivariable analysis = X2=38.76; p<0.0001						

pCR: Pathologic complete response; OR: Odds ratio; cT-stage: Clinical tumor stage; HR: Hormone receptor; HER2: Human epidermal growth factor receptor 2; CI: Confidence interval; Multiv.: Multivariable; Univ.: Univariable

disseminated disease 26±17 months and *exitus* 38.7±18 months. Death occurred in 10 cases so that OS was 90.6%. In pCR, the OS at 5 years was 100% (non-pCR 84.2%; p = 0.007). DFS showed significant differences regarding SLNB (p<0.0001), HER2 expression (p = 0.0277), tumour size (p = 0.002), and NAC-response (p<0.0001) (Figure 2).

Discussion and Conclusion

SLN identification reached the recommended value of at least 95% (16, 17). Periareolar intradermal injection can obtain better radiotracer drainage compared to intra- or peritumoral injections. It is important to highlight the absence of axillary recurrence (AR) in the cases of negative SLNB, in line with previous publications (18-20)

Table 4. Univariable analysis of predictors for negative sentinel lymph nodes after NAC

			No. of patients	Negative SLN	Negative SLN rate (%)	*p*-value
All patients			179	140	79.5	
Histology						0.166
Invasive cancer, NST			165	131	80.9	
Invasive lobular cancer and others**			14	9	64.3	
Tumour subtype						<0.001
HR-/HER+			30	28	93.3	
HR+/HER+			45	36	80	
TNBC			58	52	89.7	
HR+/HER2-			43	24	55.8	
Nottingham Grade						0.003
I and II			68	46	67.6	
III			101	86	86.9	
Unknown			9			
cT-stage						0.117
cT1			24	22	91.7	
cT2			132	103	78	
cT3			14	12	85.7	
cT4			6	3	50	
T size						0.005
≤30 mm			92	81	88	
>30 mm			84	59	70.2	
Tumour focality						0.430
Unifocal			150	121	80.7	
Multifocal/multicentric			26	19	73.1	
ypT category after NAC						<0.001
	pCR		71	69	97.2	
		ypT0	52	51	98.1	
		ypTis	19	18	94.7	
	ypT1		57	40	70.2	
		ypT1mi	2	2	100	
		ypT1a	2	1	50	
		ypT1b	13	11	84.6	
		ypT1c	40	26	65	
	ypT2		43	28	65.1	
	ypT3		5	3	60	

SLN: Sentinel lymph node; NST: No special type; HR: Hormone receptor; HER2: Human epidermal growth factor receptor 2; TNBC: Triple-negative breast cancer; NAC: Neoadjuvant chemotherapy; pCR: Pathological complete response. *metaplasia (4), mucinous (2) and apocrin (2)

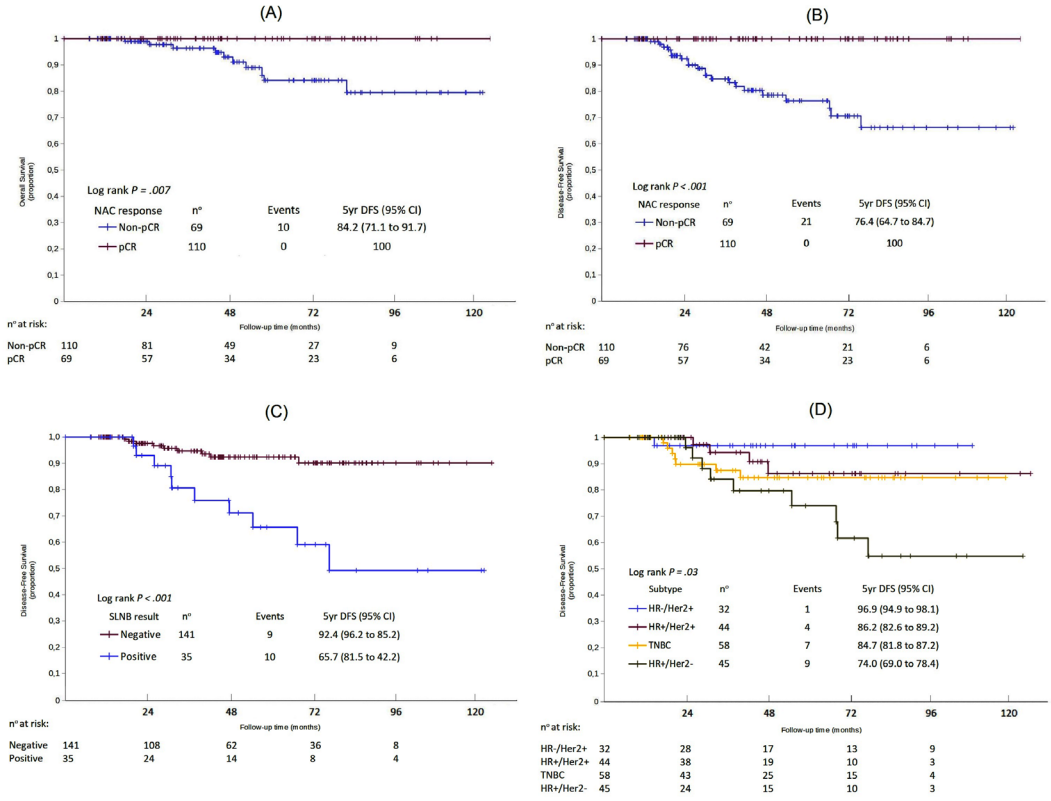

Figure 2. (A) Overall survival (OS) (y-axis) and **(B)** disease-free survival (DFS) (y-axis) plotted against time in months from cancer diagnosis (x-axis) according to NAC-response groups: pCR (red) and residual-disease (blue). **(C)** DFS (y-axis) plotted against SLNB-result: Negative (red) and positive (blue). **(D)** DFS (y-axis) plotted against tumour molecular subtypes: HR-/HER2+ (blue), HR+/HER2+ (red), TNBC (yelow) and HR+/HER2- (green). Log-rank P values for each survival graph are shown.

SLNB: Sentinel lymph node biposy; HR: Hormone receptor; HER2: Human epidermal growth factor receptor 2; TNBC: Triple-negative breast cancer; NAC: Neoadjuvant chemotherapy; pCR: Pathological complete response

suggesting that SLNB performs better than ALD. We do not attribute this to the average length of follow-up, which was longer than in other published studies (34 months) (21), nor to the interval of time until AR. Another reason for the absence of AR was the use of adjuvant systemic treatment, which lowers the risk for local and regional recurrence (22, 23). In the present study, patient selection was based on the chemotherapy course, in which all patients received hormone and anti-HER2 therapy depending on the molecular subtype, NAC was based on anthracyclines and taxanes in 91.6% of cases, of which only 5% did not complete treatment. The increase in the rate of local recurrence in these cases of high fibrosis due to good response to NAC, which translates into pCR, is a matter of concern for surgeons. In our study, we had no recurrence is any patient achieving pCR.

Tumor size and molecular subtypes are independent predictors of pCR (18-21). Some authors claim that achieving pCR does not completely rule out long-term recurrence. Thus, for the design of our study we took into account the limitation of previous studies (retrospective nature, lack of knowledge of NAC courses, Nottingham scoreing, and pathological data) to evaluate the survival results. We found an OS and a DFS at five years of 100% in the group that achieved pCR, independently of the tumour size at diagnosis and the molecular subtype. The strengths of these results lie in the well-selected patient sample, with a high homogeneity of chemotherapy scheme and an exhaustive registry of the administered cycles and the causes for treatment interruption. The presence of HR+ could negatively influence the pCR rate of the HER2+ group, whereas HR-/HER2+ achieves higher pCR rates, with impact on NAC response and OS/DFS. OS and DFS were 100% in the pCR group, probably due to well-selected patients, with homogeneous NAC protocols and anti-HER2 therapy. Furthermore, and according to literature, there could be a slight difference in prognosis with respect to the *in situ* presence of tumour after NAC (24, 25). Based on this evidence, another strength of our study is the registry of all variables of the pathological examination of the samples, which provided exact data on staging of the AJCC and RCB of Symmans after NAC. We obtained an OS and a DFS at five years of 100% in the group of women who had an *in situ* component in the samples that corresponded to the ypTis stage and the pCR category. Therefore, in our study, these women showed the same excellent results regarding OS and DFS at five and eight years as those achieving a complete pCR, categorized as ypT0.

It is worth highlighting that in our study the DFS at five years for our TNBC group, considered as a good response to NAC (pCR rate of 51.7%) was 84.7%, whereas the DFS at five and eight years for the HR+/HER2- group was 73.3% and 54.3%, with a pCR rate of only 6.8%. We explain this notable prognosis difference between groups, compared to other studies (20) by homogeneity in the NAC courses, with subsequent adjuvant chemotherapy.

Current evidence suggests that molecular criteria should be prioritized over anatomical criteria, especially in higher probability of recurrence in patients with HR+ tumours (26-28). Our OS and DFS results in the HR+/HER2- subtype suggest considering an initial surgery and a later

adjuvant treatment, omitting NAC, with the objective of removing the biggest amount of tumour tissue with low probability of response to chemotherapy as soon as possible.

Predictive factors of SLNB would permit the patient selection for omission of SLNB after NAC.

A limitation of this study is that magnetic resonance imaging was not performed (29), without radiologic complete response assessment. Nonetheless, results regarding the association of RCB index and molecular subtype show its value as a predictive tool for breast pCR and negative-SLNB rate. Significant rates of ypN0 in HR-/HER2+ and TNBC, compared to HR+ show molecular subtype as an initial criterion to select patients for omission of SLNB after NAC. Tumour subtype and breast pCR were the strongest predictive characteristics in SLNB-negativity after NAC. Omitting SLNB could be an option in TNBC and HR-/HER2+ who achieve breast pCR, with the support of correct assessment with imaging techniques (30).

The findings of this study affirm that SLNB after NAC is an appropriate, safe and effective treatment for cN0. The most important predictors of pCR were molecular subtype and tumor size. Response to NAC is the strongest predictor with better prognosis if SLNB-negativity and pCR are achieved. A categorization of molecular subtypes based on response to NAC, SLNB and survival is a priority to establish individualized therapeutic strategies after NAC. Molecular subtypes with higher pCR rates and lower SLNB-positivity rates could benefit from non-invasive axillary evaluation strategies that include omission of SLNB.

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Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: J.A.-R., S.S.-V., F.J.F.-G.; Concept: J.A.-R., S.S.-V., F.S.-P.; Design: J.A.-R., S.S.-V., F.S.-P.; Data Collection and/or Processing: J.A.-R., S.S.-V., F.J.F.-G.; Analysis and/or Interpretation: J.A.-R., S.S.-V., F.J.F.-G., F.S.-P.; Literature Search: J.A.-R., S.S.-V.; Writing: J.A.-R., S.S.-V., F.J.F.-G., F.S.-P.

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Original Article

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Turmeric Inhibits MDA-MB-231 Cancer Cell Proliferation, Altering miR-638-5p and Its Potential Targets

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ABSTRACT

Objective: Recent research suggests curcumin extracted from the turmeric plant may inhibit the proliferation of cancer cells by controlling the expression of microRNAs (miRNAs). The effect of phenolic curcumin on miR-638-5p and potential target gene expressions in the triple negative breast cancer (TNBC) cell line MDA-MB-231 was investigated in this study.

Materials and Methods: GSE154255 and GSE40525 datasets were downloaded and analyzed using GEO2R to identify dysregulated miRNAs in TNBC. To find differently expressed genes in breast cancer (BRCA), The Cancer Genome Atlas Program data was examined. Utilizing *in silico* tools, KEGG, GO, and other enrichment analyses were performed. The databases miRNet, miRTarBase v8.0, and TarBase v.8 were used for miRNA and mRNA matching. Real-time quantitative reverse transcription polymerase chain reaction was used to examine the levels of miRNA and its targets in miRNA mimic transfected/curcumin-treated MDA-MB-231 cultures and controls. The cell viability detection kit-8 method was used to assess cell viability, and the scratch assay was used to conduct migration assessment.

Results: Bioinformatics analysis showed that miR-638-5p was significantly reduced in TNBC patients. Experimental results showed that miR-638-5p was upregulated in MDA-MB-231 treated with curcumin, while the potential target genes of miR-638-5p, *CFL1*, *SIX4*, *MAZ,* and *CDH1* were downregulated. Mimic miR-638-5p transfection inhibited MDA-MB-231 cell proliferation and reduced migration and expression of *CFL1*, *SIX4,* and *MAZ* genes was decreased in mimic miR-638-5p transfected cells.

Conclusion: These findings suggest that curcumin exerts its anticancer effects on MDA-MB-231 cells by modulating the expression of miR-638-5p and its possible target genes.

Keywords: Triple negative breast cancer; bioinformatics; MDA-MB-231; curcumin; miR-638-5p

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Key Points

- This is the first study investigating the curcumin/miR-638-5p/potential target genes in triple negative breast cancer (TNBC) cell line MDA-MB-231.
- The relationship between TNBC and miRNAs/genes was studied using bioinformatics tools and *in vitro* experiments, and many important miRNAs and genes have been identified.
- MiR-638-5p may play an important role in the cancer process through its potential target genes *CFL1, SIX4*, and *MAZ*.

Introduction

The molecular tumor complexity of breast cancer (BRCA) is an important obstacle to the treatment. Even though there are many useful therapies (surgery, radiation therapy, or hormone therapy), BRCA metastases, drug resistance, and relapse result in poor patient survival. Curcumin is the most prominent polyphenol component extracted from the turmeric (rhizomes of Curcuma longa). Vogel and Pelletier of the Harvard College Laboratory first identified curcumin in 1815 (1). Much subsequent research has demonstrated that curcumin is extremely beneficial to health (2). Its cytotoxic efficacy in several cancer cell lines, including BRCA, has been demonstrated. The pleiotropic action of curcumin in cancer cell inhibition is due to its numerous targets, which include signaling pathways, proteins/

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enzymes, and microRNAs (miRNAs) (3). miRNAs are single-stranded RNA molecules with around 18-22 nucleotides that act as master regulators of gene expression by binding to their target mRNAs in the cells (4, 5). In 271 species, 38,589 mature miRNAs have been identified, including 2654 mature human miRNAs (6). By recognizing matching sequences at the 3' UTR region of the target mRNA, a single miRNA may affect thousands of genes (7, 8). Many studies, especially in the last 10 years, have demonstrated that dysregulation of miRNA expression is associated with almost every kind of cancer, including BRCA (9, 10). Cancer hallmarks, such as maintaining cell proliferative signaling, apoptosis avoidance, stimulating invasion and metastasis, and triggering angiogenesis have been linked to altered miRNAs (11). Studies show that many natural dietary supplements, including curcumin, have important roles in various cellular processes (12, 13). The results suggest that these may make important contributions to the fight against cancer in the future (14).

This study was conducted to investigate the relationship between BRCA, miRNAs, mRNA and curcumin using *in silico* and *in vitro* methods. Briefly, geo datasets were used to identify miRNAs and genes that may be linked to BRCA. *In silico* tools were used to match the detected miRNAs and target genes. Enrichment analyses of selected miRNAs and genes were performed using various bioinformatics tools. The relationship between the selected miRNA and the target genes were then confirmed in *in vitro* evaluation, and the expression levels of the relevant miRNA and genes were investigated in curcumin-treated cells.

Materials and Methods

Identification of Triple Negative Breast Cancer (TNBC)-Associated miRNAs

Overlapping miRNAs between GSE154255 and GSE40525 datasets, which met the criteria of logFC >2 and logFC >1, respectively, and $p<0.05$ for both datasets, were identified. This was carried out because the GSE154255 dataset contains very few miRNAs with a logFC >2 value, the logFC >1 value was used for this dataset.

Identification of the Effect of Overlapping miRNAs on Overall Survival in BRCA and TNBC

Whether overlapping miRNAs were effective on overall survival (OS) in both BRCA and TNBC was investigated in METABRIC data using kmplot (https://kmplot.com/analysis/) and a significant miRNA was selected for further *in silico* and *in vitro* analysis.

The Detection of Overexpressed Genes in BRCA

To identify significant genes in BRCA, The Cancer Genome Atlas Program (TCGA) BRCA data were searched through the GEPIA2 (http://gepia2.cancer-pku.cn/) web tool. Among the overexpressed genes in TCGA BRCA data, genes that met LogFC >1 and $p<0.05$ criteria were identified.

In silico Investigation of Potential Target Genes of the Selected miRNA

In silico potential target genes of the selected miRNA were identified using the databases miRNet (https://www.mirnet.ca/), miRTarBase v8.0 (https://mirtarbase.cuhk.edu.cn/) and TarBase v.8 (https://dianalab.e-ce.uth.gr/) tools.

Detection of Overlapping Genes Between *in silico* Target Genes of the Selected miRNA and Significant Genes in TCGA BRCA Data

The overlapping genes between the *in silico* potential targets of the selected miRNA and the genes overexpressed in TCGA BRCA and meeting the LogFC >1 and $p<0.05$ criteria were determined.

Enrichment Analysis of Overlapping Genes

The diseases, hub proteins, and pathways most associated with overlapping genes were identified using the Enrichr (https://maayanlab.cloud/Enrichr/) and ShinyGO 0.77 (http://bioinformatics.sdstate.edu/go/) tools.

Identification of Genes Associated With BRCA Overall Survival

Employing the kmplot tool, it was determined whether overlapping genes were associated with BRCA OS.

In vitro Studies

Cell Culture

For cell culture, the TNBC cell line, MDA-MB-231, was cultivated in Dulbecco's Modified Eagle's Medium (DMEM) (EcoTech Biotechnology, Erzurum, Turkey) with 1% penicillin (Invitrogen, Thermo Fisher Scientific Inc., Waltham, MA, USA) 10% fetal bovine serum (FBS) (EcoTech Biotechnology, Turkey) in a humidified incubator (Sanyo) with 5% CO_2 at 37 °C.

Curcumin Treatment

Highly purified curcumin (Bio Basic Inc., Canada) was dissolved in dimethyl sulfoxide (DMSO) (1 mg/mL). Curcumin was prepared at different concentrations (1 µM, 3 µM, 5 µM, 10 µM, 30 µM and 50 µM). As curcumin was dissolved in DMSO, the control cells were treated with DMSO at the same quantities as the experimental groups. Cells were maintained in 6-well or 96-well plates (Nest Biotechnology Co., China) for 24 hours at 37 °C. The 50% inhibition concentration (IC50) value of curcumin was determined (10 µM). For further investigation, this value was used to treat MDA-MB-231 cells.

miR-638-5p Mimic Transfection

MDA-MB-231 cells were seeded at sixty percent confluency into 96-well or 6-well cultivation plates. Afterward, using the supplier's protocol for transient overexpression of miR-638-5p, cells were transfected with 30 pM miR-638-5p mimic (5′-AGGGAUCGCGGGCGGGUGGCGGCCU-3′) (Thermo Fisher Scientific), or non-targeting (NT) control miRNA using lipofectamine 2000 (Invitrogen). Following 24 hours of culture, transfected cells were used for functional assays.

RNA Isolation, cDNA Synthesis Process, and Quantitative Real-time PCR

Total RNA was extracted from curcumin-treated and miR-638-5p transfected cells and control cultures using TRIzol (Invitrogen). A NanoDrop spectrophotometer (Thermo) was used to assess the quality and quantity of the RNA samples. To examine the expression of selected genes or miRNAs, equal amounts of RNA from the specimens were reverse-transcribed into cDNA using a cDNA Reverse Transcription Kit (Invitrogen, Thermo Fisher Scientific) or TaqMan Kit (Invitrogen, Thermo Fisher Scientific), respectively. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) reactions were carried out via 5x HOT FIRE qPCR Mix Plus (Solis Bio-Dyne Co,

Estonia) or TaqMan Advanced Master Mix (Invitrogen, Thermo Fisher Scientific). Table 1 shows the primer sequences used for qRT-PCR experiments. *B-actin* or RNU43 expression were used to normalize gene or miRNA expression. All reactions were performed at least twice. The $2^{-\Delta\Delta Ct}$ method was employed to calculate the relative expressions of the genes and miRNAs that were investigated.

Detection of Cell Viability Using Cell Viability Detection Kit-8

Cell viability was determined via the cell viability detection kit-8 (CVDK-8) assay (EcoTech Biotechnology) MDA-MB-231 cells (3 x 10^3 cells per well) were seeded into 96-well plates in five replicates and incubated for 24 hours. Then the cells were transfected with lipofectamine 2000 (Invitrogen) reagent to express miR-638-5p mimic or NT miRNA. After 24 hours, each well was treated with CVDK-8 reagent, and the plates were incubated for three hours. A Multiskan spectrophotometer (Thermo Fisher Scientific) was used to measure absorbance at 450 nm.

Detection of Cell Proliferation Using the Viability Imaging Method

After enzyme-linked immunoabsorbance (ELISA) evaluation for cell viability, the 96 well plate was inverted and the liquid was removed. Then the wells were washed with PBS. After removal of the PBS, a light microscope image was taken at x10 and recorded.

Scratch Assay

5 x10^5 MDA-MB-231 cells in DMEM with 10% FBS were seeded in 6-well plates. When the cells reached 95% confluency, scratches were made with a 10 µL pipette tip. After removing the medium from the plate and washing with PBS, the attached cells were cultured in DMEM. The cells that migrated to the "wound area" were measured from multiple microscopic areas, and images were captured at 0 and 24 hours with a light microscope at x100 magnification.

Statistical Analysis

Publicly available data were used in part of the bioinformatics the study, and in the miRNA analysis, those with logFC >2 for GSE154255, logFC >1 for GSE40525, and $p<0.05$ for both datasets were selected. For genes, among the TCGA BRCA data, those with logFC >+1 and $p<0.05$ values were considered significant. In terms of the *in vitro* studies, all data is shown as the mean ± standard deviation of a minimum of two independent experiments that yielded comparable results. Student's t-test was used to analyze significant differences using GraphPad Prism 7.0. A difference that was statistically significant was indicated by $p<0.05$.

Results

Bioinformatics Analysis

TNBC-Associated miRNAs

The geo dataset analysis revealed 16 downregulated miRNAs to be common in both geo datasets (Table 2).

The Prognostic Importance of Selected miRNA

The KMplot survival evaluation revealed that decreased expressions of miR-638-5p and miR-139-3p had an effect on the OS of BRCA patients in general and also for the TNBC subtype of BRCA (Figure 1). As miR-638-5p was found to be more closely associated with BRCA on literature review, it was chosen for the remaining *in silico* analyses and the *in vitro* study.

Overexpressed Genes in TCGA BRCA Data

Analysis of TCGA BRCA data identified 248 genes which met the LogFC >+2 and $p<0.05$ criteria.

Detection of Potential Target Genes of the Selected miRNA

Using miRNet (miRTarBase v8.0 and TarBase v.8), it was found that miR-638-5p could potentially target 1416 genes (Figure 2).

Detection of Overlapping Genes

Thirteen genes were found to overlap between the TCGA data and potential *in silico* targets of miR-638-5p (Table 3).

Enrichment Analysis Results

It was found that the thirteen overlapping genes were linked to various cancers, particularly BRCA, and that these genes are associated with cancer-related pathways, such as those involved in cell division and chromosome segregation, as well as being related to hub proteins which are closely associated with BRCA (Figure 3 and Figure 4).

The Prognostic Importance of Selected Genes

The prognostic importance of four hub genes in BRCA patient survival was investigated. It was revealed that the differential expression of *SIX4* and *CDH1* influenced patient survival (Figure 5).

In vitro Investigations

Cell Viability Assay Results

Both ELISA absorbance measurement results and the viability imaging method results showed that curcumin treatment or miR-638-5p mimic transfection significantly reduced the proliferation of MDA-MB-231 cells at 24 hours ($p<0.01$) (Figure 6 and Figure 7).

Table 1. Primer sequences for qRT-PCR

Gene	Forward	Reverse	Ref.
CDH1	5'-AGAACGCATTGCCACATACA-3'	5'-TGCTTAACCCCTCACCTTGA-3'	(30)
MAZ	5'-GGATCACCTCAACAGTCACGTC-3'	5'-GGCACTTTCTCCTCGTGTCGTA-3'	(31)
SIX4	5'-AGCAGCTCTGGTACAAGGC-3'	5'-CTTGAAACAATACACCGTCTCCT-3'	(25)
CFL1	5'-TGCTGCCAGATAAGGACTGC-3'	5'-CTCTTAAGGGGCGCAGACTC-3'	(32)
SMC1A	5'-TGATGCTGCCTTGGATAACA-3'	5'-TTCGACCTCACCAAGTACCC-3'	(33)
β-actin	5'-GCCTCGCCTTTGCCGATC-3'	5'-CCCACGATGGAGGGGAAG-3'	(34)

Primer list of selected putative target genes of miR-638-5p for in vitro study. Ref.: Reference. β-actin gene was used internal control (housekeeping gene); qRT-PCR: Real-time quantitative reverse transcription polymerase chain reaction

Scratch Assay Results

Scratch assay results showed that curcumin treatment at a concentraton of 10 μM significantly diminished the cell migration of MDA-MB-231 compared to the untreated control group at 24 hours of evaluation. Furthermore, at 24 hours, miR-638-5p mimic transfection reduced cell migration compared to the NT miRNA mimic group (Figure 8).

qRT-PCR Results

The effect of curcumin treatment or miR-145-5p mimic transfection on the expression of the selected genes were investigated using qRT-PCR. The selected *CDH1*, *MAZ*, *SIX4*, *CFL1*, and *SMC1A* genes were quantified using the primers shown in Table 1. To normalize gene expression, the β-*actin* housekeeping gene was used. It was observed that the expression of *CFL1*, *SIX4*, and *MAZ* genes decreased

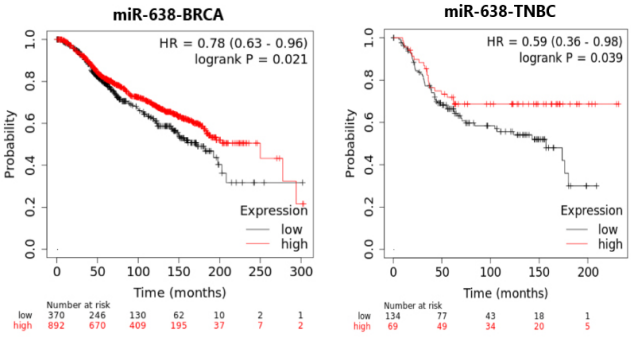

Figure 1. Survival effect of miR-638-5p on BRCA and TNBC. OS analysis was performed via kmplot using METABRIC data (Including 2509 BRCA patients and 300 TNBC patients)

BRCA: Breast cancer; TNBC: Triple negative breast cancer

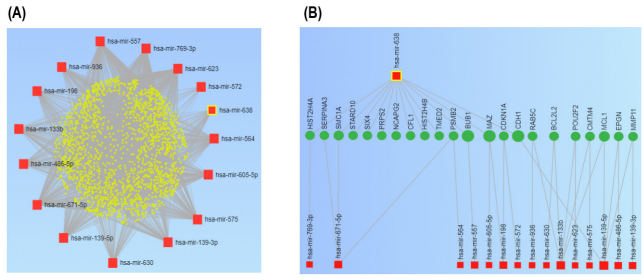

Figure 2. (A) Overlapping miRNAs in the GSE154255 and GSE40525 datasets. Red squares represent 16 overlapping miRNAs, including miR-638-5p, yellow circle shapes indicate probable miRNA targets, and lines illustrate interactions (1856 edge) between miRNAs (16) and genes (1416). **(B)** Relationship between miR-638-5p and 15 other miRNAs and potential more associated genes

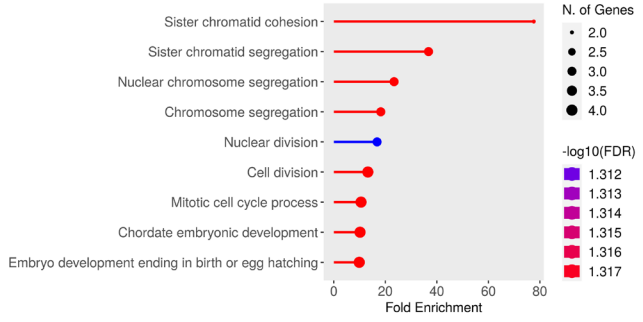

Figure 3. Enrichment analysis of selected possible miR-638-5p 13 targets. Many of these selected genes are related to critical biological events like cell division or mitotic cell cycle process

Table 2. Overlapping miRNAs in the GSE154255 and GSE40525 datasets, which matched the requirements of LogFC >2 and LogFC >1 respectively and $p<0.05$

GSE154255				GSE40525		
Adj. p-value	***p*-value**	**logFC**	**miRNAs**	**logFC**	***p*-value**	**Adj. *p*-value**
0.010161	1.27E-04	-8.61	**hsa-miR-486-5p**	-2.66	3.14e-04	0.018947
0.0012138	6.17E-06	-7.46	**hsa-miR-139-5p**	-2.16	5.21e-04	0.02344
0.0013373	8.00E-06	-7.01	**hsa-miR-557**	-1.78	6.74e-04	0.023942
0.0599677	1.08E-03	-6.66	**hsa-miR-936**	-1.67	2.29e-02	0.1619
0.0148909	2.00E-04	-5.79	**hsa-miR-198**	-1.64	8.49e-03	0.100838
0.0976094	7.36E-02	-5.14	**hsa-miR-564**	-1.55	5.74e-03	0.083076
0.2128058	1.75E-01	-4.97	**hsa-miR-630**	-1.46	1.33e-03	0.034503
0.1205795	9.20E-02	-4.64	**hsa-miR-671-5p**	-1.44	4.21e-02	0.241701
0.0691052	2.08E-02	-4.24	**hsa-miR-572**	-1.32	8.22e-04	0.026259
0.0691052	1.34E-02	-3.73	**hsa-miR-638-5p**	-1.29	1.59e-02	0.139233
0.2189317	1.81E-01	-3.47	**hsa-miR-139-3p**	-1.24	2.24e-02	0.159857
0.0906878	6.80E-02	-3.22	**hsa-miR-575**	-1.22	6.34e-03	0.085311
0.1792597	1.46E-01	-3.19	**hsa-miR-623**	-1.05	6.02e-02	0.285467
0.1457456	1.14E-01	-3.01	**hsa-miR-769-3p**	-1.04	3.87e-03	0.069472
0.4032279	3.70E-01	-2.06	**hsa-miR-133b**	-1.01	7.46e-03	0.091564
0.4032279	3.70E-01	-2.06	**hsa-miR-605**	-1.01	1.05e-01	0.410721

hsa-miR-638-5p and hsa-miR-139-3p that may be more closely associated with BRCA in GSE154255 and GSE40525 are highlighted in red. Adj. *p*-value: Adjusted *p*-value

Table 3. Overlapping 13 genes between miRNet and TCGA BRCA data

Gene symbol	Gene name	LogFC	Adj. *p*-value
BUB1	BUB1 mitotic checkpoint serine/threonine kinase	2.650	1.11e-163
STARD10	StAR related lipid transfer domain containing 10	2.481	2.21e-84
HIST2H4A	H4 clustered histone 14	1.743	1.11e-50
CDH1	Cadherin 1	1.729	1.42e-27
MAZ	MYC associated zinc finger protein	1.611	7.77e-197
HIST2H4B	H4 clustered histone 15	1.560	2.98e-54
SIX4	SIX homeobox 4	1.542	3.97e-65
SERPINA3	Serpin family a member 3	1.391	8.62e-9
TMED2	Transmembrane P24 trafficking protein 2	1.302	7.54e-73
PRPS2	Phosphoribosyl pyrophosphate synthetase 2	1.266	7.59e-60
NCAPG2	Non-SMC condensin II complex subunit G2	1.224	7.85e-68
CFL1	Cofilin 1	1.046	7.84e-143
SMC1A	Structural maintenance of chromosomes 1A	1.046	9.27e-19

13 overlapping genes were identified with logFC >1 and $p<0.05$ values. TCGA: The Cancer Genome Atlas Program; BRCA: Breast cancer; Adj. *p*-value: Adjusted *p*-value

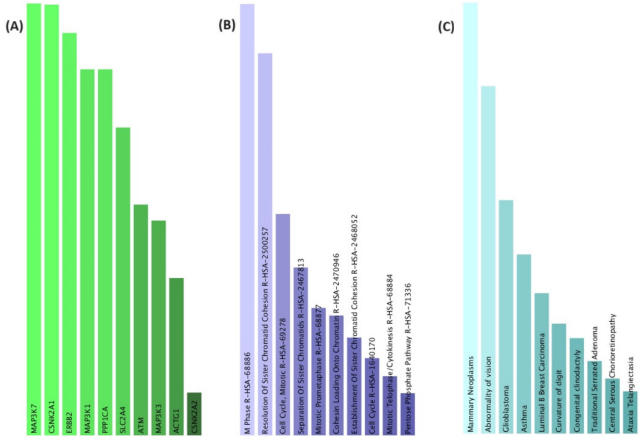

Figure 4. Enrichment analysis of the selected 13 potential miR-638-5p targets in TCGA BRCA samples. **(A)** The hub proteins of the 13 miR-638-5p targets according to huMAP ($p<0.05$). **(B)** Pathways associated with the 13 target genes according to Reactome 22. **(C)** Most related diseases of the 13 target genes in DisGeNet database

BRCA: Breast cancer; TCGA: The Cancer Genome Atlas Program

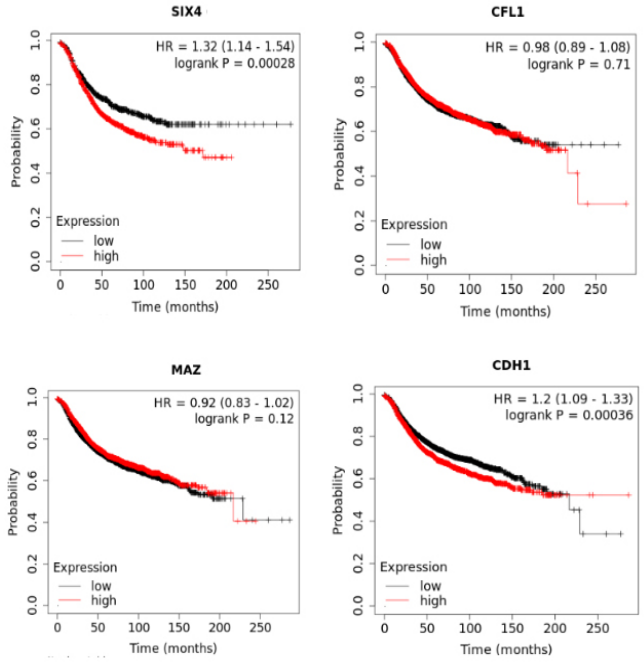

Figure 5. The effect of selected genes' overexpression on BRCA patients OS. Overexpression of the *SIX4* and *CDH1* genes was associated with OS, but not the *CFL1* and *MAZ* genes

HR: Hazard ratio; BRCA: Breast cancer; OS: Overall survival

significantly in both MDA-MAB-231 cells administered curcumin and MDA-MB-231 cells transfected with the miR-638-5p mimic (Figure 9).

Discussion and Conclusion

Numerous studies have shown that curcumin inhibits cancer cell proliferation, increases apoptosis, and disrupts migration via its effect on miRNAs. It has been reported that curcumin inhibits the progression of colorectal cancer cells by regulating the *CDCA3*/*CDK1* pathway via miR-134-5p (15). Curcumin has been shown to inhibit cell growth in BRCA through the miR-21/*PTEN*/Akt pathway. Liang et al. (16) reported that curcumin suppressed the survival, migration, and invasion of papillary thyroid cancer cells by modulating the miR-301a-3p/*STAT3* axis.

In the bioinformatics section of the present study, we found that only miR-638 and miR-139-3p had a significant effect on the OS of both BRCA, and specifically TNBC, patients among the overlapping miRNAs in the GSE154255 and GSE40525 datasets (Figure 1).

MiR-638-5p was chosen for the *in vitro* study based on the data obtained from the literature review, as miR-638-5p may be more closely associated with BRCA. However, taking the current study's bioinformatics data and literature results into account, we would like to emphasize that miR-139-3p may also be closely related to BRCA and that more comprehensive studies on the relationship between this miRNA and BRCA and specifically TNBC, are required.

To the best of our knowledge, there is no previous study into the effects of curcumin on cancer processes in BRCA cells specifically via miR-638 and its target genes. Using TCGA data and *in silico* tools, 13 potential miR-638-5p target genes were identified. Five of these 13 genes (*CFL1*, *SIX4*, *MAZ*, *CDH1*, and *SMC1A*) were chosen for *in vitro* examination. In the *in vitro* study, MDA-MB-231 cells were treated with curcumin, and it was found that the expression of miR-638-5p increased in the curcumin-supplemented group compared to the control group, while the expression of *CFL1*, *SIX4*, *MAZ*, and *CDH1* genes decreased. Subsequently, MDA-MB-231 cells were transfected with a miR-638-5p mimic. The expression of *CFL1*, *SIX4*, and *MAZ* genes was found to be reduced in the transfected group (Figure 9B).

miR-638-5p is a tumor suppressor miRNA that has been linked to a variety of cancers (17, 18). Zheng et al. (19) showed that miR-638-5p acts as a tumor suppressor in glioma by regulating *HOXA9*. Another study found that miR-638-5p inhibited cell proliferation in human osteosarcoma by repressing *PIM1* expression (20).

On November 28, 2023, a Pubmed search with the keywords "miR-638, breast cancer" yielded 19 results. Studies into BRCA, specifically investigating the role of miR-638-5p, have revealed that miR-638-5p expression was reduced in BRCA, and that miR-638-5p may be connected with resistance to various chemotherapeutics, radiotherapy, and ultraviolet (UV) sensitivity. He et al. (21) showed that CircNCOR1 regulates the efficacy of radiotherapy in BRCA through the miR-638-5p/*CDK2* axis. Wang et al. (22) reported that miR-638-5p could be used as a biomarker for 5-fluorouracil sensitivity in BRCA treatment. Another study revealed that miR-638-5p/BRCA1 regulation affects DNA repair, as well as sensitivity to UV and cisplatin in TNBC.

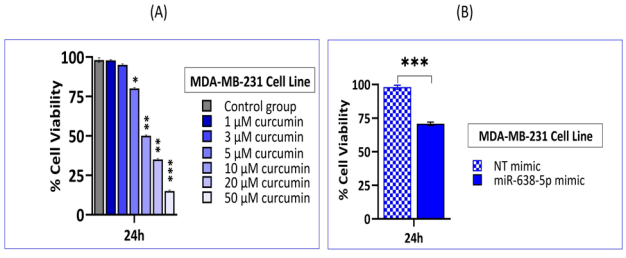

Figure 6. Effect of curcumin treatment and miR-638-5p mimic transfection on MDA-MB-231 cell viability. **(A)** It was observed that the viability of cells treated with different concentrations of curcumin decreased significantly depending on increasing doses. **(B)** Ectopic expression of miR-638-5p was observed to significantly reduce cell viability

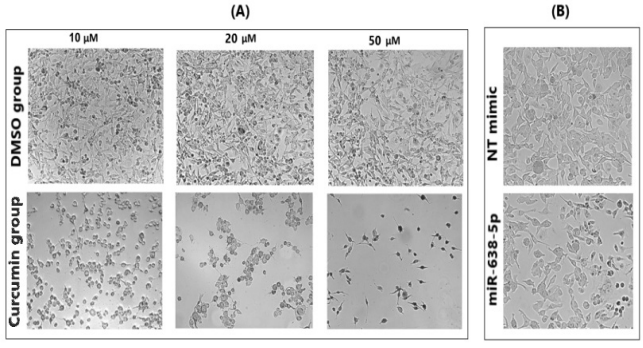

Figure 7. (A) Images of MDA-MB-231 cells treated with 10 µM, 30 µM, and 50 µM curcumin under a 10X light microscope. It has been determined that curcumin significantly reduces cell viability due to increasing concentrations. **(B)** The viability of miR-638-5p mimic transfected cells was significantly reduced compared to NT mimic transfected cells

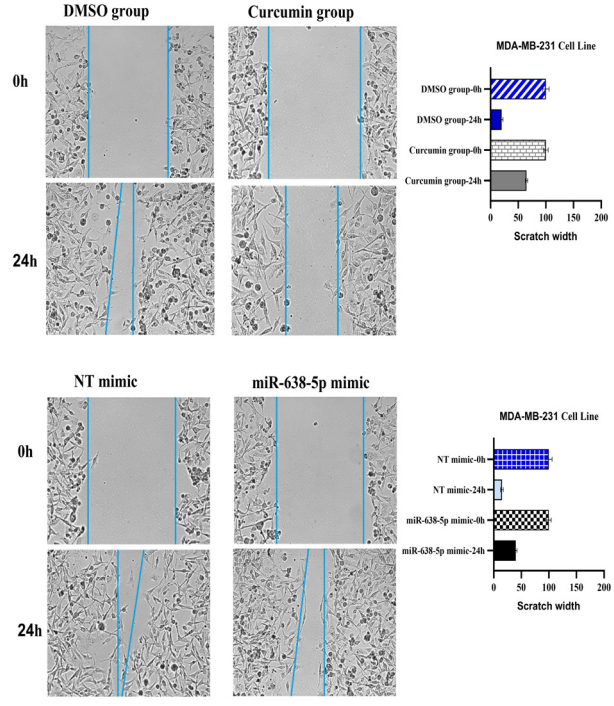

Figure 8. Effect of 10 µM curcumin and miR-638-5p mimic on MDA-MB-231 cell migration. Curcumin treatment and miR-638-5p overexpression inhibited cell migration

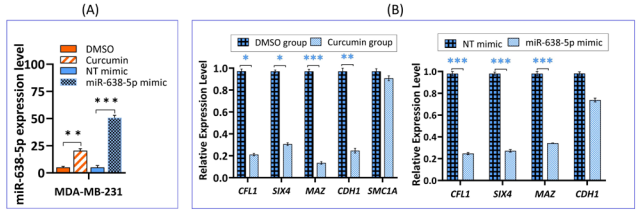

Figure 9. (A) Curcumin treatment and miR-638-5p mimic transfection significantly increased miR-638-5p expression. **(B)** When curcumin was introduced to MDA-MB-231 cells, the expression of *CFL1*, *SIX4*, *MAZ*, and *CDH1* genes decreased significantly, whereas *SMC1A* gene expression did not change. *CFL1*, *SIX4*, and *MAZ* gene expression decreased significantly in MDA-MB-231 cells transfected with miR-638-5p mimic, while there was no significant change in *CDH1* gene expression

The enrichment analysis performed in the present study on the potential target genes of miR-638-5p revealed that the disease was most likely to be associated with the identified genes is mammary neoplasms (Figure 4). Cell cycle and chromosome segregation are two biological events in which these genes may play important roles that are closely related to the cancer process. All of these suggest that further research into the relationship between miR-638-5p and the candidate genes may assist in understanding BRCA, and specifically TNBC, biology.

The decreased expression of all three selected miR-638-5p potential target genes (*CFL1*, *SIX4,* and *MAZ)* in both the curcumin-added and miR-638-5p transfected groups is an important clue about the functioning of the curcumin/miR-638/target gene axis. Despite the fact that no studies have been conducted to explain the relationship between miR-638-5p and *CFL1* in BRCA, it has been demonstrated that *CFL1* may contribute to the BRCA process by changing its expression via different miRNAs. miR-342 has been shown to inhibit the growth, migration, and invasion of BRCA cells by targeting *CFL1* (23). Another study demonstrated that miR-200b-3p and miR-429-5p inhibit the growth and motility of BRCA cells by targeting the *LIMK1*/*CFL1* pathway (24).

Although *SIX4* is a gene linked to some cancers, including BRCA, there are fewer details about it compared to other selected miR-638-5p targeted genes. *SIX4* promotes metastasis in BRCA via *STAT3* induction, according to one of the few studies (25). Wu et al. (26) reported elevated *SIX4* expression in BRCA that serves an oncogenic role by reducing the immune response, particularly in luminal subtypes, and is associated with diminished promoter methylation levels.

MAZ represents one of the genes involved in gene expression regulation and development of tumors. *MAZ* dysregulation has been related to the progression of many tumors, involving BRCA (27). *MAZ*-regulated *SIPL1* has been shown to promote tumor progression in TNBC, and dysregulation of this *MAZ* expression may be associated with a poor prognosis in TNBC (28).

In the present study, using *in silico* and *in vitro* methods, curcumin was shown to affect cancer processes in MDA-MB-231 cells by altering the expressions of miR-638-5p and its potential target genes. Numerous studies have suggested that miRNAs may be potential therapeutic molecules in cancer in the future (29). However, research into the complicated interactions between miRNAs and their target genes is currently incomplete. The findings of the present study will contribute to the existing literature. It should be noted, however, that the expression of the selected genes was determined at the mRNA level. Therefore it is recommended that in future studies, the findings obtained using *in silico* and *in vitro* approaches should be validated in BRCA tissue samples and with other *in vivo* methods.

The findings of this study showed that curcumin appears to inhibit MDA-MB-231 cancer cell proliferation and migration by altering the expression of miR-638-5p and its potential target genes *CFL1*, *SIX4*, and *MAZ*. It is suggested that miR-638-5p and its target gene axis in BRCA, should be investigated further in future studies.

Ethics Committee Approval: Because of this study was prepared using publicly available bioinformatics data and *in vitro* study utilizing MDA-MB-231 cell line it does not require ethical approval.

Informed Consent: Because of this study was prepared using publicly available bioinformatics data and in vitro study utilizing MDA-MB-231 cell line it does not require informed consent.

Authorship Contributions

Concept: M.K., A.A., S.O.; Design: M.K., A.A., S.P.; Data Collection and/or Processing: M.K., A.A., I.S., S.P., Analysis and/or Interpretation: M.K., S.P., K.C., S.O.; Literature Search: M.K., I.S., S.E., F.A.; Writing: M.K., A.A., S.E., F.A., S.P., S.O.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Article

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Histopathological Features Predicting Neuroendocrine Morphology in Primary Breast Tumors: A Retrospective Analysis

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ABSTRACT

Objective: Neuroendocrine neoplasms of primary breast tumors are rare compared to locations, such as the respiratory system and gastrointestinal system, where they are frequently observed. The diagnostic criteria for primary neuroendocrine tumors of the breast have been changed since first description. Morphological and immunohistochemical features helpful in their diagnosis, which vary due to the heterogeneous nature of these tumors, are highlighted in this retrospective study. The purpose was to determine specific histopathological features that can identify neuroendocrine morphology in primary breast tumors.

Materials and Methods: Cases diagnosed with invasive breast carcinoma from resection materials in a single center between 2011 and 2022 and in which neuroendocrine markers were investigated were included. Demographic information, initial histopathological diagnosis, presence of tumor in another organ, tumor location, size and surgical details of the cases were obtained from the hospital database and pathology reports. The slides were re-evaluated in terms of tumor growth pattern, cribriformity, tubule formation, nuclear features, prominence of nucleoli, palisading and basal location of nuclei, presence of grooves, cytoplasmic features and evidence of cytoplasmic border.

Results: The presence of basally located nuclei, absence of tubule formation, inconspicuous nucleoli, fine nuclear chromatin, granular cytoplasm and inconspicuous cytoplasmic borders were frequent findings in tumors with neuroendocrine features ($p<0.05$). These features may help differentiate primary breast tumors with neuroendocrine features from other breast carcinomas.

Conclusion: The histopathological features that are different from the specific features seen in classical neuroendocrine tumors, the absence of specific clinical and radiological findings, the inability to study neuroendocrine markers in every laboratory and the need to prove that the breast tumor is not a metastasis all create diagnostic difficulties for primary breast neuroendocrine neoplasms. We believe that the results of this study may help diagnose and identify more specific histomorphological features that help determine neuroendocrine morphology in primary breast tumors.

Keywords: Breast; neuroendocrine carcinoma; neuroendocrine neoplasia; neuroendocrine tumor; primary

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Key Point

- The histopathological features that are different from the specific features seen in classical neuroendocrine tumors, the absence of specific clinical and radiological findings, the inability to study neuroendocrine markers in every laboratory and the need to prove that the breast tumor is not a metastasis are all conditions that create diagnostic difficulties for primary breast neuroendocrine neoplasms. This study might help to understand and define the clinicopathological features of these rare tumors.

Introduction

Neuroendocrine neoplasms, which can occur in various locations, and are particularly common in the respiratory and gastrointestinal system, constitute less than 1% of all breast tumors (1).

Primary breast neuroendocrine tumors, which were first defined as "breast carcinoma with a carcinoid growth pattern" by Feyrter and Hartmann in 1963, were first included in the World Health Organization (WHO) classification in 2003 (2). Various changes have been made in the diagnostic criteria since the 2003 WHO classification (3rd edition, 2003) which are now present in the current classification (5th edition, 2019). In the latest classification, the diagnosis should be made by evaluating the expression rate of cells with neuroendocrine features and neuroendocrine markers. Thus, tumors showing neuroendocrine features and neuroendocrine

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marker expression of more than 90% are defined as neuroendocrine neoplasms. Based on further evaluation of histological features, such as whether they show histology of a small or large cell neuroendocrine carcinoma (NEC), they may either be defined as a neuroendocrine tumor (NET) or NEC. Tumors with equivocal histological features and neuroendocrine marker expression are classified as invasive breast carcinoma of no special type (IBC-NST) with neuroendocrine differentiation. Although expressing neuroendocrine markers, solid papillary carcinoma and the hypercellular variant of mucinous carcinoma are tumors that are not classified as neuroendocrine neoplasms of the breast (3).

In this retrospective study, morphological and immunohistochemical features helpful and essential in the diagnosis, which vary due to the heterogeneous nature of these tumors, are highlighted. The main purpose of the study was to investigate specific histological features that can help identify neuroendocrine morphology in primary breast tumors.

Materials and Methods

Cases diagnosed with IBC from resection materials (lumpectomy, segmental mastectomy, modified radical mastectomy or breast-conserving mastectomy) in a single center between January 2011 and October 2022 and in which neuroendocrine markers (Synaptophysin and Chromogranin-A) were studied were included. Cases in which the slides were not suitable for re-evaluation or not accessible, all cases diagnosed with another classification than IBC-NST (including solid papillary carcinoma with synaptophysin and/or chromogranin immunoreactivity and hypercellular mucinous carcinoma) and metastatic NETs were excluded.

Demographic information, initial pathological diagnosis, presence of tumor in an organ other than breast, tumor location, tumor size and surgical information of the cases were obtained from the hospital database and pathology reports.

Hematoxylin and eosin (H&E) stained slides with a thickness of 4–5 micrometers and slides stained with Synaptophysin (Cell Margue, clone MRQ–40, Roche) and Chromogranin -A (Ventana, clone LK2H10, Roche) were re-evaluated by two independent pathologists based on the 2019 WHO Breast Tumors Classification. Tumors showing focal (<10%) neuroendocrine marker expression were defined as IBC-NST with neuroendocrine differentiation, while those with diffuse staining (>90%) were accepted as NET/NEC (Figure 1). The distinction between NET and NEC was made based on the histological features required for the diagnosis of small cell neuroendocrine carcinoma (SCNEC) and large cell neuroe ndocrine carcinoma (LCNEC) (3).

Initial microscopic examination of the tumors from H&E stained slides, included evaluation of the growth pattern, cribriformity, tubule formation, nuclear features, prominence of nucleoli, palisading and basal location of nuclei, presence of grooves, cytoplasmic features, evidence of cytoplasmic borders, tumor grade, presence of venous invasion, lymphatic invasion, perineural invasion, peritumoral desmoplastic reaction, percentage of tumor infiltrating lymphocytes (TIL), presence of tumor necrosis, and microcalcification. Evaluation of the immunohistochemistry slides was the second step of the microscopic examination.

Ethics approval for the study, dated November 10, 2022 and numbered 2022-17/29 was obtained from the Uludag University Faculty of Medicine Clinical Research Ethics Committee.

Statistical Analysis

If continuous variables were normally distributed, they were described as mean ± standard deviation (SD) if the $p>0.05$ in Kolmogorov-Smirnov test or Shapiro-Wilk test ($n<30$), and if the continuous variables were not normal, they were described as median (range). To calculate prevalence, data commands were used. Comparisons between groups were made using Kruskall-Wallis tests for non-normally distributed data. Categorical variables were compared between the groups using the chi-square test or Fisher's exact test.

The level for statistical significance was predetermined at $p<0.05$.

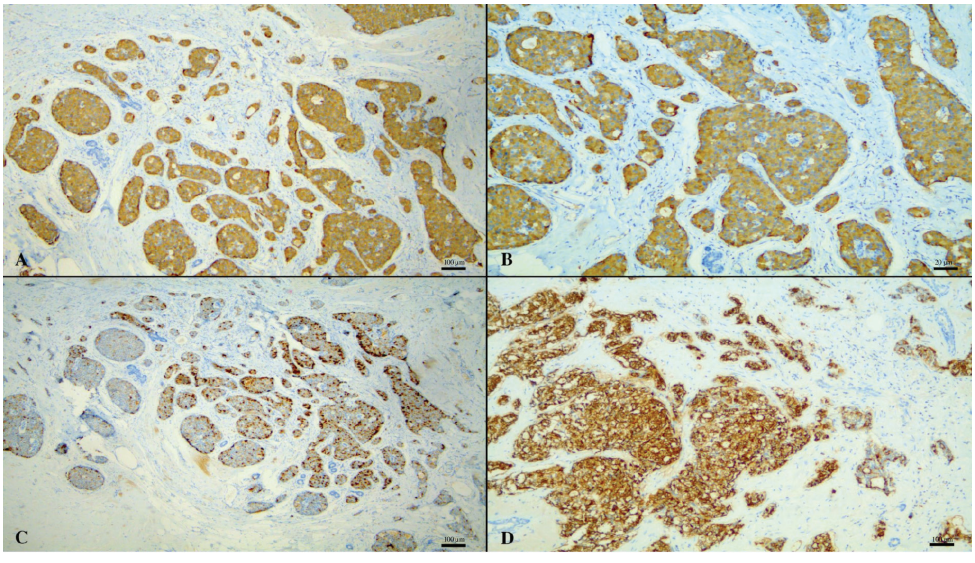

Figure 1. A-B) Diffuse, strong staining with synaptophysin in NET (x40 and x200). **C)** Focal staining with chromogranin in NET (x40). **D)** Synaptophysin staining observed in another NET (x40)

NET: Neuroendocrine tumor

Results

The retrospective study group consisted of 186 cases with available H&E and immunohistochemistry slides. Of the 186 cases, 185 (99.4%) were female and 1 (0.6%) was male. The mean ± SD age was 56.6±11.9 years, ranging from 30 to 85 years. The median age of patients diagnosed with IBC-NST was 55 (30–85) years and of patients with tumors showing neuroendocrine features, median age was 59 (31–83) years. There was no significant difference between the groups in terms of age (p = 0.113).

When histological and immunohistochemical features were re-evaluated, based on 2019 WHO Breast Tumors Classification, 54.8% of the cases were diagnosed as IBC-NST, while neuroendocrine features were found in 45.2%. Of the 84 tumors showing neuroendocrine features, 37 (19.9%) were IBC-NST with neuroendocrine differentiation, 44 (23.7%) were NET and 3 were LCNEC.

Median tumor size was 2.2 (0.5–9.0) cm. Median tumor size was 2.5 (0.6–9.0) cm in tumors with neuroendocrine features and 2.1 (0.5–8.5) cm in tumors without neuroendocrine features. Tumor diameter was significantly larger in tumors with neuroendocrine features (p = 0.029).

Of the tumors with neuroendocrine features, 48 (57.1%) were located in the left breast, 34 (40.5%) were located in the right and 2 (2.4%) were bilateral. According to the Modified Bloom and Richardson System, tumor grade was 1 in 6 (7.1%) cases, grade 2 in 42 (50%) and grade 3 in 36 (42.9%). Venous invasion was observed in 1 (1.2%), lymphatic invasion in 27 (32.1%) and perineural invasion in 19 (22.6%) tumors. Necrosis was present in 41 (48.8%) and microcalcification was present in 44 (52.4%) cases. There was no significant difference between tumor groups in terms of location, tumor grade, venous invasion, lymphovascular invasion, perineural invasion, necrosis and microcalcification (p>0.05).

Peritumoral desmoplastic reaction was mild in 24 (28.6%) of 84 tumors with neuroendocrine features, moderate in 35 (41.7%) and prominent in 25 (29.8%) cases. TIL was not observed in 31 (36.9%) tumors, while it was mild in 40 (47.6%), moderate in 7 (8.3%), and prominent in 6 (7.1%) cases. Peritumoral desmoplastic reaction and magnitude of TIL were significantly lower in tumors with neuroendocrine features (p = 0.0001 and p = 0.0001).

When the two groups were compared in terms of molecular subtyping, the distribution was significantly different (p = 0.003). Tumors with neuroendocrine features were predominantly in the luminal subgroup. Tumors without neuroendocrine features were predominantly in the Luminal B subgroup (48%), but showed a diffuse distribution. 25.5% of IBC-NSTs were Luminal A, 48% were Luminal B, 3.9% were HER2 positive and 22.5% were triple negative, while in the other group, these rates were 31%, 63.1%, 1.2% and 4.8%, respectively. Of the tumors with neuroendocrine features 54.8% showed a growth pattern of large solid islands (islands containing more than about 100 cells) and 57.8% of tumors diagnosed as IBC-NST showed a pattern of small solid islands. When growth patterns were compared, large solid islands were significantly more common in tumors with neuroendocrine features (p = 0.0001). There was no significant difference between tumor groups in terms of cribriformity, palisading or grooves (p<0.05). The presence of basally located nuclei, absence of tubule formation, inconspicuous nucleoli, fine chromatin, granular cytoplasm and indefinite cytoplasmic borders were detected more frequently and significantly more common in tumors with neuroendocrine features (p<0.05) (Figure 2). It was thought that these features may help differentiate primary breast tumors with neuroendocrine features from other breast carcinomas (Table 1).

The results were also evaluated by univariate and multivariate analysis in logistic regression tests. There was no significant difference between the groups in terms of gender, tumor lateralization, grade, lymphovascular invasion, perineural invasion, necrosis or microcalcification. A significant difference was detected between the groups in terms of peritumoral desmoplastic reaction, peritumoral lymphocytic reaction, molecular subtypes and growth patterns. The difference between groups in terms of peritumoral desmoplastic reaction was solely due to IBC-

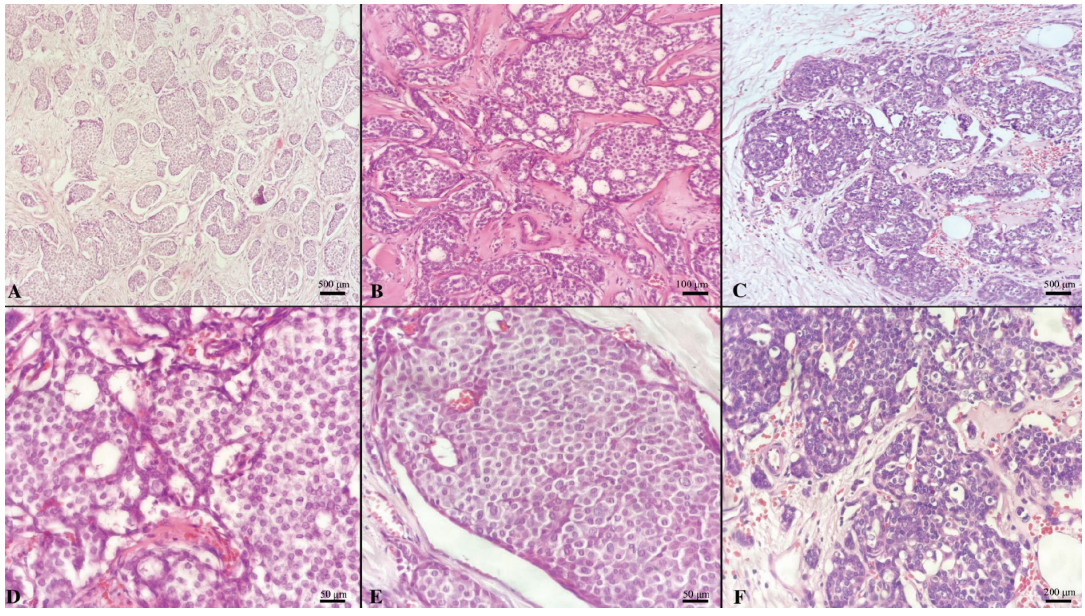

Figure 2. A-B) NET showed a growth pattern of solid islands (H&E x40 and H&E x100). **C)** Absence of tubule formation in NET (H&E x40). **D-E)** Nucleoli are not prominent in tumor cells and fine chromatin is observed (H&E x400). **F)** Indefinite cytoplasm borders in NET (H&E x200)

H&E: Hematoxylin and eosin; NET: Neuroendocrine tumor

Table 1. Intergroup comparisons

	Histopathological diagnosis			
	IBC-NST	**Neuroendocrine differentiation**	**Neuroendocrine tumor**	***p***
Peritumoral desmoplastic reaction				
Mild	8 (7.4)	7 (18.9)	15 (34.1)	
Moderate	36 (33.3)	16 (43.2)	19 (43.2)	**0.0001**
Prominent	64 (59.3)	14 (37.8)	10 (22.7)	
Peritumoral lymphocytic reaction				
Absent	1 (0.9)	7 (18.9)	21 (47.7)	
Mild	44 (40.7)	20 (54.1)	20 (45.5)	**0.0001**
Moderate	44 (40.7)	6 (16.2)	1 (2.3)	
Prominent	19 (17.6)	4 (10.8)	2 (4.5)	
Molecular subtype				
Luminal A	27 (25.0)	8 (21.6)	18 (40.9)	
Luminal B	53 (49.1)	27 (73.0)	23 (52.3)	**0.008**
HER-2	4 (3.7)	0 (0)	1 (2.3)	
Triple negative	24 (22.2)	2 (5.4)	2 (4.5)	
Growth pattern				
Infiltrative	13 (12.0)	1 (2.7)	4 (9.1)	
Large solid islands	11 (10.2)	12 (32.4)	32 (72.7)	
Small solid islands	66 (61.1)	15 (40.5)	4 (9.1)	**0.0001**
Solid	18 (16.7)	7 (18.9)	3 (6.8)	
Trabecular	0 (0)	2 (5.4)	1 (2.3)	
Palisading	14 (13.0)	2 (5.4)	13 (29.5)	0.006
Basally located nuclei	11 (10.2)	5 (13.5)	12 (27.3)	0.026
Groove	11 (10.2)	0 (0)	4 (9.1)	0.134
Presence of tubules	25 (23.1)	6 (16.2)	4 (9.1)	0.119
Presence of nucleoli	64 (59.3)	22 (59.5)	9 (20.5)	**0.0001**
Pleomorphism				
Mild	1 (0.9)	0 (0)	1 (2.3)	
Moderate	28 (25.9)	7 (18.9)	15 (34.1)	0.454
Prominent	79 (73.1)	30 (81.1)	28 (63.6)	
Mitosis				
1	55 (50.9)	17 (45.9)	28 (63.6)	
2	20 (18.5)	7 (18.9)	12 (27.3)	0.053
3	33 (30.6)	13 (35.1)	4 (9.1)	
Nuclear details				
Fine	33 (30.6)	14 (37.8)	19 (43.2)	
Coarse	36 (33.3)	13 (35.1)	5 (11.4)	
Hyperchromatic	11 (10.2)	3 (8.1)	9 (20.5)	**0.041**
Fine peripheral	11 (10.2)	5 (13.5)	8 (18.2)	
Coarse peripheral	17 (15.7)	2 (5.4)	3 (6.8)	

Table 1. Continued

	Histopathological diagnosis			
	IBC-NST	**Neuroendocrine differentiation**	**Neuroendocrine tumor**	***p***
Cytoplasmic details				
Eosinophilic	65 (60.2)	25 (67.6)	14 (31.8)	
Granular	19 (17.6)	10 (27.0)	25 (56.8)	**0.0001**
Clear	24 (22.2)	2 (5.4)	5 (11.4)	
Cell borders				
Conspicous	66 (61.1)	17 (45.9)	14 (31.8)	**0.004**
Inconspicous	42 (38.9)	20 (54.1)	30 (68.2)	

IBC-NST: Invasive Breast Carcinoma of No Special Type; HER-2: Human epidermal growth factor receptor-2

NST cases as the proportion of cases showing prominent peritumoral desmoplastic reaction was significantly higher in this group (p = 0.0001). The difference between the groups in terms of peritumoral lymphocytic reaction was also due to cases of IBC-NST. Peritumoral lymphocytic infiltration was significantly less common in the cases showing neuroendocrine features (p = 0.0001). In terms of molecular subtypes, those diagnosed with IBC-NST were most commonly triple negative tumors. The most common cases in the IBC-NST group showing neuroendocrine differentiation were luminal B, and the cases in the NET group were Luminal A and Luminal B. However, the significant difference was again due to the IBC-NST group (p = 0.008). When the distribution of the growth pattern was evaluated, the significance was due to the NET group and the growth pattern of large solid islands was significantly more common in this group (p = 0.0001). The presence of nucleoli was significantly less common in the NET group (p = 0.0001). Additionally, there was a statistically significant difference between the NET group and other groups in terms of fine chromatin, granular cytoplasm and inconspicuous cell borders (p = 0.041, p = 0.0001 and p = 0.004, respectively).

Discussion and Conclusion

Primary neuroendocrine neoplasms of the breast, which are divided into two groups, NET and NEC (SCNEC and LCNEC) in the 2019 WHO Classification of Breast Tumors, are a heterogeneous group of tumors with different clinical behaviors and prognosis. One of the most important stages of making a correct diagnosis is to keep this diagnosis in mind and to be aware of the histomorphological findings. Considering this, histomorphological features that distinguish these tumors were evaluated in the present study.

Primary NETs of the breast are most commonly seen in women in the 6th and 7th decades, but have also been reported at earlier ages and in male patients (3, 4). Through their analysis of the National Cancer Database including 1389 cases of primary breast NETs, Martinez et al. (5) found that 82.9% of the cases were over 50 years of age and 97.9% were female. When compared to IBC-NST, primary breast NET was significantly more common over the age of 70 and the incidence was twice as high in males (5). In the present study, all patients with tumors showing neuroendocrine features were female and the mean age was 59 years, which was not different from the other tumors considered in the study.

The usual clinical presentation is palpable painless mass and no distinguishing features from other breast cancers has been reported. In addition to features similar to other breast cancers on radiological studies, findings that may suggest neuroendocrine neoplasms, such as well-defined, hyperdense, rounded contours on mammography and hypervascular, homogeneous, irregular or microlobular hypoechoic solid masses, may be detected on ultrasonography (6, 7). Kayadibi et al. (8) found that, in the mammographic evaluation, architectural distortion, axillary lymphadenopathy and calcification were more common findings with breast tumors that did not show neuroendocrine features. On magnetic resonance and ultrasonographic evaluation, tumors in this group had irregular shape with more spiculated contours.

The correct diagnosis of a primary NET of the breast is based on a detailed clinical, radiological and histological evaluation. Around 0.2–1.1% of breast malignant tumors are metastatic tumors originating from non-mammary solid organs and only 1–2% of these metastatic neoplasms originate from NECs (3, 4). Metastatic neuroendocrine neoplasms may also show histological features similar to primary breast carcinomas. Treatment protocols and patient management are completely different in these tumors and therefore it is important to examine clinical history of the patient in detail and make a detailed radiological evaluation for an *in situ* component and the primary tumor focus (3, 4, 9). During the archive search we conducted within the scope of case selection, we identified three NEC cases that metastasized to the breast. Of these three, there was no history of malignancy in two, but the absence of *in situ* carcinoma component, a suspicious mass lesion in the lung found in the detailed clinic-radiological evaluation, and the immunohistochemical studies aided the diagnosis.

Cytomorphological features have been described in detail in tumors that develop in locations, such as the respiratory and gastrointestinal systems, where NETs are frequently seen. That the features observed in primary neuroendocrine neoplasms of the breast are not always typical and some features overlap with tumors that do not show neuroendocrine features may give rise to diagnostic difficulties. This is also one of the main reasons that the true prevalence of primary NETs of the breast cannot be determined.

In the histomorphological evaluation, low or medium grade tumors consisting of spindle-shaped, plasmacytoid or polygonal-shaped cells with eosinophilic, granular or clear cytoplasm showing a growth pattern in the form of trabecular and/or cellular solid islands should be evaluated for neuroendocrine features. Thin fibrovascular stroma, rosette formation and peripheral palisading are other histomorphological features that can be observed in these tumors (3, 10). The presence of intracellular and/or extracellular mucin, no prominent rosette formation, palisading and salt-and-pepper chromatin, absence of monotonous round-oval nucleoli, conspicuous nucleoli, plasmacytoid morphology and organoid growth pattern are observed in primary NETs of the breast but are not frequently expected features in NETs arising in other locations (11, 12). Kelten Talu et al. (13) compared primary breast carcinomas with and without neuroendocrine features and found that higher histological and nuclear grade, lymphovascular invasion, comedo-type ductal carcinoma *in situ*, and the presence of tumor-related microcalcification were significantly less common in tumors with neuroendocrine features. In the present study, a growth pattern in the form of large solid islands, absence of cribriformity, absence of tubule formation, absence of nucleoli, presence of fine chromatin, eosinophilic granular cytoplasm and cells with inconspicuous cytoplasmic borders were found significantly more frequently in tumors with neuroendocrine features. However, no histomorphological feature alone is sufficient to diagnose neuroendocrine neoplasms.

In the study of Bogina et al. (14), neuroendocrine features were considered by histomorphology in only 34% of tumors with neuroendocrine features. Thus immunohistochemical studies are mandatory for the exact diagnosis. Synaptophysin, Chromogranin-A, CD56, neuron specific enolase (NSE) and protein gene product 9.5 (PGP 9.5) are the main immunohistochemical stains used for NETs. Second generation markers, such as insulinoma-associated protein (INSM1) and syntaxin-1 (STX1) have been claimed to have higher sensitivity and specificity (15). The sensitivity and specificity of NSE and CD56 immunohistochemistry are lower than synaptophysin and chromogranin A (16). Razvi et al. (17) investigated the use of INSM1 immunohistochemical stain as a neuroendocrine marker in luminal B breast cancers. When synaptophysin, chromogranin, CD56 and INSM1 were used in double and quadruple combinations, INMS1 showed higher sensitivity compared to Chromogranin A and CD56.

In terms of molecular subtyping, primary neuroendocrine neoplasms of the breast are frequently of the luminal B type. These tumors are usually estrogen and/or progesterone receptor positive and almost always human epidermal growth factor receptor-2 (HER-2) negative. However, recently studies also describe HER-2 positive NETs of the breast (18, 19). In the present study, tumors with neuroendocrine features were commonly in the luminal B subgroup (63.1%), while only one case was HER-2 positive.

Primary neuroendocrine neoplasms of the breast are tumors that can cause diagnostic difficulties, considering their low incidence, and non-specific clinical and radiological features. Although the histological features observed in NETs of other organs are also observed in NETs of the breast, similar features can also be observed in *in situ* or IBCs that do not show neuroendocrine features. The absence of specific clinical and radiological findings, the inability to study neuroendocrine markers in every laboratory, and the need to prove that the breast tumor is not a metastasis are all conditions that create diagnostic difficulties. We believe that the results of this study may help diagnose and identify the more specific histomorphological features that help determine neuroendocrine morphology in primary breast tumors.

Ethics Committee Approval: Ethics approval for the study, dated November 10, 2022 and numbered 2022-17/29 was obtained from the Uludag University Faculty of Medicine Clinical Research Ethics Committee.

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: K.S., S.T., M.S.G.; Concept: M.O., S.T.; Design: M.O., S.T., T.E.; Data Collection and/or Processing: M.O., S.T., M.S.G., T.E.; Analysis and/or Interpretation: M.O., S.T.; Literature Search: M.O., S.T.; Writing: M.O., S.T.

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Original Article

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The Relationship of Pathological Response and Visceral Muscle and Fat Volume in Women With Breast Cancer Who Received Neoadjuvant Chemotherapy

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ABSTRACT

Objective: Differences in individual muscle/fat volumes may change the effectiveness of chemotherapy. In this study, the relationship between trunkal muscle and fat volume and body mass index (BMI) obtained before receiving neoadjuvant chemotherapy (NCT) in patients with breast cancer and complete pathological response (pCR) was investigated.

Materials and Methods: The volumes of psoas, abdominal and paraspinal muscles, and trunkal subcutaneous and visceral fat were calculated using CoreSlicer AI 2.0 opensource program from the F-18 fluorodeoxyglucose positron emission tomography/computed tomography (CT) and CT images before NCT and postoperative pCR rates to NCT were recorded. Muscle/fat volumes and BMI prior to NCT were compared in terms of pathological pCR rates. Patients were followed up regularly for recurrence and survival.

Results: Ninety-three patients were included with median (range) values for age, BMI, and body weights of 48 (28–72) years, 27 (16.8–51.6) kg/m^2, and 71.94 (43–137) kg, respectively. The median follow-up time was 18.6 (6.7–59.6) months. No significant correlation was found between total muscle or fat volumes of patients with and without pCR. BMI [26.2 (16.8–51.6) kg/m^2 *vs.* 24.6 (20.3–34.3) kg/m^2, p = 0.03] and pCR rates in patients with low right-psoas muscle volume [11.74 (7.03–18.51) *vs.* 10.2 (6.71–13.36), p = 0.025] were significantly greater. A significant relationship was found between right psoas muscle volume and disease-free survival (DFS) (11.74 cm^3 (7.03–18.51) vs. 10.2 cm^3 (6.71–13.36), p = 0.025). However, no significant relationship was detected between total muscle-fat volume, BMI and overall survival and DFS (p>0.05).

Conclusion: This is the first published study investigating the relationship between the pCR ratio and body muscle and fat volume measured by CoreSlicer AI 2.0 in patients with breast cancer who received NCT. No correlation was found between the pCR ratio and total muscle plus fat volume. However, these results need to be validated with larger patient series.

Keywords: Breast cancer; FDG-PET-CT; pCR; total muscle-fat volume; BMI; neoadjuvant chemotherapy

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Key Points

- This is the first published study in the to analyze the trunkal muscle/fat volumes from a single section obtained at a single level in computed tomography and to investigate the relationship between measurements and neoadjuvant chemotherapy (NCT).
- Muscle-Fat volume was measured by CoreSlicer AI 2.0 in patients with breast cancer who received NCT. In patients with pathological response (pCR), mean body mass index (BMI) and right psoas muscle volume was significantly less.
- Mean BMI and right psoas muscle volume were found to be significantly lower in patients with pCR. Total muscle volumes were higher in patients with pCR, but the difference was not statistically significant.
- Local/systemic recurrence occurred in 18 patients during the follow-up period, and no correlation was found between body muscle/fat volume and pCR in these patients.

Introduction

It is known that excess adipose tissue is a risk factor for the development of breast cancer (BC) by inducing insulin resistance, chronic inflammation, and hormonal changes (1). Obesity increases the risk of BC and decreases the effectiveness of neoadjuvant chemotherapy (NCT) (2, 3). Complete pathological response (pCR) and disease-free survival (DFS) rates after NCT are generally lower in obese patients (2, 4). Body mass index (BMI) is the most common measure for classifying weight and has been extensively studied to explore the relationship between obesity and survival in BC (5). A study that examined the effects of high visceral adipose tissue (VAT) on the survival of patients with BC showed that high VAT shortened DFS due to increased insulin levels, and increased insulin resistance (6). There is evidence that visceral fat plays a more significant role in the homeostasis of cancer cells than other adipose tissues. In addition, it has been reported that high visceral fat levels significantly affect chemosensitivity (7).

Sarcopenia and adiposity measurements obtained from abdominal computed tomography (CT) images in nonmetastatic BC patients provide more prognostic information than BMI and help to predict survival outcomes (5, 8). However, a low BMI can mask excessive fat while a high BMI can mask low muscle mass. It has also been shown that low muscle radiodensity increases the risk of mortality (5).

There is increasing interest in using body composition analysis to treat patients with BC (9, 10). CoreSlicer is the first open-source, web-based, medical image analysis software specifically designed and optimized for analytical morphometry, measuring specific biomarkers of body composition from CT images (11). The present study investigated the relationship between muscle/fat volume, BMI, and pCR using the CoreSlicer AI 2.0 program in patients who received NCT after diagnosis with BC.

Materials and Methods

Patients with a diagnosis of stage 2-3 BC in Istanbul Florence Nightingale Breast Health Center who received NCT (4 cycles of adriamycin/epirubicin + cyclophosphamide (AC/EC) + 4 cycles of taxane ± anti-Her-2 therapies) and whose data and follow-up were complete were included. The patient's demographic, clinical, pathological, and follow-up results were retrospectively evaluated (Graphic 1, Table 1). Patients younger than 18 years, receiving adjuvant chemotherapy, and metastatic patients with missing follow-up were excluded from the study. The pCR is defined as the absence of residual invasive cancer on hematoxylin and eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy. The patients' body muscle and fat volumes were measured by a radiologist (K.Y.) with more than 10 years experience in abdomen imaging before NCT treatment using the CoreSlicer AI 2.0 (Figure 1) opensource software program (11). The volumes of the left and right psoas muscles, bilateral abdominal and paraspinal muscles, and subcutaneous and visceral fat were measured from the L3 v reference point.

Before the diagnosis, body weight and height measurements were made using SECA® (Medizinische Messsysteme und Waagen, Hamburg, Deutschland), BMI was calculated, and the results were evaluated according to the World Health Organization classification (12).

Statistical Analysis

SPSS for Windows, version 20.0 was used for statistical analysis (IBM Inc., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to determine the distribution of variables, the Mann-Whitney U test for the comparison of non-normally distributed parameters (non-parametric), the chi-square test for the comparison of qualitative data, and two-way Pearson correlation test for determining the relationship between quantitative variables. The level of significance was set as $p<0.05$ in all analyses.

Results

The median age, follow-up time, BMI, and weight values of all patients were 48 (28–72), 18.6 months (6.7–59.6), 27 kg/m^2 (16.8–51.6), and 71.94 kg (43–137), respectively (Graphic 1).

Of nineteen patients acheiving pCR, 2 (7.7%) were Luminal A, 7 (17.9%) were Luminal B, 6 (50%) were human epidermal growth factor receptor type 2 (HER2), and 4 (25%) were triple-negative breast cancer. The pCR rates were significantly lower in the luminal A and B groups (p = 0.039) (Table 1). Local/systemic recurrence was observed in 18 patients during follow-up and no correlation was found between body muscle-fat volume and pCR in these patients ($p>0.05$).

In patients with pCR, median BMI and right psoas muscle volume were significantly lower than in patients not acheiving pCR (Table 1). Furthermore, total muscle [114.47 (43.54–155.82) *vs.* 106.65 (80.56–139.24), p = 0.08], and total fat [334.98 (21.77–878.58) *vs.* 309.22(111.32–595.51), p = 0.36] volumes tended to be lower in patients with pCR but were not sigificantly different.

Considering the effect of muscle-fat volumes on overall survival (OS) and DFS, a significant correlation was found between right psoas volume and DFS [11.74 cm^3 (7.03–18.51) *vs.* 10.2 cm^3 (6.71–13.36), p = 0.025]. It was calculated that each unit increase in right psoas volume increases the risk of recurrence or death by 1.2 times. No significant correlation was found between total muscle-fat volume, BMI, OS, and DFS ($p>0.05$).

No significant difference was found when BMI and psoas, abdominal and paraspinal muscles, and subcutaneous and visceral fat volumes were examined in pre-and post-menopausal patients (p>0.05). In addition, no significant relationship was found between menopausal status and DFS vs. pCR (p>0.05).

Discussion and Conclusion

This is the first published study to calculate body muscle/fat volume by examining fluorodeoxyglucose- positron emission tomography (PET) CT images with CoreSlicer AI 2.0 open-source software web tool kit in patients with BC who received NCT and investigate the relationship with pCR. CoreSlicer is the first open-source web-based medical imaging analysis designed and optimized for analytical morphometric assessment, designed to include artificial intelligence (11). Previous studies evaluated body compositions only as area, tissue, or mass (5, 13, 14). BMI, a more commonly used method, only measures the ratio between height and weight, and does not distinguish between muscle and adipose tissue, and cannot account for body fat distribution and type differences when used alone (5, 7). Thus, body composition-specific biomarkers obtained from CT images may be used instead of BMI in clinical evaluations (10, 15).

The relationships between BC and obesity, BMI, and body composition have been extensively studied (5, 6, 7, 9, 10, 16, 17-20). However, different results were obtained in studies investigating the relationship between muscle/fat tissue data obtained from CT images of these patients and BMI and survival (5, 13). Some studies have shown that body composition data obtained by BMI and CT are not associated with DFS (7, 10, 17). However, in a study by Iwase et al. (18) in 248 patients receiving NCT, decrease DFS was associated negatively with molecular subtypes, tumor stage, and high BMI. Some studies have shown muscle/fat volume measurements are more effective than BMI in determining survival. DFS is associated with visceral adiposity, insulin level, and insulin resistance (5, 6, 7, 9, 19, 20). In the present study, however, a significant relationship was found only between right psoas muscle volume and DFS, such that each unit increase in

Table 1. The correlation between age, BMI, muscle and fat volumes, molecular subtypes, and pCR

	pCR (-) Median (min-max)	pCR (+) Median (min-max)	p
Age (years)	48 (29–72)	44 (28–67)	0.27[a]
BMI (kg/m²)	**26.2 (16.8–51.6)**	24.6 **(20.3–34.3)**	**0.03[a]**
Left-psoas volume (cm³)	11.69 (7.09–19.78)	11.53 (8.66–13.68)	0.3[a]
Right-psoas volume (cm³)	**11.74 (7.03–18.51)**	**10.2 (6.71–13.36)**	**0.025[a]**
Subcutaneous fat volume (cm³)	244.48 (10.88–703.55)	195.94 (91.11-382.61)	0.15[a]
Visceral fat volume (cm³)	84.38 (8.27–357.11)	81.5 (20.21–302.2)	0.74[a]
Abdominal muscle volume (cm³)	48.58 (10.88–75.24)	45.68 (35.18–65.6)	0.16[a]
Paraspinal muscle volume (cm³)	43.04 (10.88–56.75)	37.62 (27.18–53.36)	0.12[a]
TFV (cm³)	334.98 (21.77–878.58)	309.22 (111.32–595.51)	0.36[a]
TMV (cm³)	114.47 (43.54-155.82)	106.65 (80.56–139.24)	0.08[a]
Total fat/total muscle volume (cm³)	3.07 (0.5-7.1)	2.8 (1.1–4.8)	0.65[a]
Molecular Subtypes			
Luminal A (n = 26)	**24 (92.3%)**	**2 (7.7%)**	
Luminal B (n = 39)	**32 (82.1%)**	**7 (17.9%)**	**0.039[b*]**
HER2 (n = 12)	**6 (50%)**	**6 (50%)**	
TNBC (n = 17)	**12 (75%)**	**4 (25%)**	

[a]: Mann-Whitney U, [b]: Chi-square; pCR: Complete pathological response; BMI: Body mass index; TFV: Total fat volume; TMV: Total muscle volume; HER2: human epidermal growth factor receptor type 2; TNBC: Triple negative breast cancer

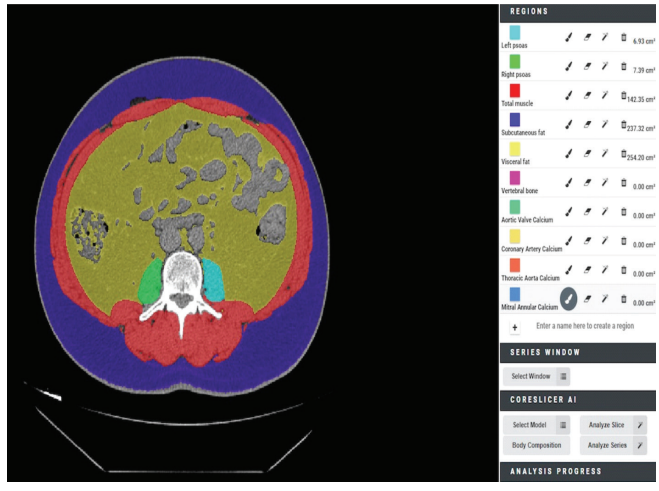
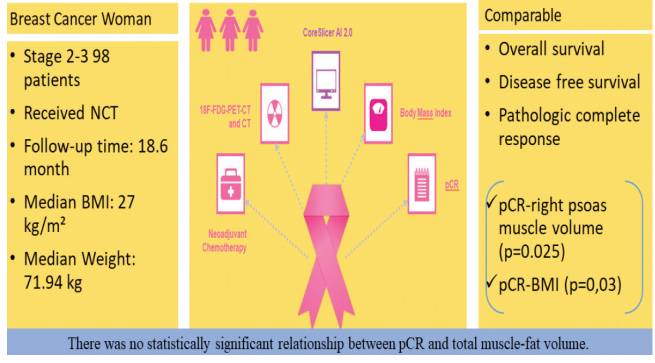


Figure 1. AI volume measurement image with coreslicer

Red: Total muscle, Yellow: Visceral fat, Purple: Subcutaneous fat, Green: Right psoas muscle, Turquoise: Left psoas muscle, AI: Artificial intelligence



Graphic 1. The relationship of pathological response and visceral muscle and fat volume in women with breast cancer who received neoadjuvan chemotherapy

right psoas muscle volume increased the risk of recurrence or death by 1.2 times. However, no relationship was found between BMI and OS/DFS, which may be due to the low number of patients and low recurrence rate.

In patients with BC, body composition determines the dose, toxicity, and efficacy of NCT and is a determinant in improving the prognosis (7, 9, 20). A lower pCR rate and shorter progression-free survival times have been demonstrated in overweight patients treated with NCT (13). In another study, while no relationship could be found between pCR and BMI, it was found that high visceral fat volume and fatty liver were negative factors for acheiving pCR (7). A study by Trestini et al. (21) showed that body composition parameters did not affect pCR. Still, an increase of ≥10 % of VAT during NCT was associated with shorter DFS. In a study comparing body composition parameters measured using PET CT before NCT and response to NCT, no significant relationship was found between them. Still, a very weak correlation was found between superficial adipose tissue and pCR (22). In the present study, no significant relationship between muscle/fat volume and pCR was identified, but BMI was significantly lower in pCR-positive patients (p = 0.03).

There are significant changes in body fat distribution in the postmenopausal period (21). A study conducted with postmenopausal women with BC showed that these patients were more overweight and had a higher visceral fat area (VFA) and more fatty liver than premenopausal patients (7). In the present study, no significant relationship was found when postmenopausal status and BMI and CoreSlicer images were compared.

In a study examining menopausal status in patients with BC in detail, no relationship could be shown between body composition parameters and pCR. At the same time, distant DFS was found to be lower in the high VFA group (p<0.05) (20). In the present study, when BMI and CoreSlicer images were compared in pre-and postmenopausal patients, no significant difference was found between them. In addition, no significant relationship was found between menopausal status and DFS vs. pCR.

The fact that changes in body composition affect treatment outcomes differently in BCs with different molecular types is another matter for debate (23). In postmenopausal HER2+ and luminal BC patients, obesity increases mortality and morbidity (24). In addition, patients with Luminal type and BC with axillary lymph node metastases have been shown to have higher BMI and VAT levels (24). In the present study, pCR rates were significantly lower in patients in Luminal A and B groups, as expected. However, no significant correlation was observed between total muscle volume (TMV) and total fat volume and molecular subtypes.

Higher rates of pCR were obtained in patients in the present study with low total muscle volume, and this difference was close to significant (p = 0.08). Thus, it is possible that calculating TMV only using these volumetric techniques, independent of height and weight characteristics, may not be an objective criterion. Therefore, muscle volume distributions were calculated in proportion to the body surface area and BMIs of the patients and re-analyzed to examine whether there was a relationship between these ratios and the treatment response. Although the relationship between TMV/BMI and pCR was insignificant, it was found that there was a tendency close to numerical significance (p = 0.065). These results suggested that standardized muscle volume analyses need to be further evaluated in studies with larger sample sizes.

Study Limitations

The strengths of the present study include being the first study in which body composition was calculated by a single radiologist with artificial intelligence-based volumetrically accurate and standardized measurements. The limitations include the small number of patients, the retrospective nature and the single-center design.

In conclusion, this is the first study to analyze the body muscle/fat volume from a single imaging section obtained at a single level in CT and to investigate the relationship between measurements and outcomes of NCT. There was no significant relationship between pCR and total muscle/fat volume. However, these results must be validated by prospective studies with more extensive patient series.

Ethics Committee Approval: The study protocol was approved by Istanbul Bilgi University Ethics Committee (date and approval number: 2021-40034-49).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: S.İ., V.Ö.; Concept: T.K.T., F.Ç., K.Y., E.Ö., S.K., A.Ö., V.Ö.; Design: T.K.T., F.Ç., G.A., N.A., Ç.O., E.Ö., T.D., V.Ö.; Data Collection and/or Processing: T.K.T., K.Y., F.A., V.Ö.; Analysis and/or Interpretation: S.İ., G.S., N.A., Ç.Ü., Z.İ., V.Ö.; Literature Search: T.K.T.; Writing: T.K.T., F.Ç., G.S., E.Ö., V.Ö.

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Original Article

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Radiomics Analysis of Contrast-Enhanced Breast MRI for Optimized Modelling of Virtual Prognostic Biomarkers in Breast Cancer

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ABSTRACT

Objective: Breast cancer clinical stage and nodal status are the most clinically significant drivers of patient management, in combination with other pathological biomarkers, such as estrogen receptor (ER), progesterone receptor or human epidermal growth factor receptor 2 (HER2) receptor status and tumor grade. Accurate prediction of such parameters can help avoid unnecessary intervention, including unnecessary surgery. The objective was to investigate the role of magnetic resonance imaging (MRI) radiomics for yielding virtual prognostic biomarkers (ER, HER2 expression, tumor grade, molecular subtype, and T-stage).

Materials and Methods: Patients with primary invasive breast cancer who underwent dynamic contrast-enhanced (DCE) breast MRI between July 2013 and July 2016 in a single center were retrospectively reviewed. Age, N-stage, grade, ER and HER2 status, and Ki-67 (%) were recorded. DCE images were segmented and Haralick texture features were extracted. The Bootstrap Lasso feature selection method was used to select a small subset of optimal texture features. Classification of the performance of the final model was assessed with the area under the receiver operating characteristic curve (AUC).

Results: Median age of patients (n = 209) was 49 (21–79) years. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the model for differentiating N0 vs N1-N3 was: 71%, 79%, 76%, 74%, 75% [AUC = 0.78 (95% confidence interval (CI) 0.72–0.85)], N0-N1 vs N2–N3 was 81%, 59%, 24%, 95%, 62% [AUC = 0.74 (95% CI 0.63–0.85)], distinguishing HER2(+) from HER2(-) was 79%, 48%, 34%, 87%, 56% [AUC = 0.64 (95% CI 0.54–0.73)], high nuclear grade (grade 2–3) vs low grade (grades 1) was 56%, 88%, 96%, 29%, 61% [AUC = 0.71 (95% CI 0.63–0.80)]; and for ER (+) vs ER(-) status the [AUC=0.67 (95% CI 0.59–0.76)]. Radiomics performance in distinguishing triple-negative vs other molecular subtypes was [0.60 (95% CI 0.49–0.71)], and Luminal A [0.66 (95% CI 0.56–0.76)].

Conclusion: Quantitative radiomics using MRI contrast texture shows promise in identifying aggressive high grade, node positive triple negative breast cancer, and correlated well with higher nuclear grades, higher T-stages, and N-positive stages.

Keywords: Breast cancer; radiomics; texture analysis; biomarkers; predictive models

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Key Point

- The precision of the presented radiomics model is 75% when distinguishing between N0 and N1-N3 cases, and 62% for differentiating between N0-N1 and N2-N3 cases. Furthermore, the model achieved an area under the curve of 71% when identifying high nuclear grade (grades 2–3) versus low grade (grade 1) cases.

Introduction

Breast cancer is the most commonly diagnosed cancer and leading cause of cancer deaths among women (1). Multiple factors impact prognosis, including patient age, tumor size, type and grade, and lymph node status (2-5). In recent years, estrogen receptor (ER), progesterone receptor (PR) and human endothelial growth factor receptor 2 (HER2) status have emerged as important molecular biomarkers in staging breast cancer and guiding treatment decisions regarding hormonal and targeted therapies, neoadjuvant chemotherapy, or upfront surgery (2, 6). Triple negative breast cancer (TNBC) is associated with poor prognosis and decreased survival (7, 8), while targeted therapies in receptor positive breast cancer improve outcomes (9, 10). The status of ER, PR and HER2 is determined by immunohistochemistry analysis of individual biopsy samples via well-established protocols (11, 12). However, due to intra-tumoral heterogeneity within the primary lesion and inter-tumoral heterogeneity between the primary cancer and its metastases, incisional biopsy results may not be representative of the whole tumor (13, 14). A non-invasive method for evaluating tumor biomarkers may be useful for detecting heterogeneity and assist as a clinical decision

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support tool throughout the continuum of patient care from detection to adjuvant therapies.

Metastatic status of axillary lymph nodes is an important prognostic marker, guiding therapy in newly diagnosed breast cancer (6). Sentinel lymph node biopsy (SLNB) is the mainstay method for evaluating axillary lymph node metastasis but SLNB is invasive and associated with morbidity (15). Implementation of a preoperative assessment of axillary lymph nodes with imaging may help avoid SLNB in some cases (15-20). Physical examination, mammography, breast ultrason and fine needle aspiration biopsy provide limited sensitivity and specificity in assessment of axillary lymph nodes and cannot reliably exclude the need for SLNB (15, 17-22). Dynamic contrast-enhanced (DCE)-magnetic resonance imaging (MRI) has been reported to be the most accurate method of evaluating disease extent (23-25). DCE-MRI also allows assessment of the axillary and internal mammary nodes for metastatic disease. However, it also has modest sensitivity and negative predictive value (NPV) at 80% and 60%, respectively, for detection of axillary lymph node metastasis (15, 20, 25, 26).

Textural kinetics are quantitative imaging features that describe the dynamic variation of textural features of breast lesions during contrast material uptake and can outperform standard morphologic, static texture, and kinetic intensity features in the differentiation of benign and malignant lesions (27). Textural heterogeneity on MRI correlates with histopathological tumor heterogeneity and shows a positive trend for correlation with prognostic markers such as ER, PR or HER2 positivity, and prognostic scores such as Oncotype Dx or PAM50 (28, 29). Several studies have shown that MRI imaging features are associated with molecular breast cancer subtypes (28-32). The results of these studies offer a possible framework in which to explore textural features as biomarkers of clinically relevant prognostic indicators.

In this study, we aimed to investigate the potential role of DCE-MRI texture radiomics for identification of virtual prognostic biomarkers for ER, PR and HER2 expression, tumor grade, molecular subtype, clinical T and N stage.

Materials and Methods

In this institutional review board-approved and HIPAA-compliant (Health Insurance Portability and Accountability Act) study, consecutive patients with primary invasive breast cancer who underwent breast DCE-MRI between July 2013 and July 2016 in our institution were retrospectively reviewed. Age, tumor size (T1–4), regional nodal metastasis (cN1, cN2, cN3) and tumor stage (I-IV) information was collected from electronic medical records. Treatments that each patient received, response to treatment (pathological complete response vs partial response vs stable disease) and residual cancer burden (I-III) were collected. Tumor grade (grade 1, 2 or 3), ER status (ER positive or ER negative), HER2 status (HER2 amplified vs HER2 non-amplified) and Ki-67 (low, intermediate, or high) status was obtained from pathology reports. American Society of Clinical Oncology/College of American Pathologists criteria were followed in the assessment of ER, PR, HER2 and Ki-67 positivity. Molecular subtypes were defined, based on previously published criteria: Luminal A (ER+ and/or PR+, Ki-67<14%), Luminal B (LuminalB-HER2-: ER+ and/or PR+, Ki-67≥14%; LuminalB-HER2+: ER+ and HER2+ regardless of Ki-67), HER2+ (ER and PR-, HER2+), and TNBC (TN, or ER-, PR-, HER2-). The significance of PR expression in the absence of ER expression in tumors is unclear as ER+ ty dominates tumor biology and prognosis. In addition, ER expression is the predominant determinant of tumor molecular subtype per The American Joint Committee on Cancer (AJCC) 8th edition classification of tumor subtype and stage (AJCC 8th ed) (33).

DCE-MRI Technique

All MRI studies were performed with the patients lying prone in a 1.5 T scanner (OptimaTM MR450w; GE Healthcare, Milwaukee, WI, USA) using a dedicated 8–channel breast array coil (MRI Devices Corporation, Pewaukee, WI, USA). A single pre-contrast and four serial bilateral dynamic VIBRANT sagittal image sets, which were obtained before and immediately after rapid intravenous bolus infusion of 0.1 mmol/kg gadopentetate dimeglumine contrast medium (Magnevist; Bayer HealthCare Pharmaceuticals Inc., Wayne, NJ, USA) at a rate of 3 mL/s with a power injector (Spectris Solaris MR Injector; MEDRAD, Warrendale, PA, USA), with an average dynamic temporal resolution of 90 s/phase (range 60-120 s, depending on patient size and full bilateral breast coverage), TR/TE 5.59–7.2/1.7–18 ms, field of view 18–26 cm, matrix 256 × 256, FA 10, and slice thickness/gap 1.8/0.9 mm.

Image Processing and Extraction of Texture Features

All MRI images were loaded into Horos with OsiriX plugin (Pixmeo SARL, Geneva, Switzerland), on a secured dedicated research computer. The series was de-identified using the RSNA Clinical Trial Processor (34) and stored in a research PACS (iPACS, Invicro, Boston, MA, USA). Lesions were segmented using regions-of-interest (ROIs). When multiple cancers were present, the index lesion, which was used to clinically stage the patient, was used. ROIs were drawn manually by a breast imaging fellow with 1 year of experience in MRI imaging and interpretation, supervised by a fellowship-trained breast imager with 16 years of MRI imaging experience to indicate the lesion of interest. When possible, the ROIs were centered in each slice on areas of contrast uptake with no visible necrotic areas. Necrotic areas were excluded from the texture analysis, as only metabolically active regions of tumor are of interest in comparing prognostic subtypes. The ROI size was chosen individually to balance the need for sufficient voxel statistics and maximum lesion coverage. The stack of ROIs was also used to generate morphological measures of the lesion. Haralick texture features were extracted using MATLAB (2015, version 8.5, R2015a, The MathWorks Inc., Natick, MA, USA). For Haralick texture features, distance was set at 1 pixel and features were averaged across all angles under the isotropic assumption.

Statistical Analysis

All features were grouped with an unsupervised Principal Component analysis (PCA)-like procedure. Similar features were grouped into disjoint clusters with a linear combination (corresponding to first principal component). The relationship between lesion and patient characteristics were investigated by Pearson correlation test and correlation is shown as a heat map (Figure 1).

A soft version of the Bootstrap Lasso (Bolasso) feature selection method was used. Specifically, 500 replicates of the data with simple random sample with replacement was generated. In each replicate, features were selected using Lasso with regularization parameter rho = 0.8. The importance of features was evaluated by the selection frequencies over the bootstrap samples. The final selected model consisted of features that were present in at least 80% of the bootstrap replications and was evaluated by a receiver operator curve (ROC) analysis under leave-one-out (LOO) cross validation. A cut-off on the ROC curve was proposed

by maximizing the Youden index. Corresponding accuracy, sensitivity, specificity, positive predictive value (PPV) and NPV were calculated with 95% confidence intervals.

ROC analysis was used to compare associations between the cluster components and clinical outcomes adjusted for age and race. Area under the curve (AUC) values were calculated with LOO cross validation. Diagnostic performance of the cut-off was calculated. Statistical software used was SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA). The significance level was 0.05 and Bonferroni correction was used for multiple comparisons, when necessary.

Results

Patient and Lesion Characteristics

Two hundred and eight patients with breast cancer underwent breast MRI and are included in the study. Median (range) patient age was 49.8 (21–79) years. Median T, N and M stages of the lesions were T2, N0 and M0 respectively (46.6%, 54.3% and 83.7%). Mean Ki-67 expression was 42.2%. Further patient (race, age) and lesion [grade, classification of malignant tumors (TNM) stage, Ki-67 expression, ER and HER2 status] characteristics are summarized in Table 1.

Texture Parameter Clustering

Texture parameters that had highest correlation with prognostic factors, determined by Pearson correlation test, were grouped under three main clusters. Each cluster included the following parameters:

• Cluster 1 Total, correlation, sum of entropy, entropy.

• Cluster 2 Angular second moment, correlation difference variance, difference entropy and information measure of correlation 2.

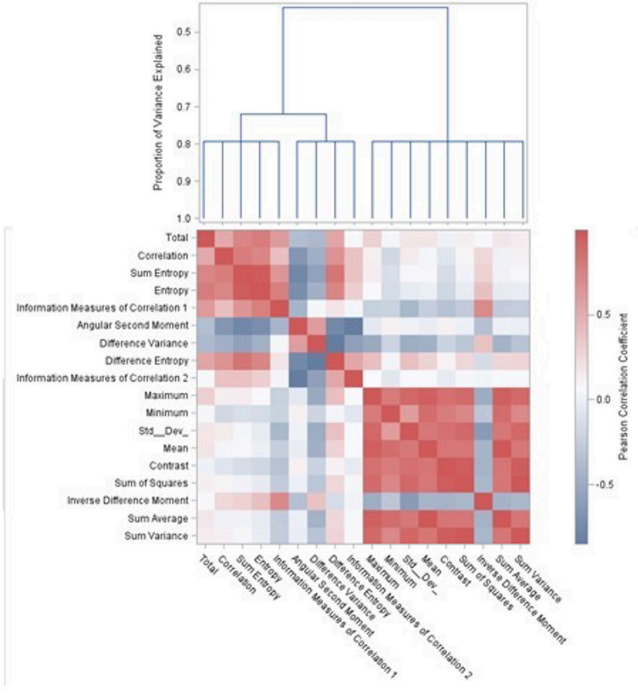

Figure 1. Principle component analysis is performed and Pearson Correlation Coefficients between parameters are depicted above. Linear combination is used to create clusters grouping similar features

• Cluster 3 Maximum, minimum, standard deviation, mean, contrast, sum of squares, inverse difference moment, sum average and sum variance.

Further correlations between the parameters are shown as a heat map in Figure 1.

Prediction of Tumor Grade and Stage

The Cluster 1 model showed the highest performance in predicting tumor grade, clinical nodal stage, and T stage of breast tumors (AUC = 0.709, 0.782 and 0.789 respectively). T stage of the tumors [T1–T2 (n = 138) vs T3–T4 (n = 70)] is predicted with 58% sensitivity, 90% specificity, 75% PPV, 80% NPV and 79% accuracy with this model [AUC = 0.789, 95% confidence interval (CI) 0.718–0.860]. Moderate-to-high sensitivity (71%), specificity (79%), PPV (76%), NPV (74%) and accuracy (75%) was observed in predicting the presence of clinically evident regional lymph node metastasis on the optimal cut-off point of the Cluster 1 model [cN0 (n = 113) vs cN1–3 (n = 95)] (AUC = 0.782, 95% CI 0.715–0.850). High-grade tumors (grade 2 or 3, n = 171) can be detected with the Cluster 1 model with high specificity (88%) and PPV (96%), but sensitivity (56%), NPV (29%) and accuracy (61%) were moderate, at best (AUC = 0.709, 95% CI 0.626–0.792) at the optimal cut-off points (Figure 2). Nodal metastasis (N0 vs N1 3) was predicted with 71% sensitivity, 79% specificity, 76% PPV and 74% NPV and 75% accuracy (AUC = 0.782, 95% CI 0.715–0.849). Higher sensitivity (81%) and NPV (95%) can be achieved for N0–N1 vs N2–N3 (AUC = 0.739, 95% CI 0.632–0848) (Figure 3).

Prediction of Molecular Biomarker Expression and Molecular Subtype

The Cluster 1 model also had the best performance in detecting ER, HER2 and Ki-67 expressions of breast tumors (AUC = 0.670, 0.636 and 0.589, respectively), compared to the Cluster 2 and 3 models. In predicting ER positive disease (n = 150), the model had 67% sensitivity, 67% specificity, 85% PPV, 43% NPV and 67% accuracy (AUC = 0.670, 95% CI 0.585–0.755). HER2 positivity (n = 50) in the tumor can be detected with moderate-to-high sensitivity (79%) and NPV (87%), and moderate-to-low specificity (48%), PPV (34%) and accuracy (56%) (AUC = 0.636, 95% CI 0.523–0.729). However, the Cluster model 1 was not a significant predictor for Ki-67 expression (n = 73) (<14% *vs.* >14%) in breast cancer, with low sensitivity (54%) and specificity (68%) (AUC = 0.589, 95% CI 0.486–0.692).

Cluster 1 had 74% sensitivity, 63% specificity and 94% NPV for distinguishing Luminal A tumors (n = 31) from other molecular subtypes (AUC = 0.658, 95% CI 0.556–0.759), whereas it was not a significant predictor for Luminal B (n = 119), TNBC (n = 40) or HER2+ (n = 18) molecular subtypes.

Prediction of Tumor Aggression

The Cluster 1 model showed the best performance in detecting late-stage, aggressive breast cancer (grade 2–3+T3-4+HER2+/Triple negative vs grade 1, T1–2, Luminal A or B) (AUC = 0.820 and 0.724 respectively). In detecting high grade, HER2 positive disease with lymph node metastases (grade 2–3+HER2+, and N1–3) it showed 78% sensitivity, 74% specificity, 94% NPV and 74% accuracy (AUC = 0.820 95% CI 0.728–0.913). In distinguishing high-grade TNBC with nodal metastases (biologically aggressive) from other subtypes, the Cluster 1 model had 100% sensitivity and NPV, with moderate-to-low specificity (42%), PPV (11%) and accuracy (46%) (Figure 4).

Table 1. Lesion and patient characteristics

		Frequency (n = 208)
Grade	1	37 (17.8%)
	2	77 (37%)
	3	94 (45.2%)
T-stage	T1	41 (19.7%)
	T2	97 (46.6%)
	T3	47 (22.6%)
	T4	23 (11.1%)
N-stage	N0	113 (54.3%)
	N1	65 (31.3%)
	N2	13 (6.3%)
	N3	17 (8.2%)
M-stagea	M0	174 (83.7%)
	M1	13 (6.3%)
Molecular subtype	HER2 positive	18 (8.7%)
	Luminal A	31 (14.9%)
	Luminal B	119 (57.2%)
	Triple negative	40 (19.2%)
Ki-67 status	Mean	42.2% (SD: 26.2%)
	0–15%	50 (24%)
	16–25%	26 (13%)
	26–35%	26 (13%)
	36–45%	21 (10%)
	>46%	85 (41%)
ER status	Negative	58 (27.9%)
	Positive	150 (72.1%)
HER2 status	Negative	158 (76%)
	Positive	50 (24%)
Patient race	Asian	7 (3.4%)
	Black	66 (31.7)
	Hispanic	110 (52.9%)
	Non-hispanic white	25 (12%)
Patient age	Mean	49.8 (SD: 10.8)
Upfront surgeryb	No	124 (59.6%)
	Yes	82 (39.4%)

HER2: Human epidermal growth factor receptor 2, ER: Estrogen receptor, PR: Progesterone receptor, SD: Standard deviation

Diagnostic performance of Cluster 1 model in predicting various prognostic parameters at the selected cut-off points is further summarized in Table 2.

Discussion and Conclusion

These results show that quantitative radiomic models can be helpful in excluding clinically aggressive disease and in predicting tumor stage and grade, which can potentially help with clinical management decisions. N-stage is one of the most important markers to be able to predict, as reliable pre-operative image-based prediction of N-stage can help to avoid SLNB, an invasive procedure. A positive nodal status will also change the management significantly, indicating an axillary lymph node dissection. An additional clinical scenario may be the use of this technology as a "tie-breaker" in the setting of high surgical risk (co-morbidities, age, etc.). Our model achieved 81% sensitivity and 95% NPV in predicting advanced nodal stage (N0–1 *vs.* N2–3) of breast cancer. Determining nodal status requires dedicated imaging and needle guided biopsy which incurs extra cost and procedure-related morbidity for the patient. Our model shows promise as a practical clinical decision support tool. Using our model, 67/95 (71%) of the

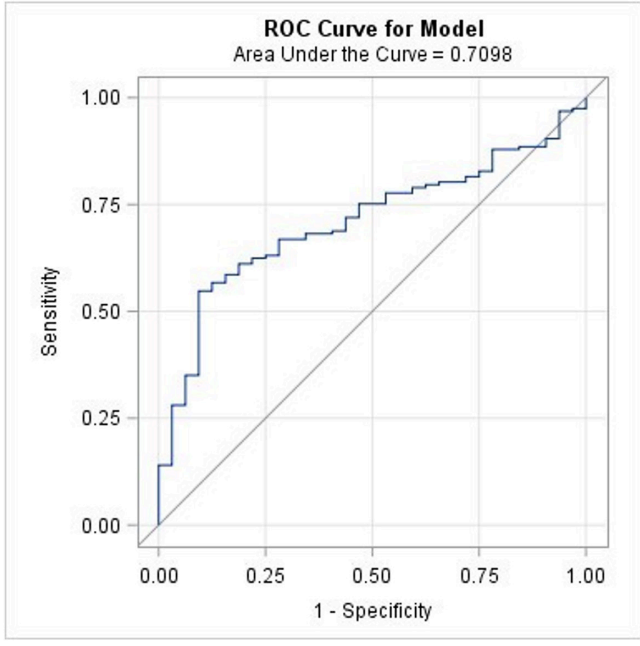

Figure 2. ROC (blue line) was performed for Cluster 1 model, and the figure shows the performance of our model in predicting tumor grade (grade 1 *vs.* 2–3). The area under curve is 0.7098 with $p<0.05$

ROC: Receiver operator curve

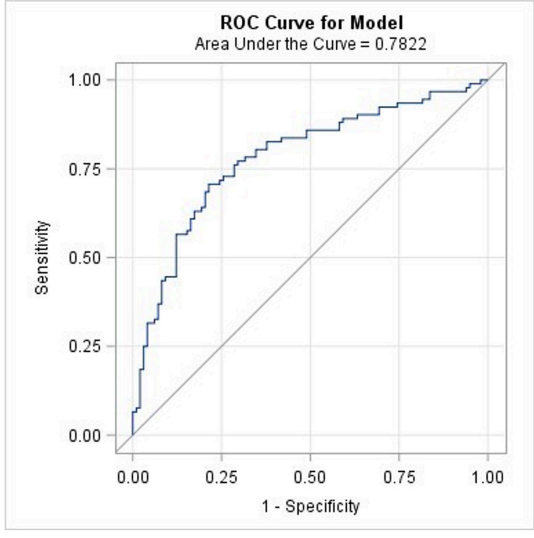

Figure 3. ROC (blue line) was performed for Cluster 1 model, and the figure shows the performance of our model in predicting nodal metastasis (N0 *vs.* N1–3). The area under curve is 0.7822 with $p<0.05$

ROC: Receiver operator curve

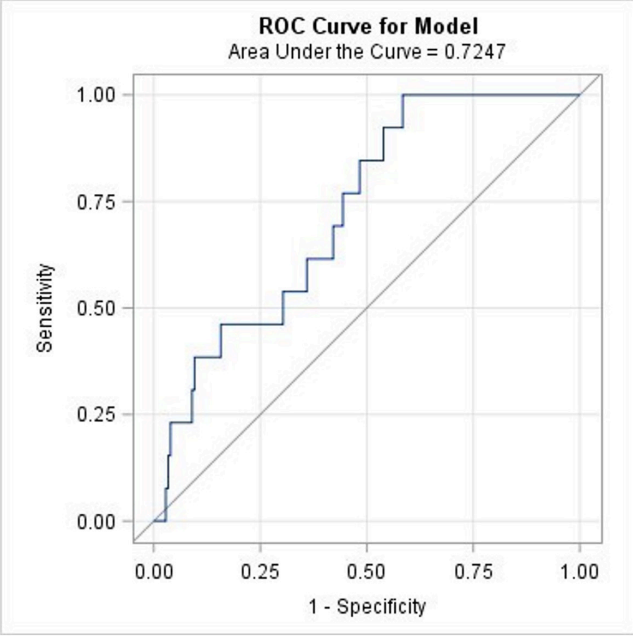

Figure 4. ROC (blue line) was performed for Cluster 1 model, and the figure shows the performance of our model in differentiating clinically aggressive tumor subtype (high-grade triple negative with lymph node metastasis) *vs.* other subtypes. The area under curve is 0.7247 with $p<0.05$

ROC: Receiver operator curve

clinically node-positive (N1–3) patients could be accurately diagnosed without the need for additional imaging or biopsy.

Clinically aggressive disease (grade 2–3+ Triple-negative+ N1–3) was detected with 100% sensitivity and 100% NPV with our model (AUC = 0.724). High sensitivity (78%), specificity (73%) and NPV (96%) were also achieved in predicting high-grade HER2+ breast cancer with nodal metastasis (grade 2–3+HER2+, and N1–3). Our results are promising and can be further developed as a reliable pre-operative decision support tool, which may help guide management decisions at initial diagnosis and throughout the treatment continuum.

Our model showed good performance in predicting T-stage (AUC = 0.789) and grade (AUC = 0.709) of breast tumors. For instance, high specificity and PPV (88% and 96% for grade and 90% and 75% for T-stage) were demonstrated in differentiating low (grade 1) vs high-grade (grade 2–3) breast cancers and T-stage. In combination with nodal stage, these results can further contribute to the preoperative management decision making, particularly chemotherapy versus upfront surgery.

Evaluation of molecular marker expression in breast cancer with MRI texture analysis may allow monitoring of changes in biomarker expression over time or after interventions, such as neoadjuvant chemotherapy, as well as resolving the problems related to tumor heterogeneity. Our model had moderate performance in detecting ER (AUC = 0.670), HER2 (AUC = 0.636) and Ki-67 (AUC = 0.589) expression in breast cancer and detecting molecular subtypes (AUC = 0.658 for luminal A) but was not a significant predictor for Luminal B, HER2+ or TNBC, and needs improvement on this aspect.

In the landscape of current research, several studies have yielded results akin to ours through the application of machine learning techniques across larger cohorts of patients (34). However, a common limitation among these studies is their exclusive focus on early-stage cancers, which narrows their applicability in the diverse spectrum of real-world clinical settings. Furthermore, while some research has successfully predicted the presence of specific biomarkers, such as HER2, or concentrated on singular molecular subtypes, like TNBC, these approaches do not fully encompass the complexity of breast cancer diagnosis and treatment (35, 36). In contrast, the present study is unique in presenting a model that mirrors the intricacies of actual clinical practice. It achieves this by incorporating a comprehensive range of molecular subtypes, spanning all cancer stages, and considering a wide array of significant biomarkers.

Table 2. AUC of Cluster 1 model in predicting tumor grade, stage and prognostic markers and sensitivity, specificity, NPV, PPV, accuracy of the model at optimal cut-off point

Parameter	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
Tumor grade (1 *vs.* 2–3)	0.709	56%	88%	96%	29%	61%
T stage (T1–2 *vs.* T3–4)	0.7896	58%	90%	75%	80%	79%
N stage (N0 *vs.* N1–3)	0.7822	71%	79%	76%	74%	75%
N stage (N0–1 *vs.* N2–3)	0.7399	81%	59%	24%	95%	62%
ER+ *vs.* ER-	0.6702	67%	67%	85%	43%	67%
HER2+ *vs.* HER2-	0.6362	79%	48%	34%	87%	56%
Ki-67<14% *vs.* >14%	0.5895	54%	68%	90%	22%	57%
Luminal A *vs.* others	0.6581	74%	63%	25%	94%	65%
Luminal B *vs.* others	0.463					
HER2+ *vs.* other	0.570					
TNBC *vs.* others	0.6005	42%	79%	30%	87%	73%
Aggressive disease (High-grade, node positive TNBC) *vs.* others	0.724	100%	42%	11%	100%	46%

DCE images were segmented and Haralick texture features were extracted, AUC: Area under receiver operating characteristic curve, HER2: Human epidermal growth factor receptor 2, ER: Estrogen receptor, PR: Progesterone receptor, TNBC: Triple negative breast cancer, PPV: Positive predictive value, NPV: Negative predictive value

This holistic approach not only enhances the model's relevance but also significantly broadens its utility in clinical decision-making, offering a more nuanced tool for healthcare professionals navigating the multifaceted landscape of breast cancer treatment. The retrospective design of the study was one of the limitations. Including MRI images from different vendors may improve the real-life application of the model. As a drawback, images were analyzed in 2D. Volumetric texture parameters with a 3D model may result in better performance. In addition to conventional statistical methods, such as cluster analysis, novel machine or deep learning can be used to train a model for further improvement. Including more demographic parameters, such as patient age and history, and radiologic parameters such as lesion size, may increase the sensitivity and performance of the model. The proposed cut-off was purely based on sensitivity and specificity without considering changes in prevalence in different population or analyzing cost to specific population or institutions as part of the clinical management process. More comprehensive decision analysis into operating this model would take account of the cost (either financially or in terms of population level welfare such as quality adjusted life-year) is necessary.

In addition, the most common indications for breast MRI are suspected multifocal/centric disease, size discrepancy between clinical exam and imaging, or between mammography and ultrasound; ER-negative disease or larger ER+ cancers with anticipated pre-operative systemic therapy, suspected anterior chest wall/nipple involvement and cancers identified in high-risk screening populations at supplemental screening. Due to these indications, there is a possibility that the cancers reported in our series are biased toward advanced disease or those patients who are likely to get neoadjuvant therapy. While we acknowledge this bias, we believe our series is representative of cancers imaged with MRI nationally, and hence from whom texture features can be extracted.

Our findings support earlier studies, which have reported correlation between breast cancer TNM stage and MRI imaging characteristics with similar ROC values and with the advantage of larger patient samples. The results of the present study indicate that whole tumor MRI texture analysis shows promise as a potential tool that can be integrated into clinical decision-making, in conjunction with histopathological markers, to distinguish low risk disease with high NPV.

Ethics Committee Approval: The protocol was approved by the Ethics Committee of University of Texas Southwestern Medical Center. IRB number is STU 092016-006 (date: 05.09.2023).

Informed Consent: The need for informed consents are waived per IRB #STU 092016-006.

Authorship Contributions

Concept: D.S.P., Y.X., K.H., M.L., B.E.D.; Design: D.S.P., Y.X., K.H., M.L., B.E.D.; Data Collection and/or Processing: D.S.P., Y.X., K.H., M.L., B.E.D.; Analysis and/or Interpretation: D.S.P., Y.X., K.H., M.L., B.E.D.; Literature Search: D.S.P., Y.X., K.H., M.L., B.E.D.; Writing: D.S.P., Y.X., K.H., M.L., B.E.D.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Article

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Evaluation of Breast Health Promotion Intervention Among Catholic Nuns in Lake Zone 'Tanzania

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ABSTRACT

Objective: Despite facing unique barriers, Catholic nuns in Tanzania require accessible breast health promotion. This study explores interventions to empower nuns through knowledge, improved attitudes, and positive practices, ultimately promoting well-being and early detection for better breast cancer outcomes.

Materials and Methods: A quasi-experimental design study guided by the Health Belief Model was conducted to monitor the implementation of a breast health intervention program aimed at increasing breast cancer screening knowledge among 385 Catholic nuns aged 20 to over 60 years old within Lake Zone, Tanzania. Data were collected at two-time points: pre-intervention (baseline) and implementation phase intervention (after three months). The intervention consisted of a 2-hour educational session. Participants had opportunities to ask questions and provide feedback.

Results: The breast health promotion intervention was well-received by Catholic nuns, with 339 (88%) expressing strong motivation to learn and promote awareness. The training effectively increased knowledge and positive attitudes towards breast cancer screening. Researcher assistants successfully delivered the program, and 354 (92%) of participants expressed interest in continued education and support. The intervention addressed cultural barriers and empowered nuns to take charge of their health, though some challenges remain meanwhile 158 (41%) had limited prior knowledge, 81 (21%) hesitated to discuss breast health due to religious beliefs, and some faced difficulty applying the learnings.

Conclusion: Overall, the breast health promotion intervention had a positive outcome on the Catholic nuns' awareness and knowledge of breast health. However, addressing the identified barriers and challenges is crucial to further enhance the intervention's effectiveness and sustainability.

Keywords: Breast cancer; breast cancer screening; Catholic nuns; knowledge; attitudes; breast health promotion intervention

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Key Points

- Breast cancer
- Breast cancer screening
- Catholic nuns
- Knowledge
- Attitudes
- Breast health promotion intervention

Introduction

Despite substantial progress in medical treatments, breast cancer (BC) continues to be a major cause of cancer-related mortality among women (1). Like any other woman, Catholic nuns face a risk of developing BC, and early detection and treatment can significantly enhance survival rates (2). The previous studies focused on knowledge, beliefs, and attitudes towards breast health promotion and factors associated with BC screening practice among Catholic nuns in Lake Zone, Tanzania. These studies identified gaps in Catholic nuns' knowledge, beliefs, and attitudes toward breast health promotion and

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BC screening practices. These studies also revealed nuns' low perceived susceptibility, low seriousness and inability to perceive the benefit of breast self-examination (BSE). In response to these findings, we implemented a breast health intervention designed to address these gaps and improve nuns' uptake of BC screening practices.

Breast health promotion interventions, grounded in the Health Beliefs Model (HBM), effectively address individuals' perceptions of health threats like BC (susceptibility, severity), the benefits of prevention, and factors influencing action (barriers, cues to action, self-efficacy) (3). These interventions align with HBM principles and target identified gaps by increasing knowledge, reducing barriers, and promoting positive attitudes and behaviors (4). The HBM proved to be a valuable tool for implementing breast health promotion intervention among Catholic nuns. The model helped us to understand the nuns' beliefs and motivations, and it provided a framework for designing an intervention that was effective in increasing BC screening rates and improving overall breast health (5).

However, evaluating breast health interventions, including the training program mechanism, is crucial to identify potential barriers and areas for improvement (6). Process evaluation assesses implementation, pinpoints factors affecting intervention success, and uncovers potential implementation barriers (7). Breast health promotion interventions empower Catholic nuns to safeguard their health and reduce BC risk by providing education, support, and resources (8).

Breast health promotion interventions, include BSE, clinical breast examination (CBE), and mammography (9). All of these have contributed to reduced mortality and improved survival rates by facilitating early detection of BC (7). Numerous studies on BC screening (BCS) performance have been conducted among women worldwide, including in sub-Saharan Africa (10). However, only a limited number of studies have focused on BCS practices among Catholic nuns (11). Likewise, several studies have been conducted in Tanzania to assess the effectiveness of breast health interventions in improving BC awareness and screening practices. These studies have found that interventions tailored to the specific needs of Tanzanian women and incorporating cultural sensitivity can be effective in increasing BSE practice and uptake of CBEs (12). Also, studies have shown that breast health interventions can be effective in increasing BSE practice and uptake of CBEs (10). Notably, interventions focusing on BCS are less common among Catholic nuns in general and absent among Tanzanian Catholic nuns in particular.

Therefore, implementing this breast health intervention program among Catholic nuns could be an effective means to reduce BC mortality rates within this group and transmit the knowledge and skills to other congregations. Furthermore, the current study intends to identify the potentials and barriers involved during the training of Catholic nuns within the Lake Zone in Tanzania on breast health promotion. This intervention provides them with knowledge and enhances their beliefs about BC and BCS practice.

Materials and Methods

A quasi-experimental design study guided by the HBM was conducted to monitor the breast health intervention program during implementation aimed at increasing BC screening knowledge among Catholic nuns. Data were collected at two time points: pre-intervention (baseline) and implementation phase intervention (after three months). The discussions were guided by a semi-structured interview guide that explored the nuns' experiences with the breast health intervention program, their perceptions of its strengths and weaknesses, and their suggestions for improvement regarding the intervention. The 2-hour educational session, delivered by an Assistant researcher to the participants, was designed to increase knowledge about BC and reduce the factors associated with BC practice. Interventions were conducted in a safe and supportive environment, with informed consent obtained and confidentiality assured. Participants had opportunities to ask questions and provide feedback.

Inclusion Criteria

Catholic nuns aged between 20 and above years from Lake Zone Congregations, also, include those who participated in the intervention phase, who had no previous cancer diagnosis, and willing to participate in the study. All study participants provided written consent after receiving detailed information about the breast health program intervention.

The Intervention

The intervention was introduced as an educational intervention on BC prevention and BC screening. This intervention was grounded in the HBM and developed based on the American Cancer Society (13), and the International Agency for Research on Cancer. This intervention was prepared and designed to bridge the gap in BC knowledge and to modify beliefs related to BC (14). Table 1 gives an outline of the educational intervention on BCS along with the application of the HBM concepts in the educational intervention.

The educational intervention consisted of four units. Unit one provides general information on the anatomy and physiology of a normal breast so that the participants have a clear understanding of the topic. Unit two provides information and knowledge about BC. It further explores BC symptoms, BC stages, and BC risk factors to increase the participants' knowledge of BC. We addressed the susceptibility of BC and the importance of early detection of the disease. Unit three explain two different methods of BCS (clinical breast exam and mammography). The effectiveness of mammography and CBE in early detection, the safety of the mammography procedure (radiation exposure), unnecessary concerns of discomfort about the procedures, and the availability of breast screening procedures to encourage participants to adopt and practice these approaches. Unit Four explains the BSE procedure to raise the participants' awareness of BC symptoms and motivate them to follow this procedure.

Text in printed materials was brief and easy to understand, with a large and clear typeface. The graphics in the materials were realistic and reflected the lives of Catholic nuns. Considering a possibly low reading ability of study subjects. The printed educational materials were designed to be bright and luminous.

Pretest

A pretest breast health intervention was carried out involving 50 participants in the same convent where the questionnaire was pretested. The face and content validity of the educational materials were approved by four professional expert panelists. The educational intervention consisted of a two-hour session delivered to groups of Catholic nuns by a trained assistant researcher. The intervention included a PowerPoint presentation, a short video about BSE, and a training session on BSE practice on a silicone breast model. At the

Table 1. Tool for breast health intervention

Sessions	Topics	HBM constructs	Area of target	Intervention/educational delivery materials
The normal breast	- Structure of the breast - Physiology of breast development	Perceived susceptibility	Awareness/knowledge of breast cancer	- PowerPoint presentation - Booklet
Knowledge of breast cancer	- What is breast cancer - Symptoms of breast cancer	Perceived susceptibility	Knowledge/awareness of breast cancer	- PowerPoint presentation,
	Breast cancer stages Breast cancer risk factors	- Perceived severity		leaflets
Breast cancer screening	- Clinical breast examination - Mammography Breast self-examination	- Perceived benefits - Perceived barriers - Health motivation (Cue to action	Knowledge, attitudes, and beliefs on acceptability and uptake of breast cancer screening	- PowerPoint presentation - Booklet - BC logo sticker - Short reminder
Breast health awareness	Breast health awareness - Acceptability for BSE performance	Perceived benefits Perceived barriers. perceived Cue to action Perceived Self efficacy	- Knowledge and beliefs on breast health awareness - Practice of BSE	- PowerPoint presentation - BSE film - Booklet - CD BC logo sticker short reminder SMS
Breast cancer screening uptake practice	- Practice of BSE	- Perceived benefits Perceived barriers - Perceived Cue to action Perceived Self-efficacy	-Practice of BSE, Clinical breast examination, and mammography	BSE practice on a silicon model - BSE film - Booklet CD - BC logo sticker - Short reminder SMS

BSE: Breast self-examination; HBM: Health Belief Model

end of the intervention, participants were given a copy of a booklet containing all the information covered in the intervention and a BC logo sticker.

Intervention Fidelity

To ensure the consistent and effective delivery of the educational intervention, standardized training and certification for intervention facilitators were implemented. Intervention manuals and checklists were utilized to guarantee adherence to the intended intervention and coverage of all key components. Regular monitoring and feedback sessions, along with ongoing observation and evaluation of facilitators' performance, enabled the identification and correction of any deviations from the intended intervention delivery. These strategies collectively aimed to promote participant adherence and engagement, thereby maximizing the intervention's effectiveness.

Intervention Implementation

The breast health promotion intervention was implemented three months after the baseline survey. To ensure consistent and faithful delivery of the intervention, the assistant researcher adhered to standardized educational intervention protocols across all participating groups. The assistant researcher diligently followed these protocols to ensure uniformity in intervention delivery. Participants were encouraged to ask questions and receive comprehensive answers during the training sessions. They were also given ample opportunities to practice their newly acquired skills and apply the intervention techniques in simulated scenarios. The researcher, acting as an observer throughout the sessions, provided participants with immediate and constructive feedback.

At the end of the training, important points were repeated in order to improve learning, and the brochures on breast health and psychological adjustment were given to the participants so that they could review them later. To evaluate the quality of the intervention execution, the principal investigator watched the Assistant researcher performance during the intervention and completed an evaluation form at the end of the intervention. BSE training was planned and rehearsed by the Assistant researcher by using a role-playing technique with the participants. Other possible means of enhancing intervention fidelity include the monthly short reminder text messages and the use of the BC logo stickers. At the end of sessions, a short test was given to the session participants to ensure that they had learned what had been taught in the sessions.

Table 2. Participants' socio-demographic characteristics

Respondents' age (n = 385)	Frequency	Percentage
20–30	89	23.1
31–40	55	14.3
41–50	101	26.2
51–60	69	17.9
Above 60	71	18.9
Education level		
Primary	76	19.7
Secondary	154	40.0
Degree	139	36.1
Master/Ph.D.	13	3.4
Others	3	0.8
Occupation		
Primary teacher	11	2.9
Secondary teacher	122	31.7
Pastoral religious	50	13.0
Cooker	34	8.8
Spiritual counselor	28	7.3
Nurse	40	10.4
Doctor	19	4.9
Clinical officer	38	9.9
Others like accountants, secretaries	43	11.2
Working period (years)		
01–10	100	26
10–15	67	17.4
15–20	81	21
Above 20	137	35.6
Source of information about breast cancer and breast cancer screening		
Family members	44	11.4
Sisters within congregation	125	32.5
Health workers	92	23.9
TV media	11	28.8
Others like internet	13	3.4

Statistical Analysis

The implementation of the breast health intervention was evaluated using a checklist tool and a structured observation method. The checklist tool was used to assess whether the intervention was conducted as planned, and the structured observation method was used to assess the participants' level of engagement and the competence of the Assistant Research. Both methods were guided by the HBM, and the responses were classified under the respective categories and sub-categories of the HBM.

Ethical Consideration

Ethical approval was granted by the joint Catholic University of Health and Allied Sciences/Bugando Medical Centre Review Board (approval number: CREC/552/2022; date: 12.05.2022). All participants signed a written informed consent before participating in the study. Participants were assured that their participation in the study training is voluntary, and they have the full right to withdraw from the study training at any time they feel to do so without.

Results

Socio-Demographic Characteristics

As summarized in Table 2, a total number of 385 nuns participated in this study, with an age range of 20 to 60 years, and a mean age of 45.8±15.4 years. The leading age group was 41–50 years old which constituted a total of 101 (26.2%) participants. A total number of 154 (40%) participants had secondary education, 122 (31.7%) of the participants were secondary school teachers, and 50 (13.0%) participants had a pastoral religious qualification. A total number of 125 (32.5%) Catholic nuns obtained breast health information from fellow nuns, and only 13 (3.4%) of the participants obtained the information from the Internet.

Breast Health Promoting Intervention Among Catholic Nuns

The breast health promotion intervention was positively received by Catholic nuns, with a majority of 339 (88%) participants expressing high motivation to learn and promote breast health awareness among their peers. During process evaluations, the research assistant exhibited strong performance in delivering the interventions, engaging over 342 (89%) of participants. More than 354 (92%) of the study participants seemed interested in education on breast health and emotional adjustment, respectively. Notably, 354 (92%) of participants expressed interest in breast health education and emotional adjustment support. Findings revealed increased awareness of BC risk factors, symptoms, and screening procedures. The training program, led by knowledgeable facilitators, effectively enhanced participants' knowledge and attitudes towards BCS. The assistant researcher performed very well on the interventions for more than 342 (89%) of the sessions.

While the intervention successfully addressed cultural barriers and empowered nuns to take charge of their breast health, certain challenges were identified. Approximately 157 (41%) of participants had limited prior knowledge about breast health, while 81 (21%) of participants hesitated to openly discuss breast health due to religious beliefs and cultural practices, and some participants faced difficulties applying the training program's lessons to their own lives and the lives of the nuns they served.

The HBM-based breast health intervention effectively influenced the nuns' perception of susceptibility to BC. The intervention provided information about the risk factors for BC and its prevalence among Catholic nuns, enhancing the nuns' understanding of their vulnerability to the disease and enhanced the nuns' belief in the seriousness of BC. By providing information about the consequences of BC and the importance of early detection, the intervention helped the nuns grasp the severity of the disease and the urgency of timely action. Furthermore, the intervention promoted the nuns' perception of the benefits of BC screening. By dispelling myths surrounding BC screening and providing information about its advantages, the

intervention helped the nuns recognize the value of screening in early detection and prevention. Lastly, the intervention bolstered the nuns' self-efficacy for BC screening. By providing practical information on accessing screening services and assisting the nuns in overcoming barriers to screening, the intervention empowered them to take charge of their breast health.

The checklist tool showed that the intervention was conducted as planned. All of the intervention components were delivered as planned, and the intervention materials were of high quality. The structured observation method showed that the participants were actively engaged in the training. They asked questions, participated in discussions, and completed all of the activities. The Assistant Research was also competent and knowledgeable. They delivered the intervention material effectively and answered all of the participants' questions.

Discussion and Conclusion

The health education intervention program utilized a behavioral change model and integrated teaching principles tailored to the learning preferences of Catholic nuns. This approach demonstrated remarkable effectiveness in increasing the participants' awareness of breast health issues and diminishing their hesitancy towards practicing BSE. These positive outcomes resonate with those observed in previous studies, revealed that health behavior change education training effectively enhanced the knowledge, proficiency, and frequency of BSE performance among educated women (15).

Another study conducted by Parashar et al. (16) revealed that a training program effectively enhanced the participants' knowledge of breast health and BSE methods, and it also encouraged them to practice BSE more frequently. Also a similar study done in Saudi Arabia by Yakout et al. (17). The outcomes of the study indicated that the training program successfully increased the participants' understanding of breast health and BSE procedures, and it also promoted more regular practice of BSE. These studies provide strong evidence that breast health intervention programs can be effective in promoting BSE among women including Catholic nuns. The findings of these studies are consistent with the findings of the current study, which found that the training program was well-designed and delivered by knowledgeable and experienced facilitators.

This study underscores the importance of culturally sensitive interventions tailored to specific populations, particularly underserved communities like Catholic nuns. By addressing cultural beliefs and practices, the intervention effectively overcame barriers and empowered nuns to take control of their breast health. This study bears similarities to research conducted among African American women by Rivers et al. (18). Their findings revealed that the program significantly enhanced the participants' knowledge of BC, alleviated their fears associated with the disease, and increased their intention to undergo mammograms. Another study conducted among Hispanic women by Livaudais et al. (19) yielded similar results. The findings demonstrated that the program effectively enhanced the participants' knowledge of BC, reduced their fear of BC, and increased their intention to undergo mammograms. These studies provide strong evidence that culturally sensitive interventions tailored to specific populations can be effective in promoting BC awareness and screening. The findings of these studies are consistent with the findings of the current study, which highlights the significance of culturally sensitive interventions tailored to underserved communities like Catholic nuns. By addressing cultural beliefs and practices, the intervention was able to overcome barriers and empower nuns to take charge of their breast health.

Our study and previous research have identified several challenges to breast health intervention and awareness among women in various cultural settings. In the current study, we found that some Catholic nuns faced challenges including limited prior knowledge about breast health, hesitation to discuss breast health openly due to religious beliefs and cultural practices, and difficulty applying what was learned in the training program to their own lives and the lives of the nuns they served. Additionally, the training program was time-intensive and required the nuns to travel to a central location of similar challenge have been reported in other studies conducted in different cultural settings. For instance, Saeed et al. (20) found that Arabian women faced similar challenges to those in the current study, including limited prior knowledge about breast health, hesitation to discuss breast health openly due to cultural practices, and difficulty applying what was learned in the training program to daily life. Additionally Kissal and Beşer (21) found that Turkish women faced challenges including limited prior knowledge about breast health, cultural taboos surrounding BC, and difficulty accessing breast health care services in rural areas. Collectively, these findings highlight the need for culturally sensitive and context-specific breast health education programs that address the unique needs and challenges of women in different cultural settings. By understanding and addressing these barriers, we can promote better breast health awareness and outcomes among women worldwide.

A similar study done in low-income countries including South Asia by Saini et al. (22) revealed that an HBM-based breast health intervention was effective in increasing BC screening rates among low-income women. The intervention provided information about the risk factors for BC, the benefits of screening, and how to get screened. Another study done in Guilan, Iran by Eghbal et al. (23) found that an HBM-based breast health intervention was effective in increasing BC screening rates among Hispanic women. The intervention provided information about the risk factors for BC, the benefits of screening, and how to get screened in Spanish. Also, a study done in China by Zhang et al. (24) found that an HBM-based breast health intervention was not effective in increasing BC screening rates among Chinese women. The authors of the study suggest that this may be due to a lack of access to screening services. The findings of the similarity studies suggest that HBM-based breast health interventions can be effective in increasing BC screening rates among underserved populations. However, the findings of the different studies suggest that there may be cultural and religious factors that can affect the effectiveness of these interventions.

Study Limitations

Our study had some limitations. The study lacked a control group, making it difficult to determine whether the observed changes in breast health awareness and practices were solely attributed to the interventions or other factors. Additionally, the sample was drawn from congregations within Lake Zone, limiting the generalizability of the findings to all Catholic nuns in Tanzania. Finally, the study did not assess the long-term impact of the interventions on breast health outcomes. Despite these limitations, we believe that our study provides valuable insights into the potential benefits of breast health interventions for Catholic nuns and increased awareness of BC risk factors, symptoms, and screening procedure. Future research should also assess the long-term impact of the interventions on breast health

outcomes. The findings can be used to inform the development of more effective and culturally appropriate breast health interventions for this population.

This study highlighted the effectiveness of culturally sensitive breast health promotion interventions tailored to specific populations, particularly underserved communities like Catholic nuns. The intervention effectively improved knowledge, attitudes, and perceptions related to BCS, demonstrating its potential to promote positive breast health behaviors among Catholic nuns. The HBM-based approach effectively addressed the nuns' perceptions of susceptibility, seriousness, benefits, and self-efficacy regarding BCS, empowering them to take charge of their breast health.

Ethics Committee Approval: Ethical approval was granted by the joint Catholic University of Health and Allied Sciences/Bugando Medical Centre Review Board (approval number: CREC/552/2022; date: 12.05.2022).

Informed Consent: All participants signed a written informed consent before participating in the study.

Authorship Contributions

Surgical and Medical Practices: G.M., K.M., R.L.; Concept: G.M., K.M., R.L.; Design: G.M., K.M., R.L.; Data Collection and/or Processing: G.M., J.M.; Analysis and/or Interpretation: G.M., K.M., R.L., J.M., P.A.S., P.R.; Literature Search: G.M.; Writing: G.M., K.M., R.L., J.M., P.A.S., P.R.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Article

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Need for Staging Investigations in Newly Diagnosed Breast Cancer: Establishing Local Guidelines for Radiological Staging in Bahrain

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ABSTRACT

Objective: Staging workup and detection of distant metastases is important in newly diagnosed breast cancer in order to make treatment decisions and establish the prognosis. There is wide variation in current recommendations for staging investigations in breast cancer. Routine staging is performed for all patients in Bahrain because of lack of consistent guidelines. Optimization of the criteria for staging is important for identification of metastases, while minimizing harm and costs. The aim of this study was to evaluate factors associated with distant metastases in newly diagnosed patients with breast cancer, in order to establish local guidelines for proper selection of patients for systemic staging.

Materials and Methods: Patients with newly diagnosed breast cancer at Salmaniya Medical Complex in Bahrain who underwent staging investigations between January 2016 and December 2022 were identified from a pathology database. Patients with previous history of cancer, synchronous tumors, bilateral breast cancer and ductal carcinoma *in situ* were excluded. Clinical, radiological and pathological data were retrospectively analyzed.

Results: A total of 593 patients underwent staging computed tomography and bone scans or a PET scan. Distant metastases were identified in 20.7% of cases. M1 disease was significantly associated with multifocality/multicentricity, high grade tumors, hormone receptor-negative cancers, high Ki67 index, advanced tumor stage, node-positive disease, triple-negative breast cancer, use of PET scans and those who underwent neoadjuvant chemotherapy. Age was not associated with identification of distant metastases.

Conclusion: The prevalence of distant metastases in this population of newly diagnosed patients with breast cancer was higher than previously reported. Routine staging of all patients at presentation was not indicated, especially for asymptomatic patients with early breast cancer. This study identified certain groups of patients with a higher risk of distant metastasis, in whom metastatic workup should be performed. These findings may allow for the development of a local guideline that addresses the question of which breast cancer patients need staging investigations for distant metastases.

Keywords: Body image; breast cancer; breast carcinoma; early breast cancer; metastasis

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Key Points

- Identification of distant metastases in breast cancer is important for decision-making when considering treatment options and establishing the prognosis.
- Routine baseline imaging of all patients with breast cancer at presentation is not indicated, especially asymptomatic cases with early breast cancer.
- Metastatic workup in patients with locally advanced breast cancer and those with symptoms of metastases is appropriate, with consideration to be given to those with abnormal axillary lymph nodes, aggressive molecular subtypes and before starting neoadjuvant chemotherapy.
- This study allows for the development of a local guideline for staging investigations of patients with newly diagnosed breast cancer.

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Introduction

Breast cancer is the second most common cancer worldwide and the most frequent among females. In Bahrain, it accounts for 37.2% of all female cancers and 20% of all new cancer cases, which is considered the highest in all Gulf Cooperation Council countries and among the highest in the world (1). Staging and identification of distant metastases in breast cancer is important, both in decision-making when considering treatment options and establishing the prognosis. The presence of metastatic disease at the time of breast cancer diagnosis is very low, with a reported incidence of 4% (2).

Many guidelines advise against baseline imaging of asymptomatic patients with early breast cancer, however, these recommendations are quite disparate and lack consistency (3). For example, the National Comprehensive Cancer Network (NCCN) guidelines advocate the use of imaging only in patients with signs and symptoms suggestive of distant metastases, locally advanced disease, significant axillary nodal burden or workup prior to neoadjuvant systemic therapy (4). Other European guidelines include T3 disease and tumors with aggressive biology as justification for staging investigations, due to higher prevalence of occult distant metastases in these patients (5).

Despite there being no clear evidence to support routine universal staging in all patients, many patients undergo extensive imaging at the time of diagnosis (6). In Bahrain, all patients newly diagnosed with breast cancer are screened for distant metastases using computed tomography (CT) and bone scans or positron emission tomography (PET) scans. However, overuse of staging investigations can lead to inappropriate use of resources, increased healthcare costs, patient anxiety and psychological distress and delay in treatment (7). Nevertheless, the failure to identify distant metastases during initial workup may also lead to increased morbidity including unnecessary treatment, such as inappropriate breast surgery, radiation therapy and systemic treatment (3). The aim of this study was to assess the necessity for staging imaging investigations by evaluating factors predictive of distant metastases at presentation, in order to establish local guidelines and identify appropriate patients for systemic staging in breast cancer in Bahrain.

Materials and Methods

The study protocol was approved by the Research Ethics Committee of Government Hospitals Bahrain (approval no: 129261223; date: 26.12.2022). All female patients who were newly diagnosed with breast cancer and underwent staging investigations at Salmaniya Medical Complex between January 2016 and December 2022 were included and retrospectively reviewed. Patients with a previous history of extramammary malignancy, synchronous cancers, bilateral breast cancer, ductal carcinoma *in situ*, male patients and those treated for local recurrence were excluded. The following data were collected from electronic medical records of the clinical notes, radiology and pathology reports: age at diagnosis, tumor laterality, tumor type, histological grade, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor 2 (HER2) receptor status, Ki67 index, lymphovascular invasion (LVI), history of neoadjuvant therapy, tumor size, T-stage, nodal status, presence of distant metastases, site of metastasis and type of imaging modalities used for detection of metastases.

All patients with breast cancer were evaluated by triple assessment. All patients were investigated with breast ultrasound, mammogram and tru-cut biopsy. For the T-stage, the largest tumor size (on radiological imaging or after surgical excision) was considered for the analysis. On axillary ultrasonography, if abnormal or suspicious lymph nodes were identified, standard practice was to perform ultrasound-guided biopsy of the nodes. Biopsy- proven metastatic lymph nodes or patients with positive sentinel lymph node biopsy (SLNB) were considered node-positive (N+), whereas benign appearing or absence of suspicious lymph nodes on imaging and negative SLNB were considered node-negative (N0). For patients who underwent axillary dissection, the pathological nodal status was considered for the analysis. For patients who underwent neoadjuvant therapy, the most advanced T and N stages (clinical or pathological) were used for the analysis.

The imaging modalities that were used for staging included CT and bone scan or a PET scan. Results of staging investigations were classified as: no distant metastases (M0), presence of distant metastases (M1) or indeterminate findings (Mx). Patients with indeterminate lesions underwent a follow-up CT scan within three months or further investigations, such as magnetic resonance imaging (MRI) or PET scans. Patients with indeterminate lung or liver nodules that were later found to be unchanged on follow-up CT or not metastatic on MRI or PET scans were classified as M0, whereas those indeterminate features later proven to be distant metastases were labelled as M1. Staging was determined according to the eighth edition of the tumor, node, metastasis system of the American Joint Committee on Cancer staging manual (8).

Statistical Analysis

Descriptive analyses were used to summarize the data and evaluate rates of distant metastases. The chi-square or Fisher's exact test was performed to test for an association between clinicopathological characteristics and the presence of distant metastases. A $p<0.05$ was considered statistically significant. Statistical analyses were performed using the Statistical Package for the Social Sciences, version 29.0 (SPSS, IBM Corp, Chicago, IL, USA).

Results

In total, 593 patients with newly-diagnosed invasive breast cancer who fulfilled the inclusion criteria were identified and retrospectively reviewed. Clinicopathological characteristics of the study population are summarized in Table 1. The mean age at diagnosis was 54 (range 23–94) years. Just over half of patients had left- sided breast cancer (51.1%). Most had unifocal disease (78.6%) and only 9.84% of patients had multicentric disease. Invasive ductal carcinoma was the most common histological tumor subtype (84.3%). Most tumors were reported as grade 2 (54.1%). The majority of patients had T2 tumors (46.7%).

LVI was present in 22.1%. Of the cohort, the majority of cases were node-positive (59.2%). Most cases were ER and PR positive (78.6% and 68.6%, respectively). Moreover, 29.2% of tumors were found to be HER2 positive and 62.3% of patients had a Ki67 index >20%. Of the molecular subtypes, the most common tumor biology was Luminal B breast cancer (62.1%). Out of all the patients, 33.6% underwent neoadjuvant systemic therapy.

CT scan of the chest, abdomen, and pelvis along with a bone scan were the imaging modalities of choice (77.1%) for staging, compared to 22.9% of patients who underwent a PET scan. Distant metastases were detected in 20.7% of all patients, with bones being the most frequent site of metastases (47.9%). When univariate and multivariate analysis were performed, the following variables were identified as

predictors for distant metastases: multifocality/multicentricity, high grade tumors (grade 2–3), ER/PR-negative tumors, Ki67 index >20%, neoadjuvant therapy, advanced T stage (T3/T4 tumors), node-positive tumors, triple-negative breast cancer and use of PET scans (Table 2). Although metastatic disease tended to be more frequent in younger (<50 years) individuals and patients with HER2-positive tumors, these findings were not significant.

Discussion and Conclusion

It is important to optimize the criteria for radiological staging in breast cancer in order to identify distant metastases, while avoiding potential harm, such as unnecessary radiation exposure, patient anxiety, false-positives, increased healthcare costs and delay in starting treatment (5). Although guidelines do exist, which address the question of staging investigations for distant metastases, breast cancer patients in Bahrain undergo intensive staging by radiological imaging modalities. One study found that most patients with early breast cancer would prefer having staging imaging investigations, even though this is against the current guidelines (9). This, as well as the demand by our local oncologists and tumor board for comprehensive staging, may explain why all patients in Bahrain undergo routine metastatic workup for newly diagnosed breast cancer.

Table 1. Clinicopathological characteristics of the study population

Age		**ER status**	
Mean	54	Positive	466 (78.6%)
Median	53	Negative	127 (21.4%)
Range	23-94		
Tumour laterality		**PR status**	
Right breast	290 (48.9%)	Positive	407 (68.6%)
Left breast	303 (51.1%)	Negative	186 (31.4%)
Disease focality		**HER2 status**	
Unifocal	466 (78.6%)	Positive	173 (29.2%)
Multifocal	69 (11.6%)	Negative	420 (70.8%)
Multicentric	58 (9.8%)		
Tumour type		**Ki67 index**	
Invasive ductal carcinoma	500 (84.3%)	≤20%	224 (37.8%)
Invasive lobular carcinoma	61 (10.3%)	>20%	369 (62.2%)
Other	32 (5.4%)		
Tumour grade		**Imaging modality**	
Grade 1	116 (19.6%)	CT and bone scan	457 (77.1%)
Grade 2	321 (54.1%)	PET scan	136 (22.9%)
Grade 3	156 (26.3%)		
Tumour stage		**Molecular subtype**	
T1	133 (22.4%)	Luminal A	108 (18.2%)
T2	277 (46.7%)	Luminal B	368 (62.1%)
T3	118 (19.9%)	HER2-enriched	64 (10.8%)
T4	65 (11.0%)	Basal-like	53 (8.9%)
Lymph node status		**Site of metastasis**	
N0	242 (40.8%)	Bone	59 (48.0%)
N1	247 (41.7%)	Lung	15 (12.2%)
N2	73 (12.3%)	Liver	11 (8.9%)
N3	31 (5.2%)	Multiple sites	38 (30.9%)
LVI		**Neoadjuvant therapy**	
Yes	131 (22.1%)	Yes	199 (33.6%)
No	462 (77.9%)	No	394 (66.4%)

ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor 2; CT: Computed tomography; PET: Positron emission tomography

Table 2. Association of clinicopathological risk factors and distant metastases

Age	Distant metastases	*p*-value (univariate)	*p*-value (multivariate)
≤50 years	24.3%	0.116	
>50 years	18.6%		
Disease focality			
Unifocal	18.1%	**0.023**	**0.005**
Multifocal/ multicentric	33.9%		
Tumour type			
Ductal	21.6%		
Lobular	21.3%	0.115	
Other	6.25%		
Tumour grade			
Grade 1	7.76%		
Grade 2	24.1%	**<0.001**	**<0.001**
Grade 3	22.7%		
Tumour stage			
T1	1.78%		
T2	8.72%	**<0.001**	**<0.001**
T3	30.7%		
T4	63.5%		
Lymph node status			
N0	8.94%	**0.031**	**0.027**
N+	28.1%		
LVI			
Present	21.5%	0.807	
Absent	20.4%		
ER status			
Positive	18.4%	**0.013**	**0.001**
Negative	29.3%		
PR status			
Positive	17.9%	**0.016**	**0.010**
Negative	26.8%		
HER2 status			
Positive	23.2%	0.371	
Negative	19.6%		
Ki67 index			
≤20%	15.2%	**0.006**	**0.003**
>20%	24.2%		
Imaging modality			
CT and bone scan	17.7%	**0.002**	**<0.001**
PET scan	30.8%		
Molecular subtype			
Luminal A	11.1%		
Luminal B	20.9%	**0.009**	**<0.001**
HER2-enriched	26.5%		
Basal-like	32.1%		
Neoadjuvant therapy			
Yes	28.3%	**0.002**	**<0.001**
No	17.2%		

ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor 2; LVI: Lymphovascular invasion; CT: Computed tomography; PET: Positron emission tomography

Our results suggest that the prevalence of occult metastases (20.7%) is approximately three times higher than in previous studies, as the overall prevalence of metastatic disease in newly diagnosed breast cancer is reported to be around 7% in other populations (10). In terms of tumor stage, our findings indicate that the rate of distant metastases T1-T2 cases is 10.5%, meaning that one in every 10 patients with early breast cancer will have metastases on staging workup. This rate is relatively high (around 2.5 times as high) compared to the low incidence (4%) previously reported (5). One explanation for higher rates of metastases in our cohort is heterogeneity between different populations with breast cancer and tumor characteristics, as a significant proportion of patients in Bahrain have aggressive tumors compared to Western countries (11). Another cause may be variations in the imaging modalities used for staging, which might affect the diagnostic ability of scans to identify small metastatic deposits. In the present study, PET scans had a significantly greater likelihood to detect distant metastases, but they are also more expensive and not readily available in all institutions (2). As reported in the present study, skeletal metastases represent the most common site of metastasis in patients undergoing baseline staging scans (12). It has been reported that more than 50% of patients with breast cancer in Bahrain have axillary lymph node metastasis at the time of presentation (1, 11). This finding was confirmed in the present study, as 59.2% of patients had positive nodes, and this high rate of nodal positivity is very likely associated with the high prevalence of distant metastases.

Our data indicated an increased likelihood of metastatic disease identification at presentation in those with multifocal or multicentric disease. This is because these cancers have larger tumor dimensions, greater metastatic rate to axillary lymph nodes and a high Ki67 proliferation index (13). As seen in the present study, a high Ki67 index is significantly correlated with a risk of distant metastases and adverse prognostic factors, since it is associated with high-grade and ER/PR-negative tumors (14).

Abnormal axillary lymph nodes on initial imaging have an increased risk of distant metastases, especially for larger tumors (15). In the present study, compared with N0 tumors, the risk of distant metastases was significantly greater for node-positive tumors. Triple-negative and HER2-positive cancers are typically more aggressive than tumors with hormone receptor-positive profiles (5). There is some controversy about the role of molecular subtypes in predicting

distant metastases, where some authors described a relationship (16), while others reported no association (2). Data from the present study, however, showed a significantly higher rate of distant metastases in patients with TNBC and HER2-enriched disease (26.5% and 32.1%, respectively). In the present study, patients who underwent neoadjuvant chemotherapy had an increased likelihood of metastatic disease identification at presentation. Although patient selection for neoadjuvant chemotherapy depends on several factors, such as tumor size, lymph node involvement and receptor status, consideration of CT staging in this group of patients seems reasonable (17).

On the basis of current NCCN guidelines, the accepted criteria for staging in newly diagnosed breast cancer with CT, bone scan or PET scan to detect distant metastatic disease in patients with signs and symptoms of possible metastases, ipsilateral recurrence and T4 disease continue to be appropriate (5). In addition, based on the results of the present study, consideration should also be given to patients with T3 tumors, abnormal axillary lymph nodes and aggressive tumor biology. Furthermore, CT staging is indicated prior to commencing neoadjuvant chemotherapy, in those not meeting the above criteria. However, routine metastatic workup should not be performed for patients with early breast cancer in the absence of symptoms.

Potential limitations of this study include its retrospective nature, patients enrolled from a single institution and a relatively small sample size compared to the literature. Some patients had a CT and bone scan, while others had a PET scan for systemic staging. Lack of standardization of the imaging modality used for detection of distant metastases might have affected our results. Nevertheless, to the best of our knowledge, this is the first study from the Middle East that evaluates the appropriateness of metastatic workup in newly diagnosed breast cancer in order to establish local guidelines for staging in Bahrain.

Although there was a higher prevalence of distant metastases in Bahrain than reported from elsewhere, the routine use of CT scans to screen for distant metastases does not appear to be indicated in all patients with newly diagnosed breast cancer. We identified subgroups of patients with a higher risk of distant metastases in whom a full metastatic workup could be indicated. Overall, our findings confirm that radiological staging of asymptomatic patients with early breast cancer is not warranted as a routine practice in Bahrain. Together with existing guidelines, our findings will help the adoption of a local policy in Bahrain for staging of patients with newly diagnosed breast cancer, with the hope of maximizing detection of metastases while minimizing harmful side effects and costs.

Ethics Committee Approval: The study protocol was approved by the Research Ethics Committee of Government Hospitals Bahrain (approval no: 129261223; date: 26.12.2022).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: R.E., H.A.A.; Concept: R.E., H.A.A.; Design: A.A.A., N.F.A., A.Z.S.; Data Collection and/or Processing: A.A.A., S.A.A., T.H.A., W.Z.A., H.M.H., A.A.Als., N.A.A., F.A.A.; Analysis and/or Interpretation: A.A.A., N.F.A.; Literature Search: A.A.A., H.A.A.; Writing: A.A.A., N.F.A., A.Z.S., S.A.A., T.H.A., W.Z.A., H.M.H., A.A.Als., N.A.A., F.A.A., R.E., H.A.A.

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Original Article

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Comparison of Volume Measurements and Bioimpedance Spectroscopy Using A Stand-on Device for Assessment of Unilateral Breast Cancer-Related Lymphedema

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ABSTRACT

Objective: Breast cancer related lymphedema (BCRL) may be assessed through objective measurement of limb swelling with common techniques including volumetric measurement using a tape measure or perometry, and measurement of extracellular water using bioimpedance spectroscopy (BIS). This study aimed to evaluate the performance of a stand-on BIS device for detection of BCRL, introduce a novel graphical method to compare volumetric and BIS methods alongside traditional specificity and sensitivity analysis, and determine and compare BIS thresholds with those published previously.

Materials and Methods: Female participants with indocyanine green lymphography confirmed unilateral arm lymphedema (n = 197) and healthy controls (n = 267) were assessed using a cross-sectional study design. BIS and volumetric measures were obtained in a single session.

Results: The BIS lymphedema index (L-Dex) method had a significantly higher sensitivity than the excess volume approach (area under the curve = 0.832 vs. 0.649, p = 0.0001). A threshold of L-Dex 6.5 had a higher true positive rate (70.6%) than L-Dex 10 (68.5%) although false positive rate increased from 0.4% to 2.6%. A threshold of 5% excess volume improved the true positive rate (68.5%) compared with 10% excess volume (49.7%) however the false positive rate increased to an unacceptable 47%. The L-Dex ranges in this study were not significantly different from previously published ranges.

Conclusion: BIS was superior for identifying BCRL compared with volume measurements, reaffirming the value of this technique. However, it is recommended that BIS be used in conjunction with comprehensive evaluation of symptoms and clinical presentation. The proposed graphical method provides a simple and easily interpretable approach to compare and define concordance between the two commonly used methods for BCRL assessment namely limb volume and BIS L-Dex indices. The existing BIS (L-Dex) thresholds for presence of BCRL were also validated.

Keywords: Lymphedema; bioimpedance spectroscopy; impedance; L-Dex

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Key Points

- BIS was superior for detecting BCRL compared with volume measurements.
- The current BIS (L-Dex) thresholds for lymphoedema presence were validated by this study.
- It is recommended that BIS be used as part of a comprehensive assessment of symptoms and clinical presentation.

Introduction

Breast cancer related lymphedema (BCRL) is a dysfunction of the lymphatic system resulting from treatment for breast cancer (1). The precise etiology of the condition may vary due to direct surgical damage to the lymphatics through to damage due to radiation treatment rather than damage due to the presence of a tumour *per se* (2). BCRL is characterised by swelling of the arm on the side of treatment due to accumulation of excess lymph through compromised lymph transport. The precise incidence of BCRL is uncertain, with estimates varying from 3 to 65% following surgery (3). Presentation may occur at any time but first occurrence is more prevalent within the first 2 years following treatment.

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It is generally recognised that the treatment of BCRL is most effective when commenced at the earliest opportunity (4). Early detection of BCRL is frequently by the patient first noting symptoms of early limb of heaviness and swelling, e.g. clothing or jewellery no longer fitting. However, limb swelling is not definitively diagnostic for BCRL. Confirmation of BCRL is best measured by assessing lymphatic function, e.g., by indocyanine green (ICG) lymphography coupled with full clinical appraisal (5). ICG lymphography is not, however, widely available and in addition to clinical assessment, the presence of BCRL is routinely assessed by objective but not consistent measurement of limb swelling. A wide variety of techniques are available for this purpose (6) but most commonly are firstly, simple volumetric measurement, either from geometric calculation from manual arm dimensional measures using a tape measure or by opto-electronic devices such as the Perometer™ and secondly, measurement of extracellular water (ECW) volume of which lymph is a principal component, by bioimpedance spectroscopy (BIS) (7). Neither arm volume nor BIS assessments measure lymph accumulation directly. The former measures overall limb volume and typically, the excess volume of the affected or at-risk arm is compared to that of the contralateral unaffected limb. Excess volume thresholds vary but typically an increase in limb size of 5% or larger is considered abnormal swelling and, in conjunction with clinical picture, is considered indicative of the presence of BCRL in an at-risk limb of affected individual (8). Bioimpedance spectroscopy measures the electrical impedance (resistance at zero frequency, R0) of the limbs and, as with volumetric measurements, compares the resistance of the affected limb to that of the unaffected limb, typically as a ratio or as a linearized ratio, the L-Dex score (9). Thresholds indicative of BCRL for BIS have been established based on the normal distribution of values seen in a healthy control population (10). These thresholds were determined using first generation BIS devices with measurements performed while the individual was in supine. Current model BIS instruments are stand-on devices with measurements made while the individual is standing (11). In addition, owing to the different postures, electrode locations are slightly different. Comparative studies have demonstrated that, while measurements with the two devices are highly correlated, they are not entirely interchangeable (11). Both volumetric and BIS methods exhibit high sensitivity and specificity although no consensus exists as to which method is optimal for BCRL assessment (12).

The current study aimed to assess the performance of the current stand-on BIS device for detection of BRCL. Secondarily, a novel graphical method for comparison of volumetric and BIS methods was developed as an adjunct to conventional specificity and sensitivity analysis [receiver operating characteristic (ROC) curves]. Additionally, BIS thresholds were determined and compared to existing published thresholds.

Materials and Methods

Participants

Female participants (n = 197) with unilateral BCRL were recruited from those attending the Australian Lymphoedema Education, Research and Treatment Centre (ALERT) at Macquarie University. All participants underwent clinical evaluation by experienced lymphedema therapists and the existence of lymphedema was confirmed by ICG lymphography. All measurements were obtained in a single session by trained research assistants.

Healthy control women (n = 267) with no history of BCRL were drawn from a number of sources. Firstly, participants were recruited from the Macquarie University staff and students. Measurements were obtained in a single attendance session at the ALERT clinic. Secondly, healthy controls were drawn from a database of comparable measurement data maintained by the authors and were drawn from participants in two previously published studies (13, 14).

All participants were female aged between 18 and 83 years of age. Exclusion criteria were minimal reflecting the general population; participants fitted with an implantable device, e.g., a pacemaker or were pregnant (determined by self-attribution) were excluded as these are contraindicated for BIS measurements. Additionally, participants were excluded if they reported a health condition or medication that might affect body water status as this would confound BIS measurements.

Originating research studies providing data for the current analysis were all approved by their respective institutions; Macquarie University (11) and University of Queensland (13) and abided by the Helsinki Declaration governing human experimentation. All participants provided informed written consent.

Measurements

Demographic Characteristics

Information was obtained at interview for each participant and included self-described medical history (for participants with BCRL this included type of cancer, adjuvant treatments, and lymphedema history) and self-ascribed limb dominance. Height was measured standing without shoes using a stadiometer (to the nearest 0.1 cm) and weight in light clothing using electronic scales to the nearest 0.1 kg. For participants with BCRL confirmed by ICG lymphography (5), the arm on the side of cancer treatment was deemed as "affected".

Volumetric

Arm volumes were determined using a number of different methods reflecting current clinical practice. In 30 (11.2%) of control participants, circumferential measurements at 4 cm or 10 cm intervals proximally from the wrist were obtained and arm volume for each segment calculated according to frustum cone geometry and total volume calculated as the sum of the segments (15). In the remaining 237 (88.8%) of control participants, whole arm volume was assessed from dual energy X-ray absorptiometry (DXA) measurements of limb composition (bone mineral, fat and lean masses) as described previously (13). DXA-derived masses were converted to their equivalent volumes using the coefficients of Wilson et al. (16) and whole arm volume calculated as the sum of the individual tissue volumes. For participants with BCRL, limb volume was calculated from circumferentially-derived geometric calculations as described above (n = 71, 36.1%) with the remaining 126 (63.9%) assessed using perometry (17).

Bioimpedance Spectroscopy (BIS)

Whole arm BIS measurements were obtained with either an ImpediMed SFB7/U400 impedance spectroscopy device or an ImpediMed SOZO® impedance spectroscopy device (ImpediMed Ltd., Brisbane). The SFB7 device is a lead-type device primarily designed for supine measurements. Measurements in standing were obtained using a bespoke footplate fitted with stainless steel electrodes and hand-grips with stainless steel electrodes mimicking the SOZO® electrode arrangement. Comparative studies showed no significant

difference in measurements between the two systems. In one-fifth (n = 55) control participants, measurements were available for the SFB7 in supine only (13). These measurements were converted to standing equivalent values using regression equations determined previously (11). All measurements were obtained following manufacturer's recommendations for participant preparation and measurement protocol as described previously (11).

For all BIS measurements, whole-arm impedance data were analysed according to Cole theory (18) using Bioimp software (Bioimp v4.12, ImpediMed Ltd. Brisbane) to provide estimates of resistance at zero frequency (R0) for each arm as described previously (9, 11) L-Dex scores were those provided by the device manufacturer and are calculated according to limb dominance (9).

Statistical Analysis

The absolute differences in volumes between the affected (BCRL) or dominant (control) arms and the respective contralateral arms were calculated and these volume differences expressed as % of the unaffected or non-dominant arm for the BCRL and control participants respectively. The ratio of R0 resistances between the two arms was calculated as unaffected R0: affected R0 for participants with BCRL and as the non-dominant R0 : dominant R0 as originally described (19). L-Dex scores, provided by the device manufacturer, represent the R0 ratios linearized with reference to the normal distribution of ratios observed in a healthy control population where an L-Dex value of 0 represents the mean R0 ratio; L-Dex 6.5, the mean + 2 standard deviation (SD) and L-Dex 10, the mean + 3 SD. The control reference values are proprietary information of the manufacturer.

Descriptive statistics are presented as mean ± SD and the range of values. Statistical significance of difference between BCRL and control data was assessed using independent t-tests and between arms using paired t-tests with Medcalc v22.007 (MedCalc Software Ltd, Ostend, Belgium). The normal distribution of R0 ratios was calculated using Medcalc and distributions compared using the *Z* statistic. Sensitivity and specificity of the volumetric and BIS methods was assessed using receiver operating characteristic curves (20) constructed using Medcalc and the Youden index (21) with significance of difference being assessed by the *Z* statistic for correlated variables (22).

Volumetric and BIS approaches for BCRL assessment were compared graphically using an adaptation of error grid analysis (23). The proportions of false negatives and positives were calculated for each method and compared using a *Z* test for proportions.

Results

Characteristics of Participants

Participant characteristics are presented in Table 1. The BCRL group was significantly older and heavier although there was no difference in height. The control cohort were generally classed in the healthy BMI range (70% <25 kg/m^2); in contrast, only 25% of participants with BCRL were in the healthy range and 39.6% having a BMI >30 kg/m^2. The R0 of the dominant arm in the control group was on average 3.1% and significantly (p<0.0001) smaller than the contralateral non-dominant arm concomitant with a mean 4.6% larger volume (p<0.0001). The resistance of the affected arm for the participants with BCRL was, on average, 15% smaller than the unaffected arm again reflecting the same % volume excess of the affected limb; this difference being highly significant (p<0.0001). The computed mean L-Dex scores were -5.5 and 22.8 for control and participants with BCRL respectively. The mean value for the controls is within the -10 to +10 L-Dex range for a healthy population without excess ECW. However, the mean value for the BCRL group was 22.8 which is in excess of the L-Dex 10 (3SD) threshold indicative of excess ECW. Notably, the range in values was markedly larger for the participants with BCRL than for the controls reflecting the different lymphedema stages. Both groups include negative values indicating that either the non-dominant or unaffected arm was larger than the contralateral limb; an observation confirmed by negative absolute volumes.

Distribution of R0 Ratios and L-Dex Scores

The frequency distribution of R0 ratios for the control participants is presented in Figure 1. Values were normally distributed around a mean value of 1.033 (Non-dominant: Dominant ratio). The ranges of ±1, 2 and 3 SD are also shown with the 2 and 3 SD ranges being equivalent to L-Dex thresholds of 6.5 and 10 units respectively. Table 2 presents a comparison of the present control distribution, as L-Dex ranges, with previously published ranges. The ranges were not significantly different and were combined to provide overall average values.

Sensitivity and Specificity Analysis

There was highly significant difference (p<0.0001) between the volume-based ROC curve and the L-Dex ROC curve (Figure 2). The respective area under the curve (AUC) values, a measure of overall sensitivity, were 0.649 and 0.832; an AUC value greater than 0.8 is considered to exhibit excellent diagnostic accuracy with values below this having marginal acceptability (24). Youden J values were 0.375 and 0.800 for the excess volume and L-Dex methods respectively.

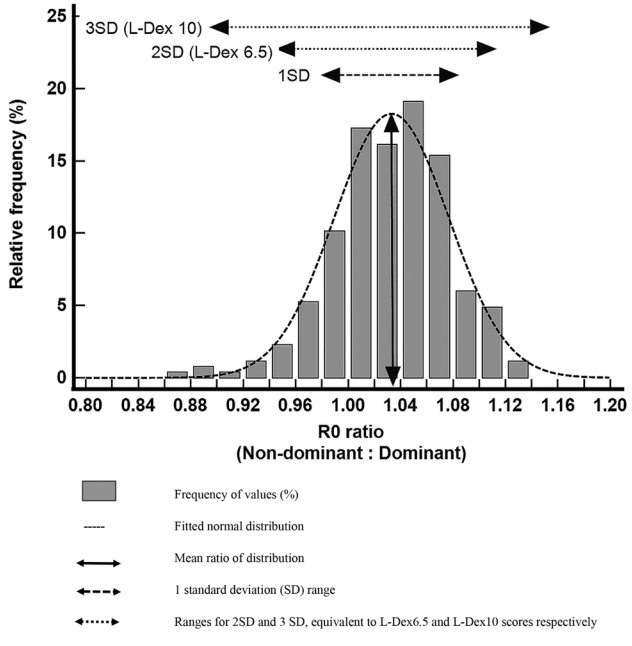

Figure 1. Distribution of ratios of arm R0 (Non-dominant: Dominant) in control population

L-Dex: Lymphedema index

Table 1. Participant characteristics

	Group		
Characteristic	**Control**	**BCRL**	***P***
Number	267	197	
Dominance (R:L)	249:18	200:12	ns
At risk (R:L)		103:109	
Age (y)	50.8±14.1 (18.3 to 83.0)	58.1±11.7 (32.0 to 82.0)	0.001
Height (cm)	162.4±7.5 (142.0 to 183.5)	163.1±6.4 (144.0 to 178.0)	ns
Weight (kg)	62.5±10.8 (39.0 to 104.7)	76.9±15.0 (46.2 to 149.8	0.0001
Body mass index (kg/m^2)	23.7±3.7 (17.1 to 36.4)	28.9±5.4 (18.7 to 50.3)	0.0001
R0 dominant arm (ohm)	410±45[a] (298 to 538)		
R0 non-dominant arm (ohm)	423±46[b] (311 to 561)		
R0 ratio (non-dominant: dominant)	1.033±0.041 (0.870 to 1.133)		
R0 unaffected arm (ohm)		361±43[a] (269 to 488)	
R0 affected arm (ohm)		302±63[b] (147 to 462)	
R0 ratio (unaffected: affected)		1.234±0.248 (0.915 to 2.226)	
L-Dex score	-5.5±4.8 (-15.2 to 11.0)	23.1±24.1 (-8.1 to 116.5)	0.001
Dominant arm volume (mL)	2867±718[a] (1222 to 5275)		
Non-dominant arm volume (mL)	2746±708[b] (1163 to 4858)		
Excess volume (mL)	125±160 (-599 to 782)		
Excess volume (%)	4.7±5.7 (-19.8 to 20.8)		
Unaffected arm volume (mL)		2679±727[a] (1346 to 5769)	
Affected arm volume (mL)		3068±913[b] (1528 to 5826)	
Excess volume (mL)		389±511 (-1902 to 2292)	
Excess volume (%)		14.9±17.5 (-32.9 to 81.6)	0.0001

Data presented as mean ± SD (range); difference statistically significant: [a]: versus; [b]: $p<0.0001$; SD: Standard deviation, BCRL: Breast cancer related lymphedema; L-Dex: Lymphedema index

Table 2. L-Dex thresholds indicative of excess extracellular water

	Number	Dominant at risk					Non-dominant at risk				
					Threshold					Threshold	
		Mean	SD	1SD	2SD (L-Dex 6.5)	3SD (L-Dex 10)	Mean	SD	1SD	2SD L-Dex 6.5)	3SD (L-Dex 10)
Cornish et al. (19)	60	1.037	0.034	1.071	1.102	1.102	0.964	0.034	0.998	1.032	1.066
Ridner et al. (34)	32	1.024	0.027	1.051	1.078	1.105	0.986	0.027	1.013	1.040	1.067
Ward et al. (10)	172	1.014	0.040	1.054	1.094	1.134	0.986	0.040	1.026	1.066	1.106
Wang et al. (35)	391	1.018	0.045	1.063	1.108	1.153	0.984	0.045	1.029	1.074	1.119
This study	267	1.033	0.041	1.072	1.114	1.156	0.972	0.041	1.013	1.055	1.097
Weighted average	922	1.022	0.042	1.064	1.106	1.145	0.980	0.042	1.021	1.063	1.105

Statistical analysis: There was no significant difference in ranges between studies; owing to the larger difference in sample sizes mean values were calculated weighted according to sample size; SD: Standard deviation; L-Dex: Lymphedema index

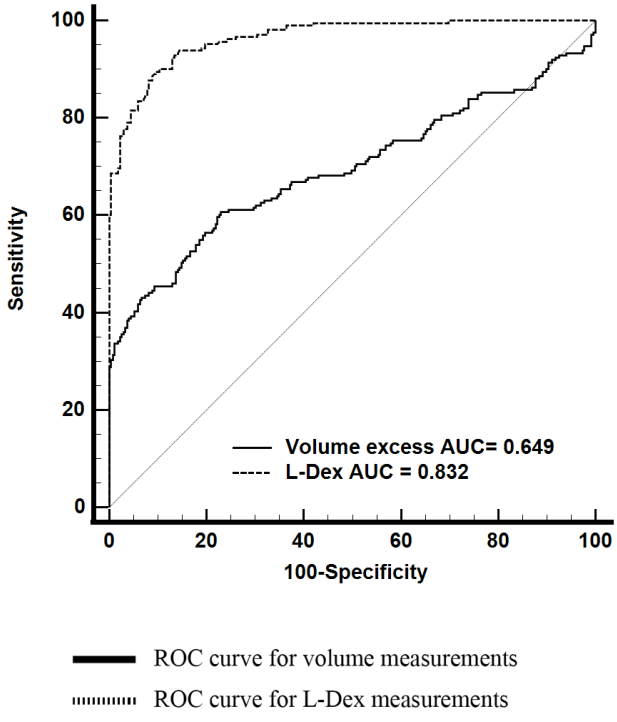

Figure 2. Receiver operator characteristic (ROC) curves for lymphedema assessment by either L-Dex or excess volume measurements

L-Dex: Lymphedema index

Graphical Comparison of Methods

A method comparison plot is presented in Figure 3. The plot presents L-Dex scores for all participants plotted against their excess limb volume. The vertical line represents either a 10% volume difference (Panel A) or 5% volume difference (Panel B) between arms, commonly used indices of presence of lymphedema, with values that fall to the right of this line being deemed positive for lymphedema. The horizontal line is either the L-Dex 10 threshold (Panel A) or L-Dex 6.5 threshold (Panel B) with data points that fall above this line being indicative of BCRL. Consequently, data points that fall in the upper right quadrant representing participants that are deemed positive for BCRL by both methods. Notably, only one control participant exceeded the L-Dex 10 threshold while 39 (14.6%) of participants exceeded the 10% volume difference threshold (false positives). Ninety-nine participants with BCRL (50.3%) were below this threshold (false negatives); the comparable figure for L-Dex 10 was 62 participants (31.5%); the corresponding true positive rates were 49.7% and 68.5% for volume and L-Dex respectively. These differences were significant (Table 3). Eighty-eight participants with BCRL (44.7%) were positive by both criteria, L-Dex >10 and excess volume >10%. If the more liberal thresholds of >5% excess volume and L-Dex 6.5 are used, then agreement between methods increase only slightly to 91 (46.2%) despite the number of BCRL positive subjects increasing to 135 (68.5%) and 139 (70.6%) for volume and L-Dex measurements respectively.

Discussion and Conclusion

The present study confirmed that both excess volume and BIS can discriminate women with BCRL from healthy controls although with different degrees of sensitivity and specificity. In addition, the different methods do not always identify the same individuals. The present study found that the BIS (L-Dex) method had a higher true positive rate with a smaller false negative rate than the excess volume approach with sensitivity similar to that observed in other studies (9). The more liberal threshold of L-Dex 6.5 had the higher sensitivity than L-Dex 10 in accord with the findings of others (25) although false positive rate increased 6-fold albeit still only 2.6% of participants. By contrast,

using a threshold of 5% excess volume improved sensitivity to almost the same as L-Dex but with an unacceptable false positive rate of 47%.

The relative merits of volumetric and impedance assessments for BCRL have been studied previously with different findings and conclusions being drawn. Barrio et al. (26) in a prospective study found volumetric assessment (10% volume excess threshold) and BIS (L-Dex 10) demonstrated poor correlation with, as observed here, inconsistent overlap of measurements between methods in individuals. Similarly, Spitz et al. (27) found poor sensitivity of BIS for detection of BCRL. In contrast, a number of studies have found that the BIS method is a reliable and valid assessment tool that correlates well with clinical assessment and physiologic measurements of lymphatic function (28) while Borman et al. (29) found that BIS detected more and earlier patients with BCRL than circumferentially-derived volume measurements. Some studies have concluded that neither volume nor BIS approaches should be considered as definitive for BCRL detection and, appropriately, have suggested that both tools should be used in conjunction with patient symptomology and comprehensive clinical evaluation (30).

In the majority of studies comparing volume and BIS, the volume method has been set a priori as the reference method (26). In the present study, the presence of lymphedema was determined by the independent method of ICG lymphography. Consequently, the volume and BIS were analysed as independent methods against this reference assignment of BCRL rather than directly against each other with one method pre-designated as the reference method. If the presence of BCRL is defined a priori by volume change, inevitably volume change will be deemed to perform better than BIS, for example, as stated in Keeley (31). Indeed, Keeley (31) acknowledged that volume change was a "reasonable' although "imperfect" gold standard for BCRL in the absence of an international consensus of an agreed method. Notably, Varagur et al. (32) also found BIS to have high sensitivity and specificity when BCRL was assessed by the lymphatic function measure of magnetic resonance lymphangiography.

Most of the aforementioned studies have considered comparison of volume and BIS techniques in terms of sensitivity and specificity and have frequently not commented on whether true positive detections for BCRL by both methods are the same individuals. This study

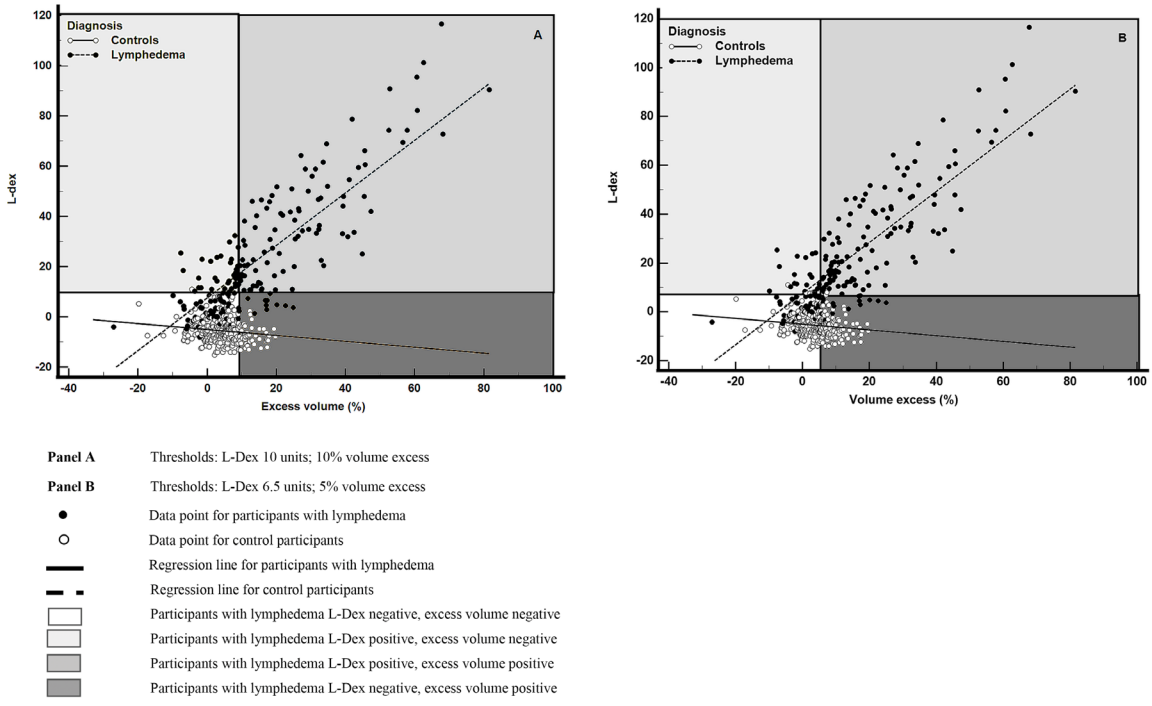

Figure 3. Relationship of excess volume measurements to L-Dex 10 scores

L-Dex: Lymphedema index

Table 3. Numbers and percentages of participants with BCRL exceeding thresholds for excess arm volume or L-Dex score

		Volume measurement		Impedance measurements	
Participants	Threshold	10% excess volume	5% excess volume	L-Dex 10	L-Dex 6.5
BCRL	> threshold	98 (49.7)[c]	135(68.5)	126 (64.0)[d]	139 (70.6)
	< threshold	99 (50.3)[c]	62 (31.5)	71 (36.0)[d]	58 (29.4)
Control	> threshold	39 (14.6)[a]	126 (47.2)[a]	1 (0.4)[b]	7 (2.6)[b]
	< threshold	228 (85.4)[a]	141 (52.8)[a]	266 (99.6)[b]	267 (98.0)[b]

Data present as n (%). Difference (volume versus L-Dex) significant: [a]: Versus [b]: p<0.0001; [c]: Versus; [d]: p<0.004; BCRL: Breast cancer related lymphedema; L-Dex: Lymphedema index

has demonstrated that concordance between methods is relatively poor. The proposed graphical presentation provides a simple way to not only assess individuals against both criteria, volume and BIS, but also to show which individuals are being identified by each method. The graphical approach also has potential for assessing the relative performance of volumetric and BIS methods when used for longitudinal BCRL assessment by tracking loci at each time-point on the grid plot.

In the present study, L-Dex thresholds were those provided by the manufacturer of the BIS device. These are proprietary information and of unknown provenance. A number of studies to date have determined the distribution of R0 ratios used to generate L-Dex thresholds but have used the older device that obtains measurements in supine, not the current stand-on model. While the two devices perform very similarly, they are not totally interchangeable (11). The present study provided the opportunity to determine R0 ratios and calculated L-Dex ranges for measurements when standing. No significant differences were observed which is perhaps not surprising since these are either directly inter-limb ratios or inter-limb L-Dex scores where presumably any physiological effects on fluid volumes due to positional change will impact similarly on each arm. This suggests that existing L-Dex thresholds are robust and may be used with confidence.

The present study has a number of strengths and weaknesses. Volumetric measurements were obtained using a number of different techniques (perometry, DXA, geometric calculation). This may be perceived as a weakness since these methods do not measure exactly the same limb volume. However, since data are expressed as inter-limb differences or ratios then any methodological differences will be mitigated. Furthermore, the use of different methods reflects lack of standardisation where different methods are used in current clinical practice. Similarly, BIS measurements were obtained using different BIS devices and for some control participants while supine and converted to their standing equivalents. It has been shown previously that there are no statistical differences in device-specific measurement of R0 when used under identical measurement conditions (33). There are differences, however, due to posture (11). The regression procedures used to interconvert supine to standing measurements exhibit high correlation (>0.93) with standard error of the estimate of <3%. The study only considered participants with ICG-confirmed BCRL and was cross-sectional with measurements at a single time-point only. Also, participants were included irrespective of lymphedema stage although BIS was originally conceived for detection of early-stage lymphedema. The study design precluded assessment of predictive performance in longitudinal prospective studies. As such it also precluded, using the preferred method of comparing either volume or BIS change relative to a baseline, ideally pre-treatment, measure.

In conclusion, BIS performed better than volume measurements for identification of women with BCRL. The study has reaffirmed the value of this technique, although its use in conjunction with patient symptomology and comprehensive clinical evaluation using other assessment tools is recommended. The proposed graphical method for presentation of both volume assessment and BIS indices of BCRL facilitates comparison of these different approaches in an easily interpretable manner. It has also conformed the validity of existing BIS (L-Dex) thresholds indicative of the presence of BCRL.

Ethics Committee Approval: The study was conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007), the CPMP/ICH Note for Guidance on Good Clinical Practice and consistent with the principles that have their origin in the Declaration of Helsinki (Macquarie University Ethics Committee - no: 5201700439; date: 30.05.2017).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: L.C.W.; Design: L.C.W., L.A.K.; Data Collection and/or Processing: L.C.W., B.T., K.G., L.A.K.; Analysis and/or Interpretation: L.C.W., B.T., K.G., L.A.K.; Literature Search: L.C.W.; Writing: L.C.W., B.T., K.G., L.A.K.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Article

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Predictors of Unilateral Arm Lymphedema in Non-obese Locoregionally Advanced Breast Cancer Patients Undergoing Neoadjuvant Chemotherapy, Modified Radical Mastectomy, and Postoperative Irradiation

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ABSTRACT

Objective: The most dreaded long-term complication of axillary lymph node dissection remains upper arm lymphedema. Our study has strategized the three most common identified causes of post treatment arm lymphedema, i.e., obesity, radiation, and neoadjuvant chemotherapy and tried to identify the histopathological and clinical or surgical factors which can predict arm lymphedema.

Materials and Methods: This is a prospective observational study was conducted at a tertiary care referral centre in India, with strict inclusion criteria of BMI <30 kg/m^2, age <75 years, presence of metastatic axillary node proven by FNAC, received anthracycline based neoadjuvant chemotherapy and postoperative nodal irradiation, and completed 24 months of regular follow-up.

Results: Total of 70 patients were included in the study. The mean age of the patients was 50.3 years (±12.9). lymphovascular invasion, total number of lymph nodes removed from level III, total number of days drain was left in situ and maximum drain output were found to be significantly ($p<0.05$) associated with arm lymphedema.

Conclusion: In patients undergoing modified radical mastectomy with level III dissection, and postoperative irradiation, the incidence of unilateral arm lymphedema is significantly influenced by several clinicopathological factors like the total number of lymph nodes removed in level III, higher maximal drain output, prolonged duration of drain placement and the presence of lymphovascular invasion.

Keywords: Axillary lymph node dissection; upper arm lymphedema; neoadjuvant chemotherapy; modified radical mastectomy; breast cancer; locoregional therapy

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Key Points

- Upper arm lymphedema is a serious long-term complication of axillary lymph node dissection.
- The study aimed to identify predictive factors for arm lymphedema in non-obese, locoregionally advanced breast cancer patients who underwent standard neoadjuvant chemotherapy and post-operative irradiation.
- Factors associated significantly with arm lymphedema are lymphovascular invasion, Total number of lymph nodes removed from level III, total number of days drain left *in situ* and maximum drain output.

Introduction

Breast cancer is the commonest cancer worldwide in women with incidence varying widely across countries and regions. It impacts over 2.1 million women each year, accounting for 25% of cancers and 15% of cancer deaths in women (1).

An age-adjusted rate as high as 25.8 and mortality up to 12.7 has been estimated per 100,000 Indian women. Besides this, young age has been identified as a major risk factor for breast cancer in the Indian subset (2). Age-standardized incidence rate is now annually increasing by 29 per cent in the world. This secular trend has been attributed to

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the changes in the population age structure (16 per cent), population growth (12 per cent), and the etiologic causes of the cancer (3).

Chemotherapy and surgery form the mainstay of treatment in early and locally advanced breast cancer. Axillary nodes are the primary draining area, hence management of the axilla is an important component of the treatment of invasive breast cancer.

Kiricuta and Tausch (4) in their seminal work and mathematical model in 1992 established that at least 10 nodes need to be dissected for proper staging. Over time, the management of the grossly uninvolved axilla has changed from complete (level I-III) lymph nodal dissection to sentinel lymph node biopsy. Even when the axilla is grossly involved, many surgeons avoid dissecting level III nodes. This change in practice was based on data that showed that level III dissection is associated with longer surgical time and morbidities without an associated improvement in overall survival (5). There is no consensus among surgeons as to what level of axillary nodes should be dissected for loco-regionally advanced breast cancer with axillary node positivity.

The National Institutes of Health consensus conference recommended level-I or level-II dissection as standard surgery and level-III dissection for patients with obviously involved level III nodes. NCCN clinical practice guidelines® recommends level III dissection when gross disease in levels I & II and/or level III is present (6, 7).

Arm lymphedema remains a dreaded long-term complication of axillary dissection affecting quality of life. The edema promotes recurrent soft tissue infections requiring intravenous antibiotics with other drastic financial and professional implications (8). Most studies, including the landmark study of Armer et al. (9) in 2019, have observed heterogeneity in the treatment of axilla, which makes it difficult to establish the factors affecting the development of lymphedema. Obesity [body mass index (BMI) >30 kg/m^2] and radiotherapy have been described as major risk factors for unilateral arm lymphedema (10).

Our study is an attempt to identify the clinicopathological factors associated with arm lymphedema in patients undergoing level III axillary lymph node dissection, over a two-year follow-up after controlling for contributing factors.

Materials and Methods

This prospective observational study was conducted at a tertiary care referral centre of the armed forces of India, where all modalities of treatment for breast carcinoma were available. Institutional Ethical Committee clearance was taken from the institutional board. Informed consent regarding the study was taken from each individual. Patients were accrued from 2018 to 2021 with the following inclusion and exclusion criteria:

Inclusion Criteria:

1. Age between 18 and 75 yrs.

2. BMI <30 kg/m^2

3. Presented with metastatic axillary node proven by following neoadjuvant chemotherapy.

4. Received anthracycline and cyclophospamide based neoadjuvant chemotherapy and taxane based adjuvant chemotherapy.

5. Received postoperative chemotherapy and regional nodal irradiation.

Exclusion Criteria:

1. Age <18 yrs or >75 yrs

2. BMI ≥30 kg/m^2

3. Clinically N0 node status

4. Did not receive chemotherapy or regional nodal irradiation as per protocol above.

5. Did not complete the mandatory follow-up of 24 months.

6. Did not consent to the study

The selected patients underwent Modified Radical Mastectomy with level I-III axillary nodal dissection. These patients were followed up at 01, 03, 06, 09, 12, 18 and 24 months after completion of nodal irradiation. The last patient completed the 2-year follow-up in June 2022.

Technique of Level III Lymph Node Dissection

The axilla was dissected from the axillary vein superiorly to the angular vein inferiorly (11). The triangular space (bound by axillary vein superiorly, thoracodorsal pedicle and tendon of latissimus dorsi laterally, Halstead ligament medially and angular vein inferiorly) was cleared of all the fibrofatty tissue. For the dissection of level III nodes, the pectoralis minor was retracted and all the fibrofatty tissue medial to its tendon was removed (12). Nerve to serratus anterior, latissimus dorsi pedicle, medial and lateral pectoral nerves were meticulously preserved. This is the standard template of dissection which in experienced hands, adds little to the morbidity (13, 14). Fat pad over the axillary vein was not removed as it leads to increased incidence of upper limb lymphedema (15).

Assessment of Lymphedema

Lymphedema was defined as a difference of more than 2 cm in the upper arm circumference between the arm ipsilateral to the axillary dissection and the contralateral arm. The upper arm circumference (in cm) at 15 cm proximal to the lateral epicondyle ipsilateral to the axilla surgery site was compared with the contralateral upper arm circumference, just as described by Veronesi et al. (16). Measurements were carried out at each follow-up visit.

Drain output was measured each morning at 0800 hours. The drain was removed once the output reached fell below 15 mL. Maximum drain output in any 24 h period and the total days that the drain was *in situ*, were recorded for each patient.

Statistical Analysis

The cumulative incidence was generated with Kaplan-Meier estimators. The incidence of lymphedema was compared across patient groups using the log-rank test. Univariate and multivariate Cox proportional hazards regression models were used to evaluate the association between baseline patient and disease characteristics and time to lymphedema. Point estimates [eg, number (percentage) of patients, hazard ratios (HRs)] and corresponding 95% confidence intervals (CIs) were used to summarize variables and associations. Statistical analysis was performed using Jamovi Software (Version 2.3.21). A *p*-value of 0.05 was considered significant.

Results

A total of 112 patients with locally advanced breast carcinoma were included in the study. However, 22 had a BMI of more than 30 kg/m^2, ten patients could not complete 24 months of follow up and ten patients did not complete the nodal irradiation protocol due to severe adverse effects. Hence, 70 patients were included in the final analysis, as depicted in Figure 1.

The mean age of the patients was 50.3 years (±12.9). The mean size of the tumour was 3.0 cm (±0.8). The cumulative incidence of arm lymphedema was 25.7% (18 out of 70). The average difference in mid-arm circumference in group A patients (patients with a midarm circumference difference of >2 cm) was 2.84 cm (95% CI; 2.51–3.17), whereas in the patients of group B (patients with a midarm circumference difference of <2 cm), it was 1.4 cm (95% CI; 1.42–1.56) (Figure 2).

On univariable analysis (Table 1), factors associated with reduced incidence of lymphedema were - hormone receptor-positive, presence of lymphovascular invasion, absence of perineural invasion or extracapsular extension, post-NACT tumour size, metastatic node to total lymph node removed ratio in level I, II and level III and total number of days the drain remained *in situ*. Factors associated with increase in lymphedema on univariate analysis were incomplete pathological response and total number of lymph nodes removed from level 3. Total number of lymph node retrieved from level I/II, age and drain output were not found to affect the lymphedema events on univariate analysis. The highest hazard rate for experiencing a lymphedema event was in patients without pathological complete response (HR: 1.86, CI: 0.54–6.42, p = 0.328) followed by patients with hormone receptors/HER2 Neu positivity (HR: 1.40, CI: 0.46–4.26, p = 0.552) and total number of lymph node nodes removed in level III (HR: 1.29, CI: 1.07–1.55, p = 0.007). In terms of protection from lymphedema events, the absence of lymphovascular invasion (HR: 0.23, CI: 0.09–0.62, p = 0.004) and low metastatic to total lymph node ratio in level III (HR: 0.25, CI: 0.04–1.44, p = 0.122) had the lowest hazard rates.

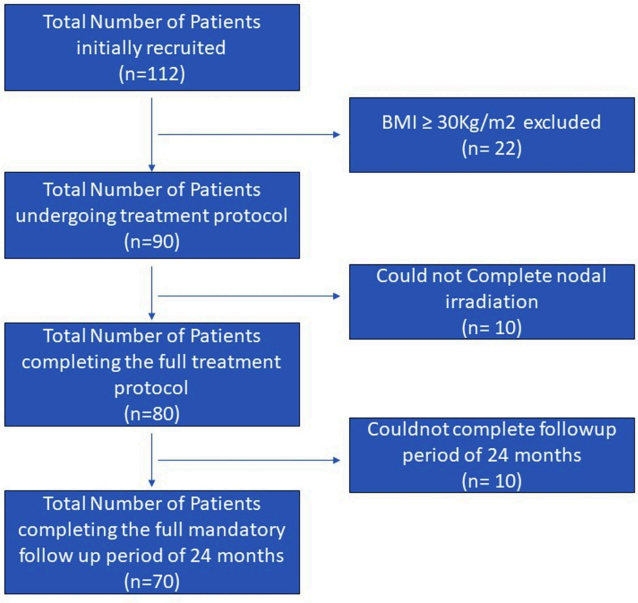

Figure 1. Patient flow chart

BMI: Body mass index

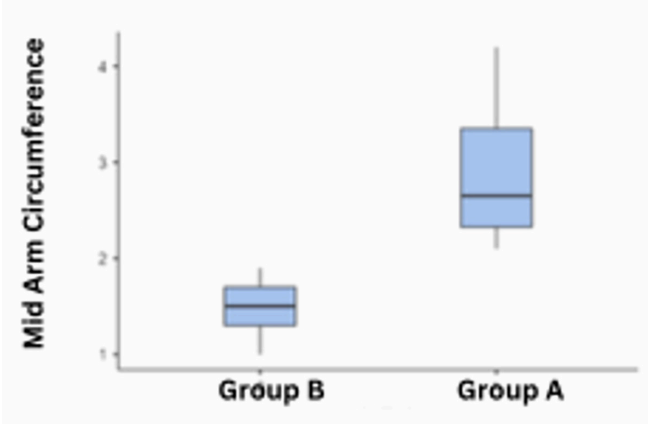

Figure 2. Box plot for arm circumference.

However, on multivariate analysis (Table 1), the effect of these factors was greatly modified. The absence of lymphovascular invasion, perineural invasion and the number of days the drain remained *in situ* retained their protective effect on lymphedema events. But, extracapsular extension, tumour size and the metastatic to total number of lymph nodes removed in level I and II ratio, lost their protective effect as its HR increased from 0.68 (CI: 0.24–1.90, p = 0.458) to 1.28 (CI: 0.18–9.85, p = 0.804), 0.66 (CI: 0.35–1.24, p = 0.192) to 1.39 (CI: 0.62–3.16, p = 0.425) and 0.71 (CI: 0.12–4.29, p = 0.711) to 1.77 (CI: 0.10–29.87, p = 0.693) respectively. The total number of lymph nodes removed in level III and the presence of hormone receptor/HER2 Neu receptor retained their effect to increase the number of lymphedema events even after multivariable analysis and in fact, the effect increased after multivariable analysis from 1.29 (CI: 1.07–1.55, p = 0.007) to 1.59 (CI: 1.23–2.06, p<0.001) and 1.40 (CI: 0.46–4.26, p = 0.552) to 3.22 (CI: 0.64–16.14, p = 0.156) respectively.

Figure 3 depicts the forest plot for the confidence intervals of the risk factors assessesed in this study. Only lymphovascular invasion, total number of lymph nodes removed from level III, total number of days drain was left *in situ* and maximum drain output were found to be significantly (p<0.05) associated with arm lymphedema.

Discussion and Conclusion

Breast cancer is ranked the number one cancer among Indian women with age adjusted rate as high as 25.8 per 100,000 women and a mortality of 12.7 per 100,000 women (3). A recent study of more than 500 patients of breast cancer at a tertiary care centre in north India concluded that the majority of the patients have advanced disease on presentation (17). Late diagnosis and advanced stage have been identified as major determinants of increased mortality. Reasons include lack of access to medical facilities, costs, poor screening programs, lack of awareness and social-cultural attitudes (2). Warmuth et al. (18) evaluated 432 patients who were free of recurrence after surgery and reported that numbness was the most frequent complication (35%), followed by pain (30%), arm swelling (15%), and limitation of arm movement (8%).

The most dreaded long-term complication of axillary lymph node dissection remains upper arm lymphedema. Our study has strategized the three most common identified causes of post treatment arm lymphedema, i.e., obesity, radiation, and neoadjuvant chemotherapy

and tried to identify the histopathological and clinical or surgical factors which can predict arm lymphedema.

To summarize, presence of hormone receptors, absence of complete response, presence of extracapsular extension, metastatic to total lymph node ratio in level I, II or III, total number of lymph node extracted in level III, and drain output were all associated with a higher risk of lymphedema events. Increasing the number of days the drain is left *in situ*, absence of LVI or PNI are associated with decreased number of lymphedema events. Age and total number of lymph nodes removed in level I/II may not have an association with lymphedema if level III node dissection is also done. However, it's important to note that only the absence of LVI, total number of lymph nodes removed in level III, the number of days the drain is left *in situ* and the maximum drain output were significantly associated with breast cancer lymphedema.

The most accurate technique of assessing lymphedema is volumetry. This is accurate but complex and hence not very practical (19). Ozcinar et al. (20) used a perimetric difference greater than 2 cm between the pre- and post-operative measures to diagnose lymphedema and this has been generally been used in routine clinical assessment. The lymphedema incidence and prevalence described in the literature vary widely, possibly due to different measurement methods and intervals between ALND and lymphedema measurement.

In a recently published meta-analysis of more than 84 studies the authors concluded that arm oedema post axillary node dissection is seen up to 30% of cases. Ethnicity (black *vs.* white), higher body mass index, increasing body weight , hypertension, higher cancer stage (III *vs.* I–II), larger tumor size, mastectomy (*vs.* breast conservation surgery), axillary lymph nodes dissection, more lymph nodes dissected, higher level of lymph nodes dissection, chemotherapy, radiotherapy, surgery complications, and higher increase in post operative volume of the limb are all positively correlated with lymphedema. Additionally, breast reconstruction surgery, and adequate finance were found to play a protective role. However, other variables such as age, number

Table 1. Univariate and multivariate analysis of various factors

Factors	Parameter considered	Number (percentage)	Hazard rate (univariable)	Hazard rate (multivariable)
Triple negative breast cancer	Yes	19 (27.1)	1.40 (0.46–4.26, $p = 0.552$)	3.22 (0.64–16.14, $p = 0.156$)
	No	51 (72.9)		
LVI[1]	Yes	25 (35.7)	0.23 (0.09–0.62, $p = 0.004$)	0.18 (0.04–0.87, $p = 0.033$)
	No	45 (64.3)		
PNI[2]	No	45 (64.3)	0.32 (0.09–1.11, $p = 0.072$)	0.67 (0.08–5.78, $p = 0.712$)
	Yes	25 (35.7)		
ECE[3]	Yes	46 (65.7)	0.68 (0.24–1.90, $p = 0.458$)	1.28 (0.18–9.85, $p = 0.804$)
	No	24 (34.3)		
Pathological complete response	No	63 (90.0)	1.86 (0.54–6.42, $p = 0.328$)	0.84 (0.08–8.91, $p = 0.886$)
	Yes	7 (10.0)		
Age	Mean (SD)	50.3 (12.9)	1.02 (0.98–1.06, $p = 0.292$)	0.98 (0.93–1.03, $p = 0.378$)
TLN2[4]	Mean (SD)	17.9 (6.2)	0.98 (0.91–1.06, $p = 0.678$)	0.94 (0.86–1.03, $p = 0.205$)
LNR[5]	Mean (SD)	0.4 (0.3)	0.71 (0.12–4.29, $p = 0.711$)	1.77 (0.10–29.87, $p = 0.693$)
Tumor size	Mean (SD)	3.0 (0.8)	0.66 (0.35–1.24, $p = 0.192$)	1.39 (0.62–3.16, $p = 0.425$)
TLN3[6]	Mean (SD)	4.9 (2.7)	1.29 (1.07–1.55, $p = 0.007$)	1.61 (1.24–2.09, $p<0.001$)
LNR3[7]	Mean (SD)	0.3 (0.3)	0.25 (0.04–1.44, $p = 0.122$)	1.11 (0.06–19.95, $p = 0.942$)
Indwell[8]	Mean (SD)	12.0 (2.0)	0.70 (0.56–0.88, $p = 0.002$)	0.46 (0.28–0.76, $p = 0.002$)
Maximum drain output	Mean (SD)	112.1 (14.0)	1.00 (0.97–1.04, $p = 0.775$)	1.12 (1.04–1.21, $p = 0.004$)

[1]Lymphovascular invasion
[2]Perineural invasion
[3]Extracapsular extension
[4]Total lymph node extracted in level I,II
[5]Positive to negative lymph node ratio in level I,II
[6]Total lymph node extracted in level III
[7]Positive to negative lymph node ratio in level III
[8]Total number of days drain was *in situ*
SD: Standard deviation; LVI: Lymphovascular invasion; PNI: Perineural invasion; ECE: Extracapsular extension; TLN2: Total lymph node extracted in level I,II; LNR2: Positive to negative lymph node ratio in level I,II; TLN3: Total lymph node extracted in level III; LNR3: Positive to negative lymph node ratio in level III; Indwell: Total number of days drain was *in situ*

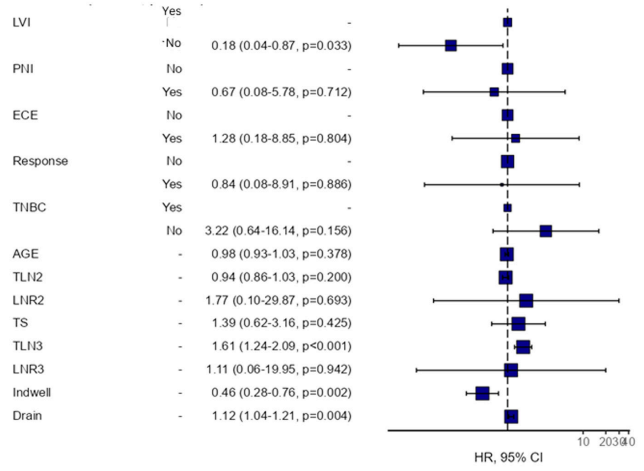

Figure 3. Forest plot depicting Hazard ratio for various factors

LVI: Lymphovascular invasion; PNI: Perineural invasion; ECE: Extracapsular extension; TLN2: Total lymph node extracted in level I,II; LNR2: Positive to negative lymph node ratio in level I,II; TLN3: Total lymph node extracted in level III; LNR3: Positive to negative lymph node ratio in level III; Indwell: Total number of days drain was *in situ*

of positive lymph nodes, and exercise were not correlated with risk of lymphedema (10).

In our study, presence of hormone receptor or Her-2/Neu was associated with increased incidence of arm lymphedema with a HR of 3.22 (0.64–16.14, p = 0.156). Morfoisse et al. (21) in their study of 2018 suggested the protective role of 17β estradiol and VEGF in breast cancer lymphangiogenesis and modulation of the fluid in the soft tissues of the arm. Since patients with hormone receptor positivity undergo anti estrogen therapy, the protective effect of these hormones is lost, resulting in increased incidence of lymphedema.

In our study, the absence of lymphovascular invasion, extracapsular extension and perineural invasion all were associated with a decreased risk of lymphedema events. This correlates well with the retrospective analysis by Invernizzi et al. (22) wherein among the patients who developed arm lymphedema, 46.8% had LVI (as compared to 29.6% in those who did not) and 74.2% had ENE as compared to 61%. Incomplete response to chemotherapy was associated with greater lymphedema events as compared to those having complete response with a HR: 1.86 (0.54–6.42, p = 0.328). This finding could be confounded by a more conservative lymph node dissection in the absence of gross lymphadenopathy (23). Guliyeva et al. (24) in their metanalysis in 2021, found that 13 studies did not find any association of age and breast cancer related lymphedema. Our study has also not demonstrated an increase or decrease in the arm lymphedema events with age [HR: 1.02 (0.98–1.06, p=0.292].

Multiple studies have found that the total number of lymph nodes removed is a significant risk factor for development of arm lymphedema (9, 10, 25). In contrast, our study did not show an increase in lymphedema events as the number of lymph nodes removed increased in level I/II. This is possibly because a significant increase in lymphedema events was observed, both on univariable and multivariable lymph nodes, as the total number of lymph nodes removed in level III increased HR: 1.61 (1.24–2.09, p<0.001).

In our study, we identified that as the metastatic to total lymph node ratio, both in level I/II & III increased, the chances of encountering a lymphedema event increased in multivariable analysis [HR: 1.77 (0.10-29.87, p = 0.693) & 1.11 (0.06–19.95, p = 0.942)]. Various studies have associated number of pathological nodes with increased risk of unilateral lymphedema. Kwan et al. (26) attempted to develop a risk model for breast cancer related lymphedema in which they included 3 patient factors (age, BMI and mammographic breast density), 1 cancer factor (number of pathological lymph nodes), and 1 treatment factor (axillary lymph node dissection) as independent prognostic variables. Zou et al. (27) in their prospective study of 387 women, found that number of positive lymph nodes (HR: 1.1, 95% CI 1.0–1.2) is an independent risk factor for development of lymphedema.

On multivariable analysis, as the post chemotherapy residual tumour size increased, the risk of having a lymphedema event also increased [HR: 1.39 (0.62–3.16, p = 0.425)]. Similar findings can be observed in the studies by Abouelazayem et al. (28), Ren et al. (29) and Aoishi et al. (30).

Suction drains are an important component of the surgical procedure of modified radical mastectomy/axillary node dissection. Drain output along with the number of days that drain remains *in situ* may be an important predictor of development of arm lymphedema (31). Ackroyd and Reed (31) in their study did not find any difference in seroma formation, lymphedema, infection rate between individuals in which drain was removed on 5th postoperative day vis-à-vis when drain output was <30 mL. We however noted a significant increase in lymphedema events as the maximum drain output increased and a decrease in risk of lymphedema events as the number of days the drain remain *in situ* is increased. This is a novel finding of our study and must be explored in further studies.

Our study was prospective with stringent follow up criteria. Strength of our study includes the fact that classical high-risk features like obesity, differences in surgery and irradiation were controlled for. Therefore, we can be confident about the association of the measured factors with the incidence of arm lymphedema.

Study Limitations

The study has many limitations some of which include the fact that it's a single institution study, surgical techniques may vary between surgeons, the use of circumferential measurement of arm as a marker of lymphedema may be less accurate, small sample size and 2 years' follow-up may be insufficient in some cases for development of lymphedema.

Future areas of research may use this study to develop nomograms or algorithms to calculate the risk of lymphedema and include novel factors like maximum drain output and total duration of days the drain remains *in situ* as important associations with breast lymphedema.

In non-obese, locoregionally advanced breast cancer patients undergoing neoadjuvant chemotherapy, modified radical mastectomy with level III dissection, and postoperative irradiation, the incidence of unilateral arm lymphedema is significantly influenced by several factors. Specifically, an increase in the total number of lymph nodes removed in level III and higher maximal drain output are associated with a higher likelihood of lymphedema events. Conversely, prolonging the duration of drain placement and the absence of lymphovascular invasion are correlated with a significant decrease in the occurrence of

lymphedema events. Further multicentric and high powered studies may be done regarding the contribution of hormone receptor positivity, lymph node ratio, and response to neoadjuvant chemotherapy towards lymphedema development.

Ethics Committee Approval: Institutional Ethical Committee clearance was taken from the institutional board (Army Hospital Ethics Committee - IEC no: 46/2018; dated: 06.08.2018).

Informed Consent: Informed consent regarding the study was taken from each individual.

Authorship Contributions

Surgical and Medical Practices: S.D., A.A., V.K.A., S.K.D., R.S., N.S.; Concept: S.D., A.A., R.S.; Design: S.D., V.K.A., N.S.; Data Collection or Processing: S.D., A.A., V.K.A., S.K.D., R.S., N.S.; Analysis or Interpretation: A.A., V.K.A., R.S., N.S.; Literature Search: A.A., V.K.A., N.S.; Writing: S.D., A.A., S.K.D., R.S.

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