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EUROPEAN JOURNAL OF BREAST HEALTH

ORIGINAL ARTICLES

NOVEL IDEA

CASE REPORTS

Turkish Federation of Breast Diseases Societies

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

Société Internationale de Sénologie | Senologic International Society

Global Federation of Breast Healthcare Societies

SIS is the official supporter of the European Journal of Breast Health

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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).

The European Journal of Breast Health indexed in PubMed Central, Web of Science-Emerging Sources Citation Index, TUBITAK ULAKBIM TR Index, Embase, EBSCO, CINAHL.

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European Journal of Breast Health

Instructions to Authors

The European Journal of Breast Health (Eur J Breast Health) is an international, open access, online-only periodical published in accordance with the principles of independent, unbiased, and double-blinded peer-review.

The journal is owned by Turkish Federation of Breast Diseases Societies and affiliated with Senologic International Society (SIS), and it is published quarterly on January, April, July, and October. The publication language of the journal is English. The target audience of the journal includes specialists and medical professionals in general surgery and breast diseases.

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- Editorial and technical processes of the journal,
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• Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,

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Original Articles: This is the most important type of article since it provides new information based on original research. The main text of original articles should be structured with "Introduction", "Materials and Methods", "Results", "Discussion and Conclusion" subheadings. Please check Table 1 for the limitations for Original Articles.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section,and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

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Review Article	5000	250	50	6	10 or total of 20 images
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

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software,and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

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When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

While citing publications, preference should be given to the latest, most up-to-date publications. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/ MEDLINE/PubMed. All authors should be listed if an article has six or less authors; it should not be represented by "et al." in articles. Arabic numbers in parentheses. References published in PubMed should have a PMID: xxxxxx at the end of it, which should be stated in parenthesis. The reference styles for different types of publications are presented in the following examples.

Journal Article: Little FB, Koufman JA, Kohut RI, Marshall RB. Effect of gastric acid on the pathogenesis of subglottic stenosis. Ann Otol Rhinol Laryngol 1985; 94:516-519. (PMID: 4051410)

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.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

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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Original Article

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The Senologic International Society Survey on Ductal Carcinoma *In Situ*: Present and Future

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ABSTRACT

Objective: Therapeutic management of ductal carcinoma *in situ* (DCIS) is heterogeneous among countries worldwide, and some treatment indications are still controversial. To investigate DCIS management in different countries; identify both consensual practices and controversial topics; and survey opinions about the future management of DCIS.

Materials and Methods: The Senologic International Society network members participated to an online survey using a questionnaire, between November 2021 and February 2022.

Results: Twenty-two responses from 20 different countries showed that organized breast cancer screening programs were present for 87% participants, and DCIS cases represented 13.7% of all breast cancers. Most participants used the grade classification (100%), the morphological classification (78%) and performed immunohistochemistry assays (73%). In case of conservative treatment, the mean re-excision rate was 10.3% and clear margins of mean 2.5 mm were considered healthy. Radical mastectomy rate was 35.5% with a breast reconstruction rate of 53%. Tumor bed boost indications were heterogeneous, and 73% of participants indicated hormone therapy for hormone-positive DCIS. Surgery and radiotherapy omission for some low-risk DCIS were considered by 73% of participants. Multigene assays were used by 43% of participants. Concerning future changes in DCIS management, participants mostly answered surgical de-escalation (48%), radiotherapy de-escalation (35) and/or active surveillance for some cases (22%).

Conclusion: This survey provided an overview of the current practices of DCIS management worldwide. It showed that some areas are rather consensual: incidence increases over time, treatment in young women, pathological classifications, definition of healthy margins, the skin-sparing mastectomy and

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immediate breast reconstruction. However, some topics are still debated and result in heterogeneous practices, such as evolution in the age of diagnosis, the benefit of de-escalation in low-risk DCIS among elderly women, indications for hormone therapy, radiotherapy omission, or multigene assays. Further evidence is needed to reach consensus on these points, and innovative approaches are still under evaluation in clinical trials. The International Senologic Society, by its members, encourages precision medicine and personalized treatments for DCIS, to avoid overtreatment and overdiagnosis, and provide better healthcare to women with DCIS.

Keywords: Ductal carcinoma *in situ*; clinical practices, survey; precision medicine; treatment de-escalation; innovative approaches

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Key Points

- Ductal carcinoma *in situ* (DCIS) is defined as a proliferation of malignant cells in the breast ducts without crossing of the basal membrane.
- Differences in DCIS characteristics, diagnosis and management exist between countries worldwide.
- The Senologic International Society (SIS) is dedicated to promoting breast health and improving the care of breast cancer patients, taking into consideration, medical, social, economic and ethical constraints. The objective of this survey was to investigate the management of DCIS though members of the SIS.
- As active members of the SIS and breast specialists, participants were invited if they wished to participate to be co-authors to the pending publication.

Introduction

Ductal carcinoma *in situ* (DCIS) of the breast is defined as a proliferation of malignant cells in the lumen of mammary ducts without visible rupture of the basement membrane on optic microscopy. This term encompasses a highly heterogeneous group of lesions that differ in their clinical presentation, histologic and biologic characteristics, and outcomes (1). DCIS is considered as an early form of breast cancer [$Tis_{(DCIS)}$ according to the 2018 Tumor-node-metastasis classification and stage 0 (TisN0M0) according to the Union for International Cancer Control (UICC) classification] (2). Breast cancer screening, whether individual or organized, has increased the diagnosis of DCIS as this pathology is mostly asymptomatic (it can nonetheless be the cause of a nipple discharge or a palpable mass). Thus, the frequency of DCIS has increased over the last 30 years (3-6).

The therapeutic management of a DCIS is aimed at preventing the development of an invasive breast cancer (IBC). Different treatments are available for DCIS: surgery; radiotherapy; and hormonal therapies. Several factors are involved in the choice of appropriate treatment plan: the age of the patient; her comorbidities and risk factors; the size of the DCIS and its prognostic factors; the clinical presentation (nipple discharge, mass); and the patient choice. Treatment indications are different among countries worldwide, and they evolved over time. This shows that some are still controversial.

Without treatment, it is estimated that about 8 to 17.6% of DCIS will progress to invasive cancer at 10 years, and this proportion has been reported to be up to 20-30% in some studies (7, 8). It therefore brings up the issue of overtreatment because more than 70% of patients diagnosed with DCIS will not develop an IBC. Current areas of concern include the need for better patient selection to identify those who will develop IBC and those who will not. Indeed, possibilities of therapeutic optimization for some cases of DCIS may be abstention from radiotherapy, or even abstention from all treatment and "active" surveillance.

The Senologic International Society (SIS), founded in 1976, affiliated to the UICC since 2019, is a unique worldwide federation of scientific societies, breast cancer patients associations and groups, located across five continents, with a priority mission: to improve breast health by constantly putting the patient in the center of its concerns. It is a society turned towards the future with a particular focus on innovation, transdiscipline inclusivity and contribution to optimization of breast cancer care (www.sisbreast.org).

In view of the current concerns, the objective of this survey was to investigate, through members of the SIS, a wide range of questions about DCIS management and national guidelines. Each participant was asked to collate the DCIS data and recommendations of their own country to answer the questionnaire, leading to the identification of both consensual practices and controversial topics, which would require further investigation and, finally, opinions about the future management of DCIS.

Materials and Methods

Members of the SIS network were invited to participate in an online survey with a Microsoft Forms questionnaire. Between the 17th of November 2021 and the 15th of February 2022, participants were invited to answer the questionnaire via email. The answers were directly recorded into an online database and only one response per participant was allowed, but more than one response was authorized from the same countries, because of regional disparities in any single country.

The online survey consisted of 27 questions. Section 1 (6 questions) was about the respondents' information, such as affiliation and medical specialty, and the number of cases of DCIS managed per year. Then, in Section 2 (2 questions) the respondents were asked about discovery mode, such as presence of a breast cancer screening program and its modalities. Section 3 was about epidemiology (4 questions) and asked about the incidence of DCIS and its evolution. After that, respondents were asked about pathology in Section 4 (3 questions)

concerning the use of different classifications (grade, morphology and immunohistochemistry assays). In Section 5 (7 questions) respondents' actual practices concerning treatment (surgery, radiotherapy, and hormonal therapy) were investigated. Finally, in Section 6 (5 questions) respondents were asked about future perspectives concerning topics such as treatment de-escalation and molecular/genetic signatures. The questionnaire is available as Supplementary Material S1 (Appendix 1).

Results

Twenty-two completed questionnaires were returned. Participants came from 20 different countries on five continents (Figure 1), with 2.7 billion inhabitants. Participants were mostly surgeons (77%, n = 17), radiologists (14%, n = 3), or radiation (5%, n = 1) or medical (5%, n = 1) oncologists. Results of the survey are shown in Table 1.

Most participants' countries had organized breast cancer screening (87%, n = 19). All of these screening programs included women between 50 and 65 years-old and most reported a recall interval of every two years (63%, n = 10).

Incidence showed that DCIS cases represented 13.7% of all breast cancers [standard deviation (SD) = 8.8%, range 2.5 – 35%]. Most participants noted that DCIS incidence had increased in the last decade (77%, n = 17), while the age of diagnosis was stable (27%, n = 6), increased (46%, n = 10) or decreased (27%, n = 6). Moreover, 64% of participants reported that the proportion of high-grade DCIS was stable over time in the last decade (n = 14) or had increased (36%, n = 8) but no respondent reported a decrease.

Concerning histopathology, all participants used the grade classification (n = 22), the majority used the morphological classification (78%, n = 17) and performed immunohistochemistry assays (73%, n = 16).

Answers in the DCIS treatment section showed that healthy margins ranged from 0 to 10 mm, with a mean of 2.5 mm (SD = 2 mm). Mean radical mastectomy rates were 35.5% (SD = 28.2%, range 3 – 100%). In case of radical mastectomy, mean breast reconstruction rate was 53% (SD = 33.8%, range 0 – 100%). Conversely, in case of conservative treatment the mean re-excision rate was 10.3% (SD = 11.3%, range 0 – 50%). Then, in case of conservative treatment, participants were asked about indications for sentinel lymph node biopsy. The most frequently reported indications were: clinical/radiological mass (26%, n = 6); high grade (22%, n = 5); and larger size (22%, n = 5). Seven (32%) participants reported that tumor bed boost in case of conservative treatment was not performed, while 7 (32%) indicated that tumor bed boost would be performed if risk factors were present and 8 (36%) performed it systematically. Finally, most participants indicated hormone therapy if hormone receptors were expressed by the tumor (73%, n = 16) and 3 (14%) reported no indication for this treatment.

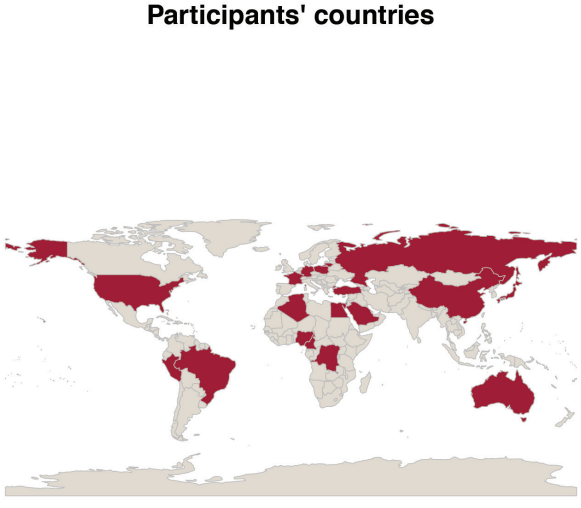

Figure 1. Participants' countries

Concerning future perspectives of DCIS management, 16 (73%) participants considered (yes or maybe) surgery and radiotherapy omission for some low-risk DCIS. The most frequent situations reported were elderly patients (41%, n = 7), low grade (24%, n = 4) and presence of comorbidities (24%, n = 4). When asked their estimate of 5-year risk of progression to an invasive form in untreated DCIS, participants mean response was 26.6% – 35.9% (mean upper and lower bounds, respectively). Concerning molecular scores and signatures, 12 participants (57%) did not use them in routine practice. When asked about future changes in DCIS management, participants mostly answered surgical de-escalation (48%, n = 11), radiotherapy de-escalation (35%, n = 8) and/or active surveillance for some cases (22%, n = 5).

Discussion and Conclusion

With its international survey, the SIS wanted to investigate the current trends and therapeutic management of DCIS worldwide. As reported in Table 2, this survey highlighted that while some points are rather consensual, other are still controversial.

Diagnosis

While some cases of DCIS are diagnosed because of a nipple discharge (typically with blood or serous liquid) or a palpable mass, most are discovered by screening which may be either individual or as part of an organized program. Most of the respondents' countries have an organized screening program, thus explaining the rate of 13.6% of DCIS among all breast cancers. Interestingly, participants' responses about evolution of the age at diagnosis were heterogeneous. This could be explained by differences in organized screening programs. Participants also reported that the proportion of high-grade DCIS had a tendency to either be stable or to increase.

Concerning pathology, the 2019 World Health Organization's (WHO) classification of breast tumors recommends the grade classification (9), which is based on cytonuclear morphology. Morphological classification was described in previous versions but not in the latest one. It has different subtypes: Comedo (comedocarcinoma) and non-comedo, further divided into cribriform, micropapillary, papillary and solid (10). Still, the latest classification considers solid papillary carcinoma *in situ* as a separate entity (9). Finally, WHO states that there is no universal agreement on the benefits of hormone receptor testing in DCIS (9) as hormone therapy is still controversial.

Surgery

Breast-Conserving Surgery

Breast-conserving surgery (BCS) is now widely performed for DCIS, and its rate increased along with the progressive discovery of small infraclinical DCIS through implementation of mammographic screening. Scientific data has demonstrated that BCS is a safe technique compared to modified radical mastectomy (MRM) (11).

Table 1. Survey results

	Question	Result	
		N / Mean	(% / SD)
Discovery mode	**Do you have organized breast cancer screening in your country?**		
	No	3	(13.6%)
	Yes	19	(86.4%)
	If yes, what are the screening modalities? (age limits and frequency)		
	Age limits (years)		
	NA	1	--
	35-69	1	(4,8%)
	35+	1	(4,8%)
	40-65	1	(4,8%)
	40-69	1	(4,8%)
	40+	6	(28,6%)
	45+	1	(4,8%)
	50-69	3	(14,3%)
	50-74	2	(9,5%)
	50+	2	(9,5%)
	Frequency		
	NA	3	--
	Annual	6	(37,5%)
	Every 2 years	10	(62,5%)
Epidemiology	**What is the proportion of DCIS in your country in relation to all breast cancers? (%)**		
	NA	3	
	Mean (SD)	13.7	(8.8)
	Range	2.5 - 35.0	
	What is the evolution of this incidence in the last decade?		
	Decrease	0	(0%)
	Stable	5	(22.7%)
	Increase	17	(77.3%)
	Has the average age of patients diagnosed with DCIS changed in the last decade?		
	Decrease	6	(27.3%)
	Stable	6	(27.3%)
	Increase	10	(45.5%)
	Has the proportion of high grade DCIS changed in the last decade?		
	Decrease	0	(0%)
	Stable	14	(63.6%)
	Increase	8	(36.4%)
Pathology	**Do you use the grade (low, intermediate, high) classification?**		
	Yes	22	(100%)
	Do you use the morphological (papillary, cribriform, massive, cliniging and comedocarcinoma) classification?		
	Don't know	1	(4.5%)
	No	4	(18.2%)
	Yes	17	(77.3%)
	Do you perform immunohistochemistry (hormone receptors, HER2) assays for DCIS?		
	No	6	(27.3%)
	Yes	16	(72.7%)

Table 1. continued

	Question	Result	
		N / Mean	(% / SD)
Treatments	**What are the margins considered healthy for DCIS in millimeters?**		
	Mean (SD)	2.5	(2.0)
	Range	0.0 - 10.0	
	What is the rate of total mastectomies you perform for DCIS? (%)		
	Mean (SD)	35.5	(28.2)
	Range	3.0 - 100	
	In case of total mastectomy, what is your reconstruction rate? (%)		
	NA	1	
	Mean (SD)	53.0	(33.8)
	Range	0.0 - 100	
	In case of conservative treatment, what is your rate of re-excisions? (%)		
	Mean (SD)	10.3	(11.3)
	Range	0.0 - 50.0	
	In case of conservative treatment, what are your indications for sentinel lymph node biopsy?		
	Clinical / radiological mass	6	(26,1%)
	Micro-invasion	2	(8,7%)
	Extensive oncoplastic surgery	1	(4,3%)
	Age < 45	1	(4,3%)
	Age > 65	1	(4,3%)
	Multifocality	1	(4,3%)
	Diagnosis on core needle biopsy alone	1	(4,3%)
	High grade	5	(21,7%)
	Large size	5	(21,7%)
	Upper outer quadrant localization	3	(13%)
	None	3	(13%)
	Comedonecrosis	2	(8,7%)
	In case of conservative treatment, do you perform a tumor bed boost in addition to breast radiotherapy?		
	No	7	(31.8%)
	Yes, if risk factor	7	(31.8%)
	Yes, systematic	8	(36.4%)
	Do you have indications (which ones) for hormone therapy in case of DCIS?		
	Age < 60 years	1	(4.5%)
	Conservative surgery	1	(4.5%)
	Hormone receptors positivity	16	(72.7%)
	No	3	(13.6%)
	Strong family history, other risk factors	1	(4.5%)

Table 1. continued

	Question	Result	
		N / Mean	(% / SD)
Perspectives	**Would you consider omitting surgery and radiotherapy for some low risk DCIS?**		
	Maybe	10	(45.5%)
	No	6	(27.3%)
	Yes	6	(27.3%)
	If yes/maybe, which ones?		
	Elderly patients	7	(41,2%)
	Low grade	4	(23,5%)
	Comorbidities	4	(23,5%)
	Small tumors	3	(17,6%)
	Low risk	3	(17,6%)
	In your opinion, what is the 5-year risk of progression to an invasive form of untreated DCIS? (% range)		
	NA	5	
	Lower bound mean (SD)	26.6	(19.2)
	Lower bound range	0.5 - 60.0	
	Higher bound mean (SD)	35.9	(21.7)
	Higher bound range	1.0 - 80.0	
	What is the place of molecular scores & signatures in case of DCIS in your practice?		
	Adjuvant radiotherapy decision	2	(8.7%)
	Emerging (not specified)	3	(13.0%)
	None	12	(56.5%)
	Risk of progression assessement	2	(8.7%)
	Routine practice (not specified)	2	(8.7%)
	Surgery decision	1	(4.3%)
	What do you think will change in the future in the management of DCIS?		
	Surgical de-escalation	11	(47,8%)
	Surgical escalation	1	(4,3%)
	Radiotherapy de-escalation	8	(34,8%)
	Active surveillance	5	(21,7%)
	Molecular signatures	4	(17,4%)
	Non-invasive treatments (high radiofrequency, ultrasound)	1	(4,3%)
	Prevention	1	(4,3%)
	Targeted therapies	1	(4,3%)

* for these open questions, some answers were multiple and therefore the total of responses can be superior to the total of participants

However, BCS raised two additional issues regarding local recurrence risk: Negative margins and adjuvant radiotherapy. Radiotherapy is discussed in the dedicated section below. Today, a margin of more than 2 mm has been found to minimize the local recurrence risk for BCS with radiotherapy (12, 13). Moreover, wider negative margins do not improve local control for DCIS (12, 14) and are not recommended by the American Society of Clinical Oncology (15).

Subsequently, new surgical techniques have been developed in conjunction with the widespread adoption of BCS and can be applied to patients with DCIS. Oncoplastic procedures include different techniques ranging from ipsilateral glandular rearrangement to contralateral reduction mammoplasties and symmetry procedures. However, data on radiotherapy toxicity in patients (with invasive or *in situ* breast cancer) undergoing oncoplastic procedures is still limited and studies are contradictory (16, 17). Thus, although these techniques may reduce radiotherapy complications associated with larger-breasted patients, they need additional procedures with their specific complications and in some case may even increase radiotherapy toxicity (18). Therefore, oncoplastic techniques can be applied in

Table 2. DCIS management consensual and debated topics

	Consensual	Debated
Discovery	Incidence increased over the last decade (mean 13.7% of all breast cancers)	None
Age	Treatment is necessary for DCIS in young women	Evolution in the age of diagnosis of DCIS Benefit of treatment de-escalation in low-risk DCIS in elderly women
Pathology	Grade classification is necessary for DCIS	Morphological classification and immunohistochemistry assays can be performed Proportion of high-grade DCIS is stable or increases
Surgery	Breast-conserving surgery: margins are considered healthy if ≥ 2 mm Skin-sparing mastectomy and immediate breast reconstruction are possible Contralateral risk-reducing mastectomy is not recommended Sentinel lymph node biopsy should be in association of a radical treatment	Oncoplastic procedures with breast-conserving surgery: benefits on radiotherapy toxicities not yet demonstrated Indications of the nipple-sparing mastectomy
Radiotherapy	Reduces local recurrence but does not affect mortality	Radiotherapy omission Genomic signatures Tumor bed boost in case of breast-conserving surgery
Hormone therapy	Can be prescribed for hormone-receptor positive DCIS	It does not reduce mortality Less benefit after 60 years-old

DCIS: ductal carcinoma *in situ*

DCIS according to specific indications to obtain a better aesthetic result. However, oncoplastic techniques should not be applied with the main aim of reducing radiotherapy toxicity as insufficient data is available.

Mastectomy

Ipsilateral MRM was once the standard treatment for DCIS. Nowadays, this technique has been replaced by BCS and, in case of diffuse DCIS, replaced by conservative mastectomies, such as the nipple-sparing mastectomy (NSM) or the skin-sparing mastectomy (SSM). MRM remains appropriate for patients who refuse or are contraindicated for reconstructive surgery.

Concerning SSM, several studies evaluated the oncologic safety of this technique. One study conducted on 199 patients (102 with SSM and 97 with MRM) found a higher 5-year recurrence rate in the SSM group (5.9% *versus* 0%, $p = 0.012$) (19). In contrast, a study on 399 patients (192 with SSM and 207 with MRM found no difference in 10-year recurrence rates (1.04% *versus* 0.97%, $p>0.05$) (19). Similarly, two cohort studies were conducted in DCIS patients with SSM only. The first included 223 and found a recurrence rate of 5.1% with a mean follow up of 82 months (20). The second included 44 DCIS patients with SSM with a follow up of at least 6 years, and found no recurrences (21).

For NSM, there was another issue to assess - the nipple recurrence rate. In a retrospective cohort of 199 NSM (22) with a median follow up of 97 months, the authors found a local recurrence rate of 4.5%, and a nipple recurrence rate of 3%. Multivariate analysis showed that negative progesterone receptor status was an independent risk factor for local recurrence rate. Surprisingly, margin status and tumor-to-nipple distance were not associated with increased risk for either local or nipple recurrence. In another retrospective cohort of 69 DCIS patients with NSM (23) and a mean follow-up 143 months, local recurrence rate was 11.6%, of which 1.4% was nipple recurrence. The disease-free survival rate was 88.4% and the overall survival rate was 98.6%. Finally, in a retrospective cohort of 41 NSM (24) the authors reported the long-term follow-up data for the remaining 19 patients (46%). In this group, they observed one local recurrence (5.3%).

Conservative mastectomies seem therefore to be oncologically safe, except for the cases in which there may already be DCIS involvement of the nipple (i.e., in the cases of nipple discharge and/or a short tumor-to-nipple distance). Immediate breast reconstruction is therefore feasible for some DCIS.

For women with DCIS, there is also a trend toward increased contralateral risk-reducing mastectomy (RRM) (25). However, benefits of contralateral RRM are not yet demonstrated and may be non-existent. Indeed, from a prospective database of 2,759 patients who had unilateral conservative surgery for DCIS between 1978 and 2011, Miller et al. (26) found a contralateral cancer rate 2.5 times lower than the ipsilateral recurrence rate, estimated at 5.8% at 10 years (compared with 14.5% ipsilateral recurrence). Therefore, the benefit of contralateral RRM would be less compared to unilateral mastectomy for DCIS, which is decreasing in the current context of surgical de-escalation for DCIS. In another retrospective study of 24,766 bilateral RRM for unilateral DCIS, the authors showed that the financial cost of this procedure is significant and has been steadily increasing since 2005 (27). For these reasons, and especially because of insufficient benefit from reduced mortality, performing contralateral RRM cannot be recommended for DCIS.

Radiotherapy

Historically, whole breast irradiation was performed in case of BCS for DCIS based on the data from studies of IBC (18). Since then, several randomized controlled trials have been published on the benefit of radiotherapy after BCS for DCIS. Two meta-analyses published in 2007 (28) and 2010 (29) showed that radiotherapy reduced 10-year local recurrence rates by 15% (even for low-risk patients) and the odds ratio (OR) of local recurrence was 0.4 [95% confidence interval (95% CI) =0.31–0.53, p<0.001]. However, these meta-analyses failed to demonstrate a significant benefit in distant recurrence rates and in survival. Conversely, one of them showed a significant increase in contralateral breast events [3.85% *versus* 2.5%, OR = 1.53 [95% CI 1.05–2.24], p = 0.03]. A more recent meta-analysis, published in 2018 (30), showed a decreased risk of local [relative risk (RR) = 0.53 (95% CI = 0.45–0.62)] and regional [RR = 0.54 (95% CI = 0.32–0.91)] recurrence, but still no benefit in distant recurrence nor mortality.

Moreover, practices are heterogeneous regarding additional tumor bed boost during radiotherapy. Participants reported different practices as some of them always did additional tumor bed boost while other never did, and some only in the presence of risk factors. A review showed that tumor bed boost was more often performed when risk factors, such as young age (<40 or 50 years), presence of clinical symptoms, tumor size >20 mm, high nuclear grade, presence of necrosis, insufficient surgical margins, associated atypical lesions, and lobular carcinoma *in situ*, were present (31).

Therefore, indications for post-operative radiotherapy have been questioned. It has been suggested that radiotherapy could be omitted for low-risk patients, such as those with low grade, small tumor size and elderly patients. Moreover, multigene assays have been developed for this purpose and are discussed below. Today, trials are currently underway to evaluate the omission of radiotherapy for some patients. For instance, the ROMANCE trial (Radiotherapy Omission in Low Risk Ductal in Situ Carcinoma Breast) is currently underway and includes patients aged 55 and older to better define the benefits/disadvantages and indications for radiotherapy. This tendency suggests that future management of DCIS would omit radiotherapy for low-risk patients. However, it is still necessary to identify those patients, whether by clinical characteristics (age), pathological features of DCIS (low grade), or multigene assays.

Hormone Therapy

The place of hormone therapy in the treatment of DCIS is still debated. Two major trials have evaluated the impact of tamoxifen after conservative surgery and radiation therapy for the adjuvant treatment of DCIS. The long-term analysis of the NSABP B-24 randomized controlled trial, which compared tamoxifen (n = 899) *versus* placebo (n = 900) in patients with DCIS treated by conservative surgery and radiotherapy, found a reduced risk of ipsilateral invasive recurrence (6.6% *versus* 9.0% at 5 years) with tamoxifen (32). Participants were both pre-menopausal (35.9%) and post-menopausal (64.1%). Moreover, addition of tamoxifen did not result in a statistically significant reduction in mortality risk [hazard ratio (HR)=0.86, 95% CI 0.66–1.11].

The UK/ANZ DCIS randomized controlled trial, which compared three groups: radiotherapy *versus* tamoxifen *versus* radiotherapy + tamoxifen in 1701 patients who had undergone surgery for DCIS, found a reduction in the risk of *in situ* recurrence (HR=0.70, 95% CI 0.51-0.86), with no change in invasive recurrence (33). Similarly, mortality was not affected by tamoxifen.

The randomized controlled trial NSABP B-35, which included 3,104 postmenopausal patients with hormone receptor-positive DCIS treated with conservative surgery and radiotherapy, compared anastrozole versus tamoxifen. The results showed a significant reduction in contralateral breast cancers with anastrozole (HR=0.64; 95% CI, 0.43–0.96; p = 0.032) compared to tamoxifen but there was no benefit of one therapy compared to the other in patients over the age of 60 (34).

Due to the uncertain benefit and the presence of toxicities (e.g., venous thromboembolic events or higher risk of endometrial cancer described with tamoxifen (34), hormone therapy has no place in the management of DCIS in many European countries. Conversely, in the United States, hormone therapy is more widely prescribed. Indeed, the National Comprehensive Cancer Network describes the option of prescribing hormone therapy for 5 years in women with hormone-receptor-expressing DCIS treated with surgery alone or conservative surgery and radiation therapy, without mentioning age limits (35). However, evidence reported above suggests that hormone therapy may have less benefit among older women. Therefore, this treatment could be considered for younger women with some DCIS subtypes, although it remains controversial. Efforts should be made to assess which women will benefit the most (i.e., improve survival) with DCIS hormone therapy.

Multigene Assays

Similarly, with what has happened in invasive breast cancer, multigene assays have been developed for DCIS that provide prognostic and predictive scores. Two are currently used: Oncotype DX DCIS and DCISionRT. These multigene assays stratify different groups according to their risk of local recurrence.

A study evaluating Oncotype DX DCIS in women treated by BCS alone (n = 571) versus BCS and radiotherapy (n = 689) showed that low-risk patients treated by BCS alone had a small benefit from radiotherapy by reducing the 10-years local recurrence, while those with a high risk had a greater benefit (36).

Similarly, in another study assessing DCISionRT in the SweDCIS randomized clinical trial cohort (504 women with DCIS) the authors showed that in the high risk group, radiotherapy significantly decreased relative 10-year local recurrence (both for *in situ* and invasive tumors) while in the low risk group there were no significant risk differences observed with radiotherapy (37). In another study of 526 women with DCIS, the authors showed that among low-risk DCIS defined with classical clinical and pathological characteristics, this signature reclassified 42% of patients into the high-risk group and showed that these patients had significant benefit from radiotherapy (38).

These findings suggest that multigene assays are a promising tool to distinguish high and low-risk DCIS. However, to date there is no data about benefits in terms of mortality. Multigene assays are emerging in routine clinical practice among the survey participants, and future insights could improve radiotherapy or hormone therapy decisions to better select patients who will benefit from it.

In conclusion, this survey provided an overview of the current practices of DCIS management worldwide. While some points are rather

consensual (such as healthy margins or pathological classifications), others are still widely debated and result in heterogeneous practices. DCIS treatments have significantly evolved over the last few decades, resulting in different surgical techniques or radiotherapy and hormone therapy indications. Further investigations are needed to reach consensus on these points. Moreover, while several clinical trials and observational studies are available, to our knowledge this is the first international survey published about DCIS management. This kind of initiative provides valuable insights about this topic as they could not be investigated otherwise. Finally, the SIS, through its members, encourages precision medicine and personalized treatments for DCIS, to avoid overtreatment and overdiagnosis, and provide better healthcare to women with DCIS.

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Appendix 1. SIS Questionnaire: Ductal Carcinoma *In Situ*

SIS Questionnaire: Ductal Carcinoma In Situ

Ductal Carcinoma In Situ (DCIS) is defined as a proliferation of malignant cells in the breast ducts without crossing of the basal membrane. Differences in DCIS characteristics, diagnosis and management exists between countries worldwide. The Senologic International Society (SIS) is dedicated to promoting breast health and improving the care of breast cancer patients, taking into consideration, medical, social, economic and ethical constraints. The objective of this survey is to investigate the management of DCIS though members of the SIS. It is composed by 6 sections: participant information; discovery mode; epidemiology; pathology; treatments and perspectives. Estimated time of completion is 15 minutes. Some questions are mandatory, however approximate/estimated answers are possible. This survey will be the subject of a publication in the PubMed-referenced peer-reviewed European Journal of Breast Health. As active members of the SIS and breast specialists, participants are invited if they wish to participate as co-authors to the pending publication.

Please let us know if there is any issue for your response.

Pr Carole Mathelin, M.D., Ph.D.

Deadline for response : 10th February 2022

* Obligatoire

Participant information

1. What is your first and last name? *

2. What is your affiliation? *

3. What is your medical specialty? *

4. How many DCIS do you manage per year? *

5. Would you like to participate in the publication of the article in the EJBH? *

- ◯ Yes
- ◯ No

6. If yes, what is your ORCID number?

Discovery mode

7. Do you have organized breast cancer screening in your country? *

- ◯ Yes
- ◯ No
- ◯ Don't know

8. If yes, what are the screening modalities? (age limits and frequency)

Epidemiology

9. What is the proportion of DCIS in your country in relation to all breast cancers? (%) *

10. What is the evolution of this incidence in the last decade? *

- ◯ Increase
- ◯ Stable
- ◯ Decrease

11. Has the average age of patients diagnosed with DCIS changed in the last decade? *

- ◯ Increase
- ◯ Stable
- ◯ Decrease

12. Has the proportion of high grade DCIS changed in the last decade? *

- ◯ Increase
- ◯ Stable
- ◯ Decrease

Pathology

13. Do you use the grade (low, intermediate, high) classification? *

- ◯ Yes
- ◯ Don't know
- ◯ No

14. Do you use the morphological (papillary, cribriform, massive, cliniging and comedocarcinoma) classification? *

- ◯ Yes
- ◯ Don't know
- ◯ No

15. Do you perform immunohistochemistry (hormone receptors, HER2) assays for DCIS? *

- ◯ Yes
- ◯ Don't know
- ◯ No

Treatments

16. What are the margins considered healthy for DCIS in millimeters? *

17. What is the rate of total mastectomies you perform for DCIS? (%) *

18. In case of total mastectomy, what is your reconstruction rate? (%) *

19. In case of conservative treatment, what is your rate of re-excisions? (%) *

20. In case of conservative treatment, what are your indications for sentinel lymph node biopsy? *

21. In case of conservative treatment, do you perform a tumor bed boost in addition to breast radiotherapy? *

22. Do you have indications (which ones) for hormone therapy in case of DCIS? *

Perspectives

23. Would you consider omitting surgery and radiotherapy for some low risk DCIS? *

- ◯ Yes
- ◯ Maybe
- ◯ No

24. If yes, which ones?

25. In your opinion, what is the 5-year risk of progression to an invasive form of untreated DCIS? *

26. What is the place of molecular scores & signatures in case of DCIS in your practice? *

27. What do you think will change in the future in the management of DCIS? *

Original Article

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The Factors for Success and Lack of Success in the Breast Cancer Patient Care Pathway: A Qualitative Study From the Health Care Staff Perspective

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ABSTRACT

Objective: To produce information about factors related to successful and unsuccessful breast cancer care pathways from the health care staff perspective.

Materials and Methods: An electronic qualitative survey was used to collect data simultaneously from hospitals located in four different countries, focusing on four professional groups: diagnostic radiographers; radiation therapists; breast cancer nurses; and biomedical laboratory scientists (n = 23). The hospitals participating in the study treat breast cancer patients and research permits were applied from all of them. Data was analysed by deductive thematic analysis.

Results: At the core of a successful breast cancer care pathway is the right content and timely information provided to the patient at the pace the patient is able to adopt. This is especially highlighted at the beginning of the treatment process. In regards to diagnostic services, rigorous execution of mammography, sampling techniques and analyses were seen as important. Staff also valued the importance of aftercare and follow-up, and highlighted the fact that the patient should be given a chance to keep in close contact with care and treatment staff, even after their active treatment process has finished.

Conclusion: Health care staff recognized the same success factors for optimal breast cancer care and treatment pathways as patients reported in previous studies, yet more emphasis was put on patient characteristics and the technical performance features of the process. Both patient and staff viewpoints should be taken into account in planning breast cancer care pathways.

Keywords: Breast cancer, care pathway, staff viewpoint, success factors

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Key Points

- Providing the right content and timely information to the patient at the pace the patient is able to adopt it is very important factor for breast cancer care and treatment success.
- Ensuring the availability of staff for counselling at the breast cancer care follow-up stage should be emphasised in breast clinics.
- Both patient and staff viewpoints should be taken into account in planning breast cancer care pathways.

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Introduction

Care pathways are used to systematically plan and follow up patient-focused care programmes (1, 2). The aim of the care pathway is to enhance the quality of care by improving risk-adjusted patient outcomes, promoting patient safety, increasing patient satisfaction, and optimizing the use of resources (3). Hansen et al. (4) suggested the concept of the patient-centred pathway to emphasize the importance of patient perspective in service planning (4).

Common breast cancer treatment involves surgery, chemotherapy, radiotherapy, hormonal and biological therapies. The breast cancer patient pathway from the patient perspective can be divided into three stages: diagnosis, treatment, and the follow-up stage (5, 6). Patient pathways are unique and dynamic, following the individual features of each patient's health status, genetics, experiences, as well as the context. This requires all involved health care professionals should have knowledge about the entire care and treatment process (4, 7).

The planning and execution of breast cancer care and various treatments should be conducted by a multidisciplinary team (8). The European Society of Breast Cancer Specialists, EUSOMA (9), reminds health care organizations to pay close attention to multidisciplinary and patient-centred breast cancer pathways, ranging from the diagnostic stage to the follow-up stage (10). Cancer detection, diagnosis and care coordination comprise appropriate care that is timely and provided by an interprofessional team including professionals from many fields (11). A multi-professional mode of working results in better breast cancer treatment in terms of clinical and process outcomes in many aspects, including patient participation in decision making, as well as cancer research (12).

Hansen et al. (4) found that at the beginning of their care pathway, patients are focused on the biological goals and conventional treatment. By biological goals, Berntsen et al. (13) mean removing the cause of the disease and relieving symptoms through biological manipulation. In contrast, Waelli et al. (14) reported that breast cancer patients with chronic conditions rated non-clinical demands to be almost as important as clinical demands. They identified five types of non-clinical patient demands in the delivery of their health care services: demands related to daily life; alternative medicine; the structure of the treatment pathway; administrative and logistic assistance; and demands related to new technologies (14).

Some studies show that patients report that health care staff do not have the diversity of competencies to optimally meet patient needs (6, 15-17). There are deficiencies in empathy and communication skills, as well as in giving adequate information throughout their care pathway. Unmet needs in the patient care pathway have the potential to affect their survival and satisfaction levels (6, 15). According to Sandager et al. (16, 17), patients and their next of kin were not satisfied with the level of their involvement in treatment decisions and the amount of information received. They also reported not being informed about the persons responsible for their care. Studies have demonstrated that women with breast cancer have too little relevant information (15, 18, 19). There is a lack of psychosocial support, individualized care and choice of treatment, as well as a lack of follow-up during their treatment process (15, 18, 19). In order to improve breast cancer patients' services and care, it is necessary to have the patient and staff viewpoints regarding the process (15, 20). This study is part of a larger project where the breast cancer patient care pathway is inspected from both of these viewpoints. In this article, the staff viewpoint is focused on.

The objective of this research was to produce information about the factors contributing to the success or lack of success in a breast cancer care pathway at the treatment phase, from the health care staff perspective. In this study, our subjects comprise staff educated at universities of applied sciences: diagnostic radiographers; radiation therapists; breast cancer nurses; and biomedical laboratory scientists. The information obtained in this study will be used in planning a web-based education platform about the topic for these groups of health care professionals. The research questions were:

1. What factors are associated with successful breast cancer care pathways at the treatment stage?

2. What factors are associated with unsuccessful breast cancer care pathways at the treatment stage?

Materials and Methods

Design, Sampling and Data Collection

The methodological approach chosen was phenomenography where the emphasis is on how people construct their views about the world. The analysis is whole group oriented since all data was analysed together with the aim of identifying possible conceptions of experience related to the phenomenon under investigation (21, 22). The data collection instrument was constructed based on the principles of critical incident methodology with the aim of identifying the factors contributing to successful and unsuccessful individual care pathways during cancer treatment and procedures, from the staff viewpoint (23).

Data was collected simultaneously at four hospitals treating breast cancer patients in four different countries, as follows: Tartu University Hospital in Estonia; Oulu University Hospital in Finland Cantonal Hospital of Freiburg in Switzerland; and Haukeland University Hospital in Norway. The target groups included diagnostic radiographers, radiation therapists, breast cancer nurses and biomedical laboratory scientists.

The convenience sampling method was used. In Estonia and Finland, the research contact person invited to participate in the study by sending an email to relevant organizations. In Norway, the contact person at each department provided a link to the invitation on the department web page. In Switzerland, there were several contact people at the hospital, who sent the invitation to their staff by email. It included the participant information letter comprising the data privacy notice and the link to the questionnaire. Criteria for the respondents were: being able to read and write in English (except in Switzerland); having at least three years of work experience with oncology patients; and working with breast cancer patients at the time of the survey. The aim was to have two or three respondents from each professional group per country. The survey was planned to be conducted from 17.05.21 to 07.06.2021. Due to an insufficient number of responses, reminders were sent midway through September for a two-week extra data collection period. The data collection was completed on 30.09.2021. Responses were obtained from seven diagnostic radiographers, eight radiation therapists, two breast cancer nurses and six biomedical laboratory scientists, comprising a total of 23 respondents.

Data Collection Instrument

Regarding the background factors, there was only one open-ended question about the profession of the respondent. The eight open-ended questions were based on the steps of the breast cancer care pathway as described by the EUSOMA quality indicators of breast cancer care (10, 24), as well as by European research studies (5, 25). In regards to the services in the care pathway, the staff were asked what they considered to be the factors leading to successful and unsuccessful service provision. The questionnaire in English was provided in Estonia, Finland and Norway to avoid bias due to translation. In Switzerland, the questionnaire was provided in French, and translated by the project group members who also translated the responses from French to English.

Pilot Study

Before applying for a research permit, the questionnaire was piloted by seven project group members from each of the countries participating in the study who had not participated in constructing of the data collection instrument but represented each of the target group professions. The data collection instrument was revised according to the comments obtained by piloting, including the addition of a question about the respondent profession and reformulation of some sentences to make them clearer.

Statistical Analysis

Data was analysed by deductive thematic analysis, using as a theoretical frame of analysis the steps of the breast cancer care pathway which also formed the organizing themes (26, 27). Firstly, one researcher became familiar with the data to identify units of analysis, which were then formed into condensed-meaning units. Then, the features of interest in the data were coded across the dataset, collating data relevant to each code. After coding, the codes were abstracted to themes and subthemes. The first author performed the preliminary coding and thematization. The coding consistency and thematization were then checked by two more researchers. No major discrepancies were found.

Ethical Issues

Research permits were obtained from every hospital participating in the study. The need for an ethics board permit was requested from the Norwegian centre for research data as the Western Norway University of Applied Sciences (HVL) was coordinating the data collection of this study. However, the Norwegian centre for research data responded that the ethics board permit was not necessary since no medical or sensitive data was collected. A data privacy notice was provided to the subjects. The only personal data collected from the participants was their professional title. However, it would be impossible to connect the subjects to their responses. The software used for data collection was Cisco AnyConnect Secure Mobility Client governed by the Western Norway University of Applied Sciences (HVL). Only nominated persons from the project group processed and analysed the data stored in the closed cloud drive and thereby protected against third party data access.

Results

Results are presented in two subchapters: a) diagnostic services comprising laboratory and mammography services; and b) treatments and therapies comprising preparation to treatment, breast surgery and reconstruction, radiotherapy, chemotherapy, endocrine and biological therapies and counselling following the treatment.

Factors Contributing to Success or Lack of Success of Diagnostic Services in the care Pathway

In mammography, patient guidance about the procedure and the capability to perform the examination in an optimal manner were seen as the factors important for success. In addition, the ability to support the patient during the procedure was also considered important. However, the pain, anxiety or fear experienced by the patient during the procedure or the inability of staff to conduct the examination in an optimal manner contributed to a suboptimal performance of diagnostic services.

"Patient anxiety about the mammogram result and the procedure." (Lack of success factor related to patient anxiety and fear).

The factors leading to success or lack of success of diagnostic services in the breast cancer patient care pathway are provided in Table 1.

Most comments obtained from health care staff were about the rigorous performance of sampling techniques and analyses.

"Carry out the analyses of patients conscientiously and following our ethics (quality control, respect of pre-analysis, respect of the deadline of results, professional conscience." (Success factor related to rigorous execution of sampling techniques and analyses).

Reliable and quick reporting of laboratory results, a short waiting time and pleasant behaviour of laboratory staff were reported as signs of optimal breast cancer pathways by the respondents. On the other hand, the lack of these signs may indicate a suboptimal performance (Table 1).

Factors Leading to Success or Lack of Success of Treatments and Therapies in the care Pathway

Regarding the preparation prior to treatment and giving the patient enough information with the right kind of content was recognized by the respondents as a success factor in the breast cancer care pathway.

"Information about procedures and psychological support. It is important to communicate well. Secure that the information is given and received." (Success factor related to giving the patient enough information).

Many respondents emphasized the importance of psychosocial support, continuity of care, proper facilities, planning and professional conduct. The factors contributing to the failure of the breast cancer care pathway involved mostly the absence of success factors mentioned above. In addition, the patient's emotional state or reactions, such as denial or fear, were also mentioned in association with the negative outcome of preparation for treatment. Furthermore, the staff lacking time to meet the patient needs were seen as inhibiting the preparations for treatment (Table 2).

The factors contributing to both successful and unsuccessful surgery and reconstruction of the breast were associated with tumour location and type, as well as with the patient's psychosocial and physical state and health.

"Some patients have had reconstructed their breast before they got irradiation. It can then be difficult to get high enough dose due to thin skin. We have to adjust the bolus, and the skin gets very sunburned." (Lack of success factor related to tumour type and size).

Table 1. Factors for the success and lack of success of diagnostic services in the breast cancer care pathway

Laboratory services	
Successful	**Unsuccessful**
• Rigorous execution of sampling techniques and analyses (5 comments) • Short waiting time, information about laboratory location (2 comments) • Reliable and quick reporting of results (2 comments) • Pleasant behaviour of the staff (2 comments)	• Failures in sample taking, handling and storing (2 comments) • Unpleasant or unhelpful behaviour of the staff (2 comments) • Long waiting time of the appointments and results of the samples (2 comments)
Mammography	
Successful	**Unsuccessful**
• Patient guidance about the examination and what it takes to get optimal mammograms (8 comments) • Being able to execute the examination under optimal conditions and in an optimal manner (5 comments) • Supporting the patient during procedure (3 comments)	• Patient feeling the procedure painful (4 comments) • Anxiety and fear of the patient (4 comments) • Being not able to execute the examination under optimal conditions and in an optimal manner (3 comments) • Other: long waiting time of mammogram results, lack of communication or information, costs of mammography

Some respondents recognized that information self-acquired over the internet and unclear decision-making were factors associated with unsuccessful surgery and reconstruction. Staff competency, patient trust in health care staff and clear communication about breast surgery contribute to successful breast cancer surgery of the patient. On the other hand, comments about unsuccessful surgery were mainly related to technical failures during surgery (Table 2).

"Poorly done operation/reconstruction".

"Failure in reconstruction technique" (Lack of success factor related to staff performance).

In radiotherapy treatment, the factors contributing to the success or failure of treatment were mainly the same as at the surgery stage, but with different weightings. Most respondents highlighted the importance of understanding all radiotherapy treatment stages by the patient, including the effects and side-effects of radiation.

"Information on the location of the radiotherapy, how it works, the risks involved (burns), the different appointments, the means of reimbursement for transport to get to the radiotherapy every day." (Success factor related to patient understanding).

However, according to respondents, radiotherapy treatment will not proceed in an optimal manner if the patient is fearful or nervous about it or shows disagreement or signs of inability to continue through the entire treatment process or gets multiple side-effects from the treatment. The most often mentioned factor in radiotherapy treatment was the staff ability to perform optimal radiotherapy treatment, as well as staff competency in general. Furthermore, the importance of multi-professional collaboration of the radiotherapy team was emphasized. Issues contributing to suboptimal radiotherapy treatment were a lack of time, lack of timely patient information and ineffective organization of appointments (Table 2).

Respondents identified informing the patient about the treatment and its possible side-effects among the success factors for chemotherapy, and endocrine and biological therapies. However, most of them agreed that an important factor for potential failure of treatment was the patient's fear about the side-effects of treatment or actual realization of them.

"Severe side-effects which occurs during therapies, treatment cancellations."

"Fear of side effects of treatment." (Lack of success factor related to fear of side-effects).

Ensuring good communication in aftercare and follow-up was seen as the most important issue. Importantly, it was recognized that the patient left alone or without any aftercare may result in the failure of the entire care pathway (Table 2).

Discussion and Conclusion

In mammography, the guidance and support given to the patient during the examination were emphasized, in addition to technical performance. Mammography is a somewhat inconvenient procedure and may be painful for some women (28). That is why helping the patient to feel peaceful and relaxed while tolerating optimal amounts of compression is also important for the optimal quality of a mammography image. When it comes to laboratory services, the staff focus seemed to be more on the technical details of the laboratory process than on the fluency of services, which is natural due to the importance of details in their professional knowledge.

Based on this study, it seems that health care staff recognize that the patient needs to be well-informed in a timely manner, especially at the beginning of the care and treatment pathway. The recognition of patient needs may also be due to the adoption of evidence-based practice where reading research studies is essential.

Table 2. Factors for the success and lack of success of treatments in the breast cancer care pathway

Preparation prior to treatment, e.g., information about procedures and psychological support, genetic counselling and preserving fertility

Successful	Unsuccessful
• Enough information with right kind of content (11 comments) • Psychosocial support available for the patient (8 comments) • Other: ensuring continuity of care, proper facilities and planning, professional conduct of staff (4 comments)	• Too little, unclear or wrong time given information to the patient and her care givers (7 comments) • Lack of psychosocial support (4 comments) • Emotional state of the patient (3 comments) • Staff not having enough time for the patient (2 comments)

Surgery and reconstruction of the breast

Successful	Unsuccessful
• Favourable type or location of the cancer or operation type (2 comments) • Good physical and psychosocial state of the patient (2 comments) • Competency of staff performing surgery (3 comments) • Trust on health care professionals (2 comments) • Clear information given to the patient of different aspects of surgery (4 comments)	• Unfavourable type or location of the cancer or operation type (2 comments) • Impaired psychosocial state of the patient, fear (3 comments) • Self-acquired information from the internet and unclear decision making (2 comments) • Some failure in performing surgery (4 comments)

Radiotherapy

Successful	Unsuccessful
• Patient understanding all the stages of radiotherapy treatment including effects of radiation and its side-effects (8 comments) • Other patient related factors: lack or fear, early-stage cancer (3 comments) • Being able to execute optimal RT techniques (5 comments) • Competent radiotherapy professionals (3 comments) • Multiprofessional co-operation of the RT team (3 comments)	• Fear or nervousness of the patient (4 comments) • Disagreement or problems in continuing through the whole treatment period (3 comments) • Side-effects of the RT treatment (2 comments) • Other patient related factors: patient smoking, patient having metastases (2 comments) • Lack of time for the patient - too little staff (4 comments) • Lack of timely information and support (3 comments) • Problems in organising or keeping appointment time (3 comments) • Other staff related factors: suboptimal co-operation of RT-team, suboptimal fixation of the patient (2 comments).

Chemotherapy, endocrine and biological therapies

Successful	Unsuccessful
• Staff is able to clearly inform about the treatment and its side-effects to the patient (5 comments) • Good response to the treatment (2 comments)	• Fear of side-effects and side-effects as such (7 comments) • Lack of information and communication (2 comments)

Aftercare and counselling following treatments

Successful	Unsuccessful
• Ensuring good communication with the patient at the follow up stage (7 comments) • Different ways of ensuring aftercare and follow up (3 comments) • Being attentive and empathetic towards the patients (2 comments) • Ensuring that the patient and her care givers understand the meaning and are involved in the follow up (2 comments)	• No follow-up, patient being left alone (5 comments) • Lack of information or communication (3 comments) • Other: fear, denial, patient returning to special care, not optimal recovery from the treatments (3 comments)

However, in studies about breast cancer patients' unmet needs, the patients and their next of kin have reported a lack of information throughout their treatment process (15-17). Current research studies clearly show that having too little timely information still seems to be a problem for women with breast cancer (16-18). In previous studies, patients have also reported a lack of psychosocial support, individualized care and choice of treatment (15, 18, 19). According to our results, respondents seemed to understand the importance of psychosocial support, especially at the treatment preparation stage where the patient may have difficulties with understanding her illness, not to mention the upcoming treatment.

Several respondents mentioned that they did not know about the success factors for treatments regarding their own specialist work area. It was a bit surprising that only three respondents named the importance of multi-professional teamwork as a success factor for optimal breast cancer care and treatment pathway at the treatment phase. All of the international quality criteria for breast cancer care (8, 10, 12) raise this as an essential element of breast cancer care and treatment quality.

The respondents agreed that for patients at the beginning of their care pathway, the focus seems to be on biological goals and conventional treatment (4). With regard to surgery, radiotherapy, chemotherapy, and endocrine and biological therapies, the staff thought that the main success factors are related to tumours and the physical condition of the patient, as well as the technical performance of therapies, which is largely dependent on staff competency. However, they also mentioned that a lot of success or failure of the process depends on the patient's physiological and psychological state and on the capacity for communication and collaboration during the treatment process. Compared to what patients have reported about supporting their breast cancer treatment pathway (4, 14, 16, 17), the respondents seemed to focus more on the above-mentioned factors. The staff recognized the factors related to tumour size and location, patient feelings, understanding, mental and physical state, health state and their behaviour and commitment to the treatments as affecting the success or contributing to suboptimal flow of the care and treatment pathway in case these issues were of a negative nature.

Performing appropriate follow-up and the availability of staff for counselling in breast cancer care at the follow-up stage are important quality criteria for breast cancer care according to the EUSOMA working group criteria (10). Respondents in our study also highlighted these aspects clearly. In a study concerning breast cancer patients' unmet support needs (18), women who had been treated for breast cancer, brought up the importance of organizing proper follow-up and aftercare. Based on patient experience, there is still space for improvement to reach the situation where no woman with breast cancer will be left alone after the active treatment process is over.

Trustworthiness and Limitations

There are several limitations in this study and the results should be interpreted with caution. Firstly, though we recruited staff members from four different countries for the study, we do not know if the data comprises all these countries. With regard to the Swiss data, which was collected with the survey form in French, we do know that there are respondents from all staff categories although the number of nurses was small compared to the other groups. Only two nurses responded to the study. In addition, the authors do not know where exactly these two nurses were working. This is a pity and somewhat surprising, since nurses are the ones participating the most in the breast cancer patient care pathway. The research group cannot explain why the nurse response rate was low. It may be due to the coronavirus disease-2019 (COVID-19) pandemic that increased their workload at the time of data collection. However, the pandemic certainly added to the workload of all professional groups that were the subjects of this study.

Although geographical generalization is not the aim of qualitative research, knowing that staff members represent all four countries participating in the study, would have added to the generalizability of the results. However, since the country of origin was not asked from the subjects, in order to collect as little as possible background information for data privacy reasons, we have no knowledge about each respondent's country of origin except for Switzerland, since the Swiss questionnaire was in French and comprised a dataset of its own.

Consistency of the results was ensured by cross-checking the analysis by two other authors in addition to the one conducting the analysis. We tried to ensure credibility by letting the project group members representing the respondent groups comment on the results and the way of interpretation. In terms of conformability, there remains some chance of bias. Although to be a qualitative study, the number of respondents was not particularly small, including four different professional groups with different kinds of viewpoints regarding the topic may have demanded a bigger group of respondents to ensure the data saturation.

In conclusion, at the core of breast cancer care and treatment success seems to be the provision of timely patient information with relevant content at the individual pace the patient is able to adopt. This is essential at the beginning of the treatment process. According to this study, there are several patient-related factors contributing to a successful or unsuccessful care pathway. Staff tend to put more emphasis on patient characteristics and technical performance features of the process compared to those identified by the breast cancer patients themselves. Common aspects for both patients and staff are understanding the importance of aftercare and follow-up, and the fact that the patient should be given a chance to maintain close contact with the care and treatment staff, even after their active treatment process has finished.

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Ethics Committee Approval: The need for an ethical board permit was requested from the Norwegian centre for research data as the Western Norway University of Applied Sciences (HVL) was coordinating the data collection of this study. However, the Norwegian centre for research data responded that the ethical board permit was not necessary since no medical or sensitive data was collected.

Informed Consent: Informed consents of the participants were obtained.

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Authorship Contributions

Concept: E.M., B.S., S.K.; Design: E.M., B.S.; Data Collection and/or Processing: E.M., T.S.S., K.S., B.S., L.M., J.A.P.J., L.R., S.K.; Analysis and/or Interpretation: E.M., B.S., M.O., S.K.; Literature Search: E.M.; Writing: E.M., T.S.S., K.S., B.S., L.M., M.O., J.A.P.J., L.R., S.K.

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Original Article

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Association Between GATA3 and Histopathological and Immunohistochemical Parameters in Early-Infiltrating Breast Carcinomas

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ABSTRACT

Objective: This study evaluated the frequency of GATA-binding protein 3 (GATA3) expression in early breast cancer and its relationship with histopathological and immunohistochemical parameters.

Materials and Methods: GATA3 was analysed by immunohistochemistry in histological sections of tumors from 105 female patients, with histological diagnosis of invasive breast carcinoma (BC), at clinical stages I, II and IIIA, who underwent primary surgical treatment. GATA3 nuclear expression was determined as the percentage of positive tumor cells and further categorized as high (positive expression in more than 95% of cells) or non-high (negative or low positive expression in up to 95% of tumor cells). GATA3 expression was analysed according to the patient age, tumor and node pathological stage, histological type, histological and nuclear grade, lymphovascular invasion, and estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), human epidermal growth factor 2 (HER2) status, and Ki-67 expression.

Results: GATA3 expression was positive in 103 cases (98.1%). High expression was significantly associated with low histological and nuclear grade, positive hormonal receptors, and less proliferative activity based on Ki-67 expression. A prominent feature was that 94.7% of the ER-positive/HER2-negative cases presented high-GATA3 expression, as 94.0% of the tumors showing high-GATA3 were ER-positive. In ER-negative/HER2-positive or ER-negative/HER2- negative, high-GATA3 was present in 25% while 75% were non-high-GATA3 compared with ER-positive/HER2- negative (4.1%) and ER-positive/HER2-positive (20%). Proliferative activity in triple-negative breast cancer tended to be higher among tumors with low-GATA3, irrespective of AR expression. In the group of ER-positive/HER2-negative tumors only three cases were low-GATA3 (85% and 80%), both with high proliferative activity.

Conclusion: High GATA3 expression is associated with favorable histopathologic and immunohistochemical BC prognostic factors.

Keywords: Breast cancer; GATA3 expression; immunohistochemistry; histopathology

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Key Points

- GATA3 is a transcription factor involved in estrogen receptor signaling in breast cancer.
- High-GATA3 expression is linked to favorable histopathological and immunohistochemical findings.
- Breast cancer responsiveness to hormone therapy is probably modulated by GATA gene expression.
- Further studies with GATA3 expression are warranted to determine its clinical role in specific situations, such as low ER tumors and ER positive/PR-negative tumors.

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Introduction

Transcription factors modulate gene expression by binding to their cognate DNA sequence and attracting the enzyme RNA polymerase II to the proper initiation site for transcription. The *GATA3* gene, at chromosome location 10p14, is a member of the GATA family with two GATA-type zinc-finger proteins and encodes the transcription factor GATA-binding protein 3 (GATA3), critical for luminal breast epithelium development and maintenance (1).

The GATA3 protein is linear, with more than 400 amino acids, that can be recognized by immunohistochemical analysis. Mutations of GATA3 and loss of the expression of its related protein are implicated in breast cancer (BC) development and aggressiveness (2, 3). GATA3 is a crucial regulator of estrogen receptor (ER) function and has been associated with a more favorable prognosis in patients with BC (4-6).

Precision oncology, with the identification of actionable genetic alterations, has progressed in the last decades. Currently, medical oncologists' decisions are based on prognostic and tumor predictive response factors, such as pathological and immunohistochemical parameters, and multigene tests. Nevertheless, additional prognostic factors are still needed. In this scenario, GATA3 is emerging as a candidate to broaden treatment options. Therefore, it is appropriate to better understand the role of GATA3 expression in BC and its relationship with other biomarkers.

The aim of this study was to assess the association between pathological and immunohistochemical parameters and GATA3 expression, in order to increase the understanding of BC development and expanding the evidence base for precision therapy.

Materials and Methods

In this prospective case series study, we analysed consecutive BC surgical specimens from patients attending a Mastology Reference Center (Clínica Prof. Alfredo Barros, São Paulo, Brazil). The research was approved by the board of the Ethics Committee of the University of São Paulo School of Medicine. Clinicopathological and immunohistochemical data from patients who fulfilled the inclusion criteria were collected from September 2017 to September 2020. All the cases were treated by the same surgeon (ACSDB) and all the pathological and immunohistochemical analysis were performed by the same pathologists (FMC and FNA).

Patients were eligible if they met these conditions: female sex, early BC (clinical stages I, II, and IIIA), and primary surgical treatment. The exclusion criterium was any patient who had received neoadjuvant treatment.

Histopathological and Immunohistochemical Analyses

All surgical specimens were fixed in a 10% buffered formaldehyde solution, embedded in paraffin, sectioned by handheld microtome, and stained with hematoxylin-eosin. The tumors were classified according to current recommendations (5). The following variables were analysed: Histologic type, nuclear grade, histologic grade, and lymphovascular invasion (LVI). LVI was defined as focal, when only one vascular space was involved, and multifocal, when more than one space had neoplastic emboli.

Estrogen receptor (ER) (clone SP1, Neomarkers, Fremont, CA, USA), progesterone receptor (PR) (PgR636, Dako, Carpinteria, CA, USA), androgen receptor (AR) (F39.4.1, Biogenex, Fremont, CA, USA), Ki-67 (MIB1, Dako, Glostrub, Denmark), and human epidermal growth factor 2 (HER2) (SP3, Thermo Scientific, Fremont, CA, USA) were determined by immunohistochemistry (IHC) staining, which was performed using Streptavidin Biotin Complex and EnVision methods (DakoCytomation). For ER, PR and AR, nuclear staining in more than 1% of the cells was considered positive. ER tumors with 1% to 10% of stained nuclei was reported as ER-low positive.

For HER2, cases with a score of 3+ by IHC, or 2 + with amplification by *in situ* hybridization method, were considered positive. The percentage of cells stained for Ki-67 was determined on the most representative area of the tumor after selecting three to five random fields within the tumor (both periphery and center), with at least 500 cells. Five fields were selected in cases with visually heterogeneous expression, while three fields were accepted for those with a homogeneous distribution of stained cells. The selected fields were transformed in digital images and percentage of Ki-67 stained cells were calculated using the software QuPath (https://qupath.github.io). The average was accepted as the final Ki-67 value.

For GATA3 staining a primary mouse monoclonal antibody was used, clone L 50-823 (Cell Marque - ref. 390 M - 16, CA, USA) at 1:1000 dilution. Nuclear staining in at least 1% of tumor cells was accepted as positive. The percentages in each case were assessed, and after observing their distribution, two GATA3 expression classes were empirically defined: high-positive (>95% of stained cells) and non-high-GATA3 (negative or positive expression in up to 95% of tumor cells).

As most of the cases were high-positive, and negativity was very rare, we grouped the results of the GATA3 expression in only two categories for statistical analysis: high and non-high (negative or low).

Statistical Analysis

The associations between GATA3 expression and qualitative variables were analysed by the chi-square test or Fisher's exact test. Correlation with quantitative variables was analysed by the Mann–Whitney test. MedCalc® Statical Software version 19.6.1 (Ostend, Belgium) was used for the analyses. A $p<0.05$ was considered significant.

Results

A total of 105 patients were included. GATA3 expression was positive in 103/105 (98.1%) cases. The percentage of stained cells ranged from 0 (n = 2 cases) to 100%, and no case presented with expression in the 1% to 19% range.

In this study the categories "negative", "low-positive", and "high-positive" for GATA3 expression were defined, and due to the very widespread expression of GATA3, the negative and low positive results were grouped together in a single category, the non-high GATA3 group. According to the proportion of stained cells, 84 cases (80%) presented >95% of positive cells (high-positive expression); 19 cases (18.0%) presented ≤95% of positive cells (low-positive expression), and two cases were negative (1.9%). Figure 1 shows examples of immunohistochemical findings.

There was a tendency to observe non-high expressions in younger patients, with a median age of 50.0 years in patients with GATA3 low or negative, and 60.5 years in the GATA3 high group ($p = 0.08$).

High-positive GATA3 expression was more frequent in samples with low histological grade and low nuclear grade (p<0.001). High-GATA3 expression was observed in all infiltrating lobular carcinomas. There was no association with tumor size, lymph node status, or LVI. These results are detailed in Table 1.

Immunohistochemical findings are shown in Table 2. Significant differences in ER, PR, AR, and Ki-67 were found when comparing the groups based on low/negative or high GATA3 expression.

As for immunohistochemical tumor subtypes classification, a prominent feature was that 94.7% of the ER-positive/HER2-negative

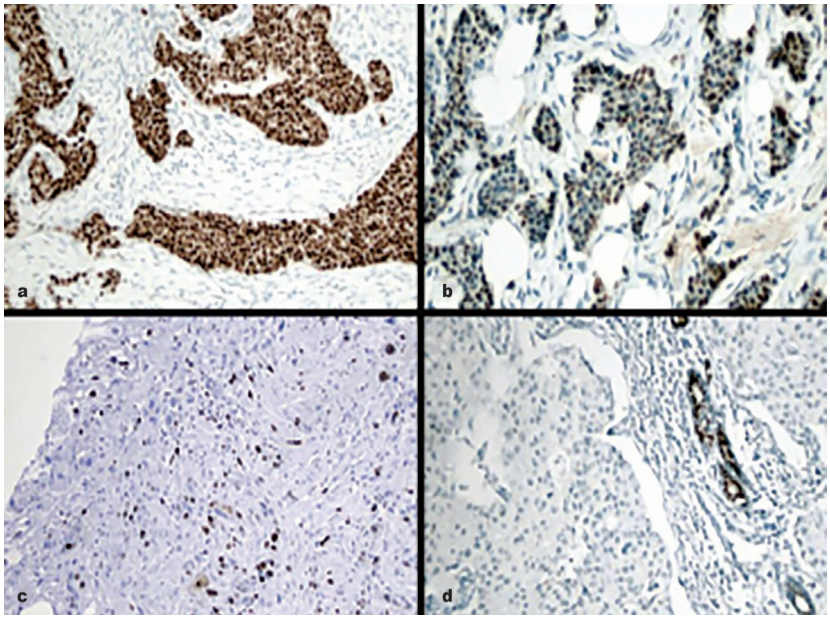

Figure 1. Immunohistochemical findings of GATA 3 expression in non-special type invasive breast carcinoma cells: **a)** high-positive with strong expression in 100% of tumor cells; **b)** low-positive with 70% of stained cells; **c)** low-positive with 20% of positive cells; **d)** negative

Table 1. Pathological parameters in the GATA3 expression categories

		Non-Hhigh	High	*p*-value
		N (%)	N (%)	
Histological type	Non-special type	17 (22.7)	58 (77.3)	0.130
	Lobular	0 (0)	14 (100)	
	Others	4 (25)	12 (75)	
Histological grade	I	1 (5.9)	16 (94.1)	<0.001
	II	4 (6.6)	57 (93.4)	
	III	16 (59.3)	11 (40.7)	
Nuclear grade	1	1 (7.7)	12 (92.3)	0.001
	2	2 (4.3)	44 (95.7)	
	3	18 (39.1)	28 (60.9)	
Lymphovascular invasion	Absent	15 (20.5)	58 (79.5)	0.423
	Focal	5 (26.3)	14 (73.7)	
	Multifocal	1 (7.7)	12 (92.3)	
pT	≤20 mm	12 (19.1)	51 (80.9)	0.286
	>20 mm e ≤50 mm	9 (25.7)	26 (74.3)	
	>50 mm	0 (0)	7 (100)	
pN	Positive	5 (13.8)	31 (86.1)	0.260
	Negative	16 (23.2)	53 (76.8)	

pT: pathological tumor size; pN: pathological node status

cases presented high-GATA3 expression, as 94.0% of the tumors showing high-GATA3 were ER-positive. in contrast, only 25% of cases with ER-negative/HER2-positive or ER-negative/HER2-negative evidenced high GATA3.

The two patients with GATA3 negative tumors (aged 50 and 30 years old) presented stage I triple-negative (TN) carcinomas, histological grade III, nuclear grade 3, AR-negative, and Ki-67 of 40% and 80%, respectively. Among the 13 TN cases, 9 (69.2%) presented with non-high-GATA3 expression, and of these 6 (66.7%) were AR-negative.

Discussion and Conclusion

Looking at clinicopathological parameters, high-GATA3 expression was associated with some favorable pathological features of BC, such as histological grade I and nuclear grade 1. In keeping with most of the previous studies, we did not find an association between GATA3 and tumor size and/or lymph node metastasis (6-8) despite one study, Querzoli et al. (9), reporting an inverse association with tumor size . A further study, Mehra et al. (10), reported low-GATA3 expression associated with lymph node metastases. Generally, our cohort findings linked high-GATA3 expression to favorable pathological features of BC.

It is well known that GATA3 plays a significant role in the normal development and function of the mammary gland, and as a marker of luminal identity in BC. As the most frequent transcription factor in luminal tumor cells, GATA3 became an important indicator of mammary differentiation in neoplasia of unknown origin, with better utility than mammaglobin and gross cystic disease fluid protein (3). Our results confirm this high GATA3 frequency in BC, as we found 98.1% positivity among all tumors. These results highlight the role of GATA3 as a reliable marker for primary tumor identification in occult BC found in lymph nodes or systemic metastases.

GATA3 is highly expressed in luminal BC, taking part in a gene set that identifies intrinsic tumor subtypes with distinct survival outcomes and is closely correlated with ER-alpha gene expression, an important fact to explain the role of GATA3 in hormone responsiveness (11-13). Notwithstanding, ER positivity is not a guarantee of a satisfactory response to hormone therapy and other factors must be considered. One of these factors is proliferative activity, evidenced by multigenic tests or by the expression of Ki-67 (14, 15). We found four patients with ER-positive/HER2-negative tumors and low-GATA3, all of them exhibiting a high-Ki-67 index. Although both *GATA3* and *ESR1* gene expression are correlated, probably other genes regulated by GATA3 are critical to hormone=responsiveness and possibly different types of mutation can impact its transcriptional function. Some of the other factors in the transcriptional apparatus include the cooperative action of the transcription factor FOXA1 and the genes *TP53*, *PIK3CA*, and *CDH1* (4, 16).

Given this complex scenario, it seems very difficult to establish a clear association between the categories of GATA3 expression–and gene mutations or epigenetic silencing (1). Probably when a strong and diffuse expression of GATA3 is observed, there is a whole transcriptional apparatus_functioning. A major challenge will be understanding the effect of differences in protein expression.

GATA3 is considered a prognostic marker in patients with biologically less aggressive tumors and is potentially useful to forecast sensitivity to anti-estrogenic treatment (17). In this regard, Parikh et al. (16)

Table 2. Immunohistochemical parameters by GATA3 expression category

		Non-Hhigh	High	*p*-value
		n (%)	n (%)	
ER	Positive	6 (7.1)	79 (92.9)	<0.001
	Negative	15 (75)	5 (25)	
ER	Negative	15 (75)	5 (25)	<0.001
	Low	2 (100)	0 (0)	
	Positive	4 (4.8)	79 (95.2)	
PR	Positive	3 (4.2)	69 (95.8)	<0.001
	Negative	18 (56.2)	14 (43.7)	
AR	Positive	10 (11.4)	77 (88.5)	<0.0001
	Negative	11 (61.1)	7 (38.9)	
HER2	Positive	8 (44.4)	10 (55.6)	0.005
	Negative	13 (14.9)	74 (85.1)	
kKi-67	≤20%	4 (8.5)	43 (91.5)	0.001
	>20%	17 (29.3)	41 (70.7)	
Subtypes	ER positive/HER2 negative	4 (5.3)	71 (94.7)	<0.0001
	ER positive/HER2 positive	2 (20)	8 (80)	
	ER negative/HER2 positive	6 (75)	2 (25)	
	ER negative/HER2 negative	9(75)	3 (25)	

ER: estrogen receptor; PR: progesterone receptor; RA: androgen receptor

remarkably observed that none of the responders to endocrine therapy was GATA3 negative, whereas 43% of the non-responders were GATA3 negative. A controversial group is the one with low expression of ER (1%-9% positive cells), which shares clinicopathological characteristics, biomarker profile, and outcomes with TN tumors (18, 19). In our cohort, we identified only two ER low positive carcinomas, both with-low GATA3 expression, a proportion closer to ER-negative rather than ER-positive cases.

Among ER-positive/HER2-negative tumors, the PR-negative subgroup has well-known lower responsiveness to endocrine therapy (20). Liu et al. (21) observed that the genomic analysis of this subgroup revealed that 16% are basal by PAM50 and present loss of GATA3. They developed an IHC-based classification-tool to discriminate non-luminal-like from luminal-like subtypes within the ER-positive/PR-negative/HER2-negative phenotype, showing that the non-luminal-like phenotype, characterized by GATA3-negative, CK5-positive and/or EGFR-positive tumors, received a limited benefit from adjuvant hormone therapy. In their turn, Tahiri et al. (22) observed that loss of PR expression correlates with higher tyrosine kinase activity in tumors that were HER2-negative.

The degree of benefit of hormone therapy in ER-positive/RP-negative cases remains debatable, and the possibility of using GATA3 for management decisions appears attractive. We had eight ER-positive/PR-negative/HER2-negative BC cases: 2 (25%) of them with GATA3 ≤95% and high Ki-67 (70% and 80%), and the other six cases with high GATA3 and Ki-67 between 5% and 30%. These data suggest that these tumors correspond to a heterogeneous group, and the therapeutic decision could be improved by additional data including the expression level of GATA3 and proliferative activity.

There is also a paucity of reliable data on the efficiency of hormone therapy in low-ER tumors. As convincing evidence remain elusive, it may be, at the moment, pragmatic to believe that the chance of good response with hormone therapy is higher in patients with low-ER tumors, but with preserved high-GATA3 expression.

Our results confirmed that high-GATA3 is much more frequent in ER-positive than in ER-negative tumors (92.9% *versus* 25%). Among our 13 cases of TN breast carcinomas, ten had low-GATA3 and, in this group, Ki-67 varied from 30% to 95%. In the three cases with high-GATA3, Ki-67 was 45%, 10%, and 18%. While all TN tumors with high-GATA3 expression were AR positive, only three low-GATA3 expressed AR, one of them with only 2% of positive cells. AR-positive TN carcinomas are associated with the luminal androgen receptor molecular subtype of these tumors. This subtype has hormonally regulated pathways that are active in the synthesis and metabolism of steroids, and share mutations with luminal carcinomas (23). These tumors are characterized by a low Ki-67 index and distinct clinicopathological presentation, and it is reasonable to conjecture that they preserve GATA3 functional integrity.

We analysed 18 HER2-positive carcinomas, ten of them ER positive and eight ER-negative. Although low-GATA3 was more frequent among ER-negative tumors (75% *versus* 20%), no statistical difference in proliferative activity, evaluated by Ki-67 expression, could be demonstrated (median 34% and 35% in both groups). This suggests that the role of GATA3 in HER2-positive carcinomas might be distinct, maybe because of the activity of tyrosine-kinase enzymes.

An association between non-high-GATA3 expression and high-grade tumors, in accordance with Lu et al. (24), was found. These characteristics are associated with poor prognosis, irrespective of molecular subtype, and Cakir et al. (6) estimated an 87% rate for GATA3-positive and a 78% rate for negative tumors in terms of five-year disease-free survival. For these authors, GATA3 was an independent factor for overall survival. In addition, Kouros-Mehr et al. (25), in an experimental model, observed that non metastatic cell lines have elevated GATA3 levels, whereas cells of metastatic lines have low GATA3 levels.

We acknowledge important limitations in our study, including the small sample size (particularly in the groups of ER-negative tumors), and the short follow-up period, which are insufficient for reliable outcome analysis. However, it opens new avenues to be explored concerning the role of GATA3 in the pathogenesis, evolution, and therapy of BC, mainly in the ER-low, ER-positive/PR negative, HER2-positive, and LAR TN subgroups. The results presented in these subgroups warrant further clinical investigation.

In conclusion, high expression of GATA3 protein in early infiltrating BC, is a surrogate marker of less aggressive disease. High-GATA3 expression is linked to greater tumoral differentiation and lower nuclear grade, lesser proliferative activity, and positivity of ER, PR, and AR. Regarding GATA3 expression in the various IHC subtypes, the highest rate was identified in luminal-like tumors (ER-positive and HER2-negative), and the lowest rate was found in basal-like tumors (ER-negative and HER2-negative). These results indicate that GATA3 expression can add important information about sensitivity and/or resistance to endocrine therapy, as well as possible chemosensitivity, especially in ER-positive tumors.

Ethics Committee Approval: The study was approved by the Ethics Committee of the University of São Paulo Medical School (CAAE: 562 69416.0.0000.0065; decision No.1.604.792).

Informed Consent: Due to the retrospective design of the research, informed consent was waived.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Conception: P.M.S., A.C.S.D.B.; Microscopic Analysis: F.M.C., F.N.A.; Data Collection: P.M.S.; Supervision D.G., F.M.C., A.C.S.D.B.; Writing: P.M.S., A.C.S.D.B., D.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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An *In Silico* Analysis Identified Members of the Pleckstrin Homology-Like Domain, Family B (PHLDB family) as Potential Prognostic and Predictive Biomarkers of Treatment Response in Breast Cancer Patients

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ABSTRACT

Objective: Breast cancer is the leading cause of morbidity and mortality in women worldwide. This malignant neoplasm can be classified into four clinically relevant subtypes according to the expression of a number of biomarkers. However, these tumors show considerable intratumoral heterogeneity and multidrug resistance. Members of the pleckstrin homology-like domain, family B (PHLDB) play a critical role in the regulation of p53 and AKT signaling pathways, important for cancer and cellular metabolism. The present study was performed to evaluate the expression pattern of PHLDB family members in breast cancer and its potential prognostic and predictive value for therapeutic response using bioinformatics tools.

Materials and Methods: This in silico analysis was performed using several online repositories, including UALCAN, GEPIA2, bc-GenExMiner, KM Plotter, PrognoScan and ROC Plotter.

Results: PHLDB family genes were found to be differentially expressed in tumor samples when compared to healthy breast tissue samples. Furthermore, epigenetic regulation may be one of the regulatory mechanisms for the expression of these markers. The PHLDB family of genes proved to be potential markers for predicting the development of lymph node metastasis ($p<0.0001$) and poor clinical outcome. All members of the PHLDB family were significantly correlated with hormone receptors. High levels of PHLDBs expression were associated with worse overall survival and recurrence-free survival in breast cancer patients. Finally, our data demonstrate that members of the PHLDB family can be promising markers in the stratification of patients who may or may not respond to different available therapies.

Conclusion: Our cumulative results demonstrate that PHLDB family members may be promising biomarkers for predicting prognosis and therapeutic response in breast cancer patients.

Keywords: Breast cancer, PHLDB, *in silico* analysis, biomarkers

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Key Points

- The pleckstrin homology-like domain, family b (PHLDB) family of genes are differentially expressed in tumor and normal breast tissues.
- Members of the PHLDB family are potential markers for predicting the development of lymph node metastasis and poor clinical outcome.
- Reduced expression of PHLDB 1, 2, and 3 mRNA was associated with decreased overall and recurrence-free survival rates in breast cancer patients.
- There is a possible relationship between PHLDB family member expression and response to endocrine therapy and to anti-HER2 antibodies.

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Introduction

Breast cancer is the malignant neoplasm with the highest rates of occurrence and mortality among women worldwide (1). Currently, it is known that breast cancer represents a phenotypically and biologically heterogeneous collection of diseases, culminating in different clinical patterns, prognosis and response to usual treatments (2).

Based on the expression of molecular biomarkers, breast cancer can be classified into four main subtypes widely accepted and used in clinical practice: Luminal A, Luminal B, human epidermal growth factor receptor 2 (HER2+) and triple negative breast cancer (TNBC) (3). The segregation of these molecular subtypes is due to genes responsible for the expression of hormone receptors for estrogen (ER) and progesterone (PR), HER2 and the cell proliferation marker, Ki-67 (4).

Although the sum of current clinical, pathological and molecular indicators favors a contribution in establishing the prognosis and predicting the therapeutic response of patients, the investigation of new, more robust, sensitive, specific and well-validated biomarkers is occurring, partially in response to the trend towards personalized medicine (5).

In this context, the Pleckstrin Homology-like Domain (PHLD) multifunctional protein class has been attracting interest for its role in the regulation of p53 and AKT signaling pathways, both of which are important for cancer and cellular metabolism (6). The PHLD protein class is organized into two separate families, PHLDA and PHLDB, each of which is composed of three members (6). All members of the PHLD families code for proteins that have a functional domain called PH (pleckstrin homology) (6). PH-like domains consist of 100 to 120 amino acid residues and are found in a wide range of proteins involved in intracellular signaling, and may also participate in cytoskeletal rearrangement and membrane trafficking (7). Furthermore, proteins with the PH domain have been well categorized as phosphatidylinositol-binding molecular modules located internally in the cell membrane, as well as other proteins with varying specificity (8, 9). The two PHLD protein families, A and B, differ from each other by the position of their PH domain in the N- or C-terminal region or in the length of the protein (6). Although identified nearly three decades ago, the PHLD class of proteins remains understudied in the oncological context, with members of the PHLDB family receiving the least attention in recent research.

Therefore, the present study was carried out to evaluate the expression pattern of PHLDB family members in breast cancer and its potential prognostic and predictive value for therapeutic response, through public datasets deposited in online repositories.

Materials and Methods

UALCAN and GEPIA2: UALCAN (http://ualcan.path.uab.edu/) is a free online platform to access and assess the expression profile of biomarkers in different types of cancers (10). UALCAN was used to investigate gene expression levels of PHLDB family members in normal and tumor samples from the breast, as well as in tumor subgroups and at different clinical stages. The level of methylation of the promoter region of the PHLDB family in breast cancer samples and normal tissues was also investigated using this same platform. Additionally, GEPIA2 (http://gepia2.cancer-pku.cn/) was accessed. GEPIA2 is a new improved web server to analyze RNA sequencing expression data from 9,736 tumors and 8,587 normal samples from the TCGA project (The Cancer Genome Atlas) and Genotype-Tissue Expression (GTEx) (11).

bc-GenExMiner: The Breast Cancer Gene-Expression Miner v4.5 (http://bcgenex.centregauducheau.fr/) is an online mining tool for properly annotated breast cancer transcriptomic data (12). For this study, we considered only the microarray data to analyze the expression of the PHLDB family with clinic pathological parameters, regarding the classic breast cancer biomarkers and the different molecular subtypes. The median expression was used as the cut-off point.

KM Plotter: The Kaplan–Meier Plotter (https://kmplot.com/analysis/) is a practical, easy-to-use survival analysis platform that hosts data from 21 different types of cancers (13). We investigated the expression of PHLDB family members according to overall survival (OS) and recurrence-free survival (RFS). The dataset included cDNA microarrays from the TCGA available in the KM Plotter online database. The validated probes were chosen according to the best automatic cut selection criteria. Follow-up time was adjusted to 120 months. Log-rank *p*-values and hazard ratio (HR) with 95% confidence interval (CI) were automatically determined.

PrognoScan: The PrognoScan online database (http://www.prognoscan.org/) provides a powerful platform to assess biological relationships between gene expression and cancer patient prognosis information, including overall survival (OS), relapse-free survival (RFS), distant metastasis-free survival (DMFS), and disease-specific survival (DSS) (14). PrognoScan includes public cDNA microarray datasets with clinical annotations of gene expression and prognosis from Gene Expression Omnibus (GEO) and ArrayExpress, for example. Cox *p*-values and hazard ratio (HR) with 95% confidence intervals (CI) were calculated automatically.

ROC Plotter: The ROC Plotter (http://www.rocplot.org/) is an interactive and user-friendly online tool (15). With transcriptomic data from 3104 breast cancer patients treated and not treated with endocrine therapy, anti-HER2 therapy, or chemotherapy. Here, we quickly evaluated the expression pattern of PHLDB family genes in the face of the treatment received by the patient.

Results

PHLDB Family Expression and Methylation Status in Samples From Breast Cancer Patients

Using TCGA data analyzed by the UALCAN platform, it was found that *PHLDB1* and *PHLDB2* had reduced expression in breast cancer tumor tissues when compared to adjacent normal tissues (Figures 1a and 2a; $p<0.0001$, respectively) and in a larger cohort the same pattern was observed (Supplementary Figures 1a and 1b; $p = 0.01$, respectively). Furthermore, hyper-methylation of the *PHLDB1* promoter region was observed in breast cancer tissues in relation to healthy tissues (Figure 1b; $p<0.0001$), indicating a possible direct relationship of this epigenetic regulatory mechanism with the reduction of expression in samples of breast cancer. Meanwhile, the highest level of methylation of the *PHLDB2* promoter region was observed in healthy breast tissues compared to tumor tissues (Figure 2b; $p<0.0001$). Contrary to what was observed for *PHLDB1* and *PHLDB2*, *PHLDB3* gene expression was higher in breast tumor samples when compared to healthy tissue samples (Figure 3a; $p<0.0001$) and, again, the same pattern was observed in a larger cohort, although this was not statistically significant (Supplementary Figure 1C). The highest level of methylation of

the *PHLDB3* promoter region was observed in breast cancer tissues compared to normal tissues (Figure 3b; $p<0.0001$).

Additionally, the expression patterns of PHLDB family members in relation to molecular classification was investigated. The Luminal type exhibited an increased transcriptional distribution in relation to the TNBC and HER2+ subtypes (Figures 1c, 2c and 3c; $p<0.0001$, respectively). Furthermore, patients with the most advanced clinical stage of breast cancer tended to express lower levels of *PHLDB1*, although this was not statistically significant when compared to the other stages of the disease (Figure 1d). However, there was no association between the differential expression of *PHLDB2* and *PHLDB3* with the different clinical stages of patients with breast tumors (Figures 2d and 3d, respectively).

Association of the Expression of PHLDB Family Members With Clinical-Pathological Characteristics

The open-source tool, bc-GenExMiner, was used for this analysis. The sample evidence suggested that there was a statistically significant association between the status of *PHLDB1* expression with all variables tested (Table 1). Significant associations were observed between *PHLDB2* expression and nodal status (p = 0.0228), Scarff-Bloom-Richardson (SBR) classification ($p<0.0001$), Nottingham Prognostic Index (NPI) (p = 0.0002), the statuses of ER ($p<0.0001$), PR (p = 0.0061), HER2 (p = 0.0147), and TP53 ($p<0.0001$) and molecular classification ($p<0.0001$) (Table 1). For the last member of the PHLDB family, statistically significant associations were found between differential expression of *PHLDB3* and patient age ($p<0.0001$), SBR classification ($p<0.0001$), NPI ($p<0.0001$), TP53 mutational status ($p<0.0001$), the expression of ER ($p<0.0001$), PR ($p<0.0001$), and HER2 ($p<0.0001$) and molecular subtype ($p<0.0001$) (Table 1).

Expression of PHLDB Family Members and Prognostic Value in Breast Cancer Patients

Next, the prognostic value of PHLDB family genes using the KM Plotter platform was investigated. Most notably, reduced levels of mRNA expression of PHLDB family members were significantly correlated with poor prognosis for overall survival (PHLDB1 p = 0.0044; PHLDB2 p = 0.0040 and PHLDB3 p = 0.0046) (Figures 4a, 4b and 4c, respectively) and recurrence-free survival (PHLDB1 $p<0.0001$; PHLDB2 p = 0.0013 and PHLDB3 $p<0.0001$) (Figures 4d, 4e and 4f, respectively). Additionally, the PrognoScan database showed that down-regulation of PHLDB family expression was significantly associated with reduction in cumulative rates of overall survival (OS), recurrence-free survival (RFS), distant metastasis-free survival (DMFS) and disease-free survival (DFS) (Table 2).

We also investigated the prognostic role of PHLDB family members in different intrinsic molecular subtypes. Kaplan-Meier curves indicated that high PHLDB1 level was significantly associated with lower cumulative rates of RFS in the TNBC subtype (p = 0.0330) and OS in the TNBC (p = 0.0330) and HER2 subtypes (p = 0.0067)

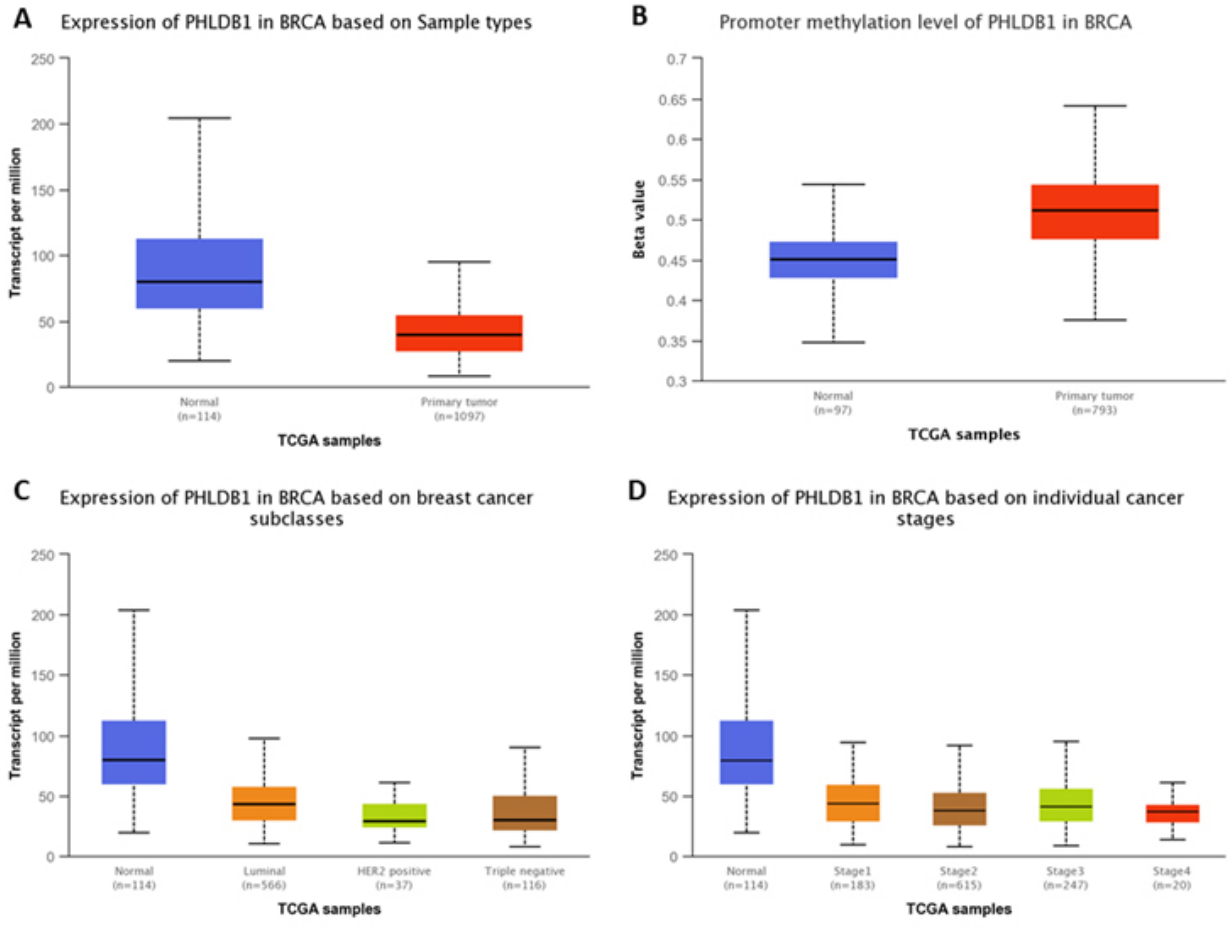

Figure 1. Expression of PHLDB1 in breast cancer patients. **a)** Expression of PHLDB1 in tumor and normal breast samples. **b)** Methylation profile of the PHLDB1 promoter region in tumor and normal breast samples. **c)** PHLDB1 expression in different molecular subtypes of breast cancer. **d)** PHLDB1 expression based on the different stages of the diseasePHLDB: pleckstrin homology-like domain family B

(Supplementary Figures 2D, 2A and 5A, respectively). Meanwhile, reduced levels of PHLDB1 showed lower RFS in Luminal A ($p < 0.0001$) and Luminal B ($p < 0.0001$) and OS in Luminal A ($p = 0.0008$) subtypes in breast cancer patients (Supplementary Figures 3D, 4D and 3A, respectively). We found that upregulation of PHLDB2 expression was significantly correlated with worse rates of RFS in the TNBC ($p = 0.0014$) and HER2 ($p = 0.0210$) subtypes and OS in the HER2 subtype ($p = 0.0240$) (Supplementary Figures 2E, 5E and 5B, respectively). In contrast, reduced levels of PHLDB2 mRNA expression were significantly correlated with reduced RFS in Luminal A ($p = 0.0002$) and Luminal B ($p = 0.0170$) and OS in Luminal A subtype ($p = 0.0025$) (Supplementary Figures 3E, 4E and 3B, respectively). Finally, the Kaplan-Meier curves indicated that the highest level of PHLDB3 correlated with preferable RFS in TNBC ($p = 0.0260$), Luminal A ($p < 0.0001$) and Luminal B ($p = 0.0009$) and OS subtypes in the Luminal A subtype ($p = 0.0260$) (Supplementary Figures 2F, 3F, 4F and 3C, respectively). Meanwhile, high PHLDB3 level was significantly associated with lower cumulative OS rates in the HER2 subtype ($p = 0.0150$) (Supplementary Figure 5C).

Predictive Value of PHLDB Family Members for Treatment Response

Considering the reports of some previous studies indicating PHLD family members as potential biomarkers for response to different treatments (16, 17), we conducted an analysis with the ROC Plotter web tool. Our results showed that among patients with hormone-dependent tumors, those who did not respond to hormone treatment had significantly reduced expression of *PHLDB1* in cases classified as Luminal A ($p = 0.040$) (Figure 5a), but there was no relationship in Luminal B tumors ($p = 0.054$) (Figure 5b). In the evaluation of PHLDB2 related to response rates to endocrine treatment, there was no statistical association in cases subtyped as Luminal A ($p = 0.300$) and Luminal B ($p = 0.054$) (Figures 6a and 6b, respectively). Finally, for the last family member, a significant relationship was found between high levels of PHLDB3 for patients who responded to endocrine treatment with tamoxifen or anastrozole (Luminal A, $p = 0.047$ and Luminal B, $p = 0.012$) (Figures 7a and 7b, respectively). Furthermore, reduced *PHLDB3* expression in HER2+ tumors was correlated with low response rates to anti-HER2 treatment ($p = 0.029$) (Figure 7c). However, PHLDB1 and PHLDB2 showed no relationship in the response rates of patients with tumors that overexpress HER2 when treated with monoclonal antibodies targeting this receptor ($p = 0.710$ and $p = 0.320$, respectively) (Figures 5c and 6c, respectively). Contrary to the effect observed for hormone-dependent and HER2-overexpressing tumors, patients with TNBC-type tumors that did not respond to chemotherapy had significantly increased rates of PHLDB1 ($p = 0.009$) (Figure 5d) and PHLDB2 ($p = 0.034$) (Figure 6d), in this particularly more aggressive form of breast cancer. However, for the third family member, no relationship between *PHLDB3* differential expression with response to chemotherapeutic treatments was observed in TNBC cases ($p = 0.730$) (Figure 7d).

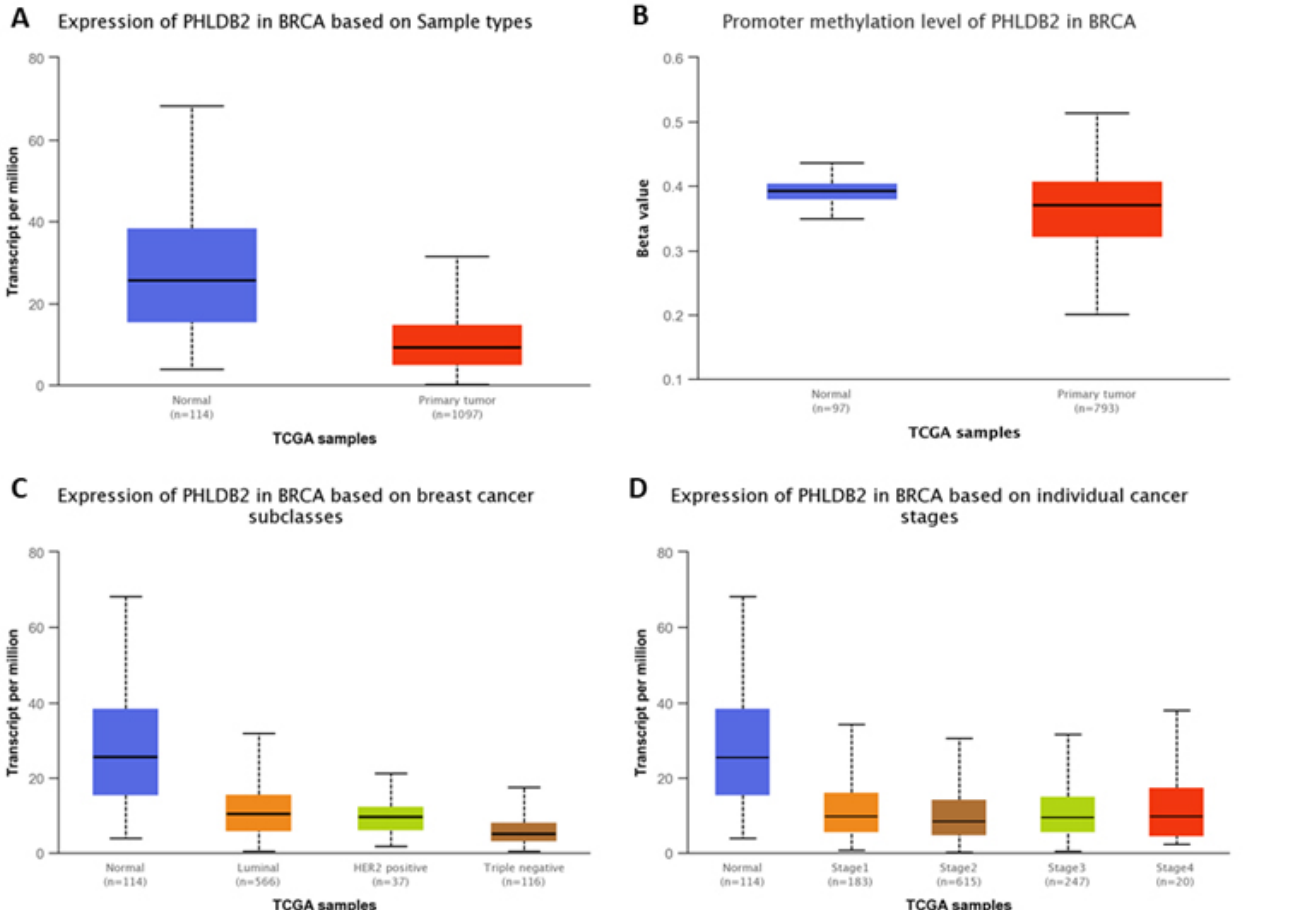

Figure 2. Expression of PHLDB2 in breast cancer patients. **a)** Expression of PHLDB2 in tumor and normal breast samples. **b)** Methylation profile of the PHLDB2 promoter region in tumor and normal breast samples. **c)** PHLDB2 expression in different molecular subtypes of breast cancer. **d)** PHLDB2 expression based on the different stages of the diseasePHLDB: pleckstrin homology-like domain family B

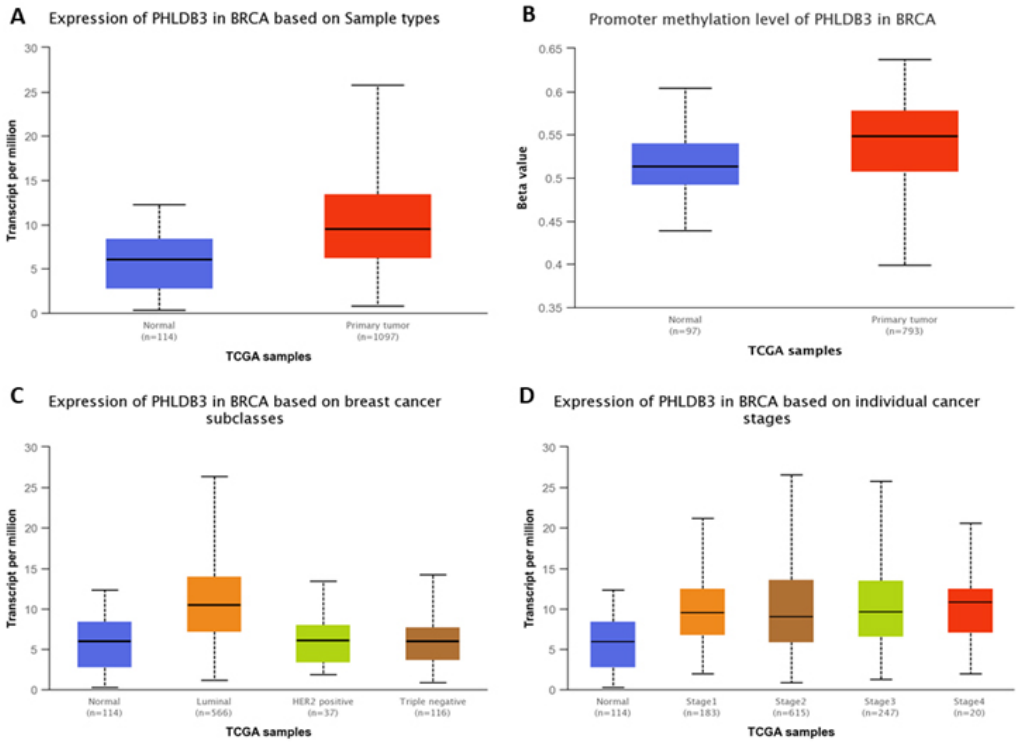

Figure 3. Expression of PHLDB3 in breast cancer patients. **a)** Expression of PHLDB3 in tumor and normal breast samples. **b)** Methylation profile of the PHLDB3 promoter region in tumor and normal breast samples. **c)** PHLDB3 expression in different molecular subtypes of breast cancer. **d)** PHLDB3 expression based on the different stages of the diseasePHLDB: pleckstrin homology-like domain family B

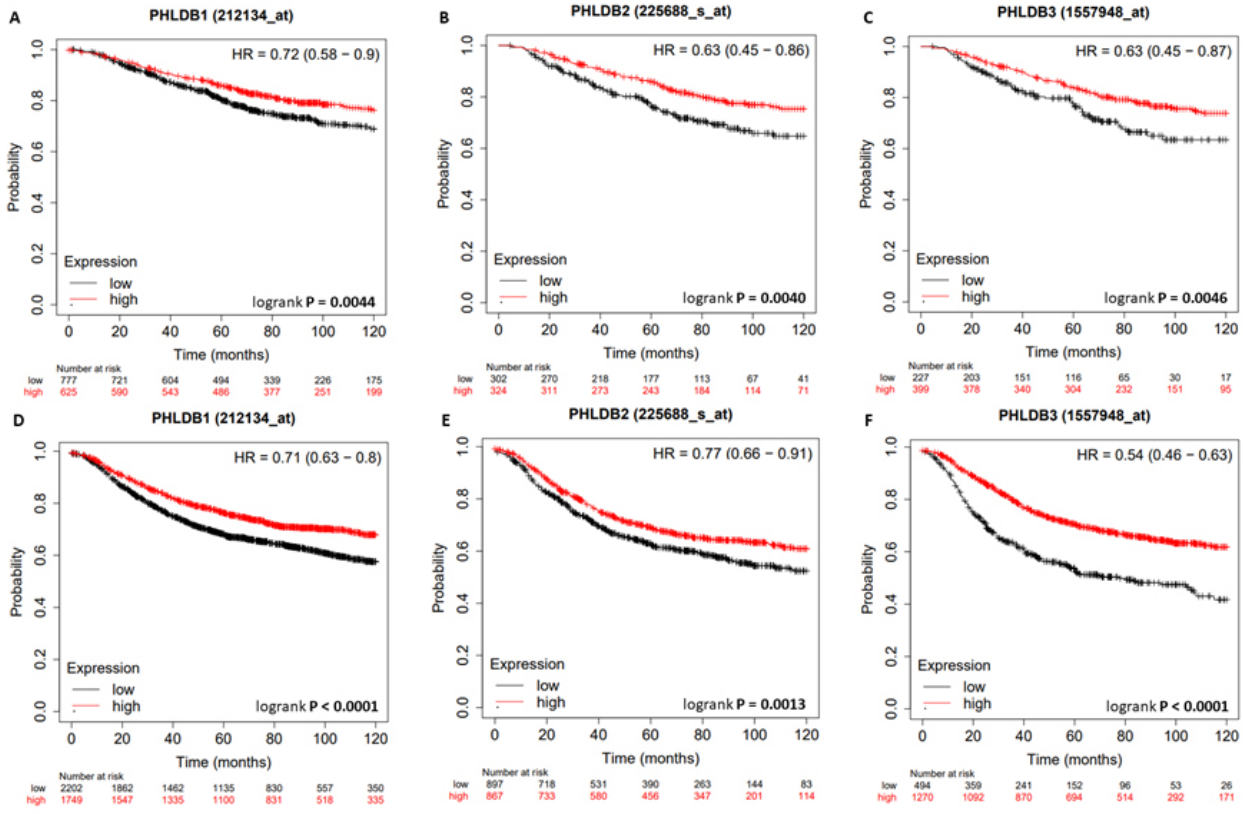

Figure 4. Survival curves derived from the Kaplan–Meier Plotter evaluating the prognostic significance of members of the PHLDB family. Overall survival for breast cancer patients stratified by expression of PHLDB1 **(a)**, PHLDB2 **(b)**, and PHLDB3 **(c)**; Relapse-free survival of patients stratified by the expression of PHLDB1 **(d)**, PHLDB2 **(e)** and PHLDB3 **(f)**PHLDB: pleckstrin homology-like domain family B

Discussion and Conclusion

Despite great advances in the diagnosis, prognosis, prevention and treatment of breast cancer, this type of malignant tumor remains the most prevalent and lethal in women globally (3). In this context, hundreds of other biomarker candidates are being studied for potential implications for improving diagnosis and personalized therapy. In view of this, our study aimed to investigate the expression profile of members of the PHLDB family and the potential prognostic and clinically useful value in breast cancer using bioinformatics tools, taking into account the limitation of studies of members of the PHLDB family in the context of breast oncology and the attractive relationship of these markers as direct and indirect targets of p53 at its transcriptional levels and as competitive modulators of AKT activity by directly interfering in the binding of this oncoprotein to phosphatidylinositol (6).

The PH domain shared by all members of the PHLD family has the ability to anchor itself transiently on the surface of the intracellular

Table 1. Relationship between the expression of PHLDB family members and clinical parameters of breast cancer patients using the bc-GenExMiner database.

Variables	Number of the patients	PHLDB1 microarray	*p*-value	Number of the patients	PHLDB2 microarray	*p*-value	Patient Number	PHLDB3 microarray	*p*-value
Age									
≤51	2813	Increased	**0.0011**	2296	-	0.1212	2209	-	**<0.0001**
>51	4692	-		4292	-		4084	Increased	
Nodal status									
Negative	4431	Increased	**<0.0001**	3259	Increased	**0.0228**	3095	-	0.1373
Positive	3457	-		3052	-		2934	-	
SBR									
1	915	-		820	-		779	-	
2	3025	Decreased		2609	Decreased		2486	Decreased	
3	3033	Decreased	**<0.0001**	2653	Decreased	**<0.0001**	2527	Decreased	**<0.0001**
NPI									
1	1234	-		998	-		917	-	
2	2119	Decreased		1823	Decreased		1714	Decreased	**<0.0001**
3	675	Decreased	**<0.0001**	662	Decreased	**0.0002**	650	Decreased	
Status TP53									
Wild-type	638	Increased	**0.0008**	578	Increased	**<0.0001**	578	Increased	**<0.0001**
Mutated	284	-		264	-		264	-	
Estrogen receptor									
Negative	2362	-	**<0.0001**	1822	-	**<0.0001**	1707	-	**<0.0001**
Positive	6531	Increased		5006	Increased		4828	Increased	
Progesterone receptor									
Negative	2509	-	**<0.0001**	2761	-	**0.0061**	2123	-	**<0.0001**
Positive	3224	Increased		2184	Increased		2712	Increased	
HER2									
Negative	4120	Increased	**0.0407**	3362	-	**0.0147**	3279	-	**<0.0001**
Positive	683	-		642	Increased		639	Increased	
Molecular subtypes									
Luminal A	3103	Increased		2517	Increased		2467	-	
Luminal B	2809	Decreased		2274	Decreased		2228	Increased	
HER2	1156	-		837	-		821	-	**<0.0001**
Triple negative	1867	-	**<0.0001**	1465	Decreased	**<0.0001**	1417	Decreased	

Significant p-values are shown in bold.

PHLDB: pleckstrin homology-like domain family B; SBR: Scarff-Bloom-Richardson; NPI: Nottingham Prognostic Index; HER2: human epidermal growth factor receptor 2

membrane and participate in multiple signal transduction processes, being the subject a number of studies (9, 18). To date, the expression pattern in patient samples and the potential prognostic and predictive value of response to different accepted therapies provided by investigating PHLDB family members remain unclear in breast cancer.

Initially, we analyzed the expression profile of the members of the PHLDB family using the UALCAN and GEPIA2 databases. PHLDB1 and PHLDB2 were expressed less in breast tumor samples when compared to healthy tissue. Meanwhile, PHLDB3 was expressed more highly in breast cancer samples. To date, no study has investigated the

Table 2. PHLDB family expression and survival data from breast cancer patients using the PrognoScan database

Gene name	Dataset	Probe name	End point	Patient number	Cox p-value	HR
PHLDB1	GSE11121	212134_at	Distant Metastasis Free Survival	200	**0.019867**	0.37 (0.16–0.86)
PHLDB1	GSE1456-GPL96	212134_at	Overall Survival	159	**0.008066**	0.21 (0.07–0.67)
PHLDB2	GSE1456-GPL97	225688_s_at	Relapse Free Survival	159	**0.011724**	0.57 (0.36–0.88)
PHLDB2	GSE1456-GPL97	225688_s_at	Disease Specific Survival	159	**0.031768**	0.56 (0.34–0.95)
PHLDB2	GSE1456-GPL97	238419_at	Relapse Free Survival	159	**0.030639**	0.68 (0.48–0.96)
PHLDB2	GSE4922-GPL97	238419_at	Disease Free Survival	249	**0.049142**	1.32 (1.00–1.73)
PHLDB3	GSE12276	236082_at	Relapse Free Survival	204	**0.034811**	0.77 (0.60–0.98)
PHLDB3	GSE12276	1557948_at	Relapse Free Survival	204	**0.001562**	0.66 (0.51–0.85)
PHLDB3	GSE1456-GPL97	236082_at	Overall Survival	159	**0.011543**	3.63 (1.33–9.87)
PHLDB3	GSE1456-GPL97	236082_at	Disease Specific Survival	159	**0.028974**	3.70 (1.14–11.97)

Significant values are shown in bold.
PHLDB: pleckstrin homology-like domain family B; HR: hazard ratio

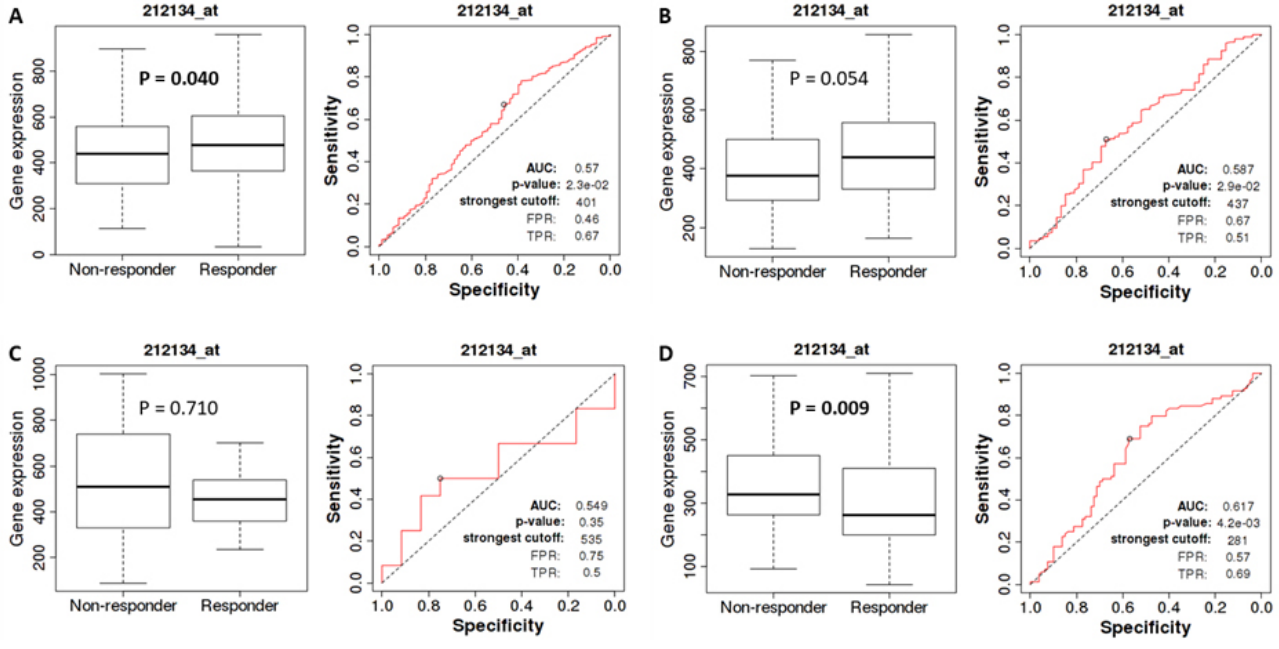

Figure 5. PHLDB1 expression pattern in patients receiving different therapies. 5-year recurrence-free survival among responders and non-responders to endocrine therapy in patients with tumors classified as Luminal A **(a)** and Luminal B **(b)**. 5-year recurrence-free survival among responders and non-responders to anti-HER2 therapy in patients with HER2+ tumors **(c)**. 5-year recurrence-free survival among chemotherapy responders and non-responders in patients with TNBC tumors **(d)**PHLDB: pleckstrin homology-like domain family B; HER2: human epidermal growth factor receptor 2

gene expression profile of the PHLDB family in healthy and tumor samples from the breast and therefore the current study is a pioneer in this sense. Furthermore, our results indicate that the methylation process can serve to repress or activate PHLDB family gene expression in breast tumor samples. It is known that the loss of balance in the methylation of specific regions of DNA can lead to increased predisposition to various diseases and abnormalities, including cancer (19). Another study identified PHLDB2 mRNA as differentially

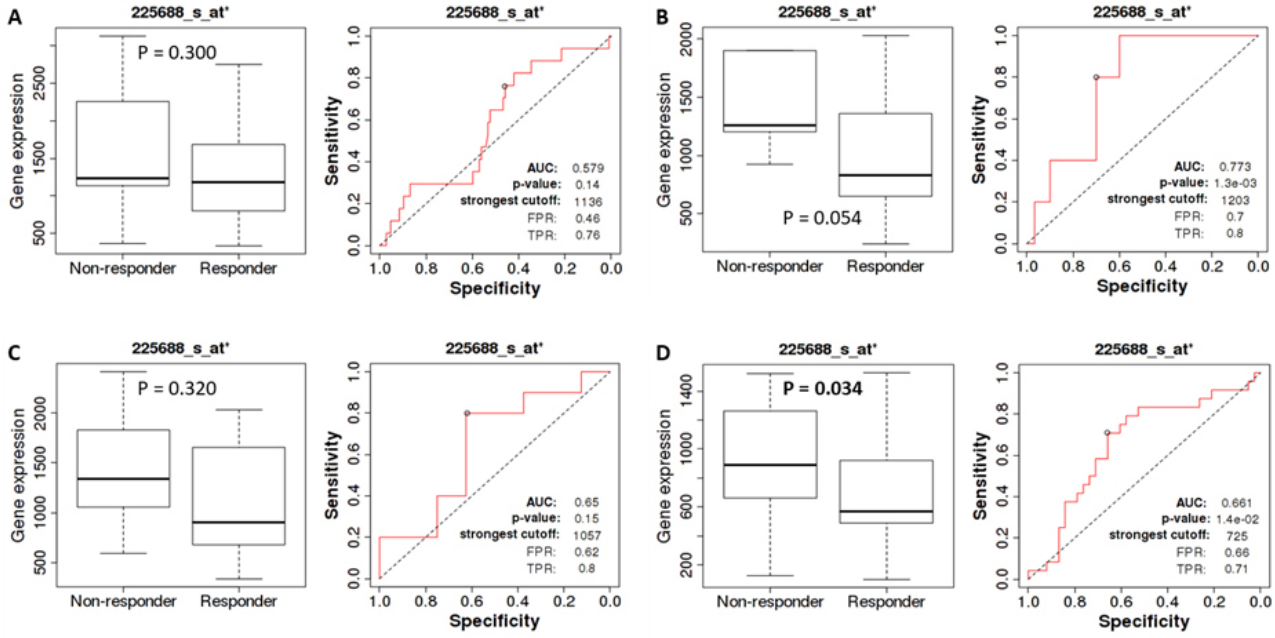

Figure 6. PHLDB2 expression pattern in patients receiving different therapies. 5-year recurrence-free survival among responders and non-responders to endocrine therapy in patients with tumors classified as Luminal A **(a)** and Luminal B **(b)**. 5-year recurrence-free survival among responders and non-responders to anti-HER2 therapy in patients with HER2+ tumors **(c)**. 5-year recurrence-free survival among chemotherapy responders and non-responders in patients with TNBC tumors **(d)**PHLDB: pleckstrin homology-like domain family B; HER2: human epidermal growth factor receptor 2

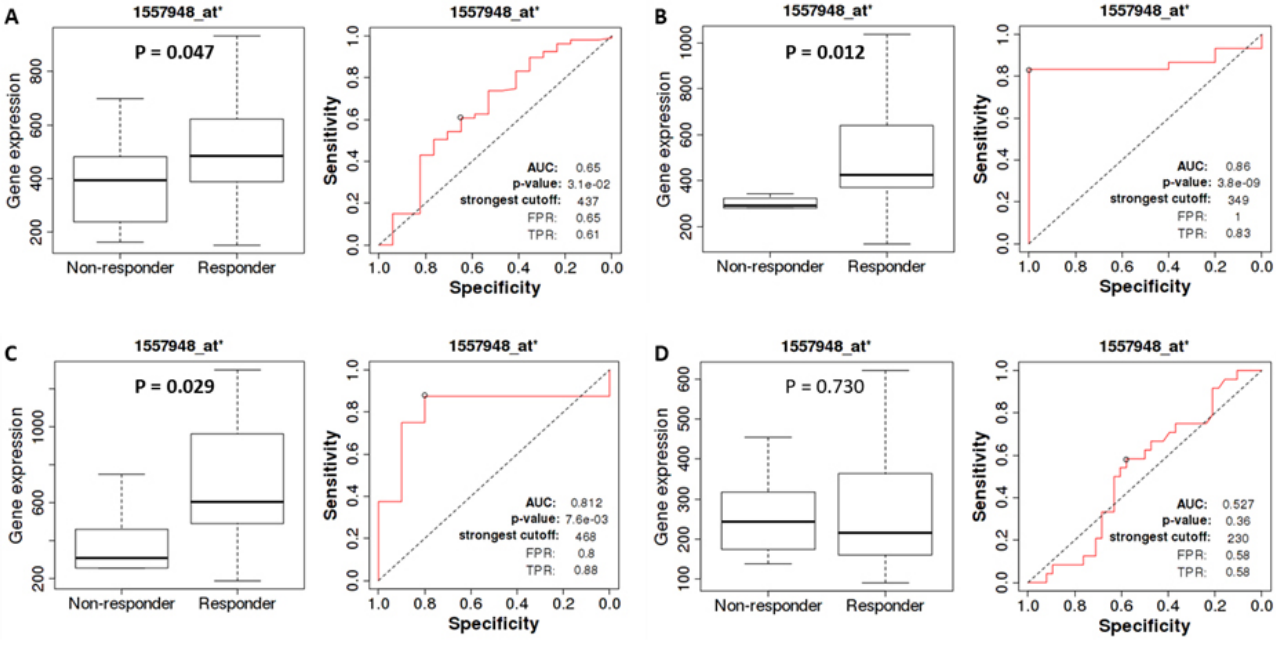

Figure 7. PHLDB3 expression pattern in patients receiving different therapies. 5-year recurrence-free survival among responders and non-responders to endocrine therapy in patients with tumors classified as Luminal A **(a)** and Luminal B **(b)**. 5-year recurrence-free survival among responders and non-responders to anti-HER2 therapy in patients with HER2+ tumors **(c)**. 5-year recurrence-free survival among chemotherapy responders and non-responders in patients with TNBC tumors **(d)** PHLDB: pleckstrin homology-like domain family B; HER2: human epidermal growth factor receptor 2

expressed, driven by methylation in uterine corpus endometrial carcinoma (UCEC) samples (20). Together, these observations may indicate that DNA methylation may be an important mechanism of epigenetic regulation of the PHLDB family in breast cancer, requiring further investigation.

Next, the relevance of the expression of PHLDB family members to different clinic pathological characteristics of breast cancer patients was analyzed. It was found that increased expression of the three members of the PHLDB family was significantly correlated with several variables, including lower rates of lymph node involvement and with the lowest degree of SBR and NPI. Routinely in clinical practice, the presence and extent of lymph node metastases are indicators of an aggressive phenotype, generally with an inverse relationship with prognosis (21). Thus, the genes of the PHLDB family, based on this *in silico* study, are shown to be potential markers for predicting the development of lymph node metastasis and unsatisfactory clinical outcome.

Additionally, our work showed a statistically significant correlation between the increased expression of *PHLDB1*, *2* and *3* with wild-type *TP53* and hormone receptor positivity (ER and PR) and, inevitably, with Luminal subtype tumors. In addition, PHLDB2 and 3 were more highly expressed in tumors with positive HER2 receptor tyrosine kinase classification, while PHLDB1 was inversely correlated compared to its paralogs. Interestingly, in addition to our findings, in previous studies it was observed that MCF-7 malignant breast cells treated with E2 (17β-estradiol) showed a large increase in the expression of *PHLDA1* transcripts compared to untreated cells (22) and that ER and NF-κB act synergistically for the direct transcriptional activation of *PHLDA1* (23). As for HER2, the picture remains unclear between the relationship between the PHLD family and this tyrosine kinase. However, previous work has already identified that *PHLDA2* expression is reduced at transcriptional and protein levels immediately and significantly by suppression of EGFR/HER2 oncogenic signaling in multiple HER2+ breast cancer cell lines (24, 25). These data indicate that members of the PHLD family can act as downstream targets of the EGFR/HER2 oncogenic signaling pathway. Finally, PHLD class proteins have been suggested as direct and indirect targets of p53 at its transcriptional levels by different studies (26, 27), demonstrating a potential critical role in tumorigenesis.

Subsequently, the prognostic significance of PHLDB family members in breast cancer was investigated using the public Kaplan–Meier Plotter and PrognoScan databases. It was found that reduced expression of PHLDB1, 2 and 3 mRNA was associated with decreased rates of OS and RFS in breast cancer patients. Supporting our previous data, the reduced expression of PHLDB family members was identified as critical for OS, RFS, DMFS and DFS reduction by the meta-analysis performed with the PrognoScan online repository. No study to date has evaluated the possible prognostic role of the PHLDB family in breast cancer. However, other works have already convincingly demonstrated that among the paralogs of the PHLDB family, members of the PHLDA family have a possible tumor suppressor role in breast cancer (28-30). Regarding the prognostic impact on different molecular subtypes, we identified that the reduced expression of PHLDB family members was associated with significantly reduced rates of OS and RFS in patients with Luminal-type tumors. For TNBC subtype tumors, an inverse role was observed, where the increased expression of PHLDB1 and 2 seems to favor a worse prognosis. Finally, among patients with tumors classified as HER2+, increased expression of PHLDB1 and 3 was responsible for worse OS. However, when evaluating these data, we have to take into account that the curves generated for OS and RFS of patients with breast cancer of molecular subtypes TNBC and HER2+ was based on smaller data sets when compared to Luminal-type tumors. Furthermore, we already know that many members of the PHLD family have a pleiotropic mechanism that will depend on the cell, tissue and molecular type and context. These findings provide evidence that PHLDB family members can serve as predictive markers for breast cancer prognosis.

Finally, our results for predicting therapeutic response showed that among patients with tumors classified as hormone-dependent and who were not responsive to endocrine treatment, these cases had lower gene expression for *PHLDB1* and *PHLDB3*. For HER2+ cases, reduced expression of *PHLDB3* was observed in samples from patients who did not respond to anti-HER2 antibody therapy. Finally, for the TNBC subtype, high expression of *PHLDB1* and *PHLDB2* was identified in samples from patients who did not respond to chemotherapeutic agents. So far, we do not know how these markers may be acting in TNBC cases, and *in vitro* studies are needed to confirm the relationship between PHLDB1 and 2 in the rates of patients' responses to chemotherapy.

Whereas, the PI3K/AKT/mTOR signaling pathway has been consistently implicated in resistance to several therapies in breast cancer (31) and that proteins with the PH domain can bind to phosphatidylinositol coupled to the surface of the intracellular membrane for suppression of this important oncogenic signaling pathway (9), we can hypothesize that PHLDB1 and 3 appear to be promising molecules to stratify patients who may or may not respond to hormone therapy and anti-HER2 agents. In addition to our findings, other studies have already demonstrated a possible relationship between the members of the PHLD family for therapeutic response in cases of Luminal and HER2+ breast cancer (16, 17, 24, 32).

In summary, this pioneering research revealed that members of the PHLDB family may be promising biomarkers for predicting prognosis and therapeutic response in breast cancer patients. It is important to highlight that *in silico* and data mining analyzes may have certain limitations, such as the extent and quality of information in publicly available databases, non-pairing of samples and, sometimes, small cohort size. However, our research was able to provide a stimulus, we hope, for possible further *in vitro* and *in vivo* studies, necessary for an application in the context of translational medicine in oncology.

Ethics Committee Approval: For this type of project, research ethics committee approval is not required.

Informed Consent: Informed consent was not required for this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: R.G.N, J.M., D.O.C.; Design: R.G.N, J.M., D.O.C.; Data Collection and/or Processing: R.G.N, J.M.; Analysis and/or Interpretation: R.G.N, D.O.C., S.J.J.; Literature Search: R.G.N, J.M., D.O.C., S.J.J.; Writing: R.G.N, J.M., D.O.C., S.J.J.

Conflict of Interest: No conflict of interest was declared by the authors.

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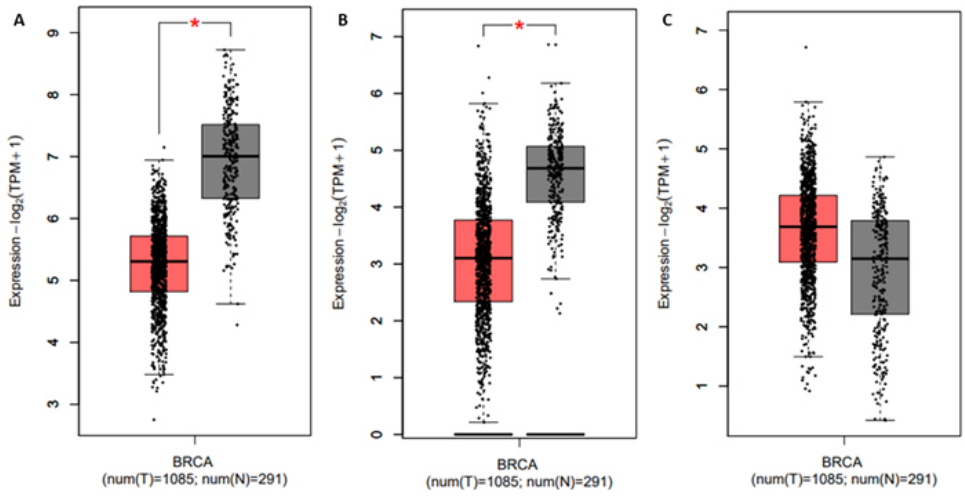
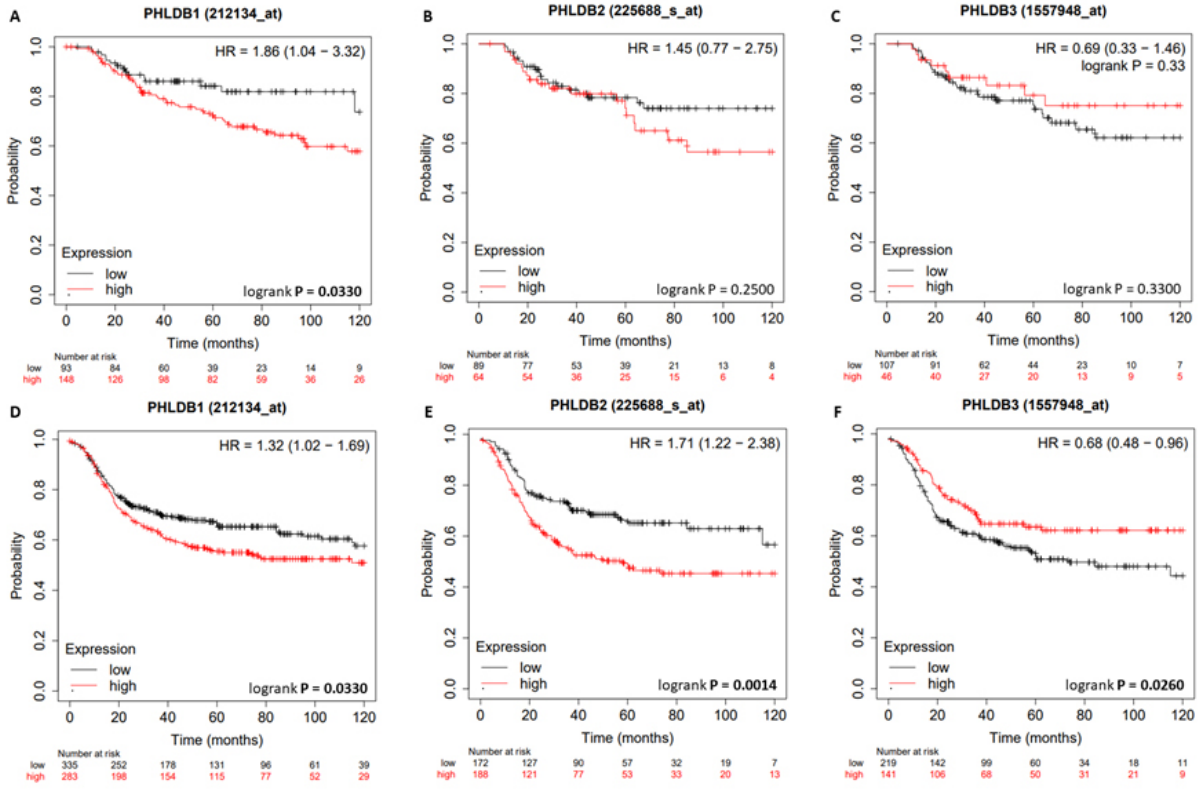
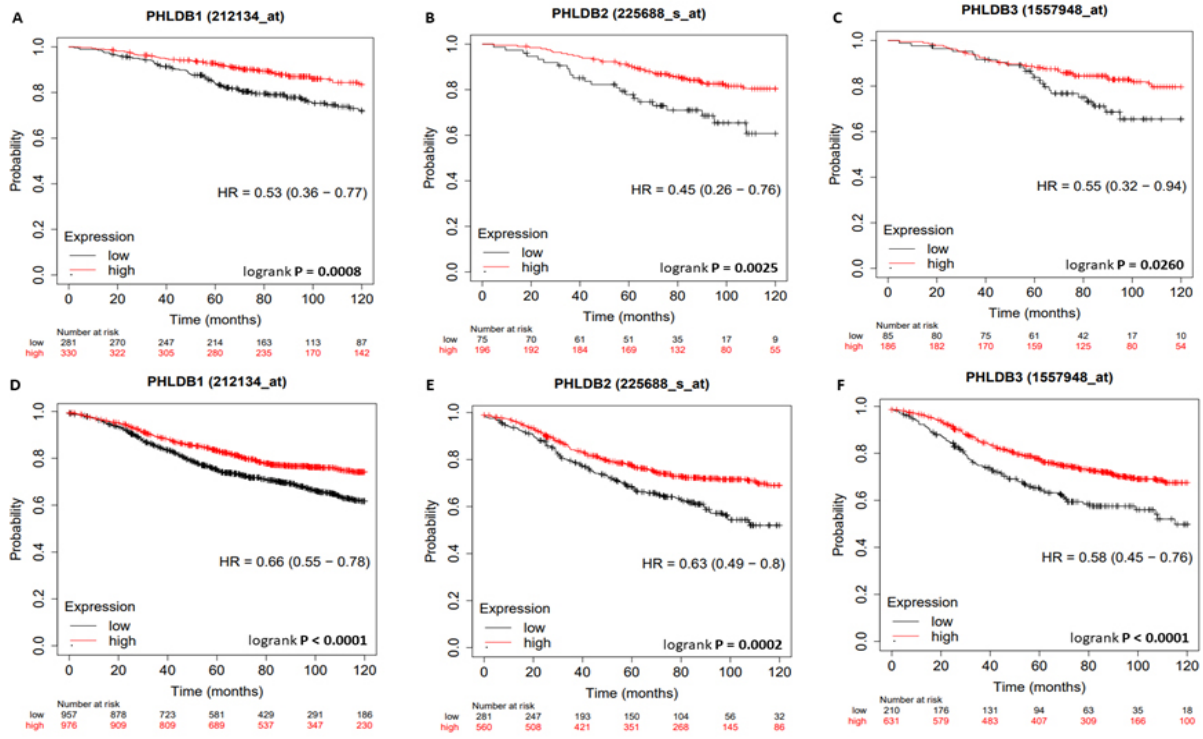


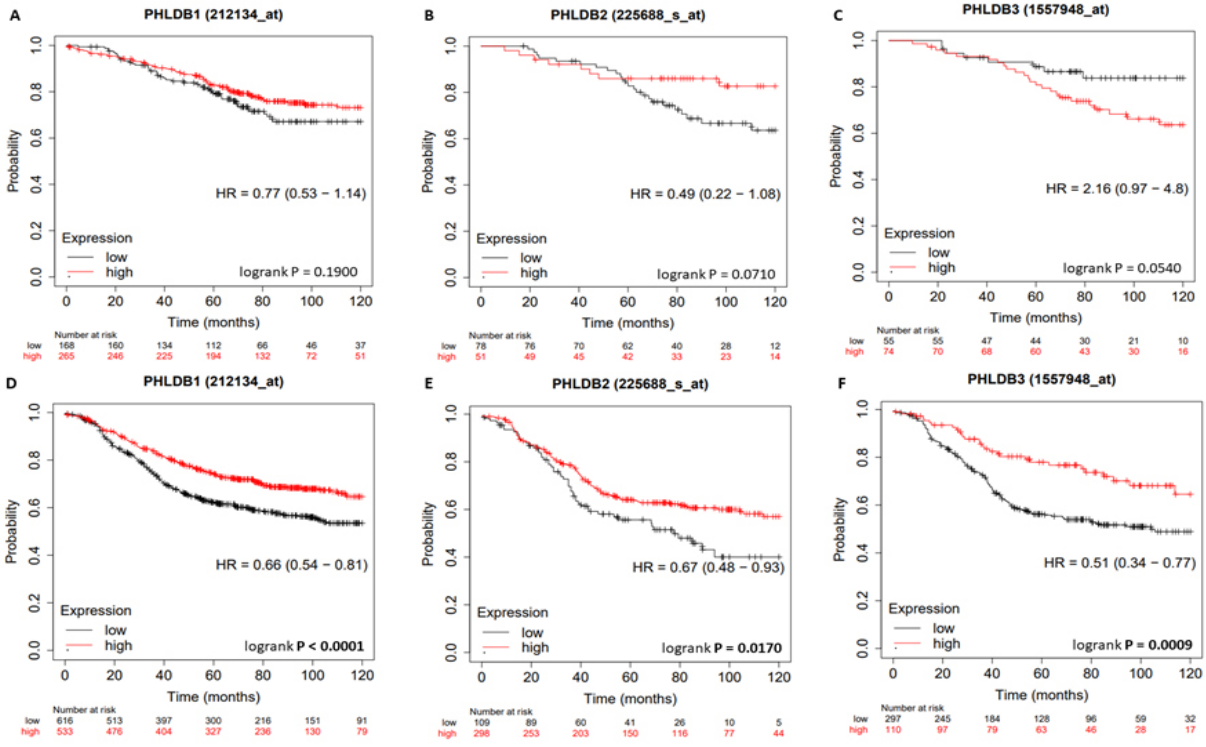
Supplementary Figure 1. Expression of PHLDB family members in normal and tumor samples of the breast. Gene expression in normal and tumor breast tissue samples for PHLDB1 **(a)**, PHLDB2 **(b)** and PHLDB3 **(c)** using the GEPIA2 databasePHLDB: pleckstrin homology-like domain family B



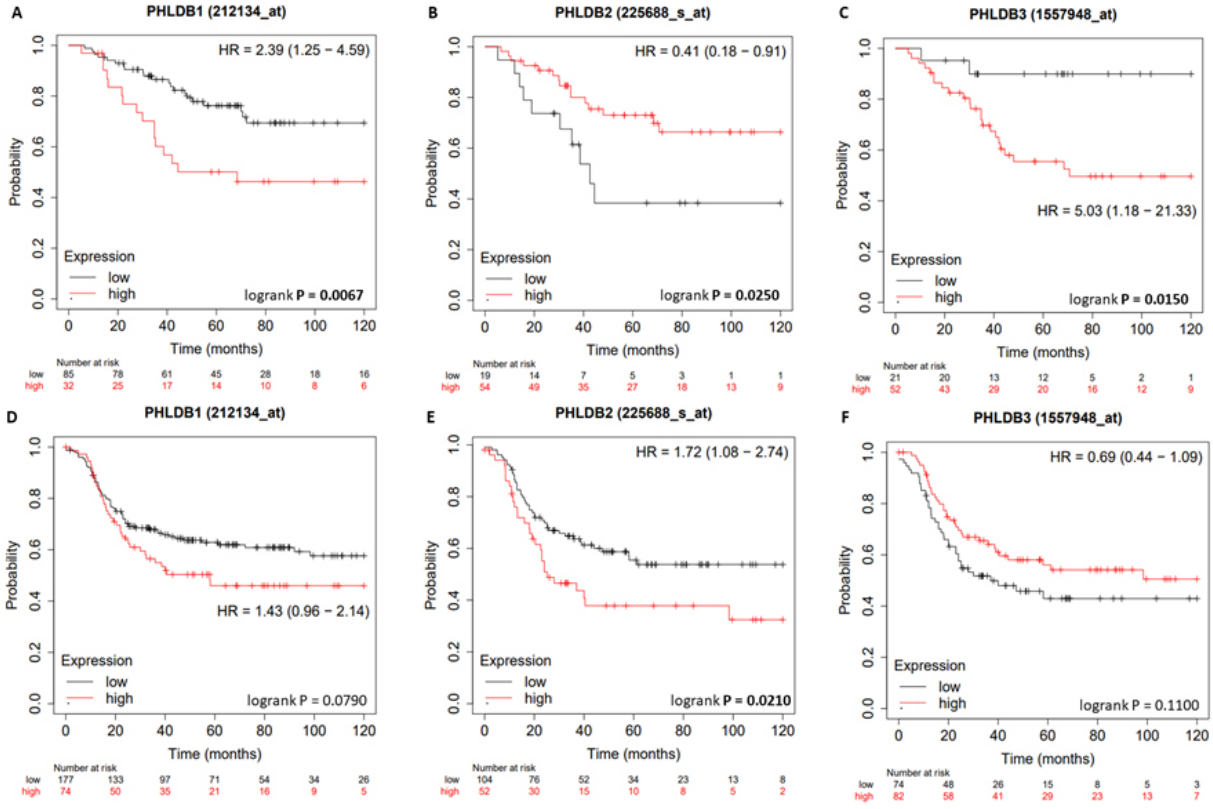
Supplementary Figure 2. Survival curves derived from the Kaplan–Meier Plotter evaluating the prognostic significance of PHLDB family members in TNBC subtype tumors. Overall survival for breast cancer patients stratified by expression of PHLDB1 **(a)**, PHLDB2 **(b)**, and PHLDB3 **(c)**; Relapse-free survival of patients stratified by the expression of PHLDB1 **(d)**, PHLDB2 **(e)** and PHLDB3 **(f)**PHLDB: pleckstrin homology-like domain family B; TNBC: triple negative breast cancer



Supplementary Figure 3. Survival curves derived from the Kaplan–Meier Plotter evaluating the prognostic significance of PHLDB family members in Luminal A subtype tumors. Overall survival for breast cancer patients stratified by the expression of PHLDB1 **(a)**, PHLDB2 **(b)** and PHLDB3 **(c)**; Relapse-free survival of patients stratified by the expression of PHLDB1 **(d)**, PHLDB2 **(e)** and PHLDB3 **(f)**PHLDB: pleckstrin homology-like domain family B



Supplementary Figure 4. Survival curves derived from the Kaplan–Meier Plotter evaluating the prognostic significance of PHLDB family members in Luminal B subtype tumors. Overall survival for breast cancer patients stratified by the expression of PHLDB1 **(a)**, PHLDB2 **(b)** and PHLDB3 **(c)**; Relapse-free survival of patients stratified by the expression of PHLDB1 **(d)**, PHLDB2 **(e)** and PHLDB3 **(f)**PHLDB: pleckstrin homology-like domain family B



Supplementary Figure 5. Survival curves derived from the Kaplan–Meier Plotter evaluating the prognostic significance of PHLDB family members in HER2+ subtype tumors. Overall survival for breast cancer patients stratified by expression of PHLDB1 **(a)**, PHLDB2 **(b)**, and PHLDB3 **(c)**; Relapse-free survival of patients stratified by the expression of PHLDB1 **(d)**, PHLDB2 **(e)** and PHLDB3 **(f)** PHLDB: pleckstrin homology-like domain family B; HER2: human epidermal growth factor receptor 2

Original Article

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Open-Label Three Arm Trial Comparing Ormeloxifene, Gamma Linolenic Acid With Methylcobalamine + Vitamin C and Placebo in Mastalgia

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ABSTRACT

Objective: We evaluated the beneficial effect of Ormeloxifene (Centchroman) versus a combination of Gamma Linolenic acid (GLA), methylcobalamine and vitamin C on mastalgia in a three-arm, open-label, placebo-controlled trial.

Materials and Methods: Patients aged above 18 years with mastalgia were recruited between January 2019 and July 2021. Patients were divided in three arms: Ormeloxifene arm, GLA arm and Placebo arm. Response was evaluated using visual analogue scale (VAS) and score below 3/10 was defined as complete relief.

Results: A total of 113 consecutive women with mastalgia were randomized to the GLA group (Group 1, n = 39 women), Ormeloxifene (Group 2, n = 36) and Placebo (Group 3, n = 38). Complete response was observed in 94% patient in Group 1, 96% in Group 2 and 87% in Group 3 at the end of 12 weeks and it was not significant (p = 0.49). Adverse events were reported by eleven patients taking Ormeloxifene, compared to none in the other two groups.

Conclusion: In this study Ormeloxifene and GLA were not superior to placebo for pain relief in mastalgia. Furthermore, there were concerning side effects associated with Ormeloxifene therapy. The role of Ormeloxifene in mastalgia needs further evaluation before recommending it as preferred therapy.

Keywords: Benign, breast disease, mastalgia

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Key Points

- Centchroman, also known as Ormeloxifene, is a relatively new drug under the class of non-steroidal selective estrogen receptor modulators which is being used to treat mastalgia.
- We evaluated the beneficial effect of Ormeloxifene (Centchroman) versus a combination of Gamma Linolenic acid (GLA), methylcobalamine and vitamin C on mastalgia in a three-arm, open-label, placebo-controlled trial.
- In this study Ormeloxifene was not superior to GLA or placebo and was also associated with concerning side effects.

Introduction

Mastalgia or breast pain is one of the commonest breast disorders in women that affects quality of life, with a reported incidence of 70% during a woman's lifetime (1, 2). Concern regarding cancer is one of the major reasons for this, impacting psychosocial well-being, prompting evaluation and treatment and hence exclusion of malignancy is the first step in treating women with mastalgia, which often relieves these symptoms leading to improved psychosocial well-being (2). Other non-pharmacological interventions, such as use reassurance with relaxation therapy and breast support, dietary supplements, and pain relief with a non-steroidal anti-inflammatory drug, gamma linolenic acid (GLA)with methylcobalamine and vitamin C have been reported to improve the quality of life in the majority of patients (3-6).

However, pharmacological interventions in the form of low dose oral contraceptives (OCP), tamoxifen, danazol and bromocriptine are required in severe and chronic mastalgia (7). Centchroman or Ormeloxifene is a relatively new drug under the class of non-steroidal selective estrogen receptor

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modulators, which is being used to treat mastalgia and fibroadenoma (8, 9). We evaluated the beneficial effect of Centchroman and GLA on mastalgia in a three-arm, open-label placebo-controlled trial.

Materials and Methods

Study Design

This was a prospective, open label, interventional study conducted between January 2019 to May 2021 on patients attending the outpatient department of General Surgery at Netaji Subhash Chandra Bose Medical College, Jabalpur, after approval from institutional ethics committee. The study was a three-arm randomized trial of Centchroman *versus* GLA *versus* Placebo in mastalgia.

Patient Eligibility and Selection

All female patients with complaint of breast pain reporting to our department were identified. All patients were evaluated as per protocol and underwent triple assessment, consisting of complete clinical examination, ultrasonography (USG) and/or X-ray mammography of both breasts and fine needle aspiration cytology (FNAC) or core needle biopsy, if indicated. Exclusion criteria were: women unwilling to participate; patients with malignant pathology; fibroadenoma >5 cm; patients taking oral contraceptive pill (OCP); pregnancy; known polycystic ovarian disease; cervical hyperplasia; recent jaundice; and females planning to conceive within 6 months.

Sample Size, Randomization, Treatment Plan and Response Evaluation

Based on a power of 80%, the aim was to recruit 36 participants in each arm to detect an intervention effect size w = 0.30. A randomization table was generated *in silico* to assign patients to three groups: Groups 1, 2 and 3. A resident, who was not involved in the study, assigned the enrolled patients to groups. Patients in Group 1 received GLA (100 mg) in combination with methyl-cobalamin (100 mg) and vitamin C (100 mg) one capsule/day for three months. In Group 2 patients received Ormeloxifene 30 mg on alternate days and Group 3 received placebo. All patients were reassured on every follow up, while dietary modification and external breast support was advised to all.

Patients were followed up at 4^{th}, 8^{th} and 12^{th} weeks. Pain severity was measured with visual analogue scale (VAS) score to assess the response to therapy (10). Patients were considered to have complete relief of pain if the VAS score fell below 3/10. Treatment was continued for a total of 12 weeks and then patients were followed up for another 12 weeks without medication to assess the continuum of relief. All drugs were stopped after three months and the last follow up was done at three months interval from stopping treatment.

Outcome Measure

Primary outcome measure for the mastalgia group was pain relief defined as

VAS score <3. If a woman also had fibroadenoma, its size was assessed by ultrasonography at baseline. No response was defined as no change in size of nodule or increase in size, partial regression was defined as decrease in size of more than 30 percent and complete regression was defined as complete disappearance of nodule. Secondary outcome measure was the occurrence of side effects of therapy.

Statistical Analysis

Demography, clinical, radiological, pathological and treatment data was collected in a pre-designated proforma. Statistical analysis was done using SPSS, version 16 (IBM Inc., Chicago, IL, USA). All analyses are reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) following intention-to-treat (ITT) procedures. Normality of data was assessed using Q-Q plot and it was normally distributed. For all statistical analyses, the significance level was set at $p<0.05$. Effectiveness of treatment arms was assessed by proportions of patients having relief from mastalgia or decrease in the size of fibroadenoma at 4^{th}, 8^{th} and 12^{th} weeks. Categorical data was analyzed by Freeman-Halton extension of Fisher's exact test or chi-square test, as required.

Results

A total of 113 consecutive women with mastalgia were enrolled. Cyclical pain was observed in 56% patients and noncyclical breast pain in 44% patients. The mean age at presentation was 40.32±11.5 (range 18–53 years) and all were pre-menopausal. The GLA group (Group 1) included 39 (34.5%) women, Ormeloxifene group (Group 2) included 36 (31.9%) and the Placebo group (Group 3) included 38 (33.6%) women. There was no significant difference with regards to age, baseline pain scores or cyclical and non-cyclical mastalgia between the three groups. On USG examination five patients had fibroadenomas and eight had diffuse fibrocystic breast disease. The majority of patients reported pain relief in both Ormeloxifene and GLA arm as compared to placebo at the end of 4 weeks of therapy (100% *versus* 94% *versus* 80%). At the end of 12 weeks of therapy, complete relief (reduction of pain to <3 on VAS and pain duration to ≤7 days/month) was observed in 92% patients in Group 1, 96% in Group 2 and 87% in Group 3, which was not significant ($p = 0.49$). At the end of follow up (three months after stopping drugs) 94% patients in Group 2 were pain free as compared to 87% in Group 1 and 82% in Group 3 (Table 1), which again was not significantly different ($p = 0.24$). Subgroup analysis for patients with cyclical and non-cyclical mastalgia was performed. For the cyclical mastalgia group (n = 63), complete response rates were 90%, 90% and 81% for Groups 1, 2 and 3 respectively ($p = 0.9$). As patients with underlying breast pathologies were very few, subgroup analysis was not done.

In terms of adverse events, there were no adverse effects observed with Group 1 (GLA arm) or the placebo arm (Group 3). However, 11% (n = 4) of patients complained of dizziness and 11% (n = 4) patients suffered from abnormal menstrual cycles and per vaginal discharge due to cervical inflammation taking Ormeloxifene (Group 2). Three patients developed cystic adnexal pathology (Table 2). Overall, eight patients were forced to discontinue Ormeloxifene before completing three months of treatment as compared to none in the GLA and placebo arms.

Discussion and Conclusion

In the present study, Ormeloxifene, 30 mg on alternate days for 12 weeks, was not superior to placebo or GLA in relieving moderate and severe mastalgia. We also found that GLA was as effective as Ormeloxifene in providing early relief (within 4 weeks) from mastalgia.

Table 1. Comparison of response at 12 weeks between three groups on mastalgia

Group	Complete response	Partial response	No response	Follow up (proportion pain free three months after stopping treatment)
Group 1 (n = 39)	36 (92.4%)	2	1	34 (87%)
Group 2 (n = 36)	34 (94.4%)	1	1	34 (94.4%)
Group 3 (n = 38)	33 (86.8%)	2	3	31 (82%)
p-value (chi-square test)	0.49	0.76	0.70	0.24

n: number

Table 2. Comparison of side effects between three groups

Group	Dizziness	Ovarian cyst	Menstrual irregularity
Group 1 (n = 39)	0	0	0
Group 2 (n = 36)	11% (4)	8% (3)	11% (4)
Group 3 (n = 38)	0	0	0

n: number

The rapid response to and early efficacy of Ormeloxifene in mastalgia was reported by Dhar and Srivastava (8) in 2007, which generated a huge interest in the drug, leading to multiple studies. They reported a response rate of 71% at the end of one week and almost all the patients were pain-free at the end of one month of Ormeloxifene therapy in this single arm study. Another study by Rathi et al. (10) reported that Ormeloxifene had a response rate of 88% at the end of 12 weeks and 85% at the end of 24 weeks in relieving mastalgia. However, neither study contained a control arm. We too observed a good response rate with Ormeloxifene (96% at the end of 12 weeks) but this was not superior to the responses reported by either the GLA arm or even the placebo arm in our study.

Kumar et al. (11) conducted a randomized, double-blind, placebo-controlled trial and reported that the mean pain level significantly reduced in the active group compared to that in the placebo group ($F = 18.66$, $p<0.0001$). The significant clinical difference in this study could be due to the use of a mean pain score instead of proportion of patients cured. Tejwani et al. (12) compared Ormeloxifene with danazol and reported significant reduction in mastalgia with Ormeloxifene as compared to danazol (89% *versus* 69%, $p = 0.001$). However, these studies did not report if other measures, such as reassurance, dietary modification and external breast support, were used along with Ormeloxifene. This information is crucial, as various studies have reported that reassurance and dietary modifications are effective in 50%–90% of mastalgia patients (13-15). A similar outcome was observed with the placebo and GLA arms in our study compared to the Ormeloxifene arm, but it should be noted that all patients were advised to modify diet and seek breast support while all patients received reassurance at all visits.

Eleven patients in the Ormeloxifene group reported side effects during the study. Dizziness was reported by 11%, menstrual irregularity by 11% and ovarian cyst by three patients. Tejwani et al. (9), reported that 75% patients receiving Ormeloxifene experienced scanty menstruation and 19% had ovarian cyst on follow up USG. Again, daily Ormeloxifene used by the authors rather than the alternate day regimen used in our study could be the cause for higher adverse events reported by Tejwani et al. (12) Gupta (16) and Rathi et al. (10) also used an alternate day regimen and reported 14% and 8% menstrual irregularity respectively, similar to that seen in our study. However, both did not have follow up USG protocol and did not report ovarian cysts. Overall, there are some concerning side effects with Ormeloxifene which needs further evaluation.

One of the limitations of the current study is that it was open label. Further studies with larger sample size with blinding are required to verify the findings of the present study.

In our study, no significant difference was found in symptom relief obtained in patients receiving Ormeloxifene, GLA or placebo in terms of proportion of women reporting pain relief. GLA was as effective as Ormeloxifene in providing early relief from mastalgia and we suggest can be given in place of Ormeloxifene. The development of ovarian cyst and menstrual irregularity in patients receiving Ormeloxifene is a matter of concern, and it needs further evaluation in larger number of cases.

In conclusion, in this study Ormeloxifene and GLA were not superior to placebo for pain relief in mastalgia. Furthermore, there were concerning side effects associated with Ormeloxifene therapy. The role of Ormeloxifene in mastalgia needs further evaluation before recommending it as preferred therapy.

Ethics Committee Approval: Ethics clearance was obtained from Institute Ethics Committee of NSCB Medical College, Jabalpur, India approval number – MS PG Thesis- Surg/1/2018.

Informed Consent: Written informed consent was taken from patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.V., D.B.S.; Design: A.V., D.B.S.; Data Collection and/or Processing: A.V., D.B.S.; Analysis and/or Interpretation: S.K.Y.; Literature Search: A.V., D.B.S., S.K.Y.; Writing: S.K.Y., D.S.; Revision and editing: D.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Clinical Significance of Radiologically Detected Small Indeterminate Extra-Mammary Lesions in Breast Cancer Patients

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ABSTRACT

Objective: Patients with breast cancer who have indeterminate extra-mammary lesions, for example in lung, liver or bone, without other metastatic lesions pose a clinical dilemma regarding subsequent management. This study aimed to investigate the prevalence, characteristics and outcomes of such lesions detected on initial staging imaging, and address the clinical significance of these incidental findings.

Materials and Methods: Medical records of patients with newly diagnosed breast cancer who underwent computed tomography scans and bone scintigraphy between January 1, 2015 and June 30, 2021 were reviewed. Patients with indeterminate extra-mammary lesions on imaging were included. Patients with obvious metastatic disease were excluded. Lesion characteristics, breast cancer staging, duration of follow-up and natural history of disease progression were analysed.

Results: The study included 52 patients with indeterminate lesions on pre-operative imaging. The median follow-up duration was 14 (range: 6–41) months. The most common site of occurrence of indeterminate lesions was the lung (60.9%) followed by the liver (26.1%). Forty-six had lesions that remained stable (88.5%), while six (11.5%) had progression to metastatic disease. Out of these six, only two (3.8%) developed metastasis in the same site as the original indeterminate lesion, whereas the remaining four developed metastases in other sites.

Conclusion: Patients with breast malignancy found to have indeterminate extra-mammary lesions without obvious distant metastasis on initial staging scans are associated with a small risk of subsequently developing metastatic disease. Although most of these lesions remain quiescent, surveillance imaging is recommended because a small but significant proportion of patients with such lesions eventually harbour actual metastatic disease.

Keywords: Breast cancer; extra-mammary; indeterminate lesion; metastatic

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Key Points

- This is the first study to evaluate the incidence and progression of indeterminate lesions in breast cancer in an Asian population.
- Most common site of occurrence of an indeterminate lesion was in the lung (60.9%).
- A small but significant proportion of these indeterminate lesions will progress to metastatic disease (3.8%).
- Routine biopsy of such lesions is not recommended, but dedicated imaging can be considered if resources permit.
- Surveillance of such indeterminate lesions is recommended.

Introduction

Breast cancer has the highest prevalence and is the leading cause of cancer-related deaths amongst women globally. There has been a 3.1% annual increase in the incidence of breast cancer from 1980 to 2010, with more than 1.6 million cases diagnosed yearly worldwide (1). In Singapore, the five-year age-standardised relative survival has increased significantly from 50.4 % in 1973–1977 to 81.4% in 2014–2018 (2). The majority of patients with breast cancer are diagnosed at an early-stage (close to 75% based on the 2018 Singapore Cancer Registry) and only 11.2% of patient present with metastatic disease (2).

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With pre-operative staging using computed tomography (CT) becoming more routine, we have observed an increasing number of patients presenting with radiologically indeterminate lesions in extra-mammary locations, such as the lung, liver and bone, without definitive evidence of metastatic disease. Indeterminate lesions are often too small to be characterised definitively. The term "indeterminate" also has varying definitions for differing organs in different studies. For instance, an indeterminate pulmonary nodule is defined as a small, focal radiographic opacity located completely within the lung measuring up to 1.5–3 cm in diameter without other abnormalities (3, 4), whereas an indeterminate liver nodule has been defined as a low-attenuating hepatocellular opacity smaller than 1.5–2 cm and visible on at least one phase of the dynamic helical CT scanning (5, 6).

In the absence of other metastatic lesions, the incidence of such lesions is reported to be in the range of 4.2% to 59% (4, 7-10). The significance of these lesions is often unknown at the time of diagnosis. Benign lesions represent the most frequent findings; however, the incidence of malignant incidental lesions have been shown to be higher in patients with a personal history of breast cancer (11). Rates of occult metastatic disease in patients with newly diagnosed breast cancer have also been shown to be low, estimated at between 5%–7% (12, 13). Hence, it is unclear whether such patients should be managed as early breast cancer with curative intent, or labelled as metastatic disease. Given the paucity of clinical data to guide management, surveillance is often recommended.

This retrospective study aimed to investigate the prevalence, characteristics and outcomes of extra-mammary indeterminate lesions detected on initial staging imaging in patients with newly diagnosed breast cancer. It also aimed to address the clinical significance of these incidental findings by evaluating if there was a higher propensity of progression to distant metastasis.

Materials and Methods

Medical records of all patients with newly diagnosed breast cancer, regardless of initial stage of disease, who underwent initial staging scans at Khoo Teck Puat Hospital, a tertiary regional hospital in Singapore, were collected retrospectively from January 1, 2015 to June 30, 2021. Routine staging imaging comprised of CT chest, abdomen and pelvis scan and also a bone scintigraphy scan.

Indeterminate lesions were defined as lesions less than 15 mm in diameter in the absence of other metastatic lesions, which were detected on contrasted single-phase CT imaging or on bone scintigraphy scan. These are lesions which could not be concluded as definitely benign or malignant, based on radiological appearances, and warrant further imaging or interval surveillance. Patients noted to have indeterminate lesions in extra-mammary locations, including lungs, liver, bone or other organs on pre-operative imaging were included. If there were greater than one indeterminate lesion noted within the same patient, they were included and lesions were recorded separately. Lesions that were characterized definitively by the radiologist as metastasis (Stage IV disease), or benign lesions without the suggestion of further radiologic follow-up, were excluded. Patients that were lost to follow-up or declined further surveillance scans were similarly excluded. All subsequent imaging scans of patients with indeterminate lesions were reviewed to assess for progression. Lesions with an increase in size or manifestation of malignant radiologic features over time were considered as likely metastasis.

Details about patient demographics, tumour characteristics [tumor-node-metastasis (TNM) staging, hormone receptor and human epidermal growth factor receptor 2 (HER2) status, grade] were abstracted from the electronic medical records. Characteristics of the indeterminate lesions on pre-operative staging scans, including site, size, location and presence of calcification, were recorded. These same lesion characteristics were similarly recorded for subsequent scans performed. The surveillance intervals and time interval to progression of these lesions were also analysed.

The statistical analysis compared the clinicopathologic and demographic data between patients with stable lesions on radiologic follow-up and patients with malignant lesions. The Mann–Whitney U test was used to compare age in the two groups. Fisher's exact probability test was used to check the association between patients' tumour histologic grade, tumour stage, lymph node status and receptor status with risk of metastatic disease progression. Statistical Package for the Social Sciences, version 25.0 (SPSS Inc., Armonk, NY, USA) was used for the analysis, and the significance level was set at 0.05.

Institutional Review Board approval was granted for this retrospective study, and a waiver of informed consent was obtained (DSRB reference number: 2020/00181).

Results

A total of 736 patients with breast cancer who were treated in Khoo Teck Puat Hospital in Singapore from January 1, 2015 to June 30, 2021 had pre-operative staging CT and bone scan performed. Fifty-two (7.1%) patients were identified as having indeterminate lesions that could not be definitively characterised.

The patients with indeterminate nodules were characterised according to patient demographics (Table 1). All patients were female and the mean age was 59.6 years. Out of 52 patients, 47 (90.4%) had invasive ductal carcinoma, two (3.8%) had invasive lobular carcinoma, one (1.9%) had a malignant phyllodes tumour and two (3.8%) had squamous cell carcinoma. Three (5.7%) were Grade 1, 21 (40.4%) were Grade 2 and 28 (53.8%) were Grade 3 tumours. When stratified according to stage of disease, 18 (34.6%) had Stage I, 22 (42.3%) had

Table 1. Patient demographics

Age	
Mean ± SD	59.6±11.8
(minimum, maximum)	(37, 86)
Sex (n = 52)	
Male	0 (0%)
Female	52 (100%)
Ethnic group (n = 52)	
Chinese	37 (71.2%)
Malay	7 (13.5%)
Indian	4 (7.6%)
Others	4 (7.6%)
Smoker (n = 52)	
Yes	5 (9.6%)
No	47 (90.4%)

SD: standard deviation

Stage II and 12 (23.1%) had Stage III disease. Further breakdown of the histopathological data of the primary breast tumour is summarised in Table 2.

Review of the imaging for the indeterminate lesions was undertaken to assess the location and characteristics of these lesions. The most common locations for indeterminate lesions were the lungs (n = 28, 60.9%), bone (n = 14, 30.4%), liver (n = 12, 26.1%), and adrenal glands (n = 6, 13.0%). There was an isolated case of an indeterminate large (8 cm) retrosternal thyroid lesion which was largely cystic with small areas of hypodensity on CT. Subsequently this lesion was worked up with an ultrasound thyroid and found to be a benign cystic lesion. There was another isolated case of a right adnexal lesion which remained stable on subsequent imaging.

Characteristics of these indeterminate lesions were also analysed. Amongst the 28 patients with indeterminate lung nodules, all had either one or two nodules with an average nodule size of 4.6 mm (range: 2 mm – 13 mm). None of the nodules were calcified and 5 (18%) had centrally located nodules, whilst 23 (82%) had peripherally located nodules. Of the 14 patients with indeterminate bony lesions, four (29%) had lytic lesions whilst 10 (71%) had sclerotic lesions. Of the 12 patients with indeterminate liver lesions, five (42%) had solid-cystic lesions compared to seven (58%) who had solid lesions. The indeterminate adrenal lesions ranged in size from 13 mm to 35 mm (mean: 22 mm), with Hounsfield units on unenhanced CT images of -22 HU up to 27 HU (mean: 6.3 HU). These patients subsequently underwent CT adrenal scan which revealed lipid-rich lesions and hence low likelihood for malignancy.

Patients were followed up for a median duration of 14 (range: 6–41) months with all patients undergoing subsequent surveillance imaging to reassess the indeterminate lesions. Amongst the cohort, 46 (88.5%) patients were shown to have stable lesions that were likely benign. Progression to metastatic disease from the original indeterminate lesion was found in only two patients (3.8%), whilst four (7.7%) developed metastases at other sites, as shown in Figure 1. The progression of the lesions in these six patients was seen on subsequent imaging performed at a median of 18 (range: 4–50) months after the initial staging CT scan. Four of these six patients subsequently died of advanced malignancy, and the rest received palliative treatment for metastatic disease.

For patients who had stable indeterminate lesions on subsequent imaging, 28 (60.1%) of them received adjuvant chemotherapy and 31 (67.4%) received adjuvant radiotherapy. There were 31 (67.4%) patients who were on endocrine therapy and 17 (37%) patients who received trastuzumab (Table 3). With regard to the two patients

Table 2. Histopathological results of the primary breast malignancy

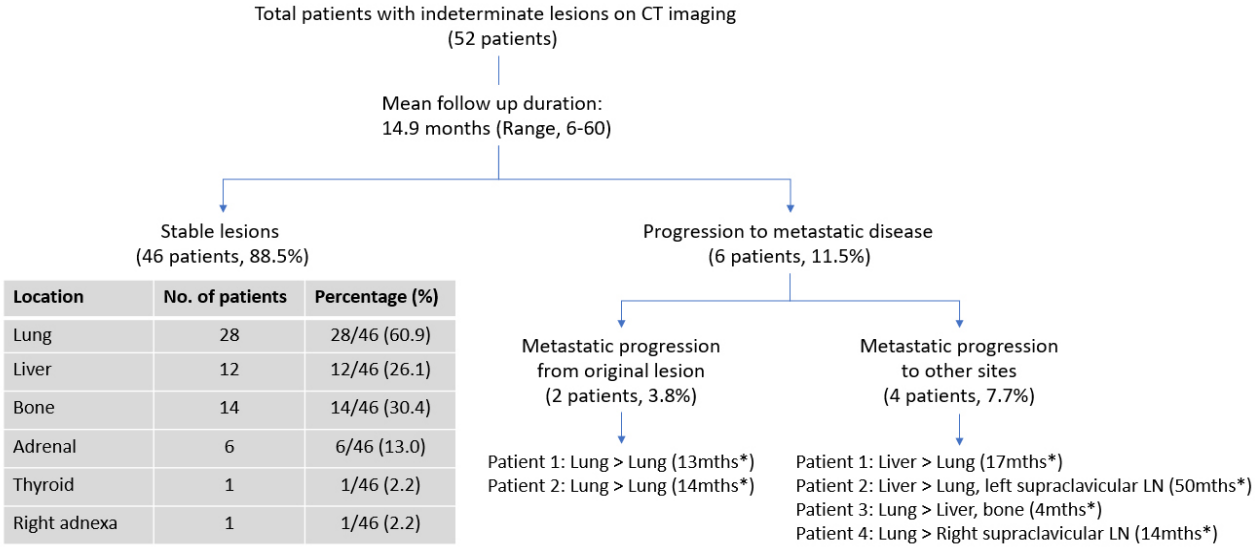
Histological grade (n = 52)	
Grade 1	3 (5.7%)
Grade 2	21 (40.4%)
Grade 3	28 (53.8%)
Number of lymph nodes involved (n = 52)	
0	36 (69.2%)
1–3	9 (17.3%)
≥4	7 (13.5%)
TMN staging (n = 52)	
T status	
T1	19 (36.5%)
T2	24 (46.1%)
T3	4 (7.7%)
T4	5 (9.6%)
N status	
N0	33 (63.5%)
N1	11 (21.2%)
N2	5 (9.6%)
N3	3 (5.7%)
Stage (n = 52)	
Stage I	18 (34.6%)
Stage II	22 (42.3%)
Stage III	12 (23.1%)
Histological subtype (n = 52)	
Invasive ductal carcinoma	47 (90.4%)
Invasive lobular carcinoma	2 (3.8%)
Squamous cell carcinoma	2 (3.8%)
Malignant phyllodes	1 (1.9%)
Receptor status (n = 52)	
Luminal A (ER+ PR+/- HER2-)	19 (36.5%)
Luminal B (ER+ PR+/- HER2+)	17 (32.7%)
HER2+ (ER- PR- HER2+)	5 (9.6%)
Triple negative (ER- PR- HER2-)	11 (21.2%)

ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; n: number

Table 3. Treatment data of patients with indeterminate lesions, with or without clinical progression

	Stable indeterminate lesion (n = 46)	Progression from original lesion (n = 2)	Progression to other sites (n = 4)
Endocrine therapy	31 (67.4%)	1 (50%)	1 (25%)
Chemotherapy in neo-adjuvant setting	12 (26.1%)	2 (100%)	0
Chemotherapy in adjuvant setting	28 (60.1%)	1 (50%)	1 (25%)
Trastuzumab	17 (37.0%)	1 (50%)	1 (25%)
Radiotherapy in adjuvant setting	31 (67.4%)	1 (50%)	2 (50%)

n: number



Location	No. of patients	Percentage (%)
Lung	28	28/46 (60.9)
Liver	12	12/46 (26.1)
Bone	14	14/46 (30.4)
Adrenal	6	6/46 (13.0)
Thyroid	1	1/46 (2.2)
Right adnexa	1	1/46 (2.2)

Figure 1. Clinical progression of patients with indeterminate lesions on CT imaging

** indicates time interval to progression*

CT: computed tomography; LN: lymph node

found to have progression to metastatic disease from the original indeterminate lesion, both had indeterminate lesions in the lung. Both patients had two, non-calcified lung nodules each within the same side of the lung. The first patient was Stage IIIc (T4N3) on presentation, with biopsy showing Grade 3, triple negative malignant phyllodes. The size of her largest lung nodule measured 12 mm. The second patient was Stage II (T2N0) on presentation. She had Grade 2, triple positive invasive ductal carcinoma. Her largest lung nodule measured 13 mm. Both patients received neoadjuvant chemotherapy, with the second patient subsequently receiving adjuvant chemoradiotherapy in the adjuvant setting as well. The second patient also received trastuzumab and endocrine therapy.

No statistical significance was found in the demographic or clinicopathologic data between patients with stable lesions and those with malignant lesions on radiologic follow-up that could predict the natural history of these indeterminate extra-mammary lesions. However, this analysis may be limited by the small number of patients.

Discussion and Conclusion

Five-year survival rates for metastatic breast cancer have been reported to be 27%, as compared to 84% in locally advanced breast cancer (14), while 20%–30% of breast cancer patients can progress to metastases after diagnosis (13). It is known that breast cancer has metastatic heterogeneity, with a propensity towards bone (63%), liver (25%) and lung (23%) (15). Metastases to other organs, such as adrenals (16), thyroid (17) and adnexa (18) are considered rare. Different molecular subtypes have also been shown in certain studies to have different preferential sites of metastasis or relapse, such as a predominance of bone metastasis in luminal A and B subtypes, compared to non-luminal HER2 subtype which had a higher propensity for liver metastasis (9, 19).

Despite the latest National Comprehensive Cancer Network (NCCN) guidelines recommending that routine systemic staging in early breast cancer patients is not required because of the low likelihood of identification of metastatic disease (20), many centers still practice routine screening for breast cancer patients for evaluation of distant metastases. This commonly includes CT scans, positron emission tomography-CT (PET-CT) scans, and/or bone scintigraphy. Consequently, lesions of indeterminate nature are often noted. In our series, they were present in 7.1% of patients who got pre-operative staging scans, and these indeterminate lesions were mostly in the lung (60.9%), followed by bone (30.4%) and liver (26.1%). In a study performed by Brothers et al. (21), which was the only other study amongst the available literature that analyzed an array of indeterminate lesions in multiple organs, the lung was similarly the most common site. The incidences of indeterminate lesions recorded in their study were lung (50%), bone (26%) and liver (39%). Additionally, they reported close to 20% incidence of lymphadenopathy, adnexal and renal lesions which were not observed in our study. Other studies found a wide range of incidences of indeterminate nodules, varying from 4.2% to 59% (4, 7-10).

To our knowledge, this study is the first to evaluate the incidence and subsequent progression of indeterminate lesions in breast cancer on staging scans in an Asian population. The majority of the lesions (88.5%) in our series were stable on follow-up surveillance imaging with no progression of disease, at a median follow-up of 14 months. Only two patients (3.8%) with indeterminate lung lesions developed actual lung metastases on follow-up. These patients had Stage II and Stage IIIc disease respectively. Compared to the study by Brothers et al. (21) who reported progression in 29 out of 127 patients (22.8%) at a median follow-up of 4.9 years in patients with abnormal initial scan findings (20), ours was significantly lower. These differences are likely due to two main factors, the first being that their study population consisted of only Stage II and III breast cancer patients, whereas our study population had 34.6% Stage I patients, with the rest being Stage II and III. Secondly, the mean follow-up duration of our study was shorter, which could have resulted in fewer lesions having progressed to metastases.

Due to our small sample size and with only two patients progressing to metastatic lung disease, it was difficult to analyze them to determine if there were any significant factors associated with development of metastatic disease. Studies on indeterminate lesions found during staging for breast cancer have been focused mainly on pulmonary nodules. These studies have shown that significant risk factors associated with the development of lung metastases included large nodules ≥10 mm, multiple nodules, clinical Stage II-III and Grade 3 tumors (4, 21). This is similar to our two patients, who both had two unilateral non-calcified lung nodules each, of which the largest nodule measured >1 cm for both patients. Both patients also had a higher clinical stage of breast cancer. A study conducted by Lee at al. (4) concluded that sub-centimeter lung nodules with no other evidence of distant metastasis posed a low risk of progression and hence, should not preclude treatment with curative intent or entry into clinical trials. Thus, we suggest that an individualized risk-stratified approach, based on the probability of malignancy should be adopted for patients with indeterminate lung lesions. We have used serial CT imaging to monitor patients with indeterminate pulmonary nodules as an alternative to more invasive testing, such as biopsy. However, if progression of these indeterminate lesions is detected on surveillance, further diagnostic investigations, such as bronchoscopy or transthoracic needle aspiration/biopsy, can aid in excluding malignancy. Although the optimal frequency of follow-up imaging is unknown for these lung lesions in the setting of breast cancer, certain surveillance protocols can be extrapolated from indeterminate lung nodules in the setting of lung malignancy. According to the Fleischner Society Guidelines, the duration and frequency of surveillance of an indeterminate lung nodule is largely guided by the original size of the lesion and the individual risk factors for lung malignancy (22). Surveillance imaging at 3-monthly intervals during the first year after incidental nodule is discovered and then 6-monthly in the following year with high-resolution CT imaging has also been recommended (3).

Bone is another common site of distant metastases from breast cancer and is the first affected site in a substantial proportion of women (23, 24). Breast cancer guidelines and consensus recommendations indicate that various imaging studies may be used for staging or evaluation of bone metastasis in women with breast cancer (25, 26). Although the first choice is often a nuclear medicine bone scan (or bone scintigraphy), as this method shows only bone metabolism, another imaging study might be needed for an accurate diagnosis. This includes plain radiography, CT, magnetic resonance imaging (MRI), single-photon-emission CT (SPECT) and F-18-Deoxyglucose or Fluorodeoxyglucose (FDG) PET-CT (27). Bone scintigraphy relies on the radiotracer Tc99m methyl diphosphonate (MDP), which allows visualisation of uptake in regions of increased bone turnover and osteoblast activity and a resulting increase in blood perfusion (28). However, they are insensitive for tumours that are predominantly lytic. Additionally, bone metastases in avascular sites of disease can also result in false-negative scans due to the lack of increased perfusion that typically accompanies osteoblastic activity (29). Hence several studies have shown that FDG PET-CT or magnetic resonance imaging (MRI) should be recommended in high-risk patients for further evaluation of indeterminate bony findings due to their higher sensitivity (30, 31).

While incidental liver lesions are often found on cross sectional imaging, they pose a particular challenge for oncology patients when they are deemed indeterminate or too small to characterise. There are no established guidelines as of yet about how extensive or aggressive workup should be. In a study done by Khalil et al. (5), the presence of at least one indeterminate liver lesion was found in 29% of women with breast cancer who had cross sectional imaging performed. More than two-thirds (69%) of these women had follow-up imaging which showed the majority of the lesions were either unchanged (92%) or had disappeared (4%). Overall, in 92.7%–96.9% of women with indeterminate liver lesions, these lesions were eventually benign, after a median follow-up of 54 weeks. The same authors also evaluated the role of MRI in breast cancer patients with liver lesions on CT imaging (32). Out of 38 patients with indeterminate liver lesions on CT, only two eventually had metastatic liver disease on MRI liver. In the women who had indeterminate lesions even on MRI liver who received further workup (n = 8) such as biopsy or surveillance imaging, all had benign disease. The authors concluded that in these patients with indeterminate liver lesions, MRI of liver offered minimal further benefit in the majority of their patients, and they did not recommend immediate work-up with MRI or biopsy. In our opinion, one further utility of the MRI would be the confirmation of a benign diagnosis early in the diagnostic work-up. This would help to ease frequency of surveillance imaging as well as patient anxiety. Hence in a centre where resources permit, an MRI liver can be considered as the subsequent follow-up imaging after an initial CT finding of an indeterminate liver lesion.

This study has several limitations. Firstly, its clinical relevance is limited by its small sample size. Larger-scaled studies will be required to determine the applicability of these findings. Secondly, this is a retrospective cohort study and there was no standard follow-up protocol for patients with these small uncharacterized extra-mammary lesions. This is due to currently limited published data on the prevalence and nature of such indeterminate lesions. Therefore, this study hopes to contribute towards future efforts in creating a standardised protocol for follow-up of such indeterminate lesions.

In conclusion, indeterminate extra-mammary lesions detected on imaging for newly diagnosed breast cancer patients pose a pertinent diagnostic challenge. Routine biopsy is generally not indicated due to the indolent nature of the majority of these lesions. However, further dedicated imaging, such as MRI or PET scan, can be considered where resources are available. Continued surveillance imaging of such lesions is recommended as a small, albeit noteworthy, proportion of them eventually harbour actual metastatic disease. Clinicopathological characteristics of the patients can also be considered in the eventual surveillance strategy. We also suggest that these patients with primary breast cancer and indeterminate extra-mammary lesions should be offered treatment with curative intent.

Ethics Committee Approval: The protocol for this research project has been approved by our institutional research board, National Healthcare Group Domain Specific Review Board and it conforms to the provisions of the Declaration of Helsinki (DSRB Reference Number: 2020/00181).

Informed Consent: Informed consent was exempted in view of minimal potential risks to research subjects and that all attempts to preserve anonymity of the data by deidentification of the patients' data had been taken.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: R.Y.C., J.T.T.G., M.W.O., Design: M.W.O., Data Collection and/or Processing: R.Y.G., H.T.L., S.C., M.W.O., Analysis and/or Interpretation: R.Y.C., M.W.O., Literature Search: R.Y.C., R.Y.G., M.W.O., Writing: R.Y.C., R.Y.G., V.K.M.T., C.L.K.C., J.T.T.G., M.W.O.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Update of the 100 Most Cited Articles on Breast Cancer: A Bibliometric Analysis

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ABSTRACT

Objective: The aim of this study was to perform a bibliometric analysis of the 100 most cited articles related to breast cancer.

Materials and Methods: The research was done on the Web of Science (WOS) database. Only research articles were included in the study. Results were obtained by typing the term "breast cancer" in the WOS Search box. The results were sorted according to the number of WOS core citations and all database citations, the first author of the article, the institution of the first author, publication year, article category, and countries.

Results: The most cited article had 10236 citations. Nearly three-quarters (70%) of the articles were from the USA and most articles were published by Harvard University. Thirty-seven percent of the articles were in the medicine, general and internal medicine categories.

Conclusion: This bibliometric analysis identified the 100 most cited research articles about breast cancer and provided a record of historical developments and trends in breast cancer research.

Keywords: Breast cancer; citation; research

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Key Points

- The result of this research about the 100 most cited articles on breast cancer may help to understand important studies on breast cancer and shed light on future studies.

Introduction

Breast cancer is the most prevalent cancer globally, as well as a leading cause of cancer-related death among women (1). Substantial support for breast cancer awareness and research funding has helped created advances in the diagnosis and treatment of breast cancer (2). Early detection, a novel personalized approach to treatment, and a better knowledge of the disease have all contributed to an improvement in breast cancer survival rates and a steady decline in the number of deaths related with the disease (3). Current guidance on preventing and treating breast cancer, as well as what might cause it, has come mainly from information discovered from research studies (4). The most significant component of the methodological qualities of studies is associated with an increase in citations and a high impact factor of the journal in which it was published (5). To the best of our knowledge, there is only one early study that has performed a bibliometric analysis of the attributes of the 100 most cited articles about studies concerning breast cancer (6). The aim of this study was to evaluate the current status of the 100 most frequently cited articles.

Materials and Methods

A Web of Science (WOS) (Clarivate Analytics, Philadelphia, PA, United States) search was used to collect the information for this investigation. The journals indexed in the Science Citation Index Expanded (SCI-E) were included. There were no restrictions on the journals. Over 9200 of the world's most influential publications from 178 scientific areas are now indexed in the Science Citation Index Expanded™. More than 53 million records and 1.18 billion cited references date from 1900 to the present (7).

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Inclusion Criteria

The term "breast cancer" was typed into the search box of WOS basic research with the selection of all the years and the search was performed on 11.02.2022. The search produced 621,351 published articles between 1978 and 2022. As filters, English language, SCI-E scope and research article type were selected, resulting in a reduction to 376,105 articles. These were then ranked in order of citation frequency, from highest to lowest. The study was conducted by generating a shortlist of the top 100 cited publications from this search list, which were classified by journal, study category, country and location where the research was published, authors, and publication date.

Exclusion Criteria

Articles in indexes other than SCI-E, published in languages other than English, and other types of articles, such as reviews, meeting abstracts, letters, book chapters, etc., were excluded. Also, cancer statistics articles were excluded, despite receiving more citations than the included research articles.

Written informed consent was not necessary because no patient data was included in the study. The study complied with the Declaration of Helsinki.

Statistical Analysis

No inferential statistical analysis was undertaken. All the data is given in percentages, numbers and charts.

Results

The articles included in the study are listed according to the total number of citations in the WOS database and in the all databases (WOS database, Arabic Citation Index, BIOSIS Citation Index, Chinese Science Citation Database, Data Citation Index, Russian Science Citation Index and SciELO Citation Index). According to our results, the most cited article was by Charles M. Perou and his colleagues, with 10,236 citations in the WOS database, and the least cited article was by Lisa A Carey and her colleagues, with 1,403 citations. Considering the number of publications, the most cited author was D.J. Slamon with 25,000 citations, followed by B. Fisher with 11,809 citations, T. Sorlie with 11,343 citations, Charles M. Perou with 10,236 citations, and N.K. Aaronson with 9247 citations (Table 1). It was evident that all articles received more than 1000 citations and all were published between 1985 and 2021. Twelve of the most cited articles were published in 2007, and there was one publication each for 1987, 1992, 1993, 1995, 2000, 2018, and 2021 among the most cited articles (Figure 1).

These most cited articles were published in 20, high-impact factor journals, with 24 articles published in the New England Journal of Medicine, 13 in Nature, 11 in the Journal of Clinical Oncology, and 11 in Science (Table 2). Seventy of the studies originated from the United States of America (USA), 13 publications from the United Kingdom (UK), six from Italy and three from Canada (Figure 2).

The articles were sourced from 51 different centers. The institution with the most publications was Harvard University with eight articles, followed by the University of Pittsburgh with six articles, the IRCCS European Institute of Oncology (IEO) with five articles, and the University of North Carolina with five articles, while 32 institutions had only one publication each (Table 3).

According to WOS publication categories, 37% of the articles were in the field of medicine, general and internal medicine, medicine, research & experimental, cell biology; pathology and surgery were the least published categories in this list. In addition, when the categories we created according to the content of the articles were examined, most articles were on genetics and drug research (47% and 24%, respectively) (Table 4).

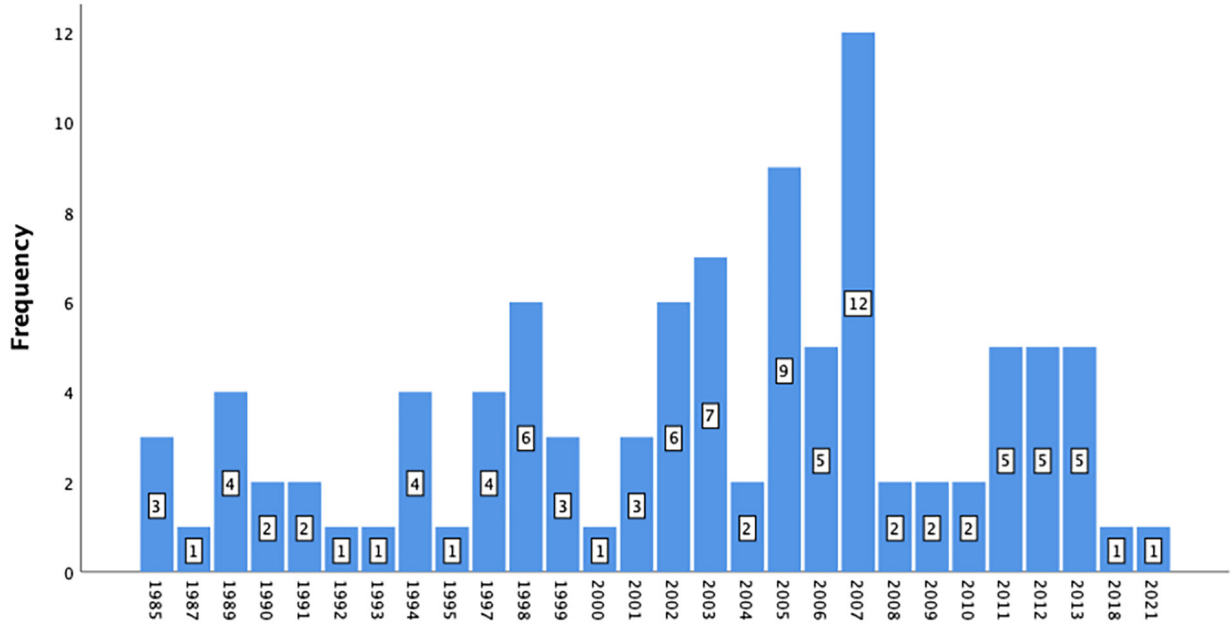

Figure 1. Distribution of the most cited articles by publication year

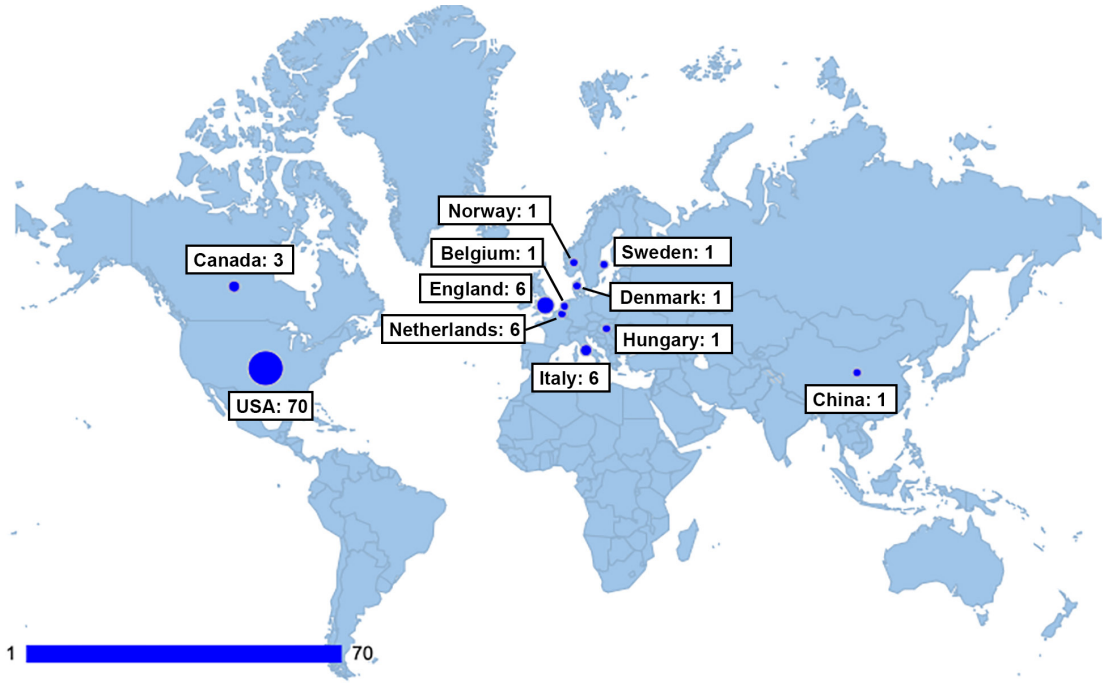

Figure 2. Countries from which publications originate

Table 1. The top 100 cited articles in breast cancer in order

No	First author	Article title	Journal	Times cited, WOS Core	Times cited, all databases
1	Perou, CM	Molecular portraits of human breast tumours	Nature. 2000 Aug 17;406(6797):747-52.	10,236	10,700
2	Slamon, DJ	Human-breast cancer correlation of relapse and survival with amplification of the her-2 neu oncogene	Science. 1987 Jan 9;235(4785):177-82.	9387	9636
3	Aaronson, NK	The european organization for research and treatment of cancer qlq-c30: a quality of life instrument for use in international clinical trials in oncology	J Natl Cancer Inst. 1993 Mar 3;85(5):365-76.	9247	9464
4	Wang, X	Effect of Capecitabine Maintenance Therapy Using Lower Dosage and Higher Frequency vs Observation on Disease-Free Survival Among Patients With Early-Stage Triple-Negative Breast Cancer Who Had Received Standard Treatment The SYSUCC-001 Randomized Clinical Trial	JAMA. 2021 Jan 5;325(1):50-58.	8914	9284
5	Slamon, DJ	Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2.	N Engl J Med. 2001 Mar 15;344(11):783-92.	7942	8211
6	Sorlie, T	Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications	Proc Natl Acad Sci U S A. 2001 Sep 11;98(19):10869-74.	7588	7798
7	Al-Hajj, M	Prospective identification of tumorigenic breast cancer cells	Proc Natl Acad Sci U S A. 2003 Apr 1;100(7):3983-8.	7419	7987
8	Koboldt, DC	Comprehensive molecular portraits of human breast tumours	Nature. 2012 Oct 4;490(7418):61-70.	7295	7404
9	Van't Veer, LJ	Gene expression profiling predicts clinical outcome of breast cancer	Nature. 2002 Jan 31;415(6871):530-6.	6840	6970

Table 1. continued

No	First author	Article title	Journal	Times cited, WoS Core	Times cited, all databases
10	Slamon, Dj	Studies of the her-2/neu proto-oncogene in human-breast and ovarian-cancer	Science. 1989 May 12;244(4905):707-12.	6050	6158
11	Weidner, N	Tumor angiogenesis and metastasis - correlation in invasive breast-carcinoma	N Engl J Med. 1991 Jan 3;324(1):1-8.	4906	5402
12	Miki, Y	A strong candidate for the breast and ovarian-cancer susceptibility gene BRCA1	Science. 1994 Oct 7;266(5182):66-71.	4753	4898
13	Van De Vijver, MJ	A gene-expression signature as a predictor of survival in breast cancer.	N Engl J Med. 2002 Dec 19;347(25):1999-2009.	4575	4672
14	Fisher, B	Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer	N Engl J Med. 2002 Oct 17;347(16):1233-41.	4375	4613
15	Elston, Cw	Pathological prognostic factors in breast-cancer .I. the value of histological grade in breast-cancer - experience from a large study with long-term follow-up	Histopathology. 2002 Sep;41(3A):154-61.	4179	4285
16	Fisher, B	Tamoxifen for prevention of breast cancer: Report of the National Surgical Adjuvant Breast and Bowel Project P-1 study	J Natl Cancer Inst. 1998 Sep 16;90(18):1371-88.	4057	4129
17	Muller, A	Involvement of chemokine receptors in breast cancer metastasis	Nature. 2001 Mar 1;410(6824):50-6.	4048	4403
18	Romond, EH	Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer	N Engl J Med. 2005 Oct 20;353(16):1673-84.	3947	4095
19	Li, J	PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer	Science. 1997 Mar 28;275(5308):1943-7.	3889	4264
20	Sorlie, T	Repeated observation of breast tumor subtypes in independent gene expression data sets	Proc Natl Acad Sci U S A. 2003 Jul 8;100(14):8418-23.	3755	3929
21	Piccart-Gebhart, MJ	Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer	N Engl J Med. 2005 Oct 20;353(16):1659-72.	3660	3790
22	Cristofanilli, M	Circulating tumor cells, disease progression, and survival in metastatic breast cancer	N Engl J Med. 2004 Aug 19;351(8):781-91.	3210	3311
23	Curtis, C	The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups	Nature. 2012 Apr 18;486(7403):346-52.	3144	3188
24	Iorio, MV	MicroRNA gene expression deregulation in human breast cancer	Cancer Res. 2005 Aug 15;65(16):7065-70.	3132	3345
25	Malkin, D	Germ line p53 mutations in a familial syndrome of breast-cancer, sarcomas, and other neoplasms	Science. 1990 Nov 30;250(4985):1233-8.	2998	3050
26	Lehmann, BD	Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies	J Clin Invest. 2011 Jul;121(7):2750-67.	2879	2974
27	Dent, R	Triple-negative breast cancer: Clinical features and patterns of recurrence	Clin Cancer Res. 2007 Aug 1;13(15 Pt 1):4429-34.	2755	2943
28	Veronesi, U	Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer	N Engl J Med. 2002 Oct 17;347(16):1227-32.	2734	2915

Table 1. continued

No	First author	Article title	Journal	Times cited, WoS Core	Times cited, all databases
29	Orimo, A	Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion	Cell. 2005 May 6;121(3):335-48.	2637	2741
30	Wolff, AC	American Society of Clinical Oncology/ College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer	J Clin Oncol. 2007 Jan 1;25(1):118-45.	2633	2746
31	Carey, LA	Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study	JAMA. 2006 Jun 7;295(21):2492-502.	2608	2754
32	Sjoblom, T	The consensus coding sequences of human breast and colorectal cancers	Science. 2006 Oct 13;314(5797):268-74.	2589	3551
33	Wooster, R	Identification of the breast-cancer susceptibility gene BRCA2	Nature. 1995 Dec 21-28;378(6559):789-92.	2550	2611
34	Parker, JS	Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes	J Clin Oncol. 2009 Mar 10;27(8):1160-7.	2547	2597
35	Wolff, AC	Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update	J Clin Oncol. 2013 Nov 1;31(31):3997-4013.	2388	2489
36	Miller, K	Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer	N Engl J Med. 2007 Dec 27;357(26):2666-76.	2383	2457
37	Geyer, CE	Lapatinib plus capecitabine for HER2-positive advanced breast cancer	N Engl J Med. 2006 Dec 28;355(26):2733-43.	2379	2451
38	Vogel, CL	Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer	J Clin Oncol. 2002 Feb 1;20(3):719-26.	2373	2471
39	Goldhirsch, A	Strategies for subtypes-dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011	Ann Oncol. 2011 Aug;22(8):1736-47.	2330	2545
40	Wood, LD	The genomic landscapes of human breast and colorectal cancers	Science. 2007 Nov 16;318(5853):1108-13.	2308	2361
41	Karnoub, AE	Mesenchymal stem cells within tumour stroma promote breast cancer metastasis	Nature. 2007 Oct 4;449(7162):557-63.	2289	2405
42	Banks, E	Breast cancer and hormone-replacement therapy in the Million Women Study	Lancet. 2003; 362: 419–27.	2255	2318
43	Gail, MH	Projecting individualized probabilities of developing breast-cancer for white females who are being examined annually	J Natl Cancer Inst. 1989 Dec 20;81(24):1879-86.	2224	2275

Table 1. continued

No	First author	Article title	Journal	Times cited, WoS Core	Times cited, all databases
44	Neve, RM	A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes	Cancer Cell. 2006 Dec;10(6):515-27.	2222	2246
45	Ford, D	Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families	Am J Hum Genet. 1998 Mar;62(3):676-89.	2174	2210
46	Cobleigh, MA	Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease	J Clin Oncol. 1999 Sep;17(9):2639-48.	2149	2219
47	Wang, YX	Gene-expression pro-files to predict distant metastasis of lymph-node-negative primary breast cancer	Lancet. 2005 Feb 19-25;365(9460):671-9.	2097	2151
48	Minn, AJ	Genes that mediate breast cancer metastasis to lung	Nature. 2005 Jul 28;436(7050):518-24.	2070	2138
49	Verma, S	Trastuzumab Emtansine for HER2-Positive Advanced Breast Cancer	N Engl J Med. 2012 Nov 8;367(19):1783-91.	2054	2124
50	Giuliano, AE	Lymphatic mapping and sentinel lymphadenectomy for breast-cancer	Ann Surg. 1994 Sep;220(3):391-8	2023	2118
51	Ma, L	Tumour invasion and metastasis initiated by microRNA 10b in breast cancer	Nature. 2007 Oct 11;449(7163):682-8.	2010	2184
52	Overgaard, M	Postoperative radiotherapy in high-risk premenopausal women with breast cancer who receive adjuvant chemotherapy. Danish Breast Cancer Cooperative Group 82b Trial	N Engl J Med. 1997 Oct 2;337(14):949-55.	1963	2016
53	Darby, SC	Risk of Ischemic Heart Disease in Women after Radiotherapy for Breast Cancer	N Engl J Med. 2013 Mar 14;368(11):987-98.	1957	2038
54	Goldhirsch, A	Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013	Ann Oncol. 2013 Sep;24(9):2206-23.	1949	2151
55	Fisher, B	Effect of preoperative chemotherapy on the outcome of women with operable breast cancer	J Clin Oncol. 1998 Aug;16(8):2672-85.	1944	2029
56	Nielsen, TO	Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma	Clin Cancer Res. 2004 Aug 15;10(16):5367-74.	1912	2069
57	Baselga, J	Everolimus in Postmenopausal Hormone-Receptor-Positive Advanced Breast Cancer	N Engl J Med. 2012 Feb 9;366(6):520-9.	1902	1977

Table 1. continued

No	First author	Article title	Journal	Times cited, WoS Core	Times cited, all databases
58	Hall, JM	Linkage of early-onset familial breast-cancer to chromosome-17q21	Science. 1990 Dec 21;250(4988):1684-9.	1898	1951
59	Giuliano, AE	Axillary Dissection vs No Axillary Dissection in Women With Invasive Breast Cancer and Sentinel Node Metastasis A Randomized Clinical Trial	JAMA. 2011 Feb 9;305(6):569-75.	1869	1956
60	Kang, YB	A multigenic program mediating breast cancer metastasis to bone	Cancer Cell. 2003 Jun;3(6):537-49.	1860	1925
61	Hammond, MEH	American Society of Clinical Oncology/ College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer	J Clin Oncol. 2010 Jun 1;28(16):2784-95.	1847	1917
62	Gyorffy, B	An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients	Breast Cancer Res Treat. 2010 Oct;123(3):725-31.	1834	1862
63	Paik, S	Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer	J Clin Oncol. 2006 Aug 10;24(23):3726-34.	1833	1877
64	Liedtke, C	Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer	J Clin Oncol. 2008 Mar 10;26(8):1275-81.	1779	1874
65	Doyle, LA	A multidrug resistance transporter from human MCF-7 breast cancer cells	Proc Natl Acad Sci U S A. 1998 Dec 22;95(26):15665-70.	1760	1866
66	Carter, CL	Relation of tumor size, lymph-node status, and survival in 24,740 breast-cancer cases	Cancer. 1989 Jan 1;63(1):181-7.	1757	1792
67	Easton, DF	Genome-wide association study identifies novel breast cancer susceptibility loci	Nature. 2007 Jun 28;447(7148):1087-93.	1751	1816
68	Howell, A	Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer	Lancet. 2005 Jan 1-7;365(9453):60-2.	1721	1777
69	Berry, DA	Effect of screening and adjuvant therapy on mortality from breast cancer	N Engl J Med. 2005 Oct 27;353(17):1784-92.	1687	1726
70	Saslow, D	American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography	CA Cancer J Clin. 2007 Mar-Apr;57(2):75-89.	1657	1702
71	Weidner, N	Tumor angiogenesis - a new significant and independent prognostic indicator in early-stage breast-carcinoma	J Natl Cancer Inst. 1992 Dec 16;84(24):1875-87.	1643	1943

Table 1. continued

No	First author	Article title	Journal	Times cited, WoS Core	Times cited, all databases
72	Slamon, D	Adjuvant Trastuzumab in HER2-Positive Breast Cancer	N Engl J Med 2011; 365:1273-1283	1621	1682
73	Schmid, P	Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer	N Engl J Med 2018; 379:2108-2121	1615	1669
74	Baselga, J	Pertuzumab plus Trastuzumab plus Docetaxel for Metastatic Breast Cancer	N Engl J Med. 2012 Jan 12;366(2):109-19.	1614	1673
75	Yu, M	Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition	Science. 2013 Feb 1;339(6119):580-4.	1595	1667
76	Qian, BZ	CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis	Nature. 2011 Jun 8;475(7355):222-5.	1578	1626
77	Harvey, JM	Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer	J Clin Oncol. 1999 May;17(5):1474-81.	1555	1601
78	Tabar, L	Reduction in mortality from breast-cancer after mass-screening with mammography	Lancet. 1985 Apr 13;1(8433):829-32.	1543	1557
79	Krag, D	The sentinel node in breast cancer - A multicenter validation study	N Engl J Med. 1998 Oct 1;339(14):941-6.	1532	1587
80	King, MC	Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2	Science. 2003 Oct 24;302(5645):643-6.	1531	1583
81	Liaw, D	Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome	Nat Genet. 1997 May;16(1):64-7.	1526	1558
82	Veronesi, U	Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph-nodes	Lancet. 1997 Jun 28;349(9069):1864-7.	1523	1571
83	Baum, M	Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial	Lancet. 2002 Jun 22;359(9324):2131-9.	1486	1528
84	Yu, F	Iet-7 regulates self renewal and tumorigenicity of breast cancer cells	Cell. 2007 Dec 14;131(6):1109-23.	1480	1633
85	Tavazoie, SF	Endogenous human microRNAs that suppress breast cancer metastasis	Nature. 2008 Jan 10;451(7175):147-52.	1476	1606
86	Spiegel, D	Effect of psychosocial treatment on survival of patients with metastatic breast-cancer	Lancet. 1989 Oct 14;2(8668):888-91.	1475	1490

Table 1. continued

No	First author	Article title	Journal	Times cited, WoS Core	Times cited, all databases
87	Veronesi, U	A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer	N Engl J Med. 2003 Aug 7;349(6):546-53.	1469	1533
88	Sotiriou, C	Breast cancer classification and prognosis based on gene expression profiles from a population-based study	Proc Natl Acad Sci U S A. 2003 Sep 2;100(18):10393-8.	1458	1511
89	Boyd, NF	Mammographic density and the risk and detection of breast cancer	N Engl J Med. 2007 Jan 18;356(3):227-36.	1448	1475
90	Hankinson, SE	Circulating concentrations of insulin-like growth factor-I and risk of breast cancer	Lancet. 1998 May 9;351(9113):1393-6.	1446	1484
91	Ford, D	Risks of cancer in brca1-mutation carriers	Lancet. 1994 Mar 19;343(8899):692-5.	1439	1461
92	Fisher, B	Five-year results of a randomized clinical-trial comparing total mastectomy and segmental mastectomy with or without radiation in the treatment of breast-cancer	N Engl J Med. 1985 Mar 14;312(11):665-73.	1433	1453
93	Dupont, WD	Risk-factors for breast-cancer in women with proliferative breast disease	N Engl J Med. 1985 Jan 17;312(3):146-51.	1432	1470
94	Wooster, R	Localization of a breast-cancer susceptibility gene, BRCA2, to chromosome 13q12-13	Science. 1994 Sep 30;265(5181):2088-90.	1429	1471
95	Cummings, SR	The effect of raloxifene on risk of breast cancer in postmenopausal women - Results from the MORE randomized trial	JAMA. 1999 Jun 16;281(23):2189-97.	1418	1460
96	Gradishar, WJ	Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer	J Clin Oncol. 2005 Nov 1;23(31):7794-803.	1417	1483
97	Cheang, Maggie C. U.	Ki67 Index, HER2 Status, and Prognosis of Patients With Luminal B Breast Cancer	J Natl Cancer Inst. 2009 May 20;101(10):736-50.	1414	1497
98	Dawson, Sarah-Jane	Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer	N Engl J Med. 2013 Mar 28;368(13):1199-209.	1413	1481
99	Bauer, Katrina R	Descriptive analysis of estrogen receptor (ER)negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype - A population-based study from the California Cancer Registry	Cancer. 2007 May 1;109(9):1721-8.	1408	1508
100	Carey, LA	The triple negative paradox: Primary tumor chemosensitivity of breast cancer subtypes	Clin Cancer Res. 2007 Apr 15;13(8):2329-34.	1403	1523

Table 2. Journals in which the most cited articles were published

Journal name	Record count	Percentage
New England Journal of Medicine	24	24.0
Nature	13	13.0
Journal of Clinical Oncology	11	11.0
Science	11	11.0
Lancet	9	9.0
Proceedings of the National Academy of Sciences of the United States of America	5	5.0
Cell	4	4.0
Jama-Journal of the American Medical Association	4	4.0
Clinical Cancer Research	3	3.0
Journal of the National Cancer Institute	3	3.0
Annals of Oncology	2	2.0
Cancer	2	2.0
Journal of the National Cancer Institute	2	2.0
American Journal of Human Genetics	1	1.0
Annals of Surgery	1	1.0
Breast Cancer Research and Treatment	1	1.0
CA-A Cancer Journal for Clinicians	1	1.0
Cancer Research	1	1.0
Histopathology	1	1.0
Journal of Clinical Investigation	1	1.0

Discussion and Conclusion

Citation analysis is used to find important papers on a certain subject. It aids in the analysis of scientific influence while also acknowledging substantial/pioneering contributions made by predecessors and noteworthy research advancement. There are numerous bibliometric article analyses conducted in various areas of medicine (8-11). To the best of our knowledge, there is only one previous article about the 100 most cited articles concerning breast cancer, and it was published in 2017 (6). Since research areas can change due to advances in science and technology, we found that the total number of citations in this study, which we aimed to evaluate the current status of the 100 most frequently cited articles, reached 280,906, an increase of approximately 1.6 times compared to 2017. This result suggests that interest in quality publications on breast cancer has increased. Also, 41 of the articles in the list were found to have changed. The vast majority of articles on the list were on chemotherapy and genetic studies.

The number of citations may be related to the time since publication. As the publication time increases, the number of citations also increases. In our study, we observed that 12 articles from 2007 and 9 articles from 2005 entered the list (Figure 1). However, many factors, such as the content of the article, its quality and the journal in which it was published, can affect the number of citations. Therefore, although it was published in 2021, the study by Xi Wang and his colleagues was the fourth most cited article (6).

As expected, the most cited articles were published in the medical journals with the highest impact factors. In the present study, most articles were published in the New England Journal of Medicine, followed by articles in Nature, the Journal of Clinical Oncology, and Science, respectively. The first three articles on the list were published in Nature, Science and the Journal of the National Cancer Institute, respectively. It is feasible to hypothesize that the audience of a general medical journal is particularly interested in the topic of breast cancer, or that authors of breast cancer research choose popular medical journals to reach more researchers and readers. One of the important points in the study was that 70% of the articles originated from the USA. Similar to our study, in the bibliographic studies in the literature, 70%–93% of the research articles were USA based (8-11). The fact that these quality studies originate from the USA can be explained by the large patient population and the presence of many well-funded cancer centers.

The first most cited article was "Molecular portraits of human breast tumours" written by Perou et al. (12) in 2000. In this study, in which they made a molecular portrait of breast cancer, they created a molecular subtype classification of breast cancer (12). Today, this molecular classification is still in use and therefore the topic of this article remains relevant.

The second most cited article was "Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene" written by Slamon et al. (13) in 1987. In this study, they showed that the *HER-2/neu* oncogene may play a role in the biological behavior and pathogenesis of human breast cancer. Also, they found that amplification of the *HER-2/neu* gene is an important predictor of both overall survival and time to relapse in patients with breast cancer, and that the *HER-2/neu* oncogene plays a role in the biological behavior and pathogenesis of human breast cancer.

Table 3. Distribution of institutions according to the number of published articles

Institution	Record count	Record percentage
Harvard University	8	8.0
University of Pittsburgh	6	6.0
IRCCS European Institute of Oncology (IEO)	5	5.0
University of North Carolina	5	5.0
University of California	4	4.0
University of Cambridge	4	4.0
University of Texas	4	4.0
David Geffen School of Medicine at UCLA	3	3.0
Johns Hopkins University	3	3.0
Massachusetts Institute of Technology (MIT)	3	3.0
Memorial Sloan Kettering Cancer Center	3	3.0
National Institutes of Health (NIH) - USA	3	3.0
University of London	3	3.0
University of Toronto	3	3.0
University of Utah	3	3.0
Merck & Company	2	2.0
Cancer Research UK	2	2.0
Stanford University	2	2.0
Vanderbilt University	2	2.0
Aarhus University	1	1.0
Allegheny General Hospital	1	1.0
American Cancer Society	1	1.0
American Society of Clinical Oncology	1	1.0
Christie NHS Foundation Trust	1	1.0
Columbia University	1	1.0
Falun Hospital	1	1.0
Hungarian Academy of Sciences	1	1.0
Indiana University	1	1.0
John Wayne Cancer Institute	1	1.0
Johnson & Johnson	1	1.0
Joyce Eisenberg Keefer Breast Center	1	1.0
Netherlands Cancer Institute	1	1.0
Northwestern University	1	1.0
Nottingham City Hospital	1	1.0
Ohio State University	1	1.0
Rush University	1	1.0
Saint James's University Hospital	1	1.0
State Key Lab Oncology South China	1	1.0
UCLA Jonsson Comprehensive Cancer Center	1	1.0
ULSS 8 Berica	1	1.0
United States Department of Energy (DOE)	1	1.0
Universite Libre de Bruxelles	1	1.0
University Maryland	1	1.0
University of Edinburgh	1	1.0
University of Miami	1	1.0
University of Michigan	1	1.0
University of Oslo	1	1.0
University of Oxford	1	1.0
University of Vermont	1	1.0
University of Washington	1	1.0
Yeshiva University	1	1.0

Table 4. WOS publication categories

WOS categories	Record count	Record percentage
Medicine, General & Internal	37	37.0
Multidisciplinary Sciences	28	28.0
Oncology	26	26.0
Biochemistry & Molecular Biology; Cell Biology	2	2.0
Genetics & Heredity	2	2.0
Oncology; Cell Biology	2	2.0
Cell Biology; Pathology	1	1.0
Medicine, Research & Experimental	1	1.0
Surgery	1	1.0
Research categories		
Genetic research	47	47.0
Drug research	24	24.0
Basic research	16	16.0
Surgery	8	8.0
Radiology	4	4.0
Quality of life	1	1.0

WOS: Web of science

The third most cited article was "The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology" written by Aaronson et al. (14) in 1993. The EORTC QLQ-C30 questionnaire was developed as a reliable and valid measure of cancer patients' quality of life in multicultural clinical research settings in this multicenter survey performed by the European Organization for Research and Treatment of Cancer.

The other most cited articles are in the fields of chemotherapy, gene expression, tumor genetics, pathology, and surgery. Research in these areas has made important contributions to the understanding of breast cancer. According to WOS categories, 91% of the articles were in the field of general internal medicine, multidisciplinary sciences was the second most common category, and cancer research came third. Surgery was one of the least-published fields. As the biological behavior and pathogenesis of breast cancer are better understood, studies on chemotherapy drugs have come to the fore. A possible reason for the increase in these types of studies is the increase in funding for drug research in the treatment of breast cancer. For this reason, research on surgery may have lagged behind.

Although citation analysis is a useful method that can provide insight into trends in the literature, it is not without flaws. Only the WOS database was used in this study. Thus, publications that may be indexed in other databases, such as Scopus and Google Scholar, were not included in the list of this study. Also, self-citations, lectures and textbooks were not evaluated. A search was made by typing only the term "breast cancer" in the WOS search box. Other terms that may be related to breast cancer, such as "breast, breast neoplasm, breast surgery, etc.," were not searched. Another limitation was that the research area was examined according to the research categories determined by WOS. A more detailed investigation could not be made.

In conclusion, in this study, in which a bibliographic analysis of the 100 most cited articles in WOS on breast cancer was performed, it was observed that the number of citations increased by 1.6 times in the last 5 years. It was found that the most cited articles were published in high impact factor journals, especially the New England Journal of Medicine, most publications were from 2007, and the most cited articles were from the USA and Harvard University. Most studies focused on gene expression and chemotherapy. The result of this research may help to understand important studies on breast cancer and shed light on future studies.

Ethics Committee Approval: No ethical approval was obtained because this study did not involve a prospective evaluation, did not involve laboratory animals and only involved non-invasive procedures (e.g. faecal samples, voided urine etc).

Informed Consent: N/A

Peer-review: Externally peer-reviewed.

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Original Article

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An Overview of the Impact of Body Mass Index on Pathological Complete Response Following Neoadjuvant Chemotherapy in Operable Breast Cancer in a Tertiary Care Centre in South India

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ABSTRACT

Objective: The incidence of female breast cancer in the world is 11.7% with a mortality rate of 6.9%. According to Globocon 2020, breast cancer is the most commonly diagnosed cancer (24.5%) and the leading cause of cancer-related death amongst women worldwide. The purpose of this study was to analyze the impact of Body Mass Index (BMI) on pathological complete response (pCR) rates for operable breast cancer after neoadjuvant chemotherapy (NACT). The primary endpoint was to assess histopathological features of the surgical specimen in response to NACT and to investigate the relationship with pre-chemotherapy BMI taking into account the various molecular subtypes of breast cancer.

Materials and Methods: Patients with biopsy-proven breast carcinoma who underwent surgery after NACT between January 2017 and May 2021 were included. All patients were initially divided into three groups depending on their pre-chemotherapy BMI. With BMI <22.9 as normal or underweight category, BMI of 23-27.4, was taken as overweight category and BMI ≥27.5 as obese category.

Results: The study included 184 patients. Normal weight patients had the highest rate of pCR (75%) and the lowest was seen in the obese category (33.75%). Furthermore, the subtype most likely to achieve pCR was HER2+/ER negative followed by triple negative BC with odds ratios of 3.46 and 2.21, respectively.

Conclusion: This retrospective study established that overweight and obese patients suffering from breast carcinoma had a lessened pCR rate following NACT in comparison with those who were under-/normal weight.

Keywords: Body mass index; breast carcinoma; invasive ductal carcinoma; molecular subtypes of breast carcinoma; neoadjuvant chemotherapy; pathological complete response

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Key Points

- The endpoint of the study was to assess histopathological features of the surgical specimen as a response to neoadjuvant chemotherapy and investigate its relation with pre-chemotherapy body mass index with regard to the subtype of breast cancer.
- This study showed that overweight and obese breast cancer patients had a lower pathological clinical response rate following neoadjuvant chemotherapy compared to those with under-/normal weight.
- The pathological clinical response rate was highest in the HER2/neu enriched patients followed by those with the triple-negative subtype of breast cancer.

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Introduction

The incidence of female breast cancer globally is 11.7% with a mortality rate of 6.9% (1). According to Globocon 2020, breast cancer is the most commonly diagnosed cancer (24.5%) and the leading cause of cancer-related death amongst women worldwide (1). Various studies have established the risk associated with obesity and the development of malignancies, such as endometrial, ovarian and breast cancers (2). Obesity is a well-known risk factor for the development of hormone receptor-positive breast cancer in postmenopausal women (3, 4). Furthermore, it is linked with an advanced stage at the time of the breast carcinoma diagnosis along with a higher rate of recurrence risk, post-treatment (5, 6). Obesity is associated with poor outcomes in both premenopausal and postmenopausal breast cancer patients (7). However, the exact mechanism leading to the association between obesity and breast cancer risk and outcome remain obscure. Assessing the connection between obesity and pathological complete response (pCR) after neoadjuvant chemotherapy (NACT) will increase the understanding of the effect of obesity in patients with breast cancer. NACT offers a unique setting to assess whether there may be a link between obesity and response to chemotherapy *in vivo* (8).

Overweight is defined by the World Health Organization (WHO) as a Body Mass Index (BMI) ≥25 and <30 kg/m^2) and obesity as (BMI ≥30 kg/m^2) (9). Nonetheless, the definition of obesity differs with ethnicities because certain populations have a higher percentage of body fat or a preferential visceral fat accumulation. So lower BMI thresholds are recommended for black African, African-Caribbean, and Asian individuals so that overweight in these ethnicities is defined as BMI 23.0 to 27.4 kg/m^2 and obesity as BMI >27.5 kg/m^2 (9), (Table 1).

Various molecular subtypes of breast cancer were defined in accordance with the St. Gallen's surrogate definition of intrinsic subtypes of breast cancer. These are: luminal A [ER + and/or PR+, Ki-67 <14% and human epidermal growth factor receptor 2 (HER2) -]; luminal B (estrogen receptor (ER) + and/or progesterone receptor (PR)+, Ki-67 high and/or HER2+); HER2-positive (ER-, PR- and HER2+); and triple-negative (ER-, PR-, HER2-) (10). Patients are accepted as ER/PR-positive if receptor expression is >1%.

The purpose of this study was to analyze the impact of BMI on pathological complete response (pCR) rates for operable breast cancer after NACT. The primary endpoint was to assess histopathological features of the surgical specimen as a response to NACT and study its relation with pre-chemotherapy BMI, considering various molecular subtypes of breast cancer.

Materials and Methods

After institutional review and ethical board approval, we retrospectively analyzed the medical records of 184 biopsy-proven breast carcinoma patients who had undergone surgery, post neoadjuvant chemotherapy at Manipal Comprehensive Cancer Centre, a tertiary care centre in South India, between January 2017 and May 2021.

All patients were initially divided into three groups depending on their pre-chemotherapy BMI. With BMI <22.9 as normal or underweight category, BMI: 23–27.4, was taken as overweight category and BMI ≥27.5 as obese category (Table 1). These categories were in coherence with WHO standards of BMI classification for Asian populations (9). Various molecular subtypes of breast cancer were defined in accordance with the St. Gallen's surrogate definition of intrinsic subtypes of breast cancer as luminal A, luminal B, HER2-positive and triple-negative, as described above. In our study patients who were hormone receptor-positive and HER-2 positive were classified as HER-2 positive luminal B, whereas those who were HER-2 positive and ER/PR negative were classified as HER-2 enriched. HER-2 positive status was indicated by evidence of protein overexpression on immunohistochemical staining or gene amplification on fluorescence *in sit*u hybridisation (FISH). Immunohistochemical overexpression with a score of 3 was accepted as positive. Borderline expression of score 2 was validated using FISH.

Written informed consent was taken from each patient after explaining the nature of the procedure with its advantages, disadvantages, expected results, and possible re-excision rates. As per our institutional protocol, all patients recruited were those who had received NACT followed by surgery, which was further followed by adjuvant treatment depending upon the final histopathology and nature of surgery. In our institute, we used a chemotherapy regimen of four courses of Anthracycline and cyclophosphamide, followed by four courses of taxanes. (11, 12). Additionally, carboplatin and trastuzumab were added for HER2 positive disease along with taxane in a 3-weekly schedule for six consecutive cycles preoperatively (13, 14). Pertuzumab was not added to patient treatment regimens in our study. The duration of neoadjuvant chemotherapy was 12–14 weeks.

Following NACT, patients were evaluated and planned for breast conservation surgery (BCS) with sentinel lymph node biopsy (SLNB) or mastectomy with sentinel lymph node biopsy (SLNB) with or without axillary lymph node dissection (ALND), depending upon the stage of the tumour at the time of detection and its response to NACT and frozen section report of SLNB. Patients who underwent BCS were then continued for adjuvant radiotherapy. This adjuvant treatment plan was structured in accordance with the recommendations proposed during the tumour board discussions.

A comparison was made for pathological response post NACT, between the various BMI category groups. Moreover, an analysis of the association between BMI and pCR in various subtypes of breast cancer, based on hormone receptors and HER2 status was performed.

pCR was defined according to the Lancet trial of 2014 as ypT0/Tis ypN0, ypT0/Tis, or ypN0 (15).

Statistical Analysis

Data were first summarized by as mean, standard deviation, median, and range for continuous variables and frequency and proportion for categorical variables. A Spearman correlation coefficient was calculated to evaluate the relationship between BMI, age, and body

Table 1. BMI subgroups

Ethnicity	Normal	Overweight	Obese
Asians, Black Africans	<22.9	23–27.4	>27.5
Other population	18–24.9	25–29.9	>30

composition measurements. A univariate logistic regression model was fitted to evaluate the association between clinical characteristics and the probability of pCR and those showing a *p*-value <0.25 were further considered for multivariate analysis. Further, using the forward stepwise method the best method was chosen. For all the logistic regression models, parameter estimates, standard error of estimates, odds ratios, 95% confidence intervals and *p*-values of each factor were computed. Akaike information criterion (AIC) and Residual Deviance of models were compared. All statistical tests were performed using R software (R Foundation). Statistical significance was set at a 95% level of significance ($p<0.05$).

Results

In total the records of 184 patients were retrospectively reviewed. The median age of the whole cohort was 52 years and most of the patients (58%) were postmenopausal. BMI of the overall cohort was 26.19 kg/m^2. All demographic data and data specific to the type and stage of breast cancer for the study population are shown in Table 2. Approximately one fifth of patients were underweight/normal, one third were overweight and the remainder were obese according to BMI categories for Asians.

A total of 176 (95.6%) had infiltrating ductal carcinoma and the remaining eight (4.35%) had lobular carcinoma. Most of the patients had stage II (n = 90; 49.18%), followed by stage III (n = 88; 48.09%) disease. A total of 79 (43.17%) patients had achieved pCR.

A total of 176 patients had a tumour that was infiltrating ductal carcinoma and the rest eight patients had a tumour that was lobular carcinoma (Table 2).

Our study is limited by the fact that there are fewer patients in the low BMI group compared to the overweight and obese groups.

A univariate logistic regression model (Table 3) was conducted for the primary outcome of the pathological stage (pCR and non pCR) with all the variables. The model showed a strong association between BMI categories and type of surgery with the pathological stage ($p<0.01$). The rest of the variables were found to be non-significant ($p>0.05$).

The highest pCR rate was seen in normal-weight patients (75 %) and the lowest in the obese category (33.75%) (Graph 1). The odds ratio of achieving pCR of 0.21 (0.08, 0.52) for overweight and 0.20 (0.08, 0.49) for the obese group in the overall cohort using the underweight/normal patients as reference indicate that the higher the BMI then the lower the chance of achieving pCR (Table 4).

Multivariate analysis was carried out for primary outcome pCR for the variables which had *p*-value ≤0.25 in the univariate analysis. These variables were: menopausal status; BMI; quadrant; type of surgery; and Luminal type. Following further optimization of the model using the stepwise method, the final model was obtained. The final model showed that the variables BMI (category), type of surgery and Luminal type, were associated with the pathological stage (Table 4).

Analysis showed that, based on the odds ratio (OR) value with respect to Luminal A (OR = 1 as reference), the trend of achieving pCR, was in favour of HER2+/ER negative and TNBC with odds ratios of 3.46 (0.92, 14.38) and 2.21 (0.62, 8.58), respectively. These were found to be independent factors affecting pCR (Table 3).

Analysis also revealed that patients undergoing MRM and BCS + ALND were less likely to achieve pCR, with an OR of 0.54 (0.25, 1.11) and 0.18 (0.05, 0.54) compared to patients who underwent BCS + SLNB in our center (OR = 1 as reference).

The final model was found to be superior to the preliminary multivariate model with AIC 222.03 *versus* 225.46. The residual deviance was found to be 204.03 (degree of freedom = 172) and lack of fit insignificant (*p*-value = 0.051). Hosmer and Lemeshow goodness of fit (GOF) test *p*-value = 0.3327. Hence the final model is a good fit and can be considered over the preliminary multivariate model.

Discussion and Conclusion

The impact of high BMI on breast malignancy patients undergoing NACT is a topic of uncertainty and controversy. Therefore, to develop an improved perspective in this topic, we investigated the influence of BMI on pathological response rates after NACT, in operable carcinomas of the breast. The results showed that overweight and

Table 2. Demographic and baseline characteristics of study population (n = 184)

Variables	BMI classification n (%)			
	Underweight/normal BMI <22.9 kg/m^2	Overweight BMI: 23–27.4 kg/m^2	Obese BMI ≥ 27.5 kg/m^2	Overall cohort n (%)
No of patients	40 (21.86)	63 (34.43)	80 (43.72)	184 (100)
Age (years)				
Median (min, max) mean	53.00 (29.00, 71.00) 51.41±12.53	52.00 (26.00, 72.00) 51.48±11.54	52 (29.00, 84.00) 51.99±11.30	52.00 (26.00, 84.00) 51.41±11.65
Menopausal, n (%)				
Pre	17 (42.5)	26 (41.27)	33 (41.25)	76 (41.53)
Post	23 (57.5)	37 (58.73)	47 (58.75)	107 (58.47)
BMI	21.36 (19.14, 22.31) IQR (20.57, 22.21)	24.91 (22.52, 27.34) IQR (42.00, 25.73)	31.01 (27.55, 48.98) IQR (29.28, 33.32)	26.14 (19.14, 48.98) IQR (23.33, 30.12) 27.22±5.12

Table 2. continued

Variables	BMI classification n (%)			
	Underweight/normal BMI <22.9 kg/m²	Overweight BMI: 23–27.4 kg/m²	Obese BMI ≥ 27.5 kg/m²	Overall cohort n (%)
Stage, n (%)				
I	1 (2.50)	1 (1.59)	0	2 (1.09)
II	24 (60.00)	33 (52.38)	33 (41.25)	90 (49.18)
III	15 (37.50)	27 (42.86)	45 (56.25)	88 (48.09)
IV	1 (2.86)	1 (1.59)	2 (2.50)	4 (2.19)
Quadrant, n (%)				
UO	23 (57.50)	47 (74.60)	53 (66.25)	124 (67.76)
LO	7 (17.50)	6 (9.52)	15 (18.75)	28 (15.30)
UI	1 (2.86)	8 (12.70)	5 (6.25)	14 (7.65)
LI	5 (12.50)	1 (1.59)	4 (5.00)	10 (5.47)
Central	4 (10.00)	1 (1.59)	3 (3.75)	8 (4.37)
Side, n (%)				
Right	23 (57.50)	34 (53.96)	37 (46.25)	95 (51.91)
Left	17 (42.50)	29 (46.03)	43 (53.75)	89 (48.63)
Type				
IDC	37 (92.50)	62 (98.41)	76 (95.00)	176 (96.18)
Lobular	3 (7.50)	1 (1.59)	4 (5.00)	8 (4.37)
Luminal, n (%)				
A	3 (7.50)	6 (9.52)	9 (11.25)	18 (9.84)
B	18 (45.00)	23 (36.51)	37 (46.25)	78 (42.63)
TNBC	10 (25.00)	22 (34.92)	19 (23.75)	52 (28.42)
HER 2+/ER NEG	9 (22.50)	8 (12.70)	12 (15.00)	29 (15.85)
HER2+/ER POS	0	4 (6.3)	3 (3.75)	7 (3.83)
Type surgery, n (%)				
BCS + SLNB	19 (47.50)	32 (50.79)	31 (38.75)	82 (44.81)
MRM	19 (47.50)	20 (31.75)	32 (40.00)	72 (39.34)
BCS + ALND	2 (5.00)	9 (14.29)	17 (21.25)	28 (15.30)
Grade, n (%)				
2	29 (68.57)	43 (72.06)	51 (62.96)	124 (67.39)
3	11 (31.43)	20 (27.94)	29 (37.04)	60 (32.61)
Pathological stage, n (%)				
No pCR	10 (25.00)	41 (65.08)	53 (66.25)	104 (56.83)
pCR	30 (75.00)	22 (34.92)	27 (33.75)	79 (43.17)
SLNB, n (%)				
Negative	10 (25.00)	39 (61.91)	53 (66.25)	144 (78.69)
Positive	30 (75.00)	22 (34.92)	27 (33.75)	38 (20.77)

UO: upper outer quadrant; LO: lower outer quadrant; UI: upper inner quadrant; UO: upper outer quadrant; TNBC: triple negative breast cancer; BCS: breast conservation surgery; IDC: infiltrating ductal carcinoma; IQR: interquartile range; BMI: Body Mass Index; IDC: invasive ductal carcinoma; BCS: breast conservation surgery; SLNB: sentinel lymph node biopsy; MRM: modified radical mastectomy; ALND: axillary lymph node dissection; pCR: pathological complete response; HER2: human epidermal growth factor receptor 2; ER: estrogen receptor; TNBC: triple-negative breast cancer; NEG: negative; POS: positive; min: minimum; max: maximum; n: number

obese breast cancer patients were less likely to achieve a pCR to NACT which was consistent with a meta-analysis carried out by Wang et al. (16) in 2021.

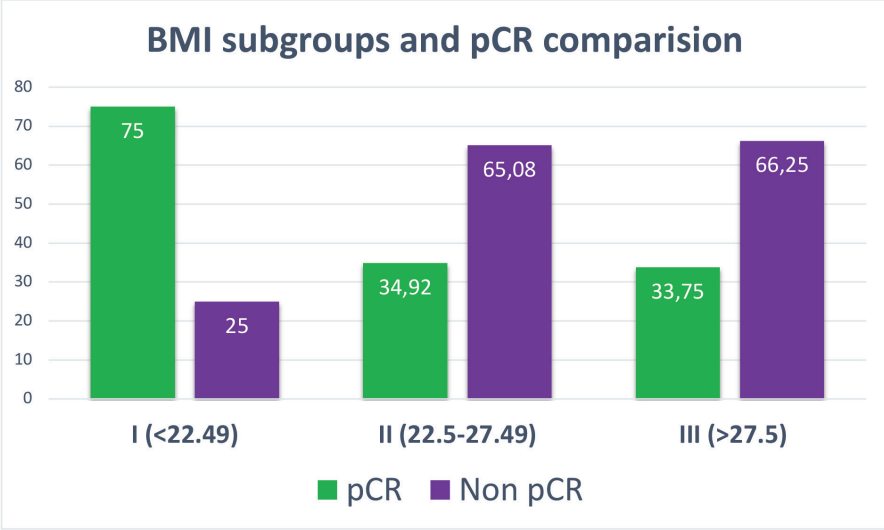
Additionally, we attempted to investigate the relationship between BMI and various subtypes of breast cancer, based on hormone receptors and HER2 status. A study by Warner et al. (17), explored this concept, and there was a significant inverse association between BMI and pCR in ER+/HER2+ patients (p-trend = 0.01) whereas in contrast, in ER–/HER2+ patients pCR rates were higher in overweight (71.3%; 62/87), obese women (60.7%; 74/122) and underweight women (83.3%; 10/12) women compared to normal-weight women (54.4%; 49/90),

Table 3. Univariate models

Variables		Estimate ± SD	OR (95%CI)	*p*-value
Age		-0.002±0.01	1.00 (0.97, 1.02)	**0.861**
Menopausal	**Pre**	Reference	1	
	Post	0.37±0.31	1.45 (0.80, 2.65)	0.2272
BMI	Underweight/normal <22.9	Reference	1	
	Overweight = 23–27.4	-1.67±0.45	0.19 (0.08, 0.44)	**0.0002**
	Obese ≥27.5	-1.77±0.44	0.17 (0.07, 0.39)	**4.58e-05**
Stage	I	Reference	1	
	II	-16.48±1696.73	$6.98x10^{-8}$ (NA, $3.01x10^{108}$)	0.992
	III	-17.23±1696.73	$3.31x10^{-8}$ (NA, $1.40x10^{108}$)	0.992
	IV	-33.13±2399.54	$4.08x10^{-15}$ (NA, $1.32x10^{105}$)	0.989
Quadrant	UO	Reference	1	
	LO	0.12 ±0.44	1.12 (0.47, 2.63)	0.11
	UI	-0.16±0.59	0.85 (0.25, 2.61)	
	LI	2.62±1.07	13.78 (2.48, 258.03)	
	Central	0.94±0.75	2.55 (0.60, 12.89)	
Side	Right	Reference	1	
	Left	0.61±0.30	1.85 (1.02, 3.36)	0.04263
Type	IDC	Reference	1	
	Lobular	-16.38±848.37	$7.68x10^{-08}$ (NA, $1.19x10^{24}$)	0.985
Ki-67		2.08e-05±8.27e-05	1.00002 (0.99986, 1.0002)	0.8015
Luminal	A	Reference	1	
	B	0.22±0.55	1.25 (0.44, 3.92)	0.16
	TNBC	0.65±0.57	1.92 (0.64, 6.26)	
	HER 2+/ER NEG	1.28 ±0.64	3.60 (1.07, 1.33)	
	HER2+/ER POS	-15.87 ±906.94	$1.28x10^{-07}$ ($2.46x10^{-152}$,$1.63 x10^{07}$)	
Type of surgery	BCS + SLNB	Reference	1	
	MRM	2.10±0.65	8.14 (2.58, 36.10)	<0.0001
	BCS + ALND	4.80±0.77	121.13 (30.88, 662.81)	
Grade	2	1	Reference	
	3	-0.11±0.32	0.90 (0.48, 1.68)	0.740
SLNB	0	Reference	1	
	1	-18.84±994.69	$6.57x10^{-09}$ ($9.28x10^{-150}$, $3.52x10^{10}$)	0.985

Significant values are shown in bold.

UO: upper outer quadrant; LO: lower outer quadrant; UI: upper inner quadrant; UO: upper outer quadrant; TNBC: triple negative breast cancer; BCS: breast conservation surgery; SLNB: sentinel lymph node biopsy; IDC: infiltrating ductal carcinoma; BMI: body mass Index; IDC: invasive ductal carcinoma; BCS: breast conservation surgery; SLNB: sentinel lymph node biopsy; MRM: modified radical mastectomy; ALND: axillary lymph node dissection; pCR: pathological complete response; HER2: human epidermal growth factor receptor 2; ER: estrogen receptor; NEG: negative; POS: positive; min: minimum; max: maximum; SD: standard deviation; OR: odds ratio; CI: confidence interval; n: number



Graph 1. Comparison of BMI categories and pCR rates in breast carcinoma post NACT

I: Normal BMI, II: Overweight, III: Obese, pCR is in percentage; BMI: Body Mass Index; pathological complete response

Table 4. Multivariate analysis

Variables OR (95%CI)		Initial model		Final model	
		p-value	OR (95%CI)	*p*-value	
Menopausal	Pre	1		-	
	Post	1.25 (0.60, 2.61)	0.55202	-	0.55202
BMI	Underweight/normal <22.9	1		1	
	Overweight = 23-27.4	0.25 (0.09, 0.67)	**0.00672**	0.21 (0.08, 0.52)	**0.001139**
	Obese BMI ≥ 27.5	0.22 (0.08, 0.55)	**0.00152**	0.20 (0.08, 0.49)	**0.00152**
Quadrant	UO	1		-	-
	LO	1.16 (0.43, 3.12)	0.76784	-	-
	UI	0.54 (0.14, 1.95)	0.35870	-	-
	LI	5.91 (0.93, 116.01)	0.11158	-	-
	Central	2.55 (0.44, 20.52)	0.31780	-	-
Luminal	A	1		1	
	B	1.35 (0.42, 4.72)	0.6189	1.35 (0.43, 4.63)	0.6158
	TNBC	2.21 (0.62, 8.58)	0.23316	2.21 (0.62, 8.58)	0.233871
	HER 2+/ER NEG	2.98 (0.77, 12.65)	0.12298	3.46 (0.92, 14.38)	**0.07449**
	HER2+/ER POS	$1.64x10^{-07}$ ($2.75x10^{-152}$, $5.97x10^{06}$)	0.98561	$1.87x10^{-07}$ ($1.22x10^{-142}$, $4.01x10^{07}$)	0.985842
Type of surgery	BCS + SLNB	1		1	
	MRM	0.47 (0.21, 1.01)	**0.057**	0.54 (0.25, 1.11)	**0.0978**
	BCS + ALND	0.18 (0.05, 0.57)	**0.0061**	0.18 (0.05, 0.54)	**0.0046**

Significant values are shown in bold.

UO: upper outer quadrant; LO: lower outer quadrant; UI: upper inner quadrant; UO: upper outer quadrant; TNBC: triple negative breast cancer; BCS: breast conservation surgery; SLNB: sentinel lymph node biopsy; ALND: axillary lymph node dissection; BMI: body mass Index; IDC: invasive ductal carcinoma; BCS: breast conservation surgery; SLNB: sentinel lymph node biopsy; MRM: modified radical mastectomy; ALND: axillary lymph node dissection; pCR: pathological complete response; HER2: human epidermal growth factor receptor 2; ER: estrogen receptor; TNBC: triple-negative breast cancer; NEG: negative; POS: positive; min: minimum; max: maximum; SD: standard deviation; OR: odds ratio; CI: confidence interval; n: number

resulting in a non-significant positive association between BMI and pCR (p-trend = 0.82) for that subtype in their study (17). In our study, the highest pCR rate was seen in Normal-weight patients (75%) and the lowest was found in the obese category (33.75%). Also, in our cohort the trend of achieving pCR, was in favour of HER2+/ER negative and TNBC compared to the other molecular subtypes.

Despite the molecular mechanisms being unclear, there have been hypotheses concerning the relationship between raised BMI and worsened breast cancer outcomes. It has been noted that a higher level of adipose tissue contributes to an elevation in estrogen production, thus leading to significant levels of circulating estrogen (18). Besides, it is seen that obese individuals have a higher level of insulin-like growth factor (IGF) and raised insulin resistance. This could activate the tumour cell survival pathways (19, 20). Studies have proven that patients having high insulin levels have been associated with higher breast malignancy incidence and, importantly, mortality (21). Another contributing pathological process may be low-grade chronic inflammation which is initiated and exaggerated by hypoxia in adipose tissues of obese patients (22). This is associated with an increased level of adipocytokines, such as interleukin 1β (IL-1β), IL-6, IL-8, leptin, and vascular endothelial growth factor (VEGF)(20). The low-grade chronic inflammation in obese adipose tissue is activated and maintained by the nuclear factor kappa B (NF-κB) pathway (23). Chronic NF-κB activation in obese adipose tissue maintains a micro-environment that also leads to stimulation of breast cancer cell proliferation, invasion, angiogenesis, and metastasis (24).

It has been reported that the overall pCR rate varied widely between 9.6% and 40.3% in numerous studies in a meta-analysis of 18,702 women with biopsy-proven breast cancer who had received NACT (16).

Breast cancer is a heterogeneous disease, and each St. Gallen subtype has different mechanisms of molecular carcinogenesis. Our study shows that, by considering BMI as a variable, different subtypes demonstrate variable responses to NACT. Our data suggest that maximum pCR is seen in HER2 positive patients, followed by triple-negative subtype and lastly the hormone receptor-positive sub-type. The study by Warner et al. (17) found significantly worse BMI related pCR in ER-positive/HER2 positive subtype.

Our study is limited by the fact that there are fewer patients in the low BMI group compared to the overweight and obese groups. So, there is a need for further, larger studies with similar sized subgroups and uniform neoadjuvant regimes to prove correlation.

In conclusion, this retrospective study established that overweight and obese South Asian patients suffering from breast carcinoma had a lower pCR rate following NACT in comparison with those who were under-/normal weight. Crucially, this holds true even for Asian populations, wherein obesity is defined by BMI >27.5. Taking BMI as a variable, various subtypes of breast malignancies exhibited differing responses to NACT.

It is notable that a high rate of pCR was detected in HER2+/ER negative patients, then the patients with triple-negative sub-type followed by the hormone receptor-positive sub-types (HER2+/ER positive, Luminal A and Luminal B). Studies should continue to investigate the mechanisms related to lower pCR rates, particularly in relation to patients with breast cancer who are overweight or obese, especially given the increasing trends for overweight in national populations globally.

Also, the need for further studies with comparable size subgroups and larger cohorts and uniform neoadjuvant regimes to prove co-relations, which was the limitation of our study.

Ethics Committee Approval: This original study was exempted by the ethics committee of our institution.

Informed Consent: Written informed consent was obtained from the patient for publication of this original study and accompanying images and tables.

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Authorship Contributions

Surgical and Medical Practices: S.P.S., R.J., B.C.A., K.R.A., R.K., A.R., S.R.; Concept: S.P.S., R.J., K.R.A.; Design: S.P.S., R.J., K.R.A., R.K., H.I.; Data Collection and/or Processing: S.P.S., R.J., H.K.K., A.P., N.Y.; Analysis and/or Interpretation: S.P.S., R.J., R.K., K.R.A., N.Y.; Literature Searching: S.P.S., R.J., A.P., P.P.; Writing: S.P.S., R.J., R.K., K.R.A., H.I., H.K.K.

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Novel Idea

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Stereotaxic Core-Needle Biopsy in Assessing Intraductal Pathologic Findings at Ductography

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ABSTRACT

Objective: The purpose of this study was to analyze the capabilities of ductography (DG) to navigate stereotactic core-needle biopsy (sCNB) for localizing and differentiating intraductal benign and malignant proliferations of the breast in patients with pathological nipple discharge (PND).

Materials and Methods: Patients underwent physical, radiological, ultrasound, endoscopic and histopathological examinations.

Results: The study included 183 patients. In 51, traditional DG was performed and in eight patients DG was performed using endoscopic mammoductoscopy (EMDS). A routine ductectomy labeled with methylene blue or propylene thread was performed in 81 patients. In 77 cases, a ductectomy was performed after double wire marking of intraductal proliferations (IDP) through the nipple and through the skin. In 26 patients, a preoperative sCNB under guidance of DG was performed. After sCNB 23/26 patients had benign IDP and three (11.5%) had invasive cancer. Breast surgery confirmed histology to be the gold standard in all patients, with the exception of 7 (26.9%) under the age of 45 years with benign IDP. These patients had watchful waiting and after 35 months of follow-up no signs of malignant growth were detected.

DG was characterized by high (87.9%) sensitivity and low (33.3%) specificity. False positive rate was 25.9% and the cause was peripheral location of IDP (>3 cm from the nipple) in 57.1% and inadequate excision with leaving them outside the resection.

Conclusion: This initial study on sCNB under the guidance of traditional or selective DG reports promising findings. Further studies are needed to determine whether preoperative histological assessment of pathologic intraductal lesions at DG would reduce the number of open surgeries with benign histology at sCNB.

Keywords: Breast; intraductal proliferations; ductography; stereotactic core-needle biopsy

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Key Points

- DG is characterized by frequent false results and low (33.3%) specificity
- 57.1% cause of the false-positive results of DG is the unsuccessful excision of peripherally located IDN
- To maximize the elimination of false results and increase the diagnostic efficiency of DG, we proposed selective contrasting of the ducts under the control of EMDS, as well as the use of images obtained as a result of traditional and / or selective DG for navigating sCNB.

Introduction

Ductography (DG) is a method of X-ray visualization of intraductal proliferations (IDP) of the breast after contrasting the milk ducts with pathological nipple discharge (PND) which was first described in the 1930s (1). Today, DG has been described as a technically incomplete, non-standardized procedure, which is accompanied by additional radiation exposure and is less specific than magnetic resonance imaging (MRI) and/or high-resolution ultrasonography (US) (2, 3).

In contrast, some authors consider DG to be the gold standard and are confident that its high (up to 95.0%) sensitivity allows identification of IDP and providing supporting evidence for surgical intervention (4, 5). According to the criteria of the American College of Radiology (ACR),

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DG is not a mandatory procedure, but DG may be used by surgeons who need additional information about the topography of IDP (6). However, the simple detection of IDP and routine ductectomy no longer corresponds to the current level of oncomammology, since with IDP the cancer frequency reaches 20.0%–23.0% with a tendency to decrease to 5.0%–6.0% when there are negative results on MG and/or US (7, 8).

In order to avoid unnecessary surgery in benign conditions, and in cases of cancer, to establish its invasive and molecular-genetic characteristics, histopathological verification and a reliable histological characterization of IDP is recommended, which may be "benign", "lesions of undetermined oncological potential", "high risk lesions" and/or "malignant lesions". This approach allows the personalization of therapies including neoadjuvant, targeted, and/or immune therapies, perform breast-conserving and/or oncoplastic surgery, and the use of alternative ablation procedures (9-11). At the same time, the utility of minimally invasive, visually controlled biopsies for histopathological verification are limited; 38.0%–85.0% of IDP cannot be identified by MG, 35.0%–71.0% is not visualized by US and 16.3%–22.7% is not detected by endoscopic mammoductoscopy (EMDS) (7, 12).

Navigation of a biopsy under the control of MRI requires complex, expensive equipment, is not technically developed and has not yet become widespread (13). Therefore, currently, routine ductectomy retains its utility, although the detection of cancer by means of a traumatic open biopsy is not clinically effective nor cost-effective, given that about 20.0% of IDP confirmed with the help of DG or EMDS are not detected by histopathology after surgery (14).

Some reports have shown that a more successful excision of IDP is possible after wire marking under the control of the DG. However, the evidence is poor as these studies are single, contain few observations and do not consider alternatives to routine ductectomy (15, 16).

To the best of our knowledge, there are no reports concerning the performance of a minimally invasive stereotactic core needle biopsy (sCNB) under the control of DG for preoperative histopathological assessment of IDP.

The purpose of this study was to analyze the capabilities of DG for orientation navigating sCNB when there is radiological evidence of possible IDP after standard or selective contrast of milk ducts and to compare the results with alternative approaches that did not use preoperative sCNB.

Materials and Methods

This study was approved by the Commission on Bioethics at the National Cancer Institute of the Ministry of Health of Ukraine and complies with the principles of the Helsinki Declaration (protocol no: 77, date: 12/09/2015). All patients received verbal informed consent.

The criteria for inclusion in this study were: female gender; adulthood; and the presence of PND. According to the recommendations of ACR, the main clinical signs of PND were considered to be bloody, amber-colored or watery discharge, which was unilateral and spontaneous and , persistent (6). Exclusion criteria were: bilateral lactorrhea, not associated with childbirth; severe somatic or mental conditions; acute galactophoritis; and allergy to iodinated contrast agents.

In 183 patients, physical, radiological, ultrasound, endoscopic, and histopathological studies were performed. For X-ray studies, digital mammography systems, the "Mammomat 3000 Nova" (Germany) and Hologic M-4 (Fort Myers, Florida, USA) were used, equipped with stereotactic puncture attachments. Standard DG (n = 51) was performed under aseptic conditions under local application anesthesia with EMLA® (Recipharm Karlskoga AB, Sweden). A SteryLab® device (Italy) with a tip diameter of 30G and the contrast agent Ultravist® (Bayer Pharma AG, Germany) were used. Selective DG (n = 8) under the control of EMDS was performed according to our own method (Ukrainian patent 106064). For this, if it is impossible to introduce a ductoscope into the secreting milk duct of second, third and fourth order of magnitude, it was intubated with a flexible microcapillary tube and a contrast agent was introduced through it (17). Ductograms were evaluated as technically inadequate (insufficient filling of the duct, extravasation, air bubbles); with normal duct structure; with ductectasia (>0.2 cm); with filling defects; with lines of "amputation"; or with the presence of filling defects and "amputation" lines simultaneously (18).

US was performed using high-frequency transducers on modern scanners, which were the EnVisor (Netherlands), Prosound-6, and Aplio SSA-780A (Japan) in the B-mode gray scale. Additional examination techniques were used, including the rolled-nipple, peripheral compression, and two-handed compression (19). Both MG and US results were assessed according to the assessment categories of ACR® Breast Imaging Reporting and Data System (BI-RADS) (20).

For EMDS, a rigid two-channel ductoscope from Karl Storz (Germany) with a tube length of 12.0 cm and an outer diameter of 0.13 cm (16G) was used. The results were evaluated in accordance with the recommendations of the Japanese Association of Mammary Ductoscopy and four types of lesions were distinguished: solitary; multiple; superficial; and mixed, as described (21).

After a comprehensive diagnostic process, 81 patients underwent a routine ductectomy with a marking of the secreting duct with indigo carmine or propylene thread. In 77 patients, a ductectomy was performed according to our own method after double wire marking of IDP through the nipple under the control of EMDS and through the skin under the control of US (Ukrainian patent 116603) (22).

In 26 patients, sCNB under the guidance of DG, was performed as described in detail by Ukrainian patent 119847. To do this, traditional or selective contrasting of the secreting milk duct was carried out, characteristic radiological signs of IDP (filling defect/amputation line) were identified and sCNB was performed. When there was a filling defect, the biopsy needle (G14) was introduced directly to the center of the filling defect. In the presence of an amputation line, the biopsy needle was aimed at the adjacent target next to the amputation line, but not further than 0.1 cm in the direction from the nipple (23).

Examination by light microscopy of 5 μm preparations stained with hematoxylin and eosin was chosen as the reference method. If necessary, immunohistochemical staining was used. Biological markers that were investigated included human epithelial growth factor receptor 2 (HER-2 neu), estrogen and progesterone receptors, and the marker of cellular proliferation, Ki-67).

Statistical Analysis

Data were analyzed by Microsoft Office Excel 2007 for Windows (Microsoft Corporation, Redmond, Washington, USA). Statistical indicators of sensitivity, specificity, positive and negative predictive values were calculated according to standard formulas, based on the

number of true and false positives, and true and false negative results of diagnostic tests. Histopathological findings confirming the presence/absence of benign or malignant IDP were used as the gold standard for all imaging tests.

Results

The performance indicators of the diagnostic tests are presented in Table 1. Paradoxically, DG, as a selective test intended exclusively for the diagnosis of IDP with high (87.9%) sensitivity is characterized by low (33.3%) specificity.

We found that IDP, diagnosed pre-operatively by DG and EMDS after ductectomy and using localization with indigo carmine or propylene thread, were histopathologically proven in only 31 (38.3%) cases. Thus, removal was unsuccessful in the remaining 50 (61.7%) cases. In contrast, after double marking with wire, all IDPs were adequately excised (Table 2).

In a detailed analysis of the causes of the 14 false-positive DG results, it was found that in 8 (57.1%) cases, IDP was located at a distance of at least 3 cm from the nipple and could remain outside the resection tissue and was thus not removed. Thorough histopathological analysis revealed that the remaining six (42.9%) false-positive DG results were due X-ray artefacts simulating IDP (Figure 1), which were described histopathologically as pseudopapillary intraductal structures with proliferation, apocrinization and desquamation of the ductal epithelium in the presence of chronic inflammation (Figure 2).

These results show that the low (33.3%) specificity of DG is associated with frequent (25.9%) false-positive results due to unsuccessful excision of peripherally located IDP, as well as the formation of pseudopapillary intraductal structures against the background of chronic inflammation.

To increase the diagnostic efficiency of DG, selective contrasting of secreting milk ducts of the second, third and fourth orders, under endoscopic control is proposed. The possible use of selective DG, under the control of EMDS, is illustrated in Figures 3 and 4. It is also proposed to use ductographic images obtained as a result of traditional and/or selective contrasting to navigate sCNB, which was done in 26 patients, the main clinical and pathological characteristics of which are given in Table 3. sCNB orientation and navigation using a ductographic image was carried out as follows. In a patient with PND and negative MG, conventional or endoscopically controlled DG was performed, a characteristic radiological sign of an IDP, such as a filling

Table 1. The effectiveness of diagnostic tests

Tests Parameters	MG (n = 64)	US (n = 82)	DG (n = 54)	EMDS (n = 158)
Results (amt., %)				
True positive	9 (14.0)	16 (19.5)	29 (53.7)	98 (62.1)
True negative	14 (21.9)	20 (24.4)	7 (13.0)	16 (10.1)
False positive	3 (4.7)	10 (12.2)	14 (25.9)	34 (21.5)
False negative	38 (59.4)	36 (43.9)	4 (7.4)	10 (6.3)
Indicators (%)				
Sensitivity	19.1	30.8	87.9	90.7
Specificity	82.4	66.7	33.3	32.0
PPV	75.0	61.5	67.4	74.2
NPV	26.9	35.7	63.6	61.5

MG: mammography; US: ultrasound; DG: ductography; EMDS: endoscopic mammoductoscopy; PPV: positive predictive value; NPV: negative predictive value, Amt: amount

Table 2. The results of histopathological studies after ductectomy

Histopathological diagnosis	Marking	
	Indigo carmine or propylene thread (n = 81)	Dual (n = 77)
Multiple papillomas	12 (14.8%)	40 (51.9%)
Solitary papillomas	11 (13.6%)	30 (39.0%)
Atypical ductal hyperplasia	2 (2.5%)	5 (6.5%)
Invasive carcinoma	6 (7.4%)	2 (2.6%)
Fibroadenomatosis	40 (49.4%)	-
Inflammation	7 (8.6%)	-
Ductectasia	3 (3.7%)	-

defect (Figure 5), was revealed, which was used to guide the biopsy needle (Figure 6).

After sCNB and histopathological analysis, benign processes were detected in 23 (88.5%) cases and invasive carcinomas in three (11.5%) cases. In 16 patients aged 45 years and older with benign sCNB results, ductectomies were performed and these showed complete concordance with the histopathological diagnosis, both before and after the operation. In seven patients under the age of 45 years with benign IDP, monitoring was carried out for up to 35 months. Signs of malignant growth were not found in them. In three women, aged 48, 59, and 60 years, poorly-differentiated (G3) invasive carcinomas of the luminal B subtype were detected: Her-2/neu positive in one, and pronounced positive reaction to estrogen receptors in the other two with high proliferative activity in all patients. Comprehensive treatment was given to all three women, in accordance with modern protocols. There was no relapse of the disease at follow up in 1.5, 2.5 and 3 years.

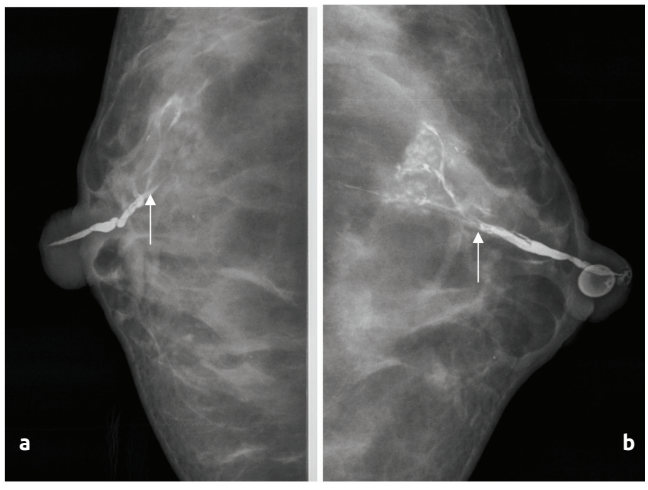

Figure 1. Traditional DG in a 55-year-old patient: a) craniocaudal and b) mediolateral projections; defective filling indicated by arrow

DG: ductography

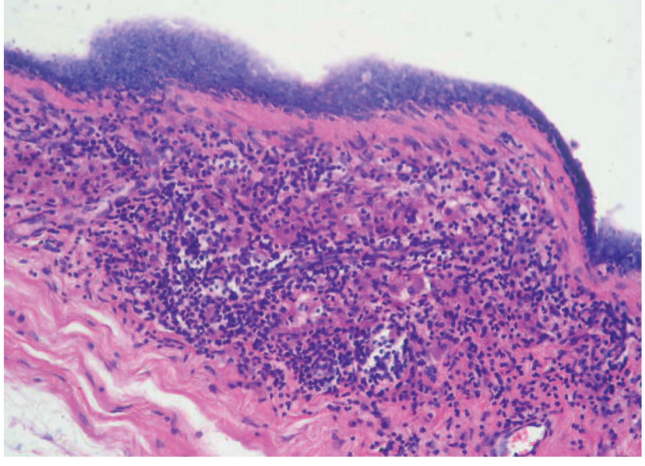

Figure 2. Histopathological examination in the same patient as in Figure 1. The formation of a pseudopapillary intraductal structure against a background of chronic inflammation. H&E x200

H&E: hematoxylin and eosin stain

Discussion and Conclusion

Current trends in oncological surgery of breast IDP are to favor the avoidance of unnecessary surgery in benign processes and to personalize therapy for invasive carcinomas. This approach requires confident preoperative histopathological verification with accurate assessment of the degree of invasiveness and molecular-genetic subtyping of the tumor.

Minimally invasive, visually guided biopsy has clear advantages over open biopsy, in the form of routine ductectomy with labeling of the secretory ducts of the first order with indigo carmine or propylene thread. However, as our studies have shown, without ductography, only 15.4% and 30.8% of the IDP are visible on mammography (MG) or US, respectively.

The highest sensitivity was demonstrated by EMDS (90.7%) and DG (87.9%), but intraductal biopsy does not yet have sufficient technical support and has not yet become widespread (24), and the usefulness of DG for orientation and navigation during biopsy are virtually unstudied. Paradoxically DG, as a focused procedure designed

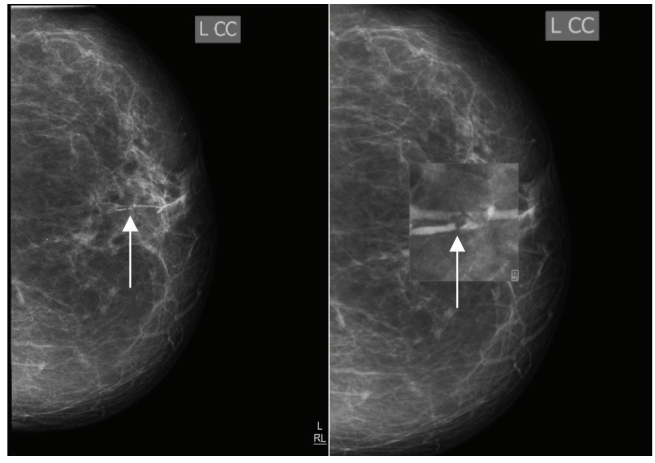

Figure 3. The ductogram after selective contrasting of the duct of the second order under the control of endoscopy (filling defect is indicated by an arrow)

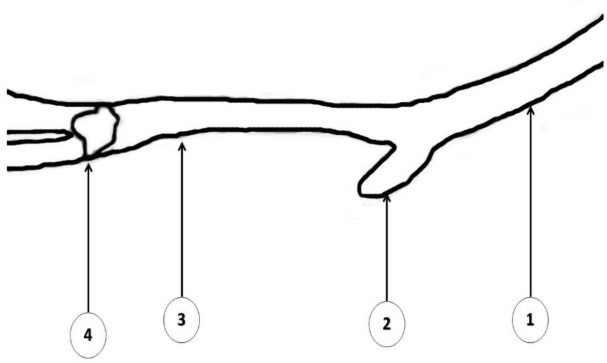

Figure 4. The scheme of ductogram after selective contrasting of the duct of the second order under the control of endoscopy: 1) duct of the second order; 2) lower branch of a duct of the third order; 3) upper branch of a duct of the third order; 4) filling defect due to intraductal proliferation

exclusively for the diagnosis of IDP, is characterized by frequent false results and low (33.3%) specificity.

Jiang et al. (25) reported the development of a system for assessing and classifying DG, taking into account some X-ray signs of IDP, which showed improvement in the differentiation of benign and malignant processes. However, there is insufficient evidence to support this use routinely and the final differentiation continues to rely on histopathological verification.

We found that the leading (57.1%) cause of false-positive results after DG was the unsuccessful excision of peripherally located IDP, in accordance with the findings of Istomin et al. (14). To eliminate false positives as far as possible and increase the diagnostic efficiency of DG, we proposed selective contrasting of the ducts under the control of EMDS, as well as the use of images obtained as a result of traditional and/or selective DG for orientation and navigation during sCNB.

The first experience of using the proposed methods showed their full technical reproducibility and safety. The combined use of well-known

Table 3. The main characteristics of patients undergoing sCNB under the control of DG (n = 26)

Indicator	Amt. (%)
Laterality: right/left	9 (34.6%)/17 (65.4%)
The nature of the discharge	
Bloody;	16 (61.5%)
Transparent;	6 (23.1%)
Amber colored	4 (15.4%)
MG results (BI-RADS®-2–4 categories)	4 (15.4%)
US results (BI-RADS®-2–4 categories)	8 (30.8%)
DG results	
Lines of "amputation";	10 (38.5%)
Filling defects;	9 (34.6%)
A combination of these symptoms;	7 (26.)
Distance from the nipple (less than 3 cm/3 cm or more);	8 (30.%)/18 (69.2%)
IDP dimensions in cm (min/max/mean)	0.2/6.0/0.9

MG: mammography; US: ultrasonography; BI-RADS: Breast Imaging Reporting and Data System; min: minimum; max: maximum; n: number, Amt: amount

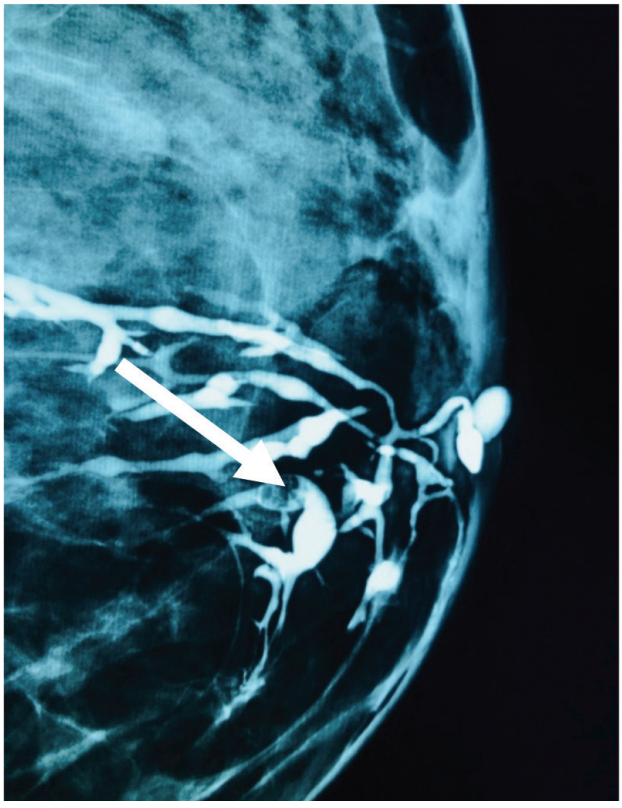

Figure 5. Ductogram after the traditional introduction of a contrast medium (filling defect is indicated by an arrow)

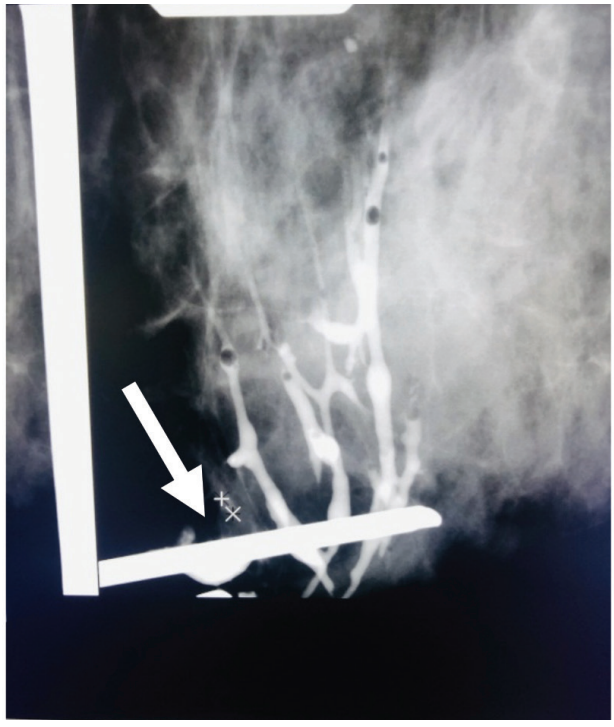

Figure 6. sCNB under the control of traditional DG. The position of the puncture needle after taking the material (filling defect is indicated by the arrow). Note: Multiple round filling defects due to air bubbles are also visible

DG: ductography; sCNB: stereotactic core-needle biopsy

techniques, DG and sCNB, means that there are no learning curves to deal with and there should be no new complications, except for the well-known and already described for DG and sCNB.

Selective contrasting of the second, third and fourth order ducts under the control of EMDS provided additional visualization and created the conditions for the navigation of sCNB when the IDP is peripherally located, and undetectable on X-ray and US, when endoscopic revision is impossible.

The advantages of sCNB under the control of traditional or selective DG are that it is less traumatic to obtain a complete biopsy for a reliable histopathological analysis, which opens up the possibility of planning further treatment tactics, including surgery, depending on the benign or malignant nature of IDP. An additional advantage of sCNB under the control of DG is the presence of a puncture channel and hemorrhage around it, which can be used as a kind of marker before routine ductectomy, and which is economically beneficial for medical institutions with a limited budget (26). The possibilities of sCNB under the control of DG are limited by well-known circumstances. The combined technique is technically not feasible in patients with flat breasts (thickness <2.5–2.7 cm after compression on a mammograph) and is potentially dangerous if the critical location of the IDP is near the ribs, pleura, large blood vessels and nerves (27). Furthermore, the feasibility of performing sCNB under the control of DG in cases of large, X-ray positive and echopositive IDP is debatable, but, in our opinion, the contrasting of the secreting duct allows precise selection of the area of the tumor that most closely matches the nature of the lesion.

This study has some limitations. These include a small number of observations and a lack of randomization which do not allow for a complete statistical analysis and, therefore, to safely draw robust conclusions from the findings.

In conclusion, further study of the possibilities of sCNB under the control of traditional or selective DG is promising in terms of minimizing the number of open biopsies (routine ductectomy) for preoperative verification of the benign or malignant nature of IDP of the breast.

Ethics Committee Approval: Ethics committee approval was received for this study from the Commission on Bioethics at the National Cancer Institute of the Ministry of Health of Ukraine (protocol no. 77 dated 12/09/2015).

Informed Consent: Verbal informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: G.A.B., N.A.S., A.A.A., E.G.A.; Design: G.A.B., N.A.S., A.A.A., E.G.A.; Data Collection and/or Processing: G.A.B., N.A.S., A.A.A., E.G.A.; Analysis and/or Interpretation: G.A.B., N.A.S., A.A.A., E.G.A.; Literature Search: G.A.B., N.A.S., A.A.A., E.G.A.; Writing: G.A.B., N.A.S., A.A.A., E.G.A.; Critical Review: G.A.B., N.A.S., A.A.A., E.G.A.

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Case Report

Eur J Breast Health 2022; 18(3): 286-288
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Three Dimensional Modelling in the Optimisation of Chest Wall Resection and Reconstruction Following Metastatic Breast Cancer

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ABSTRACT

Two-dimensional computed tomography scans no longer offer the level of detail that many surgeons desire for more accurate and precise surgical intervention. Computed tomography image reconstruction into three dimensional (3D) virtual models with interactive capability is providing an enhanced understanding of the patient's anatomy and pathology allowing the surgeon to create tailored intraoperative plans, minimizing complications and maximizing the intended therapeutic outcome. In this case report we demonstrate the use of 3D image reconstruction software in the management of a 36-year-old female with metastatic breast cancer affecting the chest wall.

Keywords: Bone metastasis, breast imaging, metastasis

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Key Points

- Three-dimensional interactive modelling can enhance precision in anterior chest wall resection and limit the extent of the post-procedural chest wall deformity. Its use as an adjunct to other pre-operative modalities should be considered to support targeted resection.

Introduction

The surgical management of breast cancer patients must often accommodate psychosocial issues, in addition to surgical challenges. This is because surgical resection can often leave patients with significant chest wall deformity, suboptimal function, and aesthetics. It is therefore essential to ensure resection is limited to therapeutic benefit. This can be achieved by mapping exact tissue margins, ensuring maximal preservation of tissue whilst minimizing the risk of tumour recurrence. 3D modelling supports this process by enhancing pre-operative planning by using existing conventional computed tomography (CT) images to create 3D virtual reconstructions.

These models can aid in localising the exact extent of the chest wall tumour, guiding intraoperative resection. We report the case of a 36-year-old female who underwent an anterior chest wall resection for recurrent breast cancer. We highlight the use of interactive 3D image reconstruction software in the surgical management of this patient.

Case Presentation

A 36-year-old female presented with metastatic relapse of her breast cancer. This was on the background of a grade 3, oestrogen receptor and HER-2 positive, right-sided breast carcinoma, previously treated with chemotherapy, mastectomy, axillary node clearance and chest wall radiotherapy, followed by a delayed deep inferior epigastric perforators (DIEP) flap reconstruction.

In addition to metastatic deposits in the sternum, the cancer was also contiguous with the medial aspect of the DIEP flap. A CT scan revealed lesions in both the sternum and right breast extending from the skin to the chest wall, illustrated in Figures 1a and b. An ultra-high definition (HD) fluorodeoxyglucose (FDG) positron-emission tomography (PET) scan confirmed localised disease with no distant metastatic disease,

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illustrated in Figure 1c. The patients only symptoms were a palpable erythematous lesion below the skin.

Due to the age of the patient and the localised nature of recurrence, it was decided to offer a partial anterior chest wall resection and reconstruction.

Preoperative Planning

To limit resection to therapeutic benefit and minimize resection of the chest wall, virtual 3D planning was used. This used existing CT scans of the patient to reconstruct a virtual model, highlighting both the site of the tumours and the extent of the planned en-bloc resection site informing the subsequent reconstruction strategy. Figures 2a and b illustrate the model and the planned resection site and size.

Operation

A curvilinear incision was planned and made, encompassing the sternum and anterior chest wall medial to the right nipple (Figure 3a). This was followed by en-bloc excision (Figure 3b and c) of the medial aspect of the left clavicle and all left sided ribs at the articulating point with the sternum. The right sternal edge was disarticulated in a similar fashion, except ribs 3, 4 and 5 which were divided laterally beyond the mid-clavicular line to achieve adequate clearance, as per the pre-operative planning, illustrated in Figure 2.

The area was reconstruction with a prolene mesh sandwich containing gentamicin cement (Figure 3d). The extent and shape of this was as per the planned pre-operative model. To complete reconstruction, a left latissimus dorsi myocuteanous flap was raised and moved to the midline subpectoral plane (Figure 3e and f).

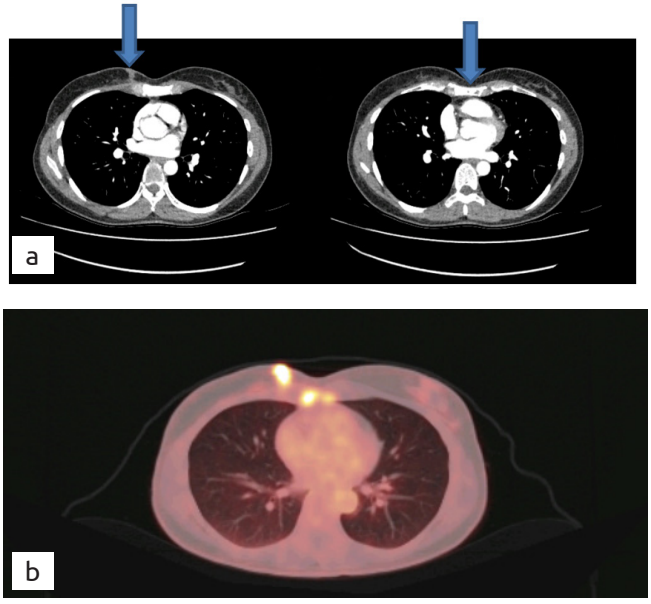

Figure 1. (a) Right breast nodule measuring 17x7 mm extending from the skin to the chest wall. **(b)** Extensive sclerotic lesions within the sternum. **(c)** PET scan showing multifocal hypermetabolic metastases within the right chest wall, right breast, and sternum

PET: positron emission tomography

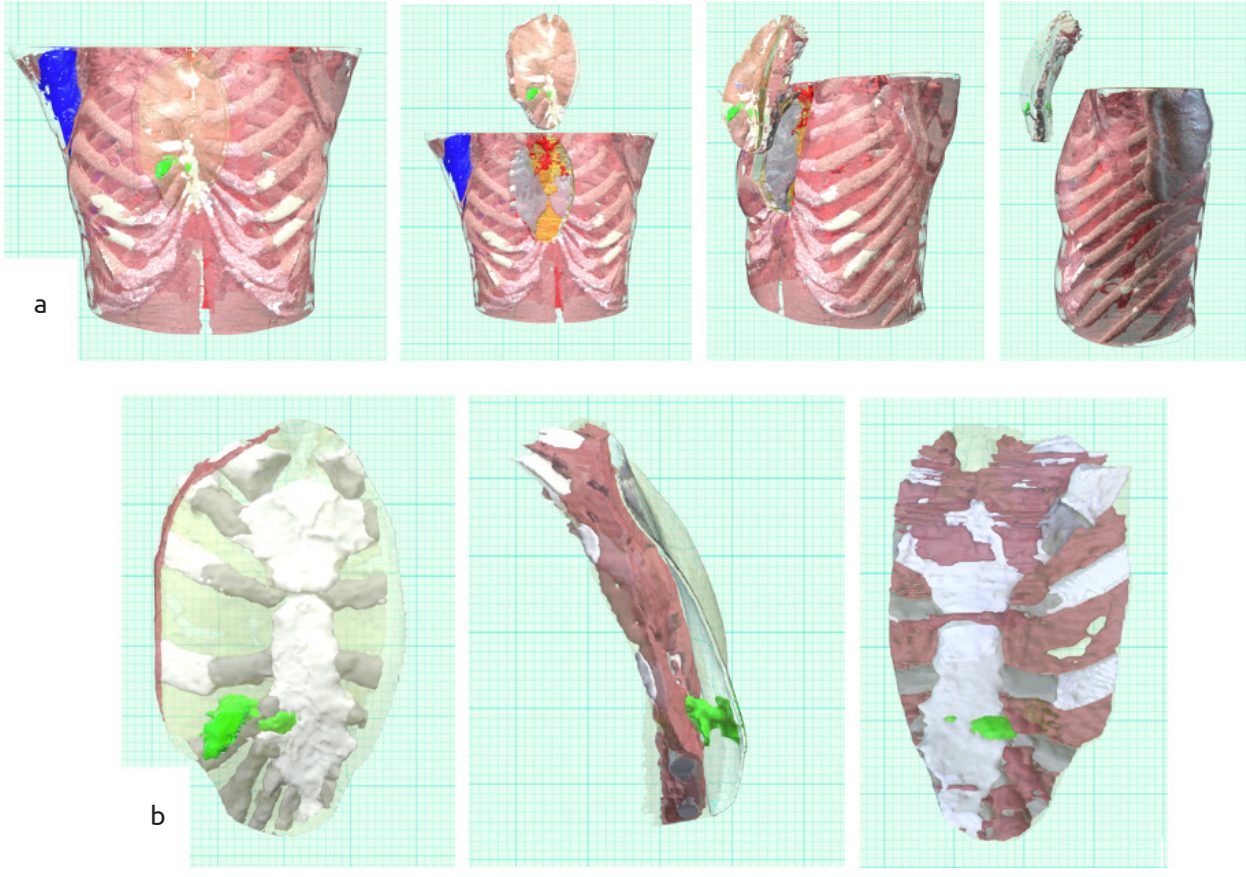

Figure 2. (a) 3D view of the chest wall, tumour (green) and planned resection site. **(b)** 3D view of the front, side and back of the planned resection site with tumour (green) *in situ*

3D: three dimentional

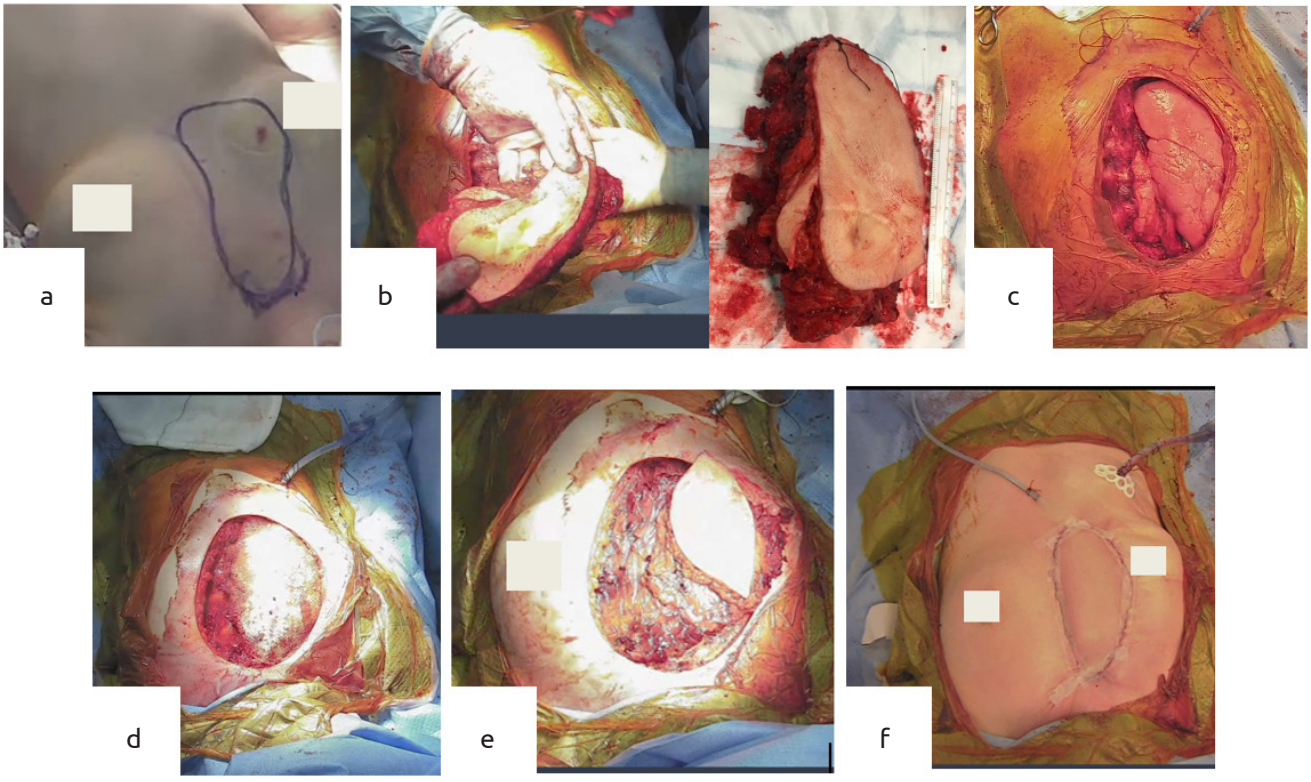

Figure 3. (a) Planned excision site. **(b)** Extraction of anterior chest wall encompassing tumour site. **(c)** Post chest wall resection with right lung exposed. **(d)** Gentamicin cement sandwiched between a prolene mesh filling defect site **(e)** Left latissimus dorsi myocuteanous flap raised and moved to midline subpectoral plane. **(f)** Completion of resection and reconstruction

The patient's post-operative recovery was uneventful with a healthy wound showing no evidence of collections, or infection. She was subsequently referred to the plastics and reconstructive team to begin her right breast reconstruction.

Discussion and Conclusion

Preoperative 3D modelling has a wide range of possible applications in thoracic surgery, ranging from the planning and localization of lesions and anatomical structures in lobectomy or segmentectomy to the evaluation of thoracic anatomical deformities in children (1, 2).

In this case report we demonstrated its use in oncological anterior chest wall resection and reconstruction. The patient's CT scan images were reconstructed by medical technology company Axial 3D. In addition to the 3Dimages, we were able to insert our desired margins for resection which allowed for the creation of virtual interactive models pre- and post-resection, which can be found here https://sketchfab.com/3d-models/p02332-sketchfab-2f311f9284e242be8baf87836f69aeb5 and https://sketchfab.com/3d-models/p02332-resection-02adfbf94d7541f293c88449a8dc5ca9. This allowed us to limit the resection area to maximize therapeutic benefit and minimise chest wall deformity.

Our experience with the 3D modelling software highlighted the potential for us to go a step further in future resections by utilising the 3D models to create 3D printed prosthetics for more precise filling of the defect site.

In conclusion, 3D interactive modelling can enhance precision in anterior chest wall resection and limit the extent of the post-procedural chest wall deformity. Its use as an adjunct to other pre-operative modalities should be considered to support targeted resection.

Informed Consent: It was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.K., A.A.; Design: M.K., A.A.; Supervision: A.A.; Data Collection and/or Processing: H.A.; Literature Search: H.A.; Writing: H.A.; Critical Review: H.A., M.K., A.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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Case Report

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Mucormycosis of the Breast in a Patient With Breast Carcinoma After COVID-19 Pneumonia

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ABSTRACT

Mucormycosis is a rare, but potentially fatal, fungal infection which is caused by mucormyctes. These forms of fungi are typically known to infect immunocompromised individuals but are rare in immunocompetent individuals. Herein, we report the case of a 52 year-old female who was diagnosed with right breast carcinoma in Manipal Hospital, a tertiary cancer care center. The patient was a known diabetic and hypertensive and who had recently recovered from coronavirus disease-2019 (COVID-19) pneumonia. In the due course of management, she developed mucormycosis infection at the operative site in her right breast where she had a radiation therapy-induced wound. This patient was successfully treated with an aggressive regimen of early surgical debridement along with administration of systemic amphotericin B.

Keywords: Axillary dissection, breast carcinoma, chemotherapy, COVID-19, mucormycosis, neoadjuvant chemotherapy

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Key Points

- Mucormycosis is an uncommon but potentially fatal fungal infection that usually affects patients with altered immunity, such as in diabetes, post COVID-19 pneumonia and in those with a history of corticosteroid intake.
- Tissue necrosis, is a hallmark of mucormycosis and is often a late sign.
- In this case aggressive treatment with early surgical debridement with the administration of systemic amphotericin B oral antifungal treatment led to a favorable response.

Introduction

Mucormycosis is a rare, but potentially fatal, invasive fungal infection which is caused by mucormyctes. It typically affects patients with immune-compromising conditions, such as hematologic malignancy, stem cell or solid organ transplantation, or uncontrolled diabetes. (1). The prevalence of mucormycosis varies from 0.005 to 1.7 per million population but in India its prevalence is nearly 80 times higher (0.14 per 1000) owing to the high number of coronavirus disease-2019 (COVID-19) cases, as reported in a recent estimate for the year 2019–2020 (2). Globally, the highest number of cases are reported from India (2).

In a recent systematic review, it was stated that the predominant sufferers of mucormycosis were hyperglycemic (83.3%). Furthermore, carcinoma (3%) was indicated as the second leading co-morbidity in these patients. A history of corticosteroid intake for the treatment of COVID-19 was present in a striking 76.3% of cases of mucormycosis. The authors declared diabetes mellitus (DM) as an independent risk factor for both severe COVID-19 and as well as mucormycosis (3). In recent times, COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has gained global attention due to its high rates of infectivity and mortality. Furthermore, these COVID-19 infected patients are frequently noted to be pre-disposed to a wide range of opportunistic bacterial and fungal infections (4). A large majority of the accounted

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cases of fungal co-infection have been reported to be due to two groups of fungal pathogens, *Aspergillus* and *Candida* (5). To date, no case of breast mucormycosis in a case of breast carcinoma after COVID-19 pneumonia has been published. Therefore, we believe our case is unprecedented and will contribute by providing an effective strategy for management in such patients.

Case Presentation

A 52 year-old, Indian, post-menopausal female presented to the department of surgical oncology, in Manipal Hospitals comprehensive cancer centre, with a suspicious lump in the upper outer quadrant of her right breast. She had a pre-exisiting diagnosis of DM and hypertension. Moreover, she had recently recovered from COVID-19 pneumonia. Histopathology reported an infiltrating ductal carcinoma (IDC) of the right breast, cT2N3bM0, Stage III C, grade 2, Luminal B with Ki-67 of 70%.

Thereafter, following a metastatic work-up, the patient was planned for neoadjuvant chemotherapy (NACT) to downsize the tumour. Post NACT, she underwent right breast conservation surgery with axillary lymph node clearance with local oncoplasty after giving written informed consent. Following surgery, she had an uneventful recovery.

The histopathological report documented a complete pathological response at the primary tumor site and 2/10 lymph nodes were reported to be positive. Therefore, as per protocol, she received adjuvant radiation therapy, following which she developed a radiation-induced wound at the operative site that developed into a chronic nonhealing ulcer (Figures 1 and 2).

So, a multidisciplinary team meeting was held and it was thought to be a non-healing wound secondary to ischemia, and the decision was taken to proceed with hyperbaric oxygen therapy (HBOT). Regular dressings were done and she was subjected to two weeks of HBOT. Regrettably, the wound did not show signs of healing, and consequently she developed a high-grade fever. In her best interest, she was admitted and managed conservatively with intravenous medications.

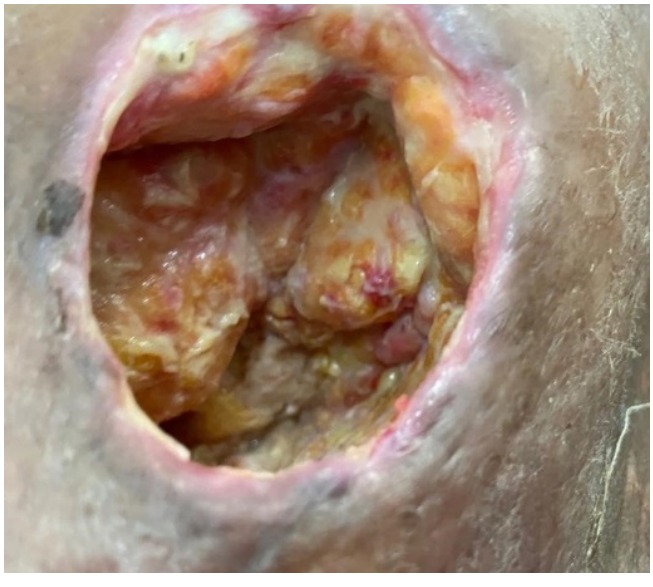

Figure 1. Infected breast wound

Following this, the decision was taken for wound debridement and wound tissue was sent for histopathological examination, to accurately identify the cause of the non-healing nature of her wound. The histopathological examination demonstrated an extensive necrosis of the breast parenchyma with polymorphonuclear infiltration, hemorrhage, and thrombosed blood vessels, and many broad aseptate hyaline fungal hyphae branching at 90° suggestive of mucormycosis (Figures 3 and 4). Subsequently, an aggressive approach was adopted for her treatment, consisting of wound debridement and excision of necrotic tissue, succeeded by the initiation of intravenous liposomal Amphotericin B (1 mg/kg/day in the form of infusion in 5% dextrose) for 15 days. Thereafter, she was given one month of oral fluconazole. The wound healed satisfactorily with regular dressings, antifungal treatment, and high protein nutrition over a period of 45 days (Figure 5).

Discussion and Conclusion

Aggressive treatment with early surgical debridement together with the administration of systemic amphotericin B led to a favorable response in our patient (Figure 5). In this case, many high-risk comorbidities were present, such as uncontrolled blood sugar levels, post COVID-19 pneumonia status, and an immune-compromised condition following standard chemotherapy and radiation therapy for breast carcinoma. All of these factors were addressed promptly and effectively, leading to the patient's complete recovery.

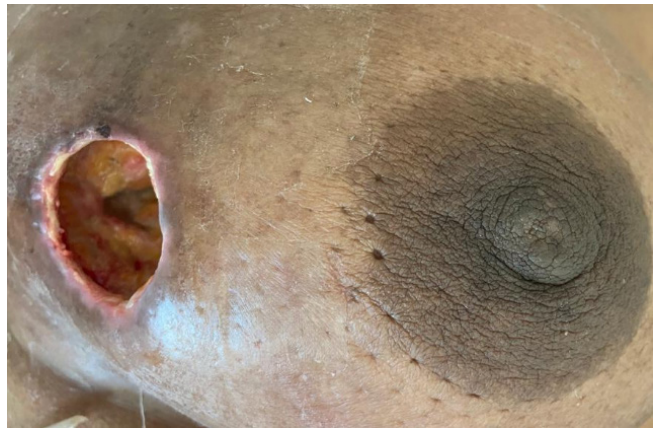

Figure 2. Infected breast wound in right breast

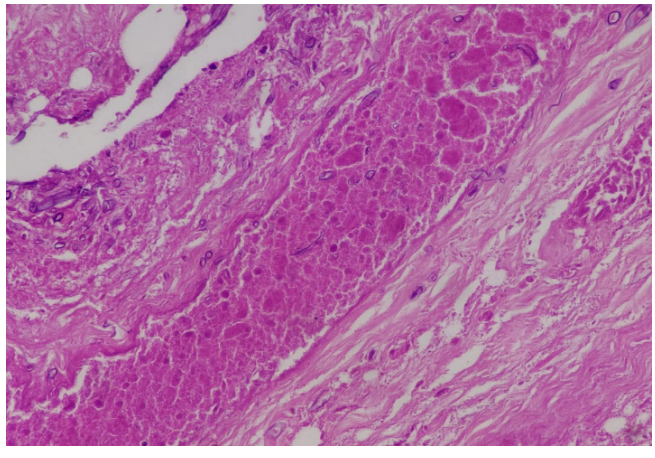

Figure 3. Histopathology showing broad aseptate hyaline fungal hyphae and angioinvasion. (Periodic acid-Schiff-diastase stain)

Available literature says that mucormycosis is an uncommon, but potentially fatal fungal infection, that usually affects patients with altered immunity. It is an angioinvasive disease caused by mold fungi of the genus *Rhizopus*, *Mucor*, *Rhizomucor*, *Cunninghamella*, and *Absidia*, of the order *Mucorales* and class *Zygomycetes* (6). *Rhizopus oryzae* is the most common type and responsible for nearly 60% of mucormycosis cases in humans (7). *Rhizopus* organisms produce an enzyme, ketone reductase, which allows them to thrive in high glucose, acidic conditions. Serum from healthy individuals inhibits the growth of *Rhizopus*, whereas serum from individuals experiencing diabetic ketoacidosis stimulates growth (8).

Tissue necrosis, a hallmark of mucormycosis, is often a late sign (9). Mucormycosis is difficult to diagnose which affects outcomes and results in a poor prognosis. The pressing priority should be the timely initiation of antifungal therapy which is proven to improve the outcome of the infection with mucormycosis. This was illustrated in a retrospective study of 70 patients with hematologic malignancy who had mucormycosis in which delayed amphotericin B therapy (starting treatment ≥6 days after diagnosis) resulted in an almost twofold increase in mortality at 12 weeks after diagnosis (83% vs. 49%) (10). It has been noted that the delay of just a week often doubles the 30-day mortality from 35% to 66%. The conundrum revolves around the poor prognosis of mucormycosis, despite accurate and aggressive treatment protocols (9). Therefore, early diagnosis and treatment are pivotal in avoiding a high risk of fatal outcome.

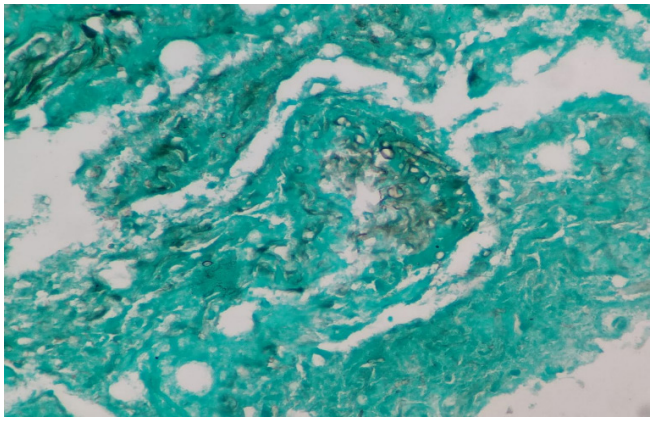

Figure 4. Histopathology showing broad aseptate hyaline fungal hyphae and angioinvasion. (Grocott Methamine silver stain)

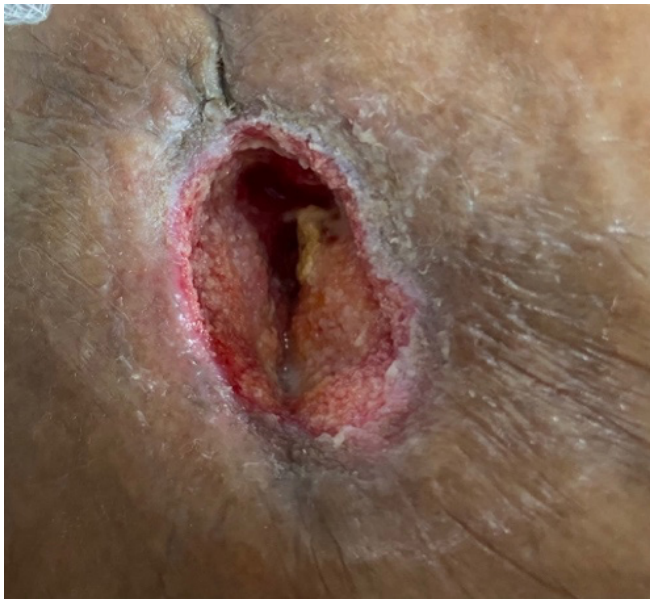

Figure 5. Wound healing post debridement and antifungals

Informed Consent: Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.P.S., R.J., B.C.A., K.R.A., R.K., A.R., S.R.; Concept: S.P.S., R.J., K.R.A., E.S.; Design: S.P.S., R.J., K.R.A., R.K., H.I.; Data Collection and/or Processing: S.P.S., R.J., H.K.K., A.P., A.F.; Analysis and/or Interpretation: S.P.S., R.J., R.K., K.R.A., S.R.; Literature Searching: S.P.S., R.J., A.P., E.S.; Writing: S.P.S., R.J., R.K., K.R.A., H.I., H.K.K.

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