E-ISSN 2587-0831

eurjbreasthealth.com

European Journal of Breast Health

Indexed in PubMed Central and Web of Science - ESCI

Turkish Federation of Breast Diseases Societies

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

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

SIS ISS SENOLOGIC INTERNATIONAL SOCIETY INTERNATIONAL SCHOOL OF SENOLOGY

NCBC National Consortium of Breast Centers

galenos Publishing House

EUROPEAN JOURNAL OF BREAST HEALTH

VOLUME: 20 • ISSUE: 3 • July 2024

eurjbreasthealth.com

European Journal of Breast Health

Société Internacionale de Sénologie
Senologic International Society

Global Federation of Breast Healthcare Societies

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

TMHDF

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

Contact
Department of General Surgery, İstanbul University İstanbul Faculty of Medicine, C Service Çapa / İstanbul
Phone&Fax : + 90 212 534 02 10

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

galenos Publishing House

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 İstanbul, Turkey
Phone: +90 (530) 177 30 97
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
Web: www.galenos.com.tr

Publisher Certificate Number: 14521
Online Publication Date: July 2024
E-ISSN: 2587-0831
International scientific journal published quarterly.

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, Scopus.

Submission Fee

The European Journal of Breast Health (Eur J Breast Health) has an open access to all articles published by itself and provides online free access as soon as it is published in the journal. We have published our journal for more than 15 years without any requests from you. But today, European Journal of Breast Health has had to charge you a low fee (50$) at the time of application to cover its increasing costs for services.

Open Access Policy

This journal provides immediate open and free access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge.

Open Access Policy is based on the rules of the Budapest Open Access Initiative (BOAI) http://www.budapestopenaccessinitiative.org/. By "open access" to peer-reviewed research literature, we mean its free availability on the public internet, permitting any users to read, download, copy, distribute, print, search, or link to the full texts of these articles, crawl them for indexing, pass them as data to software, or use them for any other lawful purpose, without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. The only constraint on reproduction and distribution, and the only role for copyright in this domain, should be to give authors control over the integrity of their work and the right to be properly acknowledged and cited.

All expenses of the journal are covered by the Turkish Federation of Breast Diseases Societies and the Senologic International Society (SIS). Potential advertisers should contact the Editorial Office. Advertisement images are published only upon the Editor-in-Chief's approval.

Statements or opinions expressed in the manuscripts published in the journal reflect the views of the author(s) and not the opinions of the Turkish Federation of Breast Diseases Societies, editors, editorial board, and/or publisher; the editors, editorial board, and publisher disclaim any responsibility or liability for such materials.

All published content is available online, free of charge at www.eurjbreasthealth.com.

OPEN ACCESS

Editor in Chief: Prof. Vahit ÖZMEN

Address: Department of General Surgery, İstanbul University İstanbul Faculty of Medicine, Çapa, İstanbul

Phone : +90 (212) 534 02 10

Fax : +90 (212) 534 02 10

E-mail : editor@eurjbreasthealth.com

Web : www.eurjbreasthealth.com

Publisher: Galenos Yayınevi

Address: Molla Gürani Mah. Kaçamak Sok. 21/1
Fındıkzade, Fatih, Istanbul, Turkey

Phone : +90 (530) 177 30 97

E-mail : info@galenos.com.tr

Web : www.galenos.com.tr/en

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.

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

Originality, high scientific quality, and citation potential are the most important criteria for a manuscript to be accepted for publication. Manuscripts submitted for evaluation should not have been previously presented or already published in an electronic or printed medium. The journal should be informed of manuscripts that have been submitted to another journal for evaluation and rejected for publication. The submission of previous reviewer reports will expedite the evaluation process. Manuscripts that have been presented in a meeting should be submitted with detailed information on the organization, including the name, date, and location of the organization.

Manuscripts submitted to the European Journal of Breast Health will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal. The Editor in Chief is the final authority in the decision-making process for all submissions.

An approval of research protocols by the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013, www.wma.net) is required for experimental, clinical, and drug studies and for some case reports. If required, ethics committee reports or an equivalent official document will be requested from the authors. For manuscripts concerning experimental research on humans, a statement should be included that shows that written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. For studies carried out on animals, the measures taken to prevent pain and suffering of the animals should be stated clearly. Information on patient consent, the name of the ethics committee, and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript. It is the authors' responsibility to protect the patients' anonymity carefully. For photographs that may reveal the identity of the patients, signed releases of the patient or their legal representative should be enclosed.

All submissions are screened by a similarity detection software (iThenticate by CrossCheck).

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/fabrication, the Editorial Board will follow and act in accordance with COPE guidelines.

Each individual listed as an author should fulfill the authorship criteria recommended by the International Committee of Medical Journal Editors

(ICMJE - www.icmje.org). The ICMJE recommends that authorship be based on the following 4 criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND

2. Drafting the work or revising it critically for important intellectual content; AND

3. Final approval of the version to be published; AND

4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

In addition to being accountable for the parts of the work he/she has done, an author should be able to identify which co-authors are responsible for specific other parts of the work. In addition, authors should have confidence in the integrity of the contributions of their co-authors.

All those designated as authors should meet all four criteria for authorship, and all who meet the four criteria should be identified as authors. Those who do not meet all four criteria should be acknowledged in the title page of the manuscript.

The European Journal of Breast Health requires corresponding authors to submit a signed and scanned version of the Copyright Transfer and Acknowledgement of Authorship Form (available for download through www.eurjbreasthealth.com) during the initial submission process in order to act appropriately on authorship rights and to prevent ghost or honorary authorship. If the editorial board suspects a case of "gift authorship," the submission will be rejected without further review. As part of the submission of the manuscript, the corresponding author should also send a short statement declaring that he/she accepts to undertake all the responsibility for authorship during the submission and review stages of the manuscript.

European Journal of Breast Health requires and encourages the authors and the individuals involved in the evaluation process of submitted manuscripts to disclose any existing or potential conflicts of interests, including financial, consultant, and institutional, that might lead to potential bias or a conflict of interest. Any financial grants or other support received for a submitted study from individuals or institutions should be disclosed to the Editorial Board. To disclose a potential conflict of interest, the ICMJE Potential Conflict of Interest Disclosure Form should be filled in and submitted by all contributing authors. Cases of a potential conflict of interest of the editors, authors, or reviewers are resolved by the journal's Editorial Board within the scope of COPE and ICMJE guidelines.

The Editorial Board of the journal handles all appeal and complaint cases within the scope of COPE guidelines. In such cases, authors should get in direct contact with the editorial office regarding their appeals and complaints. When needed, an ombudsperson may be assigned to resolve cases that cannot be resolved internally. The Editor in Chief is the final authority in the decision-making process for all appeals and complaints.

When submitting a manuscript to the European Journal of Breast Health, authors accept to assign the copyright of their manuscript to Turkish Federation of Breast Diseases Societies. If rejected for publication, the copyright of the manuscript will be assigned back to the authors. European Journal of Breast Health requires each submission to be accompanied by a Copyright Transfer and Acknowledgement of Authorship Form (available for download at www.eurjbreasthealth.com). When using previously published content, including figures,

Instructions to Authors

tables, or any other material in both print and electronic formats, authors must obtain permission from the copyright holder. Legal, financial and criminal liabilities in this regard belong to the author(s).

Statements or opinions expressed in the manuscripts published in European Journal of Breast Health reflect the views of the author(s) and not the opinions of the editors, the editorial board, or the publisher; the editors, the editorial board, and the publisher disclaim any responsibility or liability for such materials. The final responsibility in regard to the published content rests with the authors.

Submission Fee

The European Journal of Breast Health (Eur J Breast Health) has an open access to all articles published by itself and provides online free access as soon as it is published in the journal. We have published our journal for more than 15 years without any requests from you. But today, your journal has had to charge you a low fee (50$) at the time of application to cover its increasing costs for services.

The services provided in this context are the provision of systems for editors and authors, editorial work, provision of article designs, the establishment of indexing links, provision of other publishing services and support services.

You can take a look at the unbiased article evaluation process here. If you find a problem with the open access status of your article or licensing, you can contact editor@eurjbreasthealth.com

After your submission to the Eur J Breast Health evaluation system, the submission fees are collected from you or through your fund provider, institution or sponsor.

Eur J Breast Health regularly reviews the fees of submission fees and may change the fees for submission fees. When determining the costs for Eur J Breast Health submission fees, it decides according to the following developments.

- Quality of the journal,
- Editorial and technical processes of the journal,
- Market conditions,
- Other revenue streams associated with the journal

You can find the submission fees fee list here.

Article type	Price
Original articles	$50
Editorial comment	Free of charge
Review article (No application fee will be charged from invited authors)	$50
Case report	$50
Letter to the editor	Free of charge
Images in clinical practices	Free of charge
Current opinion	Free of charge
Systematic review	$50

When and How do I pay?

After the article is submitted to the Eur J Breast Health online evaluation system, an email regarding payment instructions will be sent to the corresponding author.

The editorial review process will be initiated after the payment has been made for the article.

There are two options to purchase the submission fee:

1- Making a remittance

The payment is needed to be made to the account number below. While purchasing the submission fee, please indicate your article manuscript title in the payment description section.

Account no/IBAN: TR49 0011 1000 0000 0098 1779 82 (TL)
TR17 0011 1000 0000 0098 5125 29 (USD)
TR73 0011 1000 0000 0098 5125 88 (EUR)

Account name: Meme Hastalıkları Dernekleri Federasyonu İktisadi İşletmesi

Branch code (QNB Finans Bank Cerrahpaşa): 1020

Swift code: FNNBTRISOPS

NOTE: All authors must pay the bank wire fee additionally. Otherwise, the deducted amount of the submission fee is requested from the author.

2- Virtual POS method (Credit card payment with 3D Secure)

The payment link will be sent to you for your purchase. You can contact us if you have further questions in this regard.

If you believe payment instructions are not in your email contact us via the email addresses **payment@eurjbreasthealth.com** and **journalpay@tmhdf.org.tr**

Refund policy:

The Eur J Breast Health will refund the overpayments of the submission fees for the same article or in case of multiple payments by the authors and financiers as free submission fees payment code to be used in the submission fees system.

Withdrawal of the article; There is no refund for articles whose editorial review has started in the Eur J Breast Health system. You can view article retraction policies here.

Returning the article to the author; The European Journal of Breast Health will refund the submission fees with a coupon code if the article is returned to the author. Using this code, authors can use the submission fees of different articles without making a new payment. You can view article return policies here.

Rejecting or accepting the article; Eur J Breast Health does not refund any submission fees for articles whose editorial process has started, and the process has been completed.

MANUSCRIPT PREPARATION

The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in December 2019 - http://www.icmje.org/icmje-recommendations). Authors are required to prepare manuscripts in accordance with the CONSORT guidelines for randomized research studies, STROBE guidelines for observational original research studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies, and TREND guidelines for non-randomized public behaviour.

Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at www.

Instructions to Authors

eurjbreasthealth.com. Manuscripts submitted via any other medium will not be evaluated.

Manuscripts submitted to the journal will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines. Submissions that do not conform to the journal's guidelines will be returned to the submitting author with technical correction requests.

Authors are required to submit the following:

• Copyright Transfer and Acknowledgement of Authorship Form, and

• ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors)

during the initial submission. These forms are available for download at www.eurjbreasthealth.com.

Preparation of the Manuscript

Title page: A separate title page should be submitted with all submissions, and this page should include:

• The full title of the manuscript as well as a short title (running head) of no more than 50 characters,

• Name(s), affiliations, and highest academic degree(s) of the author(s),

• Grant information and detailed information on the other sources of support,

• Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,

• Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfill the authorship criteria.

Abstract: An English abstract should be submitted with all submissions except for Letters to the Editor. The abstract of Original Articles should be structured with subheadings (Objective, Materials and Methods, Results, and Conclusion). Please check Table 1 below for word count specifications.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (https://www.nlm.nih.gov/mesh/MBrowser.html).

Key Points: All submissions except letters to the editor should be accompanied by 3 to 5 "key points" which should emphasize the most noteworthy results of the study and underline the principle message that is addressed to the reader. This section should be structured as itemized to give a general overview of the article. Since "Key Points" targeting the experts and specialists of the field, each item should be written as plain and straightforward as possible.

Manuscript Types

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).

Editorial Comments: Editorial comments aim to provide a brief critical commentary by reviewers with expertise or with high reputation in the topic of the research article published in the journal. Authors are selected and invited by the journal to provide such comments. Abstract, Keywords, and Tables, Figures, Images, and other media are not included.

Review Articles: Reviews prepared by authors who have extensive knowledge on a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. These authors may even be invited by the journal. Reviews should describe, discuss, and evaluate the current level of knowledge of a topic in clinical practice and should guide future studies. The main text should contain Introduction, Clinical and Research Consequences, and Conclusion sections. Please check Table 1 for the limitations for Review Articles.

Case Reports: There is limited space for case reports in the journal and reports on rare cases or conditions that constitute challenges in diagnosis and treatment, those offering new therapies or revealing knowledge not included in the literature, and interesting and educative case reports are accepted for publication. The text should include "Introduction", "Case Presentation", "Discussion and Conclusion" subheadings. Please check Table 1 for the limitations for Case Reports.

Letters to the Editor: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Letter to the Editor." Readers can also present their comments on the published manuscripts in the form of a "Letter to the Editor." Abstract, Keywords, and Tables, Figures, Images, and other media should not be included. The text should be unstructured. The manuscript that is being commented on must be properly cited within this manuscript.

Images in Clinical Practices: Our journal accepts original high-quality images related to the cases that we come across during clinical practices, that cite the importance or infrequency of the topic, make the visual quality stand out and present important information that should be shared in academic platforms. Titles of the images should not exceed 10 words. Images can be signed by no more than 3 authors. Figure legends are limited to 200 words, and the number of figures is limited to 3. Video submissions will not be considered.

Current Opinion: Current Opinion provides readers with a commentary of either recently published articles in the European Journal of Breast Health or some other hot topic selected articles. Authors are selected and invited by the journal for such commentaries. This type of article contains three main sections titled as Background, Present Study, and Implications. Authors are expected to describe the background of the subject/study briefly, critically discuss the present research, and provide insights for future studies.

Instructions to Authors

Table 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3500	250 (Structured)	30	6	7 or tatal of 15 images
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.

Figures and Figure Legends

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.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

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)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

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.

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be cancelled. If the submitting author(s)

believe that additional time is required, they should request this extension before the initial 30-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author,and their publication approval is requested within 2 days of their receipt of the proof.

Editor in Chief: Prof. Vahit ÖZMEN

Address: Department of General Surgery, İstanbul University İstanbul Faculty of Medicine, Çapa, İstanbul

Phone : +90 (212) 534 02 10

Fax : +90 (212) 534 02 10

E-mail : editor@eurjbreasthealth.com

Web : www.eurjbreasthealth.com

Publisher: Galenos Yayınevi

Address: Molla Gürani Mah. Kaçamak Sok. 21/1 Fındıkzade, Fatih, Istanbul, Turkey

Phone : +90 (530) 177 30 97

E-mail : info@galenos.com.tr

Web : www.galenos.com.tr

European Journal of Breast Health

Contents

European Journal of Breast Health

Contents

Systematic Review

Eur J Breast Health 2024; 20(3): 156-166
DOI: 10.4274/ejbh.galenos.2024.2023-12-16

Adjuvant and Neoadjuvant Therapy for Breast Cancer: A Systematic Review

Amal Sayfuldeen Qari[1], Ali Hussain Mowais[2], Sultan Mohammed Alharbi[2], Mohammed Jafar Almuayrifi[1], Ali Ahmed Al Asiri[4], Saud Abdulaziz Alwatid[2], Asia Ayad Aljohani[1], Rawan Mahmoud Alanazi[3], Fatma Al Thoubaity[5]

[1]Taibah University Faculty of Medicine, Medina, Saudi Arabia
[2]Alfaisal University Faculty of Medicine, Riyadh, Saudi Arabia
[3]Dar Al Uloom University Faculty of Medicine, Riyadh, Saudi Arabia
[4]Ministry of Health, Rijal Almaa General Hospital, Abha, Saudi Arabia
[5]Department of General Surgery, King Abdulaziz University Faculty of Medicine, Jeddah, Saudi Arabia

ABSTRACT

Breast cancer (BC) is the most frequent type of cancer among women. The neoadjuvant therapy was administered before surgery, and the adjuvant therapy was administered post-surgery. The goal of this systematic review is to study the effects of adjuvant and neoadjuvant BC therapy on patient outcomes and mortality. In July 2023, systematic searches were conducted through the Cochrane Library, Web of Sciences, Google Scholar, EMBASE, and PubMed databases. The search method focused on studies that included all patients with BC stages 1, 2, and 3 and excluded studies that included patients with metastatic and recurrent BC. The risk of bias in the included studies was evaluated using the Cochrane risk of bias technique. Throughout our search, 27 relevant studies with 161,552 patients were discovered. Anti-human epidermal growth factor 2 therapy (trastuzumab, pertuzumab), chemotherapy (anthracycline), endocrine therapy (tamoxifen, aromatase inhibitor), and bisphosphonates were recommended treatments for BC patients. Choices for radiotherapy included whole breast, partial breast, tumor bed boost, regional nodes, and chest wall choices after breast-conserving surgery. We discover that while the majority of treatments reduced the mortality or recurrence rates of BC, anthracycline, chemotherapy, and radiation led to an overall rise in non-BC deaths. The systemic assessment discovered several variables that impact a patient's quality of life. Based on these advantages and disadvantages, various treatment options for patients and recommendations for groups of women are made.

Keywords: Breast cancer, adjuvant, neoadjuvant, oncology

Cite this article as: Qari AS, Mowais AH, Alharbi SM, Almuayrifi MJ , Al Asiri AA, Alwatid SA, Aljohani AA, Alanazi RM, Al Thoubaity F. Adjuvant and Neoadjuvant Therapy for Breast Cancer, A Systematic Review. Eur J Breast Health 2024; 20(3): 156-166

Key Points

- The systemic assessment discovered several variables that impact a patient's quality of life.
- The majority of treatments reduced the mortality or recurrence rates of breast cancer, anthracycline chemotherapy, and radiation led to an overall rise in non-breast cancer death.

Introduction

Breast cancer (BC) is the most prevalent malignancy in the world and the main reason women die from tumor-related causes. It causes 15% of all cancer deaths and roughly 30% of cancer cases in women (1-3). A multidisciplinary team is necessary to treat a patient with BC, whether by surgery or radiation therapy, as well as systemic therapies using a variety of medications (4).

Neoadjuvant chemotherapy (NACT) was introduced in the 1970s, aiming to downstage locally advanced inoperable cancer to operable cases. NACT was subsequently extended to operable early BC, mainly to allow breast-conserving surgery (BCS), and is now widely used, particularly for large tumors (5-8). Furthermore, NACT might be more likely to eradicate micro metastatic disease than chemotherapy delayed until after surgery. Despite their adverse events, these therapies decrease BC mortality and recurrence, so highly trained clinical decision-makers are needed (9, 10). In 2016, a systemic review and meta-analysis done in Boston found that neoadjuvant endocrine therapy (NET), mainly when administered alone, has significantly decreased toxicity and is linked to response rates that are comparable to those of neoadjuvant combination chemotherapy, suggesting that NET should be given another look as a potential therapeutic option under the proper conditions. To develop logical NET combinations and prognostic biomarkers, further research is necessary to determine the optimum neoadjuvant therapy for estrogen receptor-positive BC (11).

Corresponding Author:
Asia Ayad Aljohani; asyahaljohani@gmail.com

Received: 16.01.2024
Accepted: 09.04.2024
Available Online Date: 01.07.2024

In another study done in 2021 in China, a systemic review and meta-analysis found 3842 triple-negative breast cancer (TNBC) patients, and a total of nine randomized clinical trials (RCTs) were included. Overall, disease-free survival (DFS) and overall survival (OS) were markedly improved with combined capecitabine regimens in neoadjuvant and adjuvant chemotherapy (12).

In a Switzerland study in 2022, a systemic review and meta-analysis found that the 21 RCTs with 11 regimens of neoadjuvant anti-human epidermal growth factor receptor 2 (HER2) therapy (T-DM1PC, T-DM1, and PTC_T-DM1P) had a good combination of effectiveness and safety. In contrast, the pertuzumab, trastuzumab, and chemotherapy (PTC) regimen had the greatest DFS (13).

We present a systematic review of the data required to estimate the proportional benefits and risks of modern adjuvant and neoadjuvant treatment options recommended in current clinical guidelines for and their effects on mortality and patient outcomes.

Materials and Methods

Literature Search Strategy

This systematic review was prospectively registered in PROSPERO (CRD42023446212) and adhered to the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines. A comprehensive electronic search was conducted by the Web of Sciences, Google Scholar, PubMed, Cochrane Library, and EMBASE databases for studies published between 2013 and 2023. The search strategy was designed independently by two authors and was approved by the rest of the team. An amalgamation of medical subject headings, such as "breast cancer", "adjuvant", and "neoadjuvant" was used to identify all studies inclusively. References to the selected studies were further reviewed to identify missing articles.

Inclusion/Exclusion Criteria

Two team members independently assessed each study using pre-defined inclusion and exclusion criteria-the inclusion criteria: All patients diagnosed with BC stage 1,2, and 3. Where the exclusion criteria are metastatic BC and recurrent BC, disagreements between reviewers regarding including a particular study were discussed and resolved through consensus. The studies included are RCTs, non-randomized studies, and observational studies with a control group. This process ensures that only relevant studies meeting the criteria are included in the review. The databases used to collect the included papers are Web of Sciences, Google Scholar, PubMed, Cochrane Library, and EMBASE.

Risk of Bias (Quality) Assessment

A risk-of-bias assessment was conducted to evaluate the internal validity of the included studies. More specifically, the Critical Appraisal Skills Programme (CASP) tool was used to minimize the risk of bias. Two independent reviewers conducted the assessment; they evaluated the studies for methods of randomization, treatment allocation, blinding, selection bias, performance bias, detection bias, attrition bias, and reporting bias. The risk of bias assessment was conducted at the study level.

Synthesis Methods

The criteria for data synthesis will be based on the minimum number of studies and the level of consistency. The data that will be synthesized will include outcomes related to adjuvant and neoadjuvant treatments of BC and their effects on mortality and patient outcomes.

All records resulting from the primary search were imported to Mendeley for deduplication. Then, the result was subsequently imported into Rayyan and screened by three authors for relevance based on the title and abstract. The full texts of all retained studies were then screened by all authors for final inclusion or exclusion. Disagreements at any stage of the screening process were resolved through discussion and consensus among all authors.

Data extracted for the retained studies included year, country, study sample, and study design. A meta-analysis was not possible due to the heterogeneity across the studies regarding interventions and outcomes.

Results

Study Characteristics

The 27 included studies consisting of 12 systematic reviews and meta-analyses, eight systematic reviews, three systematic reviews and network meta-analyses, two review articles, one meta-analysis, and one network of meta-analyses. All the included studies were published between 2013 and 2023. The sample size varied between studies, with a peak of 49,133. With a total of more than 215,853 participants, this systematic review covers a wide range of BC patients undergoing several BC interventions.

The stages of cancer were established to be early or late, with 11 studies emphasizing the early-stage intervention and five studies focusing only on the late-stage intervention. The remaining studies involved interventions with either mixed stages or irrespective of the cancer stage. The treatment was administered as neoadjuvant in 11 studies, nine as neoadjuvant plus adjuvant, and five as adjuvant.

Early-Stage Intervention

Different interventions have shown favorable outcomes in early-stage cancer treatment, including hormonal therapy, radiotherapy, targeted therapy, antimicrotubule agents, and chemotherapy. The findings suggest that a combination of these interventions can be beneficial in managing early-stage cancer, providing better survival rates, and reducing the risk of recurrence.

Neoadjuvant and adjuvant chemotherapy combined with capecitabine significantly improved both DFS and OS in the early stages, with a response rate of hazard ratio (HR) 0.75; 95% confidence interval (CI), 0.65–0.86 (Table 1) (12). Some interventions, such as NACT, were found to reduce mortality rates in early-stage cancer patients. Results suggest that trastuzumab plus chemotherapy may be more effective than chemotherapy alone in achieving pathological complete response (pCR) in HER2-positive BC patients undergoing neoadjuvant treatment with HER2-targeted therapies. The pooled results showed significantly higher pCR rates than chemotherapy, with a pooled relative risk (RR) of 1.81 (95% CI 1.36, 2.42) (Table 1) (13).

Targeted therapy has demonstrated promising results in improving OS and reducing the risk of recurrence (HR = 0.59, 95% CI: 0.51, 0.69), most effectively decreasing the risk of disease progression or recurrence among the comparisons (Table 2) (14).

Chemotherapy, both neoadjuvant and adjuvant, has effectively reduced mortality rates and improved survival outcomes. Significant improvement in OS was observed (HR = 0.85; 95% CI, 0.75–0.96; $p = 0.008$) (15).

Table 1. Summary of the 27 studies used for the adjuvant and neoadjuvant therapy for breast cancer systematic review

Author, year	Country	Study design	Number of participants	Stage of cancer	Type of intervention
Pinto et al. (22) 2013	Belgium	R	8,300	Early, late	Neoadjuvant + Adjuvant
Charehbili et al. (19) 2014	Netherlands	S	26 studies	Early, late	Neoadjuvant
Leal et al. (25) 2015	Brazil	SM	9 studies	Early, late	Neoadjuvant
Zhang et al. (28) 2015	China	SM	5,415	Early	Neoadjuvant
Zhang et al. (15) 2016	China	SM	9,097	Early	Neoadjuvant + Adjuvant
Spring et al. (11) 2016	USA, Boston	SM	3,490	Early, late	Neoadjuvant
Li and Shao (24) 2016	China	S	22,391	Early, late	Neoadjuvant + Adjuvant
Recht et al. (27) 2016	USA	S		Early	Adjuvant
De Felice et al. (18) 2017	Italy	SM	2,447	Late	Neoadjuvant
Pistelli et al. (33) 2018	Italy	S	6,812	Early, late	Neoadjuvant + Adjuvant
Zaheed et al. (31) 2019	Australia	R	1,695	Early	Neoadjuvant + Adjuvant
Shen et al. (14) 2019	USA, Texas	NM	13,621	Early	Adjuvant
Genuino et al. (30) 2019	Thailand	SM	10,635	Early	Adjuvant
Wang et al. (17) 2020	China	M	971	Late	Neoadjuvant
Surov et al. (35) 2020	Germany	SM	1,827	Early, late	Neoadjuvant
Huo et al. (12) 2021	China	SM	3,842	Early	Neoadjuvant + Adjuvant
Hong et al. (20) 2021	China	SM	1,028	Early, late	Neoadjuvant
Salvo et al. (34) 2021	Canada	SM	21 studies	Early	Adjuvant
Ahmed et al. (23) 2021	UK	S	3,766	Early, late	Neoadjuvant
Hickey et al. (36) 2021	USA	S	15,187	Early	
Kerr et al. (9) 2022	UK	S	13,864	Early	Neoadjuvant + Adjuvant
Nikyar et al. (26) 2022	Sweden	SM	17,224	Late	Adjuvant
Giordano et al. (16) 2022	United States	S	12,454	Late	Neoadjuvant + Adjuvant
Schettini et al. (29) 2022	Switzerland	SNM	49,133	Late	
Gunasekara et al. (13) 2022	Switzerland	SNM		Early	Neoadjuvant
Yuan et al. (21) 2022	China	SNM	12,024	Early, late	Neoadjuvant + Adjuvant
Ergun et al. (32) 2023	Turkey	SM	630	Early, late	Neoadjuvant

S: Systematic review; M: Meta-analysis; SM: Systematic review and meta-analysis; SNM: Systematic review and network meta-analysis; NM: Network of meta-analysis; R: Review articles, these 27 studies included 12 systematic reviews and meta-analyses, 8 systematic reviews, 3 systematic reviews and network meta-analyses, 2 review articles, 1 meta-analysis, and 1 network meta-analysis, with a total of more than 215,853 participants. In 11 studies, the patients were diagnosed with early-stage BC, and 5 studies focused on late-stage BC. The remaining studies involved interventions with either mixed stages or irrespective of the cancer stage. The treatment was administered as neoadjuvant in 11 studies, nine as neoadjuvant plus adjuvant, and five as adjuvant

Late-Stage Intervention

The findings contribute to understanding different interventions and their impact on outcomes in late-stage cancer, providing valuable information for clinical decision-making and treatment strategies. With emphasis on the importance of targeted therapies and antimicrotubule agents in achieving favorable outcomes for late-stage cancer patients. Trastuzumab as an adjuvant along with chemotherapy resulted in significant outcomes in terms of DFS [0.95 (95% CI, 0.71 to 1.25)] (Table 3) (16).

Additionally, the findings suggest that hormonal therapies cannot significantly improve OS or pCR. In a neo-adjuvant-based intervention study by Wang et al. (17), the rate of patients undergoing neoadjuvant hormonal therapy (NHT) was significantly lower than that of those undergoing NACT [odds ratio (OR), 0.48; 95% CI, 0.26–0.90]. Furthermore, NET and NACT had no statistically significant difference in the overall objective response rate (ORR) (pooled OR, 1.05; 95% CI, 0.73–1.52) (Table 3) (17).

Radiotherapy in late-stage cancer patients showed reduced locoregional recurrence (LRR). A study done by De Felice et al. in 2017 (18) shows that regional nodal irradiation was mainly associated with a reduction in the rate of LRR (4.3% *vs.* 6.8%) and a statistically significant improvement in 10-year DFS (82% *vs.* 77%, $p = 0.01$) and distant free survival (86.3% *vs.* 82.4%, $p = 0.03$) rates. In contrast, there was no significant difference in OS at ten years between groups (82.8% *vs.* 81.8%, $p = 0.38$) (Table 3) (18).

Aromatase inhibitors (AI) as a hormonal neoadjuvant were found to have higher response rates and better outcomes compared to tamoxifen in terms of clinical response, radiological response, and BCS. In a study

Table 2. Summary of early-stage breast cancer interventions

Author, year	Intervention	Class	Response rate	Survival rate	Recurrence rate
Zhang et al. (15) 2016	B	C	HR 0.85; (95% CI, 0.75–0.96)	DFS (HR 0.93; 95% CI, 0.85–1.02)	
Zaheed et al. (31) 2019	B	C	RR 1.15 (0.96 to 1.38)	HR 0.80, (95% CI, 0.60 to 1.08)	
Huo et al. (12) 2021	B	C	HR 0.75; (95% CI, 0.65–0.86)	HR 0.63; (95% CI, 0.53–0.77)	
Shen et al. (14) 2019	A	T	HR 0.59, (95% CI, 0.51, 0.69)		HR 0.59, (95% CI, 0.51, 0.69)
Genuino et al. (30) 2019	A	C/T		Reduce mortality by 33%	RR (95% CI, 21.6% for C/T *vs.* 29.4% for C
Kerr et al. (9) 2022	B	C/H		Reduce mortality 10–25%	RR 1.37, (95% CI, 1.17–1.61)
Salvo et al. (34) 2021	A	H			17.2% (95% CI, 14.6%–20.3%)
Recht et al. (27) 2016	A	R		DFS 21.0%, *vs.* 4.3%	45.5% without *vs.* 33.8%
Hickey et al. (36) 2021		R		Similar	RR 2.83 (95% CI, 1.23–6.51)
Zhang et al. (28) 2015	N	R		RR 0.88, (95% CI, 0.66–1.17)	RR 2.83, (95% CI, 1.23–6.51)
Gunasekara et al. (13) 2022	N	C/T	RRs (95% CI) of 1.81 (1.36, 2.42)		HR (95% CI) of 0.54 (0.32–0.91).

OS: Overall survival; HR: Hazard ratio; RR: Risk ratio; LRR: Locoregional recurrance; OR: Overall response; DFS: Disease free survival; H: Hormonal; R: Radiotherapy; T: Targeted; AM: Antimicrotubule agent; C: Chemotherapy; N: Neoadjuvant; A: Adjuvant; B: Neoadjuvant + adjuvant; CI: Confidence interval, the table summarizes various interventions and their outcomes in early-stage cancer patients. It includes information on response rates, survival rates, and recurrence rates from different stud-ies and authors. Interventions range from chemotherapy to targeted therapy, with some studies showing reductions in mortality rates and recurrence rates, while others indicate no significant difference or even increased risk

Table 3. Summary of late-stage breast cancer interventions

Author, year	Intervention	Class	Response rate	Survival rate	Recurrence rate
Wang et al. (17) 2020	N	H	OR 1.05; (95% CI, 0.73–1.52)	HR 0.92; (95% CI, 0.55–0.94)	
Nikyar et al. (26) 2022	A	R	HR 0.24; (95% CI 0.11–0.49)		LRR HR 0.59; (95% CI 0.42–0.81)
De Felice et al. (18) 2017	N	R		OS 82.8% *vs.* 81.8% (without)	LRR: 4.3% vs. 6.8% (without)
Giordano et al. (16) 2022	A	T	1.09 (90% CI, 0.97 to 1.21)	DFS [0.95 (95% CI, 0.71 to 1.25)]	
Schettini et al. (29) 2022	B	AM	OR 6.57, (95% Crl: 2.05–21.63)		

OS: Overall survival; HR: Hazard ratio; LRR: Locoregional recurrence; OR: Overall response; DFS: Disease free survival; H: Hormonal; R: Radiotherapy; T: Targeted; AM: Antimicrotubule agent; N: Neoadjuvant; A: Adjuvant; B: Neoadjuvant + adjuvant; CI: Confidence interval, the table presents interventions and their outcomes in late-stage cancer patients. It includes response rates, survival rates, and recurrence rates from various authors and studies. Interventions span from ne-oadjuvant to adjuvant therapies, with results showing varied impacts on overall survival, disease-free survival, and locoregional recurrence. Some interventions demonstrate significant improvements in sur-vival rates and recurrence rates, while others show no significant difference or even increased risk

by Spring et al. (11), there was a significantly higher clinical response rate (OR, 1.69; 95% CI, 1.36–2.10; $p<0.001$; n = 1352), radiological response rate (OR, 1.49; 95% CI, 1.18–1.89; $p<0.001$; n = 1418), and BCS rate (OR, 1.62; 95% CI, 1.24–2.12; $p<0.001$; n = 918) compared with tamoxifen (11). Furthermore, a study by Charehbili et al. (19) shows similar findings when it comes to the comparison of AI and tamoxifen in terms of a response rate of 70% for AI *vs.* 51% for Tyro3, Axl and MerTK (TAM) in a third study by Hong et al. (20). The pCR is: OR = 0.34, 95% CI = 0.04–2.85, $p = 0.318$. Leal et al. (25), 2015, reported a significant overall response and found the response rate to be OR 1.9; 95% CI 1.17–3.08 (Table 4).

Table. 4 Summary of early and late breast cancer interventions

Author, year	Intervention	Class	Response rate	Survival rate	Recurrence rate
Spring et al. (11) 2016	N	H	OR, 1.69; 95% CI, 1.36–2.10; p<0.001		3.3 for hormonal and 3.4% for chemotherapy
Ergun et al. (32) 2023	N	C/H	82% *vs.* 72.7%; OR:1.77, 95% CI, 1.20–2.62		
Charehbili et al. (19) 2014	N	H	70% for AI *vs.* 51% for TAM		
Li and Shao (24) 2016	B	C/H/T	RR 1.29; 95% CI, 1.14–1.47;	HR 0.79 (0.69–0.90)	15 years 33% for TAM *vs.* 46.2% without
Surov et al. (35) 2020	N	C	35.6% responders		
Hong et al. (20) 2021	N	H	OR 0.34, 95% CI, 0.04–2.85		
Pistelli et al. (33) 2018	B	H	HR 0.72; 95% CI, 0.60 to 0.85	DFS 92.8% with TAM	>25 BMI 50% recurrence with anastrozole
Leal et al. (25) 2015	N	H	OR 1.9; 95% CI, 1.17–3.08		
Ahmed et al. (23) 2021	N	R		61.4% to 81% at 5 years	0.8%–10% for local recurrence
Yuan et al. (21) 2022		C	RR: 0.98, 95% CI, 0.93–1.03	HR: 0.84, 95% CI, 0.73–0.97	
Pinto et al. (22) 2013	B	C/T		OS 0.66 [95% (95% CI) 0.57e0.77]	0.65 (95% CI, 0.55, 0.75)

OS: Overall survival; HR: Hazard Ratio; LRR: Locoregional recurrence; OR: Overall response; DFS: Disease free survival; H: Hormonal; R: Radiotherapy; T: Targeted; AM: Antimicrotubule agent; N: Neoadjuvant; B: Neoadjuvant + Adjuvant; AI: Aromatase inhibitor; TAM: tamoxifen BMI: Body mass index CI: Confidence interval, the table provides data on interventions and their outcomes in both early and late-stage cancer therapy. Authors and studies examine various intervention classes, including neoadjuvant, chemotherapy, hormonal therapy, and targeted therapy. Results show response rates, survival rates, and recurrence rates, with some interventions indicating significant improvements in survival and response rates while others show mixed or inconclusive results. Notably, hormonal therapies like aromatase inhibitors and tamoxifen demonstrate varied impacts on recurrence rates, with some studies suggesting significant re-ductions in recurrence

Chemotherapy in a study by Yuan et al. (21) found that trastuzumab combined with lapatinib therapy was found to be superior to standard trastuzumab therapy alone in terms of OS, DFS/event-free survival, and pathologic complete response. Illustrating the response rate to be RR: 0.98, 95% CI: 0.93–1.03, and the survival rate to be HR: 0.84, 95% CI: 0.73–0.97, Another study by Pinto et al. (22) found that trastuzumab has improved both DFS and OS in patients with early HER-2-positive BC with moderate-to-high risk of recurrence when given in combination with or in sequence with adjuvant chemotherapy. The study reports the survival rate to be OS = 0.66 [95% (CI 95%) 0.57e0.77] and the recurrence rate to be 0.66 (95% CI: 0.57–0.77) and the recurrence rate to be 0.65 (95% CI: 0.55–0.75) (Table 4).

For radiotherapy, a study by Ahmed et al. (23) shows the five-year survival to be 61.4% to 81% and the local recurrence to be 0.8–10% (Table 4).

Intervention Efficacy

AI as a neoadjuvant had higher response rates and better outcomes than tamoxifen in clinical response, radiological response, and BCS. A study done by Spring et al. (11) shows a significantly higher clinical response rate (OR, 1.69; 95% CI, 1.36–2.10; p<0.001; n = 1352), radiological response rate (OR, 1.49; 95% CI, 1.18–1.89; p<0.001; n = 1418), and BCS rate (OR, 1.62; 95% CI, 1.24–2.12; p<0.001; n = 918) compared with tamoxifen. Moreover, a study by Li and Shao (24) reports that AIs have shown superiority over tamoxifen in terms of clinical response rate and breast conservation rate in neoadjuvant therapy where OS (relative HR = 0.82; 95% CI, 0.69–0.99). A third study that compares AI to tamoxifen illustrates a reduction in BC mortality or recurrence by 10–25%. Kerr et al. (9). Lastly, a study by Leal et al. (25) reports an ORR (OR 1.9; 95% CI 1.17–3.08) (Table 5).

A study by Charehbili et al. (19) illustrates that the favorable toxicity profile of NHT makes it a very suitable treatment option for patients unfit for chemotherapy. Studies have shown that AIs, rather than tamoxifen, are the preferred agents for NHT in postmenopausal patients. Longer treatment durations demonstrated more significant clinical responses and BCS rates with acceptable tolerability (70.4% vs. 50.5%, p = 0.004).

A subgroup analysis done Hong et al. (20) showed that all three types of cyclin-dependent kinase 4 and 6 (CDK 4/6) inhibitors improved the complete clinical and pathological response rate in BC patients, with similar efficacy to NACT and a decreased risk of adverse events. Patients who received ribociclib were over ten times more likely to

Table 5. Summary of included studies

Study	Main drug	Type of intervention	Control	Response			
				Disease free survival	Recurrence	Pathological complete response	Overall response clinical/radiological
Zhang et al. (15) 2016	Cape	NACT	NA	HR: 0.93; (95% CI, 0.85–1.02; $p = 0.12$)			HR 0.85; (95% CI, 0.75–0.96; $p = 0.008$)
Spring et al. (11) 2016	AI	NHT	TAM				OR: 1.69; (95% CI, 1.36–2.10; $p<0.001$)
Wang et al. (17) 2020	Mulitple	NHT	NACT			OR: 0.48; (95% CI, 0.26-0.90)	OR: 1.05; (95% CI, 0.73–1.52)
Zaheed et al. (31) 2019	Taxanes	NHT	AC	HR: 0.84, (95% CI 0.65 to 1.09)		RR 1.15, (95% CI 0.96 to 1.38; 1280)	HR 0.80, (95% CI 0.60 to 1.08)
Huo et al. (12) 2021	Cape	NACT	Without	HR: 0.75; (95% CI, 0.65–0.86; $p<0.001$)			HR: 0.63; (95% CI, 0.53–0.77; $p<0.001$)
Shen et al. (14) 2019	Tras	AT	AC+ CP+ TAX				HR: 0.59, (95% CI, 0.51, 0.69)
Ergun et al. (32) 2023	CT+ H	NHT	NACT			(6.5% vs. 3.8%; OR:1.72, 95% CI 0.82–3.62).	ORR (82% *vs.* 72.7%; OR: 1.77, 95% CI 1.20–2.62)
Charehbili et al. (19) 2014	AN	NHT	TAM				70.4% *vs.* 50.5%, $p = 0.004$
Li and Shao (24) 2016	TAM	NHT	AI				RH 0.82; (95% CI, 0.69–0.99)
Genuino et al. (30) 2019	Tras	AT	AC		HRs: 0.65 (95% CI: 0.55, 0.75, $p<0.001$)		
Kerr et al. (9) 2022	TAM+AI	NHT	TAM		0.67 (95% CI 0.61–0.73)		
	Ribo	KI	TAM			OR 10.31, (95% CI = 3.59–29.61, $p<0.001$)	
Hong et al. (20) 2021	Palbo	KI	TAM			OR 7.39, (95% CI = 1.26–43.40, $p = 0.027$)	
	Abema	KI	TAM			OR 8.28, (95% CI = 3.41–20.11, $p<0.001$)	
Pistelli et al. (33) 2018	TAM	NHT	AN	92.8% with TAM, 92.0% with AN			
Nikyar et al. (26) 2022	NACT then ART	R	NACT		HR 0.59; (95% CI 0.42–0.81; $p<0.001$)	HR 0.24; (95% CI 0.11–0.49; $p<0.0001$)	
Leal et al. (25) 2015	AI	NHT	TAM				OR 1.9; 95% CI 1.17–3.08
Salvo et al. (34) 2021	TAM	NHT	Letrozole		17.2% (95% credible interval: 14.6–20.3%)		
Ahmed et al. (23) 2021	NRT	NRT	Multiple	61.4% to 81% at 5 years	0.8–10% for local recurrence	14% to 42%	OS 71.6% to 84.2%

Table 5. Continued

Study	Main drug	Type of intervention	Control	Response			
				Disease free survival	Recurrence	Pathological complete response	Overall response clinical/radiological
Recht et al. (27) 2016	R	ART	Without	21.0%, compared to 4.3%	45.5% without vs. to 33.8% with		
Zhang et al. (28) 2015	IORT	R	EBRT	0.88 (95% CI: 0.66–1.17)	RR for IBTR was 2.83 (95% CI 1.23–6.51)		
Giordano et al. (16) 2022	Tras+ CT	AT	Biosimilar + CT	0.95 (95% CI: 0.71 to 1.25)		1.09 (90% CI, 0.97 to 1.21)	
	PTX± Bev	AMA	CP + MTX + 5-FU			OR 6.57, (95 % CrI: 2.05–21.63)	
Schettini et al. (29) 2022	PTX± Bev	AMA	AC+-CP+ TAX			OR 3.45, 95 %CrI	
	PTX± Bev	AMA	Cape + Bev			OR 2.47, (95 %CrI:1.08–5.73)	
	Tras + CT	NAT	CT		HR (95% CI) of 0.54 (0.32–0.91).	RRs (95% CI) of 1.81 (1.36, 2.42; I2 = 0%)	
Gunasekara et al. (13) 2022	TYK + CT	NAT	Tras + CT			RR of 0.74 (95% CI 0.63, 0.87).	
	TYK+ Tras+ CT	NAT	Tras + CT			RRs of 1.26 (95% CI 1.11, 1.42)	
	TYK+ Tras+ CT	NAT	TYK+ CT			RRs 1.66 (95% CI 1.33, 2.06)	
Yuan et al. (21) 2022	Tras + TYK	AT	Tras	HR 1.22, (95% CI: 1.05–1.41, $p = 0.008$)			
	TYK	NAT	Tras				
Pinto et al. (22) 2013	Tras	AT		0.60 (95% CI 0.50–0.71, $p<0.00001$)	reduced by 40%		

OS: Overall survival; HR: Hazard ratio; LRR: Locoregional recurrence; RH: Relative hazard; OR: Overall response; DFS: Disease free survival; H: Hormonal; R: Radiotherapy; T: Targeted; AM: Antimicrotubule agent; N: Neoadjuvant; B: Neoadjuvant + Adjuvant; AI: Aromatase inhibitor; NACT: Neoadjuvant chemotherapy; NHT: Neoadjuvant hormonal therapy; AT: Adjuvant targeted therapy; KI: Kinase inhibitor; NRT: Neoadjuvant radiotherapy; ART: Adjuvant radiotherapy; AMA: Antimicrotubule agent; NAT: Neoadjuvant targeted therapy; CT: Chemotherapy; NA: Neo-adjuvant; TAM: tamoxifin; Tras: Trastuzumab; TYK: Lapatinib; PBI: Partial breast irradiation; IORT: Intraoperative radiotherapy; Bev: Bevacizumab; PTX: Paclitaxel; Ribo: Ribociclib; Palbo: Palbociclib; Abema: Abemaciclib; Cape: Capecitabine; AN: Anastrozole; WBRT: Whole breast radiotherapy; EBRT: Whole-breast external beam radiotherapy; AC: Anthracyclines; CP: Cyclophosphamide; MTX: Methotrexate; 5-FU: 5-fluorouracil; TAX: Taxane, the table provides a summary of the included studies in this systematic review, including the type of intervention, disease-free survival, recurrence, pathological complete response, and radiological response

achieve complete cell cycle arrest (CCCA) than those who did not. The 95% CI for this OR was 3.59–29.61, which means there is a high degree of certainty that the actual OR falls within this range. Similarly, the OR for palbociclib was 7.39, with a 95% CI of 1.26–43.40, and the OR for abemaciclib was 8.28, with a 95% CI of 3.41–20.11. These results suggest that all three CDK 4/6 inhibitors effectively improve CCCA, although the degree of improvement may vary (Table 5).

In a study by Nikyar et al. (26) adjuvant locoregional radiation therapy (LRRT) significantly reduced the risk of LRR in patients with N+ at diagnosis and ypN0 (HR 0.59; 95% CI 0.42–0.81). However, no statistically significant difference was found in DFS or OS. Moreover, a subgroup analysis including three studies with data on the impact of LRRT on LRR in patients with pCR (both ypT0 and ypN0) showed a statistically significant lower risk of LRR in patients who received LRRT (HR 0.24; 95% CI 0.11–0.49; $p<0.0001$) (Table 5).

Another study by Ahmed et al. (23) for patients who received neo-adjuvant radiotherapy (NRT) found that pCR values ranged from 14% to 42% in the patients who received NRT, and the 5-year DFS rates ranged from 61.4% to 81% in the patients who received NRT. Moreover, the 5-year OS rates ranged from 71.6% to 84.2% in the patients who received NRT (Table 5).

A study by Recht et al. (27) illustrates that postmastectomy radiotherapy (PMRT) reduces the risks of locoregional failure (LRF), recurrence, and BC mortality. Reporting the DFS to be 21.0% with PMRT, compared to 4.3% without the intervention. Along with a recurrence rate of 33.8% with PMRT *vs.* 45.5% without (Table 5).

Ipsilateral breast tumor recurrence (IBTR) was significantly higher in patients with intraoperative radiotherapy (IORT) compared to those with whole-breast external beam radiotherapy, with a risk ratio (RR) of 2.83 (95% CI 1.23–6.51). However, the two treatment modalities had no significant difference in overall mortality, with a pooled RR of 0.88 (95% CI: 0.66–1.17). Zhang et al. (Table 5) (28).

For antimicrotubule agents, a study done Schettini et al. (29) shows that paclitaxel + bevacizumab was likely to be significantly associated with superior ORR than several poly- chemotherapy regimens like cyclophosphamide + methotrexate + 5-fluorouracil [OR: 6.57, 95% credible intervals (CrI): 2.05–21.63], FEC (OR: 4.44, 95% CrI: 1.33–15.23), ixabepilone + capecitabine (OR: 3.45, 95% CrI: 1.02–12.03), or capecitabine + bevacizumab (OR: 2.47, 95% CrI: 1.08–5.73) (Table 5).

Results suggest that trastuzumab chemotherapy (TC) and lapatinib trastuzumab chemotherapy (LTC) may be more effective than chemotherapy (C) or lapatinib chemotherapy (LC) in achieving pCR in HER2-positive BC patients undergoing neoadjuvant treatment with HER2-targeted therapies. The pCR rates for four different treatment comparisons were TC vs. C, LC vs. TC, LTC vs. TC, and LTC vs. LC. The pooled results of a study by Gunasekara et al. (13) showed that TC had significantly higher pCR rates than C, with a pooled RR of 1.81 (95% CI 1.36, 2.42). Similarly, LTC had significantly higher pCR rates than TC and LC, with pooled RRs of 1.26 (95% CI 1.11, 1.42) and 1.66 (95% CI 1.33, 2.06), respectively. Conversely, LC had significantly lower pCR rates than TC, with a pooled RR of 0.74 (95% CI 0.63, 0.87) (Table 5).

Efficacy of different adjuvant trastuzumab-containing chemotherapy combinations for patients with early HER2-positive primary BC. For severe cardiac adverse events. A study by Shen et al. (14) based on their analysis, found that anthracycline-cyclophosphamide with concurrent trastuzumab (ACT+H) showed the best OS compared to other combinations. Compared to ACT, ACT+H (HR = 0.59, 95% CI: 0.51, 0.69) most effectively decreased the risk of disease progression or recurrence among the comparisons. In another study by Genuino et al. (30), The analysis found that combining trastuzumab with chemotherapy lowered the risks of death and relapse by one-third, with recurrence rates (95% CI) of 21.6% (16.6%, 26.5%) for the trastuzumab-chemotherapy group and 29.4% (24.6%, 34.2%) for the chemotherapy alone group. In a third study, Giordano et al. (16) found that in 2022, trastuzumab has shown efficacy in improving progression-free survival (PFS) by 1.09 (90% CI, 0.97 to 1.21), and DFS 0.95 (95% CI, 0.71 to 1.25), and OS in patients with advanced HER2-positive BC. Lastly, a study by Pinto et al. (22) in 2013 found that trastuzumab, when given in combination with or in sequence with adjuvant chemotherapy, has shown a significant improvement in disease-free and OS in women with HER2-positive BC, reducing mortality by one-third and the risk of relapse by 40% (Table 5).

In a study by Zhang et al. (15), Adding capecitabine to standard neoadjuvant regimens in early BC. Adding capecitabine did not improve DFS for all patients. DFS (HR = 0.93; 95% CI, 0.85–1.02; p = 0.12) However, a sub-analysis revealed that capecitabine provided a benefit in DFS for patients with the triple-negative subtype and extensive axillary involvement. The addition of capecitabine demonstrated a significantly superior OS in the meta-analysis (HR = 0.85; 95% CI, 0.75–0.96; p = 0.008). In another study by Huo et al. (12) in 2021, it was found that capecitabine-based regimens in neoadjuvant and adjuvant chemotherapy showed significantly improved DFS (HR = 0.75; 95% CI, 0.65-0.86; p<0.001) and OS (HR = 0.63; 95% CI, 0.53–0.77; p<0.001) in early-stage TNBC patients (Table 5).

The study by Wang et al. (17) suggests that postmenopausal HR-positive BC patients may have a better tumor response after NACT compared to NET, while the addition of endocrine therapy to chemotherapy may not provide significant clinical benefits compared to monotherapy. The pCR rate of patients undergoing NET was significantly lower than that of those undergoing NACT. (pooled OR, 0.48; 95% CI, 0.26–0.90) There was no statistically significant difference in the ORR between NET and NACT. (pooled OR, 1.05; 95% CI, 0.73–1.52) (Table 5).

The neoadjuvant studies done by Zaheed et al. (31) in 2019 suggested that the administration of taxanes first probably resulted in little to no difference in OS (HR 0.80, 95% CI 0.60 to 1.08) and DFS (HR 0.84, 95% CI 0.65 to 1.09). The administration of taxanes first also resulted in little to no difference in pathological complete response (RR 1.15, 95% CI 0.96 to 1.38) (Table 5).

In the HR-positive/HER2-negative BC study by Ergun et al. (32) in 2023, NACT significantly increased ORR without an increase in serious adverse events. Although the pCR rate increased numerically, it was not statistically significant. (6.5% *vs.* 3.8%; OR: 1.72, 95% CI 0.82–3.62). The study also reports that the NaCET arm exhibited a significantly higher ORR (82% *vs.* 72.7%; OR: 1.77, 95% CI: 1.20–2.62) (Table 5).

Discussion and Conclusion

Neoadjuvant and adjuvant treatments are recommended for BC. These therapies reduce cancer mortality and recurrence but have adverse effects. Therefore, this systematic review aims to study BC's adjuvant and neoadjuvant treatments and their effects on mortality and patient outcomes.

Anti-Human Epidermal Growth Factor 2 Therapy with Chemotherapy

Neoadjuvant and adjuvant therapies combining trastuzumab with chemotherapy have shown significant efficacy in improving DFS and OS in patients with early-stage and locally advanced HER2-positive BC. Gunasekara et al. (13) concluded that the T-DM1PC (trastuzumab emtansine + pertuzumab + chemotherapy), T-DM1 (trastuzumab emtansine), and PTC_T-DM1P (pertuzumab + trastuzumab + chemotherapy followed by T-DM1P) regimens are the most effective and safe neoadjuvant anti-HER2 therapies for early-stage and locally advanced HER2-positive BC. These regimens have the optimal balance between efficacy (pCR) and serious adverse events (SAE). The PTC regimen has the highest DFS rate among these regimens.

Additionally, Pinto et al. (22) reported that using trastuzumab in combination with or in sequence with adjuvant chemotherapy has significantly improved the DFS and OS of patients with early HER-2-positive BC with a moderate-to-high risk of recurrence. The patient outcomes were also concluded by Giordano et al. (16) that adding trastuzumab to chemotherapy as adjuvant therapy significantly improves DFS outcomes.

Moreover, Shen et al. (14) concluded that the concurrent use of anthracycline-cyclophosphamide and taxane or taxane plus carboplatin with trastuzumab resulted in the most clinical benefits for early-stage HER2-positive primary BC. Additionally, taxane and carboplatin with trastuzumab had the lowest cardiotoxicity. This is proved by Genuino et al. (30) that administering adjuvant trastuzumab in a weekly cycle concurrently with an anthracycline-taxane chemotherapy regimen appears to be a preferred option to optimize its favorable effect on improving DFS and preventing significantly higher risk for cardiotoxic effects.

Endocrine/Hormone Therapy Comparable to Chemotherapy

NHT is comparable in efficacy to NACT in hormone receptor-positive (HR+) BC patients with lower toxicity, but there is a higher risk of recurrence in node-positive patients. Spring et al. (11) concluded that NET, even as monotherapy, is associated with similar response rates as neoadjuvant combination chemotherapy but with significantly lower toxicity. It was also proved by Hong et al. (20) that the combination therapy comprising neoadjuvant CDK 4/6 inhibitors and NET demonstrated increased efficacy and toxicity compared to endocrine monotherapy. It also showed comparable efficacy and better safety than NACT. Evidence has been accrued by Li and Shao (24) of the benefits of ovarian ablation or suppression in premenopausal patients and AIs in postmenopausal patients for longer durations of adjuvant NET as well as for the clinical utility of NET.

Moreover, Leal et al. (25) reported the safety of neoadjuvant hormone therapy and reported that it could not be considered equivalent to chemotherapy. Additionally, it was reported that AIs are preferable to tamoxifen when using neoadjuvant hormone therapy due to their higher response rates. It could be proved by Pistelli et al. (33) that in premenopausal patients with HR+BC, the combination of AIs and a gonadotropin hormone-releasing hormone analog is a safe and effective treatment option. Charehbili et al. (19) reported that NHT has shown comparable efficacy to NACT in patients with HR+ BC. However, Wang et al. (17) reported that postmenopausal women with HR+ BC can respond better to tumor treatment with NACT than NET. Although neoadjuvant chemoendocrine therapy has improved prognostic outcomes compared to NET or NACT alone, such benefits may not be observed in this specific group of patients. Similar to other findings by Ergun et al. (32), the combination of NACT and NET leads to an increased ORR in patients with BC without a significant increase in SAE.

Additionally, Yuan et al. (21) reported that the effectiveness of combining pyrotinib with chemotherapy is superior to combining lapatinib with chemotherapy in the treatment of BC but has more safety risks. However, Salvo et al. (34) concluded that there is a high risk of BC recurrence, particularly among node-positive patients. Approximately 1 in 6 women with node-positive HR+/HER2-early-stage BC who undergo NET experience recurrence or death within five years of starting the treatment.

Chemotherapy

Anthracyclines and taxanes are commonly used chemotherapeutic agents for early-stage BC, but recent studies have identified risks associated with these drugs. Additionally, the benefits of combining neoadjuvant and adjuvant chemotherapy with capecitabine are mentioned for improved outcomes. Zaheed et al. (31) reported that anthracyclines and taxanes are effective chemotherapeutic agents commonly used in treating early-stage BC, either before or after surgery. Schettini et al. (29) reported that nab-paclitaxel had the highest overall response rates, while capecitabine and eribulin had the highest PFS and OS rates, respectively. It was also proved by Huo et al. (12) that combining neoadjuvant and adjuvant chemotherapy with capecitabine significantly improved DFS and OS in early-stage triple-negative BC patients with tolerable adverse events.

Moreover, Zhang et al. (15) concluded that adding capecitabine to neoadjuvant therapy did not improve DFS but OS. Furthermore, the toxicity profile of capecitabine remained favorable, and no capecitabine-related deaths were reported in the included trials. Additionally, Kerr et al. (9) reported an increased risk of leukemia associated with taxanes, while the risk of heart disease and leukemia is associated with anthracyclines. Surov et al. (35) concluded that the pretreatment apparent diffusion coefficient alone cannot predict the response to NACT in BC.

Radiotherapy

Neoadjuvant and adjuvant radiotherapy options can reduce LRR in BC, but their impact on OS varies, while IORT carries a higher risk of tumor recurrence. Ahmed et al. (23) reported that BC treatment could involve neoadjuvant radiotherapy, which can streamline oncological treatment, provide chemosensitization to enhance pCR before definitive surgery and provide treatment alternatives to ER-positive patients who are less likely to respond to chemotherapy. Moreover, De Felice et al. (18) concluded that regional nodal irradiation could reduce the LRR rate and improve disease-free and distant-free survival rates but did not significantly differ in OS at ten years. It was also proved by Nikyar et al. (26) that adjuvant LRRT after NACT can significantly reduce the risk of LRR but does not provide any survival benefit regarding DFS or OS. According to the Kerr et al. (9) study, radiotherapy options for BC include whole breast, partial breast, tumor bed boost, regional nodes after BCS, and chest wall and regional nodes after mastectomy. The study also found that anthracycline chemotherapy and radiotherapy may increase overall non-breast-cancer mortality.

Additionally, the authors identified heart disease, lung cancer, and esophageal cancer as the main radiation risks, with the risk increasing with higher doses of radiation to the heart, lungs, and esophagus, respectively. Moreover, the authors recommended bisphosphonate therapy for BC treatment. Recht et al. (27) also concluded that PMRT reduces the risks of LRF, LRR, and BC mortality for tumor BC patients with one to three positive axillary nodes. Hickey et al. (36) reported that altered fraction size regimens of radiation therapy do not have a clinically meaningful effect on local recurrence, are associated with decreased acute toxicity, and do not seem to affect breast appearance, late toxicity, or patient-reported quality-of-life measures for selected women treated with BCS. Moreover, Zhang et al. (28) concluded that IORT has a significantly higher risk of IBTR than whole-breast external beam radiotherapy. Overall mortality does not differ significantly.

Patient's Outcome Regarding Adjuvant and Neoadjuvant Therapy

AIs as neoadjuvant have higher response rates, improved outcomes, and better BCS results compared to tamoxifen. Similarly, Spring et al. (11) and Li and Shao (24) studies reported higher efficacy of AIs than tamoxifen. It could be explained by the Kerr et al. (9) study, which proved using AIs led to a reduction in BC mortality or recurrence by 10–25% as compared to tamoxifen. Leal et al. (25) also reported a higher ORR of AIs than tamoxifen in postmenopausal patients.

Moreover, Charehbili et al. (19) reported that NHT's favorable toxicity profile makes it an optimal treatment for patients unfit for chemotherapy.

In HER2-positive BC patients undergoing neoadjuvant HER2-targeted therapy, trastuzumab, and LTC may be more effective than chemotherapy or LC in achieving pCR. Gunasekara et al. (13) reported that TC had significantly higher pCR rates than chemotherapy, and LTC had significantly higher pCR rates than TC and/or LC. According to adjuvant therapy, trastuzumab-containing chemotherapy combinations showed the best OS compared to other combinations. Shen et al. (14) reported that anthracycline-cyclophosphamide with concurrent trastuzumab resulted in better OS compared to anthracycline-cyclophosphamide, reducing the risk of disease progression or recurrence. The reason could be that Genuino et al. (30) and Pinto et al. (22) studies proved that combining trastuzumab with chemotherapy showed a significant reduction of one-third in the risks of death and relapse, leading to decreased recurrence rates. Moreover, Giordano et al. (16) reported the high efficacy of trastuzumab in improving PFS, DFS, and OS in patients with advanced HER2-positive BC. In HR-positive/HER2-negative BC patients, NACT significantly increases ORR without an increase in serious adverse events, as stated by Ergun et al. (32). Moreover, Wang et al. (17) reported that NACT had a better tumor response NET in postmenopausal HR-positive BC patients.

In early-stage BC, capecitabine-based treatments in neoadjuvant and adjuvant chemotherapy showed significantly improved DFS and OS. This could be explained by the Huo et al. (12) meta-analysis study that proved these findings. However, Zhang et al. (15) reported that adding capecitabine to standard neoadjuvant regimens in early BC did not improve DFS.

Additionally, Schettini et al. (29) reported that paclitaxel + bevacizumab had superior ORR than several poly-chemotherapy regimens like ixabepilone + capecitabine or capecitabine + bevacizumab.

The adjuvant LRRT can reduce the risk of LRR. Nikyar et al. (26) stated the same finding; however, no statistically significant difference was found in DFS and OS. Neo-adjuvant radiotherapy can result in variable pCR values and 5-year survival rates, as stated by Ahmed et al. (23). Additionally, it was proved by Recht et al. (27) and Zhang et al. (28) studies that PMRT reduces the risks of LRF and LRR, and IORT is associated with a higher risk of IBTR compared to whole-breast external beam radiotherapy.

The study conducted a comprehensive search across multiple databases, including Web of Sciences, Google Scholar, PubMed, Cochrane Library, and EMBASE, which captured relevant studies and minimized selection bias. It also conducted a risk of bias assessment using the CASP tool, which helped to assess the internal validity of the included studies and provided insights into the quality of the evidence. The studies included were for the early and late stages of BC using neoadjuvant and adjuvant treatments. The studies included meta-analysis studies, which provided more precise estimates of treatment effects. The study's limitation was that metastatic and recurrent BC were excluded. Future studies should consider including metastatic and recurrent BC patients. This would help evaluate the efficacy of radiotherapy in these specific populations. Additionally, future studies should aim for more extended follow-up periods to assess the long-term effects of radiotherapy on survival, recurrence rates, and treatment-related complications.

Based on a comprehensive systematic review, AIs, as neoadjuvant therapy, were the most effective ET with a high ORR and reduced BC mortality or recurrence. Regarding anti-human epidermal growth factor 2 therapy, combining Trastuzumab with chemotherapy was the optimal treatment in HER2-positive BC patients as neoadjuvant and adjuvant therapy, with a significant reduction of one-third in the risks of death and relapse, leading to decreased recurrence rates. Additionally, capecitabine-based treatments in neoadjuvant and adjuvant chemotherapy for early-stage cancer improved the DFS and OS in BC patients. Radiotherapy had a significant role in BC treatment by reducing LRR risk (adjuvant therapy), producing variable pCR rates and 5-year survival rates (neo-adjuvant therapy), and reducing LRF and recurrence (postmastectomy therapy). However, most treatments reduced BC mortality or recurrence rates; anthracycline, chemotherapy, and radiation led to a rise in non-BC deaths overall.

Authorship Contributions

Surgical and Medical Practices: A.S.Q., S.M.A., R.M.A., A.A.A.A., F.A.T., M.J.A., A.A.A., S.A.A., A.H.M.; Concept: A.S.Q., S.M.A., A.A.A.A., A.H.M.; Design: A.S.Q., S.M.A., R.M.A., A.H.M.; Data Collection or Processing: A.S.Q., A.A.A.A., F.A.T., M.J.A., A.A.A., S.A.A.; Analysis or Interpretation: A.S.Q., M.J.A., A.A.A., S.A.A.; Literature Search: A.S.Q., S.M.A., R.M.A., A.A.A.A., F.A.T.; Writing: A.S.Q., S.M.A., A.A.A.A., F.A.T., M.J.A., A.A.A., S.A.A., A.H.M.

Conflict of Interest: No conflict of interest declared by the author.

Financial Disclosure: The author declare that this study received no financial disclosure.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin 2021; 71: 7-33. (PMID: 33433946) [Crossref]
2. Nagini S. Breast Cancer: Current Molecular Therapeutic Targets and New Players. Anticancer Agents Med Chem 2017; 17: 152-163. (PMID: 27137076) [Crossref]
3. Azamjah N, Soltan-Zadeh Y, Zayeri F. Global Trend of Breast Cancer Mortality Rate: A 25-Year Study. Asian Pac J Cancer Prev 2019; 20: 2015-2020. (PMID: 31350959) [Crossref]
4. Shien T, Iwata H. Adjuvant and neoadjuvant therapy for breast cancer. Jpn J Clin Oncol 2020; 50: 225-229. (PMID: 32147701) [Crossref]
5. Rubens RD, Sexton S, Tong D, Winter PJ, Knight RK, Hayward JL. Combined chemotherapy and radiotherapy for locally advanced breast cancer. Eur J Cancer (1965) 1980; 16: 351-356. (PMID: 7371690) [Crossref]
6. Mougalian SS, Soulos PR, Killelea BK, Lannin DR, Abu-Khalaf MM, DiGiovanna MP, et al. Use of neoadjuvant chemotherapy for patients with stage I to III breast cancer in the United States. Cancer 2015; 121: 2544-2552. (PMID: 25902916) [Crossref]
7. Clough KB, Acosta-Marín V, Nos C, Alran S, Rouanet P, Garbay JR, et al. Rates of Neoadjuvant Chemotherapy and Oncoplastic Surgery for Breast

Cancer Surgery: A French National Survey. Ann Surg Oncol 2015; 22: 3504-3511. (PMID: 25665949) [Crossref]

8. Vugts G, Maaskant-Braat AJ, Nieuwenhuijzen GA, Roumen RM, Luiten EJ, Voogd AC. Patterns of Care in the Administration of Neo-adjuvant Chemotherapy for Breast Cancer. A Population-Based Study. Breast J 2016; 22: 316-321. (PMID: 26945566) [Crossref]
9. Kerr AJ, Dodwell D, McGale P, Holt F, Duane F, Mannu G, et al. Adjuvant and neoadjuvant breast cancer treatments: A systematic review of their effects on mortality. Cancer Treat Rev 2022; 105: 102375. (PMID: 35367784) [Crossref]
10. Asselain B, Barlow W, Bartlett J, Bergh J, Bergsten-Nordström E, Bliss J, et al. Long-term outcomes for neoadjuvant versus adjuvant Chemotherapy in early breast cancer: a meta-analysis of individual patient data from ten randomized trials. Lancet Oncol 2018; 19: 27-39. (PMID: 29242041) [Crossref]
11. Spring LM, Gupta A, Reynolds KL, Gadd MA, Ellisen LW, Isakoff SJ, et al. Neoadjuvant Endocrine Therapy for Estrogen Receptor-Positive Breast Cancer: A Systematic Review and Meta-analysis. JAMA Oncol 2016; 2: 1477-1486. (PMID: 27367583) [Crossref]
12. Huo X, Li J, Zhao F, Ren D, Ahmad R, Yuan X, et al. The role of capecitabine-based neoadjuvant and adjuvant chemotherapy in early-stage triple-negative breast cancer: a systematic review and meta-analysis. BMC Cancer 2021; 21: 78. (PMID: 33468087) [Crossref]
13. Gunasekara AD, Anothaisintawee T, Youngkong S, Ha NT, McKay GJ, Attia J, et al. Neoadjuvant Treatment with HER2-Targeted Therapies in HER2-Positive Breast Cancer: A Systematic Review and Network Meta-Analysis. Cancers (Basel) 2022; 14: 523. (PMID: 35158791) [Crossref]
14. Shen Y, Fujii T, Ueno NT, Tripathy D, Fu N, Zhou H, et al. Comparative efficacy of adjuvant trastuzumab-containing chemotherapies for patients with early HER2-positive primary breast cancer: a network meta-analysis. Breast Cancer Res Treat 2019; 173: 1-9. (PMID: 30242579) [Crossref]
15. Zhang ZC, Xu QN, Lin SL, Li XY. Capecitabine in Combination with Standard (Neo)Adjuvant Regimens in Early Breast Cancer: Survival Outcome from a Meta-Analysis of Randomized Controlled Trials. PLoS One 2016; 11: e0164663. (PMID: 27741288) [Crossref]
16. Giordano SH, Franzoi MA, Temin S, Anders CK, Chandarlapaty S, Crews JR, Kirshner JJ, et al. Systemic Therapy for Patients With Advanced Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer: ASCO Clinical Practice Guideline Update. J Clin Oncol 2018; 36: 2736-2740. (PMID: 29939838) [Crossref]
17. Wang Y, He L, Song Y, Wu Q, Wang H, Zhang B, et al. The tumour response of postmenopausal hormone receptor-positive breast cancers undergoing different types of neoadjuvant therapy: a meta-analysis. BMC Womens Health 2020; 20: 17. (PMID: 32005117) [Crossref]
18. De Felice F, Ranalli T, Musio D, Lisi R, Rea F, Caiazzo R, et al. Relation between Hypofractionated Radiotherapy, Toxicity and Outcome in Early Breast Cancer. Breast J 2017; 23: 563-568. (PMID: 28252236) [Crossref]
19. Charehbili A, Fontein DB, Kroep JR, Liefers GJ, Mieog JS, Nortier JW, et al. Neoadjuvant hormonal therapy for endocrine sensitive breast cancer: a systematic review. Cancer Treat Rev 2014; 40: 86-92. (PMID: 23891267) [Crossref]
20. Hong K, Yao L, Sheng X, Ye D, Guo Y. Neoadjuvant Therapy of Cyclin-Dependent Kinase 4/6 Inhibitors Combined with Endocrine Therapy in HR+/HER2- Breast Cancer: A Systematic Review and Meta-Analysis. Oncol Res Treat 2021; 44: 557-567. (PMID: 34515204) [Crossref]
21. Yuan Y, Liu X, Cai Y, Li W. Pyrotinib versus lapatinib therapy for HER2 positive metastatic breast cancer patients after first-line treatment failure: A meta-analysis and systematic review. PLoS One 2023; 18: e0279775. (PMID: 36602979) [Crossref]
22. Pinto AC, Ades F, de Azambuja E, Piccart-Gebhart M. Trastuzumab for patients with HER2 positive breast cancer: delivery, duration and combination therapies. Breast 2013; 22 Suppl 2: S152-S155. (PMID: 24074778) [Crossref]
23. Ahmed M, Jozsa F, Douek M. A systematic review of neo-adjuvant radiotherapy in the treatment of breast cancer. Ecancermedicalscience 2021; 15: 1175. (PMID: 33680089) [Crossref]
24. Li JJ, Shao ZM. Endocrine therapy as adjuvant or neoadjuvant therapy for breast cancer: selecting the best agents, the timing and duration of treatment. Chin Clin Oncol 2016; 5: 40. (PMID: 27164856) [Crossref]
25. Leal F, Liutti VT, dos Santos VC, de Figueiredo MA, Macedo LT, Junior JA, et al. Neoadjuvant endocrine therapy for resectable breast cancer: A systematic review and meta-analysis. Breast 2015; 24: 406-412. (PMID: 25858103) [Crossref]
26. Nikyar N, Tegnelius E, Valachis A. Adjuvant locoregional radiation therapy in breast cancer patients with pathologic complete response after neoadjuvant chemotherapy: A systematic review and meta-analysis. Clin Transl Radiat Oncol 2022; 33: 45-52. (PMID: 35036587) [Crossref]
27. Recht A, Comen EA, Fine RE, Fleming GF, Hardenbergh PH, Ho AY, et al. Postmastectomy Radiotherapy: An American Society of Clinical Oncology, American Society for Radiation Oncology, and Society of Surgical Oncology Focused Guideline Update. J Clin Oncol 2016; 34: 4431-4442. (PMID: 27646947) [Crossref]
28. Zhang L, Zhou Z, Mei X, Yang Z, Ma J, Chen X, et al. Intraoperative Radiotherapy Versus Whole-Breast External Beam Radiotherapy in Early-Stage Breast Cancer: A Systematic Review and Meta-Analysis. Medicine (Baltimore) 2015; 94: e1143. (PMID: 26166124) [Crossref]
29. Schettini F, Venturini S, Giuliano M, Lambertini M, Pinato DJ, Onesti CE, et al. Multiple Bayesian network meta-analyses to establish therapeutic algorithms for metastatic triple negative breast cancer. Cancer Treat Rev 2022; 111: 102468. (PMID: 36202026) [Crossref]
30. Genuino AJ, Chaikledkaew U, The DO, Reungwetwattana T, Thakkinstian A. Adjuvant trastuzumab regimen for HER2-positive early-stage breast cancer: a systematic review and meta-analysis. Expert Rev Clin Pharmacol 2019; 12: 815-824. (PMID: 31287333) [Crossref]
31. Zaheed M, Wilcken N, Willson ML, O'Connell DL, Goodwin A. Sequencing of anthracyclines and taxanes in neoadjuvant and adjuvant therapy for early breast cancer. Cochrane Database Syst Rev 2019; 2: CD012873. (PMID: 30776132) [Crossref]
32. Ergun Y, Ucar G, Akagündüz B. Efficacy and safety of concomitant chemo-endocrine therapy in neoadjuvant treatment of hormone-positive HER2-negative breast cancer: a systemic review and meta-analysis. Expert Opin Drug Saf 2023; 22: 313-322. (PMID: 37057733) [Crossref]
33. Pistelli M, Mora AD, Ballatore Z, Berardi R. Aromatase inhibitors in premenopausal women with breast cancer: the state of the art and future prospects. Curr Oncol 2018; 25: e168-e175. (PMID: 29719441) [Crossref]
34. Salvo EM, Ramirez AO, Cueto J, Law EH, Situ A, Cameron C, et al. Risk of recurrence among patients with HR-positive, HER2-negative, early breast cancer receiving adjuvant endocrine therapy: A systematic review and meta-analysis. Breast 2021; 57: 5-17. (PMID: 33677313) [Crossref]
35. Surov A, Wienke A, Meyer HJ. Pretreatment apparent diffusion coefficient does not predict therapy response to neoadjuvant chemotherapy in breast cancer. Breast 2020; 53: 59-67. (PMID: 32652460) [Crossref]
36. Hickey BE, James ML, Lehman M, Hider PN, Jeffery M, Francis DP, et al. Fraction size in radiation therapy for breast conservation in early breast cancer. Cochrane Database Syst Rev 2016; 7: CD003860. (PMID: 27425588) [Crossref]

Review

Eur J Breast Health 2024; 20(3): 167-177
DOI: 10.4274/ejbh.galenos.2024.2024-4-7

Midkine: A Cancer Biomarker Candidate and Innovative Therapeutic Approaches

Berna Yıldırım[1], Kudret Kulak[2], Ayhan Bilir[1]

[1]Department of Histology and Embryology, İstanbul Atlas University Faculty of Medicine, İstanbul, Turkey
[2]Department of Pediatrics, İstanbul Atlas University Faculty of Medicine, İstanbul, Turkey

ABSTRACT

Midkine (MDK) is a protein that contributes to both physiological and pathological processes. Several studies provide insight into the different roles of MDK in development, tissue repair, neural plasticity, and health and disease processes. This research further examined how MDK contributed to conditions, including neurological diseases, inflammation, and ischaemia. Furthermore, MDK overexpression has been reported in many kinds of cancer and MDK is recognized as a malignancy marker. MDK stimulates pro-tumor activity by regulating a number of signaling pathways, which increase cancer cell proliferation, survival, metastasis, and treatment resistance. However, studies have shown that MDK also functions as a molecule that regulates drug resistance. Several cancer therapy techniques have been suggested to modify MDK function, including antibody-based therapies, oligonucleotides, oncolytic viruses, and small compounds. Further research and experimentation will be required to establish the therapeutic relevance and efficacy of these treatments. This review focuses on the role of MDK in cancer biology, as well as its multiple different roles in health and disease processes.

Keywords: Midkine; MDK; cancer; therapeutic target

Cite this article as: Yıldırım B, Kulak K, Bilir A. Midkine: A Cancer Biomarker Candidate and Innovative Therapeutic Approaches. Eur J Breast Health 2024; 20(3): 167-177

Key Points

- Midkine (MDK) is a protein that functions in both physiological and pathological processes.
- The role of MDK in physiological processes such as development, tissue repair and neuronal plasticity and its association with disorders such as neurodegenerative disease, inflammation and ischaemia have been investigated by many studies.
- MDK overexpression has been reported in many cancer types and identified as a biomarker in malignancy.
- MDK promotes proliferation, survival, metastasis and treatment resistance of cancer cells by activating pro-tumoral processes by activating many signalling pathways.
- MDK induces tumour formation through angiogenesis.
- Research suggests that MDK also functions as a molecule that regulates drug resistance.
- Many approaches to cancer therapy propose to target MDK.
- MDK and the proliferation of cancer cells have been suppressed by various approaches such as antibody-based therapies, oligonucleotides, oncolytic viruses and small molecules.
- However, further studies and experiments are required to determine the therapeutic relevance and efficacy of these therapies.
- This review focuses on the effects of MDK on cancer biology and its numerous roles in health and disease processes.

Introduction

Introduction to Midkine (MDK)

The Midkine (*MDK*) gene, which has a retinoic acid (RA) receptor in its promoter, is a heparin-binding growth factor or cytokine (1). MDK, a 13-kDa cysteine-rich protein, is activated by retinoic acid and generated in large quantities throughout development, especially in the nervous system (2, 3). It is generally overexpressed in adult tissues after injury, disease, and healing (2). *MDK* gene expression in individuals who are healthy has been observed in a variety of organs, including the gastrointestinal system, kidney, spleen, lungs, and thyroid (4, 5). *MDK* expression in healthy tissues is usually low and many times lower than in malignant tissue (6-10). MDK promotes ligand-dependent receptor activation, leading to a biological response (11, 12). The *MDK* promoter region contains binding sites for Hypoxia-Inducible Factor 1-alpha (HIF-1α), which, together with the retinoic acid receptor, has been associated with increased *MDK* expression in several cancers (13, 14). MDK was discovered in mouse embryonal

Corresponding Author:
Ayhan Bilir; aybilir@gmail.com

Received: 06.05.2024
Accepted: 09.06.2024
Available Online Date: 01.07.2024

cancer cells in 1988, and its molecular function is in embryonic development regulation (15, 16). MDK expression in mice increased only during the middle stages of gestation (days 8–11), after which it significantly decreased (15, 16). Only the kidneys in 15-day-old embryos showed significant MDK expression levels (15, 16). In one study, once RA was applied to cells in the early phases of embryonal cancer cell development, MDK mRNA levels increased (15, 16). The MDK family contains two members, MDK itself and pleiotropin (PTN) (16-18). These two proteins have similar receptors and physical attributes, including the ability to bind heparin (18). There are a number of evolutionarily conserved DNA sequences between MDK and PTN (17). Human MDK and PTN have been found to share around 50% sequence identity (17, 19). MDK and PTN have biological activity in processes such as fibrinolysis, anti-apoptosis, mitogenesis, and angiogenesis (18) (Figure 1). These activities suggest that growth factors play a role in cancer development (18). The increased expression of MDK and PTN in human carcinomas supports their function in cancer (18). MDK also plays a role in the pathogenesis of specific inflammatory diseases, including renal failure and vascular restenosis following angioplasty (18).

Midkine-Associated Signaling Pathways

MDK receptors include integrins, neurogenic locus notch homolog protein 2 (Notch2), anaplastic lymphoma kinase, the low-density lipoprotein receptor gene (*LRP*), and receptor type tyrosine protein phosphatase zeta (PTP-ζ) (20-24). MDK-binding integrins form α6β1 and α4β1 heterodimers (21). Syndecanes, glipican-2, PG-M/versican, and neuroglycan C are a few such protein glycans that interact with MDK (6, 25-28). MDK consists of two domains and three antiparallel β-strands containing heparin-binding sites (3, 29, 30). This structure allows MDK to form molecular complexes with proteoglycans (3, 29, 30). MDK binding to sulfated glycosaminoglycans interacts with a large number of crucial receptors, initiating a variety of signaling pathways (3, 29, 30) (Figure 2). While MDK is involved in critical processes such as development, reproduction, repair, inflammation, innate immunity, blood pressure regulation, neurite outgrowth, and angiogenesis, it also plays an important role in cancer formation and progression by stimulating cellular activity (1, 3, 25).MDK expression has been shown to be regulated by several kinds of transcription factors (30). The hormone estradiol (E2) has been shown to increase MDK mRNA levels in lung cancer cells (30). The *MDK* gene promoter region contains hypoxia response elements (14, 30). Under hypoxic conditions, HIF-1α binds to the MDK promoter region, increasing expression (13, 14). This promotes the vascularization of pulmonary arteries, the development of vasculature, and cancer cell survival (14, 30). The promoter region of *MDK* has a functional nuclear factor kappa B (NF-κB) binding site (30, 31). This leads to MDK overexpression under inflammatory conditions (31). In prostate cancer, tumor necrosis factor-α activates the NF-κB pathway (Figure 2) (31). The SP1 specificity protein 1 (*SP1*) gene is essential for embryonic development and early postnatal life (32). *SP1* expression has been reported to be more significant in human glioma tissues than in normal tissue, and it interacts with MDK in tumor development and progression (30, 32). Thyroid transcription factor 1 (TTF-1) regulates lung parenchymal growth and gene expression (30, 33). TTF-1 binds to TTF regulatory elements located in the 5′ region of the *MDK* promoter (33). TTF-null mice's lungs showed no expression of MDK (33). Various transcription factors may regulate MDK in various tissues (30). MDK could stimulate tumor development by activating TGF-β receptors, Janus kinase/signal transducers and activators of transcription (STAT), and STAT3 (34). Mitogen-activated protein kinase (MAPK) pathways promote epithelial mesenchymal transition (EMT), which regulates cancer development and metastasis (29, 35). MDK induces EMT by interacting with β-catenin through WNT signaling and the estrogen receptor (ER) (13). MDK interactions with the MAPK/phosphoinositide 3 kinase (PI3K)/AKT pathway induce proliferation and angiogenesis (35, 36) (Figure 2). Finally, MDK may inhibit Caspase-3, which decreases apoptosis (36).

Role of Midkine in Inflammation

MDK is a growth factors known to regulate inflammation since it is associated with antibacterial proteins that stimulate the innate immune system (37). MDK expression increases during inflammatory processes, which leads to increased angiogenesis (38). MDK promotes

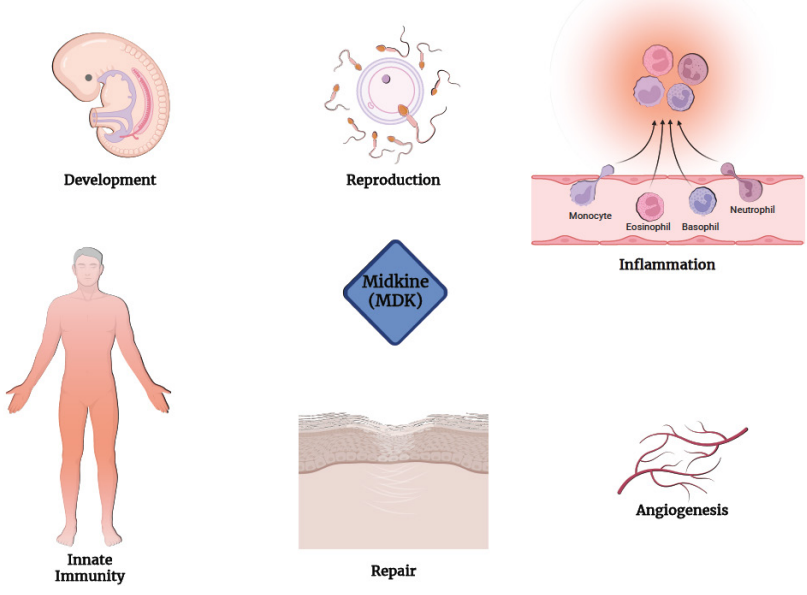


Figure 1. MDK plays a role in essential processes including as development, reproduction, repair, inflammation, innate immunity, and angiogenesis by activating various signaling pathways

*MDK: Midkine, *Created with Biorender.*

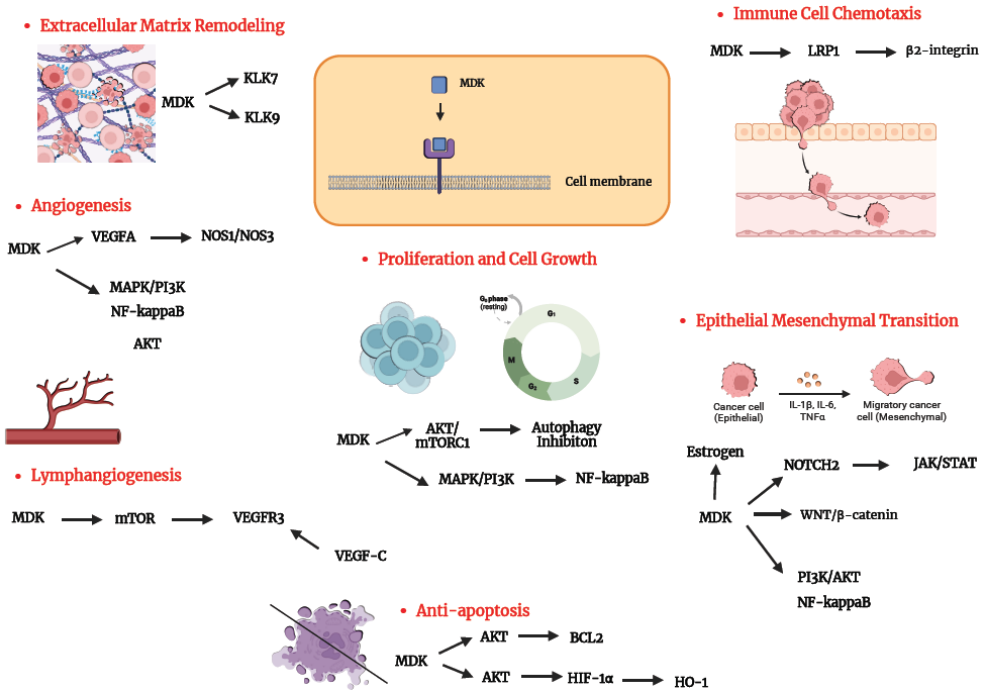


Figure 2. MDK is involved in cancer cell proliferation, survival, ECM remodeling anti-apoptosis, angiogenesis and EMT regulation through many different signalling pathways in tumor development

*MDK: Midkine; EMT: Epithelial mesenchymal transition, *Created with Biorender.*

neutrophil adhesion during angiogenesis (39). This happens through increasing the affinity of β2-integrins and suppressing LRP1 (13). It has been shown that in mice, the lack of MDK reduces neutrophil and macrophage numbers during the early stages of fracture healing (38, 40). Furthermore, the role of MDK in the inflammatory process has been attributed to its expression in endothelial cells under hypoxic conditions (14, 16). MDK is capable of maintaining tissue viability in adults after hypoxic stress (5, 25). MDK expression in tissues increases markedly following ischemia (41-45). In this case, increased MDK suppresses apoptosis and protects the tissue by decreasing cell death (5). Additionally, it promotes tissue repair through angiogenesis (46-48).Increased MDK during ischemia is characterized by an increase in blood MDK concentration (49). Serum MDK levels were shown to be significantly elevated in patients with heart failure than in the control group in studies on the treatment of the condition (49). MDK may induce neutrophil and macrophage migration to the injury site in renal ischemia-reperfusion damage, but it may decrease myocardial apoptosis in cardiac ischemia-reperfusion injury (21, 42, 50, 51). In endothelial cells, the PI3K/AKT and MAPK signaling pathways are critical for regulating vascular homeostasis and neovascularization (52, 53). AKT (protein kinase B) promotes the transcription of angiogenesis-related genes and refills tissue oxygen (54). Vascular endothelial growth factor alpha (VEGF-A) is a critical protein in the Chemokine (C-X-C motif) Ligand 1/Macrophage Inflammatory Protein 2-(CXCL1/MIP-2)-induced angiogenesis that interacts with VEGF receptor 2 (VEGFR2) (Figure 2) (16). Overexpression of MDK may affect the angiogenesis process by increasing VEGF-A levels and cellular release (Figure 2) (55, 56). These findings suggest that MDK plays an important role in angiogenesis and vascular homeostasis (16). A study suggested that MDK might be an effective therapeutic target for Th1 cell-induced autoimmune disorders such as experimental autoimmune encephalomyelitis (16). Moreover, it has been shown that MDK may promote the survival of mature B lymphocytes through an autocrine pathway (Figure 2) (16). The data suggest that MDK has multiple functions and has significant effects on many different pathophysiological processes.

Anti-Bacterial and Anti-Apoptotic Properties of Midkine

A recent study showed that MDK has significant anti-fungal and anti-bacterial effects (1, 16, 57-59). MDK contains a heparin-binding motif (Cardin-Weintraub Motif), which is a common feature of antibacterial proteins, and MDK has been proposed to have antibacterial activity by disrupting the bacterial plasma membrane, in addition to antibacterial properties, especially against gram-positive organisms (16, 57, 58). The anti-apoptotic activity of MDK has been associated with a number of pathological events, including cancer, neurogenesis, and tissue repair (16). MDK functions as an anti-apoptotic growth factor, which allows cancer cells to proliferate more efficiently (60, 61). For example, in developed G401 cells (derived from a rhabdoid tumor of the kidney), MDK has been shown to prevent apoptosis by increasing B-cell leukemia/lymphoma 2 (Bcl-2) protein expression (61). MDK is also known to suppress Caspase-3 activation through extracellular signal-regulated kinase (ERK) activation, which avoids neuronal death (62). In contrast, MDK has been shown to protect against cardiac ischemia-reperfusion (I/R) damage by increasing Bcl-2 and ERK levels, which inhibit cardiomyocyte apoptosis (42). MDK has been reported to decrease myocyte cell death by activating the PI3K/AKT signaling pathway (Figure 2) (63). MDK and Bcl-2 collaborate to suppress apoptosis, and Bcl-2 and ERK play critical roles in MDK's anti-apoptotic activity (16).

Midkine in Oxidative Stress and Cholesterol

Reactive oxygen species may cause oxidative stress due to an imbalance of pro- and anti-oxidants (64). One study found that following 5/6

nephrectomy, MDK expression increased in tubular epithelial cells and infiltrating macrophages in the kidneys of MDK+/+ mice (65). In addition, oxidative stress increased MDK expression in capillaries, lung endothelium, and alveolar-capillary endothelial cells (65). Furthermore, it was demonstrated that MDK expression increased when angiotensin I (Ang I) was converted to Ang II by angiotensin-converting enzyme (65).

MDK is expressed at extremely low levels in healthy arteries and veins (66). However, a recent study suggests an association between high blood MDK levels and serum total and low-density lipoprotein cholesterol levels (67). In apolipoprotein E (*ApoE*)-/- mice, MDK treatment has been shown to improve atherosclerotic plaque development (68). These findings suggest that MDK may have a role in the development of atherosclerosis (16, 68).

CNS and Midkine

The role of MDK in the central nervous system (CNS) has been studied extensively during development and in conditions such as traumatic brain injury (TBI) (21). MDK is expressed in the CNS during development and until mid-pregnancy, after which its mRNA levels decrease (29, 69). In mice, oligodendrocyte precursor cells express MDK before fetal astrocytes, neurons, and newly formed oligodendrocytes develop (69). In humans, fetal astrocytes are the main source of MDK in the CNS (69).

MDK is expressed early in cerebral infarction as well as in additional clinical conditions (43). MDK has been shown to function as a repairing neurotrophic factor in these situations, and its presence is recognized in damaged nerve regions (43). HIF-1α transcriptionally regulates MDK, a repair mechanism in hypoxia-induced diseases (21). Animal studies suggest that MDK mRNA and protein levels increase after short-term forebrain damage, but MDK expression increases in areas of traumatic spinal cord injury (44, 70). These findings indicate that MDK plays an important role in tissue repair in conditions such as brain damage and traumatic spinal cord injuries (70).

Secondary damage in TBI is caused by primary tissue damage, leading to disruption of the blood-brain barrier, immune cell infiltration into the brain, and neuroinflammation (71). *In vivo* studies suggested that a decrease in MDK has no effect on astrogliosis after TBI (72). Astrogliosis occurs when astrocytes respond to CNS injury by changing their transcriptional expression (73). Microglia respond to CNS injury in a similar fashion (74). However, it has been suggested that MDK may contribute to that injury in TBI by allowing immune cells to pass through the CNS (75).

Circulating MDK levels have also been shown to be significantly higher in patients with Alzheimer disease than in healthy individuals (5). This shows that MDK might be utilized as a marker in neurological disorders like Alzheimer's disease. MDK's representation in the CNS may be characterized as having a complicated process that MDK functions significantly throughout developmental stages but also in pathological situations such as TBI (21). Targeting MDK might be an effective approach for treating CNS-associated neoplastic conditions such as glioblastomas (21). These findings suggest that serum MDK levels might be used to diagnose and monitor a wide range of diseases. However, further research is required to support these findings (5).

Midkine as a Marker with Multiple Effects in Cancer

MDK overexpression has been reported in at least 20 distinct malignancies (5). Overexpression of MDK protein within tumors is a common feature of malignancy. MDK activates pro-tumoral activities in numerous cancer types via several signaling pathways (4, 12). MDK levels are frequently much greater in cancer patients than in healthy persons, and MDK expression has been shown to rise in direct proportion to the severity of illness (13, 53, 76-78). When tumors are surgically excised, circulating MDK levels often fall before increasing again when the cancer recurs (13). As a result, circulating MDK levels may act as a diagnostic, prognostic, or therapeutic marker for cancer (5).

MDK promotes cancer through a variety of processes (1). These mechanisms include cancer cell proliferation, survival, anti-apoptosis, angiogenesis, and EMT regulation (1, 79). MDK initiatives are simplified by specific receptor binding, which activates well-known downstream signaling pathways associated with tumor development and metastasis, including MAPK, PI3K/AKT, and ERK1/2 (Figure 2) (1, 79).

MDK's efficiency in promoting tumor development derives from its ability to trigger tumor angiogenesis (76). MDK is an effective pro-angiogenic factor (80, 81). Multiple studies have shown that MDK promotes angiogenesis, which enhances tumor development (13). In cancer cell culture studies with MDK overexpression, *in vitro* proliferation of endothelial cells increased, which led to angiogenesis (13, 80). The enhanced tumor development following subcutaneous MDK injection into nude mice has been attributed to increased microvessel density (76). This shows that endothelial cells are proliferating in the tumor (76). Furthermore, significant MDK expression has been found in tumor endothelial cells in human neural tumor tissues (82). A previous study focused on the interaction between the Notch2 receptor and MDK in pancreatic ductal adenocarcinoma (PDAC) cells (83). Soluble MDK has been demonstrated to stimulate Notch2 and its downstream targets, HES-1 and NF-kB/RelA (83). This suggests that MDK may regulate both phases of carcinogenesis (18).

MDK promotes tumor development partially by improving the probability of metastasis formation (84). MDK has been predicted to mediate metastasis through mitogenic, pro-inflammatory, and angiogenic activities (84-86). MDK interacts with TGF-β pathway proteins, which are necessary for EMT (Figure 2) (87-89). Furthermore, MDK regulates cell survival and proliferation through activating the PI3K and ERK signaling pathways (60, 90). MDK's interaction with the WNT/β-Catenin pathway is a key regulatory mechanism in glioma growth (91). MDK expression is increased in glioma cells (13). The *MDK* proximal promoter has a T-cell factor/lymphoid enhancer factor (TCF/LEF) family binding site that interacts with β-Catenin (91).

MDK may promote metastasis through proteolytic enzyme networks (92, 93). Expanded human kallikrein (KLKs) play an important role in these networks (94). KLKs may stimulate cancer development through extracellular hydrolysis (95). MDK has been recognized as an important protein, especially for KLK7 and KLK9 (Figure 2) (95, 96). This suggests a potential role for the KLK7/9-MDK axis in cancer development and metastasis (95, 96). MDK promotes metastatic development in melanoma through the mammalian target

of rapamycin (mTOR)/VEGFR3 signaling pathway (97). MDK regulates the mTOR signaling pathway by interacting with heparan sulfate and lymphatic endothelial cells (97). These signals promote lymphangiogenesis and metastasis in the lymph nodes and lungs (97). Silencing MDK reduces lymphangiogenesis and metastasis (97). These data suggest that MDK regulates the mTOR signaling pathway in melanoma metastasis (13). In an *in vitro* study to determine the effects of MDK concentration on drug cytotoxicity, MDK's effect on cells in the tumor microenvironment was studied in an ovarian cancer cell line (98, 99). MDK has been shown to stimulate the AKT signaling pathway in ovarian cancer cells, reducing the cytotoxic effect of cisplatin, whereas inhibiting MDK increased cisplatin cytotoxicity (98). Another study examining the role of MDK in the interaction between stromal cells and tumor cells showed that cancer-associated fibroblasts (CAFs) contribute to increased MDK levels in tumors and that CAF-derived MDK may promote cisplatin resistance (100).

MDK and Breast Cancer

Breast cancer is the world's most prevalent malignant tumor (101). Metastases in distant locations are the leading cause of mortality in breast cancer patients (102, 103). In a study comparing gene expression levels in breast cancer, it was found that *MDK* gene expression increased in tumor tissues (104). Some research has focused on the function of MDK in breast cancer. Plasma MDK levels were measured in 111 patients with primary invasive breast cancer and 25 patients with distant metastatic breast cancer (105). The results demonstrated that plasma MDK levels were markedly elevated in breast cancer patients compared to healthy controls (105). Although the mechanism is uncertain, plasma MDK levels in primary invasive carcinoma are significantly associated with the menopausal state. MDK is a substantially more effective biomarker for breast cancer than CA15-3, CEA, and NCCST-439, especially for individuals with initial invasive cancer (105). Furthermore, the MDK combination diagnoses breast cancer at significantly higher rates than the combination of two conventional tumor markers (CA15-3/CEA, CA15-3/NCCST-439, or CEA/NCCST-439) (105). As a result, MDK may be as effective, if not more so, than conventional markers in diagnosing breast cancer (105). The upstream kinases LKB1, CAMKKβ, and TAK1 phosphorylate adenosine monophosphate protein kinase (AMPK) at the Thr172 site (106). Among these kinases, the serine/threonine kinase, LKB1, regulates the conventional AMPK activation pathway, which has been clarified in cancer cells (107). It is well-established that LKB1 forms a heterotrimer with the pseudokinase STRAD and the scaffolding protein MO25 prior to self-phosphorylating at a number of amino acids to activate its own kinase activity (108). A recent study found that altering the LKB1-STRAD-MO25 complex reduced AMPKα Thr172 phosphorylation levels and AMPK activity (109-111). In another study, intracellular MDK suppressed AMPK activation by interacting with LKB1 and STRAD to depolymerise the LKB1-STRAD-MO25 complex, decreasing LKB1 activity and phosphorylation of AMPKα (112). Reducing or maintaining extracellular MDK expression caused enhanced AMPKα phosphorylation (108). Furthermore, MDK has been shown to increase cancer cell proliferation by inhibiting the LKB1-AMPK pathway, which proved to be negatively associated with LKB1/AMPK signaling pathway activity (Figure 3) (108). The treatment of locally advanced breast cancer is a combination of neoadjuvant chemotherapy (NCT), surgery, and adjuvant systemic and local treatments (113, 114). NCT increases the probability of breast-conserving surgery by minimizing the breast lesion, monitoring drug resistance, and determining prognosis and micrometastases (115). However, more than 80% of patients who get NCT do not respond effectively, causing risks that the treatment might delay surgery and drug resistance (116). As a result, early diagnosis of treatment response and resistance to chemotherapy may enhance the efficacy of NCT (117). In one study it was demonstrated that liquid biopsies, which are less invasive and less costly, were preferred, and that high levels of miR-1275 in plasma increased in response to NCT. However, reduced miR-1275 levels regulated chemoresistance in cancer stem cells by inhibiting the MDK/AKT pathway (Figure 3) (117).

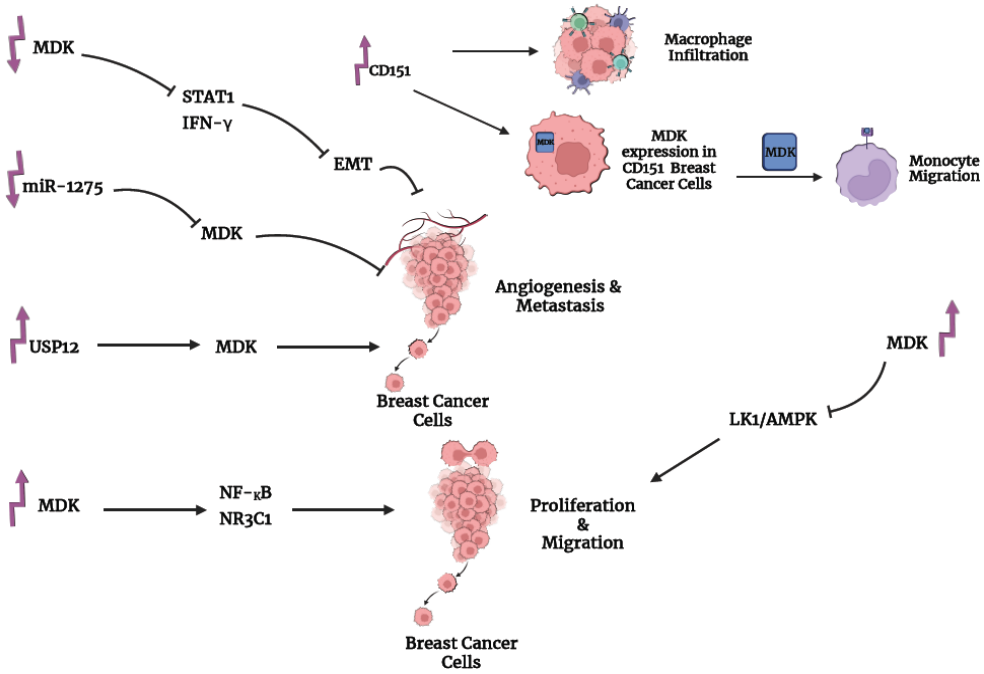


Figure 3. MDK modulates breast cancer through a variety of signaling mechanisms

*MDK: Midkine, *Created with Biorender.*

Interferons (IFNs) are a type of cytokine with antiviral, antiproliferative, and immunomodulatory properties (118). These cytokines play crucial roles in immune surveillance against cancer cells (119). IFN-γ increases anti-tumor immunity by directly targeting cancer cells (118, 120). Although IFN-γ has anti-tumor capabilities, it has been linked to an increased risk of metastasis in triple-negative breast cancer (TNBC) (121). A study showed that increasing IFN-γ levels increased MDK levels in TNBC cells (122). IFN-γ stimulates STAT1, promoting downstream signaling (123). The study demonstrated that reducing STAT1 decreased IFN-γ-induced MDK activation across all cancer cell lines. The use of midkine inhibitor (iMDK) (124), a small molecule for therapeutic use, reduced MDK levels and IFN-γ-induced EMT activation in cells (Figure 3) (122). Inhibiting MDK can inhibit IFN-γ-induced cancer migration and metastases (122).

Deubiquitination, mediated by multiple deubiquitinases (DUBs), regulates substrate protein levels by cleaving ubiquitin chains and is involved in many kinds of physiological processes (125, 126). DUBs have been found to play an important role in extracellular matrix degradation (127), epithelial-mesenchymal transition (128), angiogenesis (129, 130), and circulating tumor cell behavior (131). However, the role and mechanisms of DUBs in breast cancer metastasis are not established (103). In contrast, the ubiquitin regulator, ubiquitin specific protease 12 (USP12) is a member of the USP family that dehydrogenates and has been related to breast cancer (103). One study demonstrated that USP12 induced angiogenesis and metastasis by dehydrogenating and stabilizing the MDK protein (Figure 3) (103).

The immunological microenvironment in inflammatory breast cancer (IBC) is still undetermined, although one study demonstrated an interaction between the expression of the tetraspanin protein, CD151, and increased macrophage accumulation in malignant regions (132). It was established that higher CD151 expression and the amount of macrophages inhabiting the tumor were associated with a better response to chemotherapy in patients (132). IBC cells attract monocytes by many pathways, including CD151, MDK, integrin α6β1, and EV formation (Figure 3) (132).

NF-κB regulates several genes to enhance tumor cell proliferation, angiogenesis, differentiation, and metastasis across various cancer types (133). NR3C1, a member of the nuclear hormone receptor superfamily, encodes the glucocorticoid receptor (133). After binding to glucocorticoids, NR3C1 transfers from the cytoplasm to the nucleus and function as a transcription factor (134). In human cell lines, NR3C1/GR binds to the proximal RANKL promoter region, promoting RANKL transcription (135). High NR3C1/GR expression increased breast cancer growth and has a poor prognosis in TNBC and ER(-) subtypes (136, 137). A study showed NF-κB to be a crucial regulator, positively correlated with NR3C1 (138). Silencing MDK has been shown to inhibit breast cancer cell growth and migration (138). Transduction silencing of MDK inhibits the NF-κB pathway, resulting in reduced NR3C1 expression (138). MDK promotes breast cancer cell proliferation and migration by upregulating NR3C1 expression and activating the NF-κB pathway (Figure 3) (138).

Midkine Targeting

According to studies, MDK is a key regulator of drug resistance (15, 30). Previous studies have demonstrated that MDK protects cancer cells against cannabinoid and doxorubicin treatments and MDK expression was shown to increase the effects of chemotherapeutic drugs on lymphoblastic leukemia cells (139-142). In addition, drug-resistant gastric cancer cells were shown to express more MDK than drug-sensitive cells (143). Another study reported that decreased MDK expression enhances cisplatin resistance in oral squamous and renal carcinomas (144, 145). These findings show that MDK may produce either a drug-resistant or drug-sensitive cancer cell phenotype in different conditions (13).

MDK has been shown to be effective as a cancer biomarker at multiple stages, such as early disease identification, treatment response monitoring, and recurrence follow-up (5). Researchers are investigating ways to target MDK for cancer treatment since it plays a crucial role in tumor growth (30). An MDK antisense oligodeoxynucleotide was given to naked mice expressing rectal cancer cells, and tumor growth was significantly inhibited (146). Antisense oligonucleotides that target MDK effectively suppressed hepatocellular carcinoma (HCC) and increased its chemosensitivity to adriamycin (147).

Antibody-based therapeutics have been designed to target MDK (30). Monoclonal antibodies (mAbs) with high specificity for cell surface antigens are effective against cytotoxic pharmaceuticals (30). For example, anti-MDK mAbs combined with doxorubicin have been demonstrated to inhibit HCC proliferation (148). MDK-specific doxorubicin-conjugated single-chain variable fragments (scFv) showed similar characteristics (149). Another study showed that effective anti-MDK antibodies suppressed the growth of osteosarcoma cells (150).

In vitro viral therapy in pancreatic cancer cell lines is a different approach for treating peripheral tumors that express MDK (151). In this process, an oncolytic virus (adenovirus) containing part of the MDK promoter may eliminate tumor cells, and the process is carried out through tumor-selective replication (151). In contrast, siRNA or shRNA down-regulation of MDK has been shown to significantly inhibit PDAC growth (152). Another study found that knocking down MDK through siRNA increased cisplatin's anti-tumor activity in human gastric cancer cells (153). However, negatively charged siRNAs have limited pass-through into cells since they are not membrane-permeable and are swiftly eliminated by the kidneys due to their small size (154). They are also vulnerable to enzymatic degradation by serum endonucleases and RNAases, which may have a negative effect on their systemic distribution (154).

Metformin is utilized to treat Type II diabetes and is now being investigated as a potential anticancer drug since it affects MDK activity (30, 155). Metformin has been reported to beneficially interfere with the several MDK pathways that trigger cancer growth and its associated side effects (155). As a result, metformin may be effective as an MDK inhibitör (155).iMDK may decrease MDK expression while reducing cell growth and proliferation, possibly through blocking the PI3K signaling pathway (124). iMDK treatment of primary effusion lymphoma (PEL) cells led to cell cycle arrest in the G2/M phase in addition to a decrease in p-Cyclin-dependent kinase 1 levels (156). This might stimulate caspases, triggering PEL cell apoptosis (156). In studies of oral squamous cell carcinoma, iMDK treatment reduced CD31 expression, cell proliferation, and inhibited VEGF-induced angiogenesis (157).

Besides pharmaceuticals targeting MDK, there are many approaches for increasing MDK expression levels (21). It has been shown that cytotoxic T-cells with enhanced MDK expression may lyse tumor cells, suggesting that MDK may have potential for cancer vaccine development (13, 21). Another study used MDK RNA aptamers to activate T regulatory cells, showing that autoimmune diseases may be

prevented (158). These findings suggest that MDK-targeted treatments may be effective in inhibiting cancer formation and reducing drug resistance (30).

Discussion and Conclusion

This review discusses the effects of the MDK in cancer biology, as well as additional functions for MDK in health and disease processes. The crucial role of MDK in physiological processes, such as development, tissue repair, and neuronal plasticity, as well as its association with diseases such as neurodegenerative disorders, inflammation, and ischemia, are explained in detail. MDK's neuroprotective effects, impact on tissue regeneration, and potential effects to regulate inflammatory processes contribute to its biological importance. In this regard, a better understanding of MDK's cellular and molecular functions might lead to the development of innovative approaches for managing and treating a number of medical conditions. As a result, it is important to do more studies on MDK's role in diseases and health issues.

However, MDK's effects on cancer cell proliferation, survival, metastasis, and drug resistance have been studied. Various techniques and therapeutic approaches involving the use of MDK as a target for cancer therapy are also discussed. The approaches described include antibody-based therapy, small chemical inhibitors such as iMDK, siRNA, and RNA Aptamers. The published evidence concerning MDK's effects on cancer cell characteristics and its potential effects on cancer therapy shows that MDK plays an important biological role and that targeting it in cancer therapy has significant potential.A comprehensive understanding of the contribution of MDK to cancer biology could help in the development of innovative cancer therapies, as well as more effective cancer-fighting approaches. However, further study is required to identify these innovative approaches to cancer treatment and controlling the disease.

Authorship Contributions

Concept: A.B.; Data Collection or Processing: B.Y., A.B.; Analysis or Interpretation: A.B.; Literature Search: B.Y., K.K.; Writing: B.Y., K.K., A.B.

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

Financial Disclosure: The authors declare that this study received no financial disclosure.

References

1. Muramatsu T. Structure and function of midkine as the basis of its pharmacological effects. Br J Pharmacol 2014; 171: 814-826. (PMID: 23992440) [Crossref]
2. Winkler C, Yao S. The midkine family of growth factors: diverse roles in nervous system formation and maintenance. Br J Pharmacol 2014; 171: 905-912. (PMID: 24125182) [Crossref]
3. Christou C, Stylianou A, Gkretsi V. Midkine (MDK) in Hepatocellular Carcinoma: More than a Biomarker. Cells 2024; 13: 136. (PMID: 38247828) [Crossref]
4. Tsutsui J, Kadomatsu K, Matsubara S, Nakagawara A, Hamanoue M, Takao S, et al. A new family of heparin-binding growth/differentiation factors: increased midkine expression in Wilms' tumor and other human carcinomas. Cancer Res 1993; 53: 1281-1285. (PMID: 8383007) [Crossref]
5. Jones DR. Measuring midkine: the utility of midkine as a biomarker in cancer and other diseases. Br J Pharmacol 2014; 171: 2925-2939. (PMID: 24460734) [Crossref]
6. Ye C, Qi M, Fan QW, Ito K, Akiyama S, Kasai Y, et al. Expression of midkine in the early stage of carcinogenesis in human colorectal cancer. Br J Cancer 1999; 79: 179-184. (PMID: 10408712) [Crossref]
7. Moon HS, Park WI, Sung SH, Choi EA h, Chung HW, Woo BH. Immunohistochemical and quantitative competitive PCR analyses of midkine and pleiotrophin expression in cervical cancer. Gynecol Oncol 2003; 88: 289-297. (PMID: 12648577) [Crossref]
8. Huang Y, Cao G, Wang H, Wang Q, Hou Y. The expression and location of midkine in gastric carcinomas of Chinese patients. Cell Mol Immunol 2007; 4: 135-140. (PMID: 17484808) [Crossref]
9. Jia HL, Ye QH, Qin LX, Budhu A, Forgues M, Chen Y, et al. Gene Expression Profiling Reveals Potential Biomarkers of Human Hepatocellular Carcinoma. Clin Cancer Res 2007; 13: 1133-1139. (PMID: 17317821) [Crossref]
10. O'Brien T, Cranston D, Fuggle S, Bicknell R, Harris AL. The angiogenic factor midkine is expressed in bladder cancer, and overexpression correlates with a poor outcome in patients with invasive cancers. Cancer Res 1996; 56: 2515-2518. (PMID: 8653688) [Crossref]
11. Pedraza RC, Matsubara S, Muramatsu T. A Retinoic Acid-Responsive Element in Human Midkine Gene1. J Biochem 1995; 117: 845-849. (PMID: 7592548) [Crossref]
12. Kadomatsu K, Muramatsu T. Midkine and pleiotrophin in neural development and cancer. Cancer Lett 2004; 204: 127-143. (PMID: 15013213) [Crossref]
13. Filippou PS, Karagiannis GS, Constantinidou A. Midkine (MDK) growth factor: a key player in cancer progression and a promising therapeutic target. Oncogene 2020; 39: 2040-2054. (PMID: 31801970) [Crossref]
14. Reynolds PR, Mucenski ML, Le Cras TD, Nichols WC, Whitsett JA. Midkine Is Regulated by Hypoxia and Causes Pulmonary Vascular Remodeling. J Biol Chem 2004; 279: 37124-37132. (PMID: 15197188) [Crossref]
15. Kadomatsu K, Tomomura M, Muramatsu T. cDNA cloning and sequencing of a new gene intensely expressed in early differentiation stages of embryonal carcinoma cells and in mid-gestation period of mouse embryogenesis. Biochem Biophys Res Commun 1988; 151: 1312-1318. (PMID: 3355557) [Crossref]
16. Cai YQ, Lv Y, Mo ZC, Lei J, Zhu JL, Zhong QQ. Multiple pathophysiological roles of midkine in human disease. Cytokine 2020; 135: 155242. (PMID: 32799009) [Crossref]
17. Li YS, Milner PG, Chauhan AK, Watson MA, Hoffman RM, Kodner CM, et al. Cloning and expression of a developmentally regulated protein that induces mitogenic and neurite outgrowth activity. Science 1990; 250: 1690-1694. (PMID: 2270483) [Crossref]
18. Kadomatsu K. The midkine family in cancer, inflammation and neural development. Nagoya J Med Sci 2005; 67: 71-82. (PMID: 17375473) [Crossref]
19. Uehara K, Matsubara S, Kadomatsu K, Tsutsui J, Muramatsu T. Genomic structure of human midkine (MK), a retinoic acid-responsive growth/differentiation factor. J Biochem 1992; 111: 563-567. (PMID: 1639750) [Crossref]
20. Maeda N, Ichihara-Tanaka K, Kimura T, Kadomatsu K, Muramatsu T, Noda M. A Receptor-like Protein-tyrosine Phosphatase PTPζ/RPTPβ Binds a Heparin-binding Growth Factor Midkine. J Biol Chem 1999; 274: 12474-12479. (PMID: 10212223) [Crossref]
21. Neumaier EE, Rothhammer V, Linnerbauer M. The role of midkine in health and disease. Front Immunol 2023; 14: 1310094. (PMID: 38098484) [Crossref]
22. Huang Y, Hoque MO, Wu F, Trink B, Sidransky D, Ratovitski EA. Midkine induces epithelial-mesenchymal transition through Notch2/Jak2-Stat3 signaling in human keratinocytes. Cell Cycle 2008; 7: 1613-1622. (PMID: 18469519) [Crossref]
23. Stoica GE, Kuo A, Powers C, Bowden ET, Sale EB, Riegel AT, et al. Midkine Binds to Anaplastic Lymphoma Kinase (ALK) and Acts as a

Growth Factor for Different Cell Types. J Biol Chem 2002; 277: 35990-35998. (PMID: 12122009) [Crossref]

24. Muramatsu H, Zou K, Sakaguchi N, Ikematsu S, Sakuma S, Muramatsu T. LDL Receptor-Related Protein as a Component of the Midkine Receptor. Biochem Biophys Res Commun 2000; 270: 936-941. (PMID: 10772929) [Crossref]
25. Muramatsu T. Midkine: A Promising Molecule for Drug Development to Treat Diseases of the Central Nervous System. Curr Pharm Des 2011; 17: 410-423. (PMID: 21375488) [Crossref]
26. Kurosawa N, Chen GY, Kadomatsu K, Ikematsu S, Sakuma S, Muramatsu T. Glypican-2 binds to midkine: the role of glypican-2 in neuronal cell adhesion and neurite outgrowth. Glycoconj J 2001; 18: 499-507. (PMID: 12084985) [Crossref]
27. Zou K, Muramatsu H, Ikematsu S, Sakuma S, Salama RHM, Shinomura T, et al. A heparin-binding growth factor, midkine, binds to a chondroitin sulfate proteoglycan, PG-M/versican. Eur J Biochem 2000; 267: 4046-4053. (PMID: 10866805) [Crossref]
28. Ichihara-Tanaka K, Oohira A, Rumsby M, Muramatsu T. Neuroglycan C Is a Novel Midkine Receptor Involved in Process Elongation of Oligodendroglial Precursor-like Cells. J Biol Chem 2006; 281: 30857-30864. (PMID: 16901907) [Crossref]
29. Muramatsu T. Midkine, a heparin-binding cytokine with multiple roles in development, repair and diseases. Proc Jpn Acad Ser B 2010; 86: 410-425. (PMID: 20431264) [Crossref]
30. Saikia M, Cheung N, Singh AK, Kapoor V. Role of Midkine in Cancer Drug Resistance: Regulators of Its Expression and Its Molecular Targeting. Int J Mol Sci 2023; 24: 8739. (PMID: 37240085) [Crossref]
31. You Z, Dong Y, Kong X, Beckett LA, Gandour-Edwards R, Melamed J. Midkine is a NF-κB-inducible gene that supports prostate cancer cell survival. BMC Med Genomics 2008; 1: 6. (PMID: 18275606) [Crossref]
32. Safe S, Imanirad P, Sreevalsan S, Nair V, Jutooru I. Transcription factor Sp1, also known as specificity protein 1 as a therapeutic target. Expert Opin Ther Targets 2014; 18: 759-769. (PMID: 24793594) [Crossref]
33. Reynolds PR, Mucenski ML, Whitsett JA. Thyroid transcription factor (TTF) -1 regulates the expression of midkine (MK) during lung morphogenesis. Dev Dyn 2003; 227: 227-237. (PMID: 12761850) [Crossref]
34. Bie C, Chen Y, Tang H, Li Q, Zhong L, Peng X, et al. Insulin-Like Growth Factor 1 Receptor Drives Hepatocellular Carcinoma Growth and Invasion by Activating Stat3-Midkine-Stat3 Loop. Dig Dis Sci 2022; 67: 569-584. (PMID: 33559791) [Crossref]
35. Gowhari Shabgah A, Ezzatifar F, Aravindhan S, Olegovna Zekiy A, Ahmadi M, Gheibihayat SM, et al. Shedding more light on the role of Midkine in hepatocellular carcinoma: New perspectives on diagnosis and therapy. IUBMB Life 2021; 73: 659-669. (PMID: 33625758) [Crossref]
36. Sun B, Hu C, Yang Z, Zhang X, Zhao L, Xiong J, et al. Midkine promotes hepatocellular carcinoma metastasis by elevating anoikis resistance of circulating tumor cells. Oncotarget 2017; 8: 32523-32535. (PMID: 28430645) [Crossref]
37. Fernández-Calle R, Vicente-Rodríguez M, Gramage E, De La Torre-Ortiz C, Pérez-García C, Ramos MP, et al. Endogenous pleiotrophin and midkine regulate LPS-induced glial responses. Neurosci Lett 2018; 662: 213-218. (PMID: 29061398) [Crossref]
38. Weckbach LT, Muramatsu T, Walzog B. Midkine in Inflammation. Sci World J 2011; 11: 2491-2505. (PMID: 22235180) [Crossref]
39. Tazzyman S, Lewis CE, Murdoch C. Neutrophils: key mediators of tumour angiogenesis. Int J Exp Pathol 2009; 90: 222-231. (PMID: 19563607) [Crossref]
40. Haffner-Luntzer M, Heilmann A, Rapp AE, Beie S, Schinke T, Amling M, et al. Midkine-Deficiency Delays Chondrogenesis during the Early Phase of Fracture Healing in Mice. Awad HA, editor. PLoS One 2014; 9: e116282. (PMID: 25551381) [Crossref]
41. Horiba M, Kadomatsu K, Nakamura E, Muramatsu H, Ikematsu S, Sakuma S, et al. Neointima formation in a restenosis model is suppressed in midkine-deficient mice. J Clin Invest 2000; 105: 489-495. (PMID: 10683378) [Crossref]
42. Horiba M, Kadomatsu K, Yasui K, Lee JK, Takenaka H, Sumida A, et al. Midkine Plays a Protective Role Against Cardiac Ischemia/Reperfusion Injury Through a Reduction of Apoptotic Reaction. Circulation 2006; 14: 1713-1720. (PMID: 17015789) [Crossref]
43. Yoshida Y, Goto M, Tsutsui J ichiro, Ozawa M, Sato E, Osame M, et al. Midkine is present in the early stage of cerebral infarct. Brain Res Dev Brain Res 1995; 85: 25-30. (PMID: 7781164) [Crossref]
44. Mochizuki R, Takeda A, Sato N, Kimpara T, Onodera H, Itoyama Y, et al. Induction of Midkine Expression in Reactive Astrocytes Following Rat Transient Forebrain Ischemia. Exp Neurol 1998; 149: 73-78. (PMID: 9454616) [Crossref]
45. Sato W, Kadomatsu K, Yuzawa Y, Muramatsu H, Hotta N, Matsuo S, et al. Midkine Is Involved in Neutrophil Infiltration into the Tubulointerstitium in Ischemic Renal Injury. J Immunol 2001; 167: 3463-3469. (PMID: 11544339) [Crossref]
46. Weckbach LT, Groesser L, Borgolte J, Pagel JI, Pogoda F, Schymeinsky J, et al. Midkine acts as proangiogenic cytokine in hypoxia-induced angiogenesis. Am J Physiol Heart Circ Physiol 2012; 303: H429-H438. (PMID: 22707563) [Crossref]
47. Fukui S, Kitagawa-Sakakida S, Kawamata S, Matsumiya G, Kawaguchi N, Matsuura N, et al. Therapeutic Effect of Midkine on Cardiac Remodeling in Infarcted Rat Hearts. Ann Thorac Surg 2008; 85: 562-570. (PMID: 18222265) [Crossref]
48. Takenaka H, Horiba M, Ishiguro H, Sumida A, Hojo M, Usui A, et al. Midkine prevents ventricular remodeling and improves long-term survival after myocardial infarction. Am J Physiol-Heart Circ Physiol 2009; 296: H462-469. (PMID: 19060126) [Crossref]
49. Kitahara T, Shishido T, Suzuki S, Katoh S, Sasaki T, Ishino M, et al. Serum Midkine as a Predictor of Cardiac Events in Patients With Chronic Heart Failure. J Card Fail 2010; 16: 308-133. (PMID: 20350697) [Crossref]
50. Li X, Ma N, Xu J, Zhang Y, Yang P, Su X, et al. Targeting Ferroptosis: Pathological Mechanism and Treatment of Ischemia-Reperfusion Injury. Jiang DS, editor. Oxid Med Cell Longev 2021; 2021: 1587922. (PMID: 34745412) [Crossref]
51. Sato W, Sato Y. Midkine in nephrogenesis, hypertension and kidney diseases. Br J Pharmacol 2014; 171: 879-887. (PMID: 24106831) [Crossref]
52. Shiojima I, Walsh K. Role of Akt signaling in vascular homeostasis and angiogenesis. Circ Res 2002; 90: 1243-1250. (PMID: 12089061) [Crossref]
53. Qi M, Ikematsu S, Maeda N, Ichihara-Tanaka K, Sakuma S, Noda M, et al. Haptotactic Migration Induced by Midkine. J Biol Chem 2001; 276: 15868-15875. (PMID: 11340082) [Crossref]
54. Sluimer JC, Daemen MJ. Novel concepts in atherogenesis: angiogenesis and hypoxia in atherosclerosis. J Pathol 2009; 218: 7-29. (PMID: 19309025) [Crossref]
55. Scapini P, Morini M, Tecchio C, Minghelli S, Di Carlo E, Tanghetti E, et al. CXCL1/Macrophage Inflammatory Protein-2-Induced Angiogenesis In Vivo Is Mediated by Neutrophil-Derived Vascular Endothelial Growth Factor-A. J Immunol 2004; 172: 5034-5040. (PMID: 15067085) [Crossref]
56. Zhao SL, Zhang YJ, Li MH, Zhang XL, Chen SL. Mesenchymal stem cells with overexpression of midkine enhance cell survival and attenuate

cardiac dysfunction in a rat model of myocardial infarction. Stem Cell Res Ther 2014; 5: 37. (PMID: 24635859) [Crossref]

57. Andersson E, Rydengård V, Sonesson A, Mörgelin M, Björck L, Schmidtchen A. Antimicrobial activities of heparin-binding peptides. Eur J Biochem 2004; 271: 1219-1226. (PMID: 15009200) [Crossref]
58. Svensson SL, Pasupuleti M, Walse B, Malmsten M, Mörgelin M, Sjögren C, et al. Midkine and Pleiotrophin Have Bactericidal Properties: preserved antibacterial activity in a family of heparin-binding growth factors during evolution. J Biol Chem 2010; 285: 16105-16115. (PMID: 20308059) [Crossref]
59. Englund C, Birve A, Falileeva L, Grabbe C, Palmer RH. Miple1 and miple2 encode a family of MK/PTN homologues in Drosophila melanogaster. Dev Genes Evol 2006; 216: 10-18. (PMID: 16220264) [Crossref]
60. Ohuchida T, Okamoto K, Akahane K, Higure A, Todoroki H, Abe Y, et al. Midkine protects hepatocellular carcinoma cells against TRAIL-mediated apoptosis through down-regulation of caspase-3 activity. Cancer 2004; 100: 2430-2436. (PMID: 15160348) [Crossref]
61. Qi M, Ikematsu S, Icbihara-Tanaka K, Sakuma S, Muramatsu T, Kadomatsu K. Midkine Rescues Wilms, Tumor Cells from Cisplatin-Induced Apoptosis: Regulation of Bcl-2 Expression by Midkine. J Biochem 2000; 127: 269-277. (PMID: 10731694) [Crossref]
62. Owada K, Sanjo N, Kobayashi T, Mizusawa H, Muramatsu H, Muramatsu T, et al. Midkine inhibits caspase-dependent apoptosis via the activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase in cultured neurons. J Neurochem 1999; 73: 2084-2092. (PMID: 10537068) [Crossref]
63. Harada M, Hojo M, Kamiya K, Kadomatsu K, Murohara T, Kodama I, et al. Exogenous midkine administration prevents cardiac remodeling in pacing-induced congestive heart failure of rabbits. Heart Vessels 2016; 31: 96-104. (PMID: 25155308) [Crossref]
64. Touyz RM. Reactive oxygen species in vascular biology: role in arterial hypertension. Expert Rev Cardiovasc Ther 2003; 1: 91-106. (PMID: 15030300) [Crossref]
65. Hobo A, Yuzawa Y, Kosugi T, Kato N, Asai N, Sato W, et al. The growth factor midkine regulates the renin-angiotensin system in mice. J Clin Invest 2009; 119: 1616-1625. (PMID: 19451697) [Crossref]
66. Ito F. Polyphenols can Potentially Prevent Atherosclerosis and Cardiovascular Disease by Modulating Macrophage Cholesterol Metabolism. Curr Mol Pharmacol 2020; 14: 175-190. (PMID: 32196455) [Crossref]
67. Guzel S, S Cinemre F, Guzel E, Kucukyalcin V, Kiziler A, Cavusoglu C, et al. Midkine levels and its relationship with atherosclerotic risk factors in essential hypertensive patients. Niger J Clin Pract 2018; 21: 894-900. (PMID: 29984722) [Crossref]
68. Takemoto Y, Horiba M, Harada M, Sakamoto K, Takeshita K, Murohara T, et al. Midkine Promotes Atherosclerotic Plaque Formation Through Its Pro-Inflammatory, Angiogenic and Anti-Apoptotic Functions in Apolipoprotein E-Knockout Mice. Circ J 2018; 82: 19-27. (PMID: 28781288) [Crossref]
69. Ross-Munro E, Kwa F, Kreiner J, Khore M, Miller SL, Tolcos M, et al. Midkine: The Who, What, Where, and When of a Promising Neurotrophic Therapy for Perinatal Brain Injury. Front Neurol 2020; 11: 568814. (PMID: 33193008) [Crossref]
70. Sakakima H, Yoshida Y, Muramatsu T, Yone K, Goto M, Ijiri K, et al. Traumatic Injury-Induced Midkine Expression in the Adult Rat Spinal Cord during the Early Stage. J Neurotrauma 2004; 21: 471-477. (PMID: 15115596) [Crossref]
71. Werner C, Engelhard K. Pathophysiology of traumatic brain injury. Br J Anaesth 2007; 99: 4-9. (PMID: 17573392) [Crossref]
72. Takada S, Sakakima H, Matsuyama T, Otsuka S, Nakanishi K, Norimatsu K, et al. Disruption of Midkine gene reduces traumatic brain injury through the modulation of neuroinflammation. J Neuroinflammation 2020; 17: 40. (PMID: 31996236) [Crossref]
73. Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, et al. Genomic Analysis of Reactive Astrogliosis. J Neurosci 2012; 32: 6391-6410. (PMID: 22553043) [Crossref]
74. Streit WJ, Walter SA, Pennell NA. Reactive microgliosis. Prog Neurobiol 1999; 57: 563-581. (PMID: 10221782) [Crossref]
75. Shlosberg D, Benifla M, Kaufer D, Friedman A. Blood–brain barrier breakdown as a therapeutic target in traumatic brain injury. Nat Rev Neurol 2010; 6: 393-403. (PMID: 20551947) [Crossref]
76. Choudhuri R, Zhang HT, Donnini S, Ziche M, Bicknell R. An angiogenic role for the neurokines midkine and pleiotrophin in tumorigenesis. Cancer Res 1997; 57: 1814-1819. (PMID: 9135027) [Crossref]
77. Muramatsu H, Shirahama H, Yonezawa S, Maruta H, Muramatsu T. Midkine, A Retinoic Acid-Inducible Growth/Differentiation Factor: Immunochemical Evidence for the Function and Distribution. Dev Biol 1993; 159: 392-402. (PMID: 8405666) [Crossref]
78. Owada K, Sanjyo N, Kobayashi T, Kamata T, Mizusawa H, Muramatsu H, et al. Midkine inhibits apoptosis via extracellular signal regulated kinase (ERK) activation in PC12 cells. J Med Dent Sci 1999; 46: 45-51. (PMID: 12160213) [Crossref]
79. Kadomatsu K, Kishida S, Tsubota S. The heparin-binding growth factor midkine: the biological activities and candidate receptors. J Biochem 2013; 153: 511-521. (PMID: 23625998) [Crossref]
80. Muramaki M, Miyake H, Hara I, Kamidono S. Introduction of midkine gene into human bladder cancer cells enhances their malignant phenotype but increases their sensitivity to antiangiogenic therapy. Clin Cancer Res Off J Am Assoc Cancer Res 2003; 9: 5152-5160. (PMID: 14613994) [Crossref]
81. Gustavsson H, Jennbacken K, Welén K, Damber J. Altered expression of genes regulating angiogenesis in experimental androgen-independent prostate cancer. Prostate 2008; 68: 161-170. (PMID: 18076023) [Crossref]
82. Mashour GA, Ratner N, Khan GA, Wang HL, Martuza RL, Kurtz A. The angiogenic factor midkine is aberrantly expressed in NF1-deficient Schwann cells and is a mitogen for neurofibroma-derived cells. Oncogene 2001; 20: 97-105. (PMID: 11244508) [Crossref]
83. Güngör C, Zander H, Effenberger KE, Vashist YK, Kalinina T, Izbicki JR, et al. Notch Signaling Activated by Replication Stress–Induced Expression of Midkine Drives Epithelial–Mesenchymal Transition and Chemoresistance in Pancreatic Cancer. Cancer Res 2011; 71: 5009-5019. (PMID: 21632553) [Crossref]
84. Jono H, Ando Y. Midkine: A Novel Prognostic Biomarker for Cancer. Cancers (Basel) 2010; 2: 624-641. (PMID: 24281085) [Crossref]
85. Kishida S, Kadomatsu K. Involvement of midkine in neuroblastoma tumourigenesis. Br J Pharmacol 2014; 171: 896-904. (PMID: 24116381) [Crossref]
86. Muramatsu T, Kadomatsu K. Midkine: an emerging target of drug development for treatment of multiple diseases. Br J Pharmacol 2014; 171: 811-813. (PMID: 24460672) [Crossref]
87. Papageorgis P. TGFβ Signaling in Tumor Initiation, Epithelial-to-Mesenchymal Transition, and Metastasis. J Oncol 2015; 2015: 587193. (PMID: 25883652) [Crossref]
88. Grupp K, Melling N, Bogoevska V, Reeh M, Uzunoglu FG, El Gammal AT, et al. Expression of ICAM-1, E-cadherin, periostin and midkine in metastases of pancreatic ductal adenocarcinomas. Exp Mol Pathol 2018; 104: 109-113. (PMID: 29355490) [Crossref]
89. Katsuno Y, Lamouille S, Derynck R. TGF-β signaling and epithelial–mesenchymal transition in cancer progression. Curr Opin Oncol 2013; 25: 76-84. (PMID: 23197193) [Crossref]
90. Sandra F, Harada H, Nakamura N, Ohishi M. Midkine induced growth of ameloblastoma through MAPK and Akt pathways. Oral Oncol 2004; 40: 274-280. (PMID: 14747058) [Crossref]

91. Tang SL, Gao YL, Chen XB. Wnt/β-catenin up-regulates Midkine expression in glioma cells. Int J Clin Exp Med 2015; 8: 12644-12649. (PMID: 26550177) [Crossref]
92. Yu Y, Prassas I, Dimitromanolakis A, Diamandis EP. Novel Biological Substrates of Human Kallikrein 7 Identified through Degradomics. J Biol Chem 2015; 290: 17762-17775. (PMID: 26032414) [Crossref]
93. Filippou PS, Farkona S, Brinc D, Yu Y, Prassas I, Diamandis EP. Biochemical and functional characterization of the human tissue kallikrein 9. Biochem J 2017; 474: 2417-2433. (PMID: 28559305) [Crossref]
94. Filippou PS, Karagiannis GS, Musrap N, Diamandis EP. Kallikrein-related peptidases (KLKs) and the hallmarks of cancer. Crit Rev Clin Lab Sci 2016; 53: 277-291. (PMID: 26886390) [Crossref]
95. Haddada M, Draoui H, Deschamps L, Walker F, Delaunay T, Brattsand M, et al. Kallikrein-related peptidase 7 overexpression in melanoma cells modulates cell adhesion leading to a malignant phenotype. Biol Chem 2018; 399: 1099-1105. (PMID: 29498930) [Crossref]
96. Geng X, Liu Y, Diersch S, Kotzsch M, Grill S, Weichert W, et al. Clinical relevance of kallikrein-related peptidase 9, 10, 11, and 15 mRNA expression in advanced high-grade serous ovarian cancer. Ulrich H, editor. PLoS One 2017; 12: e0186847. (PMID: 29095848) [Crossref]
97. Olmeda D, Cerezo-Wallis D, Riveiro-Falkenbach E, Pennacchi PC, Contreras-Alcalde M, Ibarz N, et al. Whole-body imaging of lymphovascular niches identifies pre-metastatic roles of midkine. Nature 2017; 546: 676-680. (PMID: 28658220) [Crossref]
98. Rebbaa A, Chou PM, Mirkin BL. Factors secreted by human neuroblastoma mediated doxorubicin resistance by activating STAT3 and inhibiting apoptosis. Mol Med 2001; 7: 393-400. (PMID: 11474132) [Crossref]
99. Wu X, Zhi X, Ji M, Wang Q, Li Y, Xie J, et al. Midkine as a potential diagnostic marker in epithelial ovarian cancer for cisplatin/paclitaxel combination clinical therapy. Am J Cancer Res 2015; 5: 629-638. (PMID: 25973302) [Crossref]
100. Zhang D, Ding L, Li Y, Ren J, Shi G, Wang Y, et al. Midkine derived from cancer-associated fibroblasts promotes cisplatin-resistance via up-regulation of the expression of lncRNA ANRIL in tumour cells. Sci Rep 2017; 7: 16231. (PMID: 29176691) [Crossref]
101. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424. Erratum in: CA Cancer J Clin 2020; 70: 313. (PMID: 30207593) [Crossref]
102. Weigelt B, Peterse JL, van't Veer LJ. Breast cancer metastasis: markers and models. Nat Rev Cancer 2005; 5: 591-602. (PMID: 16056258) [Crossref]
103. Sheng B, Wei Z, Wu X, Li Y, Liu Z. USP12 promotes breast cancer angiogenesis by maintaining midkine stability. Cell Death Dis 2021; 12: 1074. (PMID: 34759262) [Crossref]
104. Tian Z, Tang J, Liao X, Yang Q, Wu Y, Wu G. An immune-related prognostic signature for predicting breast cancer recurrence. Cancer Med 2020; 9: 7672-7685. (PMID: 32841536) [Crossref]
105. Ibusuki M, Fujimori H, Yamamoto Y, Ota K, Ueda M, Shinriki S, et al. Midkine in plasma as a novel breast cancer marker. Cancer Sci 2009; 100: 1735-1739. (PMID: 19538527) [Crossref]
106. Goodman M, Liu Z, Zhu P, Li J. AMPK Activators as a Drug for Diabetes, Cancer and Cardiovascular Disease. Pharm Regul Aff 2014; 3: 118. (PMID: 27478687) [Crossref]
107. Woods A, Johnstone SR, Dickerson K, Leiper FC, Fryer LGD, Neumann D, et al. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. Curr Biol 2003; 13: 2004-2008. (PMID: 14614828) [Crossref]
108. Xia T, Chen D, Liu X, Qi H, Wang W, Chen H, et al. Midkine noncanonically suppresses AMPK activation through disrupting the LKB1-STRAD-Mo25 complex. Cell Death Dis 2022; 13: 414. (PMID: 35487917) [Crossref]
109. Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, Mäkelä TP, et al. Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. J Biol 2003; 2: 28. (PMID: 14511394) [Crossref]
110. Zeqiraj E, Filippi BM, Deak M, Alessi DR, van Aalten DMF. Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation. Science 2009; 326: 1707-1711. (PMID: 19892943) [Crossref]
111. Zeqiraj E, Filippi BM, Goldie S, Navratilova I, Boudeau J, Deak M, et al. ATP and MO25α Regulate the Conformational State of the STRADα Pseudokinase and Activation of the LKB1 Tumour Suppressor. PLoS Biol 2009; 7: e1000126. (PMID: 19513107) [Crossref]
112. Lin R, Elf S, Shan C, Kang HB, Ji Q, Zhou L, et al. 6-Phosphogluconate dehydrogenase links oxidative PPP, lipogenesis and tumour growth by inhibiting LKB1-AMPK signalling. Nat Cell Biol 2015; 17: 1484-1496. (PMID: 26479318) [Crossref]
113. Singletary SE, McNeese MD, Hortobagyi GN. Feasibility of breast-conservation surgery after induction chemotherapy for locally advanced breast carcinoma. Cancer 1992; 69: 2849-2852. (PMID: 1571916) [Crossref]
114. Tryfonidis K, Senkus E, Cardoso MJ, Cardoso F. Management of locally advanced breast cancer-perspectives and future directions. Nat Rev Clin Oncol 2015; 12: 147-162. Erratum in: Nat Rev Clin Oncol 2015; 12: 312. (PMID: 25668732) [Crossref]
115. Heil J, Kuerer HM, Pfob A, Rauch G, Sinn HP, Golatta M, et al. Eliminating the breast cancer surgery paradigm after neoadjuvant systemic therapy: current evidence and future challenges. Ann Oncol 2020; 31: 61-71. (PMID: 31912797) [Crossref]
116. Debled M, Mauriac L. Neoadjuvant chemotherapy: are we barking up the right tree? Ann Oncol 2010; 21: 675-679. (PMID: 20338876) [Crossref]
117. Han X, Li M, Xu J, Fu J, Wang X, Wang J, et al. miR-1275 targets MDK/AKT signaling to inhibit breast cancer chemoresistance by lessening the properties of cancer stem cells. Int J Biol Sci 2023; 19: 89-103. (PMID: 36594100) [Crossref]
118. Parker BS, Rautela J, Hertzog PJ. Antitumour actions of interferons: implications for cancer therapy. Nat Rev Cancer 2016; 16: 131-144. (PMID: 26911188) [Crossref]
119. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol 2005; 5: 375-386. (PMID: 15864272) [Crossref]
120. Du W, Frankel TL, Green M, Zou W. IFNγ signaling integrity in colorectal cancer immunity and immunotherapy. Cell Mol Immunol 2022; 19: 23-32. (PMID: 34385592) [Crossref]
121. Singh S, Kumar S, Srivastava RK, Nandi A, Thacker G, Murali H, et al. Loss of ELF5-FBXW7 stabilizes IFNGR1 to promote the growth and metastasis of triple-negative breast cancer through interferon-γ signalling. Nat Cell Biol 2020; 22: 591-602. Erratum in: Nat Cell Biol 2021; 23: 1048. (PMID: 32284542) [Crossref]
122. Zheng L, Liu Q, Li R, Chen S, Tan J, Li L, et al. Targeting MDK Abrogates IFN-γ-Elicited Metastasis inCancers of Various Origins. Front Oncol 2022; 12: 885656. (PMID: 35747815) [Crossref]
123. Chow KT, Gale M Jr. SnapShot: Interferon Signaling. Cell 2015; 163: 1808-1808.e1. (PMID: 26687364) [Crossref]
124. Hao H, Maeda Y, Fukazawa T, Yamatsuji T, Takaoka M, Bao XH, et al. Inhibition of the growth factor MDK/midkine by a novel small molecule compound to treat non-small cell lung cancer. PLoS One 2013; 8: e71093. (PMID: 23976985) [Crossref]
125. Mevissen TET, Komander D. Mechanisms of Deubiquitinase Specificity and Regulation. Annu Rev Biochem 2017; 86: 159-192. (PMID: 28498721) [Crossref]

126. Reyes-Turcu FE, Ventii KH, Wilkinson KD. Regulation and Cellular Roles of Ubiquitin-Specific Deubiquitinating Enzymes. Annu Rev Biochem 2009; 78: 363-397. (PMID: 19489724) [Crossref]

127. Hou K, Zhu Z, Wang Y, Zhang C, Yu S, Zhu Q, et al. Overexpression and Biological Function of Ubiquitin-Specific Protease 42 in Gastric Cancer. PLoS One 2016; 11: e0152997. (PMID: 27030989) [Crossref]

128. Wu Y, Wang Y, Lin Y, Liu Y, Wang Y, Jia J, et al. Dub3 inhibition suppresses breast cancer invasion and metastasis by promoting Snail1 degradation. Nat Commun 2017; 8: 14228. (PMID: 28198361) [Crossref]

129. Song IK, Kim HJ, Magesh V, Lee KJ. Ubiquitin C-terminal hydrolase-L1 plays a key role in angiogenesis by regulating hydrogen peroxide generated by NADPH oxidase 4. Biochem Biophys Res Commun 2018; 495: 1567-1572. (PMID: 29128359) [Crossref]

130. Lim R, Sugino T, Nolte H, Andrade J, Zimmermann B, Shi C, et al. Deubiquitinase USP10 regulates Notch signaling in the endothelium. Science 2019; 364: 188-193. (PMID: 30975888) [Crossref]

131. Vishnoi M, Boral D, Liu H, Sprouse ML, Yin W, Goswami-Sewell D, et al. Targeting USP7 Identifies a Metastasis-Competent State within Bone Marrow–Resident Melanoma CTCs. Cancer Res 2018; 78: 5349-5362. (PMID: 30026332) [Crossref]

132. Hayward S, Gachehiladze M, Badr N, Andrijes R, Molostvov G, Paniushkina L, et al. The CD151-midkine pathway regulates the immune microenvironment in inflammatory breast cancer. J Pathol 2020; 251: 63-73. (PMID: 32129471) [Crossref]

133. Gharesouran J, Taheri M, Sayad A, Ghafouri-Fard S, Mazdeh M, Omrani MD. The Growth Arrest-Specific Transcript 5 (GAS5) and Nuclear Receptor Subfamily 3 Group C Member 1 (NR3C1): Novel Markers Involved in Multiple Sclerosis. Int J Mol Cell Med 2018; 7: 102-110. (PMID: 30276165) [Crossref]

134. Han Z, Zhang C, Wang Q, Li L, Wang M, Li X, et al. MicroRNA-19b Downregulates NR3C1 and Enhances Oxaliplatin Chemoresistance in Colon Cancer via the PI3K/AKT/mTOR Pathway. Clin Med Insights Oncol 2021; 15: 11795549211012666. (PMID: 34017210) [Crossref]

135. Lovšin N, Marc J. Glucocorticoid Receptor Regulates TNFSF11 Transcription by Binding to Glucocorticoid Responsive Element in TNFSF11 Proximal Promoter Region. Int J Mol Sci 2021; 22: 1054. (PMID: 33494362) [Crossref]

136. Chen Z, Lan X, Wu D, Sunkel B, Ye Z, Huang J, et al. Ligand-dependent genomic function of glucocorticoid receptor in triple-negative breast cancer. Nat Commun 2015; 6: 8323. (PMID: 26374485) [Crossref]

137. Pan D, Kocherginsky M, Conzen SD. Activation of the Glucocorticoid Receptor Is Associated with Poor Prognosis in Estrogen Receptor-Negative Breast Cancer. Cancer Res 2011; 71: 6360-6370. (PMID: 21868756) [Crossref]

138. Zhang L, Song L, Xu Y, Xu Y, Zheng M, Zhang P, et al. Midkine promotes breast cancer cell proliferation and migration by upregulating NR3C1 expression and activating the NF-κB pathway. Mol Biol Rep 2022; 49: 2953-2961. (PMID: 35028860) [Crossref]

139. Xu YY, Mao XY, Song YX, Zhao F, Wang ZN, Zhang WX, et al. Midkine confers Adriamycin resistance in human gastric cancer cells. Tumour Biol 2012; 33: 1543-1548. (PMID: 22576950) [Crossref]

140. Lorente M, Torres S, Salazar M, Carracedo A, Hernández-Tiedra S, Rodríguez-Fornés F, et al. Stimulation of the midkine/ALK axis renders glioma cells resistant to cannabinoid antitumoral action. Cell Death Differ 2011; 18: 959-973. (PMID: 21233844) [Crossref]

141. Chu F, Naiditch JA, Clark S, Qiu YY, Zheng X, Lautz TB, et al. Midkine Mediates Intercellular Crosstalk between Drug-Resistant and Drug-Sensitive Neuroblastoma Cells In Vitro and In Vivo. ISRN Oncol 2013; 2013: 518637. (PMID: 24083030) [Crossref]

142. Hu R, Yan Y, Li Q, Lin Y, Jin W, Li H, et al. Increased drug efflux along with midkine gene high expression in childhood B-lineage acute lymphoblastic leukemia cells. Int J Hematol 2010; 92: 105-110. (PMID: 20544404) [Crossref]

143. Kang HC, Kim IJ, Park JH, Shin Y, Ku JL, Jung MS, et al. Identification of Genes with Differential Expression in Acquired Drug-Resistant Gastric Cancer Cells Using High-Density Oligonucleotide Microarrays. Clin Cancer Res 2004; 10: 272-284. (PMID: 14734480) [Crossref]

144. Kawai H, Sato W, Yuzawa Y, Kosugi T, Matsuo S, Takei Y, et al. Lack of the Growth Factor Midkine Enhances Survival against Cisplatin-Induced Renal Damage. Am J Pathol 2004; 165: 1603-1612. (PMID: 15509530) [Crossref]

145. Ota T, Jono H, Ota K, Shinriki S, Ueda M, Sueyoshi T, et al. Downregulation of midkine induces cisplatin resistance in human oral squamous cell carcinoma. Oncol Rep 2012; 27: 1674-1680. (PMID: 22344722) [Crossref]

146. Takei Y, Kadomatsu K, Matsuo S, Itoh H, Nakazawa K, Kubota S, et al. Antisense oligodeoxynucleotide targeted to Midkine, a heparin-binding growth factor, suppresses tumorigenicity of mouse rectal carcinoma cells. Cancer Res 2001; 61: 8486-8491. (PMID: 11731432) [Crossref]

147. Dai LC, Wang X, Yao X, Lu YL, Ping JL, He JF. Enhanced therapeutic effects of combined chemotherapeutic drugs and midkine antisense oligonucleotides for hepatocellular carcinoma. World J Gastroenterol 2007; 13: 1989-1994. (PMID: 17461503) [Crossref]

148. Inoh K, Muramatsu H, Torii S, Ikematsu S, Oda M, Kumai H, et al. Doxorubicin-Conjugated Anti-Midkine Monoclonal Antibody as a Potential Anti-Tumor Drug. Jpn J Clin Oncol 2006; 36: 207-211. (PMID: 16611663) [Crossref]

149. Zhao S, Zhao G, Xie H, Huang Y, Hou Y. A conjugate of an anti-midkine single-chain variable fragment to doxorubicin inhibits tumor growth. Braz J Med Biol Res 2012; 45: 230-237. (PMID: 22267001) [Crossref]

150. Maehara H, Kaname T, Yanagi K, Hanzawa H, Owan I, Kinjou T, et al. Midkine as a novel target for antibody therapy in osteosarcoma. Biochem Biophys Res Commun 2007; 358: 757-762. (PMID: 17506984) [Crossref]

151. Toyoda E, Doi R, Kami K, Mori T, Ito D, Koizumi M, et al. Midkine promoter-based conditionally replicative adenovirus therapy for midkine-expressing human pancreatic cancer. J Exp Clin Cancer Res 2008; 27: 30. (PMID: 18717994) [Crossref]

152. Rawnaq T, Dietrich L, Wolters-Eisfeld G, Uzunoglu FG, Vashist YK, Bachmann K, et al. The Multifunctional Growth Factor Midkine Promotes Proliferation and Migration in Pancreatic Cancer. Mol Cancer Res 2014; 12: 670-680. (PMID: 24567526) [Crossref]

153. Tian W, Shen J, Chen W. Suppression of midkine gene promotes the antitumoral effect of cisplatin on human gastric cancer cell line AGS in vitro and in vivo via the modulation of Notch signaling pathway. Oncol Rep 2017; 38: 745-754. (PMID: 28656262) [Crossref]

154. Sajid MI, Moazzam M, Kato S, Yeseom Cho K, Tiwari RK. Overcoming Barriers for siRNA Therapeutics: From Bench to Bedside. Pharmaceuticals (Basel) 2020; 13: 294. (PMID: 33036435) [Crossref]

155. Karadeniz Z, Aynacıoğlu AŞ, Bilir A, Tuna MY. Inhibition of midkine by metformin can contribute to its anticancer effects in malignancies: A proposal mechanism of action of metformin in context of endometrial cancer prevention and therapy. Med Hypotheses 2020; 134: 109420. (PMID: 31634770) [Crossref]

156. Ueno M, Kariya R, Gunya S, Cheevapruk K, Okada S. Midkine inhibitor (iMDK) induces apoptosis of primary effusion lymphoma via G2/M cell cycle arrest. Leuk Res 2022; 116: 106826. (PMID: 35316715) [Crossref]

157. Masui M, Okui T, Shimo T, Takabatake K, Fukazawa T, Matsumoto K, et al. Novel Midkine Inhibitor iMDK Inhibits Tumor Growth and Angiogenesis in Oral Squamous Cell Carcinoma. Anticancer Res 2016; 36: 2775-2781. (PMID: 27272788) [Crossref]

158. Wang J, Takeuchi H, Sonobe Y, Jin S, Mizuno T, Miyakawa S, et al. Inhibition of midkine alleviates experimental autoimmune encephalomyelitis through the expansion of regulatory T cell population. Proc Natl Acad Sci U S A 2008; 105: 3915-3920. (PMID: 18319343) [Crossref]

Original Article

Eur J Breast Health 2024; 20(3): 178-184
DOI: 10.4274/ejbh.galenos.2024.2024-3-7

Bioinformatic Investigation of Genetic Changes in Paraoxonase Genes in Breast Cancer and Breast Cancer Subtypes

Durmuş Ayan[1,2], Mehmet Ali Gül[3], Umut Karabay[4], Seyyid Mehmet Bulut[1]

[1]Niğde Ömer Halisdemir University Faculty of Medicine, Medical Biochemistry, Niğde, Turkey
[2]Nigde Ömer Halisdemir University Faculty of Medicine, Training and Research Hospital, Niğde, Turkey
[3]Amasya University Faculty of Medicine, Medical Biochemistry, Amasya, Turkey
[4]Gülhane Training and Research Hospital, Clinic of Internal Disease, Ankara, Turkey

ABSTRACT

Objective: Among women, breast cancer (BC) is the most prevalent form of cancer. Many molecular targets have been discovered for BC prognosis and treatment. However, new markers still need to be identified, as cancer pathogenesis is triggered by different mechanisms. The aim of this study was to examine the changes in the *paraoxonase* genes (*PON1*, *PON2*, and *PON 3*) involved in the pathogenesis of BC.

Materials and Methods: The characteristics of the mutations were evaluated with the Cbioportal database. Kaplan-Meier Plot evaluated recurrence-free survival (RFS). The UALCAN database determined the promoter methylation. Gene expression was evaluated by GEPIA2.0 database.

Results: PON1 harbored the most mutations. There was a significant decrease in PON3 expression levels in BC samples compared with healthy samples. PON1 and PON2 expression levels did not differ between BC tissue and normal adjacent tissue. Elevated expression levels of *PON1* and *PON2* genes were correlated with longer RFS, whereas reduced expression of the *PON2* gene showed an association with longer RFS. Moreover, the promoter regions of PON1 and PON 3 were found to be hypermethylated, while the promoter region of PON 2 was found to be hypomethylated. The PON3 promoter region was significantly hypermethylated in luminal and human epidermal growth factor receptor 2 (HER2) + BC subtypes. However, the PON3 promoter region was significantly hypomethylated in the triple negative breast cancer (TNBC) subtype.

Conclusion: These results suggest that methylation and expression status of PON3 in BC and BC subtypes (TNBC, luminal and HER2) may indicate a poor prognosis. The *PON3* gene could be a negative prognostic marker in BC. However, the results should be supported by prospective studies.

Keywords: Breast cancer; paraoxonase; bioinformatics

Cite this article as: Ayan D, Gül MA, Karabay U, Bulut SM. Bioinformatic Investigation of Genetic Changes in Paraoxonase Genes in Breast Cancer and Breast Cancer Subtypes.Eur J Breast Health 2024; 20(3): 178-184

Key Points

- Breast cancer is one of the most common cancer types among women.
- Paraoxonase 1 (PON1) enzyme has the most mutations compared to PON2 and PON3 enzymes.
- *PON3* gene expression level (downregulation) in breast tissue was significant ($p<0.05$)
- PON3 expression levels were downregulated, especially in triple negative breast cancer (TNBC)-BL1, TNBC-BL2 and TNBC-unknown subtypes
- The PON3 promoter region was significantly hypomethylated in the TNBC subtype. This may explain global DNA hypomethylation.
- Global DNA hypomethylation in breast cancer may be associated with the formation of repressive chromatin domains and gene silencing

Introduction

Breast cancer (BC) is one of the most common cancers in women (1, 2). The high mortality and morbidity rates associated with BC make it a prominent health concern among women. Despite adjuvant chemotherapy, the five-year survival rate for metastatic BC is less than 30% (3). Although the precise cause of BC is not yet fully understood, specific risk factors can heighten the probability of its occurrence. These risk factors include age (the risk increases with age), a family history of BC and genetic mutations (e.g. *BRCA1* and *BRCA2*) (4). There are five different subtypes of BC, known as luminal A [estrogen receptor (ER), progesterone receptor (PR)+, human epidermal growth factor receptor 2 (HER2)–, Ki67+ <20%], luminal B (ER/PR+ <20%, HER2–, Ki67+ ≥20%) HER2+ B2 (ER/PR+, HER2 overexpression), HER2 overexpression (ER–, PR–, HER2 overexpression) and triple

Corresponding Author:
Durmuş Ayan; durmusayan@hotmail.com

Received: 18.03.2024
Accepted: 27.03.2024
Available Online Date: 01.07.2024

negative breast cancer-basal-like (TNBC-BL) (ER–, PR–, HER2–). The most challenging of these is TNBC, where all receptors are negative, treatment options are limited, and it has an aggressive course. For this reason, the search for new treatments for this type of cancer continues (5).

The *paraoxonase (PON)* gene is located on human chromosome 7 (6). The enzyme PON is involved in antioxidant defense and is associated with lipid metabolism. There are three isoforms within the enzyme class, namely PON1, PON2 and PON3. PON1, which is mainly found in the bloodstream, is known for its ability to hydrolyse and detoxify specific harmful compounds, such as organophosphates. In addition, PON1 has an antioxidant effect and protects cells from oxidative stress caused by reactive oxygen species (ROS) (7). The antioxidant capacity of PON1 suggests that it may play a protective role against cancer development by neutralizing harmful ROS that can damage DNA and promote tumour formation.

Several studies have explored the relationship between PON and the risk and prognosis of BC, although the precise mechanisms are not yet fully understood (8). Some evidence suggests that PON1 activity may be associated with treatment outcomes in BC patients (9). Human susceptibility to BC has been studied in the context of genetic variations in the PON. Some specific single nucleotide polymorphisms in the *PON1* gene have been associated with altered enzyme activity that may affect a person's risk of developing BC (8). It has also been reported that higher levels of PON1 in BC tissue than in normal tissue indicate a possible link to cancer development (10). PON1 and PON3, which are bound to high-density lipoprotein (HDL) particles, have antioxidant and anti-inflammatory properties (11). PON2 and PON3 serve as intracellular enzymes and regulate mitochondrial superoxide anion production and endoplasmic reticulum (ER) stress-induced apoptosis (12). The pleiotropic role of PON enzymes has been studied in the context of cardiovascular and neurodegenerative diseases (13). In recent decades, researchers have identified the overexpression of PON2 and PON3 in cancer cells, leading to the suggestion that these enzymes may contribute to tumour survival and resistance to stress (11).

However, the clinical utility of PON enzymes as diagnostic or prognostic biomarkers remains unclear and requires further validation. Given the current state of knowledge, in this study we used some bioinformatics tools to analyze the association among possible mutations, expression and promotor methylation of *PON1*, *PON2* and *PON3* genes in BC subtypes, especially TNBC.

Material and Methods

Study Design

This was a bioinformatics study. The current study was based upon open-source data obtained from The Cancer Genome Atlas (TCGA, https://www.cancer.gov/tcga), which is a public database. The patients involved in the database have given ethical approval. Users can download relevant data for free for research and publish relevant articles. There are no ethical issues or other conflicts of interest. The BRCA cohort consists of a total of 996 cases. The results of these cases were obtained using the Cbioportal database. The data were accessed on July 05, 2023, from TCGA. The cbioportal database evaluated PON1, PON2, and PON3 mutations. This database accessed the amino acid in which the mutation occurred, the cancer subtype, and clinical information about the cancer. It was determined whether there was a somatic mutation or not, using the COSMIC (https://cancer.sanger.ac.uk/cosmic) database.

Survival Investigation

For recurrence-free survival (RFS) analysis, the Kaplan-Meier Plotter (KM) tool, available at https://kmplot.com/analysis/, was used to analyze the associations between gene expressions and survival rates in cancer. Moreover, this tool helped us to understand the prognostic values of the expression levels of *PON1*, *PON2* and *PON3* genes in BC patients. The KM plotter can evaluate the correlation between the expression of PON1, PON2 and PON3 (mRNA) and the survival rate in BC. The KM-Plotter used Cox proportional hazards regression and calculates the false discovery rate to analyze and evaluate the data (14).

Gene Expression and Correlation

We used Gene Expression Profiling Interactive Analysis, version 2 (GEPIA2.0) to assess the expression levels of PON1, PON2 and PON3 in both tumour and adjacent normal tissues, focusing specifically on BC. GEPIA2.0 uses the TCGA database and genotype-tissue expression (GTEx) data to perform this analysis. The screening criteria used in GEPIA2.0 were $p<0.05$, and |Log2FC| the cut-off point was 0.01. These criteria filter out genes that are not significantly differentially expressed between the two datasets (15). In addition, the correlation analysis between the expressions of the genes was examined with the GEPIA2.0 database.

Promoter Methylation and BC Subtypes Expression

UALCAN is an interactive open-access web page for OMICS data analysis (http://ualcan.Path.uab.edu/index.html). This database is built on PERL-CGI and can be used at approximately 6000 gene methylation levels (16). This study evaluated the promoter methylation level of *PON1*, *PON2*, and *PON3* genes in the BRCA data. Promoter region methylation levels of genes were also examined in BC subtypes. In addition, the change in expression levels in BC patients with BC subtypes accompanied by the TNBC subtype was examined using the UALCAN web server, since expression analysis was not available in the other web tool (GEPIA2).

Statistical Analysis

The evaluation of the study data included statistical analyses performed with the GEPIA2 database. Differential expression between BC tissue and adjacent normal tissue was measured using the One-Way ANOVA test. Correlation analysis of genes was performed by Pearson correlation analysis. The post-hoc Tukey test was favored for homogeneity of variances. Significance of difference between methylation levels was estimated by Student's t-test in UALCAN database. Significance of difference between BC subtypes was evaluated by the One-Way ANOVA test in UALCAN database. Survival status was assessed using the KM plotter with default settings, focusing on recurrence-free survival and using auto-best cut-off values and the J-best probe set. A log-rank p-value below 0.05 was considered statistically significant.

Results

Mutation Profile Interpretation

Among 996 cases, 9 cases (0.9%) of BC patients had genetic alterations in PON1, PON2 and PON3. There was a statistical significant co-occurrence of mutations in the analyzed populationamong PON1, PON2, and PON3 ($p<0.01$). The highest mutation rates were found in PON1 (55.5%), followed by PON3 (33.3%) and PON2 (11.2%).

The mutation types in the *PON1*, *PON2* and *PON3* genes in BC are listed in Table 1. In the analyzed genes of the BRCA cohort, there were eight missense mutations (88.8%) and one nonsense mutation (11.2%). None of these mutations originated in germ cells. The COSMIC database confirmed the somatic origin of the mutations.

The *PON1* gene contains two evolutionarily conserved regions. One is the arylesterase (between 168-253 amino acids) and the other is the SMP-30/gluconolaconase/LRE-like region (92-295 amino acids). There are three missense mutations in the region where the arylesterase domain of PON1 is located. All three of these mutations are located in the exon 7 region. In addition, there was a nonsense mutation (exon 4 region) and another missense mutation (exon 8 region). PON2 contains a 100% conserved arylesterase domain in the evolutionary process. This domain is located between 99 and 184 amino acid residues. There was a missense mutation in PON2 outside this region. This mutation was located in the exon 5 region. PON3 also contains an evolutionarily conserved arylesterase domain. This region is located between 167-252 amino acid residues. There was a missense mutation in this region of PON3, located in the exon 6 region. In addition, there were two further missense mutations in this gene. These mutations are located in the regions of exon-9 and exon-8.

Survival Prognosis Evaluation Results of Genes

The RFS results we obtained from the KM plotter analysis are shown in Figure 1. According to the analysis results, we found that increased expression of the *PON1* and *PON3* genes was significantly related to longer RFS (p = 7.5E-06 and p = 3.3E-05, respectively), while decreased expression of the *PON2* gene was significantly related to longer RFS (p = 0.024).

Gene Expression Results

The expression level of the *PON3* gene in tumour tissue was significantly lower when comparing tumour tissue (n = 1085) with adjacent normal tissue (n = 291) (p = 0.01), but there was no difference in the expression levels of the *PON1* and *PON2* genes (according to GEPIA2) (Figure 2A).

According to UALCAN, the expression level of PON1 in BRCA, based on major subclasses (with TNBC types) was significantly downregulated in TNBC Basal-like 1 (TNBC-BL1) (n = 13) compared to adjacent normal tissue (n = 114) (p<0.001). The expression level of PON2 in BRCA based on major subclasses (with TNBC types) was significantly upregulated in Luminal (n = 566) and downregulated in HER2+ (n = 37) compared to adjacent normal tissue (n = 114) (p<0.001). The expression level of PON3 in BRCA based on major subclasses (with TNBC types) was significantly downregulated in TNBC-BL1 (n = 13), TNBC Basal-like 2 (TNBC-BL2) (n = 11) and TNBC unspecified (TNBC-UNS) (n = 27) compared to adjacent normal tissue (n = 114) (p<0.001) (Figure 2B).

While there was a weak positive correlation between *PON1* gene expression levels and *PON2* gene expression levels (r = 0.18, p<0.001), a strong positive correlation was found between *PON1* gene expression levels and *PON3* gene expression levels (r = 0.095, p = 0.00041). In addition, there was a weak positive correlation between *PON2* gene expression levels and *PON3* gene expression levels (r = 0.28, p<0.001) (Figure 3).

Analyzes of Promoter Methylation Level

DNA methylation is an essential condition for the epigenetic modification of the genome and is closely linked to the development of cancer (17). According to the results of the analysis using the UALCAN online tool to determine the DNA methylation level, the promoter methylation level of PON1 (p = 1.62E-12) and PON3 (p = 1.62E-12) was found to be significantly higher (hypermethylation) in BC tissue compared to healthy tissue. Conversely, the methylation level of the promoter of PON2 (p = 3.79E-03) was significantly lower (hypomethylation) (Figure 4A). Moreover, compared to normal tissue, the PON3 promoter region methylation level was hypermethylated

Table 1. *PON1*, *PON2* and *PON3* gene mutation analysis

No	Gene	Nt change	AA position	Type of cancer	Subtype	American Joint Committee on Cancer Metastasis Stage Code	Neoplasm Disease Lymph Node Stage American Joint Committee on Cancer Code	Neoplasm Disease Stage American Joint Committee on Cancer Code	Diagnosed age	Overall survival (months)
1	PON1	c.743A>G	***Y248C***	BIDC	Basal like	M0	N0	STAGE IIA	69	16.4*
2	PON1	c.736C>T	***H246Y***	BIDC	Luminal B	M0	N0	STAGE I	60	13.0*
3	PON1	c.821A>T	***D274V***	BIDC	Basal like	M0	N1	STAGE IIA	40	36.3*
4	PON1	c.332C>A	***S111****	BIDC	Luminal B	M0	N1A	STAGE IIB	77	14.1*
5	PON1	c.727C>T	***H243Y***	BIDC	Basal like	MX	N1A	STAGE IIB	49	14.8*
6	PON2	c.396C>A	***N132K***	BIDC	Luminal B	M0	N0 (I-)	STAGE IIA	69	81.6
7	PON3	c.683C>T	***S222L***	BIDC	Basal like	M1	N2	STAGE IV	59	7.9*
8	PON3	c.929T>G	***L310W***	BILC	HER2+	MX	N0	STAGE IIA	65	16.6*
9	PON3	c.882C>G	***N294K***	BILC	Luminal A	M0	N2A	STAGE IIIA	72	47.0

BIDC: Breast ınvasive ductal carcinoma; BILC: Breast ınvasive lobular carcinoma; *: Livin; HER2: Human epidermal growth factor receptor 2

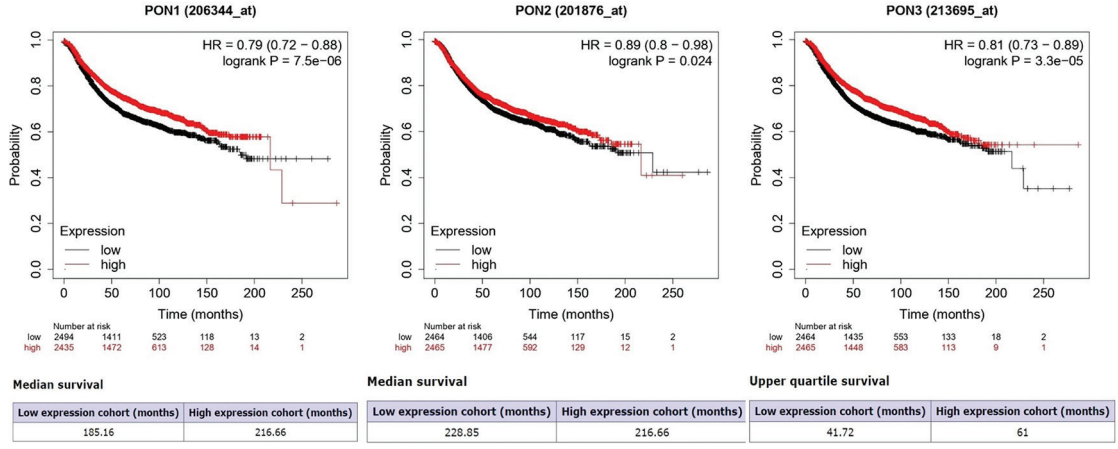


Figure 1. Different expressions of PON1, PON2 and PON3 in breast cancer (BC) patients in the recurrence-free survival curve (using the Kaplan-Meier plotter). The red line represents the survival rate curve of patients with BC who expressed the gene, and the black line represents the survival rate curve of BC patients who did not express the gene

in the luminal and HER2 positive BC subtypes (p = 1.62E-12, p = 8.83E-04, respectively), while it was hypomethylated in the TNBC (p = 3.39E-03). In BC subtypes accompanied by TNBC, PON3 expression levels were downregulated compared to normal tissue. However, significance was present only for TNBC-BCL1, TNBC-BCL1, TNBC-UNS (p = 5.44E-03, p = 1.40E-03 and p = 7.58E-03, respectively) (Figure 4B).

Discussion and Conclusion

As a multifactorial antioxidant enzyme, PON1 plays a protective role against oxidative stress, a factor thought to contribute to the development of cancer. The role of PON1 in the detoxification of cancer-causing oxidative stress has led to the evaluation of genetic variations in PON1, particularly with regard to susceptibility to BC (18). Considering the data obtained through the cBioPortal, five missense mutations in the *PON1* gene were discovered. PON1 is the gene in the paraoxonase gene family that underwent the most somatic alterations. The p.S111* nonsense changes in the exon 4 region may cause the polypeptide to terminate too early and the protein to be truncated. This probably leads to the loss of the arylesterase domain. The presence of an arylesterase domain characterizes the PON family of genes. Arylesterase enzymes, which belong to the family of conserved protein domains, are responsible for the hydrolysis of organophosphorus esters such as paraoxon. These enzymes are present both in the liver and in the blood and ensure resistance to the toxicity of organophosphates. In humans, arylesterase is associated with HDL and presumably provides protection against oxidation of low-density lipoprotein (19). There were three different (p. H243Y, p. H246Y, p.Y248C) missense mutations in this 100% conserved splice region. The result of these is likely to be dysfunctional protein. In this case, the antioxidant balance, which has an important role in the pathogenesis and development of BC, may be disrupted.

Hypermethylation in the PON1 promoter region has been reported to lead to downregulation of PON1 (20). In the BRCA cohort, the promoter region of PON1 in BC tissue was statistically hypermethylated compared to healthy tissue. In our BRCA cohort, the expression of PON1 was decreased in BC tissue compared to healthy tissue, possibly as a result of the hypermethylation of the promoter region. When we examined BC subtypes, we found that luminal and HER2+ were hypermethylated compared to normal tissue, but on the other hand, the TNBC subtype was hypomethylated. When it comes to expression levels, we found significant downregulation only in TNBC-BL1. Although this does not conform with the classical theory, it can best be explained by global DNA hypomethylation. Global DNA hypomethylation is a hallmark of human cancer, but its functional consequences remain unclear. The results of the present study suggest that global DNA hypomethylation in BC is closely linked to the formation of repressive chromatin domains and gene silencing. Therefore, this can be described as a potential epigenetic pathway for gene regulation in cancer cells (21).

Reduced PON1 expression was associated with shorter RFS in BC patients. Tumor growth and development depend on a wide variety of factors. The most prominent of these are inflammation and oxidative stress. The inflammatory and oxidative environment contributes to cell damage and may lead to cancer formation in the long-term (22). However, differential DNA methylation, detection of circulating nucleic acids, and histone-modifying enzymes are being investigated as epigenetic biomarkers for BC (21). In light of this information, an inflammatory and oxidative environment may occur as a result of the decrease in PON1 expression levels in the BRCA cohort due to the effect of epigenetic mechanisms and may cause serious prognostic effects by supporting the progression of cancer pathogenesis in patients in the long-term.

In the BRCA cohort, there was a missense mutation in the *PON2* gene. The *PON2* gene was the least somatically mutated of the *PON* genes analyzed. The promoter region of PON2 was significantly hypomethylated in BC tissue. This may explain the upregulation of PON2 in the BRCA cohort. On the other hand, the promoter region of PON2 contains an ER stress element-like sequence that regulates ER stress (6). Epigenetic changes in this region can cause ER stress in particular. There is evidence that the enzyme PON2 plays a role in cancer cell survival due to its antioxidant and anti-apoptotic activities. In addition, it is thought to play a role in the resistance of cancer cells to chemotherapy and therefore RFS (11). Thus, the upregulation of PON2 in BC tissue may be considered a survival mechanism of cancer cells. It has been observed that PON2 levels are upregulated in patients with BC (23). Although the expression of PON2 tended to

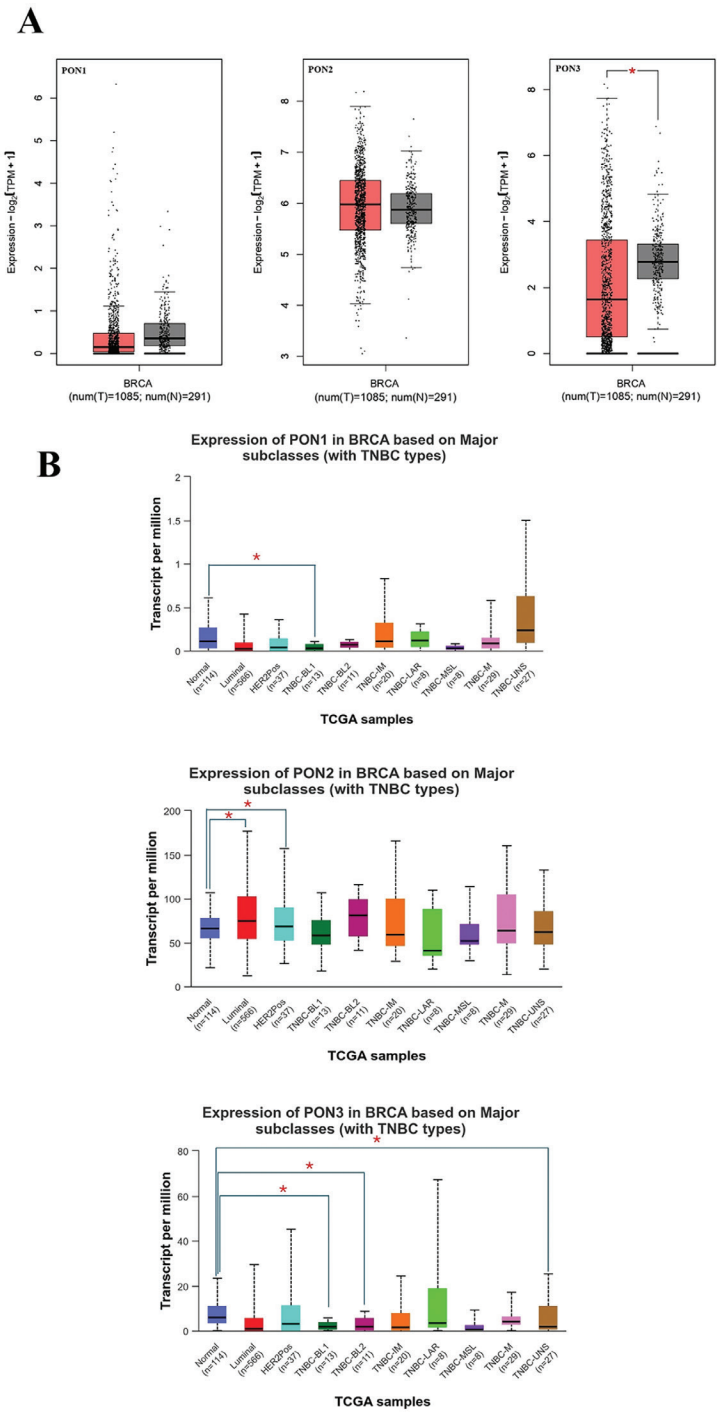


Figure 2. A. mRNA expressions of PON1, PON2 and PON3 in BC (red) and normal breast tissues (gray). **B.** The expression level of PON1, PON2 and PON3 in BRCA based on major subclasses (with TNBC types), *: $p<0.05$

TNBC-BL1: TNBC basal-like 1; TNBC-BL2: TNBC basal-like 2; TNBC-IM: TNBC immunomodulatory; TNBC-LAR: TNBC luminal androgen receptor; TNBC-MSL: TNBC mesenchymal stem-like; TNBC-M: TNBC mesenchymal; TNBC-UNS: TNBC unspecified; BC: Breast cancer; TNBC: Triple negative breast cancer

be upregulated in the BRCA cohort compared to expression in healthy tissue, there was no significant difference between these two tissues. Interestingly, higher PON2 expression levels were associated with shorter RFS in the BRCA cohort. Although this situation seems to be associated with poor prognosis in BC, the fact that the increase in PON2 levels was not significant in the BRCA cohort makes it difficult to evaluate it from a prognostic perspective.

In a study of infiltrating tumours related to BC subtypes, such as luminal A, HER2+ and TNBC, PON2 expression was significantly upregulated compared to expression levels in healthy tissue (23). TNBC is highly aggressive, prone to recurrence and early metastasis, and lacks receptors for targeted therapy, limiting treatment options to surgery, radiotherapy, and chemotherapy. It has been observed that PON2 levels are overexpressed in this BC subtype, and it has been proposed that reducing PON2 levels may be a treatment option. As a result, it was found that when PON2 expression levels were reduced, BC cell proliferation decreased and the response to chemotherapy was more effective in TNBC cells (23). In the BRCA cohort, while there was statistical difference in the PON2 expression levels of tumour tissue compare to adjacent normal tissue in luminal and HER2+ subtypes, there was no any statistically difference TNBC subtype. Furthermore, there was only one mutation in the *PON2* gene in the BRCA cohort, and the BC subtype associated with this mutation was luminal B. Therefore, more evidence is needed to consider PON2 as a treatment option in subtypes of BC.

Three missense mutations were found in the *PON3* gene in our BRCA cohort. One (p.S228L missense mutation) is a 100% conserved splice site in the arylesterase domain. Such a change in the arylesterase domain may affect the progression of cancer pathogenesis by causing a change in the antioxidant property of the protein. As with other PON family genes, increased expression levels in PON3 seem to be associated with prolonged survival.

PON3 is overexpressed in a proportion of various human tumors, just like PON2, and reduces mitochondrial superoxide formation (11). Direct interaction with coenzyme Q10 characterizes PON3, suggesting its probable function in sequestering ubisemiquinone and enhancing resistance to cell death. PON3 is situated within the ER and mitochondria, and it counteracts apoptosis triggered by DNA damage or intrinsic and extrinsic stimuli. In addition, PON3 impairs ER stress-induced apoptotic MAPK signaling and CHOP induction. In this case, the upregulation of PON3 in cancer tissue protects the cell against mitochondrial superoxide-mediated cell death (11). Studies have obtained various and different data on the regulation of PON3 in cancer tissue. One indicated that PON3 was underexpressed in 13 out of 20 cancer types, including bladder, breast, colorectal, liver, and lung cancer (24). Other studies reported that PON3 level was high in cancers such as pancreas, lung, prostate, and liver (25). In the BRCA cohort, the expression levels of the *PON3* gene in breast tissue were significantly lower when compared to healthy tissue. This decrease can be attributed to the hypermethylation of the *PON3* gene promoter region in the BRCA cohort. In addition, lower PON3 expression levels were identified to be associated with shorter RFS in the BRCA cohort. Furthermore, according to GEPIA2, tumor tissue PON3 expression levels were significantly downregulated compared to adjacent normal tissue across BC subtypes (except for the Basal Like subtype). We found very interesting results when evaluating the PON3 promoter region methylation status in BC subtypes. The PON3 promoter region was significantly hypermethylated in luminal and HER2 positive BC subtypes, and PON3 expression levels were significantly downregulated in both luminal and HER2 positive BC subtypes. This suggests gene silencing because of promoter region hypermethylation. In contrast, in our BRCA cohort, the PON3 promoter region was significantly

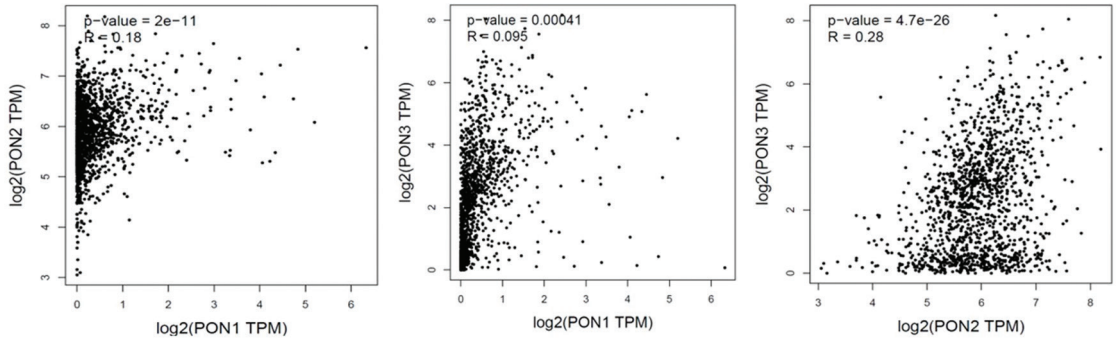


Figure 3. The correlation results of PON1, PON2 and PON3

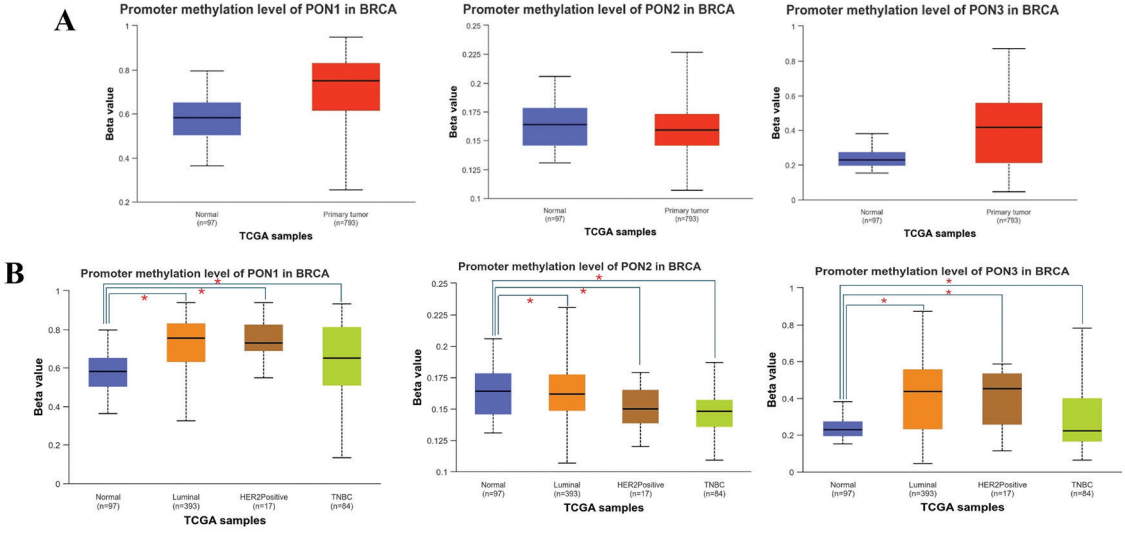


Figure 4. A. The promoter methylation level of PON1, PON2 and PON3. **B.** The promoter methylation level of *PON1*, *PON2* and *PON3* genes according to luminal, HER2+, and TNBC, *: $p<0.05$

TNBC: Triple negative breast cancer; HER2: Human epidermal growth factor receptor 2

hypomethylated in the TNBC subtype. According to classical theory, it is expected that the gene will be activated and its expression levels will increase as a result of promoter region hypomethylation (26). However, PON3 expression levels were downregulated, especially in TNBC-BL1, TNBC-BL2 and TNBC-unknown subtypes. This may again be a result of global DNA methylation and global DNA hypomethylation in BC could be associated with the formation of repressive chromatin domains and gene silencing.

Especially in TNBC, this potential epigenetic change should be evaluated in further research in terms of regulating the *PON* genes. This may help identify new pathogenetic mechanisms and treatment options. However, we believe that further, population-based studies are needed to elucidate this epigenetic pathway, which remains unclear.

Study Limitations

Although our study was well designed, there are some limitations. The study includes the results of a study conducted in a limited area of population. For this reason, priority should be given to studies to be carried out with wider participation in different populations. Additionally, since the results are obtained through the bioportal, not all data regarding the cases may be available.

Our findings suggest that, in line with the data obtained in the BRCA cohort, changes in the *PON3* gene in BC and BC subtypes appear to be a genetic risk factor for BC development and can be considered an applicable molecular biomarker for screening women with a genetic predisposition. The current study may contribute to the literature to understand the possible impact of changes in the molecular function of genes belonging to the PON family on the pathogenesis of BC. As a result of the data obtained, the methylation status of PON3 in BC and BC subtypes, especially TNBC, may indicate a poor prognosis. This data may suggest a search for new therapeutic strategies targeting PON3. However, we suggest prospective investigations with BC driver gene mutations may be required to appraise *PON* genes as a treatment target strategy. Since the mechanisms behind cancer are many, there is a need for large-scale research on this subject by adopting population-based and multidisciplinary approaches.

Ethics Committee Approval: The data used in our study were obtained from public database TCGA, therefore, ethical approval was not required.

Informed Consent: Not necessary.

Authorship Contributions

Surgical and Medical Practices: D.A., S.M.B.; Concept: D.A., M.A.G.; Design: D.A., M.A.G., U.K.; Data Collection and/or Processing: D.A., U.K., S.M.B.; Analysis and/or Interpretation: D.A., M.A.G.; Literature Search: D.A., U.K.; Writing: D.A., S.M.B.

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

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Barzaman K, Karami J, Zarei Z, Hosseinzadeh A, Kazemi MH, Moradi-Kalbolandi S, et al. Breast cancer: Biology, biomarkers, and treatments. Int Immunopharmacol 2020; 84: 106535. (PMID: 32361569) [Crossref]
2. Kocer B, Menekse E, Turan U, Namdaroglu O, Barca AN, Araz L, et al. The Relationship Between Mammographic Density and Factors Affecting Breast Cancer Risk. Sisli Etfal Hastan Tip Bul 2022; 56: 119-125. (PMID: 35515977) [Crossref]
3. Kashyap D, Pal D, Sharma R, Garg VK, Goel N, Koundal D, et al. Global Increase in Breast Cancer Incidence: Risk Factors and Preventive Measures. Biomed Res Int 2022; 2022: 9605439. (PMID: 35480139) [Crossref]
4. Ataollahi MR, Sharifi J, Paknahad MR, Paknahad A. Breast cancer and associated factors: a review. J Med Life 2015; 8: 6-11. (PMID: 28316699) [Crossref]
5. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Res 2020; 22: 61. (PMID: 32517735) [Crossref]
6. Wen Y, Huang Z, Zhang X, Gao B, He Y. Correlation between PON1 gene polymorphisms and breast cancer risk: a Meta-analysis. Int J Clin Exp Med 2015; 8: 20343-20348. (PMID: 26884950) [Crossref]
7. Ayan D, Şeneş M, Çaycı AB, Soylemez S, Eren N, Altuntaş Y, et al. Evaluation of Paraoxonase, Arylesterase, and Homocysteine Thiolactonase Activities in Patients with Diabetes and Incipient Diabetes Nephropathy. J Med Biochem 2019; 38: 481-488. (PMID: 31496913) [Crossref]
8. Manco G, Porzio E, Carusone TM. Human Paraoxonase-2 (PON2): Protein Functions and Modulation. Antioxidants (Basel) 2021; 10: 256. (PMID: 33562328) [Crossref]
9. Pan X, Huang L, Li M, Mo D, Liang Y, Liu Z, et al. The Association between PON1 (Q192R and L55M) Gene Polymorphisms and Risk of Cancer: A Meta-Analysis Based on 43 Studies. Biomed Res Int 2019; 2019: 5897505. (PMID: 31467900) [Crossref]
10. Karimian M. A common genetic variation in paraoxonase 1 and risk of breast cancer: a literature review, meta-analysis, and in silico analysis. Nucleosides Nucleotides Nucleic Acids 2023; 42: 1-16. (PMID: 35938743) [Crossref]
11. Anđelković M, Djordjevic AB, Javorac D, Baralić K, Đukić-Ćosić D, Repić A, et al. Possible role of lead in breast cancer - a case-control study. Environ Sci Pollut Res Int 2022; 29: 65211-65221. (PMID: 35488155) [Crossref]
12. Bacchetti T, Ferretti G, Sahebkar A. The role of paraoxonase in cancer. Semin Cancer Biol 2019; 56: 72-86. (PMID: 29170064) [Crossref]
13. Schweikert E-M, Devarajan A, Witte I, Wilgenbus P, Amort J, Förstermann U, et al. PON3 is upregulated in cancer tissues and protects against mitochondrial superoxide-mediated cell death. Cell Death Differ 2012; 19: 1549-1560. (PMID: 22441669) [Crossref]
14. Győrffy B. Discovery and ranking of the most robust prognostic biomarkers in serous ovarian cancer. Geroscience 2023; 45: 1889-1898. (PMID: 36856946) [Crossref]
15. Cai Y, He Q, Liu W, Liang Q, Peng B, Li J, et al. Comprehensive analysis of the potential cuproptosis-related biomarker LIAS that regulates prognosis and immunotherapy of pan-cancers. Front Oncol 2022; 12: 952129. (PMID: 35982953) [Crossref]
16. Hoffman AE, Yi CH, Zheng T, Stevens RG, Leaderer D, Zhang Y, et al. CLOCK in breast tumorigenesis: genetic, epigenetic, and transcriptional profiling analyses. Cancer Res 2010; 70: 1459-1468. (PMID: 20124474) [Crossref]
17. Huang W, Li H, Yu Q, Xiao W, Wang DO. LncRNA-mediated DNA methylation: an emerging mechanism in cancer and beyond. J Exp Clin Cancer Res 2022; 41: 100. (PMID: 35292092) [Crossref]
18. Farmohammadi A, Momeni A, Bahmani B, Ghorbani H, Ramzanpour R. Association of PON1-L55M Genetic Variation and Breast Cancer Risk: A Case-Control Trial. Asian Pac J Cancer Prev 2020; 21: 255-258. (PMID: 31983193) [Crossref]
19. Taler-Verčič A, Goličnik M, Bavec A. The Structure and Function of Paraoxonase-1 and Its Comparison to Paraoxonase-2 and -3. Molecules 2020; 25: 5980. (PMID: 33348669) [Crossref]
20. Li X, Yu Q. PON1 hypermethylation is associated with progression of renal cell carcinoma. J Cell Mol Med 2019; 23: 6646-6657. (PMID: 31400051) [Crossref]
21. Schröder R, Illert AL, Erbes T, Flotho C, Lübbert M, Duque-Afonso J. The epigenetics of breast cancer - Opportunities for diagnostics, risk stratification and therapy. Epigenetics 2022; 17: 612-624. (PMID: 34159881) [Crossref]
22. Wang R, Li J, Zhao Y, Li Y, Yin L. Investigating the therapeutic potential and mechanism of curcumin in breast cancer based on RNA sequencing and bioinformatics analysis. Breast Cancer 2018; 25: 206-212. (PMID: 29139094) [Crossref]
23. Campagna R, Pozzi V, Giorgini S, Morichetti D, Goteri G, Sartini D, et al. Paraoxonase-2 is upregulated in triple negative breast cancer and contributes to tumor progression and chemoresistance. Hum Cell 2023; 36: 1108-1119. (PMID: 36897549) [Crossref]
24. Jin Y, Li Q, Qiu J, Zhao X, Zheng C, Lv S, et al. Downregulation of paraoxonase 3 contributes to aggressive human hepatocellular carcinoma progression and associates with poor prognosis. Tumour Biol 2016; 37: 14193-14203. (PMID: 27553024) [Crossref]
25. Huang D, Wang Y, He Y, Wang G, Wang W, Han X, et al. Paraoxonase 3 is involved in the multi-drug resistance of esophageal cancer. Cancer Cell Int 2018; 18: 168. (PMID: 30386177) [Crossref]
26. Li W, Zheng X, Ren L, Fu W, Liu J, Xv J, et al. Epigenetic hypomethylation and upregulation of GD3s in triple negative breast cancer. Ann Transl Med 2019; 7: 723. (PMID: 32042739) [Crossref]

Original Article

Eur J Breast Health 2024; 20(3): 185-193
DOI: 10.4274/ejbh.galenos.2024.2024-3-6

High Levels of Superoxide Dismutase 2 Are Associated With Worse Prognosis in Patients With Breast Cancer

Daniel Rodrigues de Bastos[1], Adhemar Longatto-Filho[2,3,4], Mércia Patrícia Ferreira Conceição[1], Lara Termini[1]

[1]Department of Radiology and Oncology, São Paulo University, Institute of Cancer of São Paulo Faculty of Medicine, São Paulo, Brazil
[2]Medical Laboratory of Medical Investigation (LIM) 14, Department of Pathology, São Paulo University Faculty of Medicine, São Paulo, Brazil
[3]Life and Health Sciences Research Institute (ICVS), Minho University, Braga, Portugal
[4]Teaching and Research Institute, Molecular Oncology Research Center, Barretos Cancer Hospital - Pio XII Foundation, São Paulo, Brazil

ABSTRACT

Objective: Breast cancer is classified based on hormone receptor status and human epidermal growth factor receptor 2 (HER2) expression, including luminal, HER2+, or triple-negative (TNBC). The absence of a therapeutic target in TNBC and the resistance to treatment associated with other subtypes means that research for new biomarkers remains important. In this context, superoxide dismutase 2 (SOD2) has emerged as a potential therapeutic target due to its clinicopathological associations and its ability to predict responses in human tumors. To analyze SOD2 staining in samples obtained from individuals with breast cancer and explore its transcriptional pattern across tumor subtypes.

Materials and Methods: SOD2 staining was assessed using the immunohistochemistry (IHC) in 80 samples from breast cancer patients. To analyze the expression profile at the transcriptional level, international databases such as cBioPortal (1,980 patients) and PrognoScan were accessed.

Results: Significant differences were observed between SOD2 expression analyzed by IHC, and estrogen (p = 0.0008) and progesterone (p = 0.0003) receptors, as well as tumor subtypes (p<0.0001). These differences were found in conjunction with other associations, including clinical and pathological data, such as tumor stage (p = 0.0129), tumor size (p = 0.0296), and node metastasis (p = 0.0486). Moreover, elevated SOD2 expression correlated with an unfavorable prognosis. The *in silico* analysis revealed a similar pattern, despite operating at the transcriptional level. Moreover, notable correlations were identified between elevated SOD2 expression and worse survival.

Conclusion: These results highlight the importance of SOD2 in breast cancer, particularly in aggressive subtypes. Increased SOD2 staining correlates with poorer outcomes, suggesting it as a potential therapeutic target.

Keywords: Biomarkers; breast cancer; SOD2; superoxide dismutase 2; triple negative breast cancer

Cite this article as: Rodrigues de Bastos D, Longatto-Filho A, Conceição MPF, Termini L. High Levels of Superoxide Dismutase 2 Are Associated With Worse Prognosis in Patients With Breast Cancer. Eur J Breast Health 2024; 20(3): 185-193

Key Points

- Superoxide dismutase 2 (SOD2) is elevated in advanced stage breast tumors, lymph node metastases, negative hormone receptors, and molecular subtypes.
- SOD2 is consistently increased in triple-negative breast tumors.
- High levels of SOD2 are associated with worse clinical outcomes in patients with breast cancer.

Introduction

Breast cancer is the most incident cancer among women worldwide. The Globocan/2020 project estimated 4.4 million cancer-related deaths, with breast cancer ranking fifth in terms of mortality when considering both men and women, and first if considering only women (1).

Due to the heterogeneity of these tumors, classifications based on histopathological characteristics have emerged. The classification takes into account immunohistochemical (IHC) staining for estrogen hormone receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor type 2 (HER2). In addition, Ki67 proliferation marker analysis can be used to distinguish luminal tumors into A and B. As a result, tumors can be categorized into luminal A, luminal B, HER2-positive, and triple-negative (TNBC) (2, 3).

Corresponding Author:
Daniel Rodrigues de Bastos; danielbastos.adm@gmail.com

Received: 12.03.2024
Accepted: 16.04.2024
Available Online Date: 01.07.2024

Patient survival varies according to the tumor subtype and a better prognosis is observed in patients with luminal tumors compared to those with HER2-positive or TNBC tumors (4). Several factors contribute to the survival of the patients, ranging from early diagnosis to the existence of targeted therapies capable of assisting in the pathological response. For instance, luminal and HER2-positive breast carcinomas are responsive to hormone therapy (e.g., tamoxifen, anastrozole) and targeted therapy (e.g., trastuzumab, lapatinib), respectively. In contrast, patients with TNBC do not have a specific therapeutic target, which contributes significantly to the frequent low survival observed in this population. Thus, the search for new biomarkers to assist in prognosis and a better understanding of these tumors remains the focus of much rsearch (2, 3).

Superoxide dismutase 2 (SOD2) is a mitochondrial enzyme with an important function in modulation of reactive oxygen species (ROS). Briefly, SOD2 reacts with superoxide (O2-) and hydrogen ions (H+) to generate oxygen (O_2) and hydrogen peroxide (H_2O_2) in a physiological process of cell detoxification (5). This balance is essential for cellular homeostasis. However, in cancer cells there is an impairment of this balance, leading to resistance to conventional treatments and worse survival (6). Despite its suppressive role in certain tumors, studies have shown that high levels of SOD2 can increase cell proliferation, as well as contribute to resistance to treatment and dissemination of metastases, especially in advanced-stage tumors (6-8).

Our group has investigated SOD2 since it was observed its differential expression in response to pro-inflammatory stimulus in human papilloma virus-immortalized keratinocytes (9). Complementary studies using IHC analyses were performed to detect SOD2 expression in samples from patients with cervical and penile cancer, revealing an important clinical and pathological association. High levels of SOD2 protein are associated with severity in these tumors (10-12). Moreover, it was shown that higher SOD2 expression was associated with a very poor prognosis for squamous cell carcinoma of the uterine cervix stage IIIB in terms of treatment with chemo- and radiotherapy (13).

In breast cancer, most of the studies regarding SOD2 investigated the relation between the polymorphism of this gene, especially variant C, with treatment toxicity and efficacy. In addition, there are also studies evaluating different post-translational modifications of the enzyme (acetylated and methylated) in animal and cellular models. However, there are still few published studies that have investigated the expression of SOD2 in the context of breast cancer, particularly concerning molecular subtypes and other clinicopathological features, such as tumor stage, lymph node involvement, its relationship with hormone receptors, as well as its prognostic role (14-17). In the present study, we evaluated the expression of SOD2 in samples from patients with breast cancer and investigated its transcriptional pattern in the luminal, HER2-positive, and TNBC subtypes. Our results may contribute to assessing the value of SOD2 expression as a biomarker in breast cancer.

Materials and Methods

Ethical Approval

This study was approved by the Research Ethics Committee (REC) of the Hospital das Clínicas of the Faculty of Medicine of the University of São Paulo (HCFMUSP) with approval number 797466 (date: 11.11.2022). Samples were collected between April 2010 and January 2012, and were analyzed for this study in March 2023. The waiver of the informed consent form was approved by the REC. The patients' data were anonymized, ensuring that no identifiable information was used. Additionally, the age of all patients was over 18 years.

Immunohistochemical Analysis

IHC reactions were performed on the automated VENTANA BenchMark GX System (Roche Diagnostics, Mannheim, Germany). The anti-SOD2 polyclonal antibody produced in rabbits (Abcam, ab13533) was used at a concentration of 1:1000 according to the manufacturer's instructions. Positive reactions were visualized with a cocktail containing peroxidase-conjugated secondary anti-rabbit antibodies using the UltraView Universal 3,3'-Diaminobenzidine Detection Kit (Ventana Medical Systems Inc, Roche, Tucson, Arizona, USA) according to the manufacturer's instructions. Each of the 80 samples from breast tumors was evaluated in triplicate. Fully standardized TNBC biopsies were employed as both positive and negative controls. The negative control underwent no application of the primary antibody. The IHC results were evaluated according to the extent (E) and intensity (I) of SOD2 staining. Values from 0 to 3 were assigned to both parameters. The value 0 (zero) was assigned to samples without detectable staining for SOD2, 1 when was less than 30% of tumor cells were stained, 2 when 30 to 60% of tumor cells were stained and 3 when SOD2 staining was detected in more than 60% of tumor cells. For the intensity, the value 0 corresponds to no staining reaction, 1 for weak staining intensity, 2 for mild staining intensity and 3 for strong staining intensity. For each sample, the average between the triplicates for the variables extension and intensity was calculated, then a staining score was established by multiplying the mean values of E and I for each sample [x-E×(x-I)], resulting in a last score ranging from 0 to 9 as described by Klein and colleagues (18). Finally, the average of all scores obtained was extracted. The samples were classified as "low" when the score was below the mean value or "high" when the score was above the mean. Based on this classification, a contingency table and survival curves were constructed.

In Silico Analysis

To complement the findings of the IHC investigation, we additionally conducted an *in silico* analysis to investigate the expression pattern of SOD2 at transcriptional levels and determine its reproducibility in other patient groups. Data from the Metabric project available on the cBioPortal for Cancer Genomics were accessed. The cBioPortal (https://cbioportal.org) is a database that provides access to genomic and transcriptomic data and is hosted by the Center for Molecular Oncology at MSK (19, 20). In the present study, we accessed the SOD2 expression of 1,980 cases of patients with breast cancer. The microarray mRNA data were downloaded and correlated with the clinicopathological information of the patients. Missing data was excluded. For the construction of the contingency table, the median was initially obtained from the SOD2 expression data. Values below the median were classified as low and values above the median were defined as high. Then, a chi-square test or Fisher's exact test was applied. The low/high classification was also used for survival analysis using the Kaplan-Meier method and the logRank test.

PrognoScan is a user-friendly tool for fast access to microarray data. Using this tool, it is possible to access different genes and observe prognostic associations (21). In this study, we utilized SOD2 as the input and downloaded the relevant studies (p<0.05) for the graphical construction of the forest plot using MedCalc software (https://www.medcalc.org/).

Statistical Analysis

The assessment of Gaussian distribution was conducted to determine the most appropriate statistical test. Qualitative variables were analyzed using the chi-square test or Fisher's exact test. The t-test or Mann-Whitney test were applied to assess possible differences between two groups. ANOVA or Kruskal-Wallis was applied to analyze differences between three or more groups. Kaplan-Meier method and the log-rank test were utilized for survival analysis. Employing Cox regression, both univariate and multivariate analyses were performed. To achieve this, data from patients who underwent IHC analysis were utilized, alongside the patient cohort sourced from the Metabric database. This combined approach allowed for a comprehensive exploration of the factors influencing the outcomes under investigation. Results were considered statistically significant when $p<0.05$. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 25 (IBM Inc., Armonk, NY, USA) or GraphPad Prism v. 7 (California, USA).

Results

Immunohistochemistry

The tissue microarray (TMA) employed in this study comprised samples from 92 patients, with seven exclusions due to incomplete clinicopathological data, and an additional five exclusions resulting from sample loss during technical procedures. In this way, a total of 80 samples were used for statistical analyses. Illustrated in Figure 1 are representative images showing the SOD2 staining patterns within the analyzed samples. Notably, elevated SOD2 results manifest a distinctive punctate cytoplasmic staining pattern, while exhibiting an absence of observable membrane or nuclear staining. To facilitate subsequent analyses, samples scoring below the mean were categorized as low, while those surpassing the mean were denoted as high.

Significant associations were observed between IHC results for SOD2 and clinicopathological aspects of the study population. There were differences in ER ($p<0.0001$), PR ($p<0.0001$) levels, tumor stage ($p = 0.042$), size of the tumor ($p = 0.0296$) lymph node involvement ($p = 0.0486$) and molecular classification ($p<0.0001$). Regarding age, HER2 and metastasis status, no significant differences were found (Table 1).

In addition, regarding the TMA, we used the score to analyze the dispersion of data based on classical markers in breast cancer. We observed that ER-negative patients had a higher SOD2 score compared to those who were ER-positive ($p = 0.0008$) (Figure 2A). Similarly, PR-negative samples exhibited higher SOD2 scores ($p = 0.0003$) (Figure 2B). No differences were observed in relation to HER2 ($p = 0.083$), possibly due to the limited number of samples (Figure 2C). Regarding the tumor subtype, luminal A were found to have a lower SOD2 score, while TNBC showed significantly higher average SOD2 scores ($p<0.0001$) (Figure 2D).

In order to assess the prognostic value of SOD2 in the studied population, we conducted a survival analysis. It was observed that patients with higher SOD2 expression had a worse overall survival (hazard ratio = 2.10; $p = 0.0363$; Figure 3A). Regarding relapse-free survival (RFS), the analysis did not reveal significant differences (hazard ratio = 2.22; $p = 0.0748$; Figure 3B).

In Silico Contributions

To further confirm our observations in a larger number of samples from different centers, we conducted an *in silico* analysis. Significant differences were observed regarding patients' age and menopausal status ($p<0.0001$), cellularity ($p<0.0001$), tumor subtype ($p<0.0001$), ER ($p<0.0001$) and PR ($p<0.0001$) expression, HER2 ($p<0.0001$) expression, and according to the Neoplasm Histologic Grade ($p<0.0001$) (Table 2).

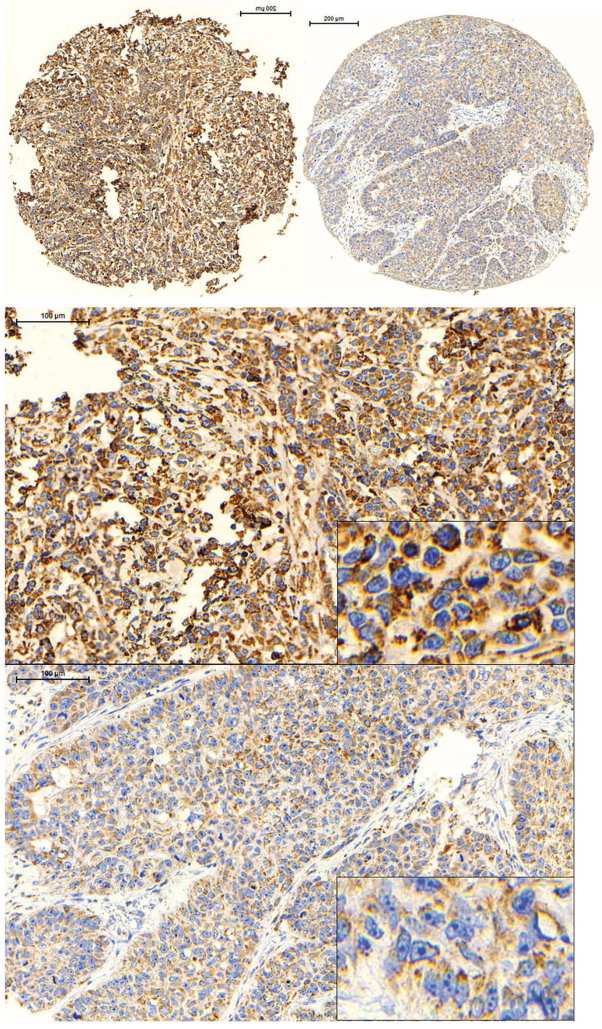


Figure 1. Representative images of SOD2 staining within breast cancer samples are presented. The images reveal **A, C.** High score and **B, D.** Low score. The upper images are captured at a 5X magnification, while the lower images are depicted at a 10X magnification. Additionally, an inset image is provided at a 20X magnification for detailed examination

SOD2: Superoxide dismutase 2

Furthermore, in relation to the patients in the Metabric study, we observed increased levels of SOD2 transcript in patients with negative staining for ER ($p<0.0001$; Figure 4A) and PR ($p<0.0001$; Figure 4B). Interestingly, with a larger number of cases, it was possible to observe a significant difference in terms of HER2 status ($p<0.0001$; Figure 4C). A similar profile was also observed regarding tumor subtypes, with lower SOD2 levels in luminal tumors and higher levels in basal tumors ($p<0.0001$; Figure 4D).

Elevated SOD2 expression demonstrated a correlation with a less favorable 120-month overall survival rate (hazard ratio = 1.40; $p<0.0001$; Figure 5A) Furthermore, it exhibited a similar association with an adverse relapse-free survival rate (hazard ratio = 1.31; $p<0.0001$; Figure 5B).

We then conducted an analysis using ROC plotter and plotter. We observed that non-responders had high SOD2 expression (p<0.0001; Figure 6A, B). The data were analyzed, and the hazard ratio along with the confidence interval observed in each study were used to construct the forest plot. Using this approach, we observed that high SOD2 expression was associated with different overall survival, relapse-free survival, metastasis-free survival, and disease-specific survival of patients with breast cancer (Figure 6C).

Finally, we conducted a univariate and multivariate analysis with the aim of exploring the factors influencing the clinical outcome of the patients. For this purpose, classical variables were employed for both the group of patients analyzed through IHC and the group

Table 1. Clinicopathological parameters and association with immunohistochemical staining for SOD2

Clinical parameters	Low (score ≤1.2)		High (score >1.2)		
	n	%	n	%	*p*-value
Age					
≤50	9	17.00	9	33.30	0.0980
>50	44	83.00	18	66.70	
ER					
Negative	6	11.50	14	56.00	*p*<0.0001
Positive	46	88.50	11	44.00	
PR					
Negative	8	15.40	18	66.70	*p*<0.0001
Positive	44	84.60	9	33.30	
HER2					
Negative	46	90.20	19	73.10	0.0930
Positive	5	9.80	7	26.90	
Tumor stage					
1	13	24.50	1	3.70	0.0129
2	27	50.90	11	40.70	
3	9	17.00	12	44.40	
4	4	7.50	3	11.10	
T					
1	16	30.20	2	7.40	0.0296
2	26	49.10	12	44.40	
3	6	11.30	7	25.90	
4	5	9.40	6	22.20	
N					
Negative	28	52.80	8	29.60	0.0486
Positive	25	47.20	19	70.40	
M					
Negative	49	92.50	24	88.90	0.6829
Positive	4	7.50	3	11.10	
Molecular classification					
Luminal A	12	26.10	2	8.00	*p*<0.0001
Luminal B	29	63.00	7	28.00	
HER2	2	4.30	5	20.00	
TNBC	3	6.50	11	44.00	

The low or high category was defined based on the average score obtained. ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor type 2; T: Tumor; N: Node; M: Metastasis; TNBC: Triple-negative breast cancer; SOD2: Superoxide dismutase 2

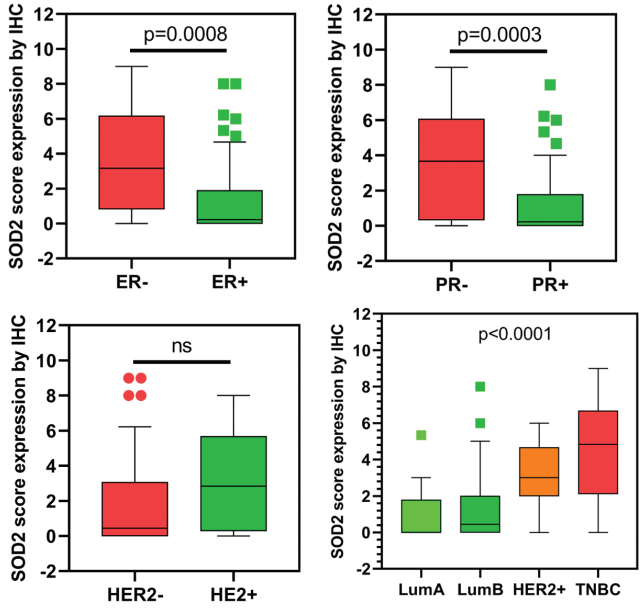


Figure 2. Scoring SOD2 after immunohistochemical technique. SOD2 score based on **A.** Estrogen receptor status; **B.** Progesterone receptor status; **C.** Human epidermal growth factor receptor type 2 status; and **D.** Molecular subtype of patients with breast cancer

SOD2: Superoxide dismutase 2

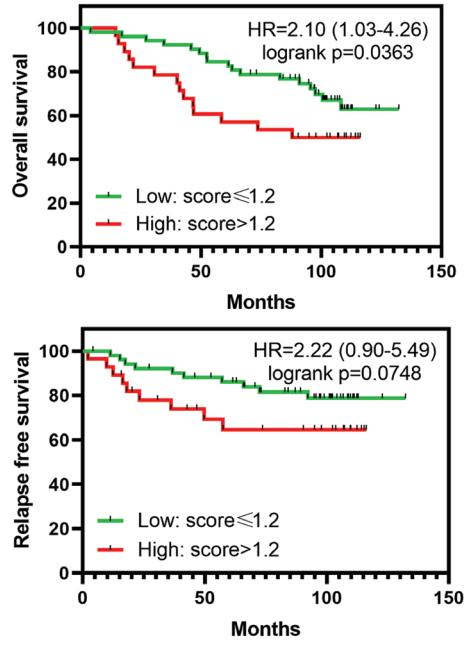


Figure 3. Survival analysis as a function of SOD2 staining. **A.** Overall survival and **B.** Relapse-free survival of patients with breast cancer based on SOD2 score

SOD2: Superoxide dismutase 2

of patients sourced from the Metabric database. In the univariate analysis, significant differences were observed in terms of ER (p = 0.002; p<0.0001) and PR (p = 0.02; p<0.0001), triple-negative and non-triple-negative status (p = 0.01; p = 0.0008), and SOD2 (p = 0.04; p<0.0001) in both patient groups (Table 3). With a larger patient sample and considering the distinct SOD2 evaluation technique, significant differences were also observed in terms of patient age (p = 0.01) and HER2 status (p<0.0001). In the multivariate analysis, no significant differences were observed concerning the group of

Table 2. Clinicopathological parameters and association with SOD2 expression of patients from the Metabric study

Clinical parameters	Low (mRNA ≤8.26)		High (mRNA >8.26)		*p*-value
	n	%	n	%	
Age					
≤50	175	17.70	249	25.20	<0.0001
>50	815	82.30	741	74.80	
Menopausal state					
Pre	175	17.70	249	25.20	<0.0001
Post	815	82.30	741	74.80	
Cellularity					
Low	112	11.70	103	10.70	0,002
Moderate	400	41.90	337	35.00	
High	443	46.40	522	54.30	
PAM50 with claudin-low subtype					
Basal	24	2.40	185	18.70	<0.0001
Claudin-low	27	2.70	191	19.30	
HER2	65	6.60	159	16.10	
Luminal A	512	52.00	188	19.00	
Luminal B	260	26.40	215	21.70	
Normal-like	97	9.80	51	5.20	
ER					
Negative	74	7.60	365	37.70	<0.0001
Positive	894	92.40	604	62.30	
PR					
Negative	335	33.80	605	61.10	<0.0001
Positive	655	66.20	385	38.90	
HER2					
Negative	918	92.70	815	82.30	<0.0001
Positive	72	7.30	175	17.70	
Neoplasm histologic grade					
1	114	12.20	55	5.80	<0.0001
2	489	52.20	282	29.50	
3	333	35.60	619	64.70	

Low or high expression according to median value. Analysis using the chi-square technique. Microarray data of breast cancer patients from the Metabric study; HER2: Human epidermal growth factor receptor type 2; SOD2: Superoxide dismutase 2

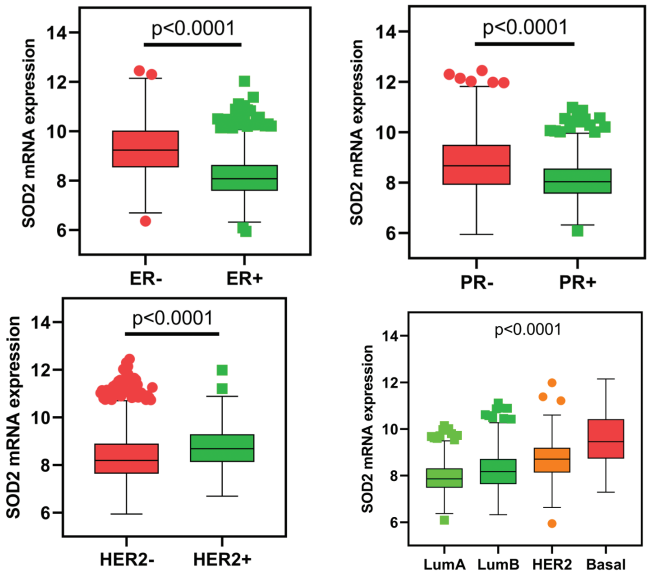


Figure 4. Transcriptional expression of SOD2. SOD2 mRNA expression based on **A.** Estrogen receptor; **B.** Progesterone receptor; **C.** Human epidermal growth factor receptor type 2; and **D.** Molecular subtype. As for the molecular subtype, clowdin-low and normal-like were excluded. Data analyzed from the Metabric study

LumA: Luminal A; LumB: Luminal B; HER2: Human epidermal growth factor receptor type 2; ER: Estrogen receptor; PR: Progesterone receptor; SOD2: Superoxide dismutase 2. Patients from the Metabric study and mRNA expression data accessed through cBioPortal

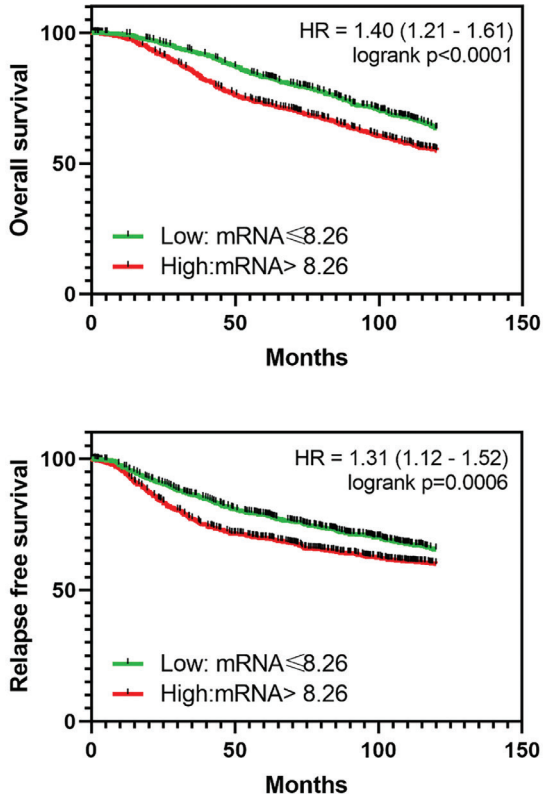


Figure 5. 120-month survival of patients with breast cancer from the Metabric study. **A.** Overall survival as a function of increased or reduced SOD2 expression. **B.** Recurrence-free survival as a function of increased or reduced SOD2 expression

SOD2: Superoxide dismutase 2

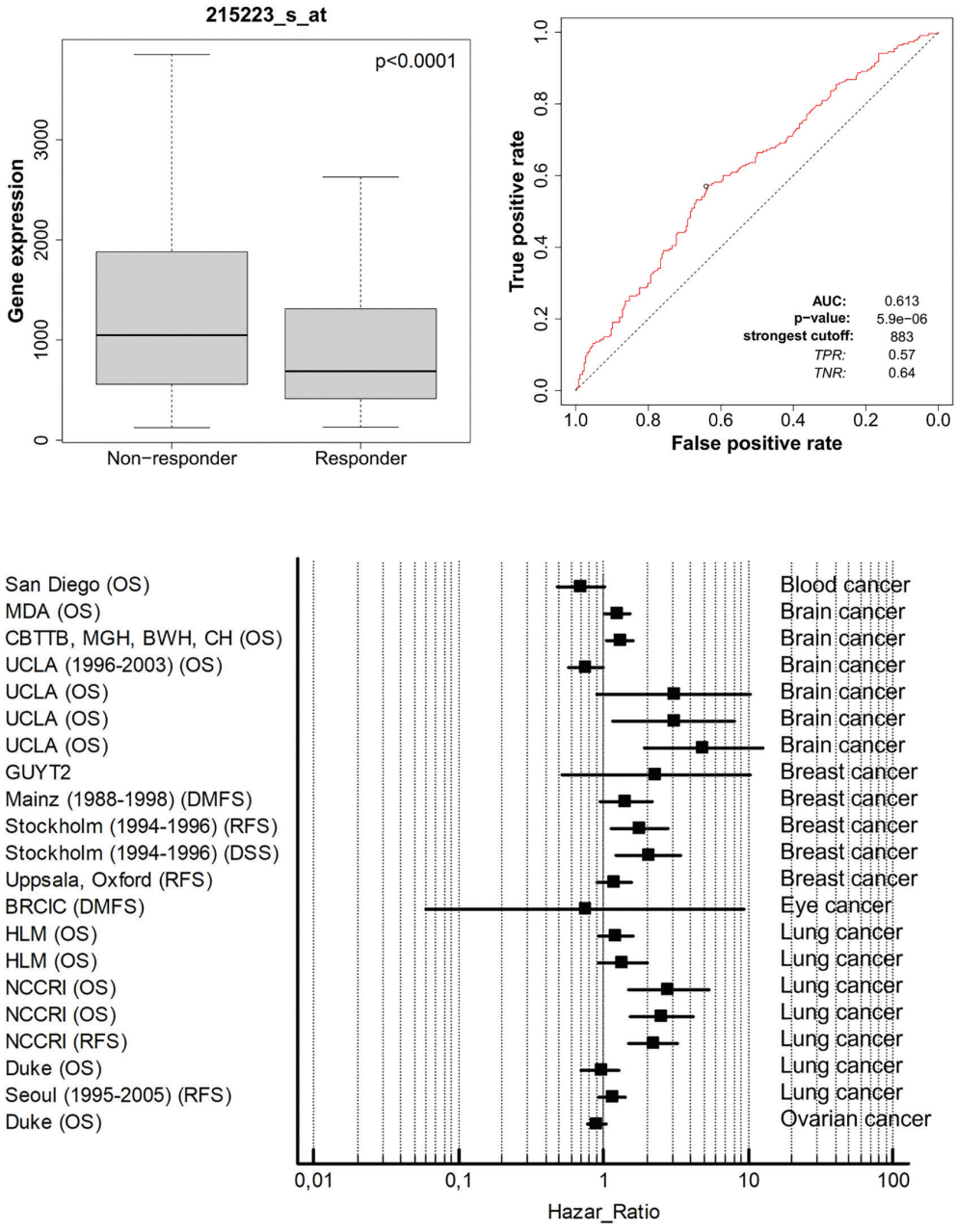


Figure 6. ROC plotter analysis and forest plot. **A.** SOD2 expression profile and **B.** ROC curve of responders and non-responders to chemotherapy treatments. **C.** Forest plot generated from the studies obtained through the PrognoScan search. The central square represents the hazard ratio (HR) of each study and the bold horizontal lines represents the confidence interval (lower and upper bounds). Studies with HR less than 1 indicate a protective factor, while HR greater than 1 indicates risk

OS: Overall survival; RFS: Relapse free survival; DMFS: Distant metastasis free survival; ROC: Receiver operating characteristic; The forest plot was constructed in MedCalc software

patients evaluated through IHC. Conversely, among patients from the Metabric cohort, significant differences were observed in terms of age (p<0.0001), ER (p<0.0001), PR (p<0.0001), HER2 (p<0.0001), and SOD2 (p<0.0001).

Discussion

Despite significant advances in cancer treatment, a considerable number of patients suffer from relapses and/or therapeutic resistance, resulting in high mortality rates. Therefore, the search for new therapeutic and prognostic targets is important. SOD2 has been investigated in several studies, including those conducted by our research group. In a previous study, using cervical tumor cell lines, our group identified alterations in the SOD2 expression pattern after treatment with tumor necrosis factor (9). In the literature, other authors have explored approaches involving drugs capable of modulating of SOD2 (22). Thus, understanding the expression pattern of SOD2 in human tumors, including its subtypes in the case of breast cancer, is essential to identify and characterize tumor profiles that are candidates for therapies that may directly or indirectly affect their transcriptional and protein levels.

In the present study, we observed elevated SOD2 staining in hormone receptor-negative breast cancer patients. These results agree with the

Table 3. Univariate and multivariate analysis of patients with breast cancer assessed by IHC and patients from the Metabric study

Variable HR (95% CI)		IHC staining		Metabric mRNA	
		p	HR (95% CI)	*p*	
Univariable					
Age	≤50	1	0.5082	1	0.0103
	>50	0.76 (0.34–1.68)		1.28 (1.06–1.54)	
ER	Positive	1	0.0025	1	<0.0001
	Negative	3.03 (1.48–6.20)		1.57 (1.34–1.85)	
PR	Positive	1	0.0181	1	<0.0001
	Negative	2.32 (1.15–4.66)		1.63 (1.41–1.88)	
HER2	Negative	1	0.2609	1	<0.0001
	Positive	1.62 (0.70–3.73)		1.75 (1.44–2.11)	
TN nTN	nTN	1	0.0115	1	0,0008
	TN	2.78 (1.26–6.13)		1.39 (1.15–1.69)	
SOD2	Negative	1	0.0407	1	
	Positive	2.10 (1.03–4.26)		1.40 (1.21–1.61)	<0.0001
Multivariable					
Age	≤50	1	0.3881	1	<0.0001
	>50	1.45 (0.62–3.41)		1.45 (1.19–1.76)	
ER	Positive	1	0.4670	1	0,0293
	Negative	2.17 (0.27–17.60)		1.24 (1.02–1.51)	
PR	Positive	1	0.9288	1	<0.0001
	Negative	0.91 (0.11–7.50)		1.37 (1.16–1.61)	
HER2	Negative	1	0.6625	1	<0.0001
	Positive	1.23 (0.48–3.15)		1.47 (1.20–1.80)	
SOD2	Negative	1	0.4268	1	0,0299
	Positive	1.43 (0.59–3.49)		1.19 (1.02–1.39)	

TN: triple negative; nTN: non triple negative; HR: Hazard ratio; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor type 2; SOD2: Superoxide dismutase 2; IHC: Immunohistochemistry; CI: Confidence interval

study conducted by Li and colleagues, who identified an elevated SOD2 transcriptional expression pattern in patients with breast cancer, compared to adjacent tissue (23). In addition, Li's group analyzed a population of 60 patients and observed higher SOD2 staining in cancer samples compared to non-tumor tissue (23). Many studies have reported that the SOD2 enzyme in the context of tumors acts as an oncogene, promoting signaling for malignant transformation, cell survival and ability to generate tumor stem cells (24). It is well known that SOD2 function cannot be replaced by others SOD family member such as SOD1. SOD2 knockout in mice model, for example, resulted in neonatal lethality revealing its importance for normal cell functionality. However, SOD2 plays a dichotomous role and is regulated differently during tumor progression depending on the tumor type. Interestingly, some cancers exhibit increased SOD2 expression compared to healthy tissues (25). Therefore, considering our data and other studies regarding SOD2 role in the context of breast cancer, we can infer that increased levels of SOD2 in tumor tissue may indicate an oncogenic role in breast tissue.

We found that increased expression of SOD2 was associated with advanced tumor stages and lymph node metastases, as well as worse overall survival (Figure 3A). These findings are supported by the study conducted by Li et al. (23), although they performed survival analysis on a different population than the one assessed by IHC. Our distinct approach included survival analysis on the same population subjected to IHC for SOD2 detection. In addition, we observed a clear differentiation between the "low" and "high" SOD2 expression curves for RFS (Figure 3B). Although there was no significant difference by the logRank test, with the *p*-value close to 0.05, a larger sample size may provide additional confirmation of this trend. Following a 60-month follow-up, only two events were observed (Figure 3B). Given that most recurrences occur within the first 60 months (26), and few events were observed in our population after this period, we decided to analyze the data adjusted for 60 months, with subsequent events censored. In this context, we observed a significant association between high SOD2 expression and increased likelihood of tumor recurrence (Supplementary Figure 1). These findings not only reinforce the importance of our initial discoveries but also underscore

the clinical potential of SOD2 as a predictive marker for identifying individuals at higher risk of tumor recurrence.

In the following assessment, it becomes apparent that elevated SOD2 expression is associated with cancer cases that are unresponsive to conventional chemotherapy agents. Moreover, by utilizing the Metabric cohort, both OS and RFS have demonstrated significance, thus solidifying our initial hypothesis. Finally, we employed Cox regression to conduct both univariate and multivariate analyses, uncovering a range of significant associations, including the classical hormonal receptors of breast cancer, triple-negative and non-triple-negative statuses, and SOD2. In summary, the present study's results indicate that various factors, including receptor status (ER, PR), triple-negative status, SOD2 levels, age, and HER2 status, can influence the clinical outcomes of patients with the analyzed condition. The impact of these factors might differ depending on the patient cohort and the evaluation techniques used (IHC or Metabric database). It is likely that with a larger sample size and the use of the IHC technique, we may observe similar results to those found in the Metabric dataset. Furthermore, the multivariate analysis suggests that some of these factors might have more pronounced effects when considered together rather than individually.

In our analysis, TNBC showcased the highest mean SOD2 scores among molecular subtypes (p<0.0001). This subgroup of tumors represents a remarkably diverse cohort, comprising approximately 12-17% of all breast cancer cases (27). Due to the absence of classical markers, such as ER, PR and HER-2, this tumor subtype does not benefit from endocrine or target-directed therapies. Although the patients initially respond to a therapeutic strategy including surgery, radiotherapy and chemotherapy, they eventually develop therapeutic resistance, leading to tumor recurrence. Therefore, our data are of paramount importance to encourage research studies of potential therapeutic targets for this population (28-30). So far, there are no specific therapies targeted at SOD2. However, its potential as a biomarker in the current context could be valuable, especially regarding its role in therapeutic resistance in patients, particularly those with TNBC. Nonetheless, additional investigations are imperative to unravel the mechanisms that underlie the elevated SOD2 expression, thereby facilitating a more comprehensive exploration of its therapeutic potential.

Conclusion

Our analyses revealed significant correlations between increased SOD2 expression and hormone receptor negativity. Moreover, consistently elevated levels of SOD2 were observed in TNBC compared to luminal and HER2 subtypes. This observation is particularly intriguing as it suggests a specific association between SOD2 and TNBC, a subtype known for its aggressiveness and treatment resistance. The observation of high SOD2 levels in these tumors may have important implications for understanding the underlying mechanisms of TNBC's aggressive biology and for developing more effective targeted therapies.

Lastly, analysis of IHC and transcriptomic data unveiled a clear association between high SOD2 levels and poorer prognosis. This link between elevated SOD2 and adverse clinical outcomes strongly suggests that this antioxidant enzyme plays a crucial role in breast cancer progression and disease course determination. The consistency of these results across different analytical platforms further confirms the clinical and prognostic importance of SOD2 in the context of breast cancer, highlighting its potential as a promising therapeutic target and valuable prognostic marker.

Acknowledgment

We would like to express our heartfelt gratitude to Prof. Dr. Maria Aparecida Nagai, Dr. Evandro Sobroza de Melo, and the Pathological Anatomy service of the Faculty of Medicine of the University of São Paulo for generously providing us with the tissue microarray samples.

Ethics Committee Approval: This study was approved by the Research Ethics Committee (REC) of the Hospital das Clínicas of the Faculty of Medicine of the University of São Paulo (HCFMUSP) with approval number 797466 (date: 11.11.2022).

Informed Consent: The waiver of the informed consent form was approved by the REC.

Authorship Contributions

Surgical and Medical Practices: D.R.d.B., A.L-F., M.P.F.C., L.T.; Concept: D.R.d.B., A.L-F., M.P.F.C., L.T.; Design: D.R.d.B., A.L-F., M.P.F.C., L.T.; Data Collection and/or Processing: D.R.d.B., A.L-F., M.P.F.C., L.T.; Analysis and/or Interpretation: D.R.d.B., A.L-F., M.P.F.C., L.T.; Literature Search: D.R.d.B., A.L-F., M.P.F.C., L.T.; Writing: D.R.d.B., A.L-F., M.P.F.C., L.T.

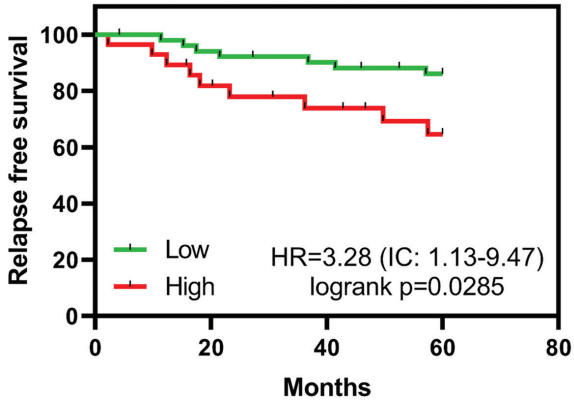
Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021; 71: 209-249. (PMID: 33538338) [Crossref]
2. Perou CM, Sørile T, Eisen MB, Van De Rijn M, Jeffrey SS, Ress CA, et al. Molecular portraits of human breast tumours. Nature 2000; 406: 747-752. (PMID: 10963602) [Crossref]
3. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001; 98: 10869-10874. (PMID: 11553815) [Crossref]
4. Ignatov A, Eggemann H, Burger E, Ignatov T. Patterns of breast cancer relapse in accordance to biological subtype. J Cancer Res Clin Oncol 2018; 144: 1347-1355. (PMID: 29675790) [Crossref]
5. Andrés CMC, Pérez de la Lastra JM, Juan CA, Plou FJ, Pérez-Lebeña E. Chemistry of Hydrogen Peroxide Formation and Elimination in Mammalian Cells, and Its Role in Various Pathologies. Stresses 2022; 2: 256-274. [Crossref]
6. Amos A, Jiang N, Zong D, Gu J, Zhou J, Yin L, et al. Depletion of SOD2 enhances nasopharyngeal carcinoma cell radiosensitivity via ferroptosis induction modulated by DHODH inhibition. BMC Cancer 2023; 23: 117. (PMID: 36737723) [Crossref]
7. Nikic P, Dragicevic D, Jerotic D, Savic S, Djukic T, Stankovic B, et al. Polymorphisms of Antioxidant Enzymes SOD2 (rs4880) and GPX1 (rs1050450) Are Associated with Bladder Cancer Risk or Its Aggressiveness. Medicina (Kaunas) 2023; 59: 131. (PMID: 36676755) [Crossref]
8. Wang R, Yin C, Li X-X, Yang X-Z, Yang Y, Zhang M-Y, et al. Reduced SOD2 expression is associated with mortality of hepatocellular carcinoma patients in a mutant p53-dependent manner. Aging (Albany NY) 2016; 8: 1184-1200. (PMID: 27221200) [Crossref]

9. Termini L, Boccardo E, Esteves GH, Hirata R, Martins WK, Colo AE, et al. Characterization of global transcription profile of normal and HPV-immortalized keratinocytes and their response to TNF treatment. BMC Med Genomics 2008; 1: 29. (PMID: 18588690) [Crossref]
10. Termini L, Fregnani JH, Boccardo E, da Costa WH, Longatto-Filho A, Andreoli MA, et al. SOD2 immunoexpression predicts lymph node metastasis in penile cancer. BMC Clin Pathol 2015; 15: 3. (PMID: 25745358) [Crossref]
11. Termini L, Filho AL, Maciag PC, Etlinger D, Alves VAF, Nonogaki S, et al. Deregulated expression of superoxide dismutase-2 correlates with different stages of cervical neoplasia. Dis Markers 2011; 30: 275–281. (PMID: 21725155) [Crossref]
12. Rabelo-Santos SH, Termini L, Boccardo E, Derchain S, Longatto-Filho A, Andreoli MA, et al. Strong SOD2 expression and HPV-16/18 positivity are independent events in cervical cancer. Oncotarget 2018; 9: 21630-21640. (PMID: 29774090) [Crossref]
13. Talarico MCR, Nunes RAL, Silva GÁF, Costa LBE da, Cardoso MR, Esteves SCB, et al. High Expression of SOD2 Protein Is a Strong Prognostic Factor for Stage IIIB Squamous Cell Cervical Carcinoma. Antioxidants (Basel) 2021; 10: 724. (PMID: 34062984) [Crossref]
14. Yao S, Barlow WE, Albain KS, Choi J-Y, Zhao H, Livingston RB, et al. Manganese superoxide dismutase polymorphism, treatment-related toxicity and disease-free survival in SWOG 8897 clinical trial for breast cancer. Breast Cancer Res Treat 2010; 124: 433-439. (PMID: 20309628) [Crossref]
15. Cronin-Fenton DP, Christensen M, Lash TL, Ahern TP, Pedersen L, Garne JP, et al. Manganese superoxide dismutase and breast cancer recurrence: a Danish clinical registry-based case-control study, and a meta-analysis. PLoS One 2014; 9: e87450. (PMID: 24498107) [Crossref]
16. Coelho DR, Palma FR, Paviani V, He C, Danes JM, Huang Y, et al. Nuclear-localized, iron-bound superoxide dismutase-2 antagonizes epithelial lineage programs to promote stemness of breast cancer cells via a histone demethylase activity. Proc Natl Acad Sci U S A 2022; 119: e2110348119. (PMID: 35858297) [Crossref]
17. Becuwe P, Ennen M, Klotz R, Barbieux C, Grandemange S. Manganese superoxide dismutase in breast cancer: from molecular mechanisms of gene regulation to biological and clinical significance. Free Radic Biol Med 2014; 77: 139-151. (PMID: 25224035) [Crossref]
18. Klein M, Vignaud J-M, Hennequin V, Toussaint B, Bresler L, Plénat F, et al. Increased expression of the vascular endothelial growth factor is a pejorative prognosis marker in papillary thyroid carcinoma. J Clin Endocrinol Metab 2001; 86: 656-658. (PMID: 11158026) [Crossref]
19. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012; 2: 401-404. (PMID: 22588877) [Crossref]
20. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013; 6: pl1. (PMID: 23550210) [Crossref]
21. Mizuno H, Kitada K, Nakai K, Sarai A. PrognoScan: a new database for meta-analysis of the prognostic value of genes. BMC Med Genomics 2009; 2: 18. (PMID: 19393097) [Crossref]
22. Jia J, Zhang L, Shi X, Wu M, Zhou X, Liu X, et al. SOD2 Mediates Amifostine-Induced Protection against Glutamate in PC12 Cells. Oxid Med Cell Longev 2016; 2016: 4202437. (PMID: 26770652) [Crossref]
23. Li J, Liu Y, Liu Q. [Expression of superoxide dismutase 2 in breast cancer and its clinical significance]. Nan Fang Yi Ke Da Xue Xue Bao 2020; 40: 1103-1111. (PMID: 32895185) [Crossref]
24. Hecht F, Pessoa CF, Gentile LB, Rosenthal D, Carvalho DP, Fortunato RS. The role of oxidative stress on breast cancer development and therapy. Tumour Biol 2016; 37: 4281-4291. (PMID: 26815507) [Crossref]
25. Kim YS, Gupta Vallur P, Phaëton R, Mythreye K, Hempel N. Insights into the Dichotomous Regulation of SOD2 in Cancer. Antioxidants (Basel) 2017; 6: 86. (PMID: 29099803) [Crossref]
26. Nakano M, Fujisue M, Tashima R, Okumura Y, Nishiyama Y, Ohsako T, et al. Survival time according to the year of recurrence and subtype in recurrent breast cancer. Breast 2015; 24: 588-593. (PMID: 26099804) [Crossref]
27. Ouyang M, Li Y, Ye S, Ma J, Lu L, Lv W, et al. MicroRNA profiling implies new markers of chemoresistance of triple-negative breast cancer. PLoS One 2014; 9: e96228. (PMID: 24788655) [Crossref]
28. Carlisi D, De Blasio A, Drago-Ferrante R, Di Fiore R, Buttitta G, Morreale M, et al. Parthenolide prevents resistance of MDA-MB231 cells to doxorubicin and mitoxantrone: the role of Nrf2. Cell Death Discov 2017; 3: 17078. (PMID: 29354292) [Crossref]
29. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 2007; 13: 2329-2334. (PMID: 17438091) [Crossref]
30. O'Reilly EA, Gubbins L, Sharma S, Tully R, Guang MHZ, Weiner-Gorzel K, et al. The fate of chemoresistance in triple negative breast cancer (TNBC). BBA Clin 2015; 3: 257-275. (PMID: 26676166) [Crossref]



Supplementary Figure 1. Overall survival analysis as a function of adjusted SOD2 staining over a 60-month period

SOD2: Superoxide dismutase 2

Original Article

Eur J Breast Health 2024; 20(3): 194-198
DOI: 10.4274/ejbh.galenos.2024.2024-3-2

A Comparative Study of Drainage of Breast Abscesses by Conventional Incision and Drainage vs Ultrasound-Guided Needle Aspiration/Re-Aspiration in A Tertiary Health Care Centre

Varsha Madhavanarayanan Totadri[1], Rishwanth Vetri[2], Surabhi Sainath[2]

[1]Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India
[2]Department of General Surgery, Stanley Medical College, Chennai, India

ABSTRACT

Objective: Breast abscesses are localized purulent collections, often arising from bacterial mastitis, and pose significant health risks, especially for lactating women. The aim of this study was to compare the efficacy and outcomes of two different treatment approaches: Traditional incision and drainage (I&D) versus ultrasound (USG)-guided aspiration in breast abscess management.

Materials and Methods: Fifty female patients with breast abscesses were enrolled and divided into two groups: Group A (n = 25, I&D) and group B (n = 25, USG-guided aspiration). Group A underwent I&D under general anaesthesia and group B underwent USG-guided aspiration under local anaesthesia. The patients were followed up for two weeks after the procedure. Patient demographics, abscess characteristics, treatment outcomes, and complications were analyzed.

Results: The mean age of patients was 36.4 and 31.8 in group A and B, respectively and the mean abscess size was 5.7 cm. The study found that USG-guided aspiration was associated with several advantages over I&D. Patients in group B experienced shorter healing times (5 days vs. 13 days, $p = 0.001$), lower rates of residual abscesses (12% *vs.* 36%, $p = 0.047$), and no recurrence after two weeks vs. 28% in group A ($p = 0.012$). Notably, the resumption of lactation was significantly greater in group B (91.67% *vs.* 20%). Importantly, patients in group B had no scarring, while 37% in group A healed with scars.

Conclusion: These results highlight that USG-guided aspiration offers a minimally invasive and effective method for managing breast abscesses, leading to quicker recovery, better cosmetic outcomes, and higher patient satisfaction compared to the traditional I&D approach. Early diagnosis and intervention with USG-guided aspiration can prevent complications and reduce the need for open surgery. Based on these findings, USG-guided aspiration is a safer and more efficient method for treating breast abscesses, particularly when initiated promptly after diagnosis.

Keywords: Abscess; benign; breast; needle aspiration; ultrasound-guided

Cite this article as: Totadri VM, Vetri R, Sainath S. A Comparative Study of Drainage of Breast Abscesses by Conventional Incision and Drainage vs Ultrasound-Guided Needle Aspiration/Re-Aspiration in A Tertiary Health Care Centre.

Key Points

- Advantages of ultrasound-guided aspiration: The results indicate several advantages associated with ultrasound-guided aspiration, including shorter healing times, lower rates of residual abscesses, no recurrence after two weeks, higher rates of lactation resumption, and minimal scarring. These findings suggest that ultrasound-guided aspiration may offer superior outcomes compared to the traditional incision and drainage approach.
- Clinical implications: The study underscores the clinical implications of adopting ultrasound-guided aspiration as a minimally invasive and effective method for managing breast abscesses.

Introduction

A breast abscess is defined as a localized collection of purulent material in the breast surrounded by a pyogenic membrane (1). Typically, breast abscesses originate from bacterial mastitis, most commonly caused by *Staphylococcus aureus*. Abscess formation is the most feared complication of parenchymal infection, predominantly observed in lactating women, and often originating from a sore or cracked nipple. The reported incidence of abscesses in lactation-related mastitis ranges from 4.8% to 11% (2). Complications may include severe necrotizing infections and sepsis (3).

The conventional approach to treating breast abscesses has been incision and drainage (I&D) with antibiotic coverage, primarily after initial unsuccessful needle aspiration. However, non-operative techniques such as percutaneous drainage using ultrasound (USG) have

Corresponding Author:
Rishwanth Vetri; rish.vetri94@gmail.com

Received: 12.03.2024
Accepted: 19.05.2024
Available Online Date: 01.07.2024

gained popularity, even in large abscesses that were once considered indications for I&D. The conventional method has major drawbacks, such as extended healing time, unfavorable cosmetic outcomes, and a higher risk of recurrence, leading to a significant shift in management protocols (4, 5).

In cases where image-guided aspiration, performed under antibiotic cover, fails to resolve the abscess or results in recurrence or increased size, surgical drainage under general anesthesia may still be necessary. I&D, along with wound debridement, may be required in cases of superficial abscesses with skin necrosis, and surgery may also be necessary when malignancy is suspected (4). The aim of this study was to compare the traditional I&D technique versus percutaneous USG-guided needle aspiration in terms of efficacy, healing time, cosmetic outcome and resumption of breast feeding. A preliminary preprint version of this article was previously posted in The Tamil Nadu Dr.M.G.R University repository on February 9, 2021.

Materials and Methods

Study Design

This was a randomized, controlled trial.

Sample Size

A total of 105 patients were referred to the Department of General Surgery at Stanley Hospital, Chennai, India, for the treatment of tender breast lumps between 2018 and 2019. All female patients underwent an initial USG examination using a commercially available portable ultrasound machine. The presumptive diagnosis of abscess was made when a homogenous or non-homogenous liquid collection was observed, often with some acoustic enhancement.

Study Criteria

All patients above the age of 12 years, diagnosed with an abscess in either breast (lactating or non-lactating), and not undergoing treatment for any other breast pathology were included in the study. Women with recurrent abscesses, a diagnosis of malignancy, or those unwilling to participate were excluded from the study.

Statistical Analysis

A data sheet was formulated to collect the data. The collected data were analysed using IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp). Frequency and percentage analysis were used for categorical variables (presence of residual abscess, recurrence rates, lactation resumption). Mean and standard deviation were used for continuous variables (patient age, abscess size, healing time). Chi-square analysis was used for categorical variables, and the independent sample t-test was used for the comparison of healing times.

Ethical Considerations

Prior to the commencement of the study, informed consent was obtained from all the participants, explaining the purpose, procedures, potential risks and benefits. Data collected, including personal details and medical records were anonymized and access was restricted to the research team. Participation was voluntary and patients were informed that their decision to participate or withdraw would not impact their medical care. The study protocol, including the research design, procedures and consent forms received approval from the Institutional Ethics Committee, Stanley Medical College, Chennai to ensure compliance with ethical guidance and standards (approval number: EC/NEW/INST/2018/461; date: 07.12.2018). The participants were also debriefed at the conclusion of the study, providing them with an understanding of the research goals and outcomes. All queries and concerns were addressed and participants were informed how their data would be used.

Procedure

Fifty-seven patients met the inclusion criteria after detailed history and examination, as well as USG. Seven patients refused to take part in the study and the remaining fifty were randomized into twenty-five patients each in groups A (I&D) and B (USG-guided aspiration). The patients were randomized using a computer generated randomization table. Written informed consent was obtained from the patients included in the study. Patients in the I&D arm were admitted and prepared for surgery under general anaesthesia in casualty theatre by the Principal Investigator. Hilton's method was used for I&D. Initial pus drained was sent for culture and sensitivity. The pus was then evacuated and loculi broken down digitally, the wound was packed with sterile gauze. Post-operatively the patient was on analgesics and appropriate antibiotics. The patient was discharged home after two days to undergo daily wound dressing at a nearby clinic until the wound healed.

Patients under the needle aspiration arm were managed in the department of interventional radiology as outpatient cases. Under aseptic condition, a small area of skin adjacent to the abscess was anaesthetized by 1% lignocaine through a 23 G needle. Aspiration was done under USG guidance using a 16 G needle and a 20 mL syringe. Initial aspirated pus was sent for culture and sensitivity against antibiotics. Aspiration was done until there was no significant residual pus. After the procedure the patient was discharged on antibiotics and analgesics. In both arms, lactating patients were advised to resume breast-feeding on both breasts as soon as they could tolerate the pain as the baby breastfeeds.

All patients were treated with appropriate empiric antibiotics and analgesics initially and were subsequently tailored according to pus culture and sensitivity results. Ultrasonogram of the breast was done on days 3.7 and 14 post operatively/post drainage to rule out residual abscess. Each patient was analyzed on the basis of residual abscess, recovery duration, recurrence of abscess and resumption of functionality for lactating mothers and the patients were followed up for a period of two weeks.

Results

The average age of the patients in group A was 36.4±10.21 years and group B was 31.8±8.01, ranging from 12 to 60 years, of whom a total of 47% were lactating mothers. Table 1 provides the age distribution of the patients.

The mean ± standard deviation abscess size was 5.7±3.4 cm (95% confidence interval, 2.8 to 8.1). Just over half (53%) of abscesses were noted in the left breast whilst the remainder were noted in the right. However, there was no significance in the size range of the abscess with respect to either breast or lactation status. There was no significant difference between group A and group B regarding age and side of breast involved. Table 2 shows the distribution of lactating mothers in each group.

Nearly all patients (94%) had a palpable breast mass. The abscess was localized to the left upper outer quadrant in 34% (n = 17), whereas whole breast involvement was present 8.6% (n = 6). Right (47%; n

= 23) and left (53%; *n* = 27) breasts were almost equally affected. Out of the 25 patients in group B, 52% (*n* = 13) were found to have undergone aspiration just once for resolution of the abscess. Table 3 shows the quadrants involved and Table 4 shows size of the abscess.

As far as symptoms were concerned, pain was present in 92% (*n* = 46) and fever in 58% (*n* = 29). On the third day after the procedure, out of 25 patients in group A, only one was normal (4%) while the rest (*n* = 24, 96%) had residual abscess, edema, minimal collection, subcutaneous edema or persistent loculations.

In comparison, in group B on the third day after the procedure, 44% (*n* = 11) returned to normal while the remaining 56% (*n* = 14) had had residual abscess, edema, minimal collection, and/or subcutaneous edema. Thirteen patients (26%) needed only a single aspiration and one week of antibiotics. Eight patients (16%) underwent aspiration twice, whereas four (8%) required three aspirations and more than one week of antibiotics.

In terms of duration of symptoms or residual abscess until the seventh post-operative day, 36% (*n* = 9) of group A patients were found to remain symptomatic while only 12% (*n* = 3) of those from group B had residual abscess <2x2 cm in size with no complaints ($p = 0.047$). Table 5 show the number of residual abscess on 7th post-operative day.

The mean healing time was 13±5.01 days in group A and 5±2.54 days in group B. The mean difference was significant ($p = 0.001$). Out of 25 patients in group B, none had recurrence after two weeks while around 28% (*n* = 7) of patients from group A returned with recurrence ($p = 0.012$). The Table 6 shows the recurrence in the two groups.

Table 1. Age distribution

Age distribution, years	I and D	USG guided aspiration
	25	25
Mean	36.4	31.8
Median	35	30
Mode	34	30
Standard deviation	10.27132	8.03119
Minimum	21	19
Maximum	56	48

I and D: Incision and drainage; USG: Ultrasonography

Table 2. Lactating patients for each group

Lactating	Management (method)		Total	Chi-square test *p*-value
	I and D	USG guided aspiration		
No	15	13	28	0.325, $p = 0.776$
Yes	10	12	22	
Total	**25**	**25**	**50**	

I and D: Incision and drainage; USG: Ultrasonography

Table 3. Quadrants involved

	Management (method)		Total
Quadrant	**I and D**	**USG guided aspiration**	
Two separate loculi	0	1	1
Diffuse	0	1	1
Diffuse, multiloculated	1	0	1
Diffuse, multiloculated with edema	1	0	1
Multiloculated	2	2	4
LLIQ (left lower inferior)	4	3	7
LLOQ (left lower outer)	3	2	5
LUIQ (left upper inferior)	2	4	6
LUOQ (left upper outer)	3	1	4
RLIQ (right lower inferior)	0	2	2
RLOQ (right lower outer)	3	4	7
RUIQ (right upper inner)	5	1	6
RUOQ (right upper outer)	1	4	5
Total	**25**	**25**	**50**

I and D: Incision and drainage; USG: Ultrasonography

Table 4. Size of the abscess in the two groups

USG finding (size)	I and D	USG guided aspiration	Total
10X8CM	1	0	1
11X10CM	0	1	1
12X13CM	1	0	1
2X2CM	0	1	1
2X3CM	0	1	1
3X2CM	0	2	2
3X4CM	1	0	1
4X2CM	1	2	3
4X3CM	3	2	5
4X4CM	4	2	6
4X5CM	2	1	3
4X6CM	1	0	1
5X2CM	1	0	1
5X3CM	3	2	5
5X4CM	1	3	4
5X5CM	2	2	4
5X6CM	1	0	1
6X3CM	0	3	3
6X5CM	1	1	2
6X6CM	1	0	1
7X3CM	0	1	1
7X4CM	1	1	2
Total	**25**	**25**	**50**

I and D: Incision and drainage, USG: Ultrasonography

There was a significant difference (p = 0.003) in the duration to resumption of lactation and comfort of re-initiating feeds which was noted to be better in group B with 91.67% resuming lactation while only 20% in group A resumed lactation. There was complete healing with no scar formation in group B compared to 37% patients in group A who healed with scarring.

Patients from group A needed hospital admission (1 to 3 days); the procedure was done under general anesthesia. Daily dressings were required for two weeks on a regular basis and most of the patients were unable to feed from the affected breast during this period, so milk was discarded by pumping.

In group B, patients continued to breastfeed, and the procedure did not require any form of general anesthesia or sedation and was carried out on an outpatient basis.

All fifty patients yielded seven aerobic and polymicrobial (14%) cultures. The isolates in decreasing order of frequency were *Staphylococcus aureus* (20 samples, 40%), *Escherichia coli* (12 samples, 24%), multiple mixed anaerobic-aerobic (7 samples, 14%), *Klebsiella pneumoniae* (5 samples, 10%) Methicillin-resistant *Staphylococcus aureus* (MRSA) (3 samples, 6%), and *Proteus* spp. (3 samples 6%).

Discussion and Conclusion

Breast abscesses, both lactational and non-lactational, are a very common clinical entity identified in daily practice. At an early stage and on initial presentation, acute mastitis may be treated conservatively with antibiotics. Once an abscess is formed, management conventionally involves I&D, but this is associated with a requirement for daily dressing, a prolonged healing time, patient apprehension regarding continuing breastfeeding, an unsatisfactory cosmetic outcome, and recurrence of breast abscess.

Table 5. Residual abscess on 7th post-treatment day

Residual abscess on 7th post–treatment day	Management (method)		Total	Chi-square test *p*-value
	I and D	USG guided aspiration		
No	16 (64%)	22 (88%)	38	3.947, p = 0.047
Yes	9 (36%)	3 (12%)	12	
Total	25	25	50	

I and D: Incision and drainage, USG: Ultrasonography

Table 6. Recurrence after two weeks

Recurrence after two weeks	Management (method)		Total	Chi-square test *p*-value
	I and D	USG guided aspiration		
No	18 (72%)	25 (100%)	43	8.857, p = 0.012
Yes	7 (28%)	0	7	
Total	25	25	50	

I and D: Incision and drainage, USG: Ultrasonography

Traditionally, this was the main modality of management but over time with further analysis, research, and trials, it has emerged that minimally invasive methods provide better results and are a more acceptable method of management. USG-guided needle aspiration under antibiotic coverage has become the latest management protocol in many institutions due to its ease and outcome.

Needle aspiration is performed with a large needle, and as much pus as possible is aspirated at each attempt at aspiration; antibiotics are also administered (2, 6, 7, 8).

Breast abscesses are most frequently located in the upper outer quadrant, which is probably because most of the breast parenchyma is found in this area (2). In the present study, 34% of the patients had abscesses in the upper outer quadrant, and 58% of the abscesses were in the left breast. The highest incidence of breast abscess during lactation has been reported within the first 12 weeks of the postpartum period (2, 9). In the present study, the mean duration of lactation before abscess formation was two months. The most common pathogen identified was *Staphylococcus aureus* (2, 10-12), which was isolated from the pus culture in 40% of cases. It is recommended that when a diagnosis of mastitis or abscess is made, antibiotics that target gram-positive bacteria should be used and milk should be drained via frequent pumping or regular nursing.

The recommended duration of antibiotic therapy is ten days for oral or intravenous antibiotics (1, 2, 7, 8, 13). A break in the skin of the nipple-areola complex may prove to be a source of infection for pathogenic organisms. This, followed by infrequent nursing due to the pain of a cracked nipple, may result in milk stasis which may further contribute to the colonisation of bacteria (14, 15). Fifty-six percent of the patients in group A were unhappy with the residual symptoms they experienced and 37% of them developed a scar.

In group B, none of the patients failed to respond to needle aspiration. The mean healing time was significantly longer in group A than in group B and fewer patients in group A were able to resume lactation following open surgery.

There are multiple advantages of using diagnostic ultrasound in the treatment protocol: The ability to differentiate simple mastitis from abscess, assessing the size of the abscess, and detecting the presence of multiple loculi. Most importantly, it helps in assessing the adequacy of drainage (15-19). Eryilmaz et al. (20) reported that breast abscesses smaller than 5 cm in diameter on physical examination can be treated with repeated needle aspirations with good cosmetic results while I&D can be reserved for patients with larger abscesses. While needle aspiration is a currently accepted minimally invasive procedure for the treatment of uncomplicated abscesses, in case of residual collections that last longer than two weeks, a surgical approach may still be considered for definitive management.

A meta-analysis of existing data and further randomized clinical studies are necessary to evaluate the benefit of USG guidance during needle aspiration in different categories of patients with breast abscesses in relation to puerperium and lactation.

There was a significant difference between group A and group B in terms of post-operative outcome and patient satisfaction, favoring group B. USG-guided guided aspiration/re-aspiration is a technically feasible and easy method of management when done under aseptic precautions and under antibiotic cover with good cosmetic and

functional results for the patient and quick recovery. We concur that I&D should be reserved for patients with skin involvement and large abscesses.

Limitations of this study include the small sample size which was taken for convenience and it was a single centre study, which may limit the generalizability of the study. Lack of blinding may introduce bias. Finally, the two week follow-up was inadequate in terms of long-term complications.

Breast abscess is a basic clinical entity that every physician or surgeon must learn to diagnose, especially in developing countries due to its common occurrence. Once diagnosed it is important to treat at the earliest, as it is a rapidly spreading condition which can involve the entire breast, skin and become multiloculated.

Our findings unequivocally support the superiority of percutaneous USG-guided aspiration over conventional I&D. The USG-guided approach demonstrated notable advantages, including faster healing times, improved cosmetic outcomes, and quicker resumption of lactation. Furthermore, it significantly reduced hospital stays, underscoring its efficiency in providing a patient-friendly and resource-effective solution. The present study also suggested that, when appropriate, multiple USG-guided aspirations can be considered before resorting to I&D, in order to optimize outcomes. Our results affirm the safety and efficacy of percutaneous USG-guided aspiration as a preferred approach for managing breast abscesses. Physicians and surgeons, particularly in resource-constrained settings, should consider the adoption of this minimally invasive technique for optimal patient outcomes.

Ethics Committee Approval: The study protocol, including the research design, procedures and consent forms received approval from the Institutional Ethics Committee, Stanley Medical College, Chennai to ensure compliance with ethical guidance and standards (approval number: EC/NEW/INST/2018/461; date: 07.12.2018).

Informed Consent: Prior to the commencement of the study, informed consent was obtained from all the participants, explaining the purpose, procedures, potential risks and benefits.

Authorship Contributions

Surgical and Medical Practices: V.M.T., R.V., S.S.; Concept: V.M.T., R.V., S.S.; Design: V.M.T., R.V., S.S.; Data Collection and/or Processing: V.M.T., R.V., S.S.; Analysis and/or Interpretation: V.M.T., R.V., S.S.; Literature Search: V.M.T., R.V., S.S.; Writing: V.M.T., R.V., S.S.

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

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Dener C, Inan A. Breast abscesses in lactating women. World J Surg 2003; 27: 130-103. (PMID: 12616423) [Crossref]
2. Marchant DJ. Inflammation of the breast. Obstet Gynecol Clin North Am 2002; 29: 89-102. (PMID: 11892876). [Crossref]
3. Boakes E, Woods A, Johnson N, Kadoglou N. Breast Infection: A review of diagnosis and management practices. Eur J Breast Health 2018; 14: 136-143. (PMID: 30123878). [Crossref]
4. Lam E, Chan T, Wiseman SM. Breast abscess: evidence based management recommendations. Expert Rev Anti Infect Ther 2014; 12: 753-762. (PMID: 24791941). [Crossref]
5. Ulitzsch D, Nyman MK, Carlson RA. Breast abscess in lactating women: US-guided treatment. Radiology 2004; 232: 904-909. (PMID: 15284435) [Crossref]
6. Colin C, Delov AG, Peyron-Faure N, Rabilloud M, Charlot M. Breast abscesses in lactating women: evidences for ultrasound-guided percutaneous drainage to avoid surgery. Emerg Radiol 2019; 26: 507-514. (PMID: 31154537) [Crossref]
7. Tan SM, Low SC. Non-operative treatment of breast abscesses. Aust N Z J Surg 1998; 68: 423-424. (PMID: 9623462) [Crossref]
8. O'Hara RJ, Dexter SP, Fox JN. Conservative management of infective mastitis and breast abscesses after ultrasonographic assessment. Br J Surg 1996; 83: 1413-1414. (PMID: 8944458) [Crossref]
9. Fetherston C. Characteristics of lactation mastitis in a Western Australian cohort. Breastfeed Rev 1997; 55-11. Erratum in: 1998; 6: 28. (PMID: 9699467). [Crossref]
10. Martin JG. Breast abscess in lactation. J Midwifery Womens Health 2009; 54: 150-151. (PMID: 19249661) [Crossref]
11. Al Benwan K, Al Mulla A, Rotimi VO. A study of the microbiology of breast abscess in a teaching hospital in Kuwait. Med Princ Pract 2011; 20: 422-426. (PMID: 21757930) [Crossref]
12. Amir LH, Garland SM, Lumley J. A case-control study of mastitis: nasal carriage of Staphylococcus aureus. BMC Fam Pract 2006; 7: 57. (PMID: 17032458) [Crossref]
13. Ferrara JJ, Leveque J, Dyess DL, Lorino CO. Nonsurgical management of breast infections in nonlactating women. A word of caution. Am Surg 1990; 56: 668-671. (PMID: 2240858) [Crossref]
14. Li D, Li J, Yuan Y, Zhou J, Xiao Q, Yang T, et al. Risk factors and prognosis of acute lactation mastitis developing into a breast abscess: A retrospective longitudinal study in China. PLoS One 2022; 17: e0273967. (PMID: 36048839) [Crossref]
15. Pileri P, Sartani A, Mazzocco MI, Giani S, Rimoldi S, Pietropaolo G, et al. Management of breast abscess during breastfeeding. Int J Environ Res Public Health 2022; 19: 5762. (PMID: 35565158) [Crossref]
16. Karstrup S, Solvig J, Nolsøe CP, Nilsson P, Khattar S, Loren I, et al. Acute puerperal breast abscesses: US-guided drainage. Radiology 1993; 188: 807-809. (PMID: 8351352) [Crossref]
17. Trop I, Dugas A, David J, El Khoury M, Boileau JF, Larouche N, et al. Breast abscesses: evidence-based algorithms for diagnosis, management, and follow-up. Radiographics 2011; 31: 1683-1699. (PMID: 21997989) [Crossref]
18. Blaivas M. Ultrasound-guided breast abscess aspiration in a difficult case. Acad Emerg Med 2001; 8: 398-401. (PMID: 11282679) [Crossref]
19. Voruganti MR, Sistu MK, Gadipudi HC. A comparative study on ultrasound guided percutaneous aspiration versus incision and drainage in the management of small breast abscesses. International Surgery Journal 2022; 9: 620-626. [Crossref]
20. Eryilmaz R, Sahin M, Hakan Tekelioglu M, Daldal E. Management of lactational breast abscesses. Breast 2005; 14: 375-379. (PMID: 16216739) [Crossref]

Original Article

Eur J Breast Health 2024; 20(3): 199-206
DOI: 10.4274/ejbh.galenos.2024.2024-3-8

Assessment of the Predictive Role of Ki-67 in Breast Cancer Patients' Responses to Neoadjuvant Chemotherapy

Ghizlane Rais[1], Rania Mokfi[2], Farah Boutaggount[2], Meryem Maskrout[2], Soundouss Bennour[2], Chaymae Senoussi[2], Fadoua Rais[3]

[1]Department of Medical Oncology, CHU Souss Massa, Biomed Laboratory, University Ibn Zohr Agadir Faculty of Medicine and Pharmacy of Agadir, Agadir, Morocco
[2]Department of Medical Oncology, CHU Souss Massa, University Ibn Zohr Agadir Faculty of Medicine and Pharmacy of Agadir, Agadir, Morocco
[3]Department of Radiation Therapy, University Hospital Center of Montreal, Montreal, Canada

ABSTRACT

Objective: Neoadjuvant chemotherapy (NAC) in breast cancer (BC) is being considered for a broader range of cases, including locally advanced tumors and situations where downstaging could reduce extensive surgery. Several trials have explored predictive markers of pathological complete response (pCR). The role of Ki-67 as a predictor of pCR has been demonstrated in studies. However, the cut-off remains vague, given the lack of standardization of measurement methods. The aim of our study was to evaluate the predictive value of Ki-67 in response to NAC and to identify the cut-off values that exhibit the strongest correlation with best response.

Materials and Methods: This retrospective study included 187 patients who had undergone surgery following NAC for BC at the CHU Souss Massa of Agadir between January 2020 and January 2023. Logistic regression was used to assess the correlation between Ki-67 and patients' characteristics. Optimal Ki-67 cutoff was identified by receiver operating characteristic curve. Kaplan-Meier curves were used to assess disease-free survival (DFS), and survival comparisons were assessed with the log-rank test.

Results: The median age was 51.8±10.7 years and 51.4% of tumors were smaller than 5 cm. Node invasion was found in 55.4%. Luminal B subtype was found in 49.7%, followed by human epidermal growth factor receptor-2 (HER-2)-positive in 27.4%, triple-negative in 14.3% and Luminal A in 8.6%. pCR occurred in 40% of patients overall. Subgroup analysis revealed a significant association between pCR and tumor size (p<0.001), lymph node involvement (p<0.001), grade 2 (p<0.001), vascular invasion (p<0.001), and positive HER-2 status (p = 0.022). In statistical analysis, pathological responses were improved in patients with Ki-67 >35% (p<0.001). DFS was 98.8% at 12 months. No statistical difference was found in DFS according to Ki-67 values and pCR status.

Conclusion: Our results indicate that Ki-67 is a predictive marker for response in the neoadjuvant setting in BC patients. Our study showed that a Ki-67 cut-off >35% predicts a better pCR rate in response to NAC. However, this cutoff value remains controversial due to the absence of a standard method of measurement, with inter- and intra-observer variability. It would be necessary to validate this cutoff in other studies.

Keywords: Breast cancer; Ki-67; neoadjuvant chemotherapy

Cite this article as: Rais G, Mokfi R, Boutaggount F, Maskrout M, Bennour S, Senoussi C, Rais F. Assessment of the Predictive Role of Ki-67 in Breast Cancer Patients' Responses to Neoadjuvant Chemotherapy.Eur J Breast Health 2024; 20(3): 199-206

Key Points

- Breast cancer
- Neoadjuvant chemotherapy
- Ki-67

Introduction

Neoadjuvant chemotherapy (NAC) in breast cancer is being considered for a broader range of cases, including locally advanced tumors and situations where downstaging may facilitate less extensive surgery. This approach allows for tailored treatment based on the tumor response before surgery. In numerous neoadjuvant analyses, patients who attained a pathological complete response (pCR) demonstrated a more favorable survival outcome (1). Several trials have explored clinical, biological and histological markers to predict pCR in breast cancer.

Corresponding Author:
Ghizlane Rais; medghiz@gmail.com

Received: 26.03.2024
Accepted: 02.06.2024
Available Online Date: 01.07.2024

Various factors have been recognized as predictors of pCR such as age, menopausal status, tumor stage, nuclear grade, lymphatic invasion, genomic signature, molecular subtype and Ki-67 value (2, 3). Ki-67 is a protein marker used to measure cell proliferation. It is expressed in the active phases of the cell cycle (G1, S, G2 and M), but not in the quiescent phase (G0) (4). The strong correlation between Ki67 and pCR has been demonstrated in numerous studies (5). However, the cut-off remains vague and inaccurate, given the lack of standardization and variability of measurement methods (6). To the best of our knowledge, only a few studies in a Moroccan population have been reported to date. The specific aim of this study was to analyze the potential role of Ki-67 in patients receiving NAC for breast cancer. In addition, we sought to identify the cut-off values of Ki-67 that exhibited the strongest correlation with the best response to NAC.

Materials and Methods

Patients

This retrospective study included 187 patients who had undergone surgery following NAC for breast cancer at the Regional Oncology Center in Agadir between January 2020 and January 2023. For inclusion in the study, patients were required to be at least 18 years old. They were enrolled only if they had completed NAC, followed by surgery. The patient selection process is shown in Figure 1.

The inclusion criteria for NAC administration were: Confirmed invasive breast cancer, from stage T2 and or lymph node involvement for human epidermal growth factor receptor-2 (HER-2)-positive or triple-negative tumors, and T4 for luminal tumors. The patient's overall health status and ability to tolerate chemotherapy were taken into consideration.

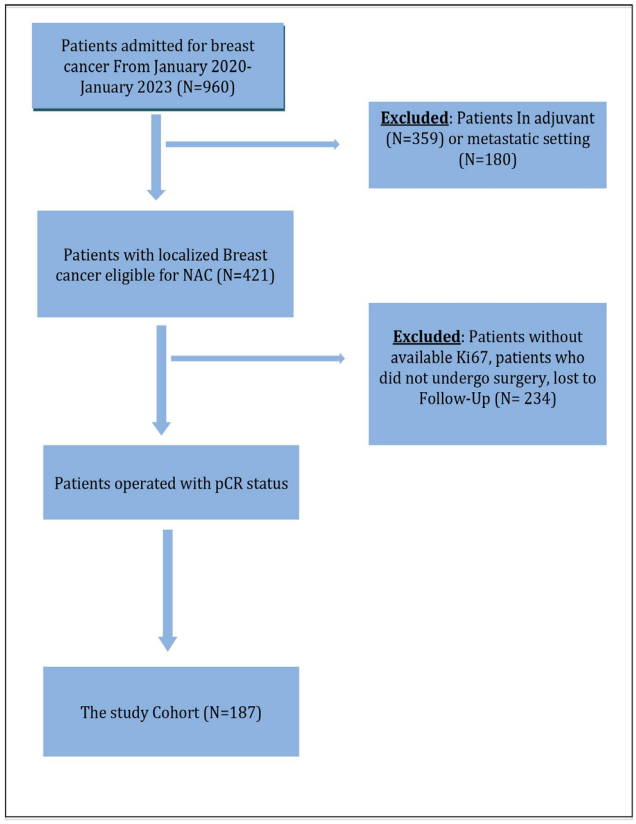


Figure 1. Patient selection

The study adhered to the principles outlined in the Declaration of Helsinki (1964) and received approval from the Local Ethics Committee (CHU Souss Massa, Biomed Laboratory, Faculty of Medicine and Pharmacy of Agadir, University Ibn Zohr Agadir, approval number: 25_01_2023, date: 25.01.2023).

Clinical Data

Patients' characteristics were selected from a database containing archived medical records. They included: Patients' age, menopausal status, disease stage, chemotherapy protocol, surgical treatment, histological results, tumor grade, molecular sub-type, and Ki-67 value. A staging assessment was carried out on all patients, which included thoraco-abdomino-pelvic computed tomography and bone scintigraphy.

Histopathological Data

Histological parameters of the tumor included the histological type, histological grade, estrogen receptor (ER) status, progesterone receptor (PR) status, HER-2 status and Ki-67 expression level, obtained from the original pathology reports. Molecular subtypes were classified according to the Saint Gallen recommendations from 2013 into four groups: Luminal-A, Luminal-B, HER-2+, and triple negative.

Molecular profiling was carried out using monoclonal mouse antibodies (for ER: ID5, 1:50 dilution; Dako, Glostrup, Denmark), (for PR (PgR636, 1:100 dilution; Dako) and HER-2 protein (CB11, 1:100 dilution; NeoMarker, Fremont, USA). Hormone receptor positivity was defined by an ER and PR cut-off value of 1%. Hormone-negative status was determined by the absence of ER and PR expression by the tumor. HER2 status was assessed using the Hercep test by immunohistochemistry (IHC). Positive results were defined as either 3+ expressions on IHC or 2+ expressions on IHC and positive results in fluorescent in situ hybridization. The Ki-67 value was evaluated by automated quantitative analysis, using a monoclonal antibody (MIB-1, 1:400 dilution; Dako, Denmark). The process typically included digitization of tissue slides byscanning to create high-resolution (×40 objectives) digital images. The images were then analyzed by computer algorithms to detect and quantify Ki-67-positive nuclei within the tissue. The percentage of Ki-67-positive cells is calculated and reported.

Treatment

Anthracyclines-based treatment followed by taxanes was administered to all patients in our study. NAC typically involved administering three to four cycles of anthracyclines as part of the standard regimen (AC60: Doxorubicin 60 mg/m² and cyclophosphamide 600 mg/m², or EC100: Epirubicin 100 mg/m² and cyclophosphamide 500 mg/m² every three weeks). The taxane-based regimens used were as follows: Weekly paclitaxel 80 mg/m² or triweekly docetaxel 100 mg/m².

For patients with HER2-positive breast cancer, anti-HER-2 targeted therapies were combined with taxanes: Either a dual HER-2 blocking therapy with pertuzumab 840 mg intravenously, followed by 420 mg and trastuzumab 600 mg subcutaneously or trastuzumab 600 mg subcutaneously every three weeks. In triple-negative breast cancer, patients received the dose-dense protocol: AC followed by weekly paclitaxel and carboplatin: Doxorubicin 60 mg/m² and cyclophosphamide 600 mg/m² every 2 weeks, followed by paclitaxel 80 mg/m² on days 1, 8 and 15, and carboplatin AUC5 (area under the curve) every 3 weeks.

The patients underwent surgery after completing NAC with a median interval of 24.7±3.68 days. All patients underwent mastectomy or breast-conserving surgery with axillary lymph node dissection. The choice of surgical procedure was discussed in a multidisciplinary consultation meeting, and it was determined by the initial stage, the optimal cosmetic results, and the patient's choice.

PCR Assessment

The pathological response to chemotherapy was assessed by analyzing surgical specimens taken from the tumor. The pCR was defined as the absence of invasive residues in the breast or nodes (ypT0 and ypN0). The pCR analysis was performed using the Sataloff and Chevalier classifications. At the time of the study, Residual Cancer Burden (RCB) classification was not used in the Moroccan centers.

Statistical Analysis

Data was collected in an Excel database. Statistical analysis was performed using Jamovi software (https://www.jamovi.org), $p<0.05$ was considered to be statistically significant.

The optimal cut-off value for Ki-67 percentage was assessed by receiver operating characteristic (ROC) curve analysis and area under the curve. The cut-off value refers to the value corresponding to maximum sensitivity and minimum 1-Specificity. The cut-off value for Ki-67 used in our series to define either high or low classification was 35%.

Student's t-test was employed for analyzing quantitative characteristics, while Pearson's chi-square test was used to contrast categorical variables. Logistic regression methods were adopted to approximate the risk ratio of achieving pCR according to the baseline factors.

Disease-free survival (DFS) was characterized as the time between surgery and relapse. Kaplan-Meier curves were used to assess DFS, and differences between survival curves were assessed with the log-rank test.

Results

Clinical and Histological Characteristics

A total of 187 patients were included in the study. The average age of patients was 518±10.7 years. Of these, 75 (40%) were premenopausal at diagnosis, and 112 were menopausal (60%). In 96 (51.4%) the tumor size <5 cm, while 91 patients (48.6%) had a tumor size >5 cm. Lymph node involvement was documented among 104 patients (55.4%). The mean CA 15-3 value was 18.6±10 U/mL. When diagnosed, 137 patients were classified as stage II (73,1%), and 50 patients were classified as stage III (26.8%) (Table 1).

The distribution of patients according to histological type was: Invasive ductal carcinoma in 171 (91.4%) and invasive lobular carcinoma in 16 (8.6%). The histological prognosis grade revealed a predominance of grade 3 in 64.4% of cases (120) and grade 2 in 35.4% of cases (67). Among the 187 patients, there were 16 patients (8.6%) with the luminal A subtype, 93 (49.7%) patients with the luminal B subtype, 51 patients (27.4%) with the HER-2 subtype, and 27 patients (14.3%) with the triple negative type. With the study cut-off value of Ki-67 >35%, 113 patients (60.2%) were considered to have a high level of Ki-67 (Table 1).

After NAC, 75 patients (40%) showed a clinical complete response (cCR), 85 patients (45.7%) showed a clinical partial response (cPR), and 27 patients (14.3%) showed clinical stable disease (cSD).

The complete absence of residual invasive carcinoma cells in the breast was confirmed by histological examination in 75 patients (40%), however, 115 (60%) patients had residual carcinoma cells in the breast or in the resected lymph nodes (Table 1).

Analysis of the Relationships Between the pCR Rate and Ki-67

As a result of a univariate analysis, clinical and pathological responses to NAC were significantly improved when Ki-67 levels were high >35% ($p<0.001$). Furthermore, a better rate of pCR was significantly associated with tumor size <5 cm ($p<0.001$), lymph node invasion ($p<0.001$), nuclear grade 2 ($p<0.001$), vascular invasion ($p<0.001$), hormone receptor positive subgroup ($p<0.001$) and HER-2-positive subgroup ($p = 0.022$) (Table 2).

Study of the Correlation Between pCR and Clinico-Pathological Factors

The multivariate logistic regression analysis of the correlation between pCR and clinicopathological factors was performed using tumor diameter, tumor grade, lymph node invasion, vascular invasion, molecular subtype (HR, HER-2), and Ki-67 expression level. It showed significant correlations between pCR and Ki-67 expression >35% ($p<0.001$), tumor size <5cm ($p<0.001$), HER2 positive status ($p = 0.023$), and lymph node invasion ($p<0.001$) (Table 3).

Progression-Free Survival

All patients were monitored until January 2024. Six (3.4%) patients presented with a local or metastatic relapse of the disease. The average

Table 1. Patient characteristics

		Value (%)
Age, years	Mean ± SD	51.8±10.7
Menopausal status	Premenopausal	75 (40%)
	Postmenopausal	112 (60%)
Tumor size (cm)	<5 cm	96 (51.4%)
	>5 cm	91 (48.6%)
Nodal status	Negative	96 (55.4%)
	Positive	91 (48.6%)
Histological type	Invasive ductal carcinoma	171 (91.4%)
	Invasive lobular carcinoma	16 (8.6%)
Nuclear grade SBR	Grade 2	67 (35.4%)
	Grade 3	120 (64.4%)
Vascular invasion	Positive	71 (38.3%)
	Negative	116 (61.7%)
Tumor subtype	Luminal A	16 (8.6%)
	Luminal B	93 (49.7%)
	HER-2 overexpression	51 (27.4%)
	Triple negative	27 (14.3%)
Ki-67 expression	≥35%	113 (60.2%)
	<35%	74 (39.4%)
Response to NCT	Non pCR	112 (60%)
	pCR	75 (40%)

SD: Standard deviation; SBR: Scarff-Bloom-Richardson; HER-2: Human epidermal growth factor receptor-2; pCR: Pathological complete response; NCT: Neoadjuvant chemotherapy

DFS period was 51.1 months (49.5-52.6) (Figure 2). No significant difference was found when comparing DFS in terms of pCR status or Ki-67 cut-off (Figures 3 and 4).

Discussion and Conclusion

Antigen Ki-67, also known as Ki-67 or Marker of Proliferation Ki-67 (MKI67), is a nuclear antigen and is closely associated with increased proliferation and a poorer prognosis in breast cancer. (7) The cellular expression level of Ki-67 can be detected using IHC and immunofluorescence (IF) methods. IHC is more frequently used (8). Measurements are conducted using various antibodies, such as mouse or rabbit monoclonal antibodies (MM1, MIB-1, SP-6) (9). The manual counting of at least 500–1000 malignant invasive cells, as proposed by the International Ki-67 in Breast Cancer Working Group, is frequently used to assess Ki-67 (10). However, counting this number of cells is a substantial, labor-intensive, and time-consuming task for histopathologists and poses challenges in terms of reproducibility (11). The evaluation and measurement methods for Ki-67 are variable, leading to inconsistencies in results. In a study by Chung et al. (12), which included 30 observers from 30 different institutions, and examined Ki-67-stained slides of 20 different breast cancers on whole sections and tissue microarray. Each observer assessed Ki-67 in two different ways: Direct counting and categorical estimation. The study concluded that inter-observer variability of the Ki-67 index for the two methods was significantly high. Tumors with hot spots had higher inter-observer variability, and restricting the measurement area resulted in lower variability.

Table 2. Patient characteristics by pCR status

		pCR (n = 75) No. (%)	Non-pCR (*n* = 112) No. (%)	*p*
Age (years)	51.8±10.7	50±10.5	52±10.8	0.353
Menopausal status	Premenopausal	32 (42.8%)	80 (71.4%)	0.051
	Menopausal	43 (51.1%)	32 (28.6%)	
Tumor size (cm)	<5 cm	45 (60%)	52 (45.7%)	<0.001
	>5 cm	30 (40%)	60 (54.3%)	
Nodal status	Positive	51 (68.6%)	52 (46.7%)	<0.001
	Negative	20 (31.4%)	60 (53.3%)	
Histological type	Invasive ductal carcinoma	62 (82.8%)	109 (97.1%)	<0.001
	Invasive lobular carcinoma	13 (17.1%)	3 (2.85%)	
Nuclear grade	Grade 3	57 (75.7%)	9 (8.6%)	<0.001
	Grade 2	18 (24.3%)	96 (91.4%)	
Vascular invasion	Positive	58 (82.8%)	10 (8.6%)	<0.001
	Negative	12 (17.1%)	102 (91.4%)	
Hormonal receptor status	Positive	63 (84.3%)	48 (42.8%)	<0.001
	Negative	12 (15.7%)	64 (57.1%)	
HER-2 status	Positive	48 (64.3%)	70 (62.8%)	= 0.022
	Negative	27 (35.7%)	42 (37.1%)	
Ki-67	<35%	11 (14.3%)	63 (56.2%)	<0.001
	>35%	64 (85.7%)	49 (43.8%)	

HER-2: Human epidermal growth factor receptor-2; pCR: Pathological complete response

Table 3. Logistic regression analysis of the correlation between pCR and clinicopathological characteristics

Parameter	Odds ratio	Confidence interval	*p*
Ki-67 >35%	5.27	2.44–11.39	<0.001
Tumor size<5cm	3.26	1.675–6.33	<0.001
Nuclear grade 3	89.18	31.48–252.62	<0.001
Hormone-positive status	91.78	12.29–685.38	<0.001
HER-2 positive status	2.23	1.12–4.45	0.023
Lymph node invasion	5.38	2.59–11.21	<0.001
Vascular invasion	341.5	71.41–1633.76	<0.001

HER-2: Human epidermal growth factor receptor-2; pCR: Pathological complete response

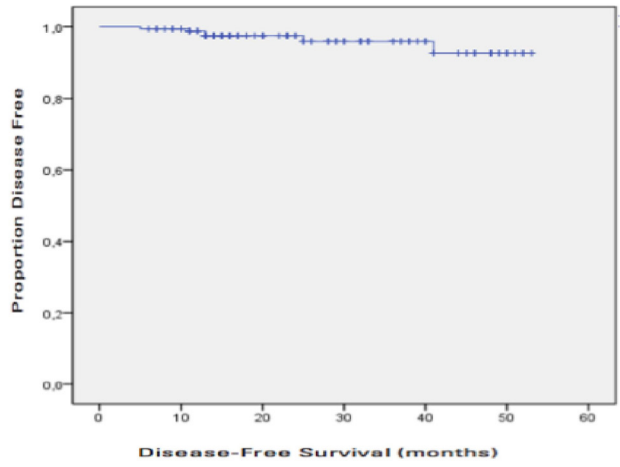


Figure 2. Disease free survival

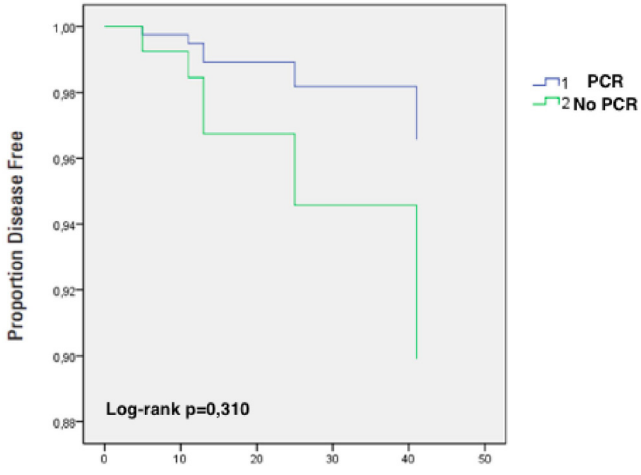


Figure 3. DFS according to PCR

DFS: Disease free survival; PCR: Pathological complete response

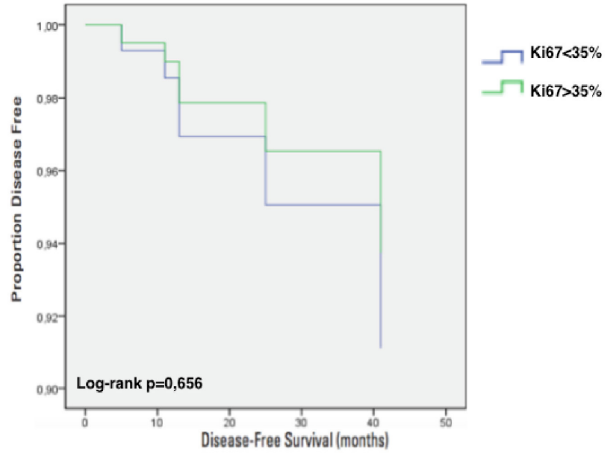


Figure 4. DFS according to Ki-67

DFS: Disease free survival

To solve this problem, automated counting using computer software may be used to objectively measure protein expression in tumor and subcellular compartments (13). It involves the processing of scanned images on whole slides by microscopy and computer analysis. It has recently been developed as a reproducible and more specific method of assessing Ki-67 than visual methods (14). Klauschen et al. (15) described encouraging results from the validation of computer-assisted Ki-67 assessment on over 1,000 breast tumors. The study concluded that it was a valid method for assessing Ki-67 and for predicting overall and progression-free survival (pOS <0.0001, pPFS <0.0002) (15).

There isn't a universally defined cut-off value for categorizing Ki-67 expressions. The Ki-67 cut points are used in various ranges, from 5% to 34% (16). In a study of prognostic factors in breast cancer, the researchers determined a cut-off value for Ki-67 at 12% (17). This value was observed in patients without recurrence, providing a rationale for their categorization. In another related study, a cutoff value of 20% was adopted (18). Kim et al. (18) determined 25% as the optimal cut-off value using ROC curve analysis. However, another study by Alba et al. (19) indicated that the Ki-67 cut-off with the greatest sensitivity and specificity values was 50%. Denkert et al. (20) concluded that Ki-67 cut-off points between 3 and 94% are predictive of pCR. Our study population of 187 patients showed a complete pathological response of 85.7% in patients with a Ki-67 value of more than 35% which was significant (p<0.001).

High Ki-67 level is correlated with an increased probability of achieving pCR in breast cancer patients undergoing NAC (21). This correlation can be explained by the fact that High Ki-67 represents rapidly dividing cells, and that NAC typically targets dividing cells, resulting in enhanced elimination of tumor cells (22). In a meta-analysis conducted by Chen et al. (23), which included 53 studies, and 10,848 patients, it was found that high Ki-67 before NAC was a predictor for pCR in the neoadjuvant setting for breast cancer patients. Indeed, a variety of cut-off points correlated with pCR were used in this meta-analysis, ranging from less than 14% to more than 50% in a total of 41 studies. In a retrospective study by Ingolf et al. (1), which included 77 patients receiving NAC for breast cancer, the study concluded that there was a significant correlation between Ki-67 and pCR as a predictive factor (p = 0.001) (1). The average Ki-67 value was 34.9% but ranged widely between 1–90%. In the group with pCR, the cutoff of Ki67 was 37.4±24%. Similar results were found in a large series by Peter et al. (5), which included 552 patients treated with NAC for invasive breast carcinoma. Ki-67 was identified as a pCR marker by using a cutoff value of more than 13% [odds ratio (OR) 3.5, p = 0.01] (5). It seems that the predictive cutoff values of Ki-67 differ based on the molecular profile according to some authors. Peter et al. (5), showed that higher cutoff values are observed in the hormone receptor-positive tumors (ranging from 36% to 40%) and the triple-negative tumors (30–40%). For the HER-2-positive tumors, Ki-67 varies between 17% and 20% (5). Across all studies, Ki-67 values between 30% and 50% were correlated with better pCR rates in all four breast cancer subtypes. In a study by Wang et al. (24), which included 240 patients, a Ki-67 value of 40% was associated with better pCR. In our series, a Ki-67 value of 35% was predictive of a complete response to NAC in all subgroups (p<0.001).

Tumor size has been considered in numerous studies as a predictor of pCR (25). Consistent with our findings, Chen et al. (25) in their study, which included 1010 BC patients, concluded that tumor sizes less than 4 cm were more likely to attain pCR (p = 0.039). The same

results were observed in the study of Peter et al. (5). The pCR rates were 34.8% in pT1 tumors and 21.5% in pT2 tumors (p<0.0001). This finding aligns with the idea that small tumors might exhibit a more robust response to treatment (5). Our study confirms similar results, with a pCR rate of 60% for tumors <5 cm versus 40% for tumors greater than 5 cm (p<0.001).

Molecular subtype is another factor that has been correlated with pCR (26). In the meta-analysis of Chen et al. (23), Ki-67 was a predictive factor for pCR in all molecular subtypes: in HR+ (n = 7; OR: 2.51), HER2+ (n = 9; OR: 2.76) and triple-negative (n = 10; OR: 2.77) (23). Moreover, the neoadjuvant GeparTrio trial showed that Ki-67 was predictive of response to NAC in nearly all molecular subtypes (21). Kim et al. (18) demonstrated in their study, which included 74 patients, that patients with HER2-positive tumors exhibited a higher rate of pCR (p = 0.040), and a similar trend was observed in ER-negative patients (p = 0.031). In the same way, Peter et al. (5) found that pCR rates were higher in tumors with HER2 over expression (p<0.00001). We also observed a significant association between pCR and HER-2 overexpression (p = 0.0023, OR = 2.227) and HR negative (p<0.001, OR = 91.777). The improvement in pCR rates in these subgroups may be due to the elevated levels of Ki-67 frequently observed in tumors with negative Hormone receptors or exhibiting HER-2 over expression (20). Nonetheless, Petit et al. (27) concluded that the absence of hormone receptor expression (ER and PR) in the high Ki-67 group was a predictor of pCR (p = 0.008 and p = 0.01, respectively). However, HER-2 overexpression was not significantly associated with achieving pCR (p = 0.99).

Tumor grade has shown a clear association with pCR status in many studies (28). In the study of Peter et al. (5) the pCR rate was significantly higher in grade 3 (45.3% compared to 10.6%) than grade 2 (p<0.00001). In another recent study performed by Jarzab et al. (28), including 353 females receiving NAC for breast cancer, increased nuclear grade demonstrated an elevated rate of pCR (31.28% in grade 3 *versus* 8.55% in grade 2, p<0.0001) (28). This finding could be explained by a direct and significant correlation between higher nuclear grades and high Ki-67. In a retrospective study involving 260 breast cancer patients, it was reported that a robust correlation existed between high Ki-67 and elevated nuclear grade (p = 0.010) (29). Furthermore, this study concluded that a higher Ki-67 index was significantly associated with positive lymph nodes and vascular invasion. This discovery may also explain the notable link between lymph node invasion, vascular invasion and pCR in patients exhibiting high Ki-67 levels (30). Our results are consistent with this finding, with pCR rates of 75.7% in nuclear grade 3 (p<0.001, OR = 89,176) and in lymph node involvement (82.8%, p<0.001, OR = 5.388).

Another question is the impact of the chemotherapy regimen on achieving pCR in patients with high Ki-67. The meta-analysis of Chen et al. (23) demonstrated that Ki-67 was also a useful predictor of pCR in patients receiving chemotherapy regimens containing anthracyclines and/or taxanes (n = 13; OR: 2.90), anthracyclines plus taxanes (n = 22; OR: 3.15), and anthracyclines (n = 5; OR: 4.67), compared to taxanes (n = 3; OR: 1.29).

pCR is generally considered a predictor of OS and DFS (31). Von Minckwitz et al. (32) presented a meta-analysis of 6.377 breast cancer patients. The authors concluded that pCR was an effective marker of survival for TNBC, luminal B and HER2-positive patients (p = 0.005). Kong et al. (33) completed a meta-analysis that included 16 studies and 3.776 patients with breast cancer. The authors indicated that pCR was prognostic for relapse-free survival (OR = 3.44), DFS (OR = 3.41), and RFS (OR = 2.45). Similar results were also reported by two large metaanalysis conducted by Cortazar et al. (34) and Spring et al. (35). Patients with TNBC who achieved pCR had significantly better DFS than those who did not (36). Moreover, some authors suggested that a decrease in Ki-67 after NAC contributed to a favorable DFS (37). Yoshioka et al. (38) demonstrated in their study, including 64 patients, that the level of Ki-67 in residual tumors after NAC was strongly associated with increased DFS and OS (p = 0.0004 and p = 0.0003, respectively) (38). Chen et al. (39) showed, in a series of 92 patients with locally advanced breast cancer, that a Ki-67 decrease of over 12.5% was consistent with a better DFS (p = 0.007).

Our study has some limitations. First, our study was conducted retrospectively. Second, the small size of our sample contributed to the variability of certain results compared to other studies. Furthermore, there was no standard cutoff value for Ki-67. It was determined, in our study, using the ROC curve, which combines sensitivity and specificity. Further studies are necessary, involving larger patient groups with analyses of cutoff values, subtypes of BC and outcomes, which will reinforce the present findings.

Our results confirm the predictive role of Ki-67 in patients' response to chemotherapy in the neoadjuvant setting. The findings of the present study suggest that this marker could help select patients who may benefit from chemotherapy. Our analysis showed that a Ki-67 cut-off >35% predicted a better pCR rate. However, this cutoff value remains controversial due to the absence of a standard method of measurement and interpretation with inter- and intra-observer variability. Validation of this cutoff value in a larger population would be desirable.

Ethics Committee Approval: The study adhered to the principles outlined in the Declaration of Helsinki (1964) and received approval from the Local Ethics Committee (CHU Souss Massa, Biomed Laboratory, Faculty of medicine and pharmacy of Agadir, University Ibn Zohr Agadir, approval number: 25_01_2023, date: 25.01.2023).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: G.R., S.B.; Concept: G.R.; Design: G.R., F.B., M.M.; Data Collection and/or Processing: R.M., F.B., S.B., C.S.; Analysis and/or Interpretation: G.R., R.M., F.B., F.R.; Literature Search: V.M.T., R.V., S.S.; Writing: G.R., R.M., F.B., M.M., F.R.

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

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Ingolf JB, Russalina M, Simona M, Julia R, Gilda S, Bohle RM,et al. Can ki-67 play a role in prediction of breast cancer patients' response to neoadjuvant chemotherapy? Biomed Res Int 2014; 2014: 628217. (PMID: 24783217) [Crossref]
2. Resende U, Cabello C, Oliveira Botelho Ramalho S, Zeferino LC. Predictors of Pathological Complete Response in Women with Clinical Complete Response to Neoadjuvant Chemotherapy in Breast Carcinoma. Oncology 2018; 95: 229-238. (PMID: 30025385) [Crossref]

3. Loibl S, von Minckwitz G, Untch M, Denkert C, German Breast Group. Predictive factors for response to neoadjuvant therapy in breast cancer. Oncol Res Treat 2014; 37: 563-568. (PMID: 25486071) [Crossref]
4. Brown JR, DiGiovanna MP, Killelea B, Lannin DR, Rimm DL. Quantitative assessment Ki-67 score for prediction of response to neoadjuvant chemotherapy in breast cancer. Lab Invest 2014; 94: 98-106. (PMID: 24189270) [Crossref]
5. Fasching PA, Heusinger K, Haeberle L, Niklos M, Hein A, Bayer CM, et al. Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. BMC Cancer 2011; 11: 486. (PMID: 22081974) [Crossref]
6. Wajid S, Samad FA, Syed AS, Kazi F. Ki-67 and Its Relation with Complete Pathological Response in Patients with Breast Cancer. Cureus 2021; 13: e16788. (PMID: 34513395) [Crossref]
7. Stathopoulos GP, Malamos NA, Markopoulos C, Polychronis A, Armakolas A, Rigatos S, et al. The role of Ki-67 in the proliferation and prognosis of breast cancer molecular classification subtypes. Anticancer Drugs 2014; 25: 950-957. (PMID: 24949917) [Crossref]
8. Varga Z, Diebold J, Dommann-Scherrer C, Frick H, Kaup D, Noske A, et al. How reliable is Ki-67 immunohistochemistry in grade 2 breast carcinomas? A QA study of the Swiss Working Group of Breast- and Gynecopathologists. PLoS One 2012; 7: e37379. (PMID: 22662150) [Crossref]
9. El Benna H, Zribi A, Laabidi S, Haddaoui A, Mlika M, Skhiri H, et al. Ki-67: role in diagnosis, prognosis and follow-up after treatment of breast cancers. Tunis Med 2015; 93: 737-741. (PMID: 27249380) [Crossref]
10. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Ki-67 in Breast Cancer Working Group. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst 2011; 103: 1656-1664. (PMID: 21960707) [Crossref]
11. Polley MY, Leung SC, McShane LM, Gao D, Hugh JC, Mastropasqua MG, et al. International Ki67 in Breast Cancer Working Group of the Breast International Group and North American Breast Cancer Group. An international Ki67 reproducibility study. J Natl Cancer Inst 2013; 105: 1897-1906. (PMID: 24203987) [Crossref]
12. Chung YR, Jang MH, Park SY, Gong G, Jung WH; Korean Breast Pathology Ki-67 Study Group. Interobserver Variability of Ki-67 Measurement in Breast Cancer. J Pathol Transl Med 2016; 50: 129-137. (PMID: 26875758) [Crossref]
13. Kadivar M, Aram F. Assessment of Ki67 in Breast Cancer: A Comparison between the Eye-10 Method, Stepwise Counting Strategy, and International System of Ki67 Evaluation. Iran J Pathol 2019; 15: 13-18. (PMID: 32095144) [Crossref]
14. Davey MG, Hynes SO, Kerin MJ, Miller N, Lowery AJ. Ki-67 as a Prognostic Biomarker in Invasive Breast Cancer. Cancers (Basel) 2021; 13: 4455. (PMID: 34503265) [Crossref]
15. Klauschen F, Wienert S, Schmitt WD, Loibl S, Gerber B, Blohmer JU, et al. Standardized Ki67 Diagnostics Using Automated Scoring--Clinical Validation in the GeparTrio Breast Cancer Study. Clin Cancer Res 2015; 21: 3651-3657. (PMID: 25501130) [Crossref]
16. Urruticoechea A, Smith IE, Dowsett M. Proliferation marker Ki-67 in early breast cancer. J Clin Oncol 2005; 23: 7212-7220. (PMID: 16192605) [Crossref]
17. Nishimura R, Osako T, Okumura Y, Hayashi M, Arima N. Clinical significance of Ki-67 in neoadjuvant chemotherapy for primary breast cancer as a predictor for chemosensitivity and for prognosis. Breast Cancer 2010; 17: 269-275. (PMID: 19730975) [Crossref]
18. Kim KI, Lee KH, Kim TR, Chun YS, Lee TH, Park HK. Ki-67 as a predictor of response to neoadjuvant chemotherapy in breast cancer patients. J Breast Cancer 2014; 17: 40-46. (PMID: 24744796) [Crossref]
19. Alba E, Lluch A, Ribelles N, Anton-Torres A, Sanchez-Rovira P, Albanell J, et al. High Proliferation Predicts Pathological Complete Response to Neoadjuvant Chemotherapy in Early Breast Cancer. Oncologist 2016; 21: 150-155. (PMID: 26786263) [Crossref]
20. Denkert C, Loibl S, Müller BM, Eidtmann H, Schmitt WD, Eiermann W, et al. Ki67 levels as predictive and prognostic parameters in pretherapeutic breast cancer core biopsies: a translational investigation in the neoadjuvant GeparTrio trial. Ann Oncol 2013; 24: 2786-2793. (PMID: 23970015) [Crossref]
21. Sivina E, Blumberga L, Purkalne G, Irmejs A. Pathological complete response to neoadjuvant chemotherapy in triple negative breast cancer – single hospital experience. Hered Cancer Clin Pract 2023; 21: 4. (PMID: 36922883) [Crossref]
22. Keam B, Im SA, Lee KH, Han SW, Oh DY, Kim JH, et al. Ki-67 can be used for further classification of triple negative breast cancer into two subtypes with different response and prognosis. Breast Cancer Res 2011; 13: R22. (PMID: 21366896) [Crossref]
23. Chen X, He C, Han D, Zhou M, Wang Q, Tian J, et al, The predictive value of Ki-67 before neoadjuvant chemotherapy for breast cancer: a systematic review and meta-analysis. Future Oncol 2017; 13: 843-857. (PMID: 28075166) [Crossref]
24. Wang J, Sang D, Xu B, Yuan P, Ma F, Luo Y, et al. Value of Breast Cancer Molecular Subtypes and Ki67 Expression for the Prediction of Efficacy and Prognosis of Neoadjuvant Chemotherapy in a Chinese Population. Medicine (Baltimore) 2016; 95: e3518. (PMID: 27149453) [Crossref]
25. Chen R, Ye Y, Yang C, Peng Y, Zong B, Qu F, et al. Assessment of the predictive role of pretreatment Ki-67 and Ki-67 changes in breast cancer patients receiving neoadjuvant chemotherapy according to the molecular classification: a retrospective study of 1010 patients. Breast Cancer Res Treat 2018; 170: 35-43. (PMID: 29480449) [Crossref]
26. Kim SI, Sohn J, Koo JS, Park SH, Park HS, Park BW. Molecular subtypes and tumor response to neoadjuvant chemotherapy in patients with locally advanced breast cancer. Oncology 2010; 79: 324-330. (PMID: 21430399) [Crossref]
27. Petit T, Wilt M, Velten M, Millon R, Rodier JF, Borel C, et al. Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. Eur J Cancer 2004; 40: 205-211. (PMID: 14728934) [Crossref]
28. Jarząb M, Stobiecka E, Badora-Rybicka A, Chmielik E, Kowalska M, Bal W, et al. Association of breast cancer grade with response to neoadjuvant chemotherapy assessed postoperatively. Pol J Pathol 2019; 70: 91-99. (PMID: 31556559) [Crossref]
29. Madani SH, Payandeh M, Sadeghi M, Motamed H, Sadeghi E. The correlation between Ki-67 with other prognostic factors in breast cancer: A study in Iranian patients. Indian J Med Paediatr Oncol 2016; 37: 95-99. (PMID: 27168707) [Crossref]
30. Shen S, Xiao G, Du R, Hu N, Xia X, Zhou H. Predictors of lymphovascular invasion identified from pathological factors in Chinese patients with breast cancer, Oncotarget 2017; 9: 2468-2474. (PMID: 29416785) [Crossref]
31. Kawajiri H, Takashima T, Aomatsu N, Kashiwagi S, Noda S, Onoda N, et al. Prognostic significance of pathological complete response following neoadjuvant chemotherapy for operable breast cancer. Oncol Lett 2014; 7: 663-668. (PMID: 24527070) [Crossref]

32. von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012; 30:1796-1804. (PMID: 22508812) [Crossref]
33. Kong X, Moran MS, Zhang N, Haffty B, Yang Q. Meta-analysis confirms achieving pathological complete response after neoadjuvant chemotherapy predicts favourable prognosis for breast cancer patients. Eur J Cancer 2011; 47: 2084-2090. (PMID: 21737257) [Crossref]
34. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 2014; 384: 164-172. (PMID: 24529560) [Crossref]
35. Spring LM, Fell G, Arfe A, Sharma C, Greenup R, Reynolds KL, et al. Pathologic Complete Response after Neoadjuvant Chemotherapy and Impact on Breast Cancer Recurrence and Survival: A Comprehensive Meta-analysis. Clin Cancer Res 2020; 26: 2838-2848. (PMID: 32046998) [Crossref]
36. Choudhary P, Gogia A, Deo SVS, Sharma D, Mathur SR, Batra A, et al. Correlation of pathological complete response with outcomes in locally advanced breast cancer treated with neoadjuvant chemotherapy: An ambispective study. Cancer Res Stat Treat 2021; 4: 611-620. [Crossref]
37. Cabrera-Galeana P, Muñoz-Montaño W, Lara-Medina F, Alvarado-Miranda A, Pérez-Sánchez V, Villarreal-Garza C, et al. Ki67 Changes Identify Worse Outcomes in Residual Breast Cancer Tumors After Neoadjuvant Chemotherapy. Oncologist 2018; 23: 670-678. (PMID: 29490940) [Crossref]
38. Yoshioka T, Hosoda M, Yamamoto M, Taguchi K, Hatanaka KC, Takakuwa E, et al. Prognostic significance of pathologic complete response and Ki67 expression after neoadjuvant chemotherapy in breast cancer. Breast Cancer 2015, 22: 185-191. (PMID: 23645542) [Crossref]
39. Chen C, Zhang Y, Huang Z, Wu J, Huang W, Zhang G. Decrease in the Ki67 index during neoadjuvant chemotherapy predicts favorable relapse-free survival in patients with locally advanced breast cancer. Cancer Biol Med 2019; 16: 575-586. (PMID: 31565486) [Crossref]

Original Article

Eur J Breast Health 2024; 20(3): 207-214
DOI: 10.4274/ejbh.galenos.2024.2024-3-9

The Effect of Informative Mobile App Use on Anxiety, Distress, and Quality of Life of Women With Breast Cancer

Filiz Salman Saraç[1], Sibel Erkal İlhan[2], Suat Kutun[3], Sevinç Kutlutürkan[4]

[1]Department of Surgical Nursing, Süleyman Demirel University Faculty of Health Sciences, Isparta, Turkey
[2]Department of Nursing, Haliç University Faculty of Health Sciences, İstanbul, Turkey
[3]Department of Surgical Oncology, Ankara Oncology Training and Research Hospital, University of Health Sciences, Ankara, Turkey
[4]Department of Nursing, Ankara University Faculty of Nursing, Ankara, Turkey

ABSTRACT

Objective: To evaluate the effect of mobile app-based educational information on anxiety, distress, and quality of life in patients with breast cancer (BC).

Materials and Methods: This mobile app was designed to assist patients before and after BC surgery. This randomized controlled study was conducted between April and August 2021. The intervention group (n = 42) received routine care and access to the mobile app for one month, while the control group (n = 40) received only routine care. Data were collected using questionnaires one week before and three weeks after surgery.

Results: The patients in the intervention group, after using the mobile app, had significantly lower anxiety and distress levels than those in the control group (p<0.05). However, there was no difference between the two groups regarding overall quality of life and subscale mean scores (p>0.05).

Conclusion: These findings suggest that using informative mobile apps starting before surgery can effectively reduce anxiety and distress in the early periods after surgery. Although the impact on overall quality of life was insignificant, such interventions may have long-term positive effects on quality of life.

Keywords: Anxiety; breast cancer; cancer care; mobile health; nursing

Cite this article as: Salman Saraç F, Erkal İlhan S, Kutun S, Kutlutürkan S. The Effect of Informative Mobile App Use on Anxiety, Distress, and Quality of Life of Women With Breast Cancer. Eur J Breast Health 2024; 20(3): 207-214

Key Points

- The anxiety and distress scores of women utilizing the informative mobile application were significantly lower in the early period after surgery.
- Although quality of life decreased during the postoperative period, no significant difference was identified between the intervention and control groups.
- Participants who received educational information through the mobile application expressed satisfaction with the provided content.

Introduction

Breast cancer (BC) is one of the most important health problems threatening women globally. According to the Cancer Statistics for 2020, female BC is the most frequently diagnosed cancer, with an estimated 2.3 million new cases (1). In Turkey, BC is also the most common cancer among women, with an incidence of 48.6 per hundred thousand (2). Following a diagnosis of BC, women commence treatments such as chemotherapy, radiotherapy, and surgical procedures.

BC patients require additional information regarding the treatment following their diagnosis (3, 4). Patients seek information on essential aspects, such as the treatment's side effects, long-term outcomes, details about aftercare, and post-treatment care (5). Due to insufficient information on the procedure, rehabilitation, recovery time, and pain management, patients harbor concerns about potential complications associated with surgery (4). Insufficient information contributes to uncertainty, distress, anxiety, and fear among BC patients. (5). In addition, any unmet need for information will increase anxiety and distress and thus decrease the quality of life (6-9). Informative training and counseling are essential in reducing the anxiety and distress experienced by women during BC surgery. Patients also express a desire for written materials, facilitating the recall of information (5).

Using accessible technologies, like mobile applications (apps), will aid in ensuring that women receive adequate information and training throughout their treatment. With their ease of use, mobile apps enable

Corresponding Author:
Filiz Salman Saraç; filizslmn@gmail.com

Received: 26.03.2024
Accepted: 02.06.2024
Available Online Date: 01.07.2024

access to much information or training via smart devices, regardless of time and place. Supportive care with mobile apps may revolutionize cancer care (10). Numerous mobile apps, whether independently developed or institutionally created, are currently accessible for BC-related purposes (11). Mobile apps continue to be produced to provide information, education, or support to patients at different stages of BC treatment (12-15). While mobile apps are designed to aid in BC care management, further studies are required to establish evidence-based data (16). Moreover, BC patients seek trustworthy mobile health apps to manage their care independently (17, 18). Implementing a mobile application that provides information and support to patients throughout the treatment process can reduce their anxiety and distress and increase their quality of life. The objective of this study was to assess the impact of a mobile app, designed to provide information to BC patients undergoing surgical treatment, on their levels of anxiety, distress, and quality of life.

Materials and Methods

Design

This study was conducted as a two-group randomized controlled trial with BC patients scheduled for elective surgery.

Participants

Patients were included if they were scheduled for elective surgery due to BC, were over 18 years old, could read and understand Turkish, were at least a primary school graduate, had internet access, and had a smartphone with an Android operating system suitable to download the mobile app. Patients who were diagnosed with active psychiatric disease, were using antidepressant medication, had vision problems that prevented them from using a mobile app, and were scheduled for reconstructive surgery using their tissue were excluded from the study. In this study, out of 162 recruited patients, 74 were excluded due to the following reasons: non-Turkish speakers (n = 5), illiteracy (n = 5), non-phone users (n = 10), lack of a phone compatible with the e-mobile application (n = 23), use of antidepressant medication (n = 12), planned additional surgeries alongside breast surgery (n = 7), and unwillingness to participate (n = 12). The participants were randomized into either the intervention group or the control group. The required sample size was determined previously using the "G. Power-3.1.7" software package after reaching ten individuals in each group. Accordingly, at least 42 individuals were identified in each group at 80% power, 0.54 effect size, and 0.05 significance level. Considering there may be drop-out during the study, 88 patients were included in the sample (Figure 1).

Randomization

Types of surgery for women who will have breast surgery for BC for the first time were breast-conserving surgery, mastectomy, and modified radical mastectomy. Intervention and control groups were determined using the bloc randomization method, stratified according to the types of surgery. The random.org (https://www.random.org/) website was used for randomization. The random assignments were conducted under the clinic nurse's supervision.

Preliminary Study

The preliminary preparation for this study was carried out in three stages, including the creation of scientific content, mobile app design, and pilot study.

The Creation of Scientific Content: Scientific content for the needs of patients was gathered under three main headings, and some content was structured with video support (Table 1). The scientific content included information and education about BC, preparations, and training before the operation, as well as post-operative care management and discharge training. The videos included training, such as breathing exercises, arm and shoulder exercises, and wound and drain care. The content validity index (CVI) of Davis (19) was used to determine the content validity of scientific content. Values with a CVI of 0.80 or above are considered valid. The overall content validity of the guide, for which ten experts were consulted, was 0.98

Mobile app Design: The mobile app had three sections: Information Forum (I), Personal Forum (notebook and reminder) (II), and Ask the Researcher (messaging) (III). The information forum is a section that enables patients to access related texts, pictures, and videos. This section, presented under three main headings, general information about BC, pre-operative information, and post-operative information, offers a comprehensive educational package to the patients (Table 1). The prepared pictures and videos were placed on the relevant subject. The personal forum is a section that allows patients to create their notes and use the necessary reminders. The Ask the Researcher section will enable patients to communicate with the researcher via messages. The participants could download the "Breast Cancer Surgery Information Guide" mobile app from the Google Play Store and install it on their phones. Users whom the researcher authenticated could access the content with an email and password.

Pilot Study: The mobile app's functionality was evaluated with three patients during the surgical treatment process, but they were omitted from the final analysis. The mobile app was finalized based on stylistic and improvement suggestions from the patients.

Data Collection

Preoperative interviews were conducted face-to-face, on average, 3–7 days before admission to the hospital. In the first interview with the patients, data were collected using the Patient Information Form and Anxiety, Distress, and Quality of Life measurement tools (see below)

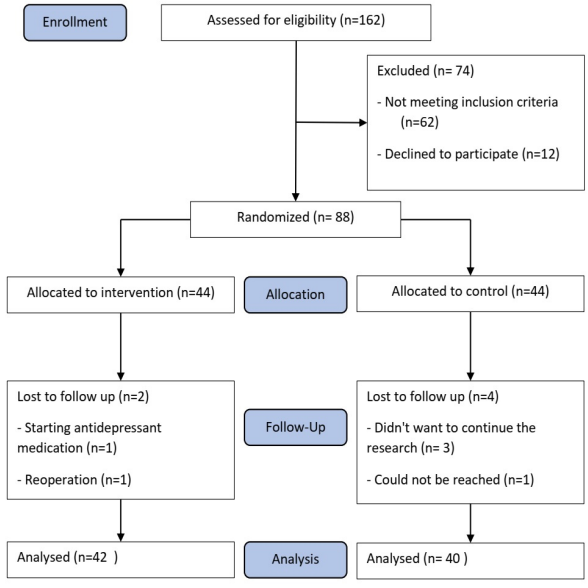


Figure 1. The CONSORT flow diagram

Table 1. Information headings and subheadings in the mobile application

General information	Preoperative information	Postoperative information
- What is Cancer? - What is Breast Cancer? - Breast Cancer Risk and Prevention - Breast Cancer Detection and Diagnosis [BSE-Video] - Types of Breast Cancer - Breast Cancer Surgery and Breast Reconstruction - Other Treatment Methods of Breast Cancer	- General information before hospitalization (information about the surgery and consent, situations that should be reported to the doctor, blood supply, pre-operative tests, private room situation, companion situation, meal and visiting hours, coping with pre-operative stress) [Video] - Preparation from the day before the surgery until the night (shower/bath, hair removal, fasting period, items to be brought to the hospital, exercises to be learned before the surgery) [Video] - Surgery Day Preparation [Video]	- Conditions to be considered in the first 24 hours after the surgery - Post-operative complications (respiratory complications, hematoma, seroma, wound complications, lymphedema) - Pain and its management - Time to discharge and recheck - Post-operative bath time - Wound care - Stitches care and removal - Surgical Drain Care [Video] - Life After Discharge (skin care, use of deodorant-perfume, avoidance of heat, nutrition, work life, home life, doing sports, traveling, sexual life, choice of clothing, breast prosthesis and pocketed bra use, psychological health after surgery) [Video] - Lymphedema and its management [Video] - Arm Shoulder Strengthening Exercises [Video]

Intervention Group: The researcher uploaded the mobile app to the Android market and downloaded it to the patients' phones. Patients were taught how to use this app and given an instruction manual. One week after the surgery, the patients were called and reminded about using the app. Data were collected three weeks after the surgery through telephone interviews with the Patient Follow-up Form, Anxiety, Distress, and Quality of Life measurement tools, the Patient Information Satisfaction Questionnaire, and the Mobile App Evaluation Form.

Control Group: The patients received routine care and training in the clinic, and no additional intervention was applied. Data were collected three weeks after the surgery through telephone interviews with the Patient Follow-up Form, Anxiety, Distress, and Quality of Life measurement tools, and the Patient Information Satisfaction Questionnaire.

After the data collection process for the mobile app was completed, the patients in the control group were informed about it. The mobile app was downloaded for the patients (n = 15) who wanted it, and the information was sent to the others (n = 25) as a PDF document. Patients in the intervention group could continue to access the mobile app.

Data Collection

Data were collected using data collection forms from patients who attended the surgical oncology clinics and outpatient clinics of a university hospital between April and August 2021.

Patient Information and Follow-Up Form: This form consists of two sections. The first section consists of questions about patients, such as age, marital status, education status, occupation, employment status, income status, smoking status, presence of chronic disease, planned hospitalization, date of surgery and discharge, the type of treatment, the performed type of the surgery, sentinel lymph node dissection, and axillary lymph node dissection status. The second section, the "Follow-up form", includes questions about the type of surgery performed and the date of hospitalization, surgery, and discharge.

Hospital Anxiety and Depression Scale: The hospital anxiety and depression scale (HAD) is a self-report scale used to diagnose anxiety and depression in a short time and to determine the risk group in patients with physical illness and applied to primary healthcare services. Only the Anxiety subscale (HAD-A) was used in this study. The anxiety subscale consists of seven items (1st, 3rd, 5th, 7th, 9th, 11th, and 13th questions). Items 1, 3, 5, 11, and 13 gradually decrease severity. The score that is obtained from the HAD-A ranges between 0 to 21 (min.-max.). The Turkish validity and reliability study of the scale was conducted by Aydemir et al. (20). In the reliability study of the scale, Cronbach's alpha value for the anxiety subscale was found to be 0.85. In this study, Cronbach's alpha value of the scale was calculated as 0.83.

The NCCN Distress Thermometer and Problem List: This scale measures psycho-social distress in cancer patients. It consists of a visual analog scale that individuals can apply independently, consisting of only one question. It is used to evaluate the stress situation patients have experienced in the last week and a list of problems. The validity and reliability study of the NCCN Distress thermometer was conducted by Özalp et al. (21) in 2006. The scale's sensitivity in the study was 0.73, and the specificity was 0.49. A list of problems was added to the distress thermometer in 2003. The problem list consists of the issues collected in five different groups (daily life, family, emotional, physical, and body problems) that cancer patients frequently experience. Patients mark the difficulties they have encountered in the last week in the list (21).

FACT-G Quality of Life Scale: Functional Assessment of Cancer Therapy-General (version 4) Quality of Life Scale consists of four dimensions: physical well-being (seven items), social/family well-being (seven items), emotional well-being (six items), and functional well-being (seven items). There were 27 questions on the scale. The scale has a 5-point Likert-type structure and is scored between 0 and 4. All questions assess the patient's quality of life in the last seven days. The scale's total score equals the sum of the sub-dimensions, and a high score indicates a high quality of life. Cronbach's alpha value calculated because of the cross-cultural use of the scale was found to be 0.88 (22). In our study, Cronbach's alpha value of the scale was determined to be 0.92.

Patient Information Satisfaction Questionnaire: This questionnaire consists of three questions about patients' satisfaction with the information they receive and the information/training they receive from doctors and nurses.

Mobile App Evaluation Form: This form consists of seven closed questions that are answered "yes, no, or partially" to evaluate the mobile information app used by the patients in the intervention group in terms of form, layout, readability, information quality, and ease of use, and two open-ended questions about the most used section on the app and suggestions for improvement (23, 24).

Ethical Considerations

Institutional permission and the University Ethics Committee approval were obtained for the study (approval number: 83264, date: 23.10.2019 - Ankara University Ethics Committee). This study was performed in compliance with the Declaration of Helsinki (as revised in Brazil 2013). Written informed consent was obtained from the patients. The permission of the authors was obtained to use the measurement tools. To protect personal data, the information used to access the mobile app was closed to third parties.

Statistical Analysis

Data were analyzed using SPSS, version 21 (IBM Inc., Armonk, NY USA). Descriptive statistics of the variables were presented with numbers (n), percentages (%), mean scores ($\bar{x}$), standard deviation (ss), median, interquartile range, and minimum-maximum values. The Shapiro-Wilk test was used to confirm the data conform to the normal distribution, the paired samples t-test was used to evaluate the repeated measurements, and the independent samples t-test was employed to compare the groups. The data obtained were evaluated at a confidence interval of 95% and a significance level of $p<0.05$. An intention to treat (ITT) analysis was performed based on the groups to which the women were initially assigned. A per-protocol analysis was used for reporting because similar main results were obtained in the ITT analysis.

Results

Demographic and Disease-Treatment Characteristics: The mean age of the patients in the intervention group was 48.8±9.0 years, and the mean age in the control group was 49.3±9.4 years. There was no statistically significant difference between the groups according to the descriptive and disease-treatment characteristics of the patients ($p>0.05$; Table 2).

The Effect of Mobile Apps on Anxiety: The mean post-test anxiety scores of the patients in the intervention and control groups were significantly lower than their pre-test scores ($p<0.05$). The mean anxiety scale scores of the patients in the intervention group after access to mobile information were significantly lower than those of the control group ($t = -2.031$, $p<0.05$; Table 3).

The Effect of Mobile App on Distress: The mean distress score of the patients in the intervention group (IG) after mobile information was significantly lower than in the pre-test score ($t = 6.50$, $p<0.01$) and control group (CG; $t = 3.642$, $p<0.01$; Table 3). The causes of patients' distress were primarily emotional problems (IG = 78.8%, CG 71.4%) in the pre-test, whereas emotional (IG = 44.5%, CG = 49.4%) and physical (IG = 38.8%, CG = 34.6%) problems were found in the post-test.

The Impact of Mobile Apps on Quality of Life: Total and sub-dimension mean quality of life scores after mobile information did not differ between the intervention and control groups ($p<0.05$). The total quality of life score and the functional well-being mean score of the patients in the control group were significantly lower than their pre-test scores (t/p = 2.123/0.04; t/p: 3.426/0.001, respectively). In both groups, the mean physical well-being score decreased ($p<0.05$), and the mean emotional well-being score increased ($p<0.05$) in the post-test, and there was no significant difference between the groups (Table 3).

Satisfaction with Patient Information: There was a significant difference between the intervention and control groups according to their satisfaction with all information/education they received ($p<0.01$). Patients in the intervention group were more satisfied with the information they received than the control group ($x^2 = 13.47$, $p = 0.001$).

Evaluation of the Mobile App: The patients' evaluations were as follows: the adequacy of information provided in the mobile app (90.5%), the quality of information (90.2%), satisfaction with the app's visual aspects (92.9%), the adequacy of the app's readability level (90.5%), and satisfaction with the app's ease of use (92.9%).

Discussion and Conclusion

The findings of this study support the research hypothesis, indicating that the implementation of an informative mobile app effectively reduced anxiety and distress levels among women undergoing surgery for BC. However, unlike our research hypothesis, the results show that this implementation did not positively impact the health-related quality of life in the early postoperative period.

The comparison between the groups demonstrated that the intervention group using the mobile app exhibited a significantly lower anxiety score, supporting the research hypothesis. Various studies with different results have been conducted examining the effect of web and mobile-based information on the anxiety level of BC patients. (25-27). Similar to this study, web-based training, which was given during the period before and after surgery, was effective in reducing the anxiety of women. However, the information that was provided with the mobile app consisted of BC biology, treatment methods, and surgical techniques and did not include the subjects that supported the care of the patient, increasing the anxiety and depression levels of the patients (25). The video-assisted information about the length of hospital stay, surgery, and adjuvant treatment given to the women in the preoperative period did not affect their anxiety levels (28). An information resource created according to women's needs, supported visually and audibly, covering care issues, and a platform where they can obtain advice about

Table 2. Descriptive and clinical characteristics of the patients

Characteristic	IG (n = 42)	CG (n = 40)	Statistical analysis	
	$\bar{x}$ ± SD	$\bar{x}$ ± SD		
Age (years)	(48.81±9.018) Min-Max (33–66)	(49.30±9.379) Min-Max (32–69)	*t* = -0.241	*p* = 0.810
	n (%)	**n (%)**	**χ^2**	***p***
Marital status				
Married	36 (85.7)	37 (92.5)	0.483	0.266
Single	6 (14.3)	3 (7.5)		
Education level				
Primary school	13 (31)	22 (55)	7.33	0.062
Secondary school	4 (9.5)	6 (15)		
High school	16 (38.1)	7 (17.5)		
University	9 (21.4)	5 (12.5)		
Job				
Worker	10 (23.8)	8 (20)	0.174	0.677
Housewife	32 (76.2)	32 (80)		
Income level				
Income less than expenses	4 (9.5)	10 (25)	3.466	0.063
Income equals expense	38 (90.5)	30 (75)		
Living city				
Ankara	31 (73.8)	27 (67.5)	0.394	0.530
Other cities	11 (26.2)	13 (32.5)		
Smoking				
Yes	13 (31)	12 (30)	0.009	0.925
No	29 (69)	28 (70)		
Chronic disease				
Yes	22 (52.4)	21 (52.5)	0.000	0.991
No	20 (47.6)	19 (47.5)		
Family history of breast cancer				
Yes	11 (26.2)	10 (25)	0.015	0.902
No	31 (73.8)	30 (75)		
Type of treatment				
Adjuvant	25 (59.5)	31 (77.5)	3.057	0.080
Neoadjuvant	17 (40.5)	9 (22.5)		
Planned type of surgery				
BCS + SLNB	14 (33.3)	15 (37.5)	0.297	0.862
Mastectomy + SLNB	20 (47.6)	19 (47.5)		
MRM	8 (19)	6 (15)		
Sentinel lymph node dissection				
Yes	34 (81)	34 (85)	0.237	0.626
No	8 (19)	6 (15)		
Axillary lymph node dissection				
Yes	24 (57.1)	18 (45)	1.209	0.272
No	18 (42.9)	22 (55)		

IG: Intervention group; CG: Control group; BCS: Breast conserving surgery; SLNB: Sentinel lymph node biopsy MRM: Modified radical mastectomy; SD: Standard deviation; Min-Max: Minimum-Maximum

Table 3. Comparison of the anxiety, distress and quality of life total and subscale scores of the patients

Characteristics		Pre-test		Post-test		Test statistics	
	Groups	**Mean ± SD**	**Median (IQR)**	**Mean ± SD**	**Median (IQR)**	**t**	***p***
Anxiety	IG	8.40±4.33	8(5)	4.74±4.037	3(5)	4.423	**0.001***
	CG	8.83±4.47	9(7)	6.55±4.038	6(6)	3.247	**0.002***
	IG *vs.*	t =-0.432		t =-2.031			
	CG	*p* = 0.667		***p* = 0.046***			
Distress	IG	5.95±2.48	6 (4)	3.81±2.25	4(3)	6.500	**0.001***
	CG	5.58±2.18	5.5 (3)	5.35±2.30	5(4)	0.621	0.538
	IG *vs.*	t = 0.783		t = -3.642			
	CG	*p* = 0.436		***p* = 0.001***			
QOL Total	IG	82.71±13.71	85.5 (22)	80.97±13.67	84 (18)	0.735	0.466
	CG	80.53±13.55	81 (17)	76.95±11.19	75 (17)	2.123	**0.04***
	IG *vs.*	t = 0.721		t = 1.45			
	CG	*p* = 0.473		*p* = 0.150			
Physical well being	IG	23.52±3.24	24 (4)	20.12±4.24	20.5 (5)	5.309	**0.001***
	CG	23.50±3.41	24 (4)	19.73±4.21	20 (7)	5.507	**0.001***
	IG *vs.*	t = 0.422		t = 0.422			
	CG	*p* = 0.675		*p* = 0.675			
Social/family well being	IG	24.56±3.25	25 (4)	24.88±2.72	25.33 (4)	-0.528	0.600
	CG	23.28±4.93	25 (6)	24.48±3.69	26 (6)	-1.824	0.076
	IG *vs.*	t = 0.563		t = 0.563			
	CG	*p* = 0.402		*p* = 0.575			
Emotional well being	IG	14.57±4.44	14.5 (5)	16.88±4.28	17.5 (6)	-3.029	**0.004***
	CG	13.9±4.34	14 (7)	15.30±3.48	15 (5)	-2.309	**0.026***
	IG *vs.*	t = 0.691		t = 1.827			
	CG	*p* = 0.491		*p* = 0.071			
Functional well being	IG	20.05±5.37	21 (8)	19.10±4.89	19.5 (7)	1.103	0.276
	CG	19.85±4.49	19 (7)	17.45±4.28	17 (6)	3.426	**0.001***
	IG *vs.*	t = 0.180		t = 1.617			
	CG	*p* = 0.858		*p* = 0.110			

*: p<0.05; IG: Intervention group; CG: Control group; QOL: Quallity of life; SD: Standard deviation; IQR: Interquartile range

their problems may have been effective in reducing women's anxiety. In the present study, an informative mobile app reduced distress levels. Similarly, Çınar et al. (13) reported that the supportive education given via a mobile app to BC patients receiving hormone therapy reduced the distress level of the patients. Studies investigating the effect of knowledge-based interventions on the level of distress are limited (13). In another study, group consultation combined with a tablet-based online app did not affect the distress level of BC patients, which may have been due to the low initial measurement distress levels of the patients (29). The increase in patients' distress levels is associated with the need for unmet information and supportive care (6, 7).Although the postoperative quality of life decreased in both groups, the quality of life score of the intervention group was better than that of the control group. In contrast to the present study's findings, research has indicated that interventions, such as self-management support, supportive education, and awareness training delivered through mobile applications and web-based educational programs positively impact the quality of life among women diagnosed with BC (13-15, 27). However, these developed applications are directed towards stages such as chemotherapy and radiotherapy rather than surgical treatment for BC. The messaging app-based rehabilitation program increased the quality of life of women who underwent breast surgery (30). In the study, interventions related to subjects, such as teaching arm-shoulder exercises, stress management, and nutrition, were applied to the women starting from the preoperative period. In the postoperative period, interventions were conducted to manage complications and life after discharge. The patient's quality of life after the surgery decreased in the first month compared with the preoperative period and increased by the sixth month (30). In this study, which evaluated the short-term quality of life of the patients, considering that other

treatment processes may have adverse effects on the quality of life, the information and support provided by the mobile app did not affect the quality of life. To evaluate the impact of informative mobile apps on the quality of life, long-term follow-ups should be performed starting from the preoperative period and after the surgery.The patients in the intervention group in the present study were satisfied with the mobile app developed and its contents. In a qualitative study evaluating the information needs of patients with BC, it was stated that women wanted the information to be given in a form that could be taken home to ensure that they could remember it easily (4). Mobile apps are technological products patients can download to their phones and quickly access information wherever and whenever they want. The mobile app we used in this study is adequate because it is easy to use and meets its purpose.

Study Limitations

The results of this study only include patients who underwent surgery for BC in the hospital where the study was conducted. Patients were operated on in three different ways based on the findings of preoperative tests, but there could be changes in the type of surgery depending on the patient's condition. Whether or not an axillary dissection would be performed on a patient scheduled for breast-conserving surgery was often clarified during the operation. Therefore, the study was conducted with patients undergoing all types of breast surgery. The mobile app used in the research was developed according to the type of smartphone used by middle-aged and older people in our country. It cannot be generalized to patients using other operating systems. Another limitation of the study is the inability to implement blinding for the researcher during the data collection. Lastly, the research was conducted when the effects of the COVID-19 pandemic had diminished, but the impact of the pandemic on the research was not investigated.

Although the informative mobile support app used in this study decreased the anxiety and distress levels of BC patients in the early postoperative period, its effect on longer term quality of life could not be determined, as there was only a one-month follow-up. This study, conducted during the pandemic, is relevant to the use of technological apps, which have been used increasingly in our daily lives and came to the fore during the pandemic. Interventions via mobile apps developed by health professionals can be used regardless of place/time. Nurses can use this application developed for BC patients in clinics to ensure continuity of patient education. Since this application can provide information to patients from the preoperative period, the unmet need for information and education may also be reduced. Moreover, supporting patient education and information with such mobile applications can enable patients to easily access reliable information or support in the post-discharge period. As a result, nurses' use of the developed mobile application in the care of patients may help reduce the anxiety and distress of BC patients. As a data source for evidence-based studies, this study will contribute to the results of using mobile informative apps in care practices for patients undergoing breast surgery.

Ethics Committee Approval: Institutional permission and the University Ethics Committee approval were obtained for the study (approval number: 83264, date: 23.10.2019 - Ankara University Ethics Committee).

Informed Consent: Written informed consent was obtained from the patients.

Authorship Contributions

Surgical and Medical Practices: S.K.; Concept: F.S.S., S.E.İ.; Design: F.S.S., S.E.İ., S.K.; Data Collection and/or Processing: F.S.S., S.K.; Analysis and/or Interpretation: F.S.S., S.K.; Literature Search: F.S.S.; Writing: F.S.S., S.E.İ., S.K., S.K.

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

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021; 71: 209-249. (PMID: 33538338) [Crossref]
2. Türkyılmaz M, Oruç Hamavioğlu Eİ, Dündar S, Kavak Ergün A, Sevinç A, Tütüncü S. Türkiye Kanser İstatistikleri 2018. Tolunay T, Kaygusuz S, Keskinkılıç B, Özsarı S, Gökler ME, editors. Ankara: Halk Sağlığı Genel Müdürlüğü Sağlık Bakanlığı; 2022. https://hsgm.saglik.gov.tr/depo/birimler/kanser-db/Dokumanlar/Istatistikler/Kanser_Rapor_2018.pdf [Crossref]
3. Findik UY. The Information Needs of Women Who Have Undergone Breast Cancer Surgery in the West of Turkey. J Cancer Educ 2017; 32: 432-437. (PMID: 26691514) [Crossref]
4. Villarreal-Garza C, López-Martínez EA, Martínez-Cannon BA, Platas A, Castro-Sánchez A, Miaja M, et al. Medical and information needs among young women with breast cancer in Mexico. Eur J Cancer Care (Engl) 2019; 28: e13040. (PMID: 31008546) [Crossref]
5. Herbert S, Wöckel A, Kreienberg R, Kühn T, Flock F, Felberbaum R, et al. To which extent do breast cancer survivors feel well informed about disease and treatment 5 years after diagnosis? Breast Cancer Res Treat 2021; 185: 677-684. (PMID: 33104958) [Crossref]
6. Barr K, Hill D, Farrelly A, Pitcher M, White V. Unmet information needs predict anxiety in early survivorship in young women with breast cancer. J Cancer Surviv 2020; 14: 826-833. (PMID: 32514909) [Crossref]
7. Lo-Fo-Wong DN, de Haes HC, Aaronson NK, van Abbema DL, den Boer MD, van Hezewijk M, et al. Risk factors of unmet needs among women with breast cancer in the post-treatment phase. Psychooncology 2020; 29: 539-549. (PMID: 31785043) [Crossref]
8. So WK, Chow KM, Chan HY, Choi KC, Wan RW, Mak SS, et al. Quality of life and most prevalent unmet needs of Chinese breast cancer survivors at one year after cancer treatment. Eur J Oncol Nurs 2014; 18: 323-328. (PMID: 24703094) [Crossref]
9. Tsuchiya M, Horn SA. An exploration of unmet information needs among breast cancer patients in Japan: a qualitative study. Eur J Cancer Care (Engl) 2009; 18: 149-155. (PMID: 19267730) [Crossref]
10. Chan RJ, Howell D, Lustberg MB, Mustian K, Koczwara B, Ng CC, et al. Advances and future directions in the use of mobile health in supportive cancer care: proceedings of the 2019 MASCC Annual Meeting symposium. Support Care Cancer 2020; 28: 4059-4067. (PMID: 32405966) [Crossref]
11. Giunti G, Giunta DH, Guisado-Fernandez E, Bender JL, Fernandez-Luque L. A biopsy of Breast Cancer mobile applications: state of the practice review. Int J Med Inform 2018; 110: 1-9. (PMID: 29331247) [Crossref]
12. Chung IY, Jung M, Park YR, Cho D, Chung H, Min YH, et al. Exercise Promotion and Distress Reduction Using a Mobile App-Based Community in Breast Cancer Survivors. Front Oncol 2020; 9: 1505. (PMID: 31998651) [Crossref]

13. Çınar D, Karadakovan A, Erdoğan AP. Effect of mobile phone app-based training on the quality of life for women with breast cancer. Eur J Oncol Nurs 2021; 52: 101960. (PMID: 33882446) [Crossref]
14. Zhu J, Ebert L, Liu X, Wei D, Chan SW. Mobile Breast Cancer e-Support Program for Chinese Women With Breast Cancer Undergoing Chemotherapy (Part 2): Multicenter Randomized Controlled Trial. JMIR Mhealth Uhealth 2018; 6: e104. (PMID: 29712622) [Crossref]
15. Öztürk ES, Kutlutürkan S. The Effect of the Mobile Application-Based Symptom Monitoring Process on the Symptom Control and Quality of Life in Breast Cancer Patients. Semin Oncol Nurs 2021; 37: 151161. (PMID: 34088557) [Crossref]
16. Jongerius C, Russo S, Mazzocco K, Pravettoni G. Research-Tested Mobile Apps for Breast Cancer Care: Systematic Review. JMIR Mhealth Uhealth 2019; 7: e10930. (PMID: 30741644) [Crossref]
17. Brennan L, Kessie T, Caulfield B. Patient Experiences of Rehabilitation and the Potential for an mHealth System with Biofeedback After Breast Cancer Surgery: Qualitative Study. JMIR Mhealth Uhealth 2020; 8: e19721. (PMID: 32687476) [Crossref]
18. Hou IC, Lan MF, Shen SH, Tsai PY, Chang KJ, Tai HC, et al. The Development of a Mobile Health App for Breast Cancer Self-Management Support in Taiwan: Design Thinking Approach. JMIR Mhealth Uhealth 2020; 8: e15780. (PMID: 32352390) [Crossref]
19. Davis LL. Instrument review: Getting the most from a panel of experts. Appl Nurs Res 1992; 5: 194-197. [Crossref]
20. Aydemir O, Güvenir T, Küey L, Kültür S. Hastane anksiyete ve depresyon ölçeği türkçe formunun geçerlilik ve güvenilirlik çalışması. Turk Psikiyatri Derg 1997; 8: 280-287. [Crossref]
21. Ozalp E, Cankurtaran ES, Soygür H, Geyik PO, Jacobsen PB. Screening for psychological distress in Turkish cancer patients. Psychooncology 2007; 16: 304-311. (PMID: 16909427) [Crossref]
22. Victorson D, Barocas J, Song J, Cella D. Reliability across studies from the functional assessment of cancer therapy-general (FACT-G) and its subscales: a reliability generalization. Qual Life Res 2008; 17: 1137-1146. (PMID: 18841493) [Crossref]
23. Levine DM, Co Z, Newmark LP, Groisser AR, Holmgren AJ, Haas JS, et al. Design and testing of a mobile health application rating tool. NPJ Digit Med 2020; 3: 74. (PMID: 32509971) [Crossref]
24. Stoyanov SR, Hides L, Kavanagh DJ, Zelenko O, Tjondronegoro D, Mani M. Mobile app rating scale: a new tool for assessing the quality of health mobile apps. JMIR Mhealth Uhealth 2015; 3: e27. (PMID: 25760773) [Crossref]
25. Foley N, O'Connell E, Lehane E, Livingstone V, Maher B, Kaimkhani S, et al. PATI: Patient accessed tailored information: A pilot study to evaluate the effect on preoperative breast cancer patients of information delivered via a mobile application. Breast 2016; 30: 54-58. (PMID: 27611236) [Crossref]
26. Korkmaz S, Iyigun E, Tastan S. An Evaluation of the Influence of Web-Based Patient Education on the Anxiety and Life Quality of Patients Who Have Undergone Mammaplasty: a Randomized Controlled Study. J Cancer Educ 2020; 35: 912-922. (PMID: 31119709) [Crossref]
27. White V, Farrelly A, Pitcher M, Hill D. Does access to an information-based, breast cancer specific website help to reduce distress in young women with breast cancer? Results from a randomised trial. Eur J Cancer Care (Engl) 2018; 27: e12897. (PMID: 30137657) [Crossref]
28. Wysocki WM, Mituś J, Komorowski AL, Karolewski K. Impact of preoperative information on anxiety and disease-related knowledge in women undergoing mastectomy for breast cancer: a randomized clinical trial. Acta Chir Belg 2012; 112: 111-115. (PMID: 22571072) [Crossref]
29. Visser A, Prins JB, Jansen L, Radema SA, Schlooz MS, van Dalen T, et al. Group medical consultations (GMCs) and tablet-based online support group sessions in the follow-up of breast cancer: A multicenter randomized controlled trial. Breast 2018; 40: 181-188. (PMID: 29906741) [Crossref]
30. Zhou K, Wang W, Zhao W, Li L, Zhang M, Guo P, et al. Benefits of a WeChat-based multimodal nursing program on early rehabilitation in postoperative women with breast cancer: A clinical randomized controlled trial. Int J Nurs Stud 2020; 106: 103565. (PMID: 32272281) [Crossref]

Original Article

Eur J Breast Health 2024; 20(3): 215-222
DOI: 10.4274/ejbh.galenos.2024.2024-3-14

Retrospective Analysis of the Clinical Usefulness of a Strut-Adjusted Volume Implant in a Single Center

Arisa Ata-Shiroshita[1], Takashi Kuwayama[1,2], Masako Kato[3], Hidenori Shinjo[3], Kazunori Miyaura[4], Aya Nagata[1], Nana Komatsu[1], Misaki Matsuyanagi[1], Haruna Sakai[1], Yuki Matsunaga[1], Sayuka Nakayama[1], Ayuha Yoshizawa[1], Murasaki Ikeda[3], Kanae Taruno[1], Hiroko Masuda[1], Terumasa Sawada[1], Naoki Hayashi[1], Yoshinori Ito[3], Chie Watanabe[1,5], Sadako Akashi-Tanaka[6], Seigo Nakamura[1,7]

[1]Division of Breast Surgical Oncology, Department of Surgery, Showa University School of Medicine, Tokyo, Japan
[2]Department of Breast Surgical Oncology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan
[3]Division of Radiation Oncology, Department of Radiology, Showa University School of Medicine, Tokyo, Japan
[4]Medical Physics Course, Showa University Graduate School of Health Science, Tokyo, Japan
[5]Showa University School of Nursing and Rehabilitation Sciences, Tokyo, Japan
[6]Department of Breast Surgery, Tokyo Women's Medical University, Tokyo, Japan
[7]Showa University Institute for Clinical Genetics and Genomics, Tokyo, Japan

ABSTRACT

Objective: Reports demonstrating the effectiveness and safety of strut-adjusted volume implants (SAVI) in Japan are limited. Therefore, this study aimed to compare the treatment outcomes of SAVI and whole-breast irradiation (WBI) at a single facility.

Materials and Methods: Data were retrospectively extracted from the medical records of patients treated with SAVI or WBI following partial mastectomy (Bp). Patients undergoing Bp, sentinel lymph node biopsy, and SAVI spacer insertion followed by brachytherapy with the SAVI device were compared to those followed with WBI. Local recurrence was assessed annually by physical examination, bilateral mammography, and breast ultrasonography.

Results: The SAVI and WBI groups comprised 53 and 113 patients, with a median age of 55 and 52 years, respectively; among them, 47 and 91 patients had a pathological tumor diameter ≤2 cm and six and 22 had a pathological tumor diameter >2 cm, respectively. Recurrence events, acute adverse events, and late adverse events were observed in the SAVI and WBI groups in 1 and 3 (p = 0.726), 24 and 79 (p = 0.01), and 24 and 18 patients (p = 0.00002), respectively, with median observation periods of 60.0 and 47.8 months, respectively. All adverse events were grades 1–2, with dermatitis being the most common in the acute phase. In the late phase, pigmentation was common in both groups.

Conclusion: The local recurrence rate does not differ between SAVI and WBI within the relatively short-term follow-up period. Longer follow-up is required to confirm our results in the Japanese population.

Keywords: SAVI; accelerated partial breast irradiation; Japanese; breast cancer; local recurrences

Cite this article as: Ata-Shiroshita A, Kuwayama T, Kato M, Shinjo H, Miyaura K, Nagata A, Komatsu N, Matsuyanagi M, Sakai H, Matsunaga Y, Nakayama S, Yoshizawa A, Ikeda M, Taruno K, Masuda H, Sawada T, Hayashi N, Ito Y, Watanabe C, Akashi-Tanaka S, Nakamura S. Retrospective Analysis of the Clinical Usefulness of a Strut-Adjusted Volume Implant in a Single Center.Eur J Breast Health 2024; 20(3): 215-222

Key Points

- The strut-adjusted volume implant (SAVI) and whole breast irradiation (WBI) groups showed similar local recurrence rates within the relatively short-term follow-up period (p = 0.726). The median follow-up periods in the SAVI and WBI groups were 60.0 and 47.8 months, respectively.
- Fewer adverse events were observed in the SAVI group than in the WBI group in the acute phase.
- In the late stage, patients in the SAVI group experienced more adverse events than those in the WBI group; however, most of them were grade 1, with no significant difference in grade 2 or higher events compared to WBI.
- In the future, tumor control, adverse events, and cosmetic outcomes in the SAVI and WBI groups should be observed and compared over a longer period.

Corresponding Author:
Takashi Kuwayama; kuwayama@med.showa-u.ac.jp

Received: 16.04.2024
Accepted: 09.06.2024
Available Online Date: 01.07.2024

Introduction

Whole-breast irradiation (WBI) is the standard treatment for local control after partial mastectomy (Bp) for breast cancer, which has been shown to reduce the risk of local recurrence and contribute to survival rates in meta-analyses of previous clinical trials (1). However, WBI usually requires long-term outpatient treatment for 5–6 weeks or even 3–4 weeks for hypofractionation, and most patients experience adverse events, primarily radiodermatitis. A clinical trial demonstrated that approximately 70% of local recurrences in the preserved breast after Bp occurred near the original tumor bed, with recurrences from other areas resembling contralateral breast cancer occurrences in terms of timing and frequency (2, 3). Therefore, control of local recurrence in the preserved breast by targeting only the tumor bed with radiation therapy may be possible.

Accelerated partial breast irradiation (APBI) is a modality that enables an increase in the per-fraction dose and a reduction in treatment duration by narrowing the irradiation target volume to only the tumor bed using several methods, including brachytherapy, intraoperative irradiation, and external irradiation. Brachytherapy involves the insertion of small, sealed sources containing radioactive isotopes into the body to directly irradiate cancerous tissues, allowing minimal damage to the surrounding normal tissues, which is a standard treatment for cervical and prostate cancers. For breast cancer, brachytherapy methods include interstitial irradiation using the multicatheter method and intracavitary irradiation using the balloon catheter method (MammoSite®) or a strut-adjusted volume implant (SAVI).

The balloon catheter method (MammoSite®) is a single-lumen balloon-type applicator, whereas the SAVI has a cage-like structure surrounding the center catheter with multiple outer catheters, which are expanded post-insertion to adhere to the resection cavity post-lesion excision. The SAVI was approved by the Food and Drug Administration in 2006 and received pharmaceutical approval in Japan in 2013.The local recurrence rate of post-irradiation using the SAVI is approximately 3.6%, showing no significant difference in treatment outcomes compared with standard treatment. It shows excellent tumor control, comparable to that of APBI, and survival with low toxicity (4). Recently, the preliminary results of a prospective clinical trial on the usefulness of brachytherapy using SAVI for Japanese patients with breast cancer have been reported (5). In this report, 44 patients were included, and Grade 2 acute toxicities were observed in 18% of the patients. This report mainly focused on dosimetry and acute adverse events; thus, clinical information regarding the local recurrence and late adverse event rates in this population is limited. Health insurance covers brachytherapy for breast cancer, including the SAVI device in Japan; however, this coverage includes brachytherapy treatments beyond those using the SAVI device. The insurance approval is not specifically focused on brachytherapy using the SAVI device, which may be a barrier to expanding brachytherapy with SAVI and might be one reason for the limited use of the SAVI device in Japan. Therefore, the clinical efficacy, usefulness, and side effects of brachytherapy with SAVI in the Japanese population should be clarified.

In this study, we compared the treatment outcomes and adverse events of SAVI with those of WBI by retrospectively examining cases of WBI and SAVI use at our institution in a single-facility setting. This study aimed to clarify the clinical data, such as local recurrence and acute/late adverse event rates, which would contribute to the new insurance coverage specifically focused on brachytherapy with the SAVI device.

Materials and Methods

Study Design

This retrospective observational study extracted data from medical records of patients treated with SAVI or WBI following breast-conserving therapy at Showa University Hospital from February 2014 to June 2019. This trial was conducted with ethical approval from Showa University Research Ethics Review Board Committee (approval no: 22-170-B, date: 17.11.2022).

Participants

Treatment was performed according to the American Society for Radiation Oncology guidelines for brachytherapy (6). The inclusion criteria were: patients aged >40 years; clinically single lesions of ≤3 cm in diameter; N0 stage ductal (invasive/non-invasive), mucinous, medullary, tubular, or lobular (invasive/non-invasive) carcinoma; no previous radiation or chemotherapy; no prior breast cancer; no synchronous bilateral breast cancer; performance status of 0–1; and the provision of informed consent and voluntarily requesting SAVI treatment. Luminal A was defined as Ki-67 ≤20% and Luminal B as Ki-67 >20%.

The SAVI treatment schedule is shown in Figure 1. The SAVI spacer was placed within the cavity and at the time of Bp and sentinel lymph

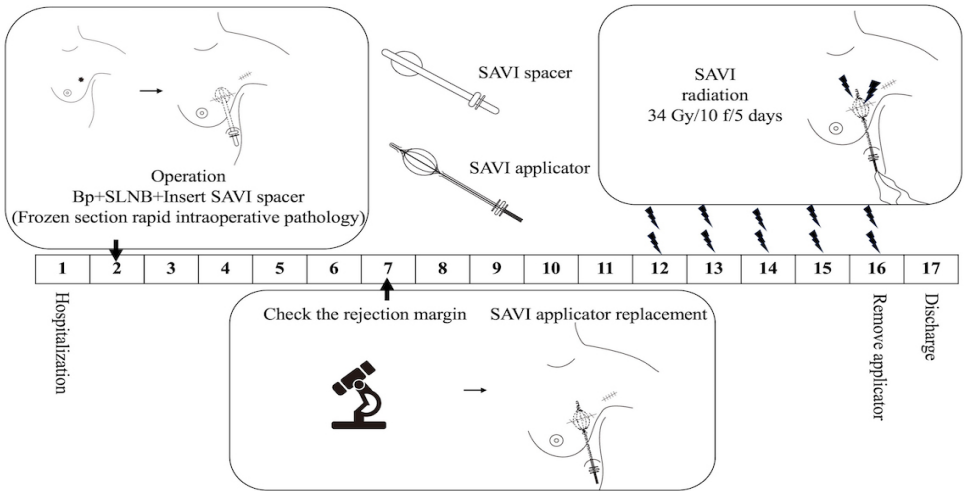


Figure 1. Treatment schedule

Bp: Partial mastectomy; SAVI: Strut-adjusted volume implant; SLNB: Sentinel lymph node biopsy

node biopsy (SLNB), where rapid pathological diagnosis confirmed a negative sentinel lymph node (SLN) metastasis. The spacer was replaced with an applicator under ultrasound guidance on postoperative day 5 after a pathological check confirmed that the surgical resection margins were free and there was no extensive intraductal spread. After computed tomography (CT)-based simulation for treatment planning, an accelerated partial irradiation dose of 34 Gy was delivered in 10 fractions, administered twice daily on 5 treatment days, starting on postoperative day 10. The planning target volume (PTV) was defined as a 1 cm expansion from the cavity's edge, which corresponds to the outer boundary of the SAVI applicator. The PTV_EVAL was subsequently determined by subtracting the volumes occupied by the chest wall, cavity, and subcutaneous tissue within 2 mm below the skin surface from the PTV. Several dosimetric constraints were established for quality assurance. These included ensuring that at least 90% of the prescribed dose covered 90% or more of the PTV_EVAL (V90% ≥90%), limiting the volume of breast tissue receiving 150% of the prescribed dose (V150%) to no more than 50 cm^3, restricting the volume of breast tissue receiving 200% of the prescribed dose (V200%) to under 20 cm^3, and maintaining the dose delivered to 1 cm^3 (D1 cm^3) of the skin within 110% of the prescribed dose. To ensure accurate treatment delivery, the position of the applicator was verified by measuring the distance between the skin and the applicator's hub, as well as through anterior-posterior and lateral CT scout views, prior to each irradiation session. If displacement of the applicator was observed, it was confirmed by CT and re-planned at the discretion of the radiologists. The applicator was removed after irradiation, and discharge was scheduled for the following day. All cases in the SAVI group had a fixed hospital stay of 17 days for surgery and brachytherapy with the SAVI device. Post-irradiation, whether using chemotherapy, hormonal therapy, or both, was at the discretion of the treating medical oncologist, based on the pathological results of each patient. The patients were followed up annually by physical examination, bilateral mammograms, and breast ultrasound to assess recurrence.

Patients with the same eligibility criteria and treated with WBI post-Bp during the same period were grouped into the WBI group, and their treatment outcomes were compared. WBI was used to deliver doses of 50 Gy in 25 total fractions or 42.56 Gy per day in 16 fractions. Boost therapy (10 or 10.64 Gy) was permitted at the discretion of the radiation oncologist.

The primary endpoint was recurrence rates, and the secondary endpoint was acute and late adverse events. Adverse events were assessed using the Common Terminology Criteria for Adverse Events version 4.0. at 1, 3, and 6 months after the end of irradiation and every six months thereafter until five years. Adverse events occurring from the end of irradiation to within three months after treatment were considered acute adverse events, and those occurring later were considered late adverse events. The maximum adverse events in each case are summarized in this study.

Statistical Analysis

For comparison between the two groups, the χ^2, Mann-Whitney U, log-rank, and Fisher's exact probability tests were performed. Statistical analyses were performed using GraphPad Prism version 7.0e (GraphPad Software, San Diego, CA, USA).

Results

The process of patient selection is illustrated in Figure 2. Sixty-one patients were subjected to Bp, SLNB, and SAVI spacer insertion. Two patients were switched to postoperative WBI treatment due to confirmation of positive SLN metastasis by frozen section. Three patients were excluded owing to positive resection margins, two patients had early removal of the SAVI after postoperative hemorrhage/infection, and one patient was ineligible because of post-augmentation mastopexy.

Fifty-three patients completed postoperative radiation treatment with the SAVI and received pharmacotherapy based on their individual pathological results. Concurrently, 143 patients underwent WBI after breast-conserving surgery. Thirteen patients were excluded owing to positive margins and 17 owing to a tumor diameter >3 cm, leaving 113 patients in the WBI group. The total radiation doses were 42.56 Gy in 68 patients and 50 Gy in 45 patients, with additional boost irradiation performed in 30 patients, including 10 Gy in seven patients and 10.64 Gy in 23 patients.

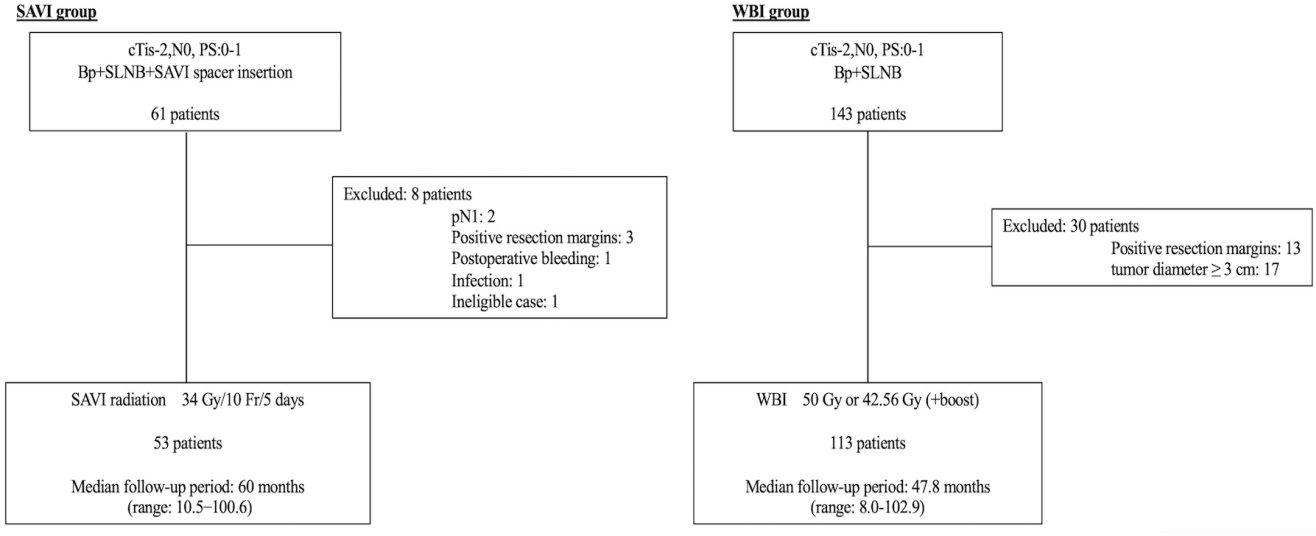


Figure 2. Trial profile

Bp: Partial mastectomy; SAVI: Strut-adjusted volume implant; SLNB: Sentinel lymph node biopsy; WBI: Whole breast irradiation

The patient demographics of each group are shown in Table 1. The median ages of the patients were 55 (range: 39–85) years and 52 (range: 40–72) years in the SAVI and WBI groups, respectively. In the SAVI group, pStage0, stage IA, stage IB, stage IIA, and stage IIB were observed in 6, 43, 1, 2, and 1 patient(s), respectively. In the WBI group, pStage0, stage IA, stage IIA, and stage IIB were observed in 11, 93, 8, and 1 patient(s), respectively.

The pathological tumor diameters (including noninvasive parts) were ≤2 cm and >2 cm in 47 (89%) and 6 (11%), as well as 91 (81%) and 22 (19%) patients in the SAVI and WBI groups, respectively. In the SAVI group, the subtypes were luminal A, luminal B, and triple-negative in 36, 8, and 1 patient(s), respectively. In the WBI group, luminal A, luminal B, estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2)+, ER+ and HER2+, and triple-negative subtypes were observed in 76, 16, 1, 6, and 1 patient(s), respectively.

Postoperative pathology showed micro-lymph node metastasis in two patients (0.3–2 mm) in the SAVI group and two patients (unknown) in the WBI group, with no significant differences in age, pStage, tumor diameter, subtype, and pN between the two groups.

Efficacy of SAVI and WBI

The Kaplan-Meier curves for local recurrence, including ipsilateral breast and lymph node recurrences, are shown in Figure 3. The median follow-up periods in the SAVI and WBI groups were 60.0 (range: 10.5–100.6) and 47.8 (range: 8.0–102.9) months, respectively. The SAVI group included one case each of local and distant recurrences, whereas the WBI group included three cases (two cases of local recurrence and one case of supraclavicular lymph node recurrence). Of note, there was no significant difference in recurrence rates between the two groups (p = 0.726).

Adverse Events After SAVI and WBI

The adverse event results of SAVI and WBI are shown in Table 2. In the SAVI group, 25 (47%) patients experienced grade 1–2 acute adverse events (within 3 months of radiotherapy start), and 25 (47%) patients experienced late adverse events (beyond 3 months), with no events of grade 3 or above. The most common adverse events were dermatitis in the acute phase and pigmentation in the late phase. Two patients experienced dermatitis of grade 2 or higher, which was improved after dermatological consultations. Two patients required antibiotic treatment due to infection of grade 2 or higher. One patient

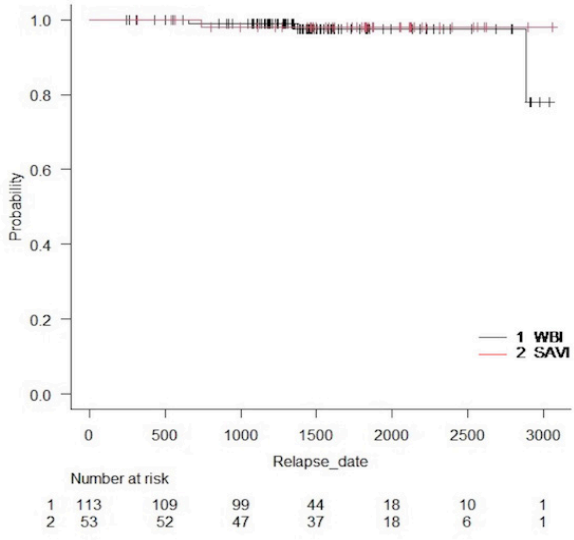


Figure 3. Kaplan-Meier curves of breast tumor recurrence

SAVI: Strut-adjusted volume implant; WBI: Whole breast irradiatio

Table 1. Patient characteristics

		SAVI group	WBI group	*p*
Number		53	113	
Age (y)		55 (39–85)	52 (40–72)	0.1
pStage	0	6 (11%)	11 (10%)	0.59
	IA	43 (81%)	93 (82%)	
	IIB	1 (2%)	0	
	IIIA	2 (4%)	8 (7%)	
	IIB	1 (2%)	1 (1%)	
Tumor diameter	≤2 cm	47 (89%)	91 (81%)	0.19
	>2 cm	6 (11%)	22 (19%)	
Subtype	Luminal A	36 (68%)	76 (67%)	0.17
	Luminal B	8 (15%)	16 (14%)	
	ER- HER2+	0	1 (1%)	
	ER+ HER2+	0	6 (5%)	
	Triple-negative	1 (2%)	1 (1%)	
pN	0	51 (96%)	111 (98%)	0.33
	Micro meta	2 (4%)	2 (2%)	
	1	0	0	

ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; SAVI: Strut-adjusted volume implant; WBI: Whole breast irradiation

Table 2. Toxicity assessment results

None		SAVI group			WBI group			*p*
		Grade 1	Grade 2	None	Grade 1	Grade 2		
Acute	Any acute adverse events	28 (53%)	23 (43%)	3 (6%)	34 (30%)	70 (62%)	9 (8%)	0.01
	Dermatitis	31 (58%)	20 (38%)	2 (4%)	34 (30%)	70 (62%)	9 (8%)	0.001
	Skin hyperpigmentation	50 (94%)	3 (6%)	0	111 (98%)	2 (2%)	0	0.33
	Dry skin	52 (98%)	1 (2%)	0	111 (98%)	2 (2%)	0	>0.99
	Skin infection	52 (98%)	0	2 (2%)	113 (100%)	0	0	0.32
	Pain	47 (89%)	6 (11%)	0	113 (100%)	0	0	0.001
	Malaise	52 (98%)	1 (2%)	0	113 (100%)	0	0	0.32
	Localized edema	52 (98%)	1 (2%)	0	113 (100%)	0	0	0.32
	Telangiectasia	53 (100%)	0	0	112 (99%)	1 (1%)	0	0.03
Late	Any late adverse events	28 (53%)	24 (45%)	1 (2%)	95 (84%)	17 (15%)	2 (2%)	0.00002
	Skin hyperpigmentation	36 (68%)	17 (32%)	0	100 (88%)	13 (12%)	0	0.002
	Superficial soft tissue fibrosis	37 (70%)	15 (28%)	1 (2%)	112 (99%)	1 (1%)	0	<0.0001
	Pain	48 (91%)	5 (9%)	0	113 (100%)	0	0	0.003
	Telangiectasia	50 (94%)	3 (6%)	0	113 (100%)	0	0	0.03
	Breast atrophy	51 (96%)	2 (4%)	0	113 (100%)	0	0	0.1
	Dry skin	52 (98%)	1 (2%)	0	109 (96%)	4 (4%)	0	>0.99
	Localized edema	52 (98%)	1 (2%)	0	113 (100%)	0	0	0.32
	Nipple deformity	52 (98%)	1 (2%)	0	113 (100%)	0	0	0.32
	Fracture	52 (98%)	1 (2%)	0	112 (99%)	1 (1%)	0	0.53
	Pneumonitis	53 (100%)	0	0	110 (97%)	1 (1%)	2 (2%)	0.55

The $\chi 2$ tests were performed on the two groups with no adverse events and those with Grade 1 and 2 adverse events, and p-values were calculated; SAVI: Strut-adjusted volume implant; WBI: Whole breast irradiation

had a rib fracture on the operated side but showed no worsening during the asymptomatic follow-up. In the WBI group, 79 (70%) patients experienced grade 1–2 acute adverse events, and 18 (16%) patients experienced late adverse events, with no events of grade 3 or higher. The most common adverse events were dermatitis in the acute phase and pigmentation in the late phase. Two patients experienced grade 2 pneumonia, of whom one showed improvement during follow-up observation, and the other required steroid treatment. One patient had a rib fracture on the operated side but showed no worsening during the asymptomatic follow-up. The χ^2 tests were performed, and *p*-values are listed in Table 2.

Discussion and Conclusion

The SAVI and WBI groups showed similar local recurrence rates. During the acute phase, significantly fewer adverse events were observed in the SAVI group than in the WBI group, whereas in the late stage, patients in the SAVI group experienced significantly more adverse events than those in the WBI group.

Comparison of Efficacy (Treatment Outcomes) and Safety Between APBI and WBI

A summary of phase III trials comparing the treatment outcomes of APBI and WBI is presented in Table 3. APBI methods, including brachytherapy, three-dimensional conformal radiation therapy (3DCRT or IMRT), and intraoperative radiation, were compared to WBI, mostly showing non-inferiority of APBI in terms of ipsilateral breast tumor recurrence (IBTR). In the National Surgical Adjuvant Breast and Bowel Project B-39/Radiation Therapy Oncology Group 0413 trial, multiple methods, such as multicatheter, 3DCRT, SAVI, and balloon catheter methods (MammoSite®), were compared to WBI. The difference in the 10-year IBTR results was not equivalent but was <1% (7). In the IMPORT LOW trial comparing intensity-modulated radiation therapy (IMRT) with WBI, the 5-year IBTR rates were 0.5 and 1.1% (IMRT and WBI), respectively, showing non-inferiority (8). Similarly, in the Florence trial comparing IMRT and WBI, the 10-year IBTR rates were 3.9 and 2.6% (IMRT and WBI), respectively, with no significant difference (9). In our study, the IBTR rate was 1.96% during a median follow-up of 60.0 months, with no significant difference from that in the WBI group, which was similar to previous reports despite different detailed conditions.

Table 4 summarizes the previous reports on SAVI. The proportion of adverse events during the acute phase was significantly lower in the SAVI group than in the WBI group, particularly dermatitis, which was observed in approximately 70% of the patients in the WBI group compared to 42% in the SAVI group. Previous research found the occurrence rate of skin complications of grade 2 or higher within 24 months post-brachytherapy with the SAVI device to be 7% (10). In the present study, the occurrence rate of adverse events of grade 2 or higher in the acute phase was equivalent to that reported previously (6%), and the severity was low grade, which was manageable on an

Table 3. Summary of phase III trials of APBI

Clinical trial	Sample size	Duration	Patient background	Treatment	APBI radiation dose/days	WBI radiation dose/ fraction	Result	Conclusions
NSABPB-39/ RTOG0413 (7)	4.214	2005–2013	≧18 years, Stage 0, I, II LN meta: 0–3 Tumor diameter ≦3 cm	Multicatheter/ MammoSite® SAVI Contura/ 3DCRT	34 Gy/10 f/5 d 34 Gy/10 f/5 d 38.5 Gy/10 f/5 d	50 Gy/25 f	10 years IBTR APBI: 4.6% *vs.* WBI: 3.9%	Not equivalent (10 years IBTR) (but the difference was <1%)
IMPORT LOW (8)	2.018	2007–2010	≧50 years, Tumor diameter ≦3 cm pT1-2, N0-1	IMRT	40 Gy/15 f/15 d	40 Gy/15 f 36 Gy/15 f	5 years IBTR IMRT: 0.5% *vs.* WBI: 1.1%	Non-inferiority (5 years IBTR)
APBI-IMRT-Florence (9)	520	2005–2013	≧40 years Tumor diameter ≦2.5 cm	IMRT	30 Gy/5 f/5 d	50 Gy/25 f (+10 Gy boost)	10 years IBTR IMRT: 3.9% *vs.* WBI: 2.6%	Non-inferiority (10 years IBTR, 10 years OS)
RAPID (11)	2.128	2006–2011	≧40 years, DCIS or N0 early-stage cancer	3DCRT	38.5 Gy/10 f/5 d	50 Gy/25 f 42.5 Gy/16 f	8 years IBTR APBI: 3.0% *vs.* WBI: 2.8%	Non-inferiority (8 years IBTR)
ELIOT (12)	1.305	2000–2007	48–75 years Tumor diameter ≦2.5 cm cN0	Electron (IORT)	21 Gy/1 f/1 d	50 Gy/25 f (+10 Gy boost)	15 years IBTR APBI: 12.6% *vs.* WBI: 2.4%	Inferiority (15 years IBTR, 15 years OS)

3DCRT: Three-dimensional conformal radiation therapy; APBI: Accelerated partial breast irradiation; IBTR: Ipsilateral breast tumor recurrence; IMRT: Intensity-modulated radiation therapy; LN: lymph node; OS: Overall survival; SAVI: Strut-adjusted volume implant; WBI: Whole breast irradiation

outpatient basis with antibiotics. In the present study, acute adverse events of grade 2 or higher within 3 months after brachytherapy with the SAVI device were not significantly different from those in recent reports on Japanese subjects (4). The RAPID Trial indicated fewer grade 2 or higher acute adverse events within 3 months post-treatment with APBI than it did with WBI, which is thought to be due to the total dose rather than the dose per session (11). Conversely, more grade 2 or higher late adverse events have been reported with APBI (12). In the present study, the incidence rates of hyperpigmentation, superficial soft tissue fibrosis, and telangiectasia were higher in the SAVI group than in the WBI group but most were grade 1, with no significant difference in grade 2 or higher events compared to WBI. The pain level was higher in the SAVI group in both the acute and late phases than in the WBI group, but it was grade 1 in all patients and controllable with analgesics. These observations suggested that SAVI showed lower acute adverse events and higher late adverse events than did WBI, and these results are comparable with the previous studies. An assessment of late adverse events of SAVI in Japanese patients has not been reported previously; therefore, this is the first report of its type.

In addition, following the results of the FAST-Forward trial (13), the European Society for Radiation Oncology (ESTRO) has recommended that an ultrafractioned dose of 26 Gy in five fractions can also be offered as standard of care or within a randomized trial or prospective cohort (14). However, the observation period has not been sufficiently long, and the outcomes of long-term follow-up and revalidation trials remain awaited. In the present study, we compared WBI in 16 or 25 fractions, the present standard of care in Japan. In terms of shortening treatment time, WBI such as ultrafractionation has similar advantages; however, APBI using the SAVI device can benefit patients such as older adults who live far from the hospital and have difficulty in making daily visits, as radiotherapy is completed during the same hospitalization period as the surgery. This suggests that APBI using the SAVI device is an alternative treatment choice for some patients. However, the application of the SAVI device remains limited for the following reasons: insurance approval is not specifically focused on brachytherapy using the SAVI device, and clinical data on brachytherapy using SAVI are limited. However, we believe that this study will contribute to evidence for the community of physicians who treat breast cancer and the expansion of APBI using the SAVI device. Going forward, we aim to compare these results with those of ultrafractionation and other APBI methods and evaluate the patient's treatment satisfaction, cosmetics, and long-term prognosis.

Comparison With Other Brachytherapy Applicators [Multicatheter and Balloon Catheter Methods (MammoSite®)]

The multicatheter method enables more precise treatment by adjusting the dose distribution according to the breast morphology, as numerous

Table 4. Summary of the previous reports on SAVI

Authors	Sample size	Duration	Patient background	Follow-up	Local recurrence rate	Adverse event (≧ grade 2)
Yashar et al. (4)	250	2007–2010	Tis-T2, N0-1	59.5 months	3.6%	Skin disorder 7% (<24 months) (erythema 2.6%, seroma 2.6%, etc.)
Amir Isbell et al.	50	2011–2015	≧18 years, Stage 0, I, II LN meta: 0–3 Tumor diameter ≦3 cm	45.6 months (3.48–56.3 months)	4%	Not observed
Yoshida et al. (5)	44	2016–2021	>40 years Tumor diameter ≦3 cm N0	Not described	Not described	Dermatitis 7% Skin infection 7% Chest wall pain 5% Breast pain 11%
Current study	53	2014–2019	>40 years Tumor diameter ≦3 cm N0	60.0 months	1.96%	Dermatitis 4% Skin infection 2% Superficial soft tissue fibrosis 2%

LN: Lymph node

applicators can be placed independently. The Groupe Européen de Curiethérapie of the European Society for Radiotherapy and Oncology APBI Trial comparing APBI and WBI using a multicatheter showed non-inferiority in the 5-year IBTR rate (WBI: 0.92% *vs.* APBI: 1.44%) (15). The benefits of the multicatheter method, especially for Asian patients with a smaller breast size compared to that of Western patients, have been reported in Japan, showing good prognoses and cosmetic outcomes (16). However, the multicatheter method has drawbacks, including greater invasiveness owing to the insertion of multiple applicators and the requirement of considerable skill for correct placement. The balloon catheter method (MammoSite®) showed excellent results in a prospective trial with an ipsilateral breast recurrence rate of 2.8% at 5 years, excellent/good cosmetic outcomes >90%, acute adverse events <10%, and almost no late adverse events (17), similar to the findings of the present study. However, this approach has not yet been approved for use in Japan. Notably, it is deemed unsuitable for smaller breasts, such as those of Japanese patients, because it can only provide a concentric dose distribution around the catheter passing through the balloon center, making fine adjustment of the dose distribution impossible when inserted close to the skin or chest wall.

This study had some limitations. First, outcome differences between the two groups may be due to selection bias or other confounding factors. Patients in the SAVI group voluntarily requested SAVI treatment. However, there are several conditions regarding therapy choice. Our institution could not simultaneously perform brachytherapy using the SAVI device for multiple patients, which is an institutional limitation. In addition, when holidays fall on weekdays, choosing brachytherapy using the SAVI device is impossible. Therefore, we should consider that these limitations may have contributed to selection bias or other confounding factors and clinical outcomes. This limitation should not be ignored, and the present results should be cautiously interpreted. Furthermore, the results should be re-evaluated in future randomized controlled trials.

Second, the observation period differed between the two groups by one year. However, the observation period of the SAVI group was longer than that of the WBI group, which had little effect on demonstrating the non-inferiority of IBTR. Therefore, controlled groups with matched conditions are required to observe long-term outcomes. Third, the surgical techniques of lumpectomy may have differed between the two groups because of the presence of a lumpectomy cavity in the SAVI group. Therefore, direct comparisons of adverse events and cosmesis may be difficult. However, previous studies have compared adverse events between SAVI and WBI, and including differences in surgical technique was deemed acceptable. Indeed, our study findings also indicated that patients who underwent WBI experienced more acute events, while those who underwent SAVI experienced more late events. Importantly, no serious complications of grade 3 or above were observed in the SAVI group, aligning with earlier reports. Thus, we are confident that our results contribute valuable insights into the feasibility of SAVI. Fourth, the occurrence rate of late adverse events differed between the two groups. As the follow-up period in the Department of Radiation Oncology was shorter in the WBI group than in the SAVI group, it is possible that late adverse events in the WBI group were underestimated. In addition, the recurrence rates in this study were only comparable over a short period of 5 years. Therefore, tumor control and adverse events in both groups should be observed and compared for a longer period in the future.

In conclusion, as a treatment option, brachytherapy with the SAVI device was not inferior to conventional WBI in terms of therapeutic efficacy and is expected to shorten the treatment time and reduce acute adverse events. Late adverse events were more frequent with SAVI than with WBI, but they were low-grade and controllable. Results,

including long-term prognoses, the presence of late adverse events, and objective evaluation of the patient's quality of life, are required.

Ethics Committee Approval: This trial was conducted with ethical approval from Showa University Research Ethics Review Board Committee (approval no: 22-170-B, date: 17.11.2022).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: A.A-S., T.K., M.K., H.S., K.M., A.N., N.K., M.M., Ha.S., Y.M., S.N., A.Y., M.I., K.T., H.M., T.S., N.H., Y.I., C.W., S.A-T., Se.N.; Concept: A.A-S., T.K.; Design: A.A-S., T.K.; Data Collection and/or Processing: A.A-S., T.K., M.K.; Analysis and/or Interpretation: A.A-S.; Literature Search: A.A-S., T.K.; Writing: A.A-S., T.K., H.S.

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

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Early Breast Cancer Trialists' Collaborative Group (EBCTCG); Darby S, McGale P, Correa C, Taylor C, Arriagada R, Clarke M, et al. Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. Lancet 2011; 378: 1707-1716. (PMID: 22019144) [Crossref]
2. Fisher ER, Anderson S, Tan-Chiu E, Fisher B, Eaton L, Wolmark N. Fifteen-year prognostic discriminants for invasive breast carcinoma: National Surgical Adjuvant Breast and Bowel Project Protocol-06. Cancer 2001; 91: 1679-1687. (PMID: 11309768) [Crossref]
3. Veronesi U, Marubini E, Mariani L, Galimberti V, Luini A, Veronesi P, et al. Radiotherapy after breast-conserving surgery in small breast carcinoma: long-term results of a randomized trial. Ann Oncol 2001; 12: 997-1003. (PMID: 11521809) [Crossref]
4. Yashar C, Attai D, Butler E, Einck J, Finkelstein S, Han B, et al. Strut-based accelerated partial breast irradiation: Report of treatment results for 250 consecutive patients at 5 years from a multicenter retrospective study. Brachytherapy 2016; 15: 780-787. (PMID: 27528591) [Crossref]
5. Yoshida M, Yoshimura RI, Notake R, Shinjo H, Miyaura K, Kuwayama T, et al. Feasibility of accelerated partial breast irradiation with strut-adjusted volume implant brachytherapy in Japan focusing on dosimetry and acute toxicity: a Japanese multi-institutional prospective study. Breast Cancer 2024; 31: 75-83. (PMID: 37865624) [Crossref]
6. Shaitelman SF, Anderson BM, Arthur DW, Bazan JG, Bellon JR, Bradfield L, et al. Partial Breast Irradiation for Patients With Early-Stage Invasive Breast Cancer or Ductal Carcinoma In Situ: An ASTRO Clinical Practice Guideline. Pract Radiat Oncol 2024; 14: 112-132. (PMID: 37984712) [Crossref]
7. Vicini FA, Cecchini RS, White JR, Arthur DW, Julian TB, Rabinovitch RA, et al. Long-term primary results of accelerated partial breast irradiation after breast-conserving surgery for early-stage breast cancer: a randomised, phase 3, equivalence trial. Lancet 2019; 394: 2155-2164. (PMID: 31813636) [Crossref]
8. Coles CE, Griffin CL, Kirby AM, Titley J, Agrawal RK, Alhasso A, et al; IMPORT Trialists. Partial-breast radiotherapy after breast conservation surgery for patients with early breast cancer (UK IMPORT LOW trial): 5-year results from a multicentre, randomised, controlled, phase 3, non-inferiority trial. Lancet 2017; 390: 1048-1060. (PMID: 28779963) [Crossref]
9. Meattini I, Marrazzo L, Saieva C, Desideri I, Scotti V, Simontacchi G, et al. Accelerated Partial-Breast Irradiation Compared With Whole-Breast Irradiation for Early Breast Cancer: Long-Term Results of the Randomized Phase III APBI-IMRT-Florence Trial. J Clin Oncol 2020; 38: 4175-4183. (PMID: 32840419) [Crossref]
10. Benitez PR, Chen PY, Vicini FA, Wallace M, Kestin L, Edmundson G, et al. Partial breast irradiation in breast conserving therapy by way of intersitial brachytherapy. Am J Surg 2004; 188: 355-364. (PMID: 15474426) [Crossref]
11. Whelan TJ, Julian JA, Berrang TS, Kim DH, Germain I, Nichol AM, et al. External beam accelerated partial breast irradiation versus whole breast irradiation after breast conserving surgery in women with ductal carcinoma in situ and node-negative breast cancer (RAPID): a randomised controlled trial. Lancet 2019; 394: 2165-2172. (PMID: 31813635) [Crossref]
12. Orecchia R, Veronesi U, Maisonneuve P, Galimberti VE, Lazzari R, Veronesi P, et al. Intraoperative irradiation for early breast cancer (ELIOT): long-term recurrence and survival outcomes from a single-centre, randomised, phase 3 equivalence trial. Lancet Oncol 2021; 22: 597-608. (PMID: 33845035) [Crossref]
13. Murray Brunt A, Haviland JS, Wheatley DA, Sydenham MA, Alhasso A, Bloomfield DJ, et al; FAST-Forward Trial Management Group. Hypofractionated breast radiotherapy for 1 week versus 3 weeks (FAST-Forward): 5-year efficacy and late normal tissue effects results from a multicentre, non-inferiority, randomised, phase 3 trial. Lancet 2020; 395: 1613-1626. (PMID: 32580883) [Crossref]
14. Meattini I, Becherini C, Boersma L, Kaidar-Person O, Marta GN, Montero A, et al. European Society for Radiotherapy and Oncology Advisory Committee in Radiation Oncology Practice consensus recommendations on patient selection and dose and fractionation for external beam radiotherapy in early breast cancer. Lancet Oncol 2022; 23: e21-e31. (PMID: 34973228) [Crossref]
15. Strnad V, Ott OJ, Hildebrandt G, Kauer-Dorner D, Knauerhase H, Major T, et al. 5-year results of accelerated partial breast irradiation using sole interstitial multicatheter brachytherapy versus whole-breast irradiation with boost after breast-conserving surgery for low-risk invasive and in-situ carcinoma of the female breast: a randomised, phase 3, non-inferiority trial. Lancet 2016; 387: 229-238. (PMID: 26494415) [Crossref]
16. Sato K, Fuchikami H, Takeda N, Shimo T, Kato M, Okawa T. Efficacy of Single-Plane Implant Technique in Partial Breast Brachytherapy in Small-Breasted Patients. Int J Radiat Oncol Biol Phys 2020; 106: 830-837. (PMID: 31785336) [Crossref]
17. Shah C, Badiyan S, Ben Wilkinson J, Vicini F, Beitsch P, Keisch M, et al. Treatment efficacy with accelerated partial breast irradiation (APBI): final analysis of the American Society of Breast Surgeons MammoSite(®) breast brachytherapy registry trial. Ann Surg Oncol 2013; 20: 3279-3285. (PMID: 23975302) [Crossref]

Original Article

Eur J Breast Health 2024; 20(3): 223-227
DOI: 10.4274/ejbh.galenos.2024.2024-3-10

Skin Staining After Injection of Superparamagnetic Iron Oxide for Sentinel Lymph Node Dissection: A Retrospective Study of Two Protocols for Injection and Long-Term Follow-Up

Marie-Pierre Mathey[1], Colin Simonson[1], Daniela Huber[1,2]

[1]Department of Gynecology and Obstetrics, Valais Hospital, Sion, Switzerland
[2]The Geneva University Hospitals, Geneva, Switzerland

ABSTRACT

Objective: Sentinel lymph node (SLN) dissection is a highly accurate surgical procedure allowing detection of lymph node invasion in patients with clinically negative axilla in early breast cancer. Superparamagnetic iron oxide (SPIO) is a marker used during SLN procedure, allowing the same detection rate as isotopes (Tc-99). A drawback of SPIO is skin staining that can occur around the injection site. The goal of this retrospective study was to assess the frequency of skin staining after oncological breast surgery with SPIO, and the impact of two different injection protocols on the rate of skin staining.

Materials and Methods: Data from breast cancer patients undergoing magnetic tracer SLN detection (SLND) procedure in a single department between 2020 and 2022 was reviewed. Injection protocol P1 consisted of retro-areolar injection of Magtrace 0.8 mL. Injection protocol P2, consisted of retro-tumoral injection with 1 mL. Presence of skin staining was assessed at day 10 after surgery. The evolution and satisfaction of the patients was assessed at six and 12 months.

Results: In total 175 sentinel lymph node biopsy procedures were performed (P1: 141/P2: 34), consisting of breast conservative surgery (BCS) (P1: 70%/P2: 53%) or mastectomy (P1: 30%/P2: 47%) with SLN. SLN detection rate was 97.7%. Skin staining was reported in 23% and occurred more often after BCS (31.6%) compared to mastectomy (6.8%). When BCS was performed, peritumoral injection was associated with a decreased risk of skin staining compared with retro-areolar injection (22.2% *vs.* 33.3%, respectively). When present skin staining persisted for 12 months, but most of the patients described only a slight discomfort. The low rate of discoloration after mastectomy, as previously reported, can be explained by the removal of skin and glandular tissue in which the tracer accumulates. Less skin staining in P2 may be because of a shorter interval between injection and surgery and the removal of the excess of SPIO during the lumpectomy.

Conclusion: SPIO injection is a safe surgical technique. After mastectomy, the rate of discoloration was low. Despite the persistent skin discoloration in 58.6% in our study, patient satisfaction was high. Deeper injection, reduced doses, massage of the injection site and peritumoral injection may reduce skin staining.

Keywords: Axillary lymph node dissection; body image; breast cancer; breast conserving surgery; mastectomy; quality of life; risk factors; sentinel lymph node dissection; surgery; women

Cite this article as: Mathey MP, Simonson C, Huber D. Skin Staining After Injection of Superparamagnetic Iron Oxide for Sentinel Lymph Node Dissection: A Retrospective Study of Two Protocols for Injection and Long-Term Follow-Up. Eur J Breast Health 2024; 20(3): 223-227

Key Points

- Axillary lymph node dissection
- Breast conserving surgery
- Sentinel lymph node dissection

Corresponding Author:
Marie-Pierre Mathey; mariepierre.mathey@gmail.com

Received: 01.04.2024
Accepted: 01.05.2024
Available Online Date: 01.07.2024

Introduction

Breast cancer is the leading form of cancer among women worldwide (1, 2). Sentinel lymph node biopsy (SLNB) has replaced axillary lymph node dissection as the standard surgical procedure for staging clinically tumor-free regional nodes in patients with early-stage breast cancer. SLNB staging considerably reduces surgical morbidity in terms of shoulder dysfunction and lymphedema, without affecting diagnostic accuracy and prognostic information (detection rate >97%) (3-6). Tracking of the sentinel lymph node (SLN) can be made by injection of different tracers such technetium-99m, blue dye, indocyanine green (ICG) or superparamagnetic iron oxide (SPIO). Radioactive agents have several disadvantages, such as the need for a nuclear radiology unit, radiation exposure, cost, and time-limited effectiveness. Meanwhile, blue dye injection can be done less than one hour before surgery, is cheap, but carries a risk of anaphylactic reaction, skin staining and tissue necrosis (7). ICG seems to be the most efficient tracer to identify metastatic lymph nodes and is detectable 10 minutes after injection, but this technique has not yet been standardized (8). Large studies reported non-inferiority of SPIO compared to standard techniques, with the same detection rate as other techniques (8-11). Detection of the SLN is feasible 20 minutes after injection, and even ip to one month after injection, which improves scheduling, benefitting both the patient and the surgical team (11, 12).

However, side effects of SPIO, such as skin staining can develop around the injection site, corresponding to a persistent gray/black skin pigmentation (11). Incidence rate of discoloration seems to be similar to blue dye injection, at 30.8%, but with a large range from 0% to 84.4%, depending on the source (12-14). Another disadvantage of SPIO is accumulation of the iron residual nanoparticles, which leads to magnetic resonance imaging (MRI) artifacts.

In this retrospective study, two protocols of two different injection sites and dilution of SPIO are described together with assocaited side effects. The goal of this retrospective study was to assess the frequency of skin staining after mastectomy or breast conserving surgery (BCS), and the impact of the two different protocols on the rate of skin staining after BCS.

Materials and Methods

Data were retrospectively analyzed from patients suffering from early breast cancer undergoing SLNB with an SPIO procedure in a single department between January 2020 and December 2022. Injection protocol P1 consisted of pre-operative retro-areolar injection of 0.8 mL Magtrace (EndoMagnetics, Cambridge, UK) from November 2020 until March 2022. In order to reduce the rate of skin staining, it was decided, from April 2022 to December 2022, to introduce injection protocol P2, which consisted of retro-tumoral injection of 1 mL Magtrace. The detector probe was Sentimag (Sysmex GmBH, Hamburg, Germany). Presence of skin staining was assessed at day 10 after surgery. If present, clinical re-evaluation of the discoloration and the satisfaction of the patients was assessed at six and 12 months postoperatively. Demographic characteristics of the patients and type of surgery (BCS vs mastectomy), were collected from patient records.

Results

In total, 175 SLNB procedures were performed [141 (80.6%) using P1, 34 (19.4%) using P2] (Table 1). Mean age was 64.7 years in P1 and 62.4 years in P2. Types of surgeries performed were BCS (70% in P1/53% in P2) versus mastectomy (30% in P1/47% in P2) with SLN. The waiting period between injection and surgery was 9.4 days in P1 and 3.6 days in P2. Sentinel node detection rate after Magtrace injection was 97.7% overall (171/175). There were four procedural failures consisting of an absence of signal detection, which led to axillary sampling.

Skin staining was reported in 23% (41/175) of the cases and occurred more often after BCS 31.6% (37/117) than after mastectomy 6.8% (4/58). When BCS was performed, peritumoral injection (P2) was associated with a decreased risk of skin staining occurring in 22.2% (4/18) compared to retro-areolar injection (P1) at 33.3% (33/99). Skin staining was less common after mastectomies and only reported in four cases, of which three did not undergo breast reconstruction, and one had a skin-sparing mastectomy with breast reconstruction.

For long-term follow-up, we were able to recall 37 of 41 patients (90.3%) in March 2024. When confirmed, skin stainings remained persistent from one month up to four years (Figure 1). The overall patient experience was good, with 73% describing no discomfort at all, 24% a slight discomfort and only one case describing a major impact on the way she perceived herself (Chart 1). While 56.8 % had persistent skin staining 2-4 years after surgery, the remaining 43.2% described complete disappearance of the stain (Chart 2). Even when skin staining remained, all these patients described a progressive fading since surgery (Figure 1).

Table 1. Demographic and surgical data

	Protocol 1 retro areolar injection	Protocol 2 peritumoral injection	Total
Number of cases	141	34	175
Mean age (years)	64.7	62.4	64.2
Proportion of tumorectomia	70.2% (99)	52.9% (18)	66.9% (117)
Proportion of mastectomies	29.8% (42)	47.1% (16)	33.1% (58)
Time lapse between injection and surgery (days)	9.4	3.6	8.9
Number developing skin staining	34	7	41
Skin staining and BCS	33.3% (33/99)	22.2% (4/18)	28.2% (37/117)
Skin staining and mastectomy	2.4% (1/42)	18.8% (3/16)	6.9% (4/58)

BCS: Breast conservative surgery

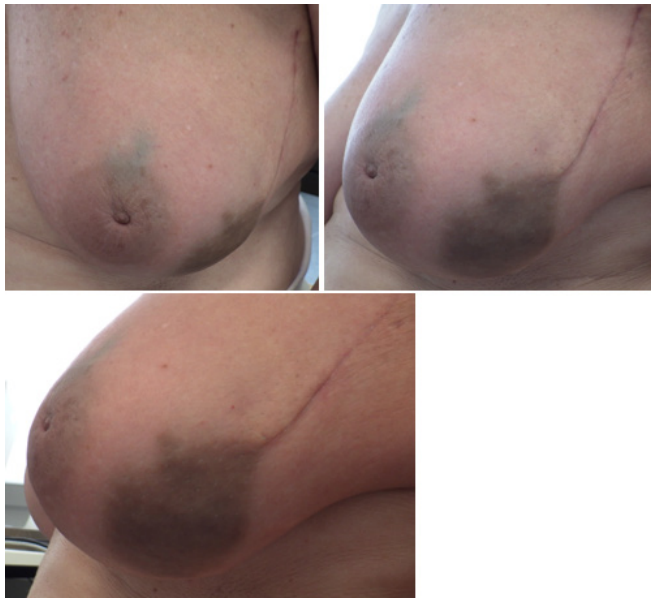


Figure 1. Picture of a skin staining, one month after surgery

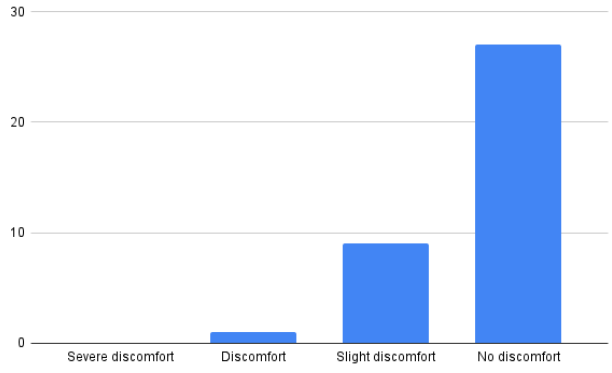


Chart 1. Self image

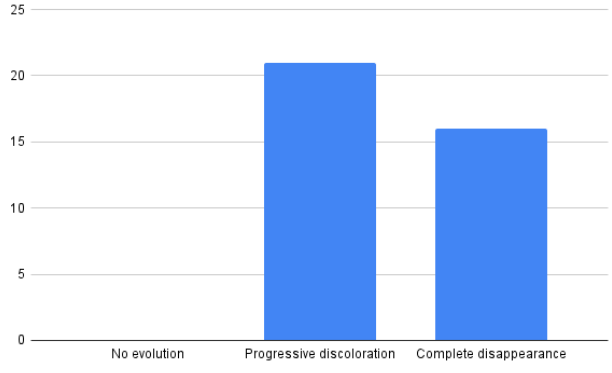


Chart 2. Clinical evolution

Discussion and Conclusion

Sentinel node identification rate after SPIO injection was similar, regardless of the injection site and was similar to that previously reported (15). Failure to detect SLN in the four patients led to axillary sampling (between 3-6 lymph nodes) without axillary dissection because they were elderly patients with low grade tumors. Although metastatic lymph nodes are associated with a lower SLN detection rate, none of the four patients with failure to detect SLN showed tumoral cells on histopathological examination (8).

The principal drawback of SPIO is skin discoloration around the injection site (16). The type of surgery, the localisation of injection and probably the time lapse between the injection and surgery may influence the rate of skin staining (14). The low rate of skin staining after mastectomy (6.8%) may have been due to the removal of the skin and the glandular tissue in which the tracer accumulated (17, 18). More skin staining was observed in P2 (18.8%) compared to P1 (2.4%) after mastectomy. This may be due to sample size effects as we collected more patients with mastectomy than BCS, and a higher dilution of SPIO. A meta-analysis performed in 2023 of 12 case series regarding skin staining concluded that 95% of skin staining were described after BCS (14). After BCS, protocol P2 was associated with a decreased risk of skin staining compared to P1. This difference, although not statistically significant, could be explained by the different injection sites, a shorter interval between injection and surgery and the removal of the excess magnetic tracer in the breast during lumpectomy.

Wärnberg et al. (17) showed that peri-tumoral injection significantly reduced the discoloration compared to retro-areolar injection in BCS (37.8% compared with 67.8%). Retro-tumoral injection could even reduce the size of the staining and lead to a more radiant regression (18). Other ways to reduce the rate of skin staining may be by injecting at least 15 mm under the skin or by reducing the dose of SPIO (21). A study by Rubio et al. (12) compared 1 mL to 1.5 and 2 mL and showed that 1 mL of SPIO significantly decreased the rate of skin discoloration (84.4% to 60%) and its size, with no effect on the SNL detection rate (8). Mirzaei et al. (19) concluded that even an ultralow dose of 0.1 mL SPIO, showed the same efficiency.

A recent meta-analysis from Pantiora et al. (14) showed that the common rate of skin staining is usually described as 30.8%, but ranged widely from 0 to 84.%. In the meta-analysis regression, taking each potential factor separately, such as the injection site, the injection volume or applying massage to the area, reported that none of these factors significantly reduced skin staining. The authors suggested that achieving less skin staining was probably only due to the combination of these factors (14).

It is known that SPIO injection may result in residual tissue nanoparticles and lead to MRI artifacts when this modality is needed for medical follow-up (20). About half of the patients showed postoperative accumulation of iron oxide particles on MRI (21). Therefore it is important to specify to the radiologist that the patient was injected with SPIO tracers. Christenhusz et al. (20) showed that a 0.1 mL intra tumoural dose did not result in MRI residual remnants and reduced the difficulty of reading subsequent MRIs. However, some specific protocols improve the MRI image quality when artifacts are observed (20, 22).

Despite persistent skin staining up to four years after surgery, a majority of the patients in the present study were satisfied with the procedure, since it only slightly affected their self-image. This has already been described by other studies with a maximum follow-up of three years (9, 14, 17). During the consultation, patients often mention less interest in the aesthetic result in comparison to the oncological issue, primarily justified by advanced patient age. Most of the time, complete disappearance takes at least a year, but it is important to reassure the patient about the fading of the stain, even if it does not totally

disappear in about half of cases. When breast cancer affects a young patient in whom BCS and SLN is planned, it seems wise to discuss the potential risk of skin discoloration and perhpas to use another type of tracer, such as a radioactive marker or ICG. SLN detection with SPIO markers shortens the preoperative care pathway, and it also seems that SPIO tends to reduce costs because of avoidance of nuclear medicine charges (10). In order to reduce medical charges and iatrogenicity, preoperative SPIO injection could also minimize unnecessary SLNB, in case of preoperative diagnosis of ductal carcinoma *in situ*, and enable SLNB to be performed later if invasive breast cancer was found post-operation (23).

SPIO injection is a safe surgical method for detecting SLN which facilitates logistics at surgery. After mastectomy, the rate of skin staining was low at less than seven percent with good patient satisfaction. In the presented series, the rate of skin staining after BCS was 31.6%. Peritumoral injection was associated with a decreased risk of skin discoloration after BCS compared to retroareolar injection. Despite the persistent skin discoloration in 58.6% of cases in the present study, patient satisfaction was high. Deeper injection in the subcutaneous tissue, reduced doses, massage of the injection area and peritumoral injection were correlated with less skin staining.

Ethics Committee Approval: No ethical statement is mandatory because the dosis and the localisation of the injection are decisions dependant on an internal service protocol, without deviating from the guidelines for respecting the use of the product.

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: M.P.M., C.S., D.H.; Concept: M.P.M., C.S., D.H.; Design: M.P.M., C.S., D.H.; Data Collection and/or Processing: M.P.M., C.S., D.H.; Analysis and/or Interpretation: M.P.M., C.S., D.H.; Literature Search: M.P.M., C.S., D.H.; Writing: M.P.M., C.S., D.H.

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

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Breast Cancer Statistics And Resources. n.d. Breast Cancer Research Foundation. Accessed February 15, 2024. https://www.bcrf.org/breast-cancer-statistics-and-resources. [Crossref]
2. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies-An Updated Review. Cancers (Basel) 2021; 13: 4287. (PMID: 34503097) [Crossref]
3. Manca G, Tardelli E, Rubello D, Gennaro M, Marzola MC, Cook GJ, et al. Sentinel lymph node biopsy in breast cancer: a technical and clinical appraisal. Nucl Med Commun 2016; 37: 570-576. (PMID: 26886421) [Crossref]
4. Lucci A, McCall LM, Beitsch PD, Whitworth PW, Reintgen DS, Blumencranz PW, et al. Surgical complications associated with sentinel lymph node dissection (SLND) plus axillary lymph node dissection compared with SLND alone in the American College of Surgeons Oncology Group Trial Z0011. J Clin Oncol 2007; 25: 3657-3663. (PMID: 17485711) [Crossref]
5. Ashikaga T, Krag DN, Land SR, Julian TB, Anderson SJ, Brown AM, et al. Morbidity results from the NSABP B-32 trial comparing sentinel lymph node dissection versus axillary dissection. J Surg Oncol 2010; 102: 111-118. (PMID: 20648579) [Crossref]
6. Mansel RE, Fallowfield L, Kissin M, Goyal A, Newcombe RG, Dixon JM, et al. Randomized multicenter trial of sentinel node biopsy versus standard axillary treatment in operable breast cancer: the ALMANAC Trial. J Natl Cancer Inst 2006; 98: 599-609. (PMID: 16670385) [Crossref]
7. Albo D, Wayne JD, Hunt KK, Rahlfs TF, Singletary SE, Ames FC, et al. Anaphylactic reactions to isosulfan blue dye during sentinel lymph node biopsy for breast cancer. Am J Surg 2001; 182: 393-398. (PMID: 11720678) [Crossref]
8. Rocco N, Velotti N, Pontillo M, Vitiello A, Berardi G, Accurso A, et al. New techniques versus standard mapping for sentinel lymph node biopsy in breast cancer: a systematic review and meta-analysis. Updates Surg 2023; 75: 1699-1710. (PMID: 37326934) [Crossref]
9. Douek M, Klaase J, Monypenny I, Kothari A, Zechmeister K, Brown D, et al. Sentinel node biopsy using a magnetic tracer versus standard technique: the SentiMAG Multicentre Trial. Ann Surg Oncol 2014; 21: 1237-1245. (PMID: 24322530) [Crossref]
10. Shams S, Lippold K, Blohmer JU, Röhle R, Kühn F, Karsten MM. A Pilot Study Evaluating the Effects of Magtrace® for Sentinel Node Biopsy in Breast Cancer Patients Regarding Care Process Optimization, Reimbursement, Surgical Time, and Patient Comfort Compared With Standard Technetium99. Ann Surg Oncol 2021; 28: 3232-3240. (PMID: 33263157) [Crossref]
11. Karakatsanis A, Christiansen PM, Fischer L, Hedin C, Pistioli L, Sund M, et al. The Nordic SentiMag trial: a comparison of super paramagnetic iron oxide (SPIO) nanoparticles versus Tc(99) and patent blue in the detection of sentinel node (SN) in patients with breast cancer and a meta-analysis of earlier studies. Breast Cancer Res Treat 2016; 157: 281-294. (PMID: 27117158) [Crossref]
12. Rubio IT, Rodriguez-Revuelto R, Espinosa-Bravo M, Siso C, Rivero J, Esgueva A. A randomized study comparing different doses of superparamagnetic iron oxide tracer for sentinel lymph node biopsy in breast cancer: The SUNRISE study. Eur J Surg Oncol 2020; 46: 2195-2201. (PMID: 32631710) [Crossref]
13. Jazrawi A, Wärnberg M, Hersi AF, Obondo C, Pistioli L, Eriksson S, et al. A Comparison of Skin Staining after Sentinel Lymph Node Biopsy in Women Undergoing Breast Cancer Surgery Using Blue Dye and Superparamagnetic Iron Oxide Nanoparticle (SPIO) Tracers. Cancers (Basel) 2022; 14: 6017. (PMID: 36497498) [Crossref]
14. Pantiora E, Tasoulis MK, Valachis A, Eriksson S, Kühn T, Karakatsanis A, et al. Evolution and refinement of magnetically guided sentinel lymph node detection in breast cancer: meta-analysis. Br J Surg 2023; 110: 410-419. (PMID: 36560842) [Crossref]
15. Karakatsanis A, Olofsson H, Stålberg P, Bergkvist L, Abdsaleh S, Wärnberg F. Simplifying Logistics and Avoiding the Unnecessary in Patients With Breast Cancer Undergoing Sentinel Node Biopsy. A Prospective Feasibility Trial of the Preoperative Injection of Super Paramagnetic Iron Oxide Nanoparticles. Scand J Surg 2018; 107: 130-137. (PMID: 29132268) [Crossref]
16. Alvarado MD, Mittendorf EA, Teshome M, Thompson AM, Bold RJ, Gittleman MA, et al. SentimagIC: A Non-inferiority Trial Comparing Superparamagnetic Iron Oxide Versus Technetium-99m and Blue Dye in the Detection of Axillary Sentinel Nodes in Patients with Early-Stage Breast Cancer. Ann Surg Oncol. 2019; 26: 3510-3516. (PMID: 31297674) [Crossref]
17. Wärnberg F, Stigberg E, Obondo C, Olofsson H, Abdsaleh S, Wärnberg M, et al. Long-Term Outcome After Retro-Areolar Versus Peri-Tumoral Injection of Superparamagnetic Iron Oxide Nanoparticles (SPIO) for Sentinel Lymph Node Detection in Breast Cancer Surgery. Ann Surg Oncol 2019; 26: 1247-1253. (PMID: 30830536) [Crossref]
18. Hersi AF, Pistiolis L, Dussan Luberth C, Vikhe-Patil E, Nilsson F, Mohammed I, et al. Optimizing Dose and Timing in Magnetic Tracer

Techniques for Sentinel Lymph Node Detection in Early Breast Cancers: The Prospective Multicenter SentiDose Trial. Cancers (Basel) 2021; 13: 693. (PMID: 33572114) [Crossref]

19. Mirzaei N, Wärnberg F, Zaar P, Leonhardt H, Olofsson Bagge R. Ultra-Low Dose of Superparamagnetic Iron Oxide Nanoparticles for Sentinel Lymph Node Detection in Patients with Breast Cancer. Ann Surg Oncol 2023; 30: 5685-5689. (PMID: 37314544) [Crossref]
20. Christenhusz A, Pouw JJ, Simonis FFJ, Douek M, Ahmed M, Klaase JM, et al. Breast MRI in patients after breast conserving surgery with sentinel node procedure using a superparamagnetic tracer. Eur Radiol Exp 2022; 6: 3. (PMID: 35083595) [Crossref]
21. Aribal E, Çelik L, Yilmaz C, Demirkiran C, Guner DC. Effects of iron oxide particles on MRI and mammography in breast cancer patients after a sentinel lymph node biopsy with paramagnetic tracers. Clin Imaging 2021; 75: 22-26. (PMID: 33486148) [Crossref]
22. Krischer B, Forte S, Niemann T, Kubik-Huch RA, Leo C. Feasibility of breast MRI after sentinel procedure for breast cancer with superparamagnetic tracers. Eur J Surg Oncol 2018; 44: 74-79. (PMID: 29217399) [Crossref]
23. Karakatsanis A, Hersi AF, Pistiolis L, Olofsson Bagge R, Lykoudis PM, Eriksson S, et al. Effect of preoperative injection of superparamagnetic iron oxide particles on rates of sentinel lymph node dissection in women undergoing surgery for ductal carcinoma in situ (SentiNot study). Br J Surg 2019; 106: 720-728. (PMID: 30839104) [Crossref]

Case Report

Eur J Breast Health 2024; 20(3): 228-230
DOI: 10.4274/ejbh.galenos.2024.2024-1-9

A Rare Case of Concurrent Lupus Mastitis and Sarcoidosis in a 62-Year-Old Female

Cheickna C. Fofana[1], Cinthia Del Toro[2], Jessica Alvarez-Lesmes[3], Cedric W. Pluguez-Turull[4]

[1]Department of Radiology, Jackson Memorial Hospital, Miami, FL, USA
[2]University of Miami, Leonard M. Miller School of Medicine, Miami, FL, USA
[3]Department of Pathology, Jackson Memorial Hospital, Miami, FL, USA
[4]Department of Radiology, University of Miami Miller School of Medicine, Sylvester Cancer Center, and Jackson Memorial Hospital, Miami, FL, USA

ABSTRACT

Systemic lupus erythematosus (SLE) and sarcoidosis are two of the most well-recognized, chronically diagnosed conditions in the United States, with a plethora of known multisystem manifestations. With regard to breast pathology, lupus mastitis is a relatively uncommon manifestation of SLE, commonly involving both the mammary gland and subcutaneous soft tissues of the breast. Sarcoidosis in the breast is a similarly, exceedingly rare manifestation of this multi-system disorder, classically presenting with non-caseating granulomas. Both present with non-specific mammographic and sonographic features. We present a 62-year-old female with known diagnosis of discoid lupus and Graves' disease who presented initially with an abnormal screening mammogram, ultimately undergoing mammographic work-up and subsequent biopsy demonstrating lupus mastitis, including vasculitis, panniculitis, and fibrosis with chronic inflammation. The patient was also found to have small non-caseating granulomas, some in a perivascular distribution, classically seen in sarcoidosis. Given the rarity of both manifestations, our case explores the coexistence of these autoimmune processes and this atypical presentation.

Keywords: Lupus mastitis; systemic lupus erythematosus; breast sarcoidosis; granulomatous disease; mammogram; breast ultrasound; ultrasound-guided core needle biopsy

Cite this article as: Fofana CC, Del Toro C, Alvarez-Lesmes J, Pluguez-Turull CW. A Rare Case of Concurrent Lupus Mastitis and Sarcoidosis in a 62-Year-Old Female.

Key Points

- Multisystem inflammatory processes occurring in the breast, albeit rare, can occur in tandem and present confounding diagnosis and clinical presentation, imaging, and pathologic diagnoses.
- Chronic inflammatory processes in the breast can present suspicious mammographic and ultrasound findings, mimicking malignancy. Further research can lend into characteristic imaging findings.
- Established treatment algorithms for chronic inflammatory processes such as sarcoid and lupus are not clearly defined. Further research can explore optimizing appropriate strategies for relief.

Introduction

Sarcoidosis is a systemic inflammatory disease of unknown etiology classically characterized by the formation of multiorgan granulomatous lesions. Existing literature has cited an immune mediated secondary response to an antigenic trigger as a potential etiology (1). In the United States, the disease is most prevalent in African Americans, with prevalence rates of up to 10 times that of white Americans (2). Extensive variability is demonstrated as far as disease prognosis, manifestations, and progression of disease. The mainstay of treatment is corticosteroids, although in severe cases immunosuppressive agents, such as methotrexate and cyclophosphamide, are used. Sarcoidosis of the breast is rare and only few cases are reported in the literature. Mammographic features are variable and may mimic both benign and malignant etiologies. The breast is involved in less than 1% of cases and can either be a primary or secondary site of presentation (3). Breast sarcoidosis can mimic carcinoma on mammographic and clinical exam, as seen in the presented case (4).

Lupus is characterized as an autoimmune disease in which a misdirected immune response generates a multisystem inflammatory response against the native tissues. Similar to sarcoidosis, the disease prognosis and manifestations are extremely variable, ranging from mild to severe. Lupus mastitis is an uncommon presentation of systemic lupus erythematosus (SLE), characterized by inflammation of the adipose fat. Lupus mastitis can present as single or multiple subcutaneous or deep breast masses, often clinically mimicking malignancy (5). The constellation of concurrent lupus and sarcoidosis in the breast has not been previously reported. Given the suspected immune mediated etiology, the intersection of concurrent pathology in our case may yield

Corresponding Author:
Cedric Pluguez-Turull; cxp932@med.miami.edu

Received: 25.01.2024
Accepted: 14.04.2024
Available Online Date: 01.07.2024

as a branch point to observe how systemic processes can present and mimic each other.

Case Presentation

A 63-year-old female with past medical history of discoid lupus and Graves' disease, with no known diagnosis of sarcoidosis at presentation, was found to have an indeterminate asymmetry in the right breast at 12 o'clock anterior depth (Figure 1), persisting on spot compression views (Figure 2). Clinically, no lesions were palpated, and the patient did not report any pain in the right breast. In addition, views with ultrasound demonstrated a 4.4 x 1.9 x 3.7 cm indistinct area of mixed echogenicity lacking significant flow on power Doppler in the right breast, corresponding to the partially persistent asymmetry on mammogram (Figure 3). A palpable second area of concern in the left breast prompted ultrasound, which revealed a 4.7 x 1.6 x 3.9 cm area of irregular, heterogenous, non-mass lesions lacking significant flow on power Doppler corresponding to a clinically reported palpable abnormality in the left breast (Figure 4). At the time, with a BI-RADS 4B classification for both areas in the breast, the decision was made to further evaluate the lesions via ultrasound-guided core needle biopsy. Pathology revealed scattered small non-caseating granulomas with giant cells on a background of chronic inflammation in the left breast with concurrent focal vasculitis and panniculitis. The right breast similarly demonstrated small non-caseating granulomas with giant cells on a background of chronic inflammation, panniculitis and more prominent focal vasculitis. These findings were seen in the context of fibrosis and chronic inflammation (Figures 5 and 6) and led to a diagnosis of lupus mastitis with recommendations for clinical correlation and consideration of sarcoidosis. The patient was subsequently referred to a rheumatology clinic and was found to have nodular lesions on the nose, which can be seen in sarcoidosis, however, with normal angiotensin converting enzyme level and serum anti-nuclear antibodies, extracatable nuclear antigen antibodies and double-stranded DNA antibody titers. Additional laboratory results showed leukopenia and neutropenia both of which were chronic and had been stable. Chest computed tomography imaging demonstrated

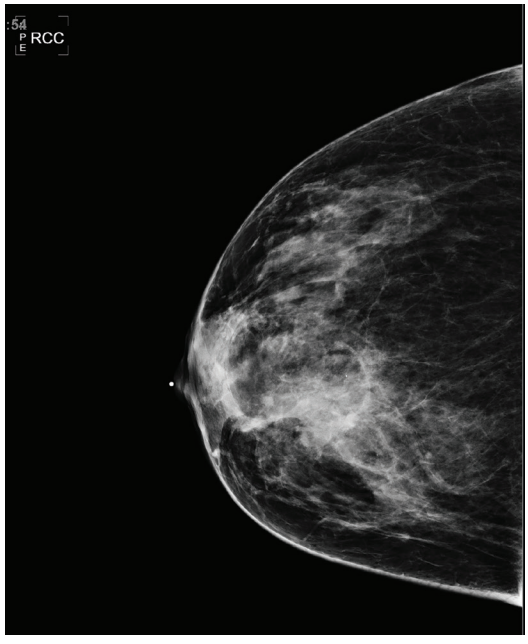


Figure 1. Initially reported indeterminate asymmetry in the right breast at 12 o'clock anterior depth

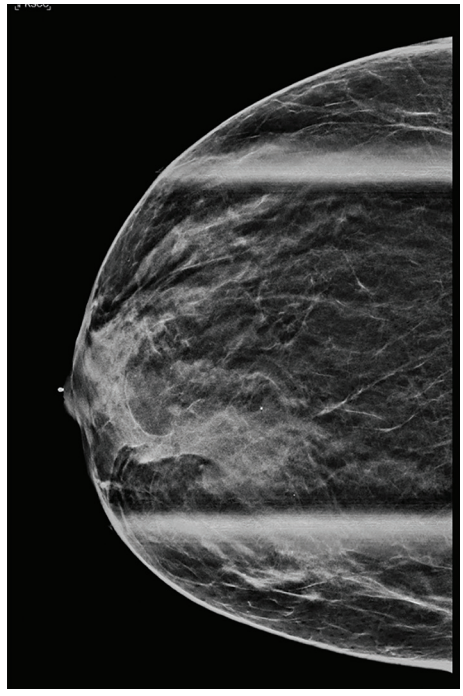


Figure 2. The previously seen focal asymmetry in the right breast anterior third at the 12:00 axis partially persists on additional views

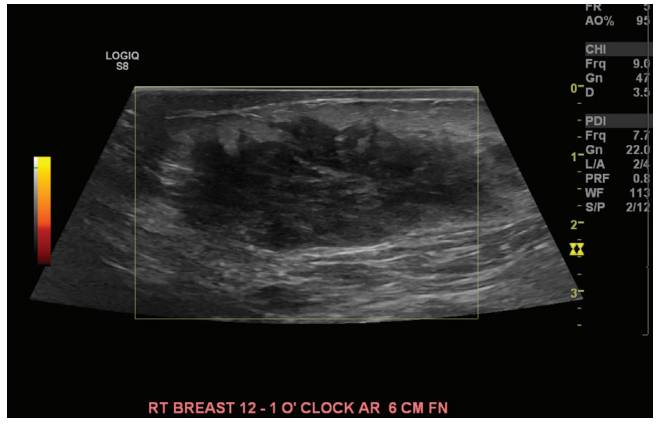


Figure 3. A 4.4 x 1.9 x 3.7 cm indistinct area of mixed echogenicity in the right breast at 1:00, 6 cm from the nipple, lacking significant flow on power doppler in the right breast, corresponding to partially persistent asymmetry on mammogram

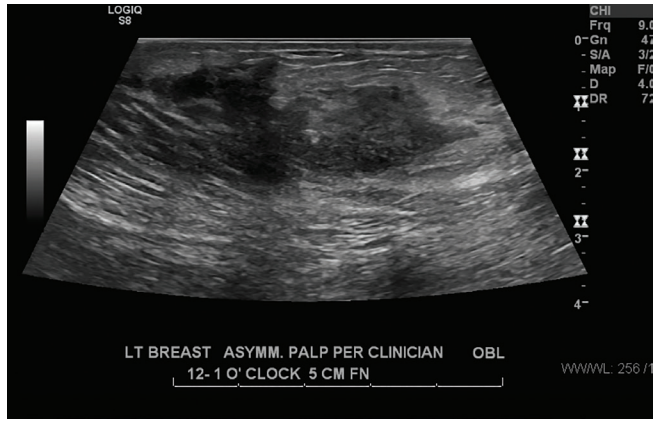


Figure 4. 4.7 x 1.6 x 3.9 cm irregular area of heterogeneity at 12:00-1:00, 5 cm from the nipple, lacking significant flow on power doppler corresponding to a palpable abnormality in the left breast

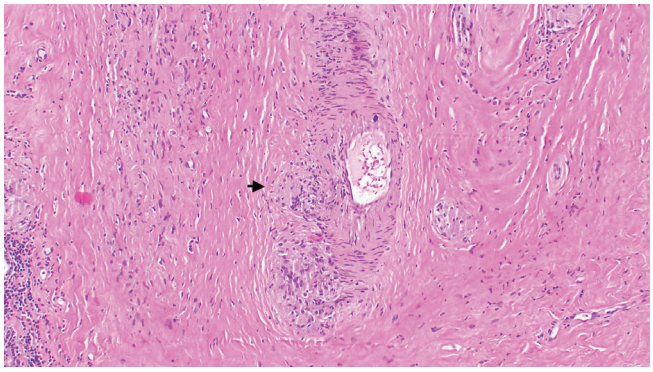


Figure 5. Focus of vasculitis with adjacent granulomatous inflammation (arrow), right breast. 100x magnification

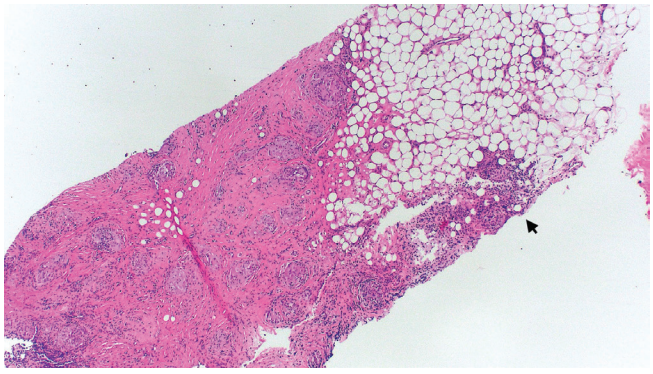


Figure 6. Benign breast parenchyma with non-caseating granulomas and giant cells (arrow) and background chronic inflammation, right breast. 40x magnification

mediastinal lymphadenopathy and multiple pulmonary nodules and fibrotic changes, which can be seen in sarcoidosis. Subsequent positron emission tomography exam demonstrated active uptake of these lesions, suggestive of active disease. These imaging findings along with the biopsy results provided enough evidence to support the diagnosis of sarcoidosis and thus a biopsy of the nose was not recommended by dermatology for cosmetic reasons. The concurrent diagnosis of sarcoidosis of the breast was made.

Outcome and Follow-up

Patient was subsequently started on 10 mg of methotrexate and folic acid and continues long-term follow-up in rheumatology clinic.

Discussion and Conclusion

The multisystem manifestations of SLE and sarcoidosis have been well-documented in the literature. However, in both systemic processes, breast manifestations are extremely rare. In addition, the concurrent presentation of both lupus and sarcoidosis has rarely been reported previously (6). Variable mammographic and sonographic imaging patterns have been seen in both processes, further complicating diagnosis and timely recognition (4). This case is atypical in that the mammographic findings ultimately yielded an unsuspected diagnosis of sarcoidosis in the setting of an asymptomatic presentation, and initiation of long-term therapy. Given the expanding research regarding risk factors for autoimmune pathology in chronic illness, our case serves as an exploration into the manifestations of autoimmune processes. The delineating factor in this case was the histopathological diagnosis, as the mammographic and sonographic findings were nonspecific. Initial sonographic findings were concerning for potential malignancy, further emphasizing the variable appearance of systemic autoimmune processes within the breast. Further research may serve to analyze risk factors for breast manifestations of these processes and treatment algorithms for appropriate management in the setting of chronic disease. Given the low incidence of breast manifestations, obtaining enough cases may serve as a research limitation. Further research may serve to analyze sonographic, mammographic and possibly histopathological features of autoimmune manifestations within the breast to assess if recurrent characteristics are visualized.

Informed Consent: Written informed consent was obtained from the patient.

Authorship Contributions

Surgical and Medical Practices: C.C.F., J.A-L., C.W.P-T.; Concept: C.C.F., C.D.T., J.A-L., C.W.P-T.; Design: C.C.F., C.D.T., J.A-L., C.W.P-T.; Data Collection and/or Processing: C.C.F., J.A-L., C.W.P-T.; Analysis or Interpretation: C.C.F., C.D.T., J.A-L., C.W.P-T.; Literature Search: C.C.F., C.D.T., J.A-L., C.W.P-T.; Writing: C.C.F., C.D.T., J.A-L., C.W.P-T.

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Loke WS, Herbert C, Thomas PS. Sarcoidosis: Immunopathogenesis and Immunological Markers. Int J Chronic Dis 2013; 2013: 928601. (PMID: 26464848) [Crossref]
2. Hena KM. Sarcoidosis Epidemiology: Race Matters. Front Immunol 2020; 11: 537382. (PMID: 33042137) [Crossref]
3. Pluguez-Turull C, Del Toro C, Tjendra Y. Sarcoidosis of the Breast. IntechOpen 2024. [Crossref]
4. Limninart N, Harvey JA, Schultz KJ, Mills AM, Noland MMB, Schroen AT, et al. "What Do You Mean It's Not Cancer?" A Review of Autoimmune and Systemic Inflammatory Diseases Involving the Breast. J Breast Imaging 2021; 3: 612-625. (PMID: 38424941) [Crossref]
5. Pimentel A, Moreira A, Silva S, Lages R, Noronha J. Lupus mastitis: A rare breast cancer differential diagnosis. Clin Case Rep 2021; 9: e04416. (PMID: 34267904) [Crossref]
6. Prieto-Peña D, Ferrer-Pargada D, Atienza-Mateo B, Mazorra-Horts R, Cifrián JM, González-Gay MA. Coexisting sarcoidosis and systemic lupus erythematosus: a case report and literature review. Acta Reumatol Port 2021; 46: 177-185. (PMID: 34243181) [Crossref]

Letter to the Editor

Eur J Breast Health 2024; 20(3): 231-232
DOI: 10.4274/ejbh.galenos.2024.2024-4-2

Artificial Intelligence in Senology: Comment

Hinpetch Daungsupawong[1], Viroj Wiwanitkit[2]

[1]Private Academic Consultant, Phonhong, Lao People's Democratic Republic
[2]Department of Research Analytics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Navi Mumbai, India

Cite this article as: Daungsupawong H, Wiwanitkit V. Artificial Intelligence in Senology: Comment.

Keywords: Big data; qualitative research; qualitative study

Dear Editor,

We would like to comment on "Artificial Intelligence in Senology - Where Do We Stand and What Are the Future Horizons?" (1). Artificial intelligence (AI), which includes deep learning, has brought interest owing to its feasibility to mimic human intelligence and further transform a number of activities and result in usefulness in industries as well as healthcare. AI-based image analysis in breast screening programs has demonstrated encouraging results in terms of reduction of workload and increasing sensitivity. ChatGPT as well as other natural language software have proven to be highly accurate in giving decisions, however, many issues with patient safety and legal requirements still require to be managed. The primary advantages of AI are the high speed and effectiveness in handling complicated works; nevertheless, there are certain drawbacks that had to be taken into concern, including cybersecurity, employment displacement in the healthcare industry, and stability of the system.

AI is still in its early phase in the subject of senology, and there is still further opportunity for performance and dependability to be upgraded. For AI systems to be safe and effective in real-world usages, the systems must be responsibly trained using high-quality data and subjected to rigorous scientific review. To reduce hazards and maintain public confidence in emerging technologies, it will be necessary to strike a balance between AI promotion and control. Introduced in December 2023, the AI Act by the European Union is an important step in creating all-encompassing legal frameworks for AI governance as well as accountability.

AI must be utilized in conjunction with human skill, empathy, and ethical considerations, even while the AI has the possibility to improve medical procedures and healthcare delivery. Achieving significant progress in senology and other medical sciences would require fusing the advantages of AI with human judgment and empathy. Sufficient research, cooperation, and regulatory monitoring are necessary for directing the conscientious and effective application of AI in healthcare and guaranteeing good consequences for both patients and healthcare providers. The importance of ethical considerations and human-machine collaboration in the development and application of AI in senology and healthcare in general have received little attention.

A further interesting area of researching is the probability ethical conundrums that the application of AI in senology may exist. There are concerns over patient privacy and clinical safety, even while AI may help improve the effectiveness and accuracy of clinical procedures. The ethical concerns should be addressed to confirm that the AI application is ethical way and do not lead to violation of the patients' rights. Furthermore, lesson learnt from the clinical practitioners who have experience in using AI in their clinical practice will be useful. Topics that should be assessed include how the AI help perform clinical decision making and affect daily clinical workload. For example, in our area in Indochina, the use of AI in clinical senology is already implemented in some private clinical center. Some private hospital already offer the additionally service including AI decision making as an alternative option for the patient in selecting their ways of breast disease therapy (see example at https://www.bumrungrad.com/th/health-blog/june-2022/using-ai-find-lung-disorders-and-breast-cancer). However, the technology is new and might be expensive and are currently limited used. The unofficial report from the private clinical setting claimed that the AI based breast radiology interpretation help early detect breast cancer in young female but there is still no publication on this issue in our area. Nevertheless, the derived data can further guide how the AI should be implemented in clinical senology practice. Accumulation of data from multi-setting about the advantages of using AI in clinical senology will help further better clinical care for the patients.

Corresponding Author:
Hinpetch Daungsupawong; hinpetchdaung@gmail.com

Received: 04.04.2024
Accepted: 01.05.2024
Available Online Date: 01.07.2024

Authorship Contributions

Surgical and Medical Practices: H.D., V.W.; Concept: H.D., V.W.; Design: H.D., V.W.; Data Collection or Processing: H.D., V.W.; Analysis or Interpretation: H.D., V.W.; Literature Search: H.D., V.W.; Writing: H.D., V.W.

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Mundinger A, Mundinger C. Artificial Intelligence in Senology - Where Do We Stand and What Are the Future Horizons? Eur J Breast Health. 2024; 20: 73-80. (PMID: 38571686) **[Crossref]**

Award Winning and Best Scored Abstracts from NCoBC 33rd Annual Interdisciplinary Breast Cancer Conference

Committee Chair: Dr. Atilla Soran
Committee Co-Chair: Dr. Terry Sarantou

Conference Date:
March 15-20, 2024
Conference Location:
Paris Hotel and Casino, Las Vegas, NV

Category I

Demographic Impact and Clinical Insights: A Comprehensive Analysis of Glycogen-Rich Carcinoma of the Breast Using National Cancer Database Data (2004-2020)

Michael Richard Demangone, Karam Abi Karam, Amaan Zafar, Daniel Lee, Suraj Puvvadi, Noureen Nakshbandi, Beau Hsia, Nicholas Heng, Patrick Sam, Will Heise, Peter Silberstein

University of Arizona, Arizona, United States

Objective: Glycogen-rich carcinoma of the breast, an exceptionally rare subtype of invasive breast carcinoma, manifests characteristic polygonal cells with abundant clear cytoplasm containing diastase-sensitive glycogen deposits. However, varied morphologies and clinical outcomes contribute to a wide range of prognoses, warranting further investigation using updated data from the National Cancer Database to explore demographic impact on prognostic factors.

Materials and Methods: A retrospective cohort investigation of the National Cancer Database (NCDB) was conducted which spanned the years 2004–2020 involving 131 patients with a confirmed diagnosis of Glycogen-rich carcinoma. Demographic factors were examined, and a regression analysis was conducted.

Results: Among the group of individuals diagnosed with Glycogen-rich carcinoma (GRC), the median age at diagnosis was 61 with a mean survival time post diagnosis of just under 12 years. Most (94%) underwent surgical procedures as their primary method of treatment for GRC, with the final status of surgical margins post-resection of the primary tumor revealing that the majority of cases (92%) had no residual tumor. As their primary treatment, most patients received radiation therapy (64%) and chemotherapy (62%), while a minority received hormone therapy (31%). A predominance of patients with GRC were female (97%), white (86%), and of non-Spanish, non-Hispanic origin (92%). Many cases came from a comprehensive community cancer program (44%) or an academic program (32.5%). At the time of diagnosis, the vast portion of patients (98%) were covered by some form of primary insurance with private insurance (50%) and Medicare services (41%) composing the majority of this cohort. The top primary site for nearly all cases was the breast (98%) with the upper-outer quadrant of breast (42%) and overlapping lesion of breast (20%) being the leading primary sites. Most patients (87%) were classified as either Stage I or Stage II analytic stage group. A large percentage of the individuals (86%) did not have any concurrent comorbidities (Charlson-Deyo = 0).

Conclusion: Following a review of the available literature, our findings suggest a possible knowledge gap in the discussion of glycogen-rich carcinoma that this novel NCDB analysis addresses. Socioeconomic factors revolving around patients living with glycogen-rich carcinoma have not yet been discussed in literature, and our findings show that a significant percentage of patients had some form of private insurance. Many were not of a minority background and a vast majority possessed few comorbidities. Given the paucity of research of this cancer, continued investigation is necessary to establish a more complete understanding of how demographics, socioeconomic factors, and treatments affect survival and outcomes of patients with glycogen-rich breast carcinoma.

Keywords: Glycogen-rich; corcinoma; treatment

Category I-A. Breast Center Office Operations Efficiency and Productivity

Implementing Standardized Ordering Pathways in a Breast Cancer Multidisciplinary Clinic Lifespan Cancer Institute, Providence RI

Francesca Rothell, Ann-Marie Galvin, Mary Anne Fenton

Lifespan Cancer Institute, Rhode Island, United States

Objective: Timeliness of cancer care from diagnosis to implementation of systemic therapy is a quality metric and may impact patient outcomes. Estrogen receptor-positive human epidermal growth factor receptor 2-negative early-stage breast cancer genomic assays including the 21 gene recurrence score (21-RS) are prognostic for chemotherapy benefit, and results impact shared medical decision making. The Lifespan Cancer Institute (LCI) Breast Cancer Steering Committee (BCSC) Quality Team noted significant variation in turnaround time (TAT) from time of surgery to 21-RS report, resulting in significant delay in care recommendation. Breast cancer patients are seen by multidisciplinary teams at 4 LCI sites which can contribute to variability in care processes. The LCI BCSC consists of a multidisciplinary team of surgeons, medical oncologists (MO), pathologists, Breast Cancer Navigators (BCN) and other members. The BCSC initiated a quality improvement project to address process variance and barriers to efficient and consistent 21-gene RS order and tissue send-out.

Materials and Methods: Two steps in 21-RS order were identified for intervention. 1. MO and BCN share an electronic medical record (EMR) results box including pathology reports. Breast surgeons will attach MO as electronic recipients to receive the surgical pathology report immediately on EMR sign out. MO and BCN will review pathology results and initiate 21-RS orders per American Society of Oncology (ASCO) guidelines. 2. Pathologists will identify appropriate tissue block for 21-RS at time of sign out to facilitate pathology staff block selection for tissue send out. Data was extracted by Exact Science (ES) from the 21-RS electronic order database. TAT from surgery to 21-RS ordering and TAT from surgery to results return were provided. We examined TAT prior to intervention (January-April 2023 n = 69 orders) and after intervention (August-October 2023 n=53 orders).

Results: The baseline average time from surgery to 21-RS order was 16 days preintervention and 10-days post-intervention, resulting in a total decrease of 6 days from surgery to order. As such, date of surgery to 21-RS report return decreased from 27 to 21 days.

Conclusion: Multidisciplinary discussion of quality issues in our LCI BCSC is an effective way to identify barriers to care and initiate a plan-do study-act quality improvement process. LCI BCSC has established a consistent streamlined process for 21-RS orders. Attaching MO to pathology reports and pre-selecting slides for send-out has reduced the internal processing time for 21-RS order results by 6 days. The process will be examined for TAT twice a year and presented to the BCSC for ongoing process improvement. This project focused on timely patient-centric care and reducing patient anxiety.

Keywords: Multidisciplinary; quality; improvement

Check Out My Rack: A Webinar Empowering Young Women Through Comprehensive Breast Health Education

Victoria Haney, Ashley Teal, Julia Kepniss, Christine B. Teal, Clare Dougherty, Andrea B. Wolf, Rachel F. Brem

The Brem Foundation to Defeat Breast Cancer, Maryland, United States

Objective: The Brem Foundation to Defeat Breast Cancer, a 501c3 non-profit organization based in the Washington, DC area, is dedicated to advancing early detection through education, access, and advocacy. While conventional guidelines recommend initiating annual breast screenings at age 40 for women of average risk, Brem underscores the importance of starting breast health education and empowerment earlier in life. Nearly 80% of young women with breast cancer find their own breast abnormality and 1 in 10 breast cancers occur in women under the age of 45, making self-exams and education essential. As part of our education programs, we aim to engage younger women and people of pre-screening age in breast cancer awareness through an annual webinar titled "Check Out My Rack".

Materials and Methods: Since 2021, Brem Foundation has hosted three annual webinars titled "Check Out My Rack". Tailored to resonate with college students and women under 40, each hour-long session shares essential breast health information, including the importance of family history, genetic testing, self-exams, and encouraging loved ones to screen. The webinars included live self-exam tutorials, provided self-advocacy tools, and featured compelling narratives from prominent young survivors. Participants were encouraged to engage with the expert educator radiologists and breast health advocates who lead the programs and to ask questions related to their breast health.

Results: In 2023, 299 people registered for Check out My Rack with a total of 130 attending the webinar, doubling attendance from the year prior. Over 48 educational materials were downloaded by participants, Brem Foundation obtained 191 new contacts for future educational engagement, and doubled the conversion rate of registrants to attendees in 2023, from 21% to 42%. Using a "collaborator" model, Brem engaged like-minded entities to market the event and grow attendance, resulting in exposure to an additional 175,000 people.

Conclusion: The Brem Foundation's commitment to empowering women with breast health education is evidenced by the growing success of the "Check Out My Rack" webinars and web-based education. Future studies will evaluate the efficacy of these increasingly popular programs in terms of breast cancer detection.

Keywords: Webinar; young women; education

Effectiveness of Community Education for Breast Cancer Screening

Ashley Teal, Victoria Haney, Julia Kepniss, Rachel Brem

The Brem Foundation to Defeat Breast Cancer, Maryland, United States

Objective: Screening based on individual risk factors results in detection of earlier, more curable breast cancer. There is expectation that improved public education about the importance of personalized screening will result in earlier diagnoses and reduced breast cancer mortality. The purpose of this study is to evaluate the effectiveness of community education on patient perceptions about risk-based screening.

Materials and Methods: This study is HIPAA compliant and institutional review board exempt. A standardized curriculum was used by radiologists and experts to conduct nine one-hour patient education sessions between 10/2018 and 1/2019 about breast cancer risk factors and screening options. Patient participants completed voluntary, anonymous pre-event and post-event surveys to determine if the presented educational program led to attitude changes. Survey results were summarized using statistical analysis including mean, median, range, and percentage of participants responding and comparison of pre- and post-event fear and anxiety.

Results: Of 336 education session participants, 59.5% (200/336) completed the pre-event and 44.3% (149/336) completed the post-event surveys, Respondents reported decreased anxiety and fear regarding breast cancer screening following educational sessions, with 36.1% (64/178) reporting anxiety pre-event compared to 23.3% (31/133) post-event, although the difference was not statistically significant (p = 0.96). Additionally, 64.7% (55/85) of participants stated they were more likely to schedule breast cancer screening based on individual risk factors and 98.0% (145/148) of participants reported increased knowledge on post-event surveys.

Conclusion: This study demonstrates the importance and effectiveness of community-based educational programs in increasing knowledge of risk-based screening and potentially reducing anxiety related to screening.

Keywords: Screening; community; education

Category I

Enabling Patient-Driven Oncoplastic Procedures Through Streamlined Oncology Centre Policy Structuring

Dom Loggerenberg

Breast Care Center of Excellence Johannesburg South Africa, Parktown West, Johannesburg

Objective: The concept of a multidisciplinary "team in oncoplastic and reconstructive surgery has resulted in the polarity of either an oncology surgeon doing both the oncological surgery and oncoplastic surgery or time time-delayed referral to a plastic and reconstructive surgeon. This long-standing quandary in breast oncology centres has provided many opportunities to study how to ensure quality oncoplastic procedures are offered to patients. Studies of the last two decades have listed a number of socio-economical factors ranging from age, resources and stage of cancer, access to affordable oncoplastic specialists which are considered as being difficult to impossible to quantify. However, other factors which are modifiable, such as referral logistics and patient education, can be addressed through the use of formal oncology centre policies, and this information provided to patients and medical practitioners outside of structured oncology centres could increase the option for patients to receive the best standard of oncoplastic support.

Materials and Methods: At the Breast Care Center of Excellence all patients have diagnosis and treatment plans discussed in a multi-disciplinary meeting (MDM) including prior and post-treatment discussions with their primary care physician. This treatment plan of action is provided by the MDM. for the clinician's follow-up consultation and includes all treatment options from surgery to systemic treatment, including oncoplastic and reconstruction discussions. The follow-up consultation is booked in conjunction with a same-day referral to an oncoplastic specialist, normally at the same centre as the primary physician.

Results: The policy implementation that 100% of patients receiving a cancer diagnosis have an oncoplastic consultation on the same day as they receive their diagnosis and potential treatment plans resulted in a 3-year average of 99.8% reconstructive procedures with >95% choosing immediate reconstruction to accompany their surgical procedure. This has resulted in over 3600 oncoplastic procedures in over 2000 patients between 2000 and 2023.

Conclusion: Studies have shown that as few as 1 in 3 patients recall discussing reconstructive options with their primary surgeons. This is further exacerbated by up to 45% of physicians saying their own inadequate experience with oncoplastic procedures negatively influenced their decisions to refer patients to plastic surgeons. The implementation of same-day referral for oncoplastic options and the offering of reconstructive procedures under a breast specialist to all patients is a viable treatment path for all patients.

Keywords: Patient-driven; oncoplastic; sameday referral

Category II

Cancer Risk Assessment and Hereditary Cancer Genetic Testing in a Community OBGYN Setting

Edith Smith, Logan Schneider, Lauren Lenz, Amanda Steele, Katherine Johansen Taber, Royce T. Adkins

Myriad Genetics, Utah, USA

Objective: American Cancer Society, American College of Obstetricians and Gynecologists, American College of Radiology, The American Society of Breast Surgeons, National Comprehensive Cancer Network, and United States Preventive Services Task Force recommend cancer risk assessment and hereditary cancer genetic testing for appropriate individuals. It has been previously estimated that approximately 24% of unaffected patients meet national guidelines for hereditary cancer testing. Given the recent expansion of hereditary cancer genetic testing guidelines and improved understanding of the impact of genetic test results on patient management, we set out to determine the percent of unaffected patients meeting updated genetic testing criteria, outline breast cancer risk assessment and genetic test results, and delineate the percent of patients in whom management change would be recommended.

Materials and Methods: This process-intervention study included the implementation of a hereditary cancer risk assessment process for patient identification at 5 unique community obstetrics and gynaecology (OBGYN) practice sites from September 2021 to November 2022. Myriad Genetics' team of certified genetic counselors provided pre-test patient education. Germline genetic testing used the MyRisk multigene panel and additional breast cancer risk stratification was based on the Tyrer-Cuzick breast cancer risk model and RiskScore. Results disclosure and care management was performed and/or coordinated though the community OBGYN provider. Descriptive statistics were used for the analysis, including genetic screening and testing completion rates.

Results: Sample Size: 5135 (4553/5135 [88.7%] provided a family history)

Met NCCN Testing Criteria: 1285/4553 (28.2%)

Patients offered Genetic Testing of those who met guidelines: 1145/1285 (89.1%)

Submitted a Sample for Genetic Testing: 515/1145 (44.97%)

Completed Testing: 439/515 (85.2%)

Number of Patients with Pathogenic Variants* – 14/439 (3.2%): 1 *BRCA2*, 2 *PALB2*, 4 *CHEK2*, 1 *MSH6*, 1 *PMS2*, 1 *BRIP1*, 1 *RAD51C*, 2 *HOXB13*, 2 *MITF*

*1 patient was a carrier of >1 pathogenic variants.

Tyrer-Cuzick and RiskScore Risk Assessment:

Overall % of women with a lifetime risk of breast cancer ≥20%: 36.5%

Conclusion: More than 28% of individuals meet national guideline criteria for genetic testing, a clinically meaningful increase from previous findings of 24%. In addition, 36.5% of patients in which pathogenic variants were not identified are also deemed to be at elevated risk for breast cancer and warrant considerations for medical management change. Comprehensive cancer risk assessment identifies patients at elevated risk and helps to ensure that medical management is tailored to the appropriate risk level for each patient.

Keywords: Risk assessment; hereditary cancer; community setting

Subcategory 2A: Nursing and Innovative Nursing Roles

Breast Implant Surgery; Risks and Health Complications

Jessica Prothe, Brenda Kozak, Paul Rozovics, Robyn Sykes, Michael Taccona

Northern Illinois University, USA

Objective: Understand the complications of breast implant surgery, breast implant illness (BII), breast implant-associated anaplastic large cell lymphoma (BIA-ALCL), and breast implant-associated squamous cell carcinoma (BIA-SCC). Understand why universal screening for implantable devices is a proposed addition to the nursing process and how utilizing this screening tool can enable illness-specific care in a timely manner. Current research on breast implant surgery demonstrates a risk of health complications: BII, BIA-ALCL, and BIA-SCC. Impacting patient safety worldwide, it is not standard healthcare practice to screen every patient at every healthcare encounter for the presence or absence of an implantable device or a history of an implantable device, specifically breast implant devices. However, by not doing so, nurses and other healthcare providers may miss the opportunity to identify systemic illness or cancer that could be related to breast implant devices. A lack of provider and nursing awareness and a knowledge gap regarding BII, BIA-ALCL, and BIA-SCC were also identified, and this is a significant patient safety concern.

Materials and Methods: To facilitate the research process, dependent and independent variables were identified. Internet database searches and widespread exploration of Medline, CINAHL, Google Scholar, and PubMed were conducted for peer-reviewed studies on this research topic.

Results: Estimates show over 300,000 women had breast implant surgery in 2019 (McKernan et al., 2021). Studies from the United States, Canada, Europe, and South America demonstrate that BII is a global phenomenon. Watad et al. (2018), in their cross-sectional study, analyzed population data from a 20-year time period that identified 24,651 women with breast implants with symptoms of BII. Lack of provider follow-up has led to underreporting of BII, BIA-ALCL, and BIA-SCC cases. The U.S. Food and Drug Administration recognizes BII, BIA-ALCL, and BIA-SCC as potential risks and health complications of breast implantation. Black box warnings have now been added to breast implants.

Framework: The IOWA model was chosen to guide the proposed evidence-based practice update and change.

Conclusion: To identify those at risk for implant-related illnesses, universal screening for implantable devices is proposed. Universal screening for implantable devices is defined as assessing all patients at every healthcare encounter for the presence of an implantable device or a history of an implantable device. Universal screening for implantable devices determines which patients are at risk for implantable device-related systemic illness or cancer and enables illness-specific care promptly. Successful evaluation occurs when healthcare providers' standard practice screens all patients for implantable devices.

Keywords: Breast implant surgery; risks and health complications of breast implant surgery; screening for implantable devices

Category II

Analysis of a South African Accredited Cancer Centre - Suspected Correlation between BMI and Biology

Dom Loggerenberg

Breast Care Center of Excellence Johannesburg South Africa, Parktown West, Johannesburg

Objective: The Breast Care Center of Excellence Johannesburg, South Africa, has been undergoing database reviews as part of its accreditation procedure since 2016; this has driven a number of research questions to arise due to database correlations. Since the drive to personalised oncology and the treatment of cancer subtypes based on the biology of cancer, the ability to search for correlations between presented biology and the associated causes of such biology rising has become possible.

Materials and Methods: Analysis of the complete database from 2016 to 2022 showed a number of patients having complete medical workups included in the initial clerk, including their body mass index (BMI); this data was correlated to their pathologically determined biology (LumA, LumB, Her2, TripNeg) and each subgroup was compared to the normal distribution of BMI based on the local population range. Further, Pearson's R assessment was performed to determine the degree of variation between these groups and the control, and the statistical significance of this variation was done via chi-squared analysis.

Results: For the Groups of Luminal A and human epidermal growth factor receptor 2 (HER2), the deviation from the statistical norm came in at less than 5%, showing that there is no significant relationship between these cancer biological subtypes and the expected normal distribution. However, for both the HER2 groups and the Luminal B group, there was a significant deviation from normative distribution, around 30% deviation towards larger BMI in the HER2 group and a 45% deviation in the Luminal B group; this incredibly large deviation shows that with a p-value of 0.05, the majority of a greater majority of Luminal B patients would fall into the category of overweight to Obese that not, and this deviates from the population norm at a rate exceeding 1 in three patients.

Conclusion: Large studies to assess the correlation between BMI and biology are required to confirm this observation; internally, this assessment with be included as a prospective review with all patients' BMI and Biology being recorded. This will prompt the assessment of the impact of hormone-sensitive cancers and their prevalence, including additional risk factors such as BMI.

Keywords: Body mass index; biology; correlation

Category II: Patient Care and Support; D Breast Cancer Genetics/Screening

Understanding the Psychological Effects of Attending Regular Cancer Screenings

Marianne Kiernan, Greg Haggerty

Mather Hospital, Northwell Health, USA

Objective: To better understand the psychological effect of breast cancer screening on patients in an outpatient Breast Center. Correlations between psychological symptomatology, worry about cancer and being screened for breast cancer were studied.

Materials and Methods: The Breast Center was established in 1995. In 2023, a total of 8,462 patients were screened for breast cancer. Upon referral to the Breast Center, patients were approached to determine their interest in participating in the research study. Participants were required to complete three instruments, as follows: 1) Demographic Form (13 items); 2) Cancer Worry scale (6 items, higher scores are indicative of more worry about cancer); and the Indices of Pathophysiology-SPECTRA Assessment Scale (96 items). The Cancer Worry scale assesses concerns about cancer recurrence and the impact of these concerns on daily functioning. SPECTRA includes 12 clinical scales providing direct measurement of conditions, such as: depression, anxiety, social anxiety, and post-traumatic stress, The Cancer Worry Scale and SPECTRA are valid measures that have been used in past research in a manner currently being used in this research study.

Results: The sample included 154 participants with an average age of 59.3 years (range 30–85; standard deviation = 12.8). The ethnicity majority was 93.4% Caucasian American. 55.2% of participants reported being currently employed. 21.4% reported a history of cancer, 9.1% reported a history of breast cancer and 33.8% reported a family history of cancer. Higher scores on the Cancer Worry Scale were related to SPECTRA scores on depression (r = 0.35, p<0.001), anxiety (r = 0.39, p<0.001), post-traumatic stress (r = 0.21, p = 0.03), and the general psychopathology index (r = 0.28, p = -0.27). There was a relationship between how treatable the participant thought cancer was and depression scores (r= 0.27, p= 0.01). Those who reported poorer overall physical health also reported more anxiety about cancer (r= 0.24, p<0.01).

Conclusion: Being screened for cancer is thought to be an anxiety provoking experience for patients and possibly more so for those with a personal or family history of cancer. Nevertheless, many patients currently do not receive regular screening tests. As a result, psychological anxiety and other forms of emotional distress go undetected; and, as a result, not treated in a timely manner. Including psychological assessment of patients being screened for cancer facilitates early intervention to help ameliorate possible psychopathology associated with cancer screening.

Keywords: Psychological assessment; breast cancer; cancer fear; anxiety; breast cancer screening

Category II

Patterns of Practice for Prophylactic Lymphedema Sleeve Prescription at a Tertiary Care Center

Anhmai Vu[1], Jessica F. Burlile[2], Yasamin Sharifzadeh[2], Meghan K. Huber[2], Daniel K. Ebner[2], Alexandra K. Bennett[2], Jennifer L. Bradt[2], Kimberly S. Corbin[2]

[1]*Mayo Clinic, Alix School of Medicine, Minnesota*
[2]*Mayo Clinic, Department of Radiation Oncology, Minnesota*

Objective: Studies have shown that prophylactic compression sleeve use following axillary lymph node dissection (ALND) for breast cancer (BC) can reduce the incidence of arm swelling in women at high risk for lymphedema. As of January 2024, Medicare Part B has expanded to include coverage of lymphedema compression treatment items, but currently, only 38% of US private insurance policies include a statement of coverage for lymphedema treatment. To understand prophylactic sleeve prescription patterns prior to this expansion, we identified potential factors associated with prophylactic sleeve prescription at our institution.

Materials and Methods: Using the electronic medical record, we identified patients who had a lymphedema therapy (LT) referral within three months of ALND between May 2022-August 2023. Patients were excluded if they did not undergo a true ALND (<10 nodes removed), if they did not have a BC diagnosis, or if they did not have complete LT records. Chi-squared and ANOVA tests were executed to assess relationships between sleeve prescription and patient factors.

Results: Eighty-two patients had a diagnosis of BC and an LT referral following ALND. Median age at diagnosis was 53 years and a median of 20 nodes were removed at ALND. Most (70%) patients underwent mastectomy, while the remainder underwent lumpectomy, chest wall wide local excision, or ALND alone. Nearly all (88%) patients had private insurance, while 8.5% had exclusively Medicaid and 3.7% had only Medicare or Tricare. About a quarter (21/80; 26.3%) of patients received prophylactic lymphedema sleeve prescriptions, and prescriptions were not associated with type of breast surgery, dominant side ALND, number of nodes removed, percentage of positive nodes, body mass index, smoking status, prior cellulitis, cup size, or type of insurance. Younger patients were more likely to receive a prophylactic compression sleeve prescription, but this did not meet statistical significance (p = 0.055).

Conclusion: None of the factors that we examined were associated with receiving prophylactic compression sleeve prescription. However, specific private insurance policies may be a barrier for patients wishing to receive prophylactic compression treatment. Given the recent lymphedema sleeve coverage expansion by Medicare, we hope to use our data to compare sleeve use before and after this change in the future. Additionally, education about the benefit of prophylactic compression sleeve use may also increase the rate of prescriptions.

Keywords: Lymphedema; prophylactic; compression sleeve

Category III: Breast Disease Diagnosis and Management/A. Diagnostic Imaging/1. Screening and Diagnostic Mammography

Performance Metrics of Screening Digital Breast Tomosynthesis Based on Age

Manisha Bahl

Massachusetts General Hospital, Harvard Medical School, Boston

Objective: The United States Preventive Services Task Force (USPSTF) recently updated its guidelines for screening mammography, recommending that all women begin screening at age 40 (rather than 50). The USPSTF also identified research gaps, one of which is that "research is needed to determine the benefits and harms of screening for breast cancer in women aged 75 years or older." The purpose of this study is to determine the performance metrics of screening digital breast tomosynthesis (DBT) based on age.

Materials and Methods: In this institutional review board-approved and HIPAA-compliant study, screening mammograms obtained from 2013 to 2019 at an academic medical center were retrospectively reviewed. All screening mammograms consisted of combined digital 2D mammography and DBT. Each mammogram was classified as a true-positive, true-negative, false-positive, or false-negative examination based on one-year follow-up data. Performance metrics were calculated according to standard definitions in the BI-RADS Atlas and compared among age groups using logistic regression models.

Results: Over the study period, 93,619 women (mean age 57 years, standard deviation 12 years) underwent 302,703 screening DBT examinations. The cancer detection rate (CDR) ranged from 2.9 per 1,000 examinations (208/70,549) in women aged 40–49, to 8.3 per 1,000 examinations (399/48,205) in women aged 70–79, to 10.6 per 1,000 examinations (127/12,021) in women aged 80+ ($p<0.001$). Positive predictive value (PPV) 1 and sensitivity were lowest in women aged 40-49 (3.2% and 72.5%, respectively). PPV1 was highest in women aged 80+ (19.2%), and sensitivity was highest in women aged 70–79 (91.7%). Abnormal interpretation rate (AIR) was highest and specificity was lowest in women aged 40–49 (9.3% and 91.0%, respectively). AIR was lowest and specificity was highest in women aged 80+ (5.5% and 95.5%, respectively).

Conclusion: CDRs increase with advancing age, while false-positive rates decrease with advancing age. The CDR is 2.9 per 1,000 examinations in women aged 40–49, which is within the American College of Radiology acceptable range of 2.5 or more cancers per 1,000 examinations. CDRs are higher than 8 per 1,000 examinations in women aged 70 and above, while AIRs are below 6%. Given that the CDRs are high in older women and the potential risks of false-positive examinations are low, our study supports guidelines recommending that screening decisions be based on individual preferences and health status rather than age alone.

Keywords: Screening; tomosynthesis; age

Category III

De-Escalation of Endocrine Therapy After Mastectomy for Ductal Carcinoma *In Situ*

K. Lupinacci, A. Hamed, E. Truong, A. Soran

Breast Surgical Oncology, Magee-Womens Hospital, UPMC

Objective: Ductal carcinoma *in situ* (DCIS) of the breast may be treated locally with either breast conservation therapy, involving segmental mastectomy followed by radiation, or total mastectomy. Per current guidelines, hormone-positive DCIS is then treated systemically with adjuvant endocrine therapy (aET). We sought to determine whether a significant benefit exists for aET following both bilateral and unilateral total mastectomy for DCIS.

Materials and Methods: We conducted a retrospective cohort study of DCIS cases treated surgically with mastectomy between 2010 and 2022 at a high-volume academic cancer center. Only patients diagnosed with pure DCIS on final surgical pathology were included in this analysis. We evaluated recurrence rates and survival outcomes after unilateral total mastectomy (UTM) or bilateral total mastectomy (BTM) performed for DCIS, with or without aET.

Results: A cohort of 290 patients were included and evaluated, 46 underwent BTM and 244 underwent UTM. In BTM group, mean age (± standard deviation) was 55.8±12 years and median follow-up duration & interquartile range (IQR) was 58 (27–96) months. For BTM, 40 patients (87%) did not receive aET, with no reported recurrences and one mortality unrelated to breast cancer. In UTM group, mean age was 61.1+12 years, and median follow-up time was 61 (IQR 37.5–94) months. For UTM, 158 patients (65%) did not receive aET, with two recurrences (1%), one mortality due to metastatic breast cancer and 13 mortalities unrelated to breast cancer. For UTM, 82 patients (35%) received aET, with one recurrence (1%), with subsequent breast cancer related mortality and five mortalities unrelated to breast cancer.

Conclusion: Our data demonstrated no significant oncologic benefit related to aET for patients with pure DCIS on final surgical pathology, following either BTM or UTM, at 5-year follow-up. This conclusion supports the current recommendation for individualized discussion of the risks related to side effects versus benefits including risk reduction in the contralateral breast in the setting of UTM and reduction of invasive systemic recurrence in this specific cohort. Further investigation examining the balance of potential side effects and impact on compliance of aET is needed to understand this risk benefit ratio and to improve treatment guidelines.

Keywords: De-escalation; mastectomy; endocrine therapy

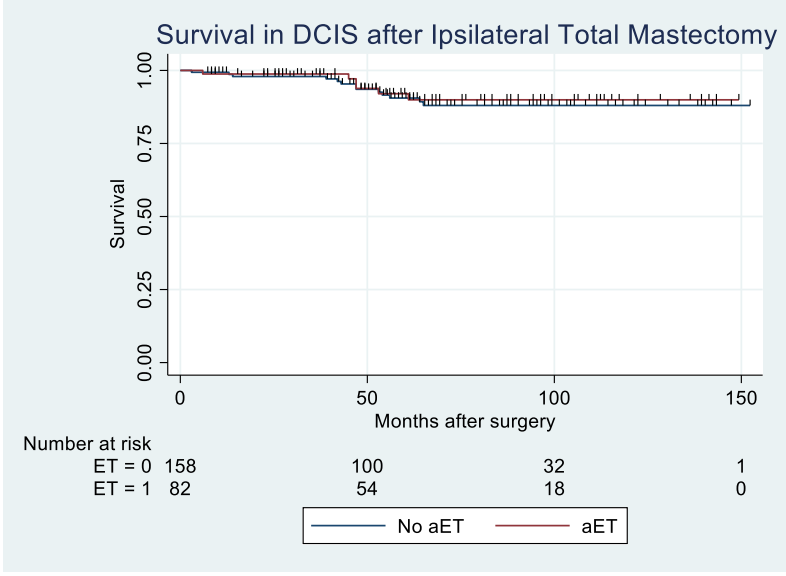


Figure 1.

Category III-A: Diagnostic Imaging 2. Ultrasound

Axillary Ultrasound of Patients With and Without Breast Cancer: Sonographic Predictors of Malignancy

J. Gu[1], E. H. Dibble[2], G. L. Baird[2], S. Ram[3]

[1]*Roger Williams Medical Center, Providence, RI*
[2]*Rhode Island Hospital/Lifespan Health System, Providence, RI*
[3]*Rochester General Hospital, Rochester, NY*

Objective: Considerable overlap exists between benign and malignant sonographic axillary nodal features, especially in patients without a known breast cancer (BC) diagnosis. Biopsy criteria, including cortical thickness cut-offs for patients without BC (woBC), are not defined. In this study, we aim to compare the malignancy risk of sonographic features of abnormal axillary lymph nodes in patients with and without a concurrent BC diagnosis.

Materials and Methods: Upon institutional review board approval, women who underwent ultrasound (US) guided axillary lymph node fine needle aspiration or core needle biopsy in community imaging sites from January 1, 2015 to October 26, 2021 were identified. Patients with a history of recent COVID-19 vaccination, non-nodal findings, nodal calcifications as lone finding, or no available reports of associated diagnostic study or subsequent pathology were excluded. Patients were categorized based on presence of concurrent BC diagnosis; sonographic and pathologic data were then collected. All statistical modeling was conducted using generalized linear modeling using a binary distribution.

Results: Final analysis included 86 women woBC and 106 women with breast cancer (wBC). For woBC patients, the odds of malignancy increased 3-fold [odds ratio (OR): 4.1, 95% confidence interval (CI) (1.501, 11.055)] when an absent hilum was noted on US relative to preserved hilum; for wBC patients, the odds increased almost 27-fold [OR: 27.9 (3.573, 218.201)], $p<0.001$. For woBC patients, the odds of malignancy increased 19% [OR: 1.19 (0.94, 1.52)] for every unit increase in cortical thickness (mm); for wBC patients, the odds increased 73% [OR: 1.7 (1.23, 2.43)] for every unit increase, $p<0.001$. For woBC patients, the odds of malignancy increased 150% [OR: 2.5 (1.3, 5.1)] for every cm increase in the short axis dimension; for wBC patients, the odds increased 300% [OR: 20.95 (4.1, 105.99)] for every unit increase, $p<0.001$. A 3 mm cortical thickness in wBC patients had a 30% likelihood of malignancy; an 11 mm cortical thickness in woBC patients had a 30% likelihood of malignancy.

Conclusion: In all patients, sonographic axillary nodal features of absent hilum, increased nodal size, and cortical thickness were found to predict malignancy; however, malignancy rates were much higher in the wBC group than in the woBC group for the same imaging findings. These results suggest it may be reasonable to use higher cut-offs for criteria such as cortical thickness when determining need for biopsy in wBC vs woBC patients.

Keywords: Axillary ultrasound; prediction; malignancy

Category III

The Effect of Sentinel Lymph Node Biopsy on Immediate Implant-Based Breast Reconstruction: A NSQIP Study

Amanda N. Awad, Samantha Scott, Hanaa Shihadeh, Ashar Ata, Christa Abraham

Albany Medical Center, New York, United States

Objective: The surgical management of breast cancer continues to evolve, and plastic surgeons continue to play an important role. The status of axillary lymph nodes is one of the most important factors impacting treatment and decision making. Sentinel lymph node biopsy (SLNB) is considered standard of care for evaluation of the axilla in patients with clinically node-negative breast cancer, but this does not come without its own morbidity. As the majority of patients who undergo immediate reconstruction choose implant-based reconstruction, it is imperative we explore how this is impacted by concurrent SLNB. The aim of this study is to use the National Surgical Quality Improvement Program (NSQIP) database to determine if SLNB at the time of mastectomy with immediate implant-based breast reconstruction affects patient outcomes.

Materials and Methods: The American College of Surgeons-NSQIP (ACS-NSQIP) data between 2016–2021 was accessed to identify patients who underwent mastectomy and immediate breast reconstruction with implant or tissue expander (CPT codes 19303 and 19340). From this cohort, patients who underwent SLNB (CPT 38525 or 38500) were divided into two groups, those who underwent SLNB at the time of mastectomy and those who did not. Rates of complications including overall morbidity, surgical site infections, wound complications, development of DVT or PE, unplanned reoperation, readmission, length of stay, and operative time were compared using the Pearson chi-squared test.

Results: 32,171 patients underwent mastectomy with immediate implant-based breast reconstruction and were included in the analysis. 22, 807 patients underwent SLNB while 12,419 patients did not. There were no significant differences in overall morbidity, surgical site infections, need for additional unplanned procedures, length of stay, or operative time between the two groups. There was a significantly higher rate of unplanned readmission in the biopsy group ($p = 0.002$).

Conclusion: SLNB performed at the time of mastectomy and immediate implant-based breast reconstruction does not increase overall morbidity, length of stay, or operative time compared to immediate breast reconstruction performed without SLNB. Unplanned readmission rates were significantly higher in the SLNB group. This NSQIP analysis of 32,171 patients suggests that performing SLNB at the time of implant-based breast reconstruction does not compromise outcomes of the operation.

Keywords: Immediate; implant-based; reconstruction; sentinel lymph node

Category III: Breast Disease Diagnosis and Management

Predictors of Axillary Complete Pathologic response in Hormone Receptor Positive, HER2 Negative Clinically Node-Positive Breast Cancer

Christina Layton, Anshumi Desai, Youley Tjendra, Eli Avisar

University of Miami, USA

Objective: Neoadjuvant therapy can downstage the axilla, reducing the need for an axillary dissection. In addition, obtaining a complete pathologic response in the axilla is associated with a better 10 year overall survival. Our institution previously reported axillary response rates in hormone positive, human epidermal growth factor receptor 2 (HER2) negative, clinically node-positive breast cancer patients undergoing neoadjuvant therapy in a small cohort of patients. The cohort however was too small for a deeper dive into potential predictors of response. In this current study, we sought to expand this cohort of patients until 2022 almost doubling the number of patients to better evaluate for potential predictors of response.

Materials and Methods: A single-institution retrospective cohort study included hormone receptor-positive, HER2 negative, clinically node-positive breast cancer patients treated with neoadjuvant therapy, either endocrine or chemotherapy, between January 2011 to December 2022. The data was divided in patients with cPR and no cPR in the axilla. The primary outcome was to identify demographic and clinicopathologic parameters that co-related to cPR in the axilla. Chi-square test or Fisher's exact test for categorical variables and t-test for continuous variables were performed. Logistic regression analysis was performed to assess clinical factors associated with the number of complications.

Results: Two hundred breast cancer patients met the inclusion criteria. They were divided into two cohorts: patients with axillary cPR rate 12% (n = 24) and no axillary cPR 88% (n = 176). The mean age was 52.17 [standard deviation 11.5]. The demographic profile of patients, tumor characteristics, and treatment is described in Table 1. Amongst the patients who underwent genomic profile (n = 25), 76% (n = 19) had Mammprint done, while 24% (n = 6) had Oncotype. There were 2 patients with cPR in the axilla who had mammaprint performed, both of which were found to be high risk. For patients who underwent chemotherapy, approximately 13.6% achieved axillary cPR. For patients who underwent hormone therapy, approximately 6.5% achieved axillary cPR (p = 0.29). A significantly higher axillary cPR rate was identified in patients with clinical stage II at diagnosis (14/70, 20%) compared with stage III (10/128, 7.8%; p = 0.013). Patients with axillary cPR had on fewer lymph nodes removed at the time of surgery, 8 *vs.* 16 (p = 0.001).

Conclusion: An axillary cPR in hormone receptor-positive, HER2 negative, clinically node-positive breast cancer patients was higher in those patients with a lower clinical stage (stage II). Patients with a cPR were able to avoid an axillary dissection. A larger cohort of patients is necessary to define more possible predictors of axillary response rate to neoadjuvant therapy.

Keywords: Predictors; complete pathologic response; luminal node positive

Table 1.

	No complete pathologic response (cPR) in axilla (n = 176)	**Complete pathologic response (cPR) in axilla (n = 24)**	***p*-value**
Age	52.5852273 [SD 11.722682]	49.125 [SD 9.71020306]	0.06019888
Menopausal status	Pre-menopausal n = 80 (45.4%) Post-menopausal n = 95 (54 %) Unknown = 1 (0.56%)	Pre-menopausal n = 15 Post-menopausal n = 9	
Race	White n = 43 (24.4%) African American n = 39 (22.15%) Hispanic n = 93 (52.8%) Asian n =1 (0.56%)	White n = 8 African American n = 5 Hispanic n = 11 Asian n = 0	
IDC	158 (89.77%)	23	
ILC	15 (8.5%)		
Others/unknown	3 (1.7%)	1	
Neo-adjuvant treatment			p = 0.29
Chemotherapy	133	21	
Hormone therapy	43	3	
Grade 1	23	4	
Grade 2	98	8	
Grade 3	49	10	p = 0.161
Unknown	6	2	
Stage 0	0	0	
Stage I	0	0	
Stage II	56	14	
Stage III	118	10	p = 0.013
Stage IV	2	0	
Mean number of nodes resected	16.14367816	8.625	p<0.001