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: 21 • ISSUE: 4 • OCTOBER 2025

eurjbreasthealth.com

# European Journal of Breast Health



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, Türkiye
**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:** September 2025
**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 (100$) 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, Türkiye

Phone : +90 (530) 177 30 97

E-mail : info@galenos.com.tr

Web : www.galenos.com.tr

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, tables, or any other material in both print and electronic formats, authors must obtain permission from

# Instructions to Authors

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 (100$) 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 | $100 |
| Editorial comment | Free of charge |
| Review article (No application fee will be charged from invited authors) | $100 |
| Case report | $100 |
| Letter to the editor | Free of charge |
| Images in clinical practices | Free of charge |
| Current opinion | Free of charge |
| Systematic review | $100 |

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

European Journal of Breast Health

# Instructions to Authors

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 İlişkiler. 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/ncidodlEID/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, Türkiye

Phone : +90 (530) 177 30 97

E-mail : info@galenos.com.tr

Web : www.galenos.com.tr

European Journal of Breast Health

# Contents

## SYSTEMATIC REVIEW



## ORIGINAL ARTICLES

European Journal of Breast Health

# Contents

## CASE REPORTS



## CLINICAL IMAGE



## LETTER TO THE EDITOR



## INDEX

**Systematic Review**

Eur J Breast Health 2025; 21(4): 285-289
**DOI:** 10.4274/ejbh.galenos.2025.2025-2-10

# Role of Radiotherapy in Elderly Patients (≥65 Years) With Triple-Negative Breast Cancer: A Systematic Review and Meta-Analysis

Eran Sharon[1,4], Igor Snast[2,4], Rinat Yerushalmi[3,4], Idit Melnik[1]

[1]Division of Breast Surgery, Department of Surgery, Rabin Medical Center - Beilinson Hospital, Petach Tikva, Israel
[2]Department of Dermatology, Rabin Medical Center - Beilinson Hospital, Petach Tikva, Israel
[3]Davidoff Cancer Center, Rabin Medical Center - Beilinson Hospital, Petach Tikva, Israel
[4]Tel Aviv University Sackler Faculty of Medicine, Tel Aviv, Israel

**ABSTRACT**

This is the first meta-analysis evaluating the benefit of adjuvant radiotherapy in older patients (≥65 years) with triple-negative breast cancer (TNBC). The medical literature was searched for all randomized controlled trials, nonrandomized controlled trials, and cohort studies with more than one treatment arm that evaluated radiation therapy for TNBC in patients aged >65 years. The primary outcome was overall survival. Four cohort studies (2015–2019) were eligible for analysis, including a total of 10,710 patients with TNBC of whom 7,209 underwent radiotherapy. Two were large retrospective population-based studies that yielded major findings on adjusted multivariable analysis. Patients who underwent radiotherapy ($n$ = 6283/8526) had a significantly better 5-year overall survival than patients who did not (77% *vs.* 55%, $p$<0.001). The addition of radiotherapy ($n$ = 815/1957) was associated with better cancer-specific survival. Of the two smaller studies, one prospective study reported similar survivability for treatment with breast-conserving surgery, chemotherapy, and radiotherapy or mastectomy with radiation, or mastectomy alone, and the other retrospective study found that adding radiotherapy had no effect on 5-year overall survival. Multivariate analyses of data from the two large retrospective population-based studies suggested that adding radiotherapy to breast-conserving surgery may improve overall and disease-free survival in elderly patients with TNBC.

**Keywords:** Breast cancer; triple-negative; radiation; elderly

**Cite this article as:** Sharon E, Snast I, Yerushalmi R, Melnik I. Role of radiotherapy in elderly patients (≥65 years) with triple-negative breast cancer: a systematic review and meta-analysis. Eur J Breast Health. 2025; 21(4): 285-289

**Key Points**

- Breast cancer
- Triple-negative
- Radiation
- Elderly

## Introduction

Breast cancer is the leading cause of cancer-related death in women worldwide. The primary risk factor is advanced age. According to the Surveillance, Epidemiology and End Results (SEER) program, the median age at breast cancer diagnosis in the United States is 68 years (1). The proportion of older women with breast cancer is expected to grow as technology and medical care continue to improve and life expectancy increases accordingly (2).

Triple-negative breast cancer (TNBC) is a subtype of breast cancer in which the tumor cells lack expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. It accounts for 15% of all breast cancers diagnosed, and is less common in elderly patients than hormone-receptor-positive breast cancer (3). The treatment of breast cancer in general, and TNBC in particular, in elderly patients is controversial. According to the National Comprehensive Cancer Network guidelines of 2015 "… there are limited data to make recommendations for those >70 years of age" (4). The problem may be largely due to under-representation of elderly patients in clinical trials from which they are often excluded because of ageism and comorbidities. Furthermore, as TNBC is unresponsive to endocrine treatment, adjuvant treatment options are limited to chemotherapy and radiotherapy, and chemotherapy is best avoided in the elderly in whom the side effects have a more substantial impact

**Corresponding Author:**
Idit Melnik MD; iditsi@clalit.org.il

**Received:** 15.02.2025
**Accepted:** 26.07.2025
**Epub:** 25.08.2025
**Available Online Date:** 25.09.2025

relative to younger patients. The SEER database shows that, among patients with node-positive stage I-II TNBC, chemotherapy was administered to 80% of those aged 67-69 years and to less than 10% of those aged more than 85 years (5). Thus, the decision to initiate adjuvant radiotherapy in the older TNBC population is a challenge.

The purpose of this systematic review and meta-analysis was to evaluate the benefit of adjuvant radiotherapy to survival in elderly patients with TNBC.

## Materials and Methods

### Search Strategy

A systematic review was conducted and reported in accordance with the PRISMA statement. The search was performed without date limits during May 2021 using PubMed. Reference lists from key trials were manually scanned for additional results. The following search criteria were used: ("breast cancer"[MeSH Terms] OR "breast cancer"[All Fields] OR "breast carcinoma"[MeSH Terms] OR "breast carcinoma"[All Fields]) AND ("triple negative"[MeSH Terms] OR "triple negative"[All Fields]) and filters: 65 and over: 65+ years.

### Eligibility Criteria

Studies that met the following criteria were included: (1) relevance - randomized controlled trials, nonrandomized controlled trials, and cohort studies with more than one treatment arm that evaluated radiotherapy for the treatment of TNBC; (2) participants - patients of both sexes aged 65 years and older with a histological diagnosis of TNBC. Although there is no clear definition of the term "elderly", we defined it as 65 years and older in accordance with other researchers (6). We excluded (a) studies not reporting our primary or secondary outcomes, and (b) studies not written in English.

### Outcome

The primary outcome was overall survival (OS). Secondary outcomes were disease-free survival (DFS) and adverse effects. If the primary and secondary outcomes were not reported, we considered other endpoints with different definitions, such as cancer-specific survival.

### Study Selection and Data Extraction

Two reviewers (I.M. and I.S.) independently screened titles and abstracts, followed by the full text of potentially eligible studies. One reviewer (I.S.) extracted the data onto an electronic form, and the other (I.M.) checked the extracted data, including the first author's name, year of publication, number of participants, mean patient age, primary *vs.* recurrent malignancy, stage, chemotherapy status, type of surgery and radiotherapy, length of follow-up, overall survival, disease-free survival, and side effects. Each reviewer independently assessed risk for observational studies using the Newcastle-Ottawa scale. Any disagreement was resolved by discussion. Further discrepancies were resolved by the first author (E.S.).

### Statistical Analysis

The OS rate was pooled using the statistical software package Comprehensive Meta-Analysis, version 3.0 (Meta-Analysis@Meta-Analysis.com, Biostat Inc., Englewood, NJ, USA). Meta-analyses were performed with the random effects model of DerSimonian and Laird because we expected considerable clinical heterogeneity. Heterogeneity was assessed by visually examining the forest plots for non-overlapping confidence intervals and by chi-square test, with $p<0.05$ indicating statistical significance and $I^2>50\%$ indicating substantial heterogeneity.

## Results

### Characteristics of Studies

Our search yielded 3167 records (Figure 1). After the exclusion process, four cohort studies were found eligible for analysis (7-10). They included three retrospective studies and one prospective study with a total of 10,710 patients, of whom 7,209 received radiotherapy. All four studies were published in peer-reviewed journals between 2015 and 2019. The largest, by Haque et al. (7) ($n$ = 8526), was based on the U.S. National Cancer Database (NCDB), 2004–2014, and the second largest ($n$ = 1957), by Zhu et al. (8), was based on the SEER database, 2010–2011. The characteristics of the included studies are detailed in Table 1.

In all studies, mean patient age was 65-70 years, and all patients were female. Across all studies, the majority of patients (≥93%) underwent surgery; however, the addition of chemotherapy and radiotherapy and the type of radiotherapy delivered varied significantly between studies. Three studies provided data on our primary outcome of OS, and one study provided data only on cancer-specific survival.

### Risk of Bias

All four cohort studies were designated high quality on risk of bias analysis (for further details see Table 1).

### Overall Survival

The largest of the three studies that provided OS data was based on the NCDB and included 8526 patients with primary stage I-II TNBC who underwent breast-conserving surgery with ($n$ = 6283) or without ($n$ = 2243) radiotherapy (7). At a median follow-up of 38 months, 5-year OS was significantly higher in the patients who received radiotherapy than in the patients who did not (77% *vs.* 55%, $p<0.001$). A higher proportion of the patients who received radiotherapy also received chemotherapy (68% *vs.* 56%). Nevertheless, the results remained significant regardless of whether or not chemotherapy was

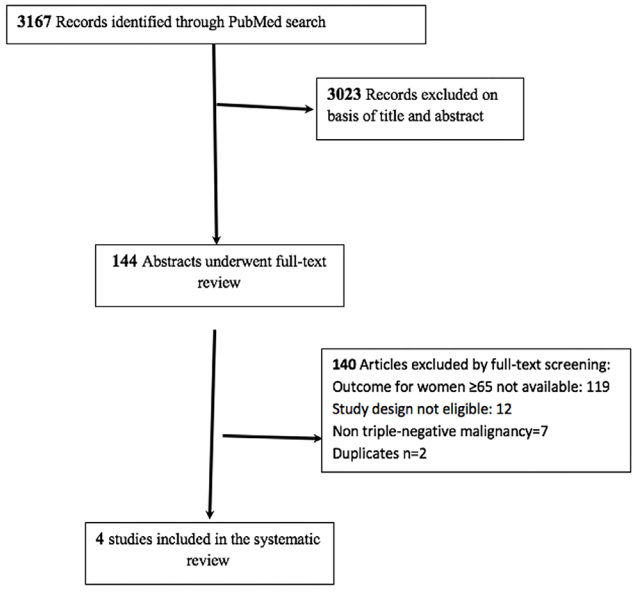



**Figure 1.** Flow chart of selection of studies for meta-analysis

**Table 1. Characteristics of radiotherapy in the four cohort studies included in the systematic review**

| Reference | Country | *n* | Mean age | Stage (%)* | Chemo-therapy (%) | Surgery (%) | Type of RT (%) | OS (%) | DFS (%) | Median FU (m) |
|---|---|---|---|---|---|---|---|---|---|---|
| Haque et al. (7) 2019 (retrospective; NCDB 2004–2014) | USA | RT: 6283<br>No RT: 2243 | ≥70 | I–II (100) | 68 *vs.* 56 | BCT (100) | Conventional fraction: 59<br>Hypofraction-ation: 22<br>Not recorded:10 | **5-year OS**<br>77 *vs.* 55[§] ($p$<0.001) | NA | 38 |
| Qiu et al. (9) 2016 (retrospective) | China | RT: 49<br>No RT: 17 | 68 | I–II (83); III (17) | Adjuvant: 38<br>Neo-adjuvant: 6[†] | Mastectomy: 86<br>BCT:14 | NA | **5-year OS**<br>92 *vs.* 94 ($p$ = 0.28) | 86 *vs.* 94 ($p$ = 0.37) | 90[a] |
| Bhoo-Pathy et al. (10) 2015 (prospective) | Malaysia, Hong Kong, Singapore | BCT+chemo+RT: 22<br>Mastectomy+RT: 40<br>Mastectomy: 99 | ≥65 | I–III (100) | 13[‡] | Mastectomy: 86<br>BCT: 14 | Variable protocols across centers | Similar survivability on crude and multiple variable adjusted analysis | NA | NA |
| Zhu et al. (8) 2015 (retrospective; SEER 2010–2011) | USA | RT: 815<br>No RT: 1142 | ≥70 | I–III (100) | NA | Mastectomy or BCT: 93 | NA | NA | RT improved cancer-specific survival (HR = 0.4, $p$<0.001, MVA) | ~ 24 |

BCT: Breast-conserving therapy; DFS: Disease-free survival; FU: Follow-up; MVA: Multivariate analysis; NCDB: National cancer database; OS: Overall survival; RT: Radiotherapy; SEER: Surveillance, Epidemiology and End Results; HR: Hazard ratio; *: All patients in studies by Haque et al. (7), Qui et al. (9), and Zhu et al. (8) had primary tumors; status not mentioned in the study of Bhoo-Pathy et al. (10)

†: Type of chemotherapy: Anthra-cycline, cyclophosphamide ± taxanes ($n$ = 30); cyclophos-phamide, methotrexate, 5-FU ($n$ = 14). Type of chemotherapy unavailable in other studies

‡: For the overall cohort which also included patients younger than 60 years

§: Results remained significant on propensity-matched analysis (5-year OS, 68% *vs.* 57%); Note: None of the studies discussed side effect

administered and on propensity-matched analysis (68% *vs.* 57%, $p$<0.001).

By contrast, a smaller retrospective study from China including 66 patients with primary stage I-III TNBC (83% I–II, 17% III) who underwent mostly (86%) breast-conserving surgery (9) found that the addition of radiotherapy had no effect on the 5-year OS or DFS. However, it was unclear if the groups treated or not treated with radiotherapy were balanced in terms of staging, chemotherapy status, and type of surgery.

Meta-analysis of these two studies revealed that the addition of radiotherapy was associated with improved 5-year survival, with borderline significance (odds ratio: 2.26, 95% confidence interval: 0.9–5.71, $I^2$=27%) (Figure 2).

The third study that evaluated OS prospectively investigated the outcome of breast-conserving surgery, chemotherapy, and radiotherapy ($n$ = 22) versus mastectomy ($n$ = 99) versus mastectomy and radiotherapy ($n$ = 40) in 161 patients with stage I-III TNBC (9). Similar survival was reported in all three groups on crude and adjusted analyses.

### Cancer-Specific Survival

The sole study that investigated cancer-specific survival was based on SEER data for 1957 patients with primary stage I–III TNBC (8). The majority (93%) underwent either mastectomy or breast-conserving surgery (Chemotherapy status is unavailable in the SEER database). On multivariate adjusted analysis, the addition of radiotherapy in 815 patients was associated with an increase in cancer-specific survival during a mean follow-up of approximately 24 months.

## Discussion and Conclusion

Many studies have recommended omitting adjuvant radiotherapy in older women with early hormone-receptor-positive breast cancer who receive endocrine therapy (11-14). However, in patients with TNBC, especially the 65+ age group, the role of adjuvant radiotherapy is still controversial (15-20). This is the first meta-analysis conducted to date to attempt to answer this question.

The two largest of the four studies evaluated, by Haque et al. (7) and Zhu et al. (8), were based on the NBSD and SEER program data, respectively. In both, patients of different

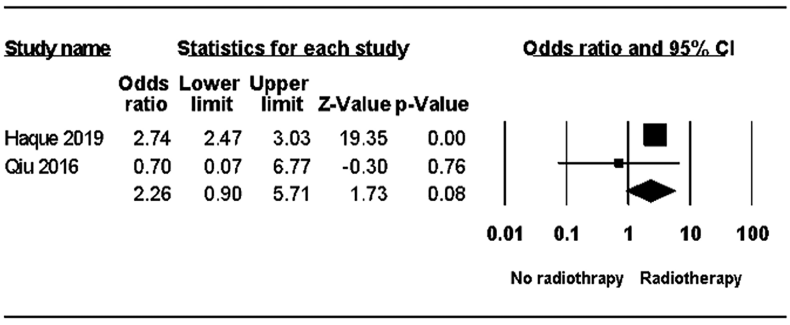



**Figure 2.** Forest plot comparing 5-year overall survival in patients with triple-negative breast cancer who received or did not receive radiotherapy

*CI: Confidence interval*

age groups who were treated or not treated with adjuvant radiation were compared for outcome using multivariate analysis. Haque et al. (7) concluded that in elderly women with T1-2N0 TNBC, omitting adjuvant radiotherapy was associated with a statistically poorer OS, regardless of age group, T-stage, or chemotherapy. However, the analysis did not correct for performance status and comorbidities. Likewise, Zhu et al. (8) suggested that the poor prognosis of elderly patients with TNBC might be associated with their lower rate of loco-regional treatment with surgery and radiation.

It is well recognized that adjuvant radiotherapy reduces the loco-regional recurrence rate and risk of breast cancer (21-25). The Early Breast Cancer Trialists' Collaborative Group study (21), including more than 10,000 women from 17 trials, concluded that adjuvant therapy is associated with a nearly 50% reduction in 10-year risk of any first recurrence compared with breast-conserving surgery alone. In addition, the patients given radiotherapy showed a reduction in 15-year risk of death from breast cancer. The improvement in prognosis might be even greater for TNBC owing to its association with the *BRCA1* mutation. Several clinical and experimental studies have suggested that tumors harboring the *BRCA1/BRCA2* mutation are more sensitive to radiotherapy (26-28). These findings prompted Trainer et al. (29) to suggest that in patients with TNBC, the presence of a *BRCA* mutation may impact the benefit of adjuvant radiotherapy.

The two other studies in our meta-analysis were conducted in Asia and included a considerably lower number of patients. Bhoo-Pathy et al. (10) found that adjuvant radiotherapy was associated with a survival gain in patients with locally advanced TNBC. Among those with early TNBC (T1-2, N0-1, and M0), the 5-year relative survival rate was highest in patients who underwent mastectomy only, followed by patients undergoing breast-conserving surgery and mastectomy with radiation. However, we believe conclusions regarding the role of adjuvant radiotherapy in early TNBC cannot be drawn on the basis of these results because patients treated with mastectomy and radiation have a worse prognosis to begin with, regardless of the addition (or not) of adjuvant radiotherapy. Therefore, they should not have been included in the early breast cancer group. Moreover, the survival gain associated with radiotherapy applied only to very young patients with TNBC.

The fourth and smallest study analyzed reached an opposite conclusion from the others. Qiu et al. (9) found that 5-year DFS and OS were significantly higher in the elderly patients even though they received significantly less radiotherapy and chemotherapy than the younger patients. The authors advised that clinicians take a more conservative and cautious approach to the decision to administer postoperative adjuvant treatment (radiotherapy, chemotherapy, or both) to elderly patients with TNBC.

Overall, the two larger studies, which were based on databases in the U.S. and evaluated the data using multivariate analysis, suggested that adjuvant radiotherapy may improve prognosis in elderly patients with TNBC. The sole study leading to a contrary conclusion used a retrospective design and a substantially smaller patient sample.

The present meta-analysis was limited by the small number of studies that met the inclusion criteria and the retrospective design of three of them. Moreover, the study population was heterogeneous in terms of age, adjuvant chemotherapy, and type of surgery.

This is the first meta-analysis to evaluate the role of adjuvant radiotherapy for TNBC in elderly patients (age ≥65 years). The weight of the evidence supports the notion that adjuvant radiotherapy has a survival advantage in this age group.

**Footnotes**

**Authorship Contributions**

Concept: R.Y., I.M.; Design: R.Y., I.M.; Data Collection or Processing: E.S., I.S.; Analysis or Interpretation: I.S.; Writing: E.S.

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

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

## References

1. National Cancer Institute Surveillance, Epidemiology, and End Results Program. Cancer stat facts: female breast cancer. Accessed 6 January 2018. [Crossref]
2 United Nations Department of Economic and Social Affairs, population division. World population ageing 2015. Accessed 6 January 2018. [Crossref]
3. Trivers KF, Lund MJ, Porter PL, Liff JM, Flagg EW, Coates RJ, et al. The epidemiology of triple-negative breast cancer, including race. Cancer Causes Control. 2009; 20: 1071-1082. (PMID: 19343511) [Crossref]
4. Gradishar WJ, Anderson BO, Balassanian R, Blair SL, Burstein HJ, Cyr A, et al. NCCN guidelines insights breast cancer, version 1.2016. J Natl Compr Canc Netw. 2015; 13: 1475-1485. (PMID: 26656517) [Crossref]

5. Schonberg MA, Marcantonio ER, Li D, Silliman RA, Ngo L, McCarthy EP. Breast cancer among the oldest old: tumor characteristics, treatment choices, and survival. J Clin Oncol. 2010; 28: 2038-2045. (PMID: 20308658) [Crossref]
6. Orimo H, Ito H, Suzuki T, Araki A, Hosoi T, Sawabe M. Reviewing the definition of "elderly". Geriatr Gerontol Int. 2006; 6: 149-158. [Crossref]
7. Haque W, Verma V, Hsiao KY, Hatch S, Arentz C, Szeja S, et al. Omission of radiation therapy following breast conservation in older (≥70 years) women with T1-2N0 triple-negative breast cancer. Breast J. 2019; 25: 1126-1133. (PMID: 31273872) [Crossref]
8. Zhu W, Perez EA, Hong R, Li Q, Xu B. Age-related disparity in immediate prognosis of patients with triple-negative breast cancer: a population-based study from SEER cancer registries. PLoS One. 2015; 10: e0128345. (PMID: 26020519) [Crossref]
9. Qiu JD, Xue XY, Li R, Wang JD. Clinicopathological features and prognosis of triple-negative breast cancer: a comparison between younger (<60) and elderly (≥60) patients. Eur J Cancer Care (Engl). 2016; 25: 1065-1075. (PMID: 26122025) [Crossref]
10. Bhoo-Pathy N, Verkooijen HM, Wong FY, Pignol JP, Kwong A, Tan EY, et al. Prognostic role of adjuvant radiotherapy in triple-negative breast cancer: a historical cohort study. Int J Cancer. 2015; 137: 2504-2512. (PMID: 26018878) [Crossref]
11. Fisher B, Bryant J, Dignam JJ, Wickerham DL, Mamounas EP, Fisher ER, et al; National Surgical Adjuvant Breast and Bowel Project. Tamoxifen, radiation therapy, or both for prevention of ipsilateral breast tumor recurrence after lumpectomy in women with invasive breast cancers of one centimeter or less. J Clin Oncol. 2002; 20: 4141-4149. (PMID: 12377957) [Crossref]
12. Pötter R, Gnant M, Kwasny W, Tausch C, Handl-Zeller L, Pakisch B, et al; Austrian Breast and Colorectal Cancer Study Group. Lumpectomy plus tamoxifen or anastrozole with or without whole breast irradiation in women with favorable early breast cancer. Int J Radiat Oncol Biol Phys. 2007; 68: 334-340. (PMID: 17363187) [Crossref]
13. Fyles AW, Manchul L, McCready D, et al. Long-term results of a randomized trial of tamoxifen with or without radiation in women over 50 years of age with T1/2 breast cancer (abstract). Int J Radiat Oncol Biol Phys. 2006; 66 (2 Suppl): S4. [Crossref]
14. Hughes KS, Schnaper LA, Bellon JR, Cirrincione CT, Berry DA, McCormick B, et al. Lumpectomy plus tamoxifen with or without irradiation in women age 70 years or older with early breast cancer: long-term follow-up of CALGB 9343. J Clin Oncol. 2013; 31: 2382-2387. (PMID: 23690420) [Crossref]
15. Abdulkarim BS, Cuartero J, Hanson J, Deschênes J, Lesniak D, Sabri S. Increased risk of locoregional recurrence for women with T1-2N0 triple-negative breast cancer treated with modified radical mastectomy without adjuvant radiation therapy compared with breast-conserving therapy. J Clin Oncol. 2011; 29: 2852-2858. (PMID: 21670451) [Crossref]
16. Wang J, Shi M, Ling R, Xia Y, Luo S, Fu X, et al. Adjuvant chemotherapy and radiotherapy in triple-negative breast carcinoma: a prospective randomized controlled multi-center trial. Radiother Oncol. 2011; 100: 200-204. (PMID: 21852010) [Crossref]
17. Parker CC, Ampil F, Burton G, Li BD, Chu QD. Is breast conservation therapy a viable option for patients with triple-receptor negative breast cancer? Surgery. 2010; 148: 386-391. (PMID: 20580045) [Crossref]
18. Adkins FC, Gonzalez-Angulo AM, Lei X, Hernandez-Aya LF, Mittendorf EA, Litton JK, et al. Triple-negative breast cancer is not a contraindication for breast conservation. Ann Surg Oncol. 2011; 18: 3164-3173. (PMID: 21947595) [Crossref]
19. Zumsteg ZS, Morrow M, Arnold B, Zheng J, Zhang Z, Robson M, et al. Breast-conserving therapy achieves locoregional outcomes comparable to mastectomy in women with T1-2N0 triple-negative breast cancer. Ann Surg Oncol. 2013; 20: 3469-3476. (PMID: 23686101) [Crossref]
20. Steward LT, Gao F, Taylor MA, Margenthaler JA. Impact of radiation therapy on survival in patients with triple-negative breast cancer. Oncol Lett. 2014; 7: 548-552. (PMID: 24396485) [Crossref]
21. 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]
22. Cuzick J, Stewart H, Peto R, Baum M, Fisher B, Host H, et al. Overview of randomized trials of postoperative adjuvant radiotherapy in breast cancer. Cancer Treat Rep. 1987; 71: 15-29. (PMID: 2856861) [Crossref]
23. Early Breast Cancer Trialists' Collaborative Group. Effects of radiotherapy and surgery in early breast cancer. An overview of the randomized trials. N Engl J Med. 1995; 333: 1444-1455. (PMID: 7477144) Erratum in: N Engl J Med. 1996; 334: 1003. [Crossref]
24. Clarke M, Collins R, Darby S, Davies C, Elphinstone P, Evans V, et al; Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005; 366: 2087-2106. (PMID: 16360786) [Crossref]
25. Ford HT, Coombes RC, Gazet JC, Gray R, McConkey CC, Sutcliffe R, et al. Long-term follow-up of a randomised trial designed to determine the need for irradiation following conservative surgery for the treatment of invasive breast cancer. Ann Oncol. 2006; 17: 401-408. (PMID: 16330517) [Crossref]
26. Rothfuss A, Schütz P, Bochum S, Volm T, Eberhardt E, Kreienberg R, et al. Induced micronucleus frequencies in peripheral lymphocytes as a screening test for carriers of a BRCA1 mutation in breast cancer families. Cancer Res. 2000; 60: 390-394. (PMID: 10667592) [Crossref]
27. Baeyens A, Thierens H, Claes K, Poppe B, Messiaen L, De Ridder L, et al. Chromosomal radiosensitivity in breast cancer patients with a known or putative genetic predisposition. Br J Cancer. 2002; 87: 1379-1385. (PMID: 12454765) [Crossref]
28. Fourquet A, Stoppa-Lyonnet D, Kirova YM, Sigal-Zafrani B, Asselain B; Institut Curie Breast Cancer Study Group; Institut Curie Breast Ovary Cancer Risk Study Group. Familial breast cancer: clinical response to induction chemotherapy or radiotherapy related to BRCA1/2 mutations status. Am J Clin Oncol. 2009; 32: 127-131. (PMID: 19307946) [Crossref]
29. Trainer AH, James PA, Mann GB, Lindeman GJ. Breast conservation versus mastectomy in triple-negative breast cancer: two steps forward, one step back? J Clin Oncol. 2011; 29: 4722-4723; author reply 4723-4724. (PMID: 22042938) [Crossref]

Original Article

Eur J Breast Health 2025; 21(4): 290-294
DOI: 10.4274/ejbh.galenos.2025.2025-4-2

# Evaluation of Tissue Expression of HMBG1 Protein in Patients With Breast Cancer

Gülden Diniz[1], İsmail Güzeliş[1], Dudu Solakoğlu Kahraman[2], Duygu Ayaz[2], Umut Varol[3], Mustafa Değirmenci[4]

[1]Department of Pathology Laboratory, İzmir Democracy University, Buca Seyfi Demirsoy Training and Research Hospital, İzmir, Türkiye
[2]Department of Pathology Laboratory, İzmir Tepecik Education and Research Hospital, University of Health Sciences Türkiye, İzmir, Türkiye
[3]Department of Medical Oncology, İzmir Democracy University Faculty of Medicine, İzmir, Türkiye
[4]Department of Medical Oncology, İzmir Tepecik Education and Research Hospital, University of Health Sciences Türkiye, İzmir, Türkiye

## ABSTRACT

**Objective:** High mobility group box 1 (HMGB1) is a nonhistone chromatin-associated protein involved in chromatin remodeling, transcription, DNA replication, and repair. The purpose of this study was to assess the relationship between tissue expression of HMGB1, clinical outcomes, and histopathological characteristics in patients with breast cancer.

**Materials and Methods:** The study included 282 patients with breast cancer. An *in vitro* diagnostic HMGB1 antibody was applied to the slides of tumor specimens.

**Results:** Overexpression of HMGB1 was found in tumor cells of 123 (43.6%) patients. HMGB1 was only expressed in the nucleus in most tumors (88.7%), while in 32 (11.3%) tumors HMBG1 expression was cytoplasmic and/or extracellular. Severe inflammatory infiltration of the peritumoral stroma was observed in 76 (27%) patients. There was a correlation between remarkable inflammatory cell infiltration in the tumor microenvironment and HMGB1 overexpression, regardless of the molecular subtype, as well as the extranuclear location of HMGB1 expression ($p$ = 0.023). HMGB1 expression was not found to be associated with overall or disease-free survival. However, axillary lymph node metastasis was significantly more common in tumors with intense inflammation ($p$ = 0.024).

**Conclusion:** The proportion of breast cancer patients with HMGB1 expression was lower in the present study than that reported previously. Furthermore, we did not detect a relationship between HMGB1 expression and prognosis. However, the relationship between HMGB1 expression and prognosis had been previously reported only in aggressive breast cancers. It is suggested that understanding the significance of HMGB1 expression in breast cancer may open new treatment opportunities, especially in aggressive and/or triple negative tumors.

**Keywords:** Breast cancer; HMGB1 protein; high mobility group box-1; tissue expression

**Cite this article as:** Diniz G, Güzeliş İ, Solakoğlu Kahraman D, Ayaz D, Varol U, Değirmenci M. Evaluation of tissue expression of HMBG1 protein in patients with breast cancer. Eur J Breast Health. 2025; 21(4): 290-294

### Key Points

- High mobility group box 1 (HMGB1) expression was mostly associated with prognosis in the most aggressive triple-negative breast cancer group.
- Since the number of triple-negative cases in our series was quite low, we may not have detected a close relationship between HMGB1 expression and prognosis.
- Understanding HMGB1 expression in breast cancers may lead to new treatment opportunities, particularly in aggressive carcinomas.

## Introduction

Non-histone nuclear proteins, known as high mobility group box 1 (HMGB) proteins, perform several important biological tasks in cells (1). Members of the HMGB protein family are HMGB1, HMGB2, and HMGB3. Only HMGB1 is widely expressed in the nuclei of almost all eukaryotic cells, but HMGB2 is mainly expressed in the thymus, and testes, and HMGB3 in hematopoietic stem cells. In contrast to its limited expression in the testes and lymphoid organs of adults, HMGB2 is also highly expressed during embryogenesis (1, 2). HMGB1 is predominantly expressed in nuclei as it is involved in chromatin remodeling, DNA replication, repair, and transcription. In immunohistochemical (IHC) studies, the nuclear expression rate of HMGB1 was generally reported to be very high. Investigations also revealed that following posttranslational changes, such as acetylation,

Corresponding Author:
Gülden Diniz MD, PhD; gulden.diniz@idu.edu.tr

Received: 10.04.2025
Accepted: 24.04.2025
Epub: 29.05.2025
Available Online Date: 25.09.2025

phosphorylation, and methylation, HMGB1 may migrate from the nucleus to the cytoplasm. HMGB1 can also be released to the extracellular environment in response to hypoxia or chemoradiotherapy, primarily through active secretion from immunocompetent cells or passive release from necrotic or apoptotic cells. When HMGB1 is released into the extracellular space, it acts as a cytokine-like molecule, signaling tissue damage and contributing to inflammation (1-3).

Overexpression of HMGB1 has been reported in various types of cancers, including breast carcinoma (4). HMGB1 may promote tumor progression through various mechanisms. By facilitating DNA repair and replication, HMGB1 may facilitate tumor cell survival and proliferation. HMGB1 may promote neoangiogenesis, which supply the tumor with nutrients and oxygen. In addition, HMBG1 may modulate the immune response, helping cancer cells evade detection and destruction by the immune system. HMGB1 may even be involved in tumor cell migration and, therefore, metastasis (1-5). High levels of HMGB1 expression in tumors have been associated with poor prognosis, higher tumor grade, and increased metastatic potential. Given these roles in cancer progression, HMGB1 is being explored as a potential therapeutic target. Strategies include blocking its extracellular signaling pathways or reducing its expression to inhibit tumor growth and spread (6-9).

Breast cancer continues to be a major global health concern for women with its high incidence rate. Even while improvements in early detection and treatment of breast cancer have somewhat reduced its mortality rate, many individuals still die from a range of intricate malignant morphologies. Abnormal HMGB1 levels have also been previously reported in breast cancer. The clinical use of HMGB1 in the detection and treatment of breast cancer has also been demonstrated by numerous investigations (5, 6). However, a deeper comprehension of dual role of HMGB1 in cancer growth is necessary due to its pro- and anti-tumoral properties. More significantly, HMGB1 has a role in controlling patients' response to radiation and chemotherapy for breast cancer. The complexity of the association between HMBG1 and the development of breast cancer has led to the continuous development of novel therapeutic approaches that target HMGB1, including the detection of putative inducers of immunogenic cell death and combination treatments with immune checkpoint inhibitors (7-10). The purpose of this study was to assess the expression of HMGB1 by IHC staining in breast cancer tissues and to investigate any correlation between HMBG1 expression and clinicopathological traits in breast cancer patients.

## Materials and Methods

The expression of HMBG1 protein was investigated in tissue samples taken from primary breast carcinoma patients who had undergone mastectomy or excisional breast biopsy between 2011 and 2018 and whose diagnoses were confirmed by IHC analysis of stained slides in our hospital's pathology laboratory. The Local Ethics Committee of Buca Seyfi Demirsoy Training and Research Hospital approved this project (reference number: 293, date: 29.05.2024). Informed consent forms were signed preoperatively by all participating patients. Archival slides stained with hematoxylin and eosin (H&E) were reassessed using the World Health Organization's 2012 categorization of breast tumors criteria. H&E stained slides were used for IHC analysis in order to detect the viable tumor regions and choose suitable paraffin blocks for study-specific IHC analysis. The 2-micron diameter paraffined cylindrical tissue samples were taken from donor blocks best suited for IHC analysis and identified first on the slide and subsequently in the block. IHC analysis was then carried out using diluted monoclonal rabbit antibodies against HMGB1 (Atlas, ATL-HPA049521, USA) at a dilution of 1:500 after several blocks had been created using mapping and addressing procedures. Histopathologists, blinded to the patients' clinical characteristics, examined the slides, and classified staining patterns based on their staining intensities. Diffuse nuclear and/or cytoplasmic staining of the tumor cells (Figure 1) was considered HMGB1 positivity, and the number of positive cells was recorded. Furthermore, invasion of the tumor tissue by inflammatory cells that were HMBG1-positive was assessed and evidence of any extracellular expression of HMGB1 was also investigated (Figure 2).

### Statistical Analysis

SPSS, version 25.0, was used for the statistical analysis (IBM Inc., Armonk, NY, USA). The chi-square test was employed to compare quantitative data. Non-parametric data were compared using the Mann-Whitney U test. The non-parametric Kruskal-Wallis test was used to compare the measurements in more than two groups. The difference in survival across groups was compared using Kaplan-Meier survival analysis. The threshold for statistical significance was set at $p<0.05$.

## Results

There were 282 patients' samples included in the study with a median (range) age of 54 (27–85) years and a mean age of 55.5±12.2 years. The mean follow-up period was 48.3±24.1 months. Two hundred and

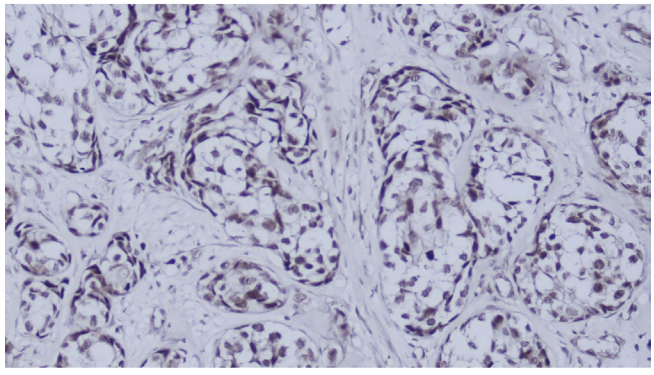


**Figure 1.** Strong nuclear expression of HMGB1 in tumor cells (DAB x 200)

*HMGB1: High mobility group box 1; DAB: diaminobenzidine*

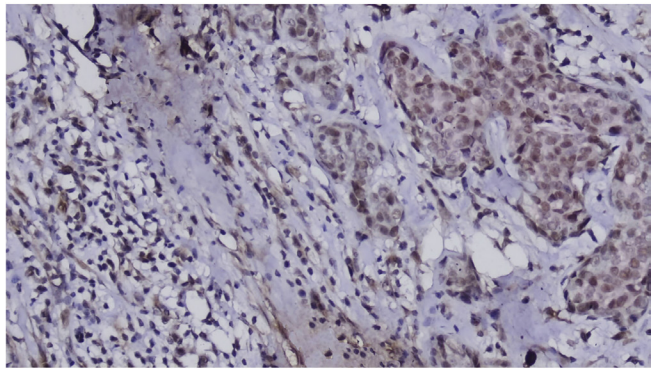


**Figure 2.** Note the weaker cytoplasmic HMGB1 expression in tumor cells compared to inflammatory cells (DAB x 200)

*HMGB1: High mobility group box 1; DAB: diaminobenzidine*

forty-eight (87.9%) patients were alive, but 34 (12.1%) patients had died at the time of data analysis. The mean overall survival time was 21 months, ranging from 1.5 to 79.9 months. The mean tumor diameter was 3.4±2.9 cm (0.4–18 cm). Clinical and histopathologic findings of the patient are shown in Table 1.

Estrogen receptor-positivity was found in 229 (81.2%) and progesterone receptor-positivity in 204 (72.4%) of the 282 patients who were part of the study. C-erbB2, which was used to assess human epidermal growth factor receptor 2 (HER2)/neu amplification, was 1+ or negative in 192 patients (68.1%), and both these groups were regarded as HER2-negative. Thirty-three cases (11.7%) were HER2-positive by combined IHC-fluorescent *in situ* hybridization examination, and each of them was treated specifically. Every case was examined using the Ki-67 proliferation index, with a mean value of 31.1±24%, ranging from 2–80%. The number of cases with luminal A ($n$ = 114; 40.4%), luminal B ($n$ = 112; 39.7%), HER2-positive ($n$ = 33: 11.7%), and triple-negative ($n$ = 23; 8.2%) were identified, based on the molecular classification. Patients in the various molecular groups had mean ages that were relatively similar to one another ($p$ = 0.603). The survival time in this series did not differ significantly between the molecular subtypes of the malignancies ($p$ = 0.178). A few inflammatory cells were identified in the peritumoral stroma of almost all tumors. However, there was severe inflammatory infiltration in the peritumoral stroma of 76 (27%) tumors. HMBG1 expression was confined to the nucleus in 250 (88.7%) tumors. However, in 32 (11.3%) tumors, there was nuclear and cytoplasmic and/or extracellular expression of HMGB1 (Table 2).

**Table 1. Clinical and histopathologic data**

| | | n | % |
|---|---|---|---|
| **Survival** | Survived | 248 | 87.9 |
| | Exited | 34 | 12.1 |
| **Tumor location** | Right breast | 134 | 47.6 |
| | Left breast | 148 | 52.4 |
| | Bilateral | - | - |
| **Diagnosis** | IDC | 200 | 70.9 |
| | ILC | 18 | 6.4 |
| | IPC | 7 | 2.5 |
| | IDC with dominant *in situ* component | 33 | 11.7 |
| | Other histologic variants | 24 | 8.5 |
| **Grade** | Grade 1 | 20 | 7.1 |
| | Grade 2 | 141 | 50 |
| | Grade 3 | 121 | 42.9 |
| **Pathologic T-stage** | pT1 | 115 | 41 |
| | pT2 | 120 | 42.4 |
| | pT3 | 34 | 12.1 |
| | pT4 | 13 | 4.5 |
| ***In situ* component** | Yes | 182 | 64.5 |
| **Type of *in situ* component (if any)** | Comedo | 26 | 14.2 |
| | Non-comedo | 76 | 41.8 |
| | Mixed | 80 | 44.0 |
| **Lymph node metastasis** | Yes | 112 | 39.7 |
| **Capsular invasion in the lymph node** | Yes | 79 | 70.5 |
| **Multifocality** | Multifocal | 22 | 7.8 |
| **Nipple involvement** | Yes | 20 | 7.1 |
| **Dermal/epidermal invasion** | Yes | 22 | 7.8 |
| **Lymphovascular invasion** | Yes | 102 | 36.2 |
| **Perineural invasion** | Yes | 71 | 25.2 |

IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; IPC: Invasive papillary carcinoma

In all cases, there were HMGB1 expressions in the nuclei of tumor cells, with a mean proportion 8.84% staining positive, but the intensity ranged widely from 1% to 90% of cells. There was no significant difference in survival rates and the presence of cytoplasmic/extracellular HMBG1 or isolated nuclear staining ($p$ = 0.295). The mean survival time was 51.1±23.2 months for the patients with a low expression rate of HMGB1 (<5%) while the mean survival time was 44.7±24.7 months for the patients with high HMGB1 expression. There was a statistically significant difference in survival time of the patients according to the HMGB1 expression rate in tumor cells ($p$ = 0.035).

**Discussion and Conclusion**

Breast cancers, like many other cancers, may exhibit IHC expression of HMGB1 in both the cytoplasm and the nuclei of breast cancer cells. While cytoplasmic and extracellular expressions of HMGB1 has been associated with its role in inflammation and the progression of cancer, its nuclear localization is related to its role in DNA-related functions. Moreover, research has demonstrated that, in contrast to normal breast tissue, HMGB1 is overexpressed in breast cancer tissues (11). Expression level of HMGB1 varies according to subtype, and stage of breast cancer. Aggressive phenotypes of breast cancer have been linked to higher levels of HMGB1 expression. For instance, HMGB1 is frequently overexpressed in cases of triple-negative breast cancers (TNBC), which are notorious for their poor prognosis and lack of targeted treatment modalities (11). Furthermore, it has been found that in individuals with breast cancer, HMGB1 overexpression is linked to a lower overall and disease-free survival rates. Thus, HMGB1 is thought to be a possible prognostic indicator, particularly for patients with more aggressive types of breast cancer. Since HMGB1 protein is secreted extracellularly or translocated to the cytoplasm, nuclear HMGB1 expression may be significantly reduced in more aggressive tumors, such as some types of colorectal cancer, gastric cancer, and advanced breast cancer, particularly TNBC. Although 40–70% of tumor cells may demonstrate nuclear expression of HMGB1, the other cells may exhibit extracellular or cytoplasmic HMGB1 expression. In contrast to earlier studies, in the present study, the highest mean nuclear HMGB1 expression rate was noted in patients with luminal A-subtype tumors, while the mean HMGB1 expression rates in the other three subtypes were similar. Furthermore, no significant correlation was found between the molecular subtype and the presence of cytoplasmic or extracellular HMGB1 expression (2, 11-13).

It was previously demonstrated that HMGB1 also plays an important role in mediating immunoregulatory functions. For instance,

**Table 2. Immunohistochemical and molecular findings**

| Parameters | | n | % |
|---|---|---|---|
| **ER status** | Positive | 229 | 81.2 |
| **PR status** | Positive | 204 | 72.4 |
| **C-erbB-2 expression (according to ASCO/CAP 2013 criteria)** | Negative or 1+ | 192 | 68.1 |
| | 2+ | 51 | 18.1 |
| | 3+ | 39 | 13.8 |
| **HER2 amplification (FISH method)** | Positive | 29 | 10.3 |
| | Negative | 23 | 8.2 |
| **Molecular subtypes** | Luminal A | 114 | 40.4 |
| | Luminal B | 112 | 39.7 |
| | HER2-positive | 33 | 11.7 |
| | Triple-negative (basal-like) | 23 | 8.2 |
| **HMBG1 expression** | Nuclear | 250 | 88.7 |
| | Cytoplasmic/extracellular | 32 | 11.3 |
| **Severe inflammation** | Yes | 76 | 27 |
| | No | 206 | 73 |

ER: Estrogen receptor; PR: Progesterone receptor; FISH: Fluorescent *in situ* hybridization; ASCO/CAP: American Society of Clinical Oncology/College of American Pathologists; HER2: Human epidermal growth factor receptor 2

extracellular HMGB1 modulates the immune response by acting as a damage-associated molecular pattern molecule. It can produce an immunosuppressive tumor microenvironment, which helps cancer cells evade the immune system. In the present study, there was a significant relationship between prominent inflammatory cell infiltration in the tumor microenvironment, regardless of the molecular subtype, and extranuclear location of HMGB1 expression ($p$ = 0.023). This appears to be further evidence of the hypothesis that extracellular or cytoplasmic expressions of HMGB1 is associated with the immune response (2, 13).

One of molecular functions HMGB1 in breast cancer is to enhance tumor cell survival by encouraging DNA repair, shielding cancer cells from the genotoxic stress that is often brought on by treatments like radiotherapy and chemotherapy. HMGB1 also promotes cell invasion and migration, which facilitates the metastatic process. In addition, it contributes to the epithelial-mesenchymal transition, a crucial step in the spread of cancer (14-19). In the present study, no relationship was found between HMGB1 protein expression and metastasis according to different cut-off values. However, axillary lymph node metastasis was seen at a significantly higher rate in tumors with intense inflammation ($p$ = 0.024). Similarly, higher extranuclear HMGB1 expression rates were found in tumors with intense inflammation, but without any difference when compared with other breast tumor subtypes ($p$ = 0.194).

HMGB1 has emerged as a possible therapeutic target because of its roles in cancer biology. Therapies could target HMBG1 directly or indirectly through signaling pathways, like the receptor for advanced glycation end products and toll-like receptor 4. Inhibiting HMGB1 may slow the growth of tumors and improve the efficacy of current therapies. By weakening HMGB1-mediated resistance mechanisms, HMGB1 inhibitors may be used in conjunction with traditional treatments such as immunotherapy, radiotherapy, or chemotherapy so as to increase their effectiveness. A growing amount of data points to a connection between the emergence of several cancers and HMGB1 overexpression. For example, *in vitro* research using gastric cancer cell lines showed that elevated HMGB1 levels were associated with cell metastasis (11). In addition, when HMGB1 was inhibited *in vivo* using short hairpin RNA, the NF-κB pathway was used to inhibit the proliferation and invasion of gastric cancer cells, indicating that HMGB1 may be a therapeutic biomarker for the disease. Similarly, HMGB1 expression was inhibited by downregulating the PI3k/Akt signaling pathway, which stopped xenograft tumor development and the proliferation and spread of hepatocellular carcinoma cells. Furthermore, inhibition of HMGB1 expression led to downstream activation of AKT signaling and a notable decrease in the growth of VCP-mediated hepatocellular carcinoma, indicating that it is a useful therapeutic target for focused intervention and enhanced cancer patient survival. Similarly, HMGB1 downregulation was related with the suppression of *in vivo* and *in vitro* development and metastasis of lung, hepatocellular, and prostate cancer cells (11, 12, 16-19).

In summary, among 282 cases of breast cancer lower overall HMGB1 expression rates were found, compared to previous studies. We suggest that this may have been due to the primary antibody we used in our study. Similarly, we did not detect a relationship between isolated nuclear or extranuclear expression of HMGB1 and prognosis. However, in previous studies, the HMGB1 expression was mostly associated with prognosis in the most aggressive TNBC group. Since the number of TNBC cases in our series was quite low, we may not have been able to detect any relationship between HMGB1 expression and prognosis. The role of HMGB1 expression in breast cancer should be fully elucidated through further larger studies. Given its apparent role in particularly aggressive breast cancer and the possible therapeutic target role for HMGB1, this research is required as quickly as possible as it may improve outcomes for some of the most severe forms of breast cancer.

**Acknowledgment**

This article was edited in English by Gürkan Kazancı who professional translator.

## Ethics

**Ethics Committee Approval:** The local Ethics Committee of Buca Seyfi Demirsoy Training and Research Hospital approved this project (reference number: 293, date: 29.05.2024).

**Informed Consent:** Informed consent forms were signed preoperatively by all participating patients.

## Footnotes

### Authorship Contributions

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

**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. Wang S, Zhang Y. HMGB1 in inflammation and cancer. J Hematol Oncol. 2020; 13: 116. (PMID: 32831115) [Crossref]
2. Tang D, Kang R, Zeh HJ, Lotze MT. The multifunctional protein HMGB1: 50 years of discovery. Nat Rev Immunol. 2023; 23: 824-841. (PMID: 37322174) [Crossref]
3. Shao LH, Zhu L, Wang M, Ning Y, Chen FQ, Gao XQ, et al. Mechanisms involved in the HMGB1 modulation of tumor multidrug resistance (Review). Int J Mol Med. 2023; 52: 69. (PMID: 37387415) [Crossref]
4. Idoudi S, Bedhiafi T, Pedersen S, Elahtem M, Alremawi I, Akhtar S, et al. Role of HMGB1 and its associated signaling pathways in human malignancies. Cell Signal. 2023; 112: 110904. (PMID: 37757902) [Crossref]
5. Jiao Y, Wang HC, Fan SJ. Growth suppression and radiosensitivity increase by HMGB1 in breast cancer. Acta Pharmacol Sin. 2007; 28: 1957-1967. (PMID: 18031610) [Crossref]
6. Dong H, Zhang L, Liu S. Targeting HMGB1: an available therapeutic strategy for breast cancer therapy. Int J Biol Sci. 2022; 18: 3421-3434. (PMID: 35637945) [Crossref]
7. Liu B, Qi X, Zhang X, Gao D, Fang K, Guo Z, et al. Med19 is involved in chemoresistance by mediating autophagy through HMGB1 in breast cancer. J Cell Biochem. 2019; 120: 507-518. (PMID: 30161287) [Crossref]
8. Amornsupak K, Thongchot S, Thinyakul C, Box C, Hedayat S, Thuwajit P, et al. HMGB1 mediates invasion and PD-L1 expression through RAGE-PI3K/AKT signaling pathway in MDA-MB-231 breast cancer cells. BMC Cancer. 2022; 22: 578. (PMID: 35610613) [Crossref]
9. Akoz G, Diniz G, Ekmekci S, Ekin ZY, Uncel M. Evaluation of human epididymal secretory protein 4 expression according to the molecular subtypes (luminal A, luminal B, human epidermal growth factor receptor 2-positive, triple-negative) of breast cancer. Indian J Pathol Microbiol. 2018; 61: 323-329. (PMID: 30004048) [Crossref]
10. Degirmenci M, Diniz G, Kahraman DS, Sahbazlar M, Koral L, Varol U, et al. Investigating the correlation between long-term response in patients with metastatic HER2+ breast cancer and the activity of regulatory T cells: a retrospective study. Breast Cancer (Dove Med Press). 2024; 16: 645-655. (PMID: 39355199) [Crossref]
11. Zhang QY, Wu LQ, Zhang T, Han YF, Lin X. Autophagy-mediated HMGB1 release promotes gastric cancer cell survival via RAGE activation of extracellular signal-regulated kinases 1/2. Oncol Rep. 2015; 33: 1630-1638. (PMID: 25652880) [Crossref]
12. Zhang J, Kou YB, Zhu JS, Chen WX, Li S. Knockdown of HMGB1 inhibits growth and invasion of gastric cancer cells through the NF-κB pathway *in vitro* and *in vivo*. Int J Oncol. 2014; 44: 1268-1276. (PMID: 24481712) [Crossref]
13. Lv G, Yang M, Gai K, Jia Q, Wang Z, Wang B, et al. Multiple functions of HMGB1 in cancer. Front Oncol. 2024; 14: 1384109. (PMID: 38725632) [Crossref]
14. Pu Z, Duda DG, Zhu Y, Pei S, Wang X, Huang Y, et al. VCP interaction with HMGB1 promotes hepatocellular carcinoma progression by activating the PI3K/AKT/mTOR pathway. J Transl Med. 2022; 20: 212. (PMID: 35562734) [Crossref]
15. Wang XH, Zhang SY, Shi M, Xu XP. HMGB1 promotes the proliferation and metastasis of lung cancer by activating the Wnt/β-catenin pathway. Technol Cancer Res Treat. 2020; 19: 1533033820948054. (PMID: 32815451) [Crossref]
16. Zhao CB, Bao JM, Lu YJ, Zhao T, Zhou XH, Zheng DY, et al. Co-expression of RAGE and HMGB1 is associated with cancer progression and poor patient outcome of prostate cancer. Am J Cancer Res. 2014; 4: 369-377. (PMID: 25057439) [Crossref]
17. Dong YD, Cui L, Peng CH, Cheng DF, Han BS, Huang F. Expression and clinical significance of HMGB1 in human liver cancer: knockdown inhibits tumor growth and metastasis in vitro and in vivo. Oncol Rep. 2013; 29: 87-94. (PMID: 23042506) [Crossref]
18. Shrivastava S, Mansure JJ, Almajed W, Cury F, Ferbeyre G, Popovic M, et al. The role of HMGB1 in radioresistance of bladder cancer. Mol Cancer Ther. 2016; 15: 471-479. (PMID: 26719575) [Crossref]
19. Ma H, Zheng S, Zhang X, Gong T, Lv X, Fu S, et al. High mobility group box 1 promotes radioresistance in esophageal squamous cell carcinoma cell lines by modulating autophagy. Cell Death Dis. 2019; 10: 136. (PMID: 30755598) [Crossref]

Original Article

Eur J Breast Health 2025; 21(4): 295-300
DOI: 10.4274/ejbh.galenos.2025.2025-5-2

# Reclassification of *BRCA1* and *BRCA2* Variants of Unknown Significance in a Turkish Cohort; A Single-Center, Retrospective Study

Leyla Özer[1], Süleyman Aktuna[1], Evrim Ünsal[2]

[1]Mikrogen Genetic Diagnosis Center, Ankara, Türkiye
[2]Yüksek İhtisas University Medical School, Department of Medical Genetics, Ankara, Türkiye

## ABSTRACT

**Objective:** Accurate classification of *breast cancer susceptibility gene (BRCA)1/2* variants is important to delineate candidates for surgical or medical treatment. We retrospectively analyzed *BRCA1/BRCA2* sequencing data and reclassified the *BRCA1/2* variants of unknown significance (VUS) in Turkish patients with breast, ovarian, pancreatic and prostate cancers.

**Materials and Methods:** *BRCA1/BRCA2* sequence data of a large cohort were retrospectively analyzed. The sequencing data were reinterpreted in the context of American College of Medical Genetics guidelines, the Evidence-based Network for the Interpretation of Germline Mutant Alleles *BRCA1/2* classification rules, and current public genomic databases.

**Results:** Among the total of 2,713 patients, 254 (9.36%) had *BRCA1* or *BRCA2* variants. A total of 264 *BRCA1/BRCA2* variants were detected. Of these, 130 (49.2%) were pathogenic variants (PV), 24 (9%) were likely pathogenic (LP) and 110 of 264 variants (41.6%) were VUS. For the 119 *BRCA1* variants, 68% (*n* = 81) were PV, 7.5% (*n* = 9) were LP, and 24.5% (*n* = 29) were VUS. Similarly, for the 145 *BRCA2* variants, 33.7% (*n* = 49) were PV, 10.3% (*n* = 15) were LP, and 55.8% (*n* = 81) were VUS. Reanalysis of the 110 *BRCA1+BRCA2* VUS variants led to 22 (20%) being reclassified. Of these 22, 45.4% (*n* = 10) were reclassified as P/LP and 54.6% (*n* = 12) were reclassified as benign/likely benign.

**Conclusion:** These results show that it may be possible to reclassify VUS, in this case *BRCA1/2* VUS, in light of changing genetic data. These results demonstrate the importance of VUS reclassification of *BRCA1/2* variants in clinical management, surgical decisions, risk counseling and screening.

**Keywords:** *BRCA1* and *BRCA2* genes; breast cancer screening; breast conserving surgery; PARP inhibitors; sequencing

**Cite this article as:** Özer L, Aktuna S, Ünsal E. Reclassification of *BRCA1* and *BRCA2* variants of unknown significance in a turkish cohort; a single-center, retrospective study. Eur J Breast Health. 2025; 21(4): 295-300

### Key Points

- *Breast cancer susceptibility gene (BRCA)1/BRCA2* sequence data was retrospectively analysed to reclassify *BRCA1/2* variants of unknown significance (VUS) in Turkish patients with breast, ovarian, and prostate cancers for improved clinical decision-making.
- Retrospective analysis of *BRCA1/2* sequencing data from 2,713 patients using American College of Medical Genetics guidelines, Evidence-based Network for the Interpretation of Germline Mutant Alleles rules, and public genomic databases.
- VUS reclassification is crucial for accurate *BRCA1/2* variant interpretation, impacting treatment, surgical planning, and genetic counseling.

## Introduction

*Breast cancer susceptibility gene 1* (*BRCA1*) and *breast cancer susceptibility gene 2* (*BRCA2*) are tumor suppressor genes that are involved in DNA repair, cell cycle regulation, and genome stability (1). Germline *BRCA1/2* gene mutations are associated with an increased risk of breast, ovarian, prostate, and several other cancers. *BRCA1/2* sequencing is increasingly being used to determine the therapeutic options, both preventive surgery in breast and ovarian cancers and medical treatments with poly (ADP) ribose polymerase inhibitors (PARPi) in breast, ovarian, prostate, and pancreatic cancers (1-3). Germline *BRCA1/2* variants may be classified into "pathogenic (P)", "likely pathogenic (LP)", "variant of unknown clinical significance (VUS)", "likely benign (LB)", or "benign (B)" (4, 5). Cases with P and LP variants will benefit from targeted treatment (surgery or chemotherapy) (1, 2). However, patients with B, LB, and VUS variants should have their treatment plans organized similarly to those who do not have P or LP variants. Diagnosing *BRCA1/2* PV guides the planning of effective surgery and chemotherapy, patient follow-up, and the consideration of prophylactic surgical options for asymptomatic

**Corresponding Author:**
Leyla Özer MD; leyla_ozer@yahoo.com

**Received:** 06.05.2025
**Accepted:** 08.06.2025
**Epub:** 25.08.2025
**Available Online Date:** 25.09.2025

individuals (2, 6). With the increased use of targeted therapy with PARPi in the treatment of *BRCA*-positive cancers, the accurate classification of *BRCA* gene variants guides treatment planning. Over the years, the improvement in next-generation sequencing (NGS) technology has led to an increase in the use of *BRCA1/2* testing and, consequently, reports of VUS variants. With the expanded use of NGS and *BRCA1/2* testing, the detection of VUS has become increasingly frequent. *BRCA1/2* VUS are a significant challenge for molecular genetic testing in specific breast, prostate, and ovarian cancers (7, 8). The rate of detection for VUS of *BRCA* was reported as 10–20% in women who were tested for *BRCA* variants (9).

Several guidelines and bioinformatic tools have been used to diminish the challenges in classifying *BRCA* variants (4, 5). Multiple guidelines and bioinformatic tools have been developed to address the challenges of variant interpretation. Among these guidelines, the American College of Medical Genetics and Genomics (ACMG) guidelines [the Association for Molecular Pathology (AMP)/ACMG 2015] are widely recognized as a reliable variant interpretation system (5). However, due to gene-specific complexities, more tailored approaches have been required to eliminate uncertainties. For this purpose, the current guideline provides detailed, *BRCA*-specific instructions to support variant curation and address discrepancies and uncertainties in variant classification. Variant classification of *BRCA1* and *BRCA2* genes depends on the current Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) consortium classification (5).

In this retrospective study, previously reported *BRCA1* and *BRCA2* VUS were reinterpreted according to the 2015 ACMG guidelines, the ENIGMA *BRCA1/2* classification rules, and current public genomic databases. This study aimed to investigate changes in *BRCA* variant classification over time and thus highlight the importance of reanalyzing VUS variants in light of changing genetic data for clinical decision-making and patient management.

## Materials and Methods

### Study Population

Sequence data (January 2018 to August 2023) of a total of 2,713 patients with breast and ovarian cancer, prostate cancer, or pancreatic tumors who were referred to the "Mikrogen Genetic Diagnosis Center" for *BRCA1/BRCA2* sequencing were retrospectively analyzed (Figure 1). This study was approved by the Yüksek İhtisas University Medical School Ethical Committee (approval number: 296, date: 14.04.2025).

### *BRCA1/2* Sequencing

QIAamp DNA Blood Kit (QIAGEN, Aarhus, Denmark) was used to isolate DNA from blood samples. NGS of *BRCA1* and *BRCA2* genes was performed on an Illumina MiSeq sequencing platform (Illumina Inc., San Diego, CA, USA) using primers covering exon/exon-intron junctions in the *BRCA1/BRCA2* genes with the Qiaseq targeted DNA panel (DHS-102Z-96) according to the manufacturer's instructions. NGS achieved a minimum 20x read depth for >98% of targeted bases. The human genome Hg19 sequence was used as a reference to identify genetic variants. FASTQ, BAM, and VCF files were obtained. The bioinformatic analysis of VCF files was performed using NextGene (SoftGenetics, State College, PA, USA), Geneticist Assistant (SoftGenetics), and Franklin Genoox (Genoox, Israel). Variant annotation and filtering were conducted using a comprehensive set of public databases, including ClinVar, the Human Gene Mutation Database, and dbSNP, as well as population frequency datasets such as the Exome Aggregation Consortium, Genome Aggregation Database (gnomAD), Turkish Variome and the 1000 Genomes Project. Functional predictions were assessed using *in silico* tools, including PolyPhen-2, SIFT, MutationAssessor, and SpliceAI (Figure 2).

### Variant Classification

All the *BRCA1/2* variants were reclassified in the context of the specific ACMG/AMP guideline for *BRCA1/2* variant classification (6, 7). This guideline uses ACMG/AMP variant classification criteria and contains additional specific updates for variant interpretation of *BRCA1/2* genes. Variant classification of *BRCA1/2* gene variants was made according to two main criteria. PV are weighted as very strong, strong, moderate, or supporting (PVS1, PS1–4, PM1–6, PP1–5), and benign variants are defined as standalone, strong, or supporting (BA1, BS1–4, BP1–6). The detected variants were classified as "P", "LP", "VUS", "LB", or "B" according to specific ACMG/AMP guidelines for *BRCA1/2* variant classification criteria.

### Statistical Analysis

Chi-square tests were used to compare reclassification patterns between *BRCA1* and *BRCA2*. We assessed whether the overall reclassification rates and the distribution of reclassification types (P/LP vs. B/LB)

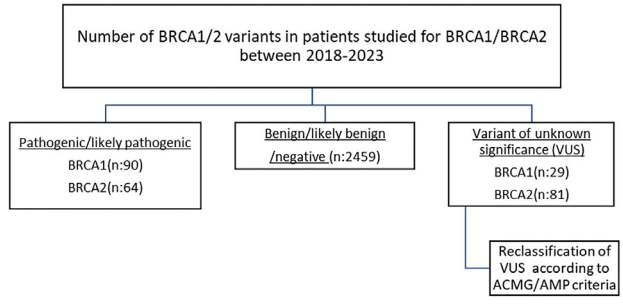



**Figure 1.** The number of reported patients and the VUS reclassification flow chart

VUS: Variant of unknown significance; ACMG: American College of Medical Genetics; AMP: Association for Molecular Pathology; *BRCA: Breast cancer susceptibility gene*

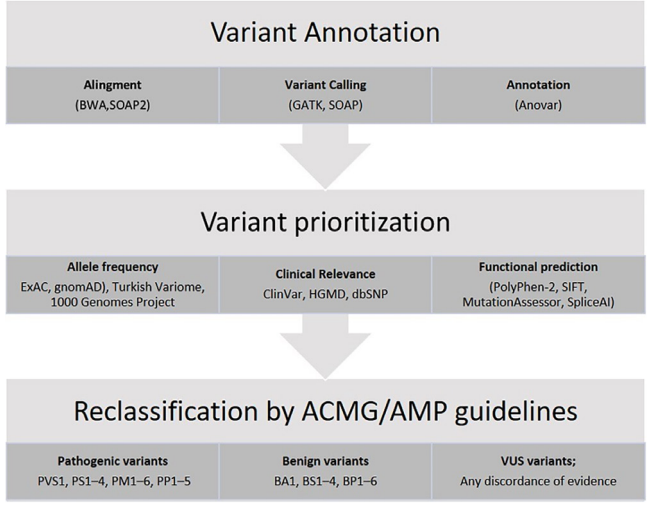



**Figure 2.** *BRCA1/BRCA2* variant reclassification workflow

ACMG: American College of Medical Genetics; AMP: Association for Molecular Pathology; *BRCA: Breast cancer susceptibility gene;* HGMD: Human Gene Mutation Database; ExAC: Exome Aggregation Consortium; gnomAD: Genome Aggregation Database

differed significantly between the two genes. All statistical tests were analyzed with a significance level of $p<0.05$. Analyses were conducted using SPSS for Windows, version 29.0 (IBM Corp., Armonk, NY, USA).

## Results

The *BRCA1/BRCA2* sequence data of 2,713 patients were retrospectively analyzed regardless of cancer type. Among these, 254 (9.36%) harbored a total of 264 had *BRCA1* or *BRCA2* variants (Figure 1). On the initial analysis, 5.7% (154/2713) with *BRCA1* or *BRCA2* variants had P and LP variants, while 3.7% (100/2713) had VUS variants. The majority of the 2,713 patients (90.6%; *n* = 2459) had no variant or only B/LB variants.

Among the patients with *BRCA1/BRCA2* variants, 51.1% (130/254) PV, 9.4% (24/254) had LP, and 39.3% (100/254) VUS. Ten patients were carriers of multiple VUSs (two *BRCA2* VUS *n* = 6; two *BRCA1* VUS *n* = 3; and one patient had one *BRCA1* and one *BRCA2* VUS). For the 119 *BRCA1* variants, 68% (*n* = 81) were PV, 7.5% (*n* = 9) were LP, and 24.5% (*n* = 29) were VUS. Similarly, for the 145 *BRCA2* variants, 33.7% (*n* = 49) were PV, 10.3% (*n* = 15) were LP, and 55.8% (*n* = 81) were VUS. The type of variants were: 105 (39.7%) missense; 97 (36.7%) frameshift; 42 (15.9%) non-sense; 9 (3.4%) splice site; 5 (1.8%) intronic; 4 (1.5%) stop gain; and 2 (0.7%) in-frame deletions.

### Reclassification of BRCA1/2 Variants

The 110 *BRCA1* and *BRCA2* VUS variants were reanalyzed, comprising 105 missense, two stop-gain, two in-frame deletions, and one intronic variant. In total, 22 (20%) were reclassified, with 40.9% (9/22) reclassified as P/LP, and 59.1% (13/22) as B/ LB. The *BRCA1* VUS rate dropped from 24.3% (29/119) to 17.6% (21/119). Among the reclassified variants, the status of 50% (4/8) of the *BRCA1* VUS variants changed to B or LB, and 50% (4/8) of them changed to P or LP. The frequencies of the specific *BRCA2* VUS changed from 56.2% (81/145) to 40.9% (59/144). The status of 64.2% (9/14) of *BRCA2* VUS variants changed to B or LB, and 35.7% (5/14) of them changed to P or LP. The VUS classification of 80% (88/110) of the *BRCA1/2* VUS did not change (Table 1).

The reclassification rate between *BRCA1* and *BRCA2* was not different ($p$ = 0.358). Similarly, the distribution of reclassification types (P/LP vs. B/LB) did not differ significantly between the two genes ($p$ = 0.838). We calculated a 95% Wilson score confidence interval based on 22 reclassified variants out of 110 to estimate the reclassification rate. The resulting confidence interval ranged 13.6% to 28.4%.

## Discussion and Conclusion

Identifying PV in *BRCA1/2* is essential when managing breast, ovarian, and prostate cancers, particularly regarding follow-up and treatment selection (10). As *BRCA1/2* testing becomes increasingly integrated into routine clinical practice, the frequency of VUS findings has also risen. The increasing presence of VUS variants complicates patient counseling and clinical management, so genetic experts strongly recommend reclassification to resolve these uncertainties. Updates in databases, the development of bioinformatics solutions, and functional studies increase the likelihood of identifying the pathogenicity of VUS variants. The reclassification of VUS variants is important in genetic diagnosis and is recommended by genetic experts. Several previous studies have reported that the rates of *BRCA1/2* PV were about 6–15% (11, 12). Several studies have reported *BRCA1/2* variant prevalence in the Turkish population. Celik Demirbas et al. (13) found P *BRCA1* and *BRCA2* variants in 7.8% and 5.4% of 3,184 hereditary breast and ovarian cancer (HBOC) patients, respectively. Bahsi and Erdem (14) reported 9.4% P, 0.3% LP, and 6.4% VUS variants among 1,419 patients with HBOC. Boga et al. (15) found 9.9% P and 5.7% VUS rates in Turkish HBOC patients.

In our cohort, the initial detection rate of *BRCA1/2* PV was 5.4%, but after the reclassification of VUS variants, the rate of PV increased to 6%, which is similar to the results of Zang et al. (16) (6%). In the current study, the *BRCA1/2* VUS dropped from 3.6% to 2.8% after reclassification, which aligns with previous reports. Several studies have reported a range of VUS rates for *BRCA* variants (3.9–22.5%) (17-20), and our VUS rate is consistent with the previous studies. Zanti et al. (21) reported the VUS rate as 23.4% for *BRCA1* and *BRCA2* genes in a case-control evidence study from 11,227 *BRCA1* and *BRCA2* variants (2025) in 96,691 female breast cancer cases and 303,925 healthy controls. VUS rates have ranged from study to study due to the types of cancers included in the study, the size of the cohort, and the bioinformatics tools and databases used during the years the study was reported. The majority of the previous studies have been reported from breast cancer cases. Our cohort consisted mainly of breast cancer patients but also included ovarian and prostate cancer patients.

The current study's rate of VUS reclassification (20%) is very similar to previous reports [Mighton et al. (18), 14.7%; Benet-Pagès et al. (22), 20%; Innella et al. (23), (20%). Our rate of VUS reclassification is higher than the rates reported by both Mersch et al. (24) (7.7%) and Macklin et al. (25) (11.3%). Several studies reported that most of the reclassified VUSs were downgraded, which is similar to our results; however, the frequency of VUSs reclassified to P is nearly the same as the frequency of VUSs reclassified to benign in our cohort (17, 18, 22, 23). There is a lack of sufficient studies on the reclassification of *BRCA1* and *BRCA2* VUS variants in the Turkish population. Özdemir et al. (26) reclassified variants identified in 26 genes, including *BRCA1* and *BRCA2*, in a cohort of 137 cancer patients. In this study, 33.6% of the variants initially classified as VUS were downgraded, while 20.83% were upgraded. However, the results are not specific to the reclassification of *BRCA1* and *BRCA2* VUS variants; they also include the reclassification outcomes of VUS variants in the other 26 genes.

Genetic authorities recommend a periodic reassessment of VUS variants to avoid uncertainty in clinical decision-making. The periodic re-evaluation of VUSs is recommended in clinical practice; however, no specific time interval has been reported regarding how often reevaluation should be performed. The recommended time interval for periodic reassessment of VUS variants changes among several studies (23, 27). AMP guidelines suggest that reassessing VUS variants every two years may be enough to show the changes in classifications (27). Although guidelines recommend re-evaluating VUS variants every two years, this period may be shorter in cases of earlier than expected recurrence, metastasis, or aggressive tumour progression. Indeed, in our study, we identified cases whose classification changed in <3 years (Table 1). Mighton et al. (18) reported that two years is an ideal time interval for the reclassification of *BRCA1/2* VUS variants. Innella et al. (23) reported that the average time from the initial classification of VUS variants to reclassification was 49.4 months for *BRCA1/2* variants. Nevertheless, they recommended 3 years for periodic reassessment of VUS variants. The present study's mean duration between the initial VUS report and the first reclassification was 33.7 months.

**Table 1. Reclassification of variants of unknown significance in *BRCA1/2* genes**

| Patient no | Gene | Variant/dbSNP number | Initial classification | Clinical significance (Clinvar) | Type of evidence | ACMG/AMP reclassification | Time between initial classification and first reclassification |
|---|---|---|---|---|---|---|---|
| 1 | *BRCA2* *NM_000059.4* | c.9857T>A rs398122624 | VUS | LB | BP4, BP6 | LB | 13 months |
| 2 | *BRCA2* | c.632-4_632-3del rs431825341 | VUS | VUS | PVS1, PM2 | LP | Recent study |
| 3 | *BRCA2* | c.8249_8251delAGA rs80359703 | VUS | VUS | PM1, PM2, PM4 | LP | Recent study |
| 4 | *BRCA2* | c.1232T>C rs79597821 | VUS | LB | BP1, BP3 BP4, PM2 | LB | 36 months |
| 5 | *BRCA2* | c.8452G>A rs80359094 | VUS | LP | PM1, PP3, PM2, PP5 | LB | 38 months |
| 6 | *BRCA2* | c.10095delCinsGAATTATATCT rs276174803 | VUS | LP | PVS1, PM2, BP6 | LP | 23 months |
| 7 | *BRCA2* | c.9934A>G rs80359254 | VUS | LB | BP4, BP6, PM2 | LB | 48 months |
| 8 | *BRCA2* | c.516G>T rs80359790 | VUS | LP | PP3, PP5, PM2 | LP | 38 months |
| 9 | *BRCA2* | c.8524C>T rs80359104 | VUS | LP | PP3, PP5, PM2 | LP | 68 months |
| 10 | *BRCA2* | c.9257G>C rs574271678 | VUS | LB | BS2, BP6, PM2 | LB | 65 months |
| 11 | *BRCA2* | c.6080G>A rs431825337 | VUS | LB | BP4, BP6, PM2 | LB | 50 months |
| 12 | *BRCA2* | c.3318C>G rs129855035 | VUS | LB | BP4, BP6, PM2 | LB | 28 months |
| 13 | *BRCA2* | c.1232T>C rs79597821 | VUS | LB | BP6, PM2 | LB | 47 months |
| 14 | *BRCA2* | c.8452G>A rs80359094 | VUS | LB | BP6, PM2, PM5 | LB | 33 months |
| 15 | *BRCA1* | c.4986+5G>A rs397509211 | VUS | P | PS3, PS4, PM2 | P | 48 months |
| 16 | *BRCA1* | c.3082C>T rs80357049 | VUS | B | BS3, BP5, BS2 | B | 54 months |
| 17 | *BRCA1* | c.5236C>A rs80357146 | VUS | LP | PM1, PM2, PM5 | LP | 9 months |
| 18 | *BRCA1* | c.53T>A rs80356929 | VUS | P | PS3, PS4, PM2, PM5 | P | 39 months |
| 19 | *BRCA1* | c.754C>T rs273902786 | VUS | LB | BS3, BP6, PM2 | LB | 24 months |
| 20 | *BRCA1* | c.4418T>C rs374519494 | VUS | LB | BP1, BP4, PM2 | LB | 12 months |
| 21 | *BRCA1* | c.1703C>T rs80356910 | VUS | LB | BP6, PM2 | LB | 12 months |
| 22 | *BRCA1* | c.5321G>C rs397509246 | VUS | P | PS3, PS4, PM2 | P | 58 months |

ACMG: American College of Medical Genetics; AMP: Association for Molecular Pathology; VUS: Variant of unknown significance; P: Pathogenic; LP: Likely pathogenic; LB: Likely benign; B: Benign; PM: Pathogenic moderate evidence; BP: Benign supporting evidence; PS: Pathogenic strong evidence; BS: Benign Strong evidence

Two of the reclassified variants are still reported as VUS by ClinVar but based on updates to databases and guidelines in this study, these variants were reclassified as LP. Some potential P or PV may be classified as VUS, according to the ClinVar database. ClinVar provides a broad collection of data on genetic variants, and this data is based on reports from different laboratories and research studies, which can sometimes lead to conflicting classifications. For example, The *BRCA2* c.632-3_632-2del(rs431825341) variant was found in one female patient with breast cancer (invasive ductal carcinoma). It was previously classified as VUS in our cohort and is still reported as VUS in the ClinVar database. After reclassification, it is upgraded to LP according to *BRCA1/2*-specific ACMG/AMP criteria. The frequency of this variant is extremely low in all databases (gnomAD (Genome); 0.0007%, gnomAD (Exome); very rare, 1000 Genomes; no observation); therefore, it was assigned as PM2 according to population data. The effect of a variant on protein is defined as loss of function due to a null variant (intronic within ±2 of a splice site) in the gene *BRCA2*, and it is assigned to PVS1. The *BRCA2* c.8249_8251del(rs80359703) variant was found in one female patient with breast cancer. The frequency of this variant is also extremely low in all databases [gnomAD (Genome); 0.0007%, gnomAD (Exome); 0%, 1000 Genomes; no observation], so it was assigned as PM2 according to population data. Protein coding length changes because of an in-frame variant in gene *BRCA2,* so it was assigned as PM4.

Over time, as the data entered into databases has increased, and more information has accumulated, the VUS rate in *BRCA1* and *BRCA2* decreased from around 13% to 2%, but VUS rates for non-*BRCA* genes are still reported as higher (20–40%) (8). The current study's *BRCA1/2* VUS rate was 3.6% before reclassification, and after reclassification, the VUS rate was revised to 2.8%.

Genetic counseling of *BRCA* VUS variants is one of the critical problems in the clinical management of cancer patients. *BRCA* VUS diagnosis causes high anxiety in both cancer and non-cancer patients (7). Limited studies have reported about the impact of VUS variants in the clinical management of patients with *BRCA1/2* VUS variants (8, 28, 29). Culver et al. (28) reported similar mastectomy rates in patients with VUS variants and *BRCA*-negative patients, but *BRCA*-negative patients have higher anxiety compared to patients with VUS variants. Welsh et al. (8) reported that the patients with *BRCA1/2* VUS variants had higher prophylactic mastectomy rates compared to *BRCA*-negative and untested patients (33% *vs.* 25%). Still, rates are lower than those of patients with *BRCA* P mutations (33% *vs.* 83%). Morgan et al. (30) reported a similar rate of prophylactic mastectomy among breast cancer cases with P *BRCA1/2* and VUS variants. In Morgan's study, VUS cases without breast cancer did not choose prophylactic mastectomy. VUS results can be confusing for patients and physicians and can cause difficulties in providing accurate information to the patient. In a study conducted among breast cancer specialists, most physicians (71%) had difficulty interpreting VUS reports, and 39% stated that they did not know how to provide counseling (28). Therefore, due to the problems in counseling, interpretation of clinical effects, and planning of patient treatment in patients with VUS variants, re-evaluation of VUS variants is essential for accurate interpretation and access to clinical geneticists would also be helpful.

### Study Limitations

The current study has some limitations. The result of the study presents a limited study population from one center and one ethnic group. However, the variant reclassification is made according to databases that mainly include European population data. There is no information about the presence of mutations in non-*BRCA* genes, and this is also another limitation of the study. Patients with VUS variants may have P/LP variants in non-*BRCA* genes. Based on some limitations of the current study, further studies are needed and should include a large study population and information about non-BRCA genes.

Improvements in bioinformatic tools and database updates may eventually diminish the reclassification rates. The current guidelines recommend reporting the *BRCA1/2* VUS variants; however, none recommend making clinical decisions according to VUS results. VUS reclassification will be necessary in the clinical management of the disease. Most VUS variants are downgraded after initial classification, preventing the patients from unnecessary surgery, therapy, and misdiagnosis. The upgraded VUS variants will facilitate surgical decisions, targeted therapy options, reproductive decision-making, and predictive at-risk family member screening.

In conclusion, this study demonstrates that the *BRCA1/2*-specific ACMG/AMP classification guidelines and current databases can be effectively used for VUS re-classification. VUS reclassification is important to avoid unnecessary treatment and to provide accurate risk management. This study supports the utility of *BRCA*-specific ACMG/AMP guidelines and updated genomic databases in improving the clinical utility of genetic testing.

### Ethics

**Ethics Committee Approval:** This study was approved by the Yüksek İhtisas University Medical School Ethical Committee (approval number: 296, date: 14.04.2025).

**Informed Consent:** All participants provided their written informed consent before undergoing molecular analysis.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: L.Ö., S.A., E.Ü.; Concept: L.Ö., S.A., E.Ü.; Design: L.Ö.; Data Collection or Processing: L.Ö., S.A., E.Ü.; Analysis or Interpretation: L.Ö., S.A., E.Ü.; Literature Search: L.Ö.; Writing: L.Ö., S.A., E.Ü.

**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. NCCN Clinical Practice Guidelines in Oncology. Genetic/Familial High-Risk Assessment: Breast, Ovarian, Pancreatic, and Prostate, Version 3.2025. [Crossref]
2. Calabrese A, von Arx C, Tafuti AA, Pensabene M, De Laurentiis M. Prevention, diagnosis and clinical management of hereditary breast cancer beyond BRCA1/2 genes. Cancer Treat Rev. 2024; 129: 102785. (PMID: 38870570) [Crossref]
3. Turner SA, Rao SK, Morgan RH, Vnencak-Jones CL, Wiesner GL. The impact of variant classification on the clinical management of hereditary cancer syndromes. Genet Med. 2019; 21: 426-430. (PMID: 29875428) [Crossref]
4. Parsons MT, de la Hoya M, Richardson ME, Tudini E, Anderson M, Berkofsky-Fessler W, et al. Evidence-based recommendations for gene-

specific ACMG/AMP variant classification from the ClinGen ENIGMA BRCA1 and BRCA2 variant curation expert panel. Am J Hum Genet. 2024; 111: 2044-2058. (PMID: 39142283) [Crossref]

5. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015; 17: 405-424. (PMID: 25741868) [Crossref]
6. Moukadem HA, Al Masry A, Atwani RW, Kreidieh F, Khalil LE, Saroufim R, et al. Genetic counseling, screening and risk-reducing surgery in patients with primary breast cancer and germline BRCA mutations: unmet needs in low- and middle-income countries. Eur J Breast Health. 2021; 18: 16-20. (PMID: 35059587) [Crossref]
7. Richter S, Haroun I, Graham TC, Eisen A, Kiss A, Warner E. Variants of unknown significance in BRCA testing: impact on risk perception, worry, prevention and counseling. Ann Oncol. 2013; 24(Suppl 8): viii69-viii74. (PMID: 24131974) [Crossref]
8. Welsh JL, Hoskin TL, Day CN, Thomas AS, Cogswell JA, Couch FJ, et al. Clinical decision-making in patients with variant of uncertain significance in BRCA1 or BRCA2 genes. Ann Surg Oncol. 2017; 24: 3067-3072. (PMID: 28766224) [Crossref]
9. Stella S, Vitale SR, Massimino M, Martorana F, Tornabene I, Tomarchio C, et al. In silico prediction of BRCA1 and BRCA2 variants with conflicting clinical interpretation in a cohort of breast cancer patients. Genes (Basel). 2024; 15: 943. (PMID: 39062721) [Crossref]
10. Fanale D, Pivetti A, Cancelliere D, Spera A, Bono M, Fiorino A, et al. BRCA1/2 variants of unknown significance in hereditary breast and ovarian cancer (HBOC) syndrome: looking for the hidden meaning. Crit Rev Oncol Hematol. 2022; 172: 103626. (PMID: 35150867) [Crossref]
11. Evans DG, van Veen EM, Byers HJ, Evans SJ, Burghel GJ, Woodward ER, et al. High likelihood of actionable pathogenic variant detection in breast cancer genes in women with very early onset breast cancer. J Med Genet. 2022; 59: 115-121. (PMID: 33758026) [Crossref]
12. Bono M, Fanale D, Incorvaia L, Cancelliere D, Fiorino A, Calò V, et al. Impact of deleterious variants in other genes beyond BRCA1/2 detected in breast/ovarian and pancreatic cancer patients by NGS-based multi-gene panel testing: looking over the hedge. ESMO Open. 2021; 6: 100235. (PMID: 34371384) [Crossref]
13. Celik Demirbas B, Kilic Erciyas S, Sukruoglu Erdogan O, Pasin O, Yalniz Kayim Z, Özgel MÇ, et al. Genetic insights into BRCA1/2 associated breast cancer in Türkiye: focus on early-onset and aggressive subtypes. Discov Oncol. 2025; 16: 746. (PMID: 40355587) [Crossref]
14. Bahsi T, Erdem HB. Spectrum of BRCA1/BRCA2 variants in 1419 Turkish breast and ovarian cancer patients: a single center study. Turkish Journal of Biochemistry. 2019; 45: 83-90. [Crossref]
15. Boga I, Ozemri Sag S, Duman N, Ozdemir SY, Ergoren MC, Dalci K, et al. A multicenter study of genotype variation/demographic patterns in 2475 individuals including 1444 cases with breast cancer in Turkey. Eur J Breast Health. 2023; 19: 235-252. (PMID: 37415649) [Crossref]
16. Zang F, Ding X, Chen J, Hu L, Sun J, Zhang J, et al. Prevalence of BRCA1 and BRCA2 pathogenic variants in 8627 unselected patients with breast cancer: stratification of age at diagnosis, family history and molecular subtype. Breast Cancer Res Treat. 2022; 195: 431-439. (PMID: 35974241) [Crossref]
17. Ha HI, Ryu JS, Shim H, Kong SY, Lim MC. Reclassification of BRCA1 and BRCA2 variants found in ovarian epithelial, fallopian tube, and primary peritoneal cancers. J Gynecol Oncol. 2020; 31: e83. (PMID: 33078592) [Crossref]
18. Mighton C, Charames GS, Wang M, Zakoor KR, Wong A, Shickh S, et al. Variant classification changes over time in BRCA1 and BRCA2. Genet Med. 2019; 21: 2248-2254. Erratum in: Genet Med. 2019; 21: 2406-2407. (PMID: 30971832) [Crossref]
19. Fanale D, Fiorino A, Incorvaia L, Dimino A, Filorizzo C, Bono M, et al. Prevalence and spectrum of germline BRCA1 and BRCA2 variants of uncertain significance in breast/ovarian cancer: mysterious signals from the genome. Front Oncol. 2021; 11: 682445. Erratum in: Front Oncol. 2022; 12: 920342. (PMID: 34178674) [Crossref]
20. So MK, Jeong TD, Lim W, Moon BI, Paik NS, Kim SC, et alJ. Reinterpretation of BRCA1 and BRCA2 variants of uncertain significance in patients with hereditary breast/ovarian cancer using the ACMG/AMP 2015 guidelines. Breast Cancer. 2019; 26: 510-519. (PMID: 30725392) [Crossref]
21. Zanti M, O'Mahony DG, Parsons MT, Dorling L, Dennis J, Boddicker NJ, et al. Analysis of more than 400,000 women provides case-control evidence for BRCA1 and BRCA2 variant classification. medRxiv [Preprint]. 2024: 2024.09.04.24313051. Update in: Nat Commun. 2025; 16: 4852. (PMID: 39281752) [Crossref]
22. Benet-Pagès A, Laner A, Nassar LR, Wohlfrom T, Steinke-Lange V, Haeussler M, et al. Reclassification of VUS in BRCA1 and BRCA2 using the new BRCA1/BRCA2 ENIGMA track set demonstrates the superiority of ClinGen ENIGMA expert panel specifications over the standard ACMG/AMP classification system. Genet Med Open. 2025; 3: 101961. (PMID: 40027238) [Crossref]
23. Innella G, Ferrari S, Miccoli S, Luppi E, Fortuno C, Parsons MT, et al. Clinical implications of VUS reclassification in a single-centre series from application of ACMG/AMP classification rules specified for *BRCA1/2*. J Med Genet. 2024; 61: 483-489. (PMID: 38160042) [Crossref]
24. Mersch J, Brown N, Pirzadeh-Miller S, Mundt E, Cox HC, Brown K, et al. Prevalence of variant reclassification following hereditary cancer genetic testing. JAMA. 2018; 320: 1266-1274. (PMID: 30264118) [Crossref]
25. Macklin S, Durand N, Atwal P, Hines S. Observed frequency and challenges of variant reclassification in a hereditary cancer clinic. Genet Med. 2018; 20: 346-350. (PMID: 29215655) [Crossref]
26. Özdemir Y, Çağ M, Seyhan S, Özkul Y, Dündar M, Konya A. Reclassification of hereditary cancer genes variants. Turk J Oncol 2022; 37: 462-467 [Crossref]
27. Deignan JL, Chung WK, Kearney HM, Monaghan KG, Rehder CW, Chao EC, et al. Points to consider in the reevaluation and reanalysis of genomic test results: a statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2019; 21: 1267-1270. (PMID: 31015575) [Crossref]
28. Culver JO, Brinkerhoff CD, Clague J, Yang K, Singh KE, Sand SR, et al. Variants of uncertain significance in BRCA testing: evaluation of surgical decisions, risk perception, and cancer distress. Clin Genet. 2013; 84: 464-472. (PMID: 23323793) [Crossref]
29. Murray ML, Cerrato F, Bennett RL, Jarvik GP. Follow-up of carriers of BRCA1 and BRCA2 variants of unknown significance: variant reclassification and surgical decisions. Genet Med. 2011; 13: 998-1005. (PMID: 21811163) [Crossref]
30. Morgan R, Brown A, Hamman KJ, Sampson J, Naik A, Massimino K. Risk management decisions in women with BRCA1 and BRCA2 mutations. Am J Surg. 2018; 215: 899-903. (PMID: 29499861) [Crossref]

**Original Article**

Eur J Breast Health 2025; 21(4): 301-306
**DOI:** 10.4274/ejbh.galenos.2025.2025-2-8

# Lymphedema and Axillary-Lateral Thoracic Vessel Juncture Irradiation: A Clinical Dilemma

Şükran Şenyürek[1], Merve Duman[1], Sena Birsen Güçlü[2], Nilüfer Kılıç Durankuş[1], Duygu Sezen[1], Yasemin Bölükbaşı[1]

[1]Department of Radiation Oncology, Koç University Faculty of Medicine, İstanbul, Türkiye
[2]Koç University Faculty of Medicine, İstanbul, Türkiye

## ABSTRACT

**Objective:** Regional nodal irradiation (RNI) is one of the main causes of breast cancer-related lymphedema (BCRL). However, studies on the relationship between the radiation dose to the axillary-lateral thoracic vessel juncture (ALTJ) region and BCRL have reported conflicting results. Based on these findings, we aimed to evaluate the clinical relevance of the dose to the ALTJ region in our patient cohort.

**Materials and Methods:** Patients diagnosed with breast cancer and who were treated at Koç University Hospital between 2016 and 2022 and received RNI were included. BCRL was defined as a difference in arm circumference between the ipsilateral and contralateral limb >2.5 cm at any single encounter or ≥2 cm on ≥2 visits. ALTJ was retrospectively contoured, and doses were recorded as equivalent dose ($\alpha/\beta = 3$).

**Results:** Of the 129 patients (median age 49 years) who met the inclusion criteria, 12 (9.3%) had lymphedema. Two-thirds of the patients (66.7%) were stage II, and one-third (33.3%) were stage III. The median follow-up was 22 months. The median (range) ALTJ $D_{mean}$ dose was 18.11 (1.87–50) Gy, the median ALTJ $D_{max}$ was 44.53 (12.8–71.1) Gy, and the median ALTJ V35 was 38% (1–100%). No significant association was determined between ALTJ parameters and BCRL.

**Conclusion:** There is insufficient data to define ALTJ as an OAR for decreasing BCRL risk. It is not appropriate to define dose and target based on ALTJ. Prospective studies with larger patient populations are needed to clarify the relationship between ALTJ and lymphedema.

**Keywords:** Breast cancer; lymphedema; radiotherapy

**Cite this article as:** Şenyürek Ş, Duman M, Güçlü SB, Kılıç Durankuş N, Sezen D, Bölükbaşı Y. Lymphedema and axillary-lateral thoracic vessel juncture irradiation: a clinical dilemma. Eur J Breast Health. 2025; 21(4): 301-306

### Key Points

- The aim of study was investigate the accosiation of reciving dose axillary-lateral thoracic vessel juncture and breast cancer related lymphedema.
- Patients were treated whole breast radiotherapy/chestwall radiotherapy and regional nodal radiotherapy were evaluated retrospectively.
- The dose axillary-lateral thoracic vessel juncture region was not detected significant factor for the development of breast cancer-related lymphedema.

## Introduction

Breast cancer-related lymphedema (BCRL) is a common and significant complication following breast cancer treatment. A meta-analysis of 84 cohort studies, including 58,358 patients, found the pooled incidence of lymphedema to be 21.9%, indicating that approximately one in five breast cancer survivors developed BCRL as a consequence of multimodal treatment (1).

The lymphedema in randomized studies evaluating the oncological results of axillary lymph node dissection (ALND) and sentinel lymph node biopsy (SLNB) showed that the risk of lymphedema in the SLNB group was between 7–11%, where this rate increases to 14–23% in patients who underwent ALND (2, 3). In a more recent meta-analysis of studies comparing SLNB and ALND, the prevalence of lymphedema was 13.7% and 24.2%, respectively (4).

There is a well-established relationship between radiotherapy and lymphedema. Specifically, the risk of lymphedema is significantly higher in breast cancer patients who receive regional nodal irradiation (RNI) compared to those who undergo whole-breast irradiation alone. This risk is more significant in patients who receive RNI following ALND, underlying the additive impact of these treatments on the lymphatic system (5).

**Corresponding Author:**
Yasemin Bölükbaşı MD; ybolukbasi@kuh.ku.edu.tr

**Received:** 27.02.2025
**Accepted:** 21.06.2025
**Epub:** 04.08.2025
**Available Online Date:** 25.09.2025

The axillary-lateral thoracic vessel juncture (ALTJ) area has been identified as a structure at risk for lymphedema in patients who have undergone lymphatic irradiation in the last five years (6-8). Notably, the three studies advocating for recognizing ALTJ as an "organ at risk" (OAR) have published different dosimetric parameters significantly associated with lymphedema risk. Besides that, the latest study by Healy et al. (9) failed to find clinically meaningful importance of the ALTJ as an OAR. This topic remains an open question in the literature, warranting further research and discussion. Therefore, we aimed to assess the controversial relationship between ALTJ and BCRL through a retrospective analysis of our patient cohort.

## Materials and Methods

### Patients Selection

Patients diagnosed with breast cancer who were treated at Koç University Hospital between 2016 and 2022 and received whole breast radiotherapy/chest wall radiotherapy and regional nodal radiotherapy were evaluated retrospectively. The study population included patients presenting with lymph node positivity and/or tumors classified as T3 or T4 according to the TNM staging system. Patients who had complete arm measurement information for lymphedema monitoring and had at least one year follow-up were included in the study. Patients who presented with lymphedema either prior to radiotherapy or at the time of their initial clinical diagnosis were excluded from the study to avoid confounding factors related to pre-existing disease. In addition, patients who developed local, regional, or distant metastases during the follow-up period were excluded, as metastatic progression and its associated treatments could independently influence the risk of lymphedema. Furthermore, patients for whom radiotherapy treatment planning data were unavailable were also excluded to ensure the accuracy and consistency of the dosimetric analysis. Patients who used adjuvant capecitabine, immunotherapy, or CDK4i during radiotherapy were excluded from the study as the effect of new chemotherapy agents on lymphedema is unknown.

### Treatment Protocol

All patients were simulated with a Siemens 4DCT scan and a 1.25 mm slice thickness. All patients were immobilized with arms upsided and customized vac-lac. The conventional (50 Gy/25 fr) or hypofraction schema (42.56 Gy/16 fr) were used for adjuvant radiotherapy of breast cancer for RNI. The most commonly used treatment technique is Field in Field (FinF).

### Axillary-Lateral Thoracic Vessel Juncture Delineation

The ALTJ area was contoured retrospectively by a single radiation oncologist (Ş.Ş.) for all patients according to the guideline of Gross et al. (6) (Figure 1a-b).

The borders were defined as:

The cranial border: One axial slice below the humeral head

The caudal border: The inferior of the axillary vessels

The anterior border: The plane defined by posterior of pectoralis major

The posterior border: The anterior surface of the subscapularis and latissimus dorsi muscles.

The lateral border: Included the axillary vessels

The medial border: The lateral border of the pectoralis minor muscle.

To validate contouring, one patient for every ten patients was randomly selected and checked by another radiation oncologist (Y.B.). The mean dose, maximum dose and V35 values of ALTJ were recorded as equivalent dose (EQD2) which $\alpha/\beta = 3$ was settled for late toxicity.

### Lymphedema Definition

Limb circumferences were taken routinely with a tape measure before and at three-month intervals for the first two years using the same landmarks to avoid excessive pressure during evaluation. The arm circumferences were measured in centimeters using a standardized flexible tape measure for all patients. Measurements were performed in the affected and unaffected limbs at 10 cm above (proximal) and below (distal) the elbow, circumference of outstretched inner hand, and wrist crease. BCRL was defined as a difference in arm circumference between the ipsilateral and contralateral limb >2.5 cm at any single encounter or ≥2 cm on ≥2 visits.

### Statistical Analysis

The primary objective was to evaluate the relationship between ALTJ dosimetric parameters and BCRL. The secondary objective

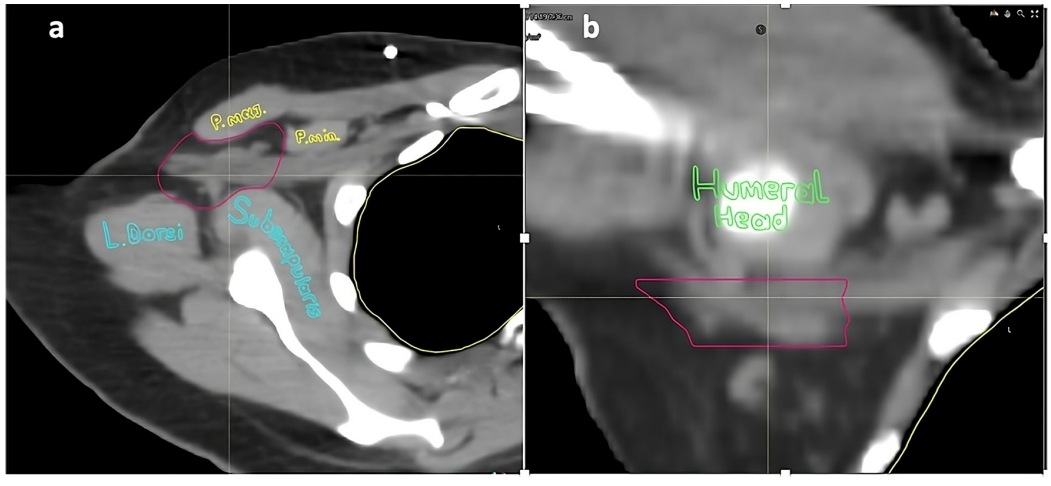



**Figure 1.** Axial plan of axila (a), coronal plan of axilla (b)

*P.min.: Pectoralis minor muscle, P. maj.: Pectoralis major muscle, L. Dorsi: Latissimus Dorsi muscle*

was to investigate the association between clinical and pathological characteristics and the development of BCRL. Cox proportional hazards regression models were used to test these associations. Univariate analyses were initially performed to assess the association between potential risk factors for BCRL, including body mass index (BMI), type of surgery, presence of axillary seroma, number of dissected lymph nodes, number of positive lymph nodes, clinical stage, radiotherapy scheme, radiotherapy technique, and ALTJ dose parameters. Variables demonstrating statistical significance ($p<0.05$) in the univariate analyses were subsequently entered into a multivariate logistic regression model to identify independent predictors of BCRL. The most optimal cut-off values for ALTJ $D_{mean}$, ALTJ $D_{max}$, and ALTJ V35 Gy were determined using receiver operating characteristic (ROC) curve analysis to investigate how they related to BCRL.

### Ethical Approval

The retrospective research design of this study (approval number: 2024.350.IRB2.149, date: 14.10.2024) was approved by the Institutional Review Board at Koç University.

## Results

Patients eligible for inclusion numbered 129, with a median (range) age of 49 (26–86) years. Of these, 86 patients had clinical stage II, and 43 patients had clinical stage III breast cancer (Table 1). The median follow-up was 22 (12–89) months. Lymphedema was observed in 12 patients (9.3%).

The majority of patients had undergone mastectomy (80.6%) and 19.4% had lumpectomy. Of the patients, ALND was performed in 58.9% and SLNB in 41.1%. Axillary seroma was observed in 20% of patients. The number of removed lymph nodes was >15 in 28% of the patients. A total of 26 patients (20.2%) had ≥4 positive lymph nodes. In 25.5% of the patients, the BMI value was 30 kg/m$^2$ or higher.

In terms of pathological receptor status, 84 patients (65.1%) identified as hormone reseptor (HR)(+)/human epidermal growth factor receptor 2HER2(-), 29 patients (22.5%) as HER2(+), and 16 patients (12.4%) as triple negative. Regarding systemic treatment, 82 patients (63.6%) received neoadjuvant chemotherapy, 36 patients (27.9%) received adjuvant chemotherapy, and 11 patients (8.5%) did not receive any chemotherapy.

Radiotherapy schemes were used in 70 patients (54.3%) whereas hypofractionated schemes were used in 59 patients (45.7%). The radiotherapy technique used was predominantly the FinF technique in 107 patients (82.9%), while 22 patients (17.1%) were treated with intensity-modulated radiotherapy (IMRT).

Dosimetric parameters of the ALTJ in EQD2 terms were: median ALTJ $D_{max}$ was 44.53 (12.8–71.1) Gy, the median ALTJ $D_{mean}$ was 18.11 (1.87–50) Gy, and the median ALTJ V35 Gy was 38% (1–100%).

In the univariate analysis, the number of removed lymph nodes, BMI and axillary dissection type were evaluated in terms of predictor of lymphedema. In the multivariate analysis, more than 15 removed lymph nodes ($p = 0.002$), ALND ($p = 0.015$), and BMI ≥30 kg/m$^2$ ($p = 0.006$) were identified as significant predictive factors for lymphedema (Table 2).

Furthermore, ROC analysis performed for ALTJ $D_{max}$, ALTJ $D_{mean}$, and ALTJ V35 Gy parameters in predicting lymphedema development did not identify any significant threshold values (Figure 2). In the patient group treated with IMRT, ALTJ doses were found to be significantly higher ($p = 0.003$) than those receiving the FinF technique, but this had no significant effect on the development of lymphedema.

## Discussion and Conclusion

In this retrospective study, we analyzed breast cancer patients who had undergone regional lymph node radiotherapy, and no significant association was detected between the dose to the ALTJ region and the development of BCRL. The results indicated that the number of removed lymph nodes (>15), the type of axillary intervention (ALND), and a high BMI (≥30 kg/m$^2$) were identified as predictive risk factors for BCRL. The current study is the first Turkish breast cancer cohort evaluating the delivered dose to the ALTJ in relation to the development of lymphedema.

**Table 1. Patients characteristics**

| | All patients (n) | All patients (%) |
|---|---|---|
| **Age, median, years** | 49 (26–86) | - |
| **Follow-up duration, months** | 22 (12–89) | - |
| **Clinical T-stage** | | |
| **T1** | 46 | 35.7 |
| **T2** | 66 | 51.2 |
| **T3** | 9 | 7 |
| **T4** | 8 | 6.1 |
| **Clinical N-stage** | | |
| **N0** | 25 | 19.4 |
| **N1** | 74 | 57.4 |
| **N2** | 15 | 11.6 |
| **N3** | 15 | 11.6 |
| **Stage groups** | | |
| II | 86 | 66.7 |
| III | 43 | 33.3 |
| **Pathological receptor status** | | |
| HR(+)/HER2(-) | 84 | 65.1 |
| HER2(+) | 29 | 22.5 |
| HR(-)/HER2(-) | 16 | 12.4 |
| **Systemic treatment** | | |
| Neoadjuvant chemotherapy | 82 | 63.6 |
| Adjuvant chemotherapy | 36 | 27.9 |
| No chemotherapy | 11 | 8.5 |
| **Lymphedema** | | |
| No | 117 | 90.7 |
| Yes | 12 | 9.3 |

HR: Hormone reseptor; HER2: Human epidermal growth factor receptor 2

The relationship between the ALTJ region and lymphedema has been established based on the distinction of breast and arm lymphatic drainage. The upper limb drainage nodes, which are identified via the axillary reverse mapping (ARM) technique, were distinctly separate lymph nodes from the sentinel nodes draining the breast in 90% of cases. A systemic review by Ahmed et al. (10) indicated an overlap rate of up to 10%; moreover, Ngui et al. (11) confirmed this low overlap rate of 9.6%. Moreover, the majority of ARM nodes (72%) were located in the upper level 1 axilla, outside the tangential whole-breast radiotherapy fields (12). One of the studies supporting distinct lymphatic drainage systems was performed by Clough et al. (13). They mapped the sentinel lymph nodes of 242 patients diagnosed with stage I breast cancer and indicated that, apart from the site of the tumor in the breast, 98.2% of sentinel lymph nodes were found in the medial part of the axilla, alongside the lateral thoracic vein.

Based on data suggesting that the use of the ARM technique in surgical series reduces BCRL (14) and the knowledge that arm and breast drainage are distinct in most patients, Gross et al. (6) proposed that there could be a significant relationship between radiotherapy dose to

**Table 2. Results of univariate and multivariate analysis for BCRL**

| Characteristics | All patients (*n* = 129) | BCRL (*n* = 12) | Univariate *p*-value | Multivariate *p*-value |
|---|---|---|---|---|
| **BMI, kg/m²** | | | | |
| <30 | 96 | 5 | 0.01 | 0.006 |
| ≥30 | 33 | 7 | | |
| **Surgery, primary** | | | | |
| Lumpectomy | 25 | 3 | 0.42 | - |
| Mastectomy | 104 | 9 | | |
| **Surgery, axilla** | | | | |
| SLND | 53 | 1 | 0.01 | 0.01 |
| ALND | 76 | 11 | | |
| **Seroma, axilla** | | | | |
| Yes | 26 | 0 | 0.58 | - |
| No | 103 | 12 | | |
| **Removed lymph nodes** | | | | |
| 15 | 93 | 4 | 0.004 | 0.002 |
| >15 | 36 | 8 | | |
| **Positive lymph nodes** | | | | |
| <4 | 103 | 9 | 0.45 | - |
| ≥4 | 26 | 3 | | |
| **Stage groups** | | | | |
| II | 67 | 6 | 0.42 | - |
| III | 50 | 6 | | |
| **Radiotherapy scheme** | | | | |
| Conventional | 70 | 7 | 0.50 | - |
| Hypofraction | 59 | 5 | | |
| **RT technique** | | | | |
| Field in Field | 107 | 12 | 0.09 | - |
| IMRT | 22 | 0 | | |
| **ALTJ $D_{max}$ (EQD2), median Gy** | 44.53 (12.8–71.1) | 42.1 (36.8–67.95) | 0.72 | - |
| **ALTJ $D_{mean}$ (EQD2), median Gy** | 18.11 (1.87–50) | 16.45 (1.87–35.57) | 0.17 | - |
| **ALTJ V35 Gy (EQD2), median (%)** | 38 (1–100) | 27 (2–74) | 0.18 | - |
| **ALTJ V35 Gy (EQD2)** | | | | |
| **≤66%** | 99 | 11 | 0.18 | - |
| **>66%** | 30 | 1 | | |

ALTJ: Axillary-lateral thoracic vessel juncture; ALND: Axillary lymph node dissection; BCRL: Breast cancer-related lymphedema; BMI: Body mass index; EQD2: Equivalent dose; N: Number of patients; SLNB: Sentinel lymph node biopsy; Gy: Gray; RT: Radiation therapy; $D_{mean}$: Mean dose; $D_{max}$: Maximum dose; V35 Gy: Percantage of volume receiving 35 Gy; IMRT: Intensity-modulated radiotherapy

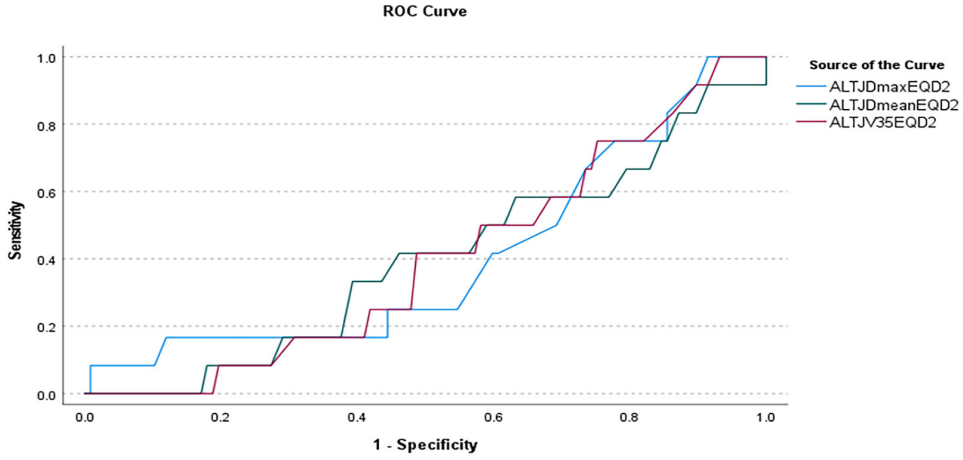



**Figure 2.** The outcomes of a receiver operating characteristic curve analysis examining the correlation between the ALTJ and EQD2 doses

*ROC: Receiver operating characteristic; ALTJ: Axillary-lateral thoracic vessel juncture; EQD2: Equivalent dose*

the upper level 1 axillary region, an area predominantly involved in arm drainage, and the development of BCRL. This study indicated that the most significant dosimetric variable and the cut-off point was ALTJ $D_{min}$ <36.8 Gy, which was associated with a 6.6-fold decrease in 3-year lymphedema rates (5.7% *vs.* 37.4%). Following this new OAR definition by Gross et al. (6), three studies have been published investigating the impact of radiotherapy doses on the ALTJ region and the risk of developing lymphedema. Two of these three studies have demonstrated that the ALTJ appears to be an OAR. Lei et al. (15), reported a significant reduction in ALTJ dose with VMAT, and we found higher ALTJ doses in patients treated with IMRT compared to the FinF technique. One of the studies validating the ALTJ as an OAR was conducted by Suk Chang et al. (7), which revealed that ALTJ V35 Gy of ≤66% in patients with ≤6 removed lymph nodes and ALTJ maximum dose of >53 Gy in patients with >15 removed lymph nodes, were identified as important factors using decision tree analysis. Another study by Park et al. (8) developed and validated a multivariable normal tissue complication probability model to predict lymphedema in breast cancer patients receiving radiation therapy. According to this model, patients were classified into three risk categories: high-risk [number of lymph nodes dissected (LNDno) >10 and ALTJ V35 >39.9%], moderate-risk (LNDno >10 and ALTJ V35 ≤39.9% or LNDno ≤10 and ALTJ V35 >39.9%), and low-risk (LNDno ≤10 and ALTJ V35 ≤39.9%). The risk of lymphedema was significantly higher in high-risk patients where both LNDno and V35 exceeded the cut-off values. In contrast to these three studies, a more recent study by Healy et al. (9) did not demonstrate a significant relationship between the dose to the ALTJ region and the development of BCRL. In the current study, which represents the fifth study in the literature analyzing the relationship between ALTJ and BCRL, no threshold value for ALTJ dose parameters was identified that could predict the risk of lymphedema. Additional analyses were conducted based on the threshold values of 39.9% and 66% for ALTJ V35 Gy reported in the literature; however, these analyses also did not yield significant results.

In patients with breast cancer, the type of surgical intervention to the axilla is among the most well-known risk factors for the development of lymphedema. In a meta-analysis of 67 studies published in 2023, focusing on upper limb morbidity associated with SLNB and ALND, it was once again shown that ALND significantly increased the risk of lymphedema compared to SLNB (13.7% and 24.2%) (4). Contrary to this clear relationship, studies investigating the relationship between the number of removed lymph nodes and the risk of lymphedema have reported conflicting results. Although Goldberg et al. (16), in their series of 600 patients, did not demonstrate a significant relationship between the number of lymph nodes removed and the risk of lymphedema, a meta-analysis of 84 studies published in 2022 by Shen et al. (1) concluded that removing more than 15 lymph nodes is a risk factor for lymphedema. In the present study, removing more than 15 lymph nodes and ALND were also significantly identified as a predictive factor for lymphedema.

High BMI is another parameter that has been identified as one of the most significant factors increasing the risk of lymphedema in breast cancer patients. Both BMI at diagnosis and weight gain in the postoperative period have been shown to contribute to this risk. In Chinese data, a BMI over 25 kg/m$^2$ has been shown to pose a risk for lymphedema due to physical differences, whereas in Western populations, this risk is observed when the BMI exceeds 30 kg/m$^2$ (1, 17). Our results demonstrated that a BMI of 30 kg/m$^2$ or higher was a significant risk factor for BCRL.

The nodal burden of the disease, age, and long follow-up duration are also risk factors for lymphedema and have been integrated into various lymphedema risk stratification models (18, 19). While our study did not demonstrate a significant association between the number of positive lymph nodes and lymphedema, presumably due to the small cohort size, the role of nodal disease burden as a strong predictive factor for lymphedema is well recognized in the literature. The progressive fibrotic changes induced by radiotherapy, compounded by age-related factors, underscore the importance of extended follow-up periods in capturing the full spectrum and incidence of lymphedema among BC survivors (20).

### Study Limitations

This study has potential limitations. Firstly, due to the retrospective nature of the study, we included only the patients with sufficient arm measurements. Secondly, even though the study explored a new concept in breast cancer treatment planning, it was limited by the small sample size. The relatively small sample size of the study may have limited statistical power, reducing the ability to detect significant

associations between variables. A lower number of patients increased the risk of type II errors, where actual differences or effects may go undetected. Thus, our findings should be considered as preliminary, necessitating confirmation through studies with larger, adequately powered populations. Thirdly, longer follow-up after 5 years could provide more meaningful and robust findings due to the high cure rates of breast cancer. Lastly, patients with lymphedema prior to radiotherapy were excluded in order to minimize the impact of surgery on lymphedema. Therefore, patients who developed acute postoperative morbidity related to surgery were not evaluated within the scope of this study and should thus be considered a separate group in terms of risk of developing lymphedema.

Although our study did not demonstrate a significant association between the radiation dose to the ALTJ and the development of BCRL, delineating the ALTJ as an OAR in clinical practice and incorporating it into treatment plan optimization, but without compromising the clinical target volume, may offer a clinically safe and beneficial strategy, particularly for long-term breast cancer survivors.

### Ethics

**Ethics Committee Approval:** The retrospective research design of this study (approval number: 2024. 350.IRB2.149, date: 14.10.2024) was approved by the Institutional Review Board at Koç University.

**Informed Consent:** Retrospective study.

### Footnotes

### Authorship Contributions

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

**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. Shen A, Lu Q, Fu X, Wei X, Zhang L, Bian J, et al. Risk factors of unilateral breast cancer-related lymphedema: an updated systematic review and meta-analysis of 84 cohort studies. Support Care Cancer. 2022; 31: 18. (PMID: 36513801) [Crossref]
2. 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]
3. Donker M, van Tienhoven G, Straver ME, Meijnen P, van de Velde CJ, Mansel RE, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981-22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. Lancet Oncol. 2014; 15: 1303-1310. (PMID: 25439688) [Crossref]
4. Che Bakri NA, Kwasnicki RM, Khan N, Ghandour O, Lee A, Grant Y, et al. Impact of axillary lymph node dissection and sentinel lymph node biopsy on upper limb morbidity in breast cancer patients: a systematic review and meta-analysis. Ann Surg. 2023; 277: 572-580. (PMID: 35946806) [Crossref]
5. Shaitelman SF, Chiang YJ, Griffin KD, DeSnyder SM, Smith BD, Schaverien MV, et al. Radiation therapy targets and the risk of breast cancer-related lymphedema: a systematic review and network meta-analysis. Breast Cancer Res Treat. 2017; 162: 201-215. Erratum in: Breast Cancer Res Treat. 2017; 162: 217. (PMID: 28012086) [Crossref]
6. Gross JP, Lynch CM, Flores AM, Jordan SW, Helenowski IB, Gopalakrishnan M, et al. Determining the organ at risk for lymphedema after regional nodal irradiation in breast cancer. Int J Radiat Oncol Biol Phys. 2019; 105: 649-658. (PMID: 31260718) [Crossref]
7. Suk Chang J, Ko H, Hee Im S, Sung Kim J, Kyung Byun H, Bae Kim Y, et al. Incorporating axillary-lateral thoracic vessel juncture dosimetric variables improves model for predicting lymphedema in patients with breast cancer: a validation analysis. Clin Transl Radiat Oncol. 2023; 41: 100629. (PMID: 37131951) [Crossref]
8. Park YI, Chang JS, Ko H, Im SH, Kim JS, Byun HK, et al. Development and validation of a normal tissue complication probability model for lymphedema after radiation therapy in breast cancer. Int J Radiat Oncol Biol Phys. 2023; 116: 1218-1225. (PMID: 36739918) [Crossref]
9. Healy E, Beyer S, Jhawar S, White JR, Bazan JG. The axillary lateral vessel thoracic junction is not an organ at risk for breast cancer-related lymphedema. Int J Radiat Oncol Biol Phys. 2023; 117: 452-460. (PMID: 37059233) [Crossref]
10. Ahmed M, Rubio IT, Kovacs T, Klimberg VS, Douek M. Systematic review of axillary reverse mapping in breast cancer. Br J Surg. 2016; 103: 170-178. (PMID: 26661686) [Crossref]
11. Ngui NK, French J, Kilby CJ, Pathmanathan N, Elder EE. Axillary reverse mapping in patients with breast cancer: is it oncologically safe? J Surg Oncol. 2016; 113: 726-731. (PMID: 27041002) [Crossref]
12. Wang W, Ward R, Jia D, Ashworth S, Estoesta E, Moodie T, et al. Location of arm draining lymph node in relation to breast cancer radiotherapy field and target volume. Radiother Oncol. 2019; 133: 193-197. (PMID: 30446320) [Crossref]
13. Clough KB, Nasr R, Nos C, Vieira M, Inguenault C, Poulet B. New anatomical classification of the axilla with implications for sentinel node biopsy. Br J Surg. 2010; 97: 1659-1665. (PMID: 20799288) [Crossref]
14. Bhimani F, Feldman S, Cavalli A, Chen Y, Obaid L, Rachofsky C, et al. Axillary reverse mapping aids in reducing the rates of breast cancer-related lymphedema in underserved ethnically diverse population. Ann Surg Oncol. 2024; 31: 5937-5946. (PMID: 38844631) [Crossref]
15. Lei R, Zhang X, Li J, Sun H, Yang R. Auxiliary structures-assisted radiotherapy improvement for advanced left breast cancer. Front Oncol. 2021; 11: 702171. (PMID: 34367986) [Crossref]
16. Goldberg JI, Wiechmann LI, Riedel ER, Morrow M, Van Zee KJ. Morbidity of sentinel node biopsy in breast cancer: the relationship between the number of excised lymph nodes and lymphedema. Ann Surg Oncol. 2010; 17: 3278-3286. (PMID: 20574774) [Crossref]
17. Wang L, Chen H, Li Y, Wang H, Liu N, Yu M, et al. Body mass index increases the risk of breast cancer-related lymphedema at 6-18 months after surgery: a retrospective study. Support Care Cancer. 2023; 31: 278. (PMID: 37074508) [Crossref]
18. Kwan JYY, Famiyeh P, Su J, Xu W, Kwan BYM, Jones JM, et al. Development and validation of a risk model for breast cancer-related lymphedema. JAMA Netw Open. 2020; 3: e2024373. (PMID: 33175175) [Crossref]
19. Lin C, Su J, Wu AJ, Lin N, Hossack MS, Shi W, et al. External validation of a 5-factor risk model for breast cancer-related lymphedema. JAMA Netw Open. 2025; 8: e2455383. (PMID: 39836421) [Crossref]
20. Shang T, Liang J, Kapron CM, Liu J. Pathophysiology of aged lymphatic vessels. Aging (Albany NY). 2019; 11: 6602-6613. (PMID: 31461408) [Crossref]

Original Article

Eur J Breast Health 2025; 21(4): 307-325
DOI: 10.4274/ejbh.galenos.2025.2025-5-6

# Prognostic Performance of the Residual Cancer Burden Index With Respect to Molecular Breast Cancer Subtypes

Emanuelle Narciso Alvarez Valente, Anke Bergman, Marcelo Adeodato Bello, Luiz Claudio Santos Thuler

Instituto Nacional do Câncer (INCA), Master Degree, Rio de Janeiro, Brazil

**ABSTRACT**

**Objective:** The use of neoadjuvant chemotherapy (NAC) has improved outcomes in breast cancer (BC). The residual cancer burden index (RCB) predicts prognosis. This study evaluated RCB as a prognosticator in BC subtypes treated with NAC.

**Materials and Methods:** A retrospective cohort of BC patients was analyzed. Five-year distant recurrence-free survival (DRFS), disease-free survival (DFS), and overall survival (OS) were analyzed. Statistical analyses included descriptive statistics, ANOVA, chi-square test, Fisher's exact test, Kaplan-Meier curves, Log-Rank test, and Cox regression.

**Results:** Among 562 women, RCB correlated with BC subtypes and predicted worse DRFS, DFS, and OS. In stratified analyses by molecular subtype, the association was significant only for luminal B and triple-negative subtypes, with inconsistent findings for luminal A and human epidermal growth factor type 2-overexpressed subtypes.

**Conclusion:** The RCB index was shown to be a prognostic marker in BC in a Brazilian population with BC. Significant associations were found only for the luminal B and triple negative subtypes. Further research is required to investigate the prognostic utility of RCB in other larger populations.

**Keywords:** Breast cancer; breast cancer subtypes; neoadjuvant chemotherapy; immunohistochemistry; prognosis

**Cite this article as:** Valente ENA, A Bergman A, Bello MA, Thuler LCS. Prognostic performance of the residual cancer burden index with respect to molecular breast cancer subtypes. Eur J Breast Health. 2025; 21(4): 307-325

**Key Points**

- The residual cancer burden (RCB) index predicts outcomes in breast cancer treated with neoadjuvant chemotherapy; notable for luminal B and triple negative breast cancer (TNBC).
- RCB-III shows poor prognosis across breast cancer subtypes, with up to a five-fold increased risk.
- Luminal B and TNBC show significant survival differences when stratified by RCB class.
- RCB index as a continuous variable forecasts distant recurrence, progression, and death.
- The present study confirms the prognostic value of RCB index in a Brazilian cohort with breast cancer but there is a need for futher subtype-specific research.

## Introduction

Breast cancer (BC) is considered a systemic disease (1) and a significant public health issue. This is the most common cancer type and responsible for the highest cancer mortality rates among women (2). Significant changes in the understanding of tumor biology and BC treatment have taken place from the end of the 20th century. Treatment has evolved from initial surgical approach with the aim of locoregional disease control into multidisciplinary management with the introduction of systemic therapy, leading to significant improvements in disease-free survival (DFS) and overall survival (OS) (3, 4).

The National Surgical Adjuvant Breast and Bowel Project studies, B-18 (5, 6) and B-27 (7), initiated a new era in the treatment of BC and demonstrated other benefits when employing neoadjuvant chemotherapy (NAC). These include the greater possibility of being able to use conservative surgery and evaluating *in vivo* treatment responses based on tumor responses (6-9).

**Corresponding Author:**
Emanuelle Narciso Alvarez Valente MD; enavalente13@gmail.com

**Received:** 30.05.2025
**Accepted:** 07.07.2025
**Epub:** 05.09.2025
**Available Online Date:** 25.09.2025

The achievement of pathological complete response (pCR) with the use of NAC has been associated with increased survival rates (8-16). Given this evidence, this treatment has been validated as a reliable prognostic marker (17). The use of an index that, through post-NAC pathological criteria, is capable of predicting the chance of disease progression and death within 5 years makes it possible to evaluate the prognosis in patients undergoing this type of initial BC treatment. The residual cancer burden (RCB) Index stratifies patients with BC undergoing NAC into four groups; the RCB Index has demonstrated differences in distant recurrence-free survival (DRFS), DFS and OS, providing more precise information and facilitating strategic indications concerning adjuvant therapies (18-21).

There is a lack of studies from Latin America, and especially from Brazil, that demonstrate the usefulness of this index in clinical practice. This is important as there are marked differences in demographic and epidemiological population characteristics from those observed on other continents (22). Thus, the aim of the present study was to evaluate the prognostic power of the RCB Index in a cohort of patients with BC undergoing NAC at the Brazilian National Cancer Institute (INCA).

## Materials and Methods

### Study Design and Location

This study comprises a retrospective cohort investigation of patients with BC followed at the Cancer Hospital III, part of the (HCIII/INCA), located in the city of Rio de Janeiro, where around 1000 treatments are provided each year for BC. The study was approved by the INCA Human Research Ethics Committee under opinion 166.838, following resolution 466/12 of the National Health Council of the Ministry of Health (date: 04.01.2013).

### Eligibility Criteria

Female patients with a histopathologically-confirmed diagnosis of BC, of any ages and with clinical staging (CS) T1-4, N0-3 and M0 and having undergone initial NAC-based treatment were included in the study. Those who did not complete NAC or who required changes to the planned NAC regimen were excluded. Patients in whom data was not sufficient to make RCB calculations (bidimensional tumor bed, % total cellularity, % *in situ* cellularity, number of positive lymph nodes and the size of the largest metastasis), had no molecular subtype specified [incomplete or no data regarding estrogen receptor, progesterone receptor, human epidermal growth factor type 2 receptor (HER-2) and Ki-67 level], or who were pregnant at diagnosis, as well as those presenting with bilateral breast carcinoma, clinical and/or cardiological contraindications to the use of chemotherapy, non-epithelial tumors, a history of previous breast carcinoma and, finally, those classified as metastatic (M1) in the imaging evaluation but were then classified as non-metastatic in the initial clinical evaluation were excluded.

### Participant Selection

Initially, a total of 935 women, presenting between 2013 and 2015, were selected from the Hospital Cancer Registry. This period was chosen to allow a sufficient follow-up period of at least 5 years in order to properly analyze the study outcomes. After applying the eligibility criteria, 562 patients were included (Figure 1).

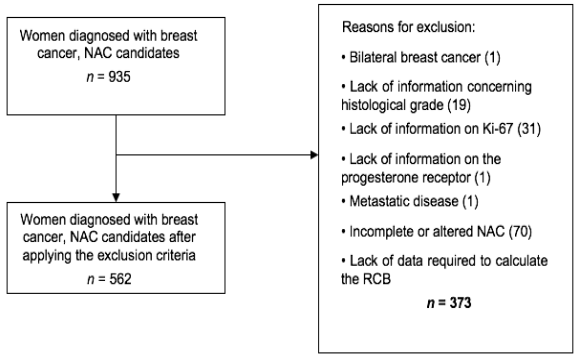



**Figure 1.** Study patient selection flowchart

*NAC: Neoadjuvant chemotherapy; RCB Residual cancer burden*

Sociodemographic, clinical, tumor, treatment and follow-up data (recurrence, metastasis and death) were obtained from electronic and physical records.

All patients underwent the same institutional treatment routine according to CS and molecular tumor profile determined by immunohistochemistry.

### RCB Index and RCB Class Calculations

The RCB index was calculated as a continuous variable, based on information obtained from the histopathological reports of the assessed surgical specimens, through data concerning the primary tumor bed (two-dimensional tumor bed, % global cellularity and % *in situ* disease) and lymph nodes (number of positive lymph nodes and diameter of the largest metastasis) (18).

The determination of global cellularity in the histopathological reports followed the INCA/HCIII Pathology Service standards, based on the sum of the cellularity of the invasive portion and the cellularity of the *in situ* portion.

This data was then used to calculate the RCB index using the formula $RCB = 1.4\ (f_{inv}d_{prim})^{0.17} + [4(1–0.75^{LN})d_{met}]^{0.17}$. The originally proposed cutoff points were applied for the data interpretation, resulting in four RCB classes, which indicate the progressive residual volume of disease, as follows: RCB-0 (RCB = 0, equivalent to pCR); RCB-1 (score >0–1.36), RCB-2 (score 1.37–3.28) and RCB-3 (score >3.28) (18). A random sample of 50 patients was included in the calculator (available at http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3) to validate the spreadsheet formula, obtaining a 100% agreement rate.

The absence of invasive breast and axilla carcinomas (pT0/pTis and pN0) was assumed to be pCR.

### Study Outcomes

To evaluate the prognostic power of the RCB index, a 5-year DRFS was proposed as the primary outcome, defined as the interval in months between the initial BC diagnosis and distant recurrence [Symmans et al. (18), 2007]. Secondary outcomes comprised DFS and 5-year OS. The DFS was considered as the time interval in months between the

pathological diagnosis and the appearance of a second primary tumor, recurrence of invasive or non-invasive disease, or death. The OS was considered as the period in months between the pathological diagnosis and the occurrence of death from any cause. Time was censored at the end of follow-up, on the date of the last institutional consultation (lost to follow-up) or December 31, 2020, the date the study ended.

**Statistical Analyses**

The descriptive statistics of the categorical variables are presented in tables, depicting their absolute and relative frequency distributions, while continuous variables are presented as central tendency measures (means and standard deviations). An analysis of variance (ANOVA) was applied to compare continuous variables, and associations between categorical variables, were verified by Pearson's chi-square test and Fisher's exact test, when indicated.

The DRFS, DFS and OS curves for the total population and each molecular subtype were constructed employing the Kaplan-Meier method and compared using the Log-Rank test. All survival curves were expressed as percentages versus time (in months).

Cox proportional hazards regression models (univariate and multiple) were used to explore the risk of distant progression, recurrence, or death according to RCB classes and the RCB index was used as a continuous variable.

Variables whose statistical significance in Cox univariate analyses exhibited $p<0.15$ values were inserted into a multiple model built sequentially using the Stepwise Forward method, beginning with the variable most strongly associated with the outcome and continuing until no other variable reached significance (23). Variables presenting with $p<0.05$ were maintained in the final model. All statistical analyses were performed using the Statistical Package for the Social Science (SPSS) for Windows, version 24 (IBM Inc., Armonk, NY, USA).

## Results

**Study Population Characteristics**

Data from the 562 patients included and presenting with the following molecular profiles were analyzed: 16.7% luminal A, 52.3% luminal B, 9.6% overexpressed HER2 and 21.4% triple negative breast cancer (TNBC). Mean patient age was 51.3±11.4 years old. Of the patients, 34.2% were Caucasian and 51.4% were menopausal. The predominant CS was III (53.2%), with 59.2% grade 1 or 2 and 92.3% of the histological findings indicated non-special type invasive carcinoma (CI-TNE). A total of 81.7% of all patients underwent mastectomies and 78.4%, axillary lymphadenectomies, while 97.5% underwent chemotherapy regimens based on anthracyclines and taxanes (Table 1).

Following NAC, the following distribution was observed between RCB classes: 12.1% pCR, 6.4% RCB-I, 51.1% RCB-II and 30.4% RCB-III. Furthermore, 11.9% still presented ypT3/T4 tumors and 40.2%, positive axilla. The post-treatment variables (RCB classes, ypT and ypN) were significantly different between the total population and the molecular BC subtypes (Table 2).

**Survival Analysis**

Supplementary Table 1 displays the calculated DRFS, DFS and OS percentages at 5 years per RCB class, both for the total population and for each molecular BC profile. Similar survival rates were observed between patients with pCR and those with RCB-I. However, RCB-II and RCB-III patients displayed progressively lower survival rates, with more evident differences in the most aggressive tumors (overexpressed HER2 and TNBC).

The Kaplan-Meier curves for DRFS, DFS and OS according to each RCB class are depicted in Supplementary Figure (SF) 1 and Figure 2. A significant difference ($p<0.001$) was observed when comparing the RCB classes for all survival curves in the total study population (SF 1A, Figure 2A and F). Concerning patients with luminal A tumors, the survival curves (SF 1B, Figure 2B and G) did not indicate significant differences when comparing RCB classes, while a significant difference in relation to the RCB classes was observed in all survival curves for those with luminal B tumors (SF 1C, Figure 2C and H) ($p<0.001$). No difference in OS was observed in patients with overexpressed HER2 tumors (Figure 2I) in relation to RCB class differences, while a significant difference was observed for DRFS and DFS (SF 1D, Figure 2D). The survival curves (SF 1E, Figure 2E and J) for patients presenting with TNBC tumors also exhibited differences in survival metrics depending on the RCB class.

**Risk Assessment According to the RCB Index and RCB Classes**

Univariate Cox regression analyses were performed (Supplementary Tables 2-4) to determine the risk of progression, recurrence, and death in the study population. Age and menopausal status were not significant in any of the analyses, nor was any difference between the RCB-0 and RCB-I classes detected.

In the general population, the adjusted Cox analysis indicated a gradual increase in the risk of DRFS, DFS and OS with increasing RCB Indices (Tables 3-5). Increases of 70, 80 and 70% were also observed concerning the risk of distant recurrence, recurrence or progression or death, respectively, with each 1-point score increase when the RCB index was used as a continuous variable.

The risks associated different RCB classes could not be calculated for patients with luminal A tumors, as no data convergence was obtained. However, when used as a continuous variable, the RCB index indicated increases of about two-fold, 2.6-fold and 5.2-fold concerning the risk of distant recurrence, recurrence or progression or death for each 1-point score increase, respectively (Tables 3-5).

In patients with luminal B tumors, the risk of DRFS, DFS and OS associated with the RCB classes increased by 4.5-fold, 4.6-fold and 4.2-fold, respectively, for RCB-III. When used as a continuous variable, the RCB index indicated an almost two-fold increase in the risk of distant recurrence, recurrence or progression or death for each 1-point score increase (Tables 3-5).

Risks associated with different RCB classes for patients with overexpressed HER-2 tumors could not be calculated, again due to a lack of data convergence. On the other hand, when the RCB index was employed as a continuous variable, a two-fold increase in the risk of distant recurrence, recurrence or progression or death was noted with each 1-point score increase ($p<0.001$) (Tables 3-5).

Finally, RCB classes exhibited independent prognostic value regarding the risk of distant recurrence, recurrence or progression and death in patients with TNBC, while an increasing risk as the RCB index increased was noted only for DRFS. The risk of distant recurrence, recurrence or progression or death increased two-fold ($p<0.001$) for each 1-point score RCB index increase (Tables 3-5).

**Table 1. Clinical characteristics of the whole study population and when stratified by molecular breast cancer subtypes at diagnosis**

| Pre-NAC variables | Total | Luminal A | Luminal B | Overexpressed HER2 | Triple negative | *p* |
|---|---|---|---|---|---|---|
| | *n* (%) | *n* (%) | *n* (%) | *n* (%) | *n* (%) | |
| | **562 (100.0)** | **94 (16.7)** | **294 (52.3)** | **54 (9.6)** | **120 (21.4)** | |
| **Age (years), mean (± SD)** | 51.3 (11.4) | 52.7 (11.1) | 51.4 (11) | 50.5 (11.6) | 50.0 (12.5) | 0.294[a] |
| **Race/skin color** | | | | | | |
| White | 192 (34.2) | 26 (27.7) | 98 (33.3) | 23 (42.6) | 45 (37.5) | |
| Non-white | 367 (65.3) | 68 (72.3) | 194 (66.0) | 31 (57.4) | 74 (61.7) | 0.452* |
| Missing | 3 (0.5) | 0 (0.0) | 2 (0.7) | 0 (0.0) | 1 (0.8) | |
| **Menopausal status** | | | | | | |
| Pre-menopausal (≤50 years old) | 273 (48.6) | 42 (44.7) | 143 (48.6) | 28 (51.9) | 60 (50.0) | 0.825 |
| Post-menopausal (>50 years old) | 289 (51.4) | 52 (55.3) | 151 (51.4) | 26 (48.1) | 60 (50.0) | |
| **Tumor size (T)** | | | | | | |
| T1/2 | 203 (36.1) | 45 (47.9) | 95 (32.3) | 25 (46.3) | 38 (31.6) | |
| T3 | 167 (29.7) | 22 (23.4) | 88 (29.9) | 16 (29.6) | 41 (34.2) | 0.05 |
| T4 | 192 (34.2) | 27 (28.7) | 111 (37.8) | 13 (24.1) | 41 (34.2) | |
| **Lymph nodes (N)** | | | | | | |
| N0 | 252 (44.8) | 52 (55.3) | 132 (44.9) | 21 (38.9) | 47 (39.2) | |
| N1 | 240 (42.7) | 31 (33.0) | 129 (43.9) | 27 (50.0) | 53 (44.2) | 0.192 |
| N2/ N3 | 70 (12.5) | 11 (11.7) | 33 (11.2) | 6 (11.1) | 20 (16.6) | |
| **Histological grade** | | | | | | |
| 1 or 2 | 389 (69.2) | 90 (95.7) | 230 (78.2) | 26 (48.1) | 43 (35.8) | |
| 3 | 164 (29.2) | 4 (4.3) | 62 (21.1) | 26 (48.1) | 72 (60.0) | **0.001*** |
| Missing | 9 (1.6) | 0 (0.0) | 2 (0.7) | 2 (3.8) | 5 (4.2) | |
| **Clinical staging** | | | | | | |
| I/ II | 263 (46.8) | 54 (57.4) | 130 (44.2) | 29 (53.7) | 50 (41.7) | 0.062 |
| III | 299 (53.2) | 40 (42.6) | 164 (55.8) | 25 (46.3) | 70 (58.3) | |
| Breast surgery | | | | | | |
| Breast conserving | 103 (18.3) | 14 (14.9) | 56 (19.0) | 7 (13.0) | 26 (21.7) | 0.428 |
| Mastectomy | 459 (81.7) | 80 (85.1) | 238 (81.0) | 47 (87.0) | 94 (78.3) | |
| **Axillary surgery** | | | | | | |
| Sentinel lymph node biopsy (SLNB) | 121 (21.5) | 22 (23.4) | 62 (21.1) | 13 (24.1) | 24 (20.0) | |
| Axillary lymph node dissection (ALND) | 388 (69.0) | 63 (67.0) | 201 (68.4) | 37 (68.5) | 87 (72.5) | 0.933 |
| SLNB + ALND | 53 (9.5) | 9 (9.6) | 31 (10.5) | 4 (7.4) | 9 (7.5) | |
| **Neoadjuvant regimen** | | | | | | |
| Anthracyclic + Taxane | 548 (97.5) | 93 (98.9) | 289 (98.3) | 52 (96.3) | 114 (95.0) | 0.173 |
| Other | 14 (2.5) | 1 (1.1) | 5 (1.7) | 2 (3.7) | 6 (5.0) | |

SD: Standard deviation; NAC: Neoadjuvant chemotherapy; HER: Human epidermal growth factor receptor-type 2; [a]: *p*-value obtained by an ANOVA analysis; *: *p*-value obtained by Fisher's exact test; Other *p* values calculated using the Pearson chi-square test; in bold, $p<0.05$

**Table 2. Characteristics of the whole study population and when stratified by molecular breast cancer subtypes by IHC following neoadjuvant chemotherapy**

| Pre-NAC variables | Total | Luminal A | Luminal B | Overexpressed HER2 | Triple negative | *p* |
|---|---|---|---|---|---|---|
| | *n* (%) | *n* (%) | *n* (%) | *n* (%) | *n* (%) | |
| | **562 (100.0)** | **94 (16.7)** | **294 (52.3)** | **54 (9.6)** | **120 (21.4)** | |
| **RCB classes** | | | | | | |
| 0 | 68 (12.1) | 2 (2.1) | 20 (6.8) | 15 (27.8) | 31 (25.8) | **0.001** |
| I | 36 (6.4) | 1 (1.1) | 25 (8.5) | 4 (7.4) | 6 (5.0) | |
| II | 287 (51.1) | 57 (60.6) | 149 (50.7) | 28 (51.8) | 53 (44.2) | |
| III | 171 (30.4) | 34 (36.2) | 100 (34.0) | 7 (13.0) | 30 (25.0) | |
| **YpT** | | | | | | |
| PCR | 105 (18.7) | 5 (5.3) | 45 (15.3) | 23 (42.6) | 32 (26.7) | **0.001*** |
| ypT1 | 193 (34.3) | 30 (31.9) | 115 (39.1) | 17 (31.5) | 31 (25.8) | |
| ypT2 | 197 (35.1) | 48 (51.1) | 99 (33.7) | 11 (20.4) | 39 (32.5) | |
| ypT3/T4 | 67 (11.9) | 11 (11.7) | 35 (11.9) | 3 (5.5) | 18 (15.0) | |
| **YpN** | | | | | | |
| ypN0 | 336 (59.8) | 47 (50.0) | 163 (55.4) | 42 (77.8) | 84 (70.0) | **0.001*** |
| ypN1 | 124 (22.1) | 30 (31.9) | 62 (21.1) | 9 (16.7) | 23 (19.2) | |
| ypN2/3 | 102 (18.1) | 17 (18.1) | 69 (23.5) | 3 (5.5) | 13 (10.8) | |

SD: Standard deviation; NAC: Neoadjuvant chemotherapy; IHC: Immunohistochemistry; HER: Human epidermal growth factor receptor-type 2; RCB: Residual cancer burden; *: *p*-value obtained by Fisher's exact test; other *p* values calculated by Pearson's chi-square test; in bold, p<0.05

## Discussion and Conclusion

This study included 562 BC patients with more than half with luminal B subtype, just over a fifth with TNBC and smaller proportions of luminal A and HER2. As the predominant CS was advanced (53.2%), most patients underwent radical surgery (81.7% mastectomies and 78.4% axillary lymphadenectomies). The most commonly used chemotherapy regimen was based on anthracyclines and taxanes (97.5%).

The survival curves analysis for the whole cohort and by molecular subtype indicated that patients of all subgroups with minimal residual disease (RD) (RCB-I) exhibit a similar prognosis at 5 years to those who achieved pCR (RCB-0). Conversely, those with extensive RD (RCB-III) exhibited a poor prognosis. Patients with an RCB-II classification, around 50% of the population, remain in need of additional investigations.

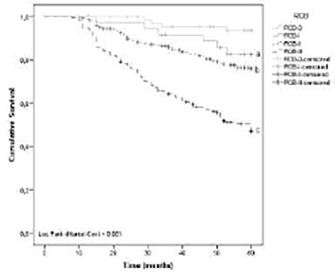
The RBC index analyzed as a continuous variable was associated with the prognosis of BC across the whole study population and in all molecular subtypes. However, when analyzed by class, this association was detected only in the total population and in patients with the luminal B and TNBC subtypes, probably due to the small sample size noted for the other profiles.

Concerning the total population, as well as in the study conducted by Hamy et al. (24) and in contrast to Symmans et al. (19), age and menopausal status did not exhibit statistically significant differences between the assessed molecular BC subtypes in both the univariate and multiple analyses. The inclusion criteria of the present study allowed for the study of patients presenting with T4, N2 and N3, similar to the study conducted by Gomes da Cunha et al. (22), who evaluated the Brazilian population, and differing from the original study carried out by Symmans et al. (18) and replicated by several other authors, such as Hamy et al. (24) and Yau et al. (25). Thus, patients presenting with more advanced CS than in the initial studies were included, with a predominance of CS III, positive hormone receptors, negative HER2, elevated Ki-67 and grades 1 and 2. The applied Kaplan-Meier analysis highlighted the significant drop in DRFS, DFS and OS with increasing RCB class and when the RCB index was analyzed as a continuous variable ($p$<0.001). The multiple analysis demonstrated an increase in the risk of distant progression, recurrence, and death by about two-fold for each 1-point increase in the RCB index ($p$<0.001), adjusted for T and Ki-67. The RCB II and III classes adjusted for T and ypN were also significantly associated with these survival parameters.

The Kaplan-Meier analyses did not indicate significant differences between RCB classes for patients with luminal A subtype. The multiple regression analysis indicated an increase in the risk of distant progression and recurrence by about 2.6-fold for each 1point increase in the continuous score, as well as an increase in the risk of death by more than five times with a one-point score increase. Such findings corroborate the results reported by Yau et al. (25), the only study demonstrating an increased risk among tumors classified as luminal and in contrast to the currently available literature.

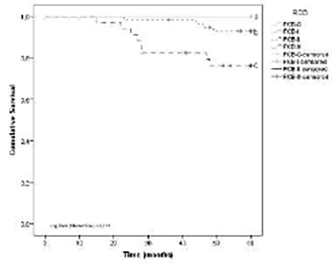
**Disease-free survival**

**A. General population**



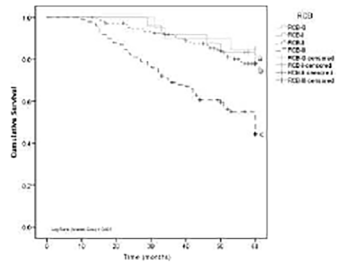
Note: a: not statistically significant in relation to the reference (RCB-0); b: not statistically significant in relation to curve a and statistically significant in relation to the reference; c: statistically significant to all prior curves.

**B. Population of patients presenting the luminal A subtype**



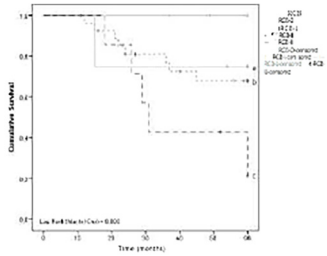
Note: a: calculation not possible due to the sample size; b: not statistically significant in relation to the reference (RCB-0) and curve a; c: statistically significant in relation to curve b.

**C. Population of patients presenting the luminal B subtype**



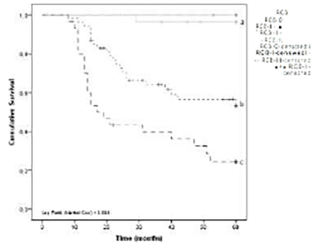
Note: a: not statistically significant in relation to the reference (RCB-0); b: not statistically significant in relation to the reference (RCB-0) and curve a; c: statistically significant in relation to all prior curves.

**D. Population of patients presenting the overexpressed HER2 subtype**



Note: a: not statistically significant in relation to the reference (RCB-0); b: statistically significant in relation to the reference and not statistically significant in relation to curve a; c: statistically significant only in relation to the reference.

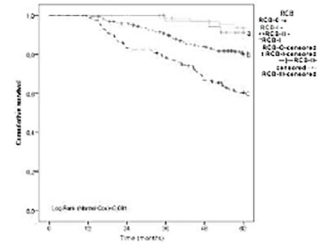
**E. Population of patients presenting the triple negative subtype**



Note: a: not statistically significant in relation to the reference (RCB-0); b: statistically significant in relation to the reference and curve a; c: statistically significant in relation to all prior curves.

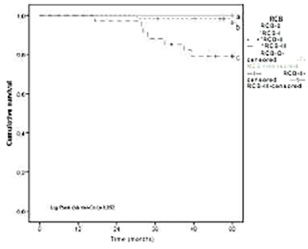
**Overall Survival**

**F. General population**



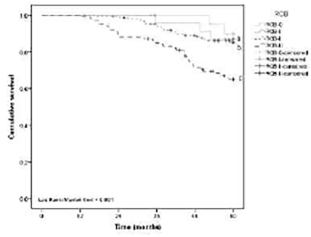
Note: a: not statistically significant in relation to the reference (RCB-0); b: statistically significant in relation to the reference and not statistically significant in relation to curve a; c: statistically significant in relation to all prior curves.

**G. Population of Patients presenting the Luminal A subtype**



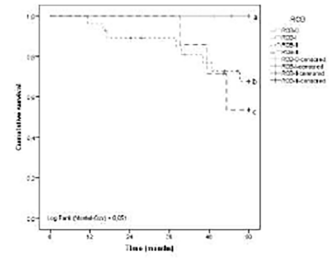
Note: a: not statistically significant in relation to the reference (RCB-0); b: statistically significant in relation to the reference and not statistically significant in relation to curve a; c: statistically significant in relation to all prior curves.

**H. Population of Patients presenting the Luminal B subtype**



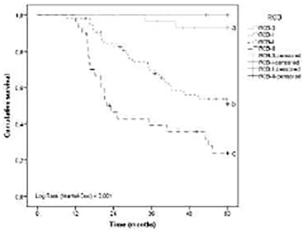
Note: a: not statistically significant in relation to the reference (RCB-0); b: not statistically significant in relation to the reference and curve a; c: statistically significant in relation to all prior curves.

**I. Population of patients presenting the overexpressed HER2 subtype**



Note: a: calculation not possible due to the sample size; b: not statistically significant in relation to the reference (RCB-0) and curve a; c: statistically significant in relation to the reference and not statistically significant in relation to curve b.

**J. Population of patients presenting the triple negative subtype**



Note: a: not statistically significant in relation to the reference (RCB-0); b: statistically significant in relation to the reference and curve a; c: statistically significant in relation to all prior curves.

**Figure 2.** Kaplan-Meier survival curves. Disease-free survival - A: general population; B: luminal A; C: luminal B; D: overexpressed HER2; E: triple negative. Overall survival - F: general population; G: luminal A; H: luminal B; I: overexpressed HER2; and J: triple negative

*RCB: Residual cancer burden; HER2: Human epidermal growth factor receptor 2*

**Table 3. Crude and adjusted Cox analysis according to RCB classes and RCB index in the whole study population and molecular breast cancer subtypes by immunhistochemistry regarding distant recurrence-free survival**

| | cHR (95% CI) | *p* | aHR (95% CI) | *p* |
|---|---|---|---|---|
| **Whole study population* (*n* = 562)** | | | | |
| RCB classes | | | | |
| 0 (*n* = 68) | Ref. | | Ref. | |
| I (*n* = 36) | 2.9 (0.8–10.4) | 0.096 | 3.1 (0.9–10.8) | 0.085 |
| II (*n* = 287) | **4.3 (1.6–11.8)** | **0.005** | 4.3 (1.6–11.7) | **0.005** |
| III (*n* = 171) | **11.7 (4.3–31.8)** | **<0.001** | 10.9 (3.9–29.6) | **<0.001** |
| RCB index (as a continuous variable) | 1.9 (1.6–2.2) | **<0.001** | 1.7 (1.1–2.5) | **<0.001** |
| **Luminal A*** (*n* = 94)** | | | | |
| RCB classes | | | | |
| 0 (*n* = 2) | Ref. | | Ref. | |
| I (*n* = 1) | £ | £ | £ | £ |
| II (*n* = 57) | £ | £ | £ | £ |
| III (*n* = 34) | £ | £ | £ | £ |
| RCB index (as a continuous variable) | 2.6 (1.3–5.2) | 0.008 | 2.6 (1.3–5.2) | 0.008 |
| **Luminal B (*n* = 294)** | | | | |
| RCB classes | | | | |
| 0 (*n* = 20) | Ref. | | Ref. | |
| I (*n* = 25) | 1.4 (0.4–6.0) | 0.627 | 1.5 (0.4–6.1) | 0.616 |
| II (*n* = 149) | 1.5 (0.5–5.0) | 0.493 | 1.5 (0.5–4.9) | 0.516 |
| III (*n* = 100) | **4.8 (1.5–15.5)** | **0.008** | 4.5 (1.4–14.4) | **0.012** |
| RCB index (as a continuous variable)*** | 1.8 (1.5–2.2) | **<0.001** | 1.8 (1.5–2.2) | **<0.001** |
| **Overexpressed HER2*** (*n* = 54)** | | | | |
| RCB classes | | | | |
| 0 (*n* = 15) | Ref. | | Ref. | |
| I (*n* = 4) | £ | 0.914 | £ | £ |
| II (*n* = 28) | £ | 0.912 | £ | £ |
| III (*n* = 7) | £ | 0.904 | £ | £ |
| RCB index (as a continuous variable) | 2.2 (1.5–3.3) | **<0.001** | 2.2 (1.5–3.3) | **<0.001** |
| **Triple negative*** (*n* = 120)** | | | | |
| RCB classes | | | | |
| 0 (*n* = 31) | Ref. | | Ref. | |
| I (*n* = 6) | 2.9 (0.8–10.4) | 0.096 | 2.9 (0.8–10.4) | 0.096 |
| II (*n* = 53) | **4.3 (1.6–11.8)** | **0.005** | 4.3 (1.6–11.8) | **0.005** |
| III (*n* = 30) | **11.7 (4.3–31.8)** | **<0.001** | 11.7 (4.3–31.8) | **<0.001** |
| RCB index (as a continuous variable) | 2.0 (1.6–2.5) | **<0.001** | 2.0 (1.6–2.5) | **<0.001** |

CI: Confidence interval; CS: Clinical staging; HER: Human epidermal growth factor receptor-type 2; RCB: Residual cancer burden; cHR: Crude hazard ratio; aHR: Adjusted hazard ratio. In bold, $p<0.05$; adjusted by: *T; **CS and ***without adjustment variables; £: Insufficient sample size for the calculation

Concerning patients with luminal B subtype, the Kaplan-Meier analyses demonstrated a significant difference between RCB classes. The multiple analysis demonstrated an increase in the risk of distant progression, recurrence and death of around two-fold for each 1-point increase in the continuous score, and when adjusted by estrogen receptor and Ki-67, only for the risk of death. The RCB class analysis for this subtype verified a significant prognostic association in RCB-III equivalent to pathological stage III, in contrast to the findings of Symmans et al. (19), probably due to the inclusion of patients with TNBC tumors in our study. These findings are, however, in line with the more recent results reported by Yau et al. (25).

**Table 4. Crude and adjusted Cox analysis according to RCB classes and RCB index in the whole study population and for molecular breast cancer subtypes regarding disease-free survival**

| | cHR (95% CI) | *p* | aHR (95% CI) | *p* |
|---|---|---|---|---|
| **Whole study population (*n* = 562)** | | | | |
| RCB classes* | | | | |
| 0 (*n* = 68) | Ref. | | Ref. | |
| I (*n* = 36) | 2.9 (0.8–10.3) | 0.099 | 3.0 (0.9–10.6) | 0.092 |
| II (*n* = 287) | **4.3 (1.6–11.9)** | **0.005** | 4.1 (1.5–11.2) | **0.007** |
| III (*n* = 171) | **11.8 (4.3–32.0)** | **<0.001** | 7.0 (2.2–21.7) | **0.001** |
| RCB index (as a continuous variable)** | 1.9 (1.6–2.2) | **<0.001** | 1.8 (1.6–2.1) | **<0.001** |
| **Luminal A[a] (*n* = 94)** | | | | |
| RCB classes | | | | |
| 0 (*n* = 2) | Ref. | | Ref. | |
| I (*n* = 1) | £ | £ | £ | £ |
| II (*n* = 57) | £ | £ | £ | £ |
| III (*n*=34) | £ | £ | £ | £ |
| RCB index (as a continuous variable) | 2.6 (1.3–5.2) | **0.008** | 2.6 (1.3–5.2) | **0.008** |
| **Luminal B (*n* = 294)** | | | | |
| RCB classes*** | | | | |
| 0 (*n* = 20) | Ref. | | Ref. | |
| I (*n* = 25) | 1.4 (0.4–6.0) | 0.632 | 1.5 (0.4–6.0) | 0.618 |
| II (*n* = 149) | 1.5 (0.5–5.0) | 0.495 | 1.5 (0.5–4.9) | 0.511 |
| III (*n* = 100) | **5.0 (1.6–15.9)** | **0.007** | 4.6 (1.5–14.7) | **0.01** |
| RCB index (as a continuous variable)[a] | 1.8 (1.5–2.2) | **<0.001** | 1.8 (1.5–2.2) | **<0.001** |
| **Overexpressed HER2 (*n* = 54)** | | | | |
| Classes de RCB | | | | |
| 0 (*n* = 15) | Ref. | | Ref. | |
| I (*n* = 4) | £ | 0.914 | £ | £ |
| II (*n* = 28) | £ | 0.912 | £ | £ |
| III (*n* = 7) | £ | 0.904 | £ | £ |
| RCB index (as a continuous variable) | 2.2 (1.5–3.3) | **<0.001** | 2.2 (1.5–3.3) | **<0.001** |
| **Triple negative (*n* = 120)** | | | | |
| Classes de RCB**** | | | | |
| 0 (*n* = 31) | Ref. | | Ref. | |
| I (*n* = 6) | £ | 0.975 | £ | 0.978 |
| II (*n* = 53) | **17.0 (2.3–126.3)** | **0.006** | 19.3 (2.6–144.2) | **0.004** |
| III (*n* = 30) | **41.7 (5.6–309.7)** | **<0.001** | 14.1 (1.5–136.5) | **0.022** |
| RCB index (as a continuous variable)[a] | 2.0 (1.6–2.3) | **<0.001** | 2.0 (1.6–2.3) | **<0.001** |

CI: Confidence interval; CS: Clinical staging; HER: Human epidermal growth factor receptor-type 2; RCB: Residual cancer burden; cHR: Crude hazard ratio; aHR: Adjusted hazard ratio. In bold, $p<0.05$; adjusted by: *ypN and T; **T; ***CS and ****ypN and [a]with no adjustment variables; £: Insufficient sample size for the calculation

With regard to patients with overexpressed HER2 BC, the Kaplan-Meier analyses only indicated a lack of differences between classes regarding OS. The multiple analysis indicated an increase in the risk of distant progression, recurrence, and death by about two-fold for each 1-point increase in the continuous score without adjustment, similar to Symmans et al. (19) and Yau et al. (25).

Finally, regarding women with TNBC, the Kaplan-Meier analyses verified a significant difference between RCB classes ($p<0.001$). The multiple analysis demonstrated an increase in the risk of distant progression, recurrence, and death of approximately double for each 1-point increase in the continuous score, again without adjustment. In the current study, RCB classes for patients with TNBC were

**Table 5. Crude and adjusted Cox analysis according to RCB classes and RCB index in the whole study population and for molecular breast cancer subtypes regarding overall survival**

| | cHR (95% CI) | *p* | aHR (95% CI) | *p* |
|---|---|---|---|---|
| **Whole study population (*n* = 562)** | | | | |
| RCB classes[a] | | | | |
| 0 (*n* = 68) | Ref. | | Ref. | |
| I (*n* =36) | 1.4 (0.3–6.3) | 0.655 | 1.5 (0.3–6.5) | 0.632 |
| II (*n* = 287) | **3.5 (1.3–9.7)** | **0.015** | 3.4 (1.3–9.5) | **0.018** |
| III (*n* = 171) | **8.1 (2.9–22.1)** | **<0.001** | 7.2 (2.6–19.8) | **<0.001** |
| RCB index (as a continuous variable)[b] | 1.8 (1.5–2.1) | **<0.001** | 1.7 (1.5–2.0) | **<0.001** |
| **Luminal A*** (*n* = 94)** | | | | |
| RCB classes | | | | |
| 0 (*n* = 2) | Ref. | | Ref. | |
| I (*n* = 1) | £ | £ | £ | £ |
| II (*n* = 57) | £ | £ | £ | £ |
| III (*n* = 34) | £ | £ | £ | £ |
| RCB index (as a continuous variable) | 5.2 (1.7–16.0) | **0.004** | 5.2 (1.7–16.0) | **0.004** |
| **Luminal B (*n* = 294)** | | | | |
| RCB classes*** | | | | |
| 0 (*n* = 20) | Ref. | | Ref. | |
| I (*n* = 25) | 1.3 (0.2–7.8) | 0.768 | 1.3 (0.2–7.8) | 0.768 |
| II (*n* = 149) | 1.6 (0.4–6.7) | 0.542 | 1.6 (0.4–6.7) | 0.542 |
| III (*n* = 100) | **4.2 (1.0–17.8)** | **0.046** | 4.2 (1.0–17.8) | **0.046** |
| RCB index (as a continuous variable)[c] | 1.7 (1.3–2.1) | **<0.001** | 1.7 (1.3–2.1) | **<0.001** |
| **overexpressed HER2*** (*n* = 54)** | | | | |
| RCB classes | | | | |
| 0 (*n* = 15) | Ref. | | Ref. | |
| I (*n* = 4) | 1.0 (*) | 1 | £ | £ |
| II (*n* = 28) | £ | 0.941 | £ | £ |
| III (*n* = 7) | £ | 0.939 | £ | £ |
| RCB index (as a continuous variable) | 1.8 (1.2–2.8) | **0.008** | 1.8 (1.2–2.8) | **0.008** |
| **Triple negative (*n* = 120)** | | | | |
| RCB classes[d] | | | | |
| 0 (*n* = 31) | Ref. | | Ref | |
| I (*n* = 6) | £ | 0.981 | £ | 0.976 |
| II (*n* = 53) | **9.0 (2.1–38.2)** | **0.003** | 10.3 (2.3–45.4) | **0.002** |
| III (*n* = 30) | **21.6 (5.1–91.9)** | **<0.001** | 6.3 (1.1–37.9) | **0.044** |
| RCB index (as a continuous variable)*** | 2.0 (1.6–2.5) | **<0.001** | 2.0 (1.6–2.5) | **<0.001** |

CI: Confidence interval; RE: Estroger receptor; ypN: Pathological axilla; HER: Human epidermal growth factor receptor-type 2; RCB: Residual cancer burden; cHR: Crude hazard ratio; aHR: Adjusted hazard ratio. In bold, $p<0.05$; adjusted by: [a]T; T and [b]Ki-67; [c]RE and Ki-67; [d]ypN and T and ***without adjustment variables; £: Insufficient sample size for the calculation

prognosticators in RCB-II and RCB-III tumors, equivalent to pathological staging II and III, and similar to that reported by Symmans et al. (19), Hamy et al. (24) and Yau et al. (25).

This study, therefore, corroborates the founding results reported by Symmans et al. (18), Symmnas et al. (19), Hamy et al. (24) and Yau et al. (25), with an increase in the chance of distant BC progression with increasing RCB scores in the whole study population and in subgroups with some different molecular BC subtypes, as well as with the risk of recurrence and death.

The survival analyses confirm the findings reported by Symmans et al. (19), Hamy et al. (24) and Yau et al. (25), indicating better survival rates in patients without RD or with minimal RD and differences

between molecular BC subtypes, with lower survival rates among patients with more aggressive BC.

As reported by Symmans et al. (18) and Romero et al. (26), positive axilla in the present study were inversely associated with DRFS. Similarly, some patients who achieved pCR following NAC evolved to recurrence or disease progression, indicating that they remained at risk of disease progression despite good responses.

### Study Limitations

This study's limitations include that it was a retrospective analysis, employing data from a single institution, with anatomopathological reports prepared by multiple pathologists. Furthermore, different chemotherapy regimens could not be assessed, as carried out by Symmans et al. (18) and Symmans et al. (19), as almost all patients (97.5%) underwent an anthracycline and taxane-based regimen. Moreover, the number of patients in the RCB classes for the luminal A and overexpressed HER-2 subtypes was insufficient to estimate specific DRFS, DFS and OS. Retrospectively obtaining the RCB did not interfere with its discriminatory power, and all patients were subjected to the same institutional routine. However, this is, to the best of our knowledge, the largest study to include patients treated in the Brazilian Unified Health System (SUS). Despite having mostly advanced CS, similar survival rates was detected, similar to the earlier studies, reinforcing previous findings and expanding the use of this tool the population served in the Brazilian SUS (18, 19, 22, 24-26).

The RCB index displayed predictive power when used as a continuous variable, comprising an independent prognostic factor for predicting distant progression, recurrence or death following NAC in the whole study population and in all molecular BC subtypes. The RCB classes in patients with luminal B subtype indicated a 4 to 5-fold increase in the risk of distant progression, recurrence, and death, which was significant only in RCB-III. In patients with TNBC, RCB classes demonstrated a 4 to 20-fold increase in the risk of distant progression, recurrence, and death, which was significant for RCB-II and RCB-III. Further investigations into the utility of RCB Index in the prognosis of patients with the luminal A and overexpressed HER-2 subtypes is required as the present study was unable to definitively demonstrate this, probably due to smaller subgroup sizes.

### Ethics

**Ethics Committee Approval:** The study was approved by the INCA Human Research Ethics Committee under opinion 166.838, following resolution 466/12 of the National Health Council of the Ministry of Health (date: 04.01.2013).

**Informed Consent:** Retrospective study.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: E.N.A.V., A.B., M.A.B., L.C.S.T.; Concept: E.N.A.V., A.B., M.A.B., L.C.S.T.; Design: E.N.A.V., A.B., M.A.B., L.C.S.T.; Data Collection or Processing: E.N.A.V., A.B., M.A.B.; Analysis or Interpretation: E.N.A.V., A.B., M.A.B., L.C.S.T.; Literature Search: E.N.A.V., A.B., M.A.B., L.C.S.T.; Writing: E.N.A.V., A.B., M.A.B., L.C.S.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.

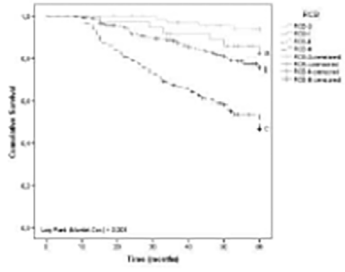
## References

1. Christian MC, McCabe MS, Korn EL, Abrams JS, Kaplan RS, Friedman MA. The National Cancer Institute audit of the National Surgical Adjuvant Breast and Bowel Project Protocol B-06. N Engl J Med. 1995; 333: 1469-1474. (PMID: 7477148) [Crossref]
2. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. Global Cancer Observatory: Cancer Today. Lyon: International Agency for Research on Cancer; 2020. Available link: https://gco.iarc.fr/today/en?utm_source=chatgpt.com [Crossref]
3. Early Breast Cancer Trialists' Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Early Breast Cancer Trialists' Collaborative Group. Lancet. 1992; 339: 1-15. (PMID: 1345950) [Crossref]
4. Ahmed MI, Lennard TW. Breast cancer: role of neoadjuvant therapy. Int J Surg. 2009; 7: 416-420. (PMID: 19524705) [Crossref]
5. Fisher B, Brown A, Mamounas E, Wieand S, Robidoux A, Margolese RG, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. J Clin Oncol. 1997; 15: 2483-2493. (PMID: 9215816) [Crossref]
6. Wolmark N, Wang J, Mamounas E, Bryant J, Fisher B. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. J Natl Cancer Inst Monogr. 2001; : 96-102. (PMID: 11773300) [Crossref]
7. Mamounas EP. NSABP protocol B-27. Preoperative doxorubicin plus cyclophosphamide followed by preoperative or postoperative docetaxel. Oncology (Williston Park). 1997; 11(6 Suppl 6): 37-40. (PMID: 9213327) [Crossref]
8. Chevallier B, Roche H, Olivier JP, Chollet P, Hurteloup P. Inflammatory breast cancer. Pilot study of intensive induction chemotherapy (FEC-HD) results in a high histologic response rate. Am J Clin Oncol. 1993; 16: 223-228. (PMID: 8338056) [Crossref]
9. Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol. 1998; 16: 2672-2685. (PMID: 9704717) [Crossref]
10. Kuerer HM, Newman LA, Smith TL, Ames FC, Hunt KK, Dhingra K, et al. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. J Clin Oncol. 1999; 17: 460-469. (PMID: 10080586) [Crossref]
11. Kaufmann M, Hortobagyi GN, Goldhirsch A, Scholl S, Makris A, Valagussa P, et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: an update. J Clin Oncol. 2006; 24: 1940-1949. Erratum in: J Clin Oncol. 2006; 24: 3221. (PMID: 16622270) [Crossref]
12. Guarneri V, Broglio K, Kau SW, Cristofanilli M, Buzdar AU, Valero V, et al. Prognostic value of pathologic complete response after primary chemotherapy in relation to hormone receptor status and other factors. J Clin Oncol. 2006; 24: 1037-1044. (PMID: 16505422) [Crossref]
13. Berry DA, Cirrincione C, Henderson IC, Citron ML, Budman DR, Goldstein LJ, et al. Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. JAMA. 2006; 295: 1658-1667. Erratum in: JAMA. 2006; 295: 2356. (PMID: 16609087) [Crossref]

14. 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. Erratum in: Lancet. 2019; 393: 986. (PMID: 24529560) [Crossref]
15. Peintinger F, Sinn B, Hatzis C, Albarracin C, Downs-Kelly E, Morkowski J, et al. Reproducibility of residual cancer burden for prognostic assessment of breast cancer after neoadjuvant chemotherapy. Mod Pathol. 2015; 28: 913-920. (PMID: 25932963) [Crossref]
16. 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]
17. Boughey JC, Alvarado MD, Lancaster RB, Fraser Symmans W, Mukhtar R, Wong JM, et al. Surgical standards for management of the axilla in breast cancer clinical trials with pathological complete response endpoint. NPJ Breast Cancer. 2018; 4: 26. Erratum in: NPJ Breast Cancer. 2019; 5: 2. (PMID: 30131975) [Crossref]
18. Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol. 2007; 25: 4414-4422. (PMID: 17785706) [Crossref]
19. Symmans WF, Wei C, Gould R, Yu X, Zhang Y, Liu M, et al. Long-term prognostic risk after neoadjuvant chemotherapy associated with residual cancer burden and breast cancer subtype. J Clin Oncol. 2017; 35: 1049-1060. (PMID: 28135148) [Crossref]
20. Masuda N, Lee SJ, Ohtani S, Im YH, Lee ES, Yokota I, et al. Adjuvant capecitabine for breast cancer after preoperative chemotherapy. N Engl J Med. 2017; 376: 2147-2159. (PMID: 28564564) [Crossref]
21. von Minckwitz G, Huang CS, Mano MS, Loibl S, Mamounas EP, Untch M, et al. Trastuzumab emtansine for residual invasive HER2-positive breast cancer. N Engl J Med. 2019; 380: 617-628. (PMID: 30516102) [Crossref]
22. Gomes da Cunha JP, Goncalves R, Silva F, Aguiar FN, Mota BS, Chequim BB, et al. Validation of the residual cancer burden index as a prognostic tool in women with locally advanced breast cancer treated with neoadjuvant chemotherapy. J Clin Pathol. 2023; 76: 239-243. (PMID: 34620608) [Crossref]
23. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. Source Code Biol Med. 2008; 3: 17. (PMID: 19087314) [Crossref]
24. Hamy AS, Darrigues L, Laas E, De Croze D, Topciu L, Lam GT, et al. Prognostic value of the residual cancer burden index according to breast cancer subtype: validation on a cohort of BC patients treated by neoadjuvant chemotherapy. PLoS One. 2020; 15: e0234191. (PMID: 32579551) [Crossref]
25. Yau C, Osdoit M, van der Noordaa M, Shad S, Wei J, de Croze D, et al. Residual cancer burden after neoadjuvant chemotherapy and long-term survival outcomes in breast cancer: a multicentre pooled analysis of 5161 patients. Lancet Oncol. 2022; 23: 149-160. (PMID: 34902335) [Crossref]
26. Romero A, García-Sáenz JA, Fuentes-Ferrer M, López Garcia-Asenjo JA, Furió V, Román JM, et al. Correlation between response to neoadjuvant chemotherapy and survival in locally advanced breast cancer patients. Ann Oncol. 2013; 24: 655-661. (PMID: 23104719) [Crossref]

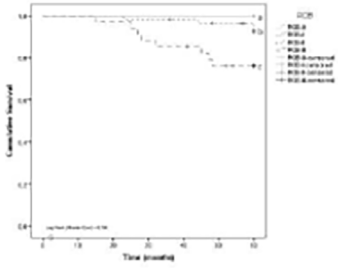
**Distant recurrence-free survival**

**A. General population**



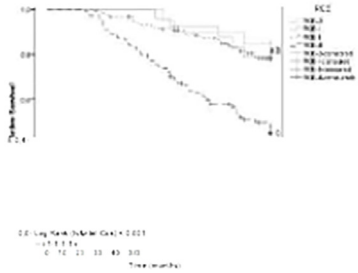
Note: a: not statistically significant in relation to the reference (RCB-0); b: not statistically significant in relation to the curve a and statistically significant in relation to the reference (RCB-0); c: statistically significant in relation to all prior curves.

**B. Population of patients presenting the luminal subtype A**



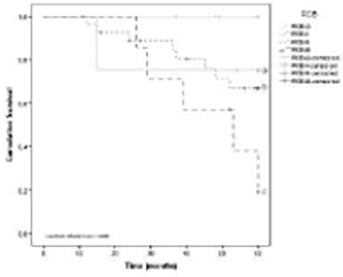
Note: a: calculation not possible due to the sample size; b: not statistically significant in relation to the reference (RCB-0) and curve a; c: statistically significant in relation to curve b.

**C. Population of patients presenting the luminal subtype B**



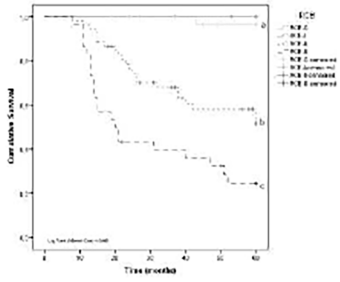
Note: a: not statistically significant in relation to the reference (RCB-0); b: not statistically significant; c: statistically significant in relation to all prior curves.

**D. Population of patients presenting the overexpressed HER2 subtype**



Note: a: not statistically significant in relation to the reference (RCB-0); b: statistically significant in relation to the reference and not statistically significant in relation to curve a and curve c; c: statistically significant only in relation to the reference.

**E. Population of patients presenting the triple negative subtype**



Note: a: not statistically significant in relation to the reference (RCB-0); b: statistically significant in relation to the reference and curve a; c: statistically significant in relation to curve a and curve b.

**Supplementary Figure 1.** Kaplan-Meier survival curves. Distant recurrence-free survival - A: general population; B: luminal A; C: luminal B; D: overexpressed HER2; E: triple negative

*RCB: Residual cancer burden; HER2: Human epidermal growth factor receptor 2*

**Supplementary Table 1. Percentage of distant recurrence-free, disease-free and overall 5-year survival in the whole study population and for molecular breast cancer subtypes**

| RCB class | % (± SD) | | | | |
|---|---|---|---|---|---|
| | Total | Luminal A | Luminal B | Overexpressed HER2 | Triple negative |
| **Distant recurrence-free survival** | | | | | |
| 0 | 93.6 (3.1) | 100.0 (0.0) | 85.0 (8.0) | 100.0 (0.0) | 93.6 (3.6) |
| I | 85.8 (5.9) | 100.0 (0.0) | 83.2 (7.7) | 75 (21.7) | 100.0 (0.0) |
| II | 77.4 (2.6) | 96.5 (2.5) | 78.0 (3.5) | 66.9 (9.7) | 58.1 (7.3) |
| III | 53.4 (3.9) | 76.2 (7.4) | 55.1 (5.1) | 38.1 (19.9) | 24.4 (8.2) |
| **Disease-free survival** | | | | | |
| 0 | 93.7 (3.1) | 100.0(0.0) | 85.0 (8.0) | 100.0 (0.0) | 96.4 (3.5) |
| I | 82.7 (6.4) | 100.0 (0.0) | 78.6 (8.5) | 75 (21.7) | 100.0 (0.0) |
| II | 76.4 (2.6) | 92.9 (3.4) | 78.0 (3.5) | 68.0 (9.4) | 56.5 (7.3) |
| III | 50.4 (3.9) | 76.3 (7.3) | 49.7 (5.1) | 42.9 (17.8) | 24.4 (8.2) |
| **Overall survival** | | | | | |
| 0 | 93.5 (3.1) | 100.0 (0.0) | 89.7 (6.9) | 100.0 (0.0) | 93 (4.8) |
| I | 91.2 (4.9) | 100.0 (0.0) | 87.0 (7.0) | 100.0 (0.0) | 100.0 (0.0) |
| II | 80.1 (2.4) | 96.3 (2.6) | 85.3 (3.0) | 67.7 (9.5) | 50.9 (7.5) |
| III | 60.3 (3.8) | 79.2 (7.0) | 64.9 (4.9) | 53.6 (20.1) | 23.8 (8.2) |

SD: Standard deviation; HER: Human epidermal growth factor receptor-type 2; RCB: Residual cancer burden

**Supplementary Table 2. Univariate risk analyzes in the general population and molecular breast cancer subtypes regarding distant recurrence-free survival**

| | Total | Luminal A | Luminal B | Overexpressed HER 2 | Triple negative |
|---|---|---|---|---|---|
| **Variables** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** |
| **Mean age** | 1.0 (1.0–1.1) 0.201 | 1.0 (1.0–1.1) 0.576 | 1.0 (1.0–1.1) 0.200 | 1.0 (1.0–1.1) 0.234 | 1.0 (1.0–1.1) 0.201 |
| **Menopausal status** | | | | | |
| Post-menopausal (>50 y) | Ref. | Ref. | Ref. | Ref. | Ref. |
| Pre-menopausal (≤50 y) | 1.2 (0.9–1.6) 0.360 | 0.6 (0.2–2.1) 0.442 | 1.4 (0.9–2.1) 0.117 | 0.6 (0.2–1.8) 0.350 | 0.9 (0.7–1.2) 0.360 |
| **Race/skin color** | | | | | |
| White | Ref. | Ref. | Ref. | Ref. | Ref. |
| Non-white | 1.0 (0.8–1.4) 0.908 | 4.2 (0.6–32.2) 0.172 | 1.1 (0.7–1.6) 0.870 | 0.5 (0.2–1.5) 0.215 | 1.0 (0.8–1.4) 0.908 |
| **Tumor size** | | | | | |
| T1/ T2 | Ref. | Ref. | Ref. | Ref. | Ref. |
| T3 | 1.5 (1.0`–2.3) **0.049** | 2.2 (0.5–10.9) 0.335 | 2.0 (1.2–3.5) **0.014** | * 0.961 | 1.5 (1.0 –2.3) **0.049** |
| T4 | 2.2 (1.5–3.3) **<0.001** | 3.5 (0.9–14.1) 0.075 | 1.9 (1.1–3.3) **0.022** | 3.0 (1.0–8.5) **0.046** | 2.2 (1.5–3.3) **<0.001** |
| **Lymph nodes** | | | | | |
| N0 | Ref. | Ref. | Ref. | Ref. | Ref. |
| N1 | 1.6 (1.1–2.2) **0.012** | 1.4 (0.4–5.1) 0.652 | 1.6 (1.0–2.4) 0.053 | 1.3 (0.4–3.8) 0.674 | 1.6 (1.1–2.2) **0.012** |
| N2/N3 | 1.6 (1.0–2.6) 0.059 | 3.2 (0.8–13.2) 0.117 | 1.4 (0.7–2.8) 0.319 | * 0.979 | 1.6 (1.0–2.6) 0.055 |
| **Estrogen receptor** | | | | | |
| Positive | Ref. | Ref. | Ref. | - | - |
| Negative | 1.6 (1.2–2.2) **0.003** | 0.1 (*) 0.802 | 2.2 (0.8–6.0) 0.127 | - | - |
| **Progesterone receptor** | | | | | |
| Positive | Ref. | Ref. | Ref. | - | - |
| Negative | 1.8 (1.3–2.4) **<0.001** | 3.1 (0.9–10.2) 0.068 | 1.7 (1.0–2.7) **0.037** | - | - |
| **HER2** | | | | | |
| Negative | Ref. | - | Ref. | - | - |
| Positive | 0.9 (0.7–1.3) 0.647 | - | 1.2 (0.7–1.8) 0.556 | - | - |
| **Ki-67** | | | | | |
| Low (≤14) | Ref. | - | Ref. | - | Ref. |
| High (>14) | 2.3 (1.4–3.6) **0.001** | - | 0.7 (0.3–1.8) 0.463 | - | 2.3 (1.4–3.6) <**0.001** |
| **Histological grade** | | | | | |
| I/ II | Ref. | Ref. | Ref. | Ref. | Ref. |
| III | 1.4 (1.0–2.0) **0.032** | 0.1 (*) 0.614 | 1.2 (0.8–1.9) 0.454 | 0.9 (0.3–2.6) 0.784 | 1.5 (1.0–2.0) **0.032** |
| **Histological type** | | | | | |
| IDC | Ref. | Ref. | Ref. | Ref. | Ref. |
| Other | 0.9 (0.5–1.6) 0.632 | 2.6 (0.8–8.5) 0.126 | 2.6 (0.8–8.5) 0.126 | 0.1 (*) 0.711 | 0.9 (0.5–1.6) 0.632 |
| **Molecular subtype** | | | | | |
| Luminal A | Ref. | - | - | - | - |
| Luminal B | 2.8 (1.5–5.1) **0.001** | - | - | - | - |
| Overexpressed HER2 | 2.5 (1.2–5.3) **0.024** | - | - | - | - |
| Triple negative | 4.2 (2.2–8.0) **<0.001** | - | - | - | - |

**Supplementary Table 2. Continued**

| | Total | Luminal A | Luminal B | Overexpressed HER 2 | Triple negative |
|---|---|---|---|---|---|
| **Variables** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** |
| **Clinical staging** | | | | | |
| I/ II | Ref. | Ref. | Ref. | Ref. | Ref. |
| III | 1.9 (1.4–2.7) **<0.001** | 1.9 (0.6–6.1) 0.265 | 1.8 (1.2–2.9) **0.007** | 1.5 (0.5–4.4) 0.440 | 1.9 (1.4–2.7) **<0.001** |
| **Breast surgery** | | | | | |
| Mastectomy | Ref. | Ref. | Ref. | Ref. | Ref. |
| Breast conserving | 0.6 (0.4–1.0) **0.040** | 0.5 (0.1–3.9) 0.516 | 0.7 (0.4–1.3) 0.263 | 1.2 (0.3–5.5) 0.799 | 0.6 (0.4–0.9) **0.040** |
| **Axillary surgery** | | | | | |
| SLNB | Ref. | Ref. | Ref. | Ref. | Ref. |
| SLNB + ALND | 1.9 (0.9–3.7) 0.086 | 1.4 (0.3–6.7) 0.644 | 1.6 (0.7–3.9) 0.280 | 2.9 (0.4–20.5) 0.172 | 1.9 (0.9–3.7) 0.086 |
| ALND | 2.6 (1.6–4.2) **<0.001** | 2.9 (0.4–20.5) 0.289 | 2.2 (1.2–4.2) **0.015** | 3.0 (0.2–48.8) 0.434 | 2.5 (1.6–4.2) **<0.001** |
| **NAC regimen** | | | | | |
| Anthracyclic + Taxane | Ref. | Ref. | Ref. | Ref. | Ref. |
| Other | 1.1 (0.4–3.0) 0.845 | 0.1 (*) 0.802 | 1.2 (0.2–4.8) 0.826 | 7.8 (0.9–71.3) 0.067 | 1.1 (0.4–3.0) 0.845 |
| **ypT** | | | | | |
| pCR | Ref. | Ref. | Ref. | Ref. | Ref. |
| T1 | 1.8 (1.0–3.5) 0.071 | * 0.957 | 1.0 (0.4–2.0) 0.863 | 9.3 (1.1–80.0) **0.042** | 1.8 (1.0–3.5) 0.071 |
| T2 | 4.3 (2.4–7.9) **<0.001** | 1.2 (0.2–9.2) 0.875 | 2.7 (1.3–5.3) **0.006** | 21.0 (2.5–176.2) **0.005** | 4.3 (2.3–7.8) <**0.001** |
| T3/T4 | 5.5 (2.9–10.6) **<0.001** | 0.5 (0.1–7.7) 0.605 | 2.7 (1.2–6.1) **0.020** | 16.6 (1.5–183.0) **0.022** | 5.5 (2.8–10.6) <**0.001** |
| **YpN** | | | | | |
| N0 | Ref. | Ref. | Ref. | Ref. | Ref. |
| N1 | 2.2 (1.5–3.2) **<0.001** | 4.4 (0.9–22.6) 0.078 | 2.2 (1.3–3.7) **0.006** | 2.3 (0.7–7.8) 0.167 | 2.2 (1.5–3.2) **<0.001** |
| N2/ N3 | 4.0 (2.8–5.8) **<0.001** | 8.3 (1.6–42.7) **0.012** | 3.7 (2.3–6.0) **<0.001** | 3.6 (0.8–17.3) 0.108 | 4.0 (2.8–5.8) <**0.001** |
| **RCB classes** | | | | | |
| RCB-0 | Ref. | Ref. | Ref. | Ref. | Ref. |
| RCB-I | 2.9 (0.8–10.4) 0.096 | 1.0 (*) 1.000 | 1.4 (0.4–6.0) 0.627 | * 0.941 | 2.9 (0.8–10.4) 0.096 |
| RCB-II | 4.3 (1.6–11.8) **0.005** | * 0.949 | 1.5 (0.5–5.0) 0.493 | * 0.940 | 4.3 (1.6–11.8) **0.005** |
| RCB-III | 11.7 (4.3–31.8) **<0.001** | * 0.940 | 4.8 (1.5–15.5) **0.008** | * 0.935 | 11.7 (4.3–31.8) **<0.001** |
| RCB index | 1.9 (1.6–2.2) **<0.001** | 2.6 (1.3–5.1) **0.008** | 1.8 (1.5–2.2) **<0.001** | 2.2 (1.4–3.3) **<0.001** | 1.9 (1.6–2.1) **<0.001** |

HR: Hazard ratio; CI: Confidence interval; NAC: Neoadjuvant chemotherapy; SLNB: Sentinel lymph node biopsy; ALND: Axillary lymph node dissection; HER2: Human epidermal growth factor receptor 2; RCB: Residual cancer burden; pCR: Pathological complete response; only valid values used; In bold, $p<0.05$; *not calculated

**Supplementary Table 3. Univariate risk analyses in the whole study population and for molecular breast cancer subtypes regarding disease-free survival**

| | Total | Luminal A | Luminal B | Overexpressed HER-2 | Triple negative |
|---|---|---|---|---|---|
| **Variables** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** |
| **Mean age** | 1.0 (1.0 –1.1) 0.201 | 1.0 (1.0–1.1) 0.614 | 1.0 (1.0–1.1) 0.191 | 1.0 (1.0–1.1) 0.231 | 1.0 (1.0–1.1) 0.756 |
| **Menopausal status** | | | | | |
| Post-menopausal (>50 y) | Ref. | Ref. | Ref. | Ref. | Ref. |
| Pre-menopausal (≤50 y) | 1.2 (0.9–1.6) 0.376 | 0.6 (0.2–2.1) 0.427 | 1.4 (0.9–2.1) 0.115 | 0.6 (0.2–1.8) 0.367 | 1.1 (0.6–1.9) 0.841 |
| **Race/skin color** | | | | | |
| White | Ref. | Ref. | Ref. | Ref. | Ref. |
| Non-white | 1.0 (0.7–1.4) 0.923 | 4.2 (0.6–32.5) 0.170 | 1.1 (0.7–1.6) 0.877 | 0.5 (0.2–1.5) 0.188 | 1.2 (0.7–2.3) 0.557 |
| **Tumor size** | | | | | |
| T1/ T2 | Ref. | Ref. | Ref. | Ref. | Ref. |
| T3 | 1.5 (1.0`–2.3) 0.059 | 2.2 (0.4–10.6) 0.352 | 2.0 (1.2–3.6) **0.014** | ***** 0.960 | 0.9 (0.4–2.0) 0.805 |
| T4 | 2.2 (1.5–3.2) **<0.001** | 3.6 (0.9–14.2) 0.073 | 1.9 (1.1–3.3) **0.020** | 2.8 (1.0–8.0) 0.059 | 2.2 (1.1–4.5) **0.027** |
| **Lymph nodes** | | | | | |
| N0 | Ref. | Ref. | Ref. | Ref. | Ref. |
| N1 | 1.6 (1.1–2.2) **0.011** | 1.4 (0.4–5.0) 0.657 | 1.6 (1.0–2.5) **0.044** | 1.3 (0.5–3.9) 0.625 | 1.3 (0.7–2.5) 0.434 |
| N2/N3 | 1.6 (1.0–2.6) 0.059 | 3.0 (0.7–12.5) 0.134 | 1.5 (0.7–2.8) 0.298 | * 0.979 | 1.8 (0.8–4.0) 0.187 |
| **Estrogen receptor** | | | | | |
| Positive | Ref. | Ref. | Ref. | - | - |
| Negative | 1.6 (1.2–2.2) **0.003** | 0.1 (*) 0.804 | 2.1 (0.8–5.7) 0.152 | - | - |
| **Progesterone receptor** | | | | | |
| Positive | Ref. | Ref. | Ref. | - | - |
| Negative | 1.8 (1.3–2.4) **<0.001** | 2.9 (0.9–9.7) 0.080 | 1.7 (1.1–2.8) **0.034** | - | - |
| **HER2** | | | | | |
| Negative | Ref. | - | Ref. | - | - |
| Positive | 0.9 (0.7–1.3) 0.635 | - | 0.9 (0.6–1.4) 0.512 | - | - |
| Ki67 | | | | | |
| Low (≤14) | Ref. | - | Ref. | - | Ref. |
| High (>14) | 2.3 (1.4–3.6) **0.001** | - | 0.8 (0.3–1.9) 0.515 | - | 2.1 (0.5–8.5) 0.324 |
| **Histological grade** | | | | | |
| I/ II | Ref. | Ref. | Ref. | Ref. | Ref. |
| III | 1.5 (1.1–2.0) **0.029** | 0.1 (*) 0.617 | 1.2 (0.8–2.0) 0.428 | 0.9 (0.3–2.5) 0.754 | 1.2 (0.6–2.3) 0.610 |
| **Histological type** | | | | | |
| IDC | Ref. | Ref. | Ref. | Ref. | Ref. |
| Outros | 0.9 (0.5–1.6) 0.597 | 2.5 (0.8–8.2) 0.142 | 0.6 (0.2–1.6) 0.263 | 0.1 (*) 0.717 | 2.6 (0.8–8.5) 0.107 |
| **Molecular subtype** | | | | | |
| Luminal A | Ref. | - | - | - | - |
| Luminal B | 2.8 (1.5–5.1) **0.001** | - | - | - | - |
| Overexpressed HER2 | 2.5 (1.2–5.3) **0.022** | - | - | - | - |
| Triple negative | 4.2 (2.2–7.9) **<0.001** | - | - | - | - |
| **Clinical staging** | | | | | |
| I/ II | Ref. | Ref. | Ref. | Ref. | Ref. |
| III | 1.9 (1.4–2.7) **<0.001** | 1.9 (0.6–6.0) 0.271 | 1.9 (1.2–2.9) **0.006** | 1.5 (0.5–4.2) 0.488 | 1.9 (1.0–3.5) 0.050 |

**Supplementary Table 3. Continued**

| | Total | Luminal A | Luminal B | Overexpressed HER-2 | Triple negative |
|---|---|---|---|---|---|
| **Variables** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** |
| **Breast surgery** | | | | | |
| Mastectomy | Ref. | Ref. | Ref. | Ref. | Ref. |
| Breast conserving | 0.6 (0.4–1.0) **0.041** | 0.5 (0.1–4.0) 0.521 | 0.8 (0.4–1.3) 0.275 | 1.2 (0.3–5.5) 0.789 | 0.3 (0.1–0.8) **0.016** |
| **Axillary surgery** | | | | | |
| SLNB | Ref. | Ref. | Ref. | Ref. | Ref. |
| SLNB + ALND | 1.9 (0.9–3.8) 0.086 | 1.5 (0.3–6.8) 0.639 | 1.6 (0.7–4.0) 0.277 | 3.5 (0.2–56.5) 0.375 | 1.5 (0.3–8.0) 0.664 |
| ALND | 2.6 (1.6–4.2) **<0.001** | 2.8 (0.4–19.6) 0.311 | 2.3 (1.2–4.2) **0.013** | 4.4 (0.6–33.9) 0.154 | 3.6 (1.3–10.0) **0.016** |
| **NAC regimen** | | | | | |
| Anthracyclic + Taxane | Ref. | Ref. | Ref. | Ref. | Ref. |
| Other | 1.1 (0.4–3.0) 0.885 | 0.1 (*) 0.804 | 1.2 (0.3–4.8) 0.827 | 5.1 (0.6–41.6) 0.131 | 0.4 (0.1–3.0) 0.371 |
| **ypT** | | | | | |
| PCR | Ref. | Ref. | Ref. | Ref. | Ref. |
| T1 | 1.9 (1.0–3.5) 0.067 | * 0.957 | 1.0 (0.5–2.1) 0.910 | 9.9 (1.2–84.8) **0.037** | 13.6 (1.8–105.4) **0.012** |
| T2 | 4.3 (2.4–7.9) **<0.001** | 1.2 (0.2–9.1) 0.882 | 2.8 (1.4–5.7) **0.005** | 17.4 (2.1–145.7) **0.008** | 21.5 (2.9–160.7) **0.003** |
| T3/ T4 | 5.5 (2.9–10.6) **<0.001** | 0.5 (0.1–7.6) 0.597 | 2.7 (1.2–6.1) **0.020** | 19.5 (1.8–215.2) **0.015** | 45.1(5.9–343.7)**<0.001** |
| **ypN** | | | | | |
| N0 | Ref. | Ref. | Ref. | Ref. | Ref. |
| N1 | 2.1 (1.5–3.1) **<0.001** | 4.3 (0.8–21.9) 0.084 | 2.1 (1.3–3.6) **0.007** | 2.6 (0.8–8.4) 0.129 | 2.9 (1.4–6.0) **0.004** |
| N2/ N3 | 4.2 (2.9–6.0) **<0.001** | 8.3 (1.6–42.9) **0.011** | 3.9 (2.4–6.3) **<0.001** | 4.9 (1.0–23.6) **0.046** | 11.0 (5.2–22.4)**<0.001** |
| **RCB** | | | | | |
| RCB-0 | Ref. | Ref. | Ref. | Ref. | Ref. |
| RCB-I | 2.9 (0.8–10.3) 0.099 | 1.0 (*) 1.000 | 1.4 (0.4 –6.0) 0.626 | * 0.913 | * 0.975 |
| RCB-II | 4.3 (1.6–1.9) **0.005** | * 0.949 | 1.5 (0.5–5.0) 0.489 | * 0.912 | 17.0 (2.3–126.3) **0.006** |
| RCB-III | 11.8 (4.3–32.0) **<0.001** | * 0.940 | 5.0 (1.6–15.9) **0.007** | * 0.904 | 41.7 (5.6–309.7) **<0.001** |
| RCB index | 1.9 (1.6–2.2) **<0.001** | 2.6 (1.3–5.2) **0.008** | 1.8 (1.5–2.2) **<0.001** | 2.2 (1.5–3.3) **<0.001** | 2.0 (1.6–2.5) **<0.001** |

HR: Hazard ratio; CI: Confidence interval; NAC: Neoadjuvant chemotherapy; SLNB: Sentinel lymph node biopsy; ALND: Axillary lymph node dissection; HER2: Human epidermal growth factor receptor 2; pCR: Pathological complete response; only valid values used; In bold, p<0.05; *not calculated

**Supplementary Table 4. Univariate risk analyzes in the whole study population and for molecular breast cancer subtypes regarding overall survival**

| | Total | Luminal A | Luminal B | Overexpressed HER-2 | Triple Negative |
|---|---|---|---|---|---|
| | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** |
| **Mean age** | 1.0 (1.0–1.1) 0.951 | 1.0 (0.9–1.1) 0.543 | 1.0 (1.0–1.1) 0.972 | 1.1 (1.0–1.1) 0.130 | 1.0 (1.0–1.1) 0.875 |
| **Menopausal status** | | | | | |
| Post-menopausal (>50 y) | Ref. | Ref. | Ref. | Ref. | Ref. |
| Pre-menopausal (≤50 y) | 1.0 (0.7–1.4) 0.918 | 0.7 (0.2–2.6) 0.527 | 1.2 (0.7–2.0) 0.466 | 0.5 (0.2–1.7) 0.246 | 1.1 (0.6–2.0) 0.704 |
| **Race/skin color** | | | | | |
| White | Ref. | Ref. | Ref. | Ref. | Ref. |
| Non-white | 1.0 (0.7–1.4) 0.777 | 3.0 (0.4– 24.0) 0.302 | 1.0 (0.6–1.7) 1.000 | 0.6 (0.2–1.9) 0.363 | 1.1 (0.6–1.9) 0.894 |
| **Tumor size** | | | | | |
| T1/ T2 | Ref. | Ref. | Ref. | Ref. | Ref. |
| T3 | 1.3 (0.8–2.2) 0.258 | 6.6 (0.7–63.3) 0.103 | 1.4 (0.7–2.8) 0.354 | * 0.963 | 1.0 (0.4–2.5) 0.806 |
| T4 | 2.4 (1.6–3.6) **<0.001** | 8.7 (1.0–74.3) **0.049** | 1.8 (1.0–3.5) 0.067 | 2.4 (0.7–7.8) 0.157 | 1.9 (1.0–3.9) 0.085 |
| **Lymph nodes** | | | | | |
| N0 | Ref. | Ref. | Ref. | Ref. | Ref. |
| N1 | 1.4 (1.0–2.1) 0.085 | 1.7 (0.4–8.3) 0.528 | 1.6 (0.9–2.7) 0.122 | 0.6 (0.2–2.0) 0.389 | 1.2 (0.7–2.4) 0.547 |
| N2/N3 | 1.9 (1.1–3.1) **0.020** | 5.2 (1.1–25.5) **0.045** | 1.6 (0.7–3.6) 0.253 | * 0.982 | 1.9 (0.9–4.3) 0.103 |
| **Estrogen receptor** | | | | | |
| Positive | Ref. | Ref. | Ref. | - | - |
| Negative | 2.5 (1.8–3.5) **<0.001** | 0.1 (*) 0.830 | 3.5 (1.3–9.5) **0.017** | - | - |
| **Progesterone receptor** | | | | | |
| Positive | Ref. | Ref. | Ref. | - | - |
| Negative | 2.4 (1.7–3.4) **<0.001** | 3.1 (0.8–12.2) 0.116 | 1.7 (0.9–3.1) 0.093 | - | - |
| **HER2** | | | | | |
| Negative | Ref. | - | Ref. | - | - |
| Positive | 0.9 (0.6–1.3) 0.391 | - | 1.0 (0.6–1.8) 0.964 | - | - |
| **Ki-67** | | | | | |
| Low (≤14) | Ref. | - | Ref. | Ref. | Ref. |
| High (>14) | 1.8 (1.1–3.0) **0.020** | - | 0.5 (0.2–1.2) 0.094 | 0.3 (0.1–1.2) 0.072 | 2.1 (0.5–8.5) 0.316 |
| **Histological grade** | | | | | |
| I/ II | Ref. | Ref. | Ref. | Ref. | Ref. |
| III | 1.5 (1.0–2.1) **0.040** | 0.1 (*) 0.666 | 1.3 (0.7–2.3) 0.430 | 0.4 (0.1–1.6) 0.193 | 0.9 (0.5–1.6) 0.677 |
| **Histological type** | | | | | |
| ICD | Ref. | Ref. | Ref. | Ref. | Ref. |
| Other | 0.9 (0.5–1.8) 0.785 | 2.5 (0.6–9.8) 0.208 | 0.7 (0.2–2.2) 0.521 | 0.1(*) 0.736 | 2.2 (0.7–7.1) 0.185 |
| **Molecular subtype** | | | | | |
| Luminal A | Ref. | - | - | - | - |
| Luminal B | 2.3 (1.1–4.6) **0.021** | - | - | - | - |
| Overexpressed HER2 | 2.4 (1.0–5.8) 0.051 | - | - | - | - |
| Triple negative | 5.8 (2.8–11.8) **<0.001** | - | - | - | - |
| **Clinical staging** | | | | | |
| I/ II | Ref. | Ref. | Ref. | Ref. | Ref. |
| III | 2.1 (1.4–3.0) **<0.001** | 2.5 (0.7–9.8) 0.208 | 2.0 (1.2–3.5) **0.015** | 1.4 (0.4–4.4) 0.629 | 2.0 (1.1–3.8) **0.030** |

**Supplementary Table 4. Continued**

| | Total | Luminal A | Luminal B | Overexpressed HER-2 | Triple Negative |
|---|---|---|---|---|---|
| | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** | **HR (95% CI) *p*** |
| **Breast surgery** | | | | | |
| Mastectomy | Ref. | Ref. | Ref. | Ref. | Ref. |
| Breast conserving | 0.5 (0.3–0.9) **0.023** | 0.4 (*) 0.422 | 0.7 (0.4–1.5) 0.379 | 0.7 (0.1–5.2) 0.691 | 0.3 (0.1–0.8) **0.014** |
| **Axillary surgery** | | | | | |
| SLNB | Ref. | Ref. | Ref. | Ref. | Ref. |
| SLNB + ALND | 1.8 (0.8–4.2) 0.204 | * 0.950 | 1.7 (0.6–5.0) 0.350 | 2.8 (0.4–22.3) 0.326 | 0.8 (0.1–7.0) 0.821 |
| ALND | 3.0 (1.7–5.4) **<0.001** | * 0.950 | 2.2 (1.0–4.8) 0.057 | 2.8 (0.2–45.2) 0.466 | 3.8 (1.4–10.7) **0.011** |
| **NAC regimen** | | | | | |
| Anthracyclic + Taxane | Ref. | Ref. | Ref. | Ref. | Ref. |
| Other | 1.5 (0.5–3.9) 0.477 | 0.1 (*) 0.830 | 0.9 (0.1–6.5) 0.910 | 12.7 (1.2–139.7) **0.038** | 0.8 (0.2–3.4) 0.793 |
| **ypT** | | | | | |
| pCR | Ref. | Ref. | Ref. | Ref. | Ref. |
| T1 | 2.0 (0.9–4.4) 0.080 | 1.0 (*) 1.000 | 0.9 (0.3–2.3) 0.767 | * 0.918 | 6.9 (1.5–31.1) **0.012** |
| T2 | 4.9 (2.4–10.2) **<0.001** | * 0.948 | 2.5 (1.0–6.0) **0.044** | * 0.911 | 11.7 (2.8–50.0) **0.001** |
| T3/ T4 | 6.8 (3.1–15.0) **<0.001** | * 0.951 | 3.2 (1.2–8.4) **0.021** | * 0.917 | 22.0 (5.0–97.8) **<0.001** |
| **ypN** | | | | | |
| N0 | Ref. | Ref. | Ref. | Ref. | Ref. |
| N1 | 1.7 (1.1–2.7) **0.018** | * 0.925 | 1.4 (0.7–2.9) 0.387 | 2.3 (0.6–9.2) 0.240 | 2.8 (1.4–5.6) **0.005** |
| N2/ N3 | 3.7 (2.5–5.4) **<0.001** | * 0.919 | 3.4 (1.9–5.9) **<0.001** | 5.7 (1.1–29.1) **0.035** | 9.3 (4.6–19.0) **<0.001** |
| **RCB class** | | | | | |
| RCB-0 | Ref. | Ref. | Ref. | Ref. | Ref. |
| RCB-I | 1.4 (0.3–6.3) 0.655 | 1.0 (*) 1.000 | 1.3 (0.2–7.8) 0.768 | 1.0 (*) 1.000 | * 0.981 |
| RCB-II | 3.5 (1.3–9.7) **0.015** | * 0.961 | 1.6 (0.4–6.7) 0.542 | * 0.941 | 9.0 ( 2.1–38.2) **0.003** |
| RCB-III | 8.1 (2.9–22.1) **<0.001** | * 0.951 | 4.3 (1.0–17.8) **0.046** | * 0.939 | 21.5 (5.1–91.9) **<0.001** |
| RCB index | 1.8 (1.5–2.1) **<0.001** | 5.2 (1.7–16.0) **0.004** | 1.7 (1.3–2.1) **<0.001** | 1.8 (1.2–2.8) **0.008** | 2.0 (1.6–2.5) **<0.001** |

HR: Hazard ratio; CI: Confidence interval; NAC: Neoadjuvant chemotherapy; SLNB: Sentinel lymph node biopsy; ALND: Axillary lymph node dissection; HER2: Human epidermal growth factor receptor 2; RCB: Residual cancer burden; pCR: Pathological complete response; only valid values used; In bold, p<0.05; *not calculated

**Original Article**

Eur J Breast Health 2025; 21(4): 326-332
**DOI:** 10.4274/ejbh.galenos.2025.2025-5-7

# Exploring Body Image Satisfaction in Post-Mastectomy Female Breast Cancer Patients

Rija Latifee[1], Usra Parvez[1], Shah Muhammad[1], Bushra Shirazi[1], Muhammad Arsalan Khan[2]

[1]Department of General Surgery, Sindh Institute of Urology and Transplantation (SIUT), Karachi, Pakistan
[2]Network for Hope, Louisville, Kentucky, USA

**ABSTRACT**

**Objective:** Mastectomy is a widely used surgical intervention for breast cancer in Pakistan, where late-stage diagnoses are common and breast-conserving options are often limited. While effective oncologically, mastectomy can significantly affect a woman's body image, emotional well-being, and social relationships. In Pakistan, sociocultural norms and limited reconstructive services further shape the post-mastectomy experience. This study aimed to assess self-perception, body image satisfaction, and related psychosocial impact in Pakistani women following mastectomy.

**Materials and Methods:** This descriptive cross-sectional study was conducted at the breast oncology clinic of the Sindh Institute of Urology and Transplantation, Karachi. A total of 159 post-mastectomy patients aged 18–65 years were surveyed using a structured, culturally adapted questionnaire based on the body image scale. Statistical analyses included chi-square testing and multinomial logistic regression to assess associations between body image perception and demographic or psychosocial variables. Internal consistency was confirmed (Cronbach's alpha = 0.863).

**Results:** While 34% reported no change in body image perception, 66% reported varying degrees of change. Strong associations were identified between negative body image perception and feelings of reduced attractiveness, mirror discomfort, and spousal relationship changes ($p$<0.001). Multinomial regression confirmed these as significant predictors of reporting major body image change. Interest in breast reconstruction was low (15.7%), and although age and education were not significantly associated, time since surgery approached significance ($p$ = 0.07).

**Conclusion:** A substantial proportion of Pakistani women experience emotional and psychosocial distress following mastectomy. These findings highlight the importance of early counseling, spousal support, and culturally sensitive body image discussions to promote long-term psychosocial recovery.

**Keywords:** Body image perception; breast cancer; mastectomy; Pakistan; patient satisfaction; psychosocial impact

**Cite this article as:** Latifee R, Parvez U, Muhammad S, Shirazi B, Khan MA. Exploring body image satisfaction in post-mastectomy female breast cancer patients. Eur J Breast Health. 2025; 21(4): 326-332

**Key Points**

- This cross-sectional study assessed body image perception and emotional impact in 159 Pakistani women following mastectomy, using an adapted body image scale.
- Two-thirds of participants reported some degree of body image disturbance, with strong associations between negative self-perception and feelings of sadness, reduced confidence, and perceived loss of femininity.
- Time since surgery showed a near-significant effect on perception, while age and education were not significantly associated.
- The findings highlight the importance of culturally sensitive counseling and open discussion of body image in breast cancer care in Pakistan.

## Introduction

Breast cancer is the most frequently diagnosed malignancy among women globally, with over 2.3 million new cases and approximately 685,000 deaths reported in 2020 (1). In Pakistan, the situation is particularly alarming, as one in nine women is projected to develop breast cancer during their lifetime (2). According to the International Agency for Research on Cancer, more than 30,000 new cases were recorded in Pakistan in 2022 alone (3). A significant proportion of these patients present with advanced-stage disease, necessitating mastectomy as a primary surgical intervention (4).

Mastectomy, while effective in managing breast cancer, often leads to profound psychological and psychosocial consequences (5). The removal of one or both breasts can significantly impact a woman's body image, self-esteem, and overall quality of life. Studies have documented that such physical alterations can result in body image disturbances, including feelings of diminished femininity and attractiveness. These

**Corresponding Author:**
Muhammad Arsalan Khan, FCPS; muhammad.a.khan@ucdenver.edu

**Received:** 02.06.2025
**Accepted:** 07.07.2025
**Epub:** 04.09.2025
**Available Online Date:** 25.09.2025

issues may persist long after treatment, affecting social reintegration and intimate relationships (6).

Research in various cultural contexts has highlighted the individualized and contextual nature of women's experiences post-mastectomy. For instance, a study conducted in Sweden investigated life satisfaction and body image among women post-mastectomy, emphasizing the importance of understanding these experiences to improve quality of life (7). Similarly, studies in other regions have identified factors associated with body image dissatisfaction and self-esteem in mastectomized breast cancer survivors (8). However, there is a notable lack of data from Pakistan, where sociocultural factors such as modesty norms, familial roles, and societal expectations significantly influence women's perceptions of illness and recovery. Open discussions about body image and psychological distress are often discouraged, potentially exacerbating the emotional burden following mastectomy. Moreover, access to breast reconstruction in Pakistan is limited due to financial constraints, lack of awareness, and insufficient surgical expertise (9).

To address this gap, our study used a quantitative survey based on the body image scale (BIS), a validated instrument designed for assessing body image in cancer patients (7, 10). The BIS, which has been translated and linguistically validated across various cultural settings (11), provides a standardized framework for evaluating subjective constructs, enabling comparative analysis with other regional and global cohorts. By adapting the BIS to the Pakistani context, we aimed to capture nuanced perspectives on body image and life satisfaction among women following mastectomy.

Understanding women's self-perception after mastectomy is important for developing culturally sensitive psychosocial interventions, particularly in Pakistan, where late-stage diagnoses and limited reconstructive options make mastectomy a frequent reality (12). This study therefore aimed to assess self-perception, including body image and life satisfaction, among Pakistani women post-mastectomy and to identify socio-demographic and clinical factors associated with negative perceptions. The findings are intended to guide tailored support services and enhance holistic, patient-centered care strategies within oncology settings.

## Materials and Methods

This descriptive cross-sectional study was conducted at the breast oncology clinic of the Sindh Institute of Urology and Transplantation (SIUT), Karachi. A total of 159 post-mastectomy patients aged 18–65 years were surveyed using a structured, culturally adapted questionnaire based on the BIS. The BIS is a 10-item validated tool for cancer patients assessing affective, behavioral, and cognitive aspects of body image. Each item is rated on a 4-point Likert scale, with higher scores indicating more severe dissatisfaction. The tool evaluated body image perception, self-confidence, emotional impact, and social relationships.

The study protocol was approved by the SIUT Institutional Review Board (approval no: SIUT-ERC-2025/A-543, date: 03.03.2025), and written informed consent was obtained from all participants prior to enrollment.

The sample size was calculated using the World Health Organization sample size calculator. Based on a previous estimate that 33.6% of patients experience body image disturbance following mastectomy [Phoosuwan and Lundberg (7)], with a 7% margin of error and a 95% confidence level, the minimum required sample size was found to be 159 participants.

Eligible participants included all consenting female patients aged 18 to 65 years who had undergone mastectomy (simple, modified radical, or radical) at SIUT and were attending regular follow-up appointments. Exclusion criteria included patients with metastatic disease, those who had undergone breast-conserving procedures (e.g., lumpectomy, quadrantectomy), male patients, individuals who were operated on outside of SIUT, and those who declined to participate.

Participants were recruited through convenience sampling and interviewed by the primary investigator using a structured, pre-tested questionnaire. The instrument collected demographic data, clinical history, and responses related to body image perception and the psychosocial impact of mastectomy on personal and interpersonal domains. To ensure data confidentiality and participant anonymity, all responses were coded, and patient identifiers were removed. Data verification processes, including double-entry techniques and secure digital storage, were employed to maintain data integrity.

### Statistical Analysis

Statistical analysis was performed using SPSS, version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables such as age and time since surgery are reported as mean ± standard deviation. Categorical variables, including educational status, employment, self-confidence, and dissatisfaction with physical appearance, are presented as frequencies and percentages.

Internal consistency of the BIS was assessed using Cronbach's alpha, which yielded a reliability coefficient of 0.863, indicating strong internal consistency. Stratification was performed based on age group, occupation, education level, and time since surgery to explore variations in outcomes. The chi-square test was used to examine associations between categorical variables, with a $p$-value of $<0.05$ considered statistically significant. We note that the chi-square test assesses statistical association (or independence) between categorical variables and is appropriate in this context (McHugh ML. The chi-square test of independence. *Biochem Med (Zagreb)*. 2013;23(2):143-149. doi:10.11613/BM.2013.018).

A multinomial logistic regression was conducted to explore factors associated with self-perceived changes in body image following breast surgery. The outcome variable was categorized into "Not at all," "A little," "Quite a bit," and "Very much" change in BI self-perception. Predictor variables included sociodemographic characteristics (e.g., age group, education level, marital and employment status, and time since surgery) and psychosocial factors (e.g., feelings of attractiveness, sadness, mirror discomfort, and spousal relationship changes). Predictors were initially assessed via univariate analyses and then entered into a multivariate multinomial logistic regression model. Odds ratios (ORs) and 95% confidence intervals were calculated, and all statistical outputs were rounded to two decimal places.

The manuscript complies with the STROBE guidelines for reporting observational original research study.

## Results

A total of 159 women who had undergone mastectomy were surveyed at the breast oncology clinic of SIUT. The highest representation was

in the 41–50 age group (35.2%), followed by 31–40 years (26.4%) and 51–60 years (20.8%). Education levels varied, with 34.6% having no formal education and 27% completing primary education. Most participants were married (76.7%) and housewives (84.3%). Regarding the time since surgery, 45.9% had undergone mastectomy 1–2 years prior, while 37.1% had surgery less than a year ago. Table 1 summarizes the sociodemographic and clinical characteristics of the participants.

Regarding body image perception, 34% of participants reported no change post-mastectomy, while 27% experienced mild change, 17% noted moderate change, and 21% indicated significant change. Feelings of decreased attractiveness were reported by 61%, while 42.1% experienced no emotional distress. Self-confidence was unaffected in 57% of women, and over half (51.6%) had no difficulty looking at themselves in the mirror. Most women (66%) did not avoid social interaction, but 13.9% did. Around 35% reported changes in spousal relationships, while only 27.7% indicated changes with friends. About 80% noticed no change in the behavior of others. Grouped Likert-scale distributions for these responses are illustrated in Figure 1.

Approximately 15.7% expressed interest in breast reconstruction, while 45.3% did not prioritize it. Importantly, 43.3% of women emphasized the value of discussing body image as part of breast cancer treatment. These comparative perceptions are presented in bar chart format in Figure 2.

Table 2 presents the associations between demographic characteristics and self-perception of body image following mastectomy. The chi-square test results showed no significant relationship between age group ($p = 0.54$) or education level ($p = 0.90$) and perceived changes in body image. However, the association between time since surgery and body image perception approached statistical significance ($p = 0.07$), suggesting a potential trend where time elapsed since mastectomy may influence how women perceive changes in their body image.

Table 3 displays the associations between psychosocial and emotional factors and post-mastectomy changes in body image perception. Strong statistical associations were observed with feeling less attractive or feminine ($p<0.001$), sadness and emotional distress ($p<0.001$), reduced self-confidence ($p<0.001$), and difficulty looking in the mirror ($p<0.001$). Additional associations were noted with social withdrawal ($p = 0.06$) and changes in spousal relationships ($p<0.001$), while shifts in the behavior of others or relationships with close friends did not reach statistical significance.

Multinomial logistic regression analysis revealed that feeling less attractive (OR = 10.07), mirror discomfort (OR = 1.78), and spousal relationship changes (OR = 1.92) were strong predictors of reporting "very much" change in body image perception. In contrast, higher education level was modestly associated with lower odds of substantial body image change (OR = 0.93), and sadness or emotional distress was also inversely associated (OR = 0.66). These results are summarized in Table 4.

**Table 1. Socio-demographic characteristics of the participants**

| Variable | Category | Frequency (n) | Percentage (%) |
|---|---|---|---|
| Age group (years) | Less than 20 | 3 | 1.9 |
| | 21–30 | 8 | 5.0 |
| | 41–50 | 56 | 35.2 |
| | 31–40 | 42 | 26.4 |
| | 51–60 | 33 | 20.8 |
| | More than 60 | 17 | 10.7 |
| Education | No formal education | 55 | 34.6 |
| | Elementary school | 43 | 27.0 |
| | Middle/high school | 37 | 23.3 |
| | College education | 21 | 13.2 |
| | Post-graduate | 3 | 1.9 |
| Marital status | Married | 122 | 76.7 |
| | Un-married | 18 | 11.3 |
| | Widowed | 15 | 9.4 |
| | Divorced | 4 | 2.5 |
| Employment | Home-maker | 135 | 84.9 |
| | Employed | 19 | 11.9 |
| | Unemployed | 5 | 3.1 |
| Time since surgery | Less than one year | 59 | 37.1 |
| | 1–2 years | 73 | 45.9 |
| | 3–5 years | 22 | 13.8 |
| | More than 5 years | 5 | 3.1 |

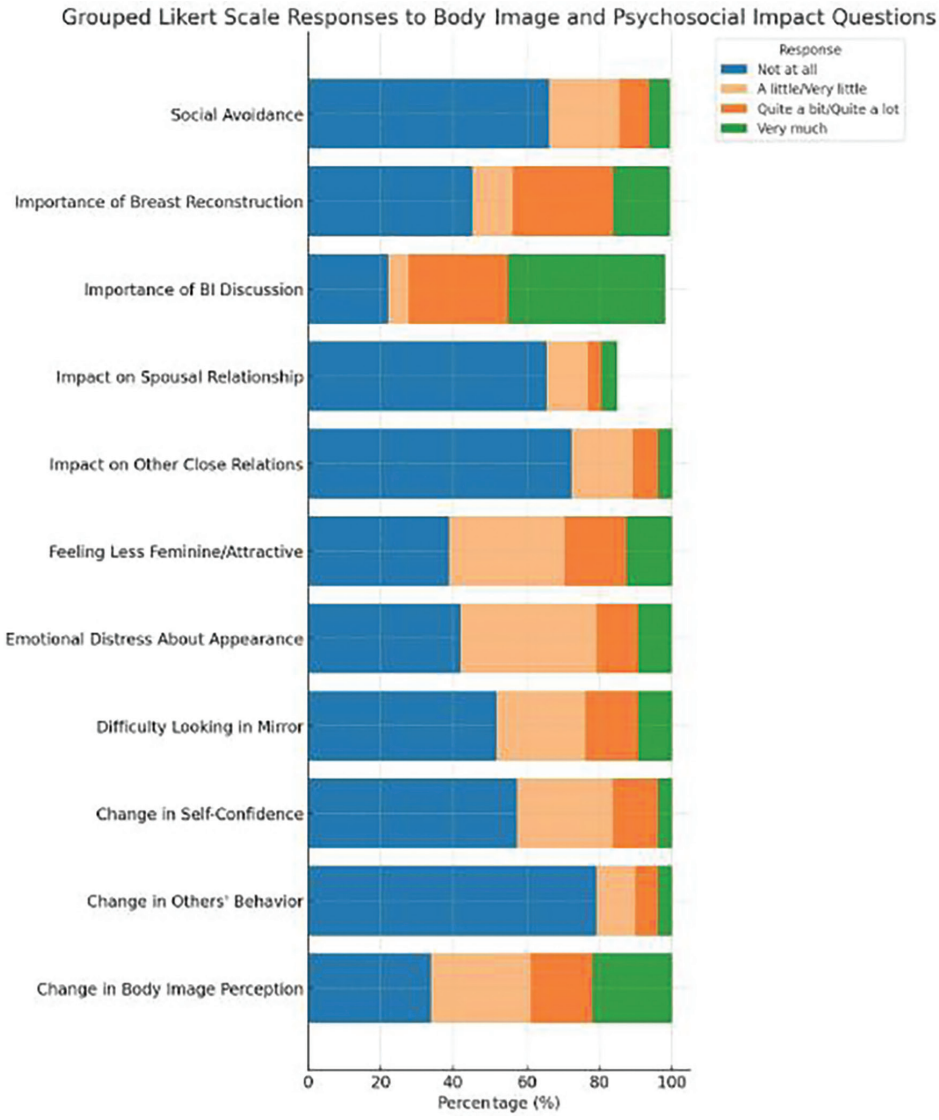



**Figure 1.** Grouped Likert scale responses to psychosocial impact questions

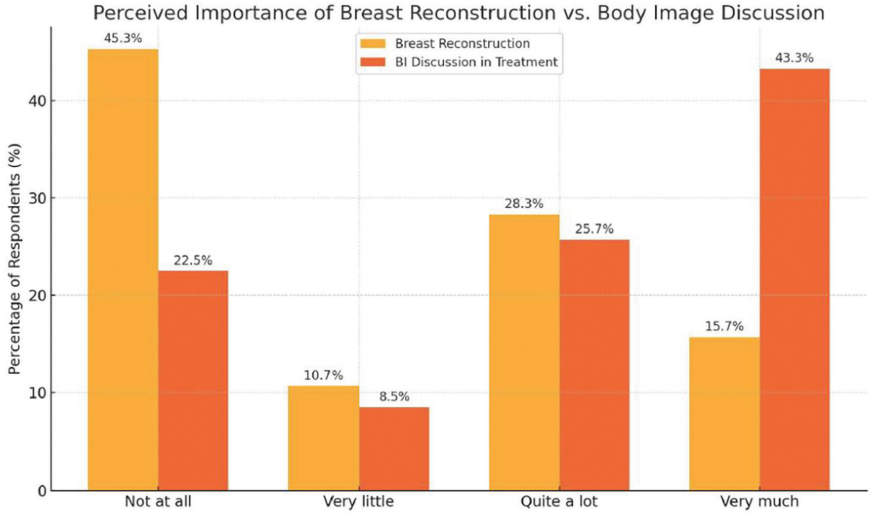



**Figure 2.** Bar chart of importance perceptions

Bar chart comparison of perceived importance of:

• Breast reconstruction

• Body image discussion during treatment across four response categories: Not at all, very little, quite a lot, very much

**Table 2. Demographic associations with body image perception**

| Variable | Chi-square statistic | Degrees of freedom | *p*-value |
|---|---|---|---|
| Age group | 23.61 | 4 | 0.54 |
| Education level | 12.26 | 3 | 0.90 |
| Time since surgery | 23.60 | 3 | 0.07 |

**Table 3. Psychosocial associations with body image perception**

| Psychosocial variable | Chi-square statistic | Degrees of freedom | *p*-value |
|---|---|---|---|
| Feeling less attractive | 151.52 | 3 | <0.001 |
| Sadness or distress | 90.32 | 3 | <0.001 |
| Low self-confidence | 67.98 | 3 | <0.001 |
| Mirror discomfort | 76.77 | 3 | <0.001 |
| Social withdrawal | 24.05 | 1 | 0.06 |
| Spousal relationship changes | 46.13 | 1 | <0.001 |

**Table 4. Predictors of body image change (regression analysis)**

| Predictor | OR (very much) | 95% CI lower | 95% CI upper |
|---|---|---|---|
| Feeling less attractive | 10.07 | 6.79 | 14.30 |
| Mirror discomfort | 1.78 | 1.20 | 2.60 |
| Spousal relationship changes | 1.92 | 1.30 | 2.90 |
| Education level | 0.93 | 0.85 | 1.02 |
| Sadness or distress | 0.66 | 0.50 | 0.85 |

OR: Odds ratio; CI: Confidence interval

## Discussion and Conclusion

This study explored body image perception and its psychosocial impact in Pakistani women following mastectomy for breast cancer, offering insights into a relatively under-researched population. Using a culturally adapted, structured questionnaire based on the BIS, the study quantitatively assessed self-perception, emotional well-being, and interpersonal relationships, while identifying demographic variables associated with these outcomes.

The findings reaffirm that mastectomy continues to be a significant psychological event in the lives of breast cancer survivors. Although 34% of women reported no change in body image perception, the majority experienced various degrees of distress, 27% mild, 17% moderate, and 21% significant, highlighting a spectrum of emotional responses. These outcomes are consistent with prior literature, including Phoosuwan and Lundberg (7), who found body image dissatisfaction closely associated with reduced life satisfaction among breast cancer patients, especially younger women and those with limited psychosocial support.

Our revised analysis showed that although age and education were not significant predictors of body image change, important trends emerged. Women with lower education were more likely to report mirror discomfort and diminished self-esteem, while younger patients tended to experience heightened emotional distress. These patterns mirror findings from studies conducted in Iran and Egypt, underscoring how educational background and life stage influence psychosocial recovery post-mastectomy (8, 13).

A notable contribution of this study is the strong statistical association found between body image change and specific emotional and interpersonal variables. Mirror discomfort, perceived loss of attractiveness, and spousal relationship changes emerged as the most robust predictors of reporting "very much" change in body image, based on multinomial logistic regression (14). These findings reaffirm that body image is not only an individual perception but is deeply embedded in interpersonal contexts (13).

The role of spousal relationships was particularly pronounced. A considerable number of participants reported changes in their intimate relationships following surgery, despite most indicating that overt behavior from others, including spouses, remained unchanged. This subtle emotional shift may reflect internalized anxieties rather than explicit rejection, a nuance that aligns with cultural norms of emotional restraint in Pakistani society (15). Familial support, in this context, may buffer visible social withdrawal while leaving underlying emotional needs unaddressed.

Reconstruction was a relatively low priority for many participants with only 15.7% expressing a strong interest, while nearly half prioritized disease remission over cosmetic or restorative goals. This finding resonates with prior studies indicating that cultural expectations, financial limitations, and fears about surgery can dampen interest in reconstruction, even when medically available (16).

Importantly, our analysis highlighted that body image is perceived less in physical terms and more through affective self-perception, with traits like character and resilience seen as core to identity. This has crucial implications for the design of psychosocial interventions in Pakistan. As Morales-Sánchez et al. (5) argue, culturally sensitive self-esteem enhancement strategies must reflect localized norms and personal identity frameworks rather than Western-centric ideals of physical recovery.

From a methodological perspective, the structured, face-to-face survey approach enabled richer data collection from a population often underserved in academic research. However, despite adapting the BIS for local use and confirming strong internal consistency, the lack of full psychometric revalidation may limit international comparability. Similarly, the absence of a preoperative psychological baseline remains a limitation in assessing true change (17).

Future studies should build on this foundation by employing longitudinal designs that track body image perception from diagnosis through long-term survivorship. Integration of fully validated tools and culturally adapted cognitive-behavioral interventions (18), especially those that include a couple's counseling and uses mirror exposure therapy, may provide further benefit. Such approaches, shown to be effective in other regions, should be tested locally to inform policy and practice.

This study highlighted that while around a third of women from a single center in Karachi, Pakistan, report stability in body image and self-confidence after mastectomy, the majority reported varying degrees of emotional and psychosocial distress. Although no significant associations were found between body image perception and age or education, emotional distress, mirror discomfort, and changes in spousal relationships were strong predictors of perceived body image deterioration. The findings underscore the importance of integrating culturally sensitive counseling, early psychosocial intervention, and patient-centered discussions about body image into breast cancer care in Pakistan. These strategies are vital for improving emotional well-being and supporting long-term recovery in resource-limited settings, like Pakistan.

### Acknowledgment

The authors thank the clinical and administrative staff at SIUT for facilitating patient recruitment and data collection. We also acknowledge the contribution of patients who generously shared their experiences for the purpose of this study.

### Ethics

**Ethics Committee Approval:** The study protocol was approved by the SIUT Institutional Review Board (approval no: SIUT-ERC-2025/A-543, date: 03.03.2025),

**Informed Consent:** Written informed consent was obtained from all participants prior to enrollment.

### Footnotes

### Authorship Contributions

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

**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. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, et al. Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast. 2022; 66: 15-23. (PMID: 36084384) [Crossref]
2. Menhas R, Umer S. Breast cancer among Pakistani women. Iran J Public Health. 2015; 44: 586-587. (PMID: 26056679) [Crossref]
3. 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 Feb 4. (PMID: 33538338) [Crossref]
4. Shirazi B, Niaz M, Khan MA. The characteristics and risk factors of breast cancer patients trend distinctive regional differences: a cross-sectional study. J Pak Med Assoc. 2024; 74: 672-676. (PMID: 38751260) [Crossref]
5. Morales-Sánchez L, Luque-Ribelles V, Gil-Olarte P, Ruiz-González P, Guil R. Enhancing self-esteem and body image of breast cancer women through interventions: a systematic review. Int J Environ Res Public Health. 2021; 18: 1640. (PMID: 33572137) [Crossref]
6. Gopie JP, Mureau MA, Seynaeve C, Ter Kuile MM, Menke-Pluymers MB, Timman R, et al. Body image issues after bilateral prophylactic mastectomy with breast reconstruction in healthy women at risk for hereditary breast cancer. Fam Cancer. 2013; 12: 479-487. (PMID: 23224779) [Crossref]
7. Phoosuwan N, Lundberg PC. Life satisfaction, body image and associated factors among women with breast cancer after mastectomy. Psychooncology. 2023; 32: 610-618. (PMID: 36670514) [Crossref]
8. Álvarez-Pardo S, De Paz JA, Montserrat Romero-Pérez E, Portilla-Cueto KM, Horta-Gim MA, González-Bernal JJ, et al. Factors associated with body image and self-esteem in mastectomized breast cancer survivors. Int J Environ Res Public Health. 2023; 20: 5154. (PMID: 36982062) [Crossref]
9. Faisal AB, Shahid F, Khalid L, Rahman MF. Advancing immediate breast reconstruction surgery in Pakistan: bridging literature gaps and meeting patient needs. Arch Plast Surg. 2024; 52: 116-118. (PMID: 40083621) [Crossref]
10. Hopwood P, Fletcher I, Lee A, Al Ghazal S. A body image scale for use with cancer patients. Eur J Cancer. 2001; 37: 189-197. (PMID: 11166145) [Crossref]
11. Belani P, Wadasadawala T, Sarin R, Pathak R, Krishnamurthy R, Syeda N, et al. Translation and linguistic validation of BIS (body image scale) for breast cancer patients in India. Indian J Surg Oncol. 2025; 16: 203-210. (PMID: 40114862) [Crossref]
12. Mehmood M, Aslam A, Ahmed W, Aman S, Ali A, Feroze H, et al. Awareness and acceptability of breast reconstruction among women with breast cancer in twin cities of Islamabad and Rawalpindi. Life and Science. 2024; 5: 9-15. [Crossref]
13. Ahmed MH, Mahmoud AB, Abd Elmoathy H. Correlation between body image, self-esteem and self-efficacy among women with mastectomy. J Nurs Sci Benha Univ. 2024; 5: 945-970. [Crossref]

14. Sheppard LA, Ely S. Breast cancer and sexuality. Breast J. 2008; 14: 176-181. (PMID: 18248559) [Crossref]

15. Konara Mudiyanselage SP, Wu YL, Kukreti S, Chen CC, Lin CN, Tsai YT, et al. Dynamic changes in quality of life, psychological status, and body image in women who underwent a mastectomy as compared with breast reconstruction: an 8-year follow up. Breast Cancer. 2023; 30: 226-240. (PMID: 36319889) [Crossref]

16. Moreira H, Silva S, Marques A, Canavarro MC. The Portuguese version of the body image scale (BIS) - psychometric properties in a sample of breast cancer patients. Eur J Oncol Nurs. 2010; 14: 111-118. (PMID: 19892597) [Crossref]

17. Boesen EH, Karlsen R, Christensen J, Paaschburg B, Nielsen D, Bloch IS, et al. Psychosocial group intervention for patients with primary breast cancer: a randomised trial. Eur J Cancer. 2011; 47: 1363-1372. (PMID: 21458989) [Crossref]

18. Vos PJ, Visser AP, Garssen B, Duivenvoorden HJ, de Haes HC. Effects of delayed psychosocial interventions versus early psychosocial interventions for women with early stage breast cancer. Patient Educ Couns. 2006; 60: 212-219. (PMID: 16442463) [Crossref]

Original Article

Eur J Breast Health 2025; 21(4): 333-338
DOI: 10.4274/ejbh.galenos.2025.2025-5-10

# Accuracy of Intraoperative Sentinel Lymph Node Evaluation by Imprint Cytology in Breast Cancer: A 12-Year Single Center Experience With 2,528 Patients

Aysel Bayram[1], Sidar Bagbudar[1], Cagla Safak Karaoglan[2], Esma Sayar[1], Baran Mollavelioglu[3], Mustafa Tukenmez[3], Hasan Karanlık[4], Semen Onder[1], Ekrem Yavuz[1]

[1]Department of Pathology, İstanbul Faculty of Medicine, İstanbul University, İstanbul, Türkiye
[2]Department of Pathology, Başakşehir Çam and Sakura City Hospital, University of Health Sciences Türkiye, İstanbul, Türkiye
[3]Department of General Surgery, İstanbul Faculty of Medicine, İstanbul University, İstanbul, Türkiye
[4]Department of Surgical Oncology Unit, Institute of Oncology, İstanbul University, İstanbul, Türkiye

## ABSTRACT

**Objective:** Sentinel lymph node biopsy (SLNB) is a key procedure for evaluating axillary lymph node status in early breast cancer, offering lower morbidity than axillary lymph node dissection. Intraoperative evaluation (IOE) of sentinel lymph nodes (SLNs) with methods like frozen section (FS) and imprint cytology (IC) aid in making immediate surgical decisions, although IOE accuracy may vary due to several factors.

**Materials and Methods:** This retrospective study involved 2,528 patients with invasive breast cancer who underwent SLNB at a single institution from 2012 to 2024. Primarily, IC was used for intraoperative assessment, while FS was selectively performed in certain cases, such as with suspicious macroscopic findings or after neoadjuvant chemotherapy (NAC). The final diagnosis relied on permanent sections with serial step-leveling and classification of metastasis size.

**Results:** IOE showed a sensitivity of 65.8% and specificity of 97% for detecting lymph node metastases. The combination of IC and FS yielded higher sensitivity (76.1%) compared to IC alone (64.1%), particularly for isolated tumor cells (ITC). Patients treated with NAC exhibited slightly lower IOE accuracy (83.8%) compared to those without NAC (85.9%). False negatives were more common in cases of micrometastasis, ITC, and invasive lobular subtype. Excluding micrometastasis and ITC significantly enhanced IOE accuracy.

**Conclusion:** The accuracy of intraoperative SLN evaluation is affected by size of the metastasis, tumor subtype, and prior NAC. While IC is acceptable for IOE, combining IC and FS is advised, especially in the setting of earlier NAC, to enhance accuracy for small metastatic foci.

**Keywords:** Breast cancer; intraoperative evaluation; neoadjuvant chemotherapy; sentinel lymph nodes

**Cite this article as:** Bayram A, Bagbudar S, Karaoglan CS, Sayar E, Mollavelioglu B, Tukenmez M, et al. Accuracy of intraoperative sentinel lymph node evaluation by imprint cytology in breast cancer: a 12-year single center experience with 2,528 patients. Eur J Breast Health. 2025; 21(4): 333-338

**Key Points**

- Parameters such as the extent of metastasis, tumor subtype, and the use of neoadjuvant chemotherapy (NAC) in the intraoperative evaluation (IOE) of sentinel lymph nodes (SLNs) affect the accuracy of the results.
- Imprint cytology (IC) is considered an acceptable method for the IOE of SLNs in a primary surgery setting.
- However, IC and frozen section are both recommended in the setting of a patient having received NAC.

## Introduction

Sentinel lymph node biopsy (SLNB) has been introduced as an effective and safe procedure to assess axillary lymph node status in patients with early breast cancer and clinically negative lymph nodes. SLNB with no further axillary lymph node dissection has been shown to reduce postoperative long-term morbidity without compromising local disease control (1-5).

The two most common methods used for intraoperative evaluation (IOE) of the sentinel lymph node include frozen section (FS) and imprint cytology (IC) (6-10). In primary surgery, detecting lymph node metastasis intraoperatively by IC alone has an estimated sensitivity of 63%, ranging from 34% to 95%. In contrast, the sensitivity for FS is 86% although this also varies widely from 44% to 99.8% (6, 11). Like any other assay, multiple factors influence the accuracy of results in

**Corresponding Author:**
Aysel Bayram MD; aysel.bayram@istanbul.edu.tr

**Received:** 15.06.2025
**Accepted:** 15.07.2025
**Epub:** 04.09.2025
**Available Online Date:** 25.09.2025

the IOE of sentinel lymph nodes. Some factors include the evaluation method, quality of the IC or FS slides, the size of the metastasis, tumor type, whether the patient received treatment before surgery, and the experience of the pathologists interpreting the slides (12, 13).

The aim of the present study was to assess the accuracy of IC and FS in the IOE of sentinel lymph nodes and investigate factors that contribute to low accuracy.

## Materials and Methods

### Patient Selection

A total of 2,528 patients with invasive breast cancer who underwent SLNB were selected through a database search at the Pathology Department of İstanbul Medical Faculty from 2012 to 2024. Clinicopathologic parameters, including patient age, histologic tumor type, pathologic tumor stage, size of nodal metastasis, and pathologic nodal stage, were obtained from patients records.

### Intraoperative Evaluation

At our institution, the pathology department has a subspecialty practice model. However, IOEs are performed by pathologists from all subspecialties, including those in the breast subspecialty. Our standard intraoperative lymph node assessment procedure was used in all cases included in this study. Lymph nodes were serially sectioned into 2- to 3- 3-mm-thick cross sections; ICs were performed by imprinting the whole cut surface on one side of all cross sections, with at least two IC slides performed on most of the lymph nodes; and tissue scraping of grossly suspicious areas was performed in some cases. Although the FS was not a part of the standard IOE procedure in our institution, in some situations, such as suspicious macroscopic findings, inadequate touch imprint preparation, and especially when the patient had undergone neoadjuvant chemotherapy (NAC), preferred to perform FSs in addition to ICs. IC and FS slides were stained with routine hematoxylin-eosin (H&E) stain and interpreted by a single pathologist assigned for FS service.

### Microscopic Evaluation

For the final diagnosis, H&E-stained, 3-step levels of the entire lymph node were assessed. An unstained level between the two H&E slides was retained for possible cytokeratin staining. The size of nodal metastasis was classified according to the eighth edition of the American Joint Committee on Cancer Staging System. Nodal metastases were classified as isolated tumor cells (ITC) (<0.2 mm), micrometastasis (>0.2 mm to <2 mm), or macrometastasis (>2 mm) (14).

### Statistical Analysis

All data were analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 21.0 (IBM Corp, Armonk, NY, USA). The $\chi^2$ and Fisher exact tests were applied for categorical variables, and the Mann-Whitney U test was used for continuous variables. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) respectively, and overall accuracy of IOE for detecting axillary lymph node metastases were calculated. A $p<0.05$ was considered significant in all comparisons. The lymph nodes with an intraoperative diagnosis of "atypical" were excluded from the statistical accuracy analysis.

## Results

The median (range) age of the 2,528 patients with invasive breast cancer who underwent SLNB was 52 (23–90) years. A total of 7,204 lymph nodes were identified from the 2,528 cases, with a median number of lymph nodes of 2.85 (1–14) lymph nodes. Among the study group, 1,757 (69.5%) cases were invasive ductal carcinoma, 164 (6.5%) cases were invasive lobular carcinoma, and 607 (24%) cases were another histological subtype. Of the patients, 645 (25.5%) were treated with NAC. The T stage of the cases (T1, T2, T3, T4) were 1,018 (40.3%), 1,302 (51.5%), 178 (7%), and 30 (1.2%), respectively. Of the patients with intraoperative pathologic evaluation, 2,238 (88.5%) were evaluated with IC, and 290 (11.5%) were assessed with IC and FS. Of the 2,528 cases that underwent IOE, 2,161 (85.5%) were evaluated by non-breast pathologists, and 367 (14.5%) were assessed by breast pathologists.

Of the 2,528 cases examined in this study, 1,713 (67.8%) were interpreted as negative and 650 (25.7) as positive. In 165 (6.5%) patients, the IOE was reported as 'atypical' rather than benign or malignant. In the final diagnosis, 1,052 (41.6%) of 2,528 cases were positive for any tumor cells. Of the metastatic cases, 773 (73.5%) contained macrometastasis, 183 (17.4%) micrometastasis, and 96 (9.1%) had ITC. The overall sensitivity, specificity, PPV, and NPV for identifying lymph node metastases was 65.8%, 99%, 97.9%, and 80.7%, respectively. When micrometastasis and ITC were excluded from the analysis, sensitivity, specificity, PPV, and NPV of IOE for identifying lymph node metastases were 81.6%, 99%, 97.7%, and 91.3%, respectively.

The sensitivity, specificity, PPV, and NPV rates of sentinel lymph nodes evaluated by IC alone were 64.1%, 99%, 97.5%, and 80.8%, respectively. In 290 (11.5%) patients evaluated by IC and FS, the sensitivity, specificity, PPV, and NPV were 76.1%, 100%, and 79.8%, respectively. In the IOEs performed only with IC, the correlation rates for macrometastasis, micrometastasis, and ITC were 80.7%, 24.1%, and 11.1%, respectively. In the examination performed with IC and FS, the correlation rates were 86.2%, 29.4%, and 37.5%, respectively. In the IOE, the use of the FS method was significantly different only when detecting ITC ($p$ = 0.037). The relationships of clinicopathological parameters with IOE and final diagnostic agreement are shown in Table 1. The distribution of cases according to the IOE method is shown in Figure 1.

IOE had a sensitivity of 67.3%, a specificity of 99%, and an accuracy of 83.8% in patients treated with NAC. In patients without NAC, IOE had a sensitivity of 65.2%, a specificity of 99%, and an accuracy of 85.9% ($p$ = 0.206). The distribution of cases according to NAC status is shown in Figure 2.

The false-negative rate was 13.1% for IOE. Among 331 false-negative results, macrometastasis, micrometastasis, and ITC were identified in 39.9%, 36.8%, and 23.3%, respectively. In sentinel lymph nodes with false negative results, micrometastasis and ITC, and invasive lobular subtype were observed at higher frequencies than in the whole cohort ($p<0.001$).

Of the 650 sentinel lymph nodes evaluated as positive in IOE, 14 were false-positive. All these patients were evaluated with IC by a non-

**Table 1. Comparison of pathologic and clinical parameters between concordant and discordant cases in intraoperative evaluation**

| | All patients** (*n* = 2,363) (%) | Concordant result (true pos+true neg) (*n* = 2,018) (%) | Discordant result (false neg+false pos) (*n* = 345) (%) | *p* |
|---|---|---|---|---|
| Age (mean) | 52.01 | 52.18 | 51.01 | 0.077 |
| Histologic subtype | | | | |
| Ductal | 1,651 (69.9) | 1,404 (85) | 247 (15) | |
| Lobular | 145 (6.1) | 112 (77.2) | 33 (22.8) | **0.002** |
| Other | 567 (24) | 502 (88.5) | 65 (11.5) | |
| Neoadjuvant chemotherapy | | | | |
| No | 1,803 (76.3) | 1,549 (85.9) | 254 (14.1) | 0.206 |
| Yes | 560 (23.7) | 469 (83.8) | 91 (16.3) | |
| T stage | | | | |
| T1 | 963 (40.8) | 832 (86.4) | 131 (13.6) | |
| T2 | 1,212 (51.3) | 1,023 (84.4) | 189 (15.6) | 0.374 |
| T3 | 161 (6.8) | 140 (86.9) | 21 (13.1) | |
| T4 | 27 (1.1) | 23 (85.2) | 4 (14.8) | |
| N stage | | | | |
| N0 | 1,443 (61.1) | 1,361 (94.3) | 82 (5.7) | |
| N1 | 690 (29.2) | 443 (64.2) | 247 (35.8) | **<0.001** |
| N2 | 175 (7.4) | 161 (92) | 14 (8) | |
| N3 | 55 (2.3) | 53 (96.4) | 2 (3.6) | |
| IOE methods | | | | |
| IC | 2,103 (89) | 1,790 (85.1) | 313 (14.9) | 0.267 |
| IC+FS | 260 (11) | 228 (87.7) | 32 (12.3) | |
| Type of metastasis | | | | |
| Macrometastasis | 716 (30.4) | 584 (81.6) | 132 (18.4) | |
| Micrometastasis | 162 (6.8) | 40 (24.7) | 122 (75.3) | **<0.001** |
| ITC | 89 (3.8) | 12 (13.5) | 77 (86.5) | |
| Evaluating pathologist | | | | |
| Breast pathologist | 346 (14.6) | 298 (86.1) | 48 (13.9) | 0.678 |
| Non-breast pathologist | 2,017 (85.4) | 1,720 (85.3) | 297 (14.7) | |

IOE: Intraoperative evaluation; IC: Imprint cytology; FS: Frozen section; ITC: Isolated tumor cells;
** Intraoperative diagnosis of "atypical" (*n* = 165) were excluded from the statistical analysis

breast pathologist. A review of false-positive lymph node IC revealed reactive changes, including histiocytes and multinucleated giant cells from a prior biopsy site or regression site associated with NAC. Finally, of the 165 (6.5%) sentinel lymph nodes interpreted as atypical, 80 (48.5%) were interpreted as negative in permanent sections. Of these 165 patients, 85 (51.5%) were treated with NAC, and 135 (81.8%) were evaluated with IC.

## Discussion and Conclusion

Intraoperative sentinel lymph node assessment to detect metastatic breast carcinoma has become the standard of care not only to avoid unnecessary axillary lymph node dissection but also to eliminate reoperation for completion of axillary lymph node dissection (14).

In previous studies, the overall sensitivity and specificity of IOE for the identification of sentinel lymph node metastases were reported to be 40–86% and 97–100%, respectively (15-19). In the present study, the overall sensitivity and specificity of IOE for identifying sentinel lymph node metastases were 65.8% and 97%, respectively.

Despite the variable accuracy, many pathologists, particularly those familiar with cytology preparations, prefer IC as it is technically easier to perform and offers a faster turnaround time. Another reason IC may be favored over FS is because it is technically challenging to cut fatty lymph nodes, and FS can deplete tissue and, therefore, possibly miss smaller metastasis. In a previous study (14), the sensitivity of IC was 37.5%, specificity was 100%, and NPV was 90.2%. In the literature,

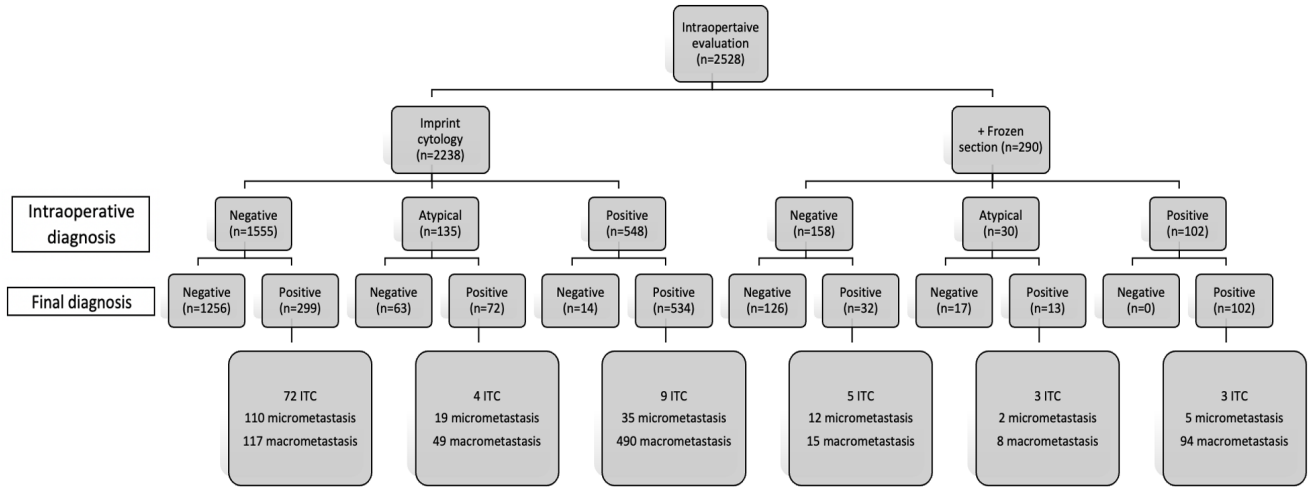



**Figure 1.** The distribution of cases according to the intraoperative evaluation method

*ITC: Isolated tumor cells*

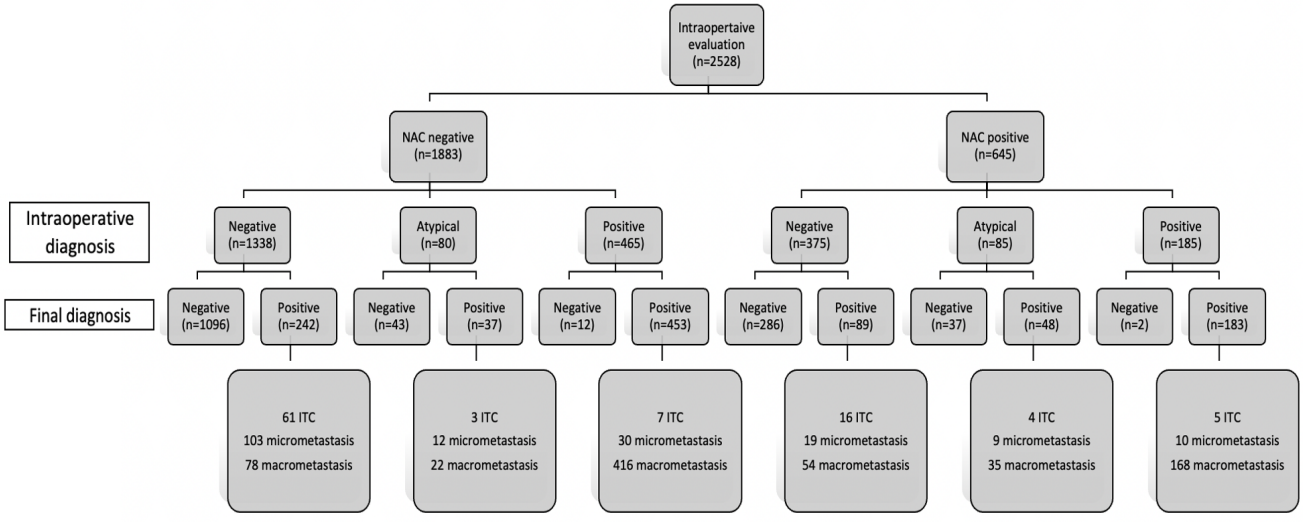



**Figure 2.** The distribution of cases according to neoadjuvant chemotherapy status

*ITC: Isolated tumor cells*

the reported sensitivity of IOE using IC varies between 34% and 95% (6, 15-19). In another study, IC alone had a sensitivity of 66.7% and specificity of 100%; FS alone had a sensitivity and specificity of 100%; and combined IC and FS had a sensitivity and specificity of 100% and 96%, respectively (20). In the present study, IC alone had a sensitivity of 64.1% and specificity of 99%; combined IC and FS had a sensitivity and specificity of 76.1% and 100%, respectively.

IOE of sentinel lymph node metastasis in patients treated with NAC can be challenging due to therapy effects. It has been suggested that the quality of IC preparations may decrease due to low cellularity and fibrosis after neoadjuvant treatment (21). In a study conducted after NAC, the sensitivity of IC was 61.8%, specificity and PPV were 100%, NPV was 82.4%, and accuracy was 86.3% (22). The sensitivity of IOE in the NAC setting in our study is within the range (38.6% to 87.9 %) reported by other studies (23-27). Our analysis revealed a lower accuracy rate for IOE of sentinel lymph nodes in patients treated with NAC (83.8%) compared with patients who had not received NAC (85.9%), even though the specificity was high in both settings.

According to clinical guidelines, patients with limited sentinel lymph node involvement may not require completion axillary lymph node dissection. Therefore, the ability to detect tumor deposits smaller than 2 mm must be balanced with its clinical benefit (28-30). The accuracy of IOE detecting metastases irrespective of the prior treatment status is much higher when micrometastasis and ITC are excluded from the analysis (23, 30). Our study also showed that IOE has a much higher

accuracy in detecting metastases when micrometastasis and ITC are excluded from the analysis.

Metastasis of invasive lobular carcinoma is known to have high false negative rates. Two previous studies have demonstrated high false negative rates for invasive lobular carcinoma for IOE of sentinel lymph node (14, 23). In general, the IOE of sentinel lymph nodes in invasive lobular carcinoma cases has lower sensitivity and accuracy than invasive ductal carcinomas in the present study.

The major strength of this study was that it included a large cohort of patients treated over a long period by varying grades of breast surgeons, and patient demographics were consistent with the breast cancer population. The limitations of our study were that it was non-randomized and retrospective.

In conclusion, parameters such as the size of the metastasis, tumor subtype, and presence of NAC in the IOE of sentinel lymph nodes affect the accuracy of the results. IC is considered an acceptable method for the IOE of sentinel lymph nodes in the primary surgery setting, while IC and FS are both recommended in the NAC setting.

### Ethics

**Ethics Committee Approval:** Not necessary.

**Informed Consent:** Retrospective study.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: B.M., M.T., H.K.; Concept: A.B., S.O., E.Y.; Design: A.B., S.O., E.Y.; Data Collection or Processing: A.B., C.S.K., E.S; Analysis or Interpretation: A.B., S.B.; Literature Search: A.B.; Writing: A.B., S.O., E.Y.

**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. Krag DN, Anderson SJ, Julian TB, Brown AM, Harlow SP, Costantino JP, et al. Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial. Lancet Oncol. 2010; 11: 927-933. (PMID: 20863759) [Crossref]
2. Alors-Ruiz J, Sanz-Viedma S, Fernández-Garcia FJ, Sendra-Portero F. Sentinel lymph node biopsy after neoadjuvant chemotherapy in cN0 breast cancer: impact of HER2-positive status on survival. Eur J Breast Health. 2024; 20: 94-101. (PMID: 38571688) [Crossref]
3. 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. Erratum in: J Natl Cancer Inst. 2006; 98: 876. (PMID: 16670385) [Crossref]
4. Canavese G, Catturich A, Vecchio C, Tomei D, Gipponi M, Villa G, et al. Sentinel node biopsy compared with complete axillary dissection for staging early breast cancer with clinically negative lymph nodes: results of randomized trial. Ann Oncol. 2009; 20: 1001-1007. (PMID: 19174453) [Crossref]
5. Lyman GH, Giuliano AE, Somerfield MR, Benson AB 3rd, Bodurka DC, Burstein HJ, et al. American Society of Clinical Oncology guideline recommendations for sentinel lymph node biopsy in early-stage breast cancer. J Clin Oncol. 2005; 23: 7703-7720. (PMID: 16157938) [Crossref]
6. Tew K, Irwig L, Matthews A, Crowe P, Macaskill P. Meta-analysis of sentinel node imprint cytology in breast cancer. Br J Surg. 2005; 92: 1068-1080. (PMID: 16106479) [Crossref]
7. Chicken DW, Kocjan G, Falzon M, Lee AC, Douek M, Sainsbury R, et al. Intraoperative touch imprint cytology for the diagnosis of sentinel lymph node metastases in breast cancer. Br J Surg. 2006; 93: 572-576. (PMID: 16550634) [Crossref]
8. Baitchev G, Gortchev G, Todorova A. Intraoperative sentinel lymph node examination by imprint cytology during breast surgery. Curr Med Res Opin. 2002; 18: 185-187. (PMID: 12201617) [Crossref]
9. Motomura K, Nagumo S, Komoike Y, Koyama H, Inaji H. Intraoperative imprint cytology for the diagnosis of sentinel node metastases in breast cancer. Breast Cancer. 2007; 14: 350-353. (PMID: 17986799) [Crossref]
10. Karamlou T, Johnson NM, Chan B, Franzini D, Mahin D. Accuracy of intraoperative touch imprint cytologic analysis of sentinel lymph nodes in breast cancer. Am J Surg. 2003; 185: 425-428. (PMID: 12727561) [Crossref]
11. Liu LC, Lang JE, Lu Y, Roe D, Hwang SE, Ewing CA, et al. Intraoperative frozen section analysis of sentinel lymph nodes in breast cancer patients: a meta-analysis and single-institution experience. Cancer. 2011; 117: 250-258. (PMID: 20818649) [Crossref]
12. Calhoun BC, Chambers K, Flippo-Morton T, Livasy CA, Armstrong EJ 3rd, Symanowski JT, et al. Breast cancer detection in axillary sentinel lymph nodes: the impact of the method of pathologic examination. Hum Pathol. 2014; 45: 2497-2501. (PMID: 25449631) [Crossref]
13. Ersoy E, Elsayad M, Pandiri M, Knee A, Cao QJ, Crisi GM. Intraoperative lymph node assessment (touch preparation only) for metastatic breast carcinoma in neoadjuvant and non-neoadjuvant settings. Arch Pathol Lab Med. 2023; 147: 149-158. (PMID: 35512225) [Crossref]
14. Ravichandran D, Kocjan G, Falzon M, Ball RY, Ralphs DN. Imprint cytology of the sentinel lymph node in the assessment of axillary node status in breast carcinoma. Eur J Surg Oncol. 2004; 30: 238-242. (PMID: 15028302) [Crossref]
15. Sauer T, Engh V, Holck AM, Sørpebøl G, Heim M, Furu I, et al. Imprint cytology of sentinel lymph nodes in breast cancer. Experience with rapid, intraoperative diagnosis and primary screening by cytotechnologists. Acta Cytol. 2003; 47: 768-773. (PMID: 14526676) [Crossref]
16. Cserni G. Effect of increasing the surface sampled by imprint cytology on the intraoperative assessment of axillary sentinel lymph nodes in breast cancer patients. Am Surg. 2003; 69: 419-423. (PMID: 12769215) [Crossref]
17. Shiver SA, Creager AJ, Geisinger K, Perrier ND, Shen P, Levine EA. Intraoperative analysis of sentinel lymph nodes by imprint cytology for cancer of the breast. Am J Surg. 2002; 184: 424-427. (PMID: 12433606) [Crossref]
18. Cserni G. The potential value of intraoperative imprint cytology of axillary sentinel lymph nodes in breast cancer patients. Am Surg. 2001; 67: 86-91. (PMID: 11206905) [Crossref]
19. Huerta-Rosario M, Mir M, Quispe-Vicuña C, Hwang H, Sarode V, Peng Y, et al. Intraoperative evaluation of sentinel lymph nodes in patients with breast cancer treated with systemic neoadjuvant therapy. J Clin Pathol. 2024; 77: 544-550. (PMID: 37258252) [Crossref]

20. Baker GM, King TA, Schnitt SJ. Evaluation of breast and axillary lymph node specimens in breast cancer patients treated with neoadjuvant systemic therapy. Adv Anat Pathol. 2019; 26: 221-234. (PMID: 31149907) [Crossref]
21. Delgado-Bocanegra RE, Millen EC, Nascimento CMD, Bruno KA. Intraoperative imprint cytology versus histological diagnosis for the detection of sentinel lymph nodes in breast cancer treated with neoadjuvant chemotherapy. Clinics (Sao Paulo). 2018; 73: e363. (PMID: 30088537) [Crossref]
22. Elliott RM, Shenk RR, Thompson CL, Gilmore HL. Touch preparations for the intraoperative evaluation of sentinel lymph nodes after neoadjuvant therapy have high false-negative rates in patients with breast cancer. Arch Pathol Lab Med. 2014; 138: 814-818. (PMID: 24878021) [Crossref]
23. Komenaka IK, Torabi R, Nair G, Jayaram L, Hsu CH, Bouton ME, et al. Intraoperative touch imprint and frozen section analysis of sentinel lymph nodes after neoadjuvant chemotherapy for breast cancer. Ann Surg. 2010; 251: 319-322. (PMID: 19864940) [Crossref]
24. Hadalin V, Pislar N, Borstnar S, Matos E, Kovac A, Dobovisek L, et al. Intraoperative touch imprint cytology in breast cancer patients after neoadjuvant chemotherapy. Clin Breast Cancer. 2022; 22: e597-e603. (PMID: 35086763) [Crossref]
25. Wu S, Wang Y, Zhang N, Li J, Xu X, Shen J, et al. Intraoperative touch imprint cytology in targeted axillary dissection after neoadjuvant chemotherapy for breast cancer patients with initial axillary metastasis. Ann Surg Oncol. 2018; 25: 3150-3157. (PMID: 30083833) [Crossref]
26. Gimbergues P, Dauplat MM, Durando X, Abrial C, Le Bouedec G, Mouret-Reynier MA, et al. Intraoperative imprint cytology examination of sentinel lymph nodes after neoadjuvant chemotherapy in breast cancer patients. Ann Surg Oncol. 2010; 17: 2132-2137. (PMID: 20155400) [Crossref]
27. Giuliano AE, Hunt KK, Ballman KV, Beitsch PD, Whitworth PW, Blumencranz PW, et al. Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. JAMA. 2011; 305: 569-575. (PMID: 21304082) [Crossref]
28. Giuliano AE, McCall L, Beitsch P, Whitworth PW, Blumencranz P, Leitch AM, et al. Locoregional recurrence after sentinel lymph node dissection with or without axillary dissection in patients with sentinel lymph node metastases: the American College of Surgeons Oncology Group Z0011 randomized trial. Ann Surg. 2010; 252: 426-432; discussion 432-433. (PMID: 20739842) [Crossref]
29. Donker M, van Tienhoven G, Straver ME, Meijnen P, van de Velde CJ, Mansel RE, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981-22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. Lancet Oncol. 2014; 15: 1303-1310. (PMID: 25439688) [Crossref]
30. Grabenstetter A, Moo TA, Hajiyeva S, Schüffler PJ, Khattar P, Friedlander MA, et al. Accuracy of intraoperative frozen section of sentinel lymph nodes after neoadjuvant chemotherapy for breast carcinoma. Am J Surg Pathol. 2019; 43: 1377-1383. (PMID: 31219817) [Crossref]

**Original Article**

Eur J Breast Health 2025; 21(4): 339-344
**DOI:** 10.4274/ejbh.galenos.2025.2025-5-1

# Evaluation of Serum STARD3 Levels in Patients With Breast and Prostate Cancer: A Case-Control Study

Almila Nazlı Korucu[1], Mine Büşra Bozkurk[2], Dilara Şevval Anasız[1], Ahmet Nihat Karakoyunlu[3], Şener Balas[4], Alpaslan Öztürk[2], Nihal İnandıklıoğlu[5]

[1]Yozgat Bozok University Faculty of Medicine, Yozgat, Türkiye
[2]Clinic of Clinical Biochemistry, Ankara Etlik City Hospital, Ankara, Türkiye
[3]Clinic of Urology, Ankara Etlik City Hospital, Ankara, Türkiye
[4]Clinic of General Surgery, Ankara Etlik City Hospital, Ankara, Türkiye
[5]Department of Medical Biology, Faculty of Medicine, Yozgat Bozok University, Yozgat, Türkiye

**ABSTRACT**

**Objective:** Cancer cells exhibit high metabolic demands and rely heavily on lipid metabolism for proliferation and membrane synthesis. Lipid transfer proteins, particularly the steroidogenic acute regulatory-related lipid transfer domain 3 (STARD3), play a significant role in intracellular cholesterol transport and may influence cancer progression. The aim of this study was to investigate serum STARD3 levels in patients with breast and prostate cancer and compare them with healthy controls, along with lipid parameters.

**Materials and Methods:** Patients with breast cancer (women) and prostate cancer (men) were recruited together with a control group matched by age-range and sex. Serum samples were collected, and STARD3 levels were measured using a commercial ELISA kit. Lipid parameters and tumor markers (carbohydrate antigen 15-3, prostate-specific antigen) were also evaluated.

**Results:** A total of 200 individuals were enrolled: 50 female breast cancer patients, 50 male prostate cancer patients, and 100 healthy controls. STARD3 levels were significantly lower in both breast cancer ($p$ = 0.045) and prostate cancer ($p$<0.001) groups compared to controls. However, no significant correlation was found between STARD3 levels and other biochemical parameters or tumor stage in either cancer group.

**Conclusion:** The results suggest that STARD3 may play a role in the pathogenesis of both hormone-related cancers, although the mechanism remains unclear. Given the limited studies evaluating STARD3 in both breast and prostate cancers simultaneously, our findings contribute novel data to the literature and may guide future research into the diagnostic or prognostic potential of STARD3 in oncology.

**Keywords:** Breast cancer; lipids; prostate cancer; STARD3

**Cite this article as:** Korucu AN, Bozkurk MB, Anasız DŞ, Karakoyunlu AN, Balas Ş, Öztürk A, et al. Evaluation of serum STARD3 levels in patients with breast and prostate cancer: a case-control study. Eur J Breast Health. 2025; 21(4): 339-344

**Key Points**

- Serum steroidogenic acute regulatory-related lipid transfer domain 3 (STARD3) levels were significantly lower in both breast and prostate cancer patients compared to healthy controls.
- No significant correlation was found between STARD3 levels and tumor markers or clinical parameters in either cancer group.
- This is the first study to evaluate serum STARD3 levels simultaneously in breast and prostate cancer.

## Introduction

Cancer is one of the leading causes of death in developed countries. Cancer cells exhibit an accelerated metabolic rate and require a continuous supply of cholesterol for cell division and membrane renewal (1). As tumors grow or metastasize, they trigger new lipid synthesis in order to adapt for future environmental conditions (2). Lipids play essential roles in metabolism, cellular homeostasis, and various biological activities. In normal tissue, lipogenesis is primarily limited to hepatocytes and adipocytes. However, cancer cells activate lipogenesis in response to their high metabolic demands or to the lack of serum-derived lipids in the tumor microenvironment, even in the presence of exogenous lipid sources (3). In addition to the metabolic and structural functions of lipids, they also act as intracellular and intercellular signaling molecules. Membrane phospholipids are hydrolyzed by phospholipases into lipid mediators (e.g., diacylglycerol, phosphatidic acid, lysophosphatidic acid, and arachidonic acid). Some of these, such as arachidonic acid, are further converted into prostaglandins and

**Corresponding Author:**
Nihal İnandıklıoğlu Assoc. Prof.; nihal.inandiklioglu@yobu.edu.tr

**Received:** 09.05.2025
**Accepted:** 15.07.2025
**Epub:** 14.08.2025
**Available Online Date:** 25.09.2025

leukotrienes through the cyclooxygenase and lipoxygenase pathways, respectively. These bioactive lipids are secreted by cancer cells and function as autocrine or paracrine mediators, regulating various cellular processes involved in tumor development and metastasis, including proliferation, migration, invasion, and angiogenesis (4, 5).

Recent studies have shown characteristic changes in lipid parameters between patients with invasive breast cancer and those with benign breast tumors. These alterations are also observed in patients with different molecular subtypes of breast cancer (6). The metastatic potential of cancer cells is influenced by processes, such as fatty acid synthesis, oxidation, and intracellular lipid storage. These metabolic changes are important for metastasis of breast and prostate cancers (7, 8). High-density lipoprotein (HDL) exhibits functions including cholesterol efflux, and has antioxidant and anti-inflammatory properties. Through these effects, HDL may reduce oxidative stress, inflammation, and cholesterol content in tumor cells, thereby affecting their proliferation (9). Cancer cells may be capable of synthesizing cholesterol or acquiring it through low-density lipoproteins (LDL). Hormone-dependent tumors, such as prostate and breast cancers require cholesterol for proliferation (10).

Lipid transfer proteins (LTPs) are involved in the distribution of cholesterol between organelles. Among LTPs, certain members of the steroidogenic acute regulatory-related lipid transfer (START) protein family regulate cholesterol transport across organelles. Changes in their expression levels are involved in various diseases, including cancers. One membrane-targeted START protein, StAR-related lipid transfer domain-3 (STARD3), has been proposed as a regulator of cholesterol accumulation in endosomes and its inter-organelle distribution (1). The possible molecular mechanism by which STARD3 contributes to tumorigenesis is through the transport of cholesterol across mitochondrial membranes (11). Some researchers have hypothesized that STARD3 may be involved in the transfer of cholesterol from late endosomes to the endoplasmic reticulum and subsequently to mitochondria via mitochondria-associated membranes (12). Although the precise molecular mechanism remains unclear, current evidence suggests that high STARD3 expression affects membrane cholesterol accumulation, which may contribute to cancer aggressiveness. Amplification or overexpression of STARD3 in cancer may induce the movement of lysosomal cholesterol to mitochondria, potentially promoting the progression of hormone-driven cancers, such as breast and prostate cancers, by triggering independent steroidogenesis (1, 13). Most cells acquire cholesterol from plasma via LDL receptor-mediated endocytosis. STARD3, one of the cholesterol transporters in late endosomes/lysosomes, contributes to the transport of late endosomal/lysosomal cholesterol to other cellular compartments (14).

As STARD3 is a key protein in cholesterol trafficking in cancer cells, assessing its activity has recently gained interest. In the present study, serum STARD3 protein levels were measured in patients diagnosed with breast and prostate cancer, as well as in healthy controls, using ELISA analysis, and the lipid profiles of these three groups were compared with a focus on STARD3.

## Materials and Methods

### Study Population

This study included 50 female patients diagnosed with breast cancer and 50 male patients diagnosed with prostate cancer between August and December 2024. The control group consisted of healthy individuals (50 females and 50 males) within the same age range. Individuals with a history of cancer, systemic chronic diseases (chronic heart disease, chronic lung disease, chronic kidney failure, and liver disease), malabsorption disorders (celiac disease or radiation enteritis), thyroid and parathyroid diseases, those receiving hormone replacement therapy, individuals with psychiatric disorders, alcohol users, and pregnant women were excluded from the control group. The participants' total cholesterol, LDL, HDL, triglyceride levels, and preoperative tumor markers, specifically carbohydrate antigen 15-3 and prostate-specific antigen were evaluated. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Yozgat Bozok University Ethics Committee (protocol code: 2024-GOKAEK-247_2024.07.17_99, date: 17.07.2024). Informed consent was obtained from all subjects involved in the study.

### ELISA Analysis

Fasting venous blood samples were taken in the morning (7:00–8:00 a.m.). Blood samples were collected in a 5 mL serum-separating vacuum tube and centrifuged at 3000 rpm for 10 minutes to separate the serum. The obtained serum samples were stored at –20 °C until further analysis. Serum STARD3 levels were measured using a commercially available ELISA kit (Cat. No. E7700Hu, Bioassay Technology Laboratory, Zhejiang, China), with a measurement range of 0.63 ng/mL to 40 ng/mL. The optical density values of the samples and standards were measured at 450 nm using the Thermo Scientific (USA) Multiscan Go Microplate Reader. The results were expressed in ng/mL.

### Statistical Analysis

Statistical analyses were performed using SPSS, version 20 (SPSS Inc., Chicago, IL, USA). The normality of continuous variables was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Categorical variables between groups were analyzed using the chi-square test or Fisher's exact test. Comparisons between groups were conducted using the Student's t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. Correlation analysis was performed using Pearson's test for normally distributed data and Spearman's test for non-normally distributed data. A $p$-value of less than 0.05 was considered statistically significant.

## Results

The demographic characteristics, laboratory results, preoperative tumor markers, and ELISA findings of the study groups are presented in Table 1 and Table 2. When the groups were statistically compared, no significant differences were found in age and cholesterol levels between the breast cancer and control groups, nor in age and glucose levels between the prostate cancer and control groups ($p>0.05$). However, other parameters showed statistically significant differences between the groups ($p<0.05$).

The breast cancer group had significantly lower levels of serum STARD3 compared to the control group ($p = 0.045$) (Table 1, Figure 1). Moreover, the prostate cancer group also had significantly lower levels of serum STARD3 compared to the control group ($p<0.001$) (Table 2, Figure 2).

**Table 1. Demographic, laboratory, tumor marker, and STARD3 results and comparisons between breast cancer patients and healthy individuals**

| | Groups | | |
|---|---|---|---|
| | **Control (*n* = 50)** | **Breast cancer (*n* = 50)** | ***p*** |
| **Age (year)** | 56.34±6.76 (57.0) | 55.67±14.75 (55.0) | 0.863 |
| **STARD3 (ng/mL)** | 15.20±7.89 (12.65) | 13.04±8.23 (11.65) | **0.045** |
| **HDL (mg/dL)** | 54.02±17.47 (48.3) | 32.69±11.97 (33.0) | **<0.001** |
| **LDL (mg/dL)** | 136.24±38.18 (129.22) | 169.13±49.86 (174.5) | **0.002** |
| **Cholesterol (mg/dL)** | 217.35±42.62 (217.1) | 217.10±56.97 (215.0) | 0.921 |
| **TG (mg/dL)** | 143.70±62.52 (138.4) | 238.77±67.09 (229.0) | **<0.001** |
| **Glucose (mg/dL)** | 110.69±49.90 (97.7) | 127.98±31.47 (130.0) | **<0.001** |
| **HbA1C (%)** | 6.22±1.13 (6.11) | 6.35±0.53 (6.4) | **0.006** |
| **CA15-3 (U/mL)** | | 58.87±65.45 (38.8) | |
| **Stage of cancer, n (%)** | | | |
| **Stage 1** | | 11 (22) | |
| **Stage 2** | | 15 (30) | |
| **Stage 3** | | 12 (24) | |
| **Stage 4** | | 12 (24) | |

STARD3: StAR-related lipid transfer domain-3; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; HbA1c: Hemoglobin A1c; CA15-3: Carbohydrate antigen 15-3. The results are expressed as frequency (%), mean±standard deviation and median (interquartile range). Significant *p* (<0.05) values are in bold

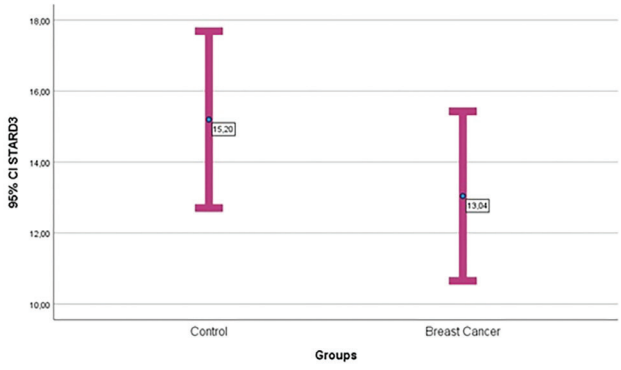



**Figure 1.** Distribution of serum STARD3 levels between the breast cancer group and the control group

*STARD3: StAR-related lipid transfer domain-3*

Correlation analysis of STARD3 serum levels in the breast and prostate cancer patient groups found no significant correlations between STARD3 and other parameters, age, lipid profile components, glucose, hemoglobin A1c or tumor markers, in either group (Tables 3, 4). The relationship between cancer stage and STARD3 was also analyzed. In the breast cancer group, since the variances were homogeneously distributed ($p$ = 0.203), a one-way ANOVA test was performed, but no significant difference was found in STARD3 levels between cancer stage groups ($p$ = 0.851). In the prostate cancer group, the variances were not homogeneously distributed ($p$<0.001), and thus the non-parametric Kruskal-Wallis test was used, which also showed no significant difference ($p$ = 0.103).

## Discussion and Conclusion

The present study is the first to evaluate serum STARD3 levels in breast and prostate cancer patients in relation to lipid parameters. We found that STARD3 levels were significantly lower in both the breast and prostate cancer groups compared to healthy controls. However, no significant association was found between lipid levels and STARD3.

In a recent study, higher levels of total cholesterol, LDL-C, and triglycerides (TG) were statistically associated with negative expression of estrogen receptor (ER) and progesterone receptor, positive human epidermal growth factor receptor 2 (HER2) status, non-luminal subtypes, and solitary lesions. Low HDL-C levels were linked specifically to negative ER expression. Tumors from patients with high LDL-C and low HDL-C levels exhibited a higher nuclear grade. Furthermore, patients with elevated TG and reduced HDL-C levels presented with more advanced disease stages, whereas total cholesterol and LDL-C levels showed no significant association with cancer stage (15). Johnson et al. (16) demonstrated through locus-specific Mendelian randomization analyses targeting HDL- and LDL-related genes that increased HDL and LDL levels may have a direct effect on breast cancer risk. Ossoli et al. (9) reported that HDL may inhibit LDL-induced cell proliferation by reducing intracellular cholesterol content in prostate cancer cell lines, suggesting that cholesterol plays a key role in prostate cancer progression. These findings highlight the therapeutic potential of targeting cellular cholesterol homeostasis as a strategy to suppress tumor growth. Another study found that high total serum cholesterol was associated with an increased risk of high-grade prostate cancer, while no association was observed between cholesterol levels and the risk of overall or low-grade prostate cancer. Interestingly, elevated serum HDL was linked to a higher risk of both

**Table 2. Demographic, laboratory, tumor marker, and STARD3 results and comparisons between prostate cancer patients and healthy individuals**

| | Groups | | |
|---|---|---|---|
| | **Control (*n* = 50)** | **Prostate cancer (*n* = 50)** | ***p*** |
| **Age (year)** | 57.95±6.49 (58.0) | 58.08±7.27 (58.0) | 0.776 |
| **STARD3 (ng/mL)** | 16.95±8.85 (13.22) | 10.79±8.65 (8.98) | **<0.001** |
| **HDL (mg/dL)** | 42.02±10.05 (39.6) | 34.92±12.14 (35.0) | **0.021** |
| **LDL (mg/dL)** | 115.35±35.17 (110.88) | 150.64±50.98 (134.0) | **0.002** |
| **Cholesterol (mg/dL)** | 191.86±39.97 (188.6) | 215.18±55.56 (220.0) | **0.025** |
| **TG (mg/dL)** | 179.06±92.02 (161.0) | 254.58±68.51 (262.5) | **<0.001** |
| **Glucose (mg/dL)** | 125.69±65.17 (104.0) | 119.92±36.06 (107.0) | 0.621 |
| **HbA1C (%)** | 5.85±0.58 (5.8) | 6.31±0.62 (6.3) | **<0.001** |
| **PSA (ng/mL)** | - | 66.29±161.35 (16.8) | - |
| **Stage of cancer, n (%)** | | | |
| **Stage 1** | | 28 (52) | |
| **Stage 2** | | 4 (8) | |
| **Stage 3** | | 6 (12) | |
| **Stage 4** | | 5 (10) | |
| **Stage 5** | | 7 (14) | |

STARD3: StAR-related lipid transfer domain-3; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; HbA1c: Hemoglobin A1c; PSA: Prostate-specific antigen. The results are expressed as frequency (%), mean ± standard deviation and median (interquartile range). Significant *p* (<0.05) values are in bold

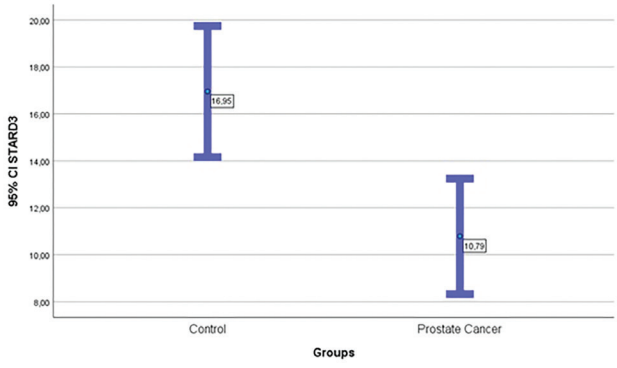



**Figure 2.** Distribution of serum STARD3 levels between the prostate cancer group and the control group

*STARD3: StAR-related lipid transfer domain-3*

overall and high-grade prostate cancer, while serum LDL levels showed no significant correlation with prostate cancer risk (17). In both breast and prostate cancer patients, LDL and TG levels were significantly higher and HDL levels significantly lower compared to healthy controls. Moreover, total cholesterol levels were notably elevated in the prostate cancer group. The significantly decreased levels of STARD3 observed in our cohort suggest that this protein may play a role in lipid metabolism and that its expression could be suppressed during tumor development.

According to the 2022 GLOBOCAN report, breast cancer is the second leading cause of cancer-related death among women in the United States (18). A significant proportion of breast cancer patients experience recurrence and frequently develop metastases. HER2 overexpression is present in approximately 15% to 20% of breast cancers and is generally associated with an increased risk of developing systemic metastases and poor survival outcomes (19). The *STARD3* gene has been reported to be co-amplified with HER2 in breast carcinoma. STARD3 is essential for cholesterol transport and metabolism in tumor cells. It has been demonstrated that STARD3 expression is significantly associated with HER2+ breast cancer tumors and breast cancer cell lines, while low levels of STARD3 mRNA and protein expression have been observed in ER-positive and triple-negative breast cancer patients (20). Lodi et al. (21) found that STARD3 expression was strongly associated with pathological complete response in a cohort of 112 patients with HER2+ breast cancer. They suggested that identifying STARD3 overexpression in baseline biopsies of HER2+ tumors may provide additional value in managing a subgroup of patients who are less likely to achieve a pathological response. Vassilev et al. (22) reported that STARD3-overexpressing cells exhibited non-adherent morphological characteristics and altered cholesterol homeostasis. In a study conducted in Finland, approximately 10% of breast cancer cases displayed high STARD3 protein levels that were strongly associated with HER2 amplification. Furthermore, the results provided evidence that STARD3 overexpression leads to increased cholesterol biosynthesis in breast cancer cells. This suggests that high STARD3 expression may contribute to the aggressiveness of breast cancer by increasing membrane cholesterol and enhancing oncogenic signaling. Analysis of a total of 136 samples obtained from 85 female breast cancer patients showed that STARD3 overexpression enhanced the prognostic power of HER2 overexpression in predicting disease-free survival (23).

**Table 3. Correlation coefficient values in the breast cancer patient group**

| | STARD3 | Age | HDL | LDL | Cholesterol | TG | Glucose | HbA1C | CA15-3 |
|---|---|---|---|---|---|---|---|---|---|
| **Age** | 0.102 | 1.000 | | | | | | | |
| **HDL** | -0.184 | -0.130 | 1.000 | | | | | | |
| **LDL** | 0.155 | 0.172 | 0.074 | 1.000 | | | | | |
| **Cholesterol** | 0.149 | 0.069 | -0.204 | -0.137 | 1.000 | | | | |
| **TG** | 0.026 | -0.246 | 0.215 | -0.178 | -0.046 | 1.000 | | | |
| **Glucose** | 0.036 | -0.142 | 0.087 | 0.021 | 0.065 | 0.101 | 1.000 | | |
| **HbA1C** | 0.128 | 0.063 | -0.117 | 0.187 | -0.017 | -0.131 | **0.492**** | 1.000 | |
| **CA15-3** | -0.231 | 0.050 | -0.093 | -0.122 | -0.260 | -0.145 | -0.138 | **0.295*** | 1.000 |
| **Stage** | 0.172 | -0.153 | -0.055 | -0.087 | 0.017 | 0.046 | -0.026 | -0.013 | 0.063 |

**: Correlation was significant at the 0.01 level (2-tailed); *: Correlation was significant at the 0.05 level (2-tailed); STARD3: StAR-related lipid transfer domain-3; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; HbA1c: Hemoglobin A1c; CA15-3: Carbohydrate antigen 15-3

**Table 4. Correlation coefficient values in the prostate cancer patient group**

| | STARD3 | Age | HDL | LDL | Cholesterol | TG | Glucose | HbA1C | PSA |
|---|---|---|---|---|---|---|---|---|---|
| **Age** | -0.068 | 1.000 | | | | | | | |
| **HDL** | -0.104 | -0.027 | 1.000 | | | | | | |
| **LDL** | -0.081 | 0.169 | 0.193 | 1.000 | | | | | |
| **Cholesterol** | -0.147 | -0.240 | 0.114 | 0.140 | 1.000 | | | | |
| **TG** | 0.054 | 0.039 | -0.103 | 0.067 | -0.194 | 1.000 | | | |
| **Glucose** | -0.034 | 0.133 | **0.329*** | 0.083 | 0.075 | -0.151 | 1.000 | | |
| **HbA1C** | 0.006 | 0.100 | **0.437**** | **0.336*** | 0.038 | -0.045 | **0.786**** | 1.000 | |
| **PSA** | 0.170 | 0.241 | -0.014 | 0.044 | 0.059 | 0.046 | 0.077 | 0.111 | 1.000 |
| **Stage** | 0.275 | 0.149 | 0.158 | -0.127 | -0.004 | **-0.385**** | 0.100 | 0.020 | 0.263 |

**: Correlation was significant at the 0.01 level (2-tailed); *: Correlation was significant at the 0.05 level (2-tailed); STARD3: StAR-related lipid transfer domain-3; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; HbA1c: Hemoglobin A1c; PSA: Prostate-specific antigen

Malignant breast cancer tissues were found to have higher levels of STARD3 immuno-expression compared to normal tissues. STARD3 is strongly correlated with HER2+ breast cancer, suggesting that it may serve as a potential biomarker for this cancer subtype (24).

The prostate is an organ regulated by androgens. Androgens exert three main effects on prostate cells: they promote proliferation; support differentiation; and inhibit programmed cell death or apoptosis (25). When reviewing the limited number of published studies concerning prostate cancer and STARD3, a linear correlation has been identified between STARD3 expression and the expression of CYP17, an enzyme involved in the steroid biosynthesis pathway. In prostate cancer, the expression of STARD3 and CYP17 may lead to steroidogenesis through continuous cholesterol transfer to the mitochondria and increase androgen biosynthesis through the catalytic activity of cytochrome CYP17. Therefore, dysregulated expression of STARD3 and CYP17 is associated with poor prognosis in prostate cancer patients (26). Another study showed that high mitochondrial cholesterol levels could inhibit apoptotic cell death in various cancer types, thereby potentially driving tumor progression (22). STARD3 overexpression in cancer may support the development of hormone-dependent cancers, such as prostate cancer, by promoting independent steroidogenesis. High STARD3 levels in prostate cancer patients are associated with metastasis, local recurrence, and shorter overall survival. For these reasons, *STARD3* is considered a potential oncogene for which the first inhibitor has already been reported (1).

**Study Limitations**

This study has certain limitations. First, it was conducted at a single center with a limited sample size, which may restrict the generalizability of the results. Second, STARD3 levels were assessed only in serum via ELISA, providing no information regarding tissue-level expression or cellular localization. Lastly, although the study evaluated the correlation between STARD3 levels and the lipid profile, other potential metabolic pathways and molecular mechanisms were not investigated.

In conclusion, the current literature on STARD3 remains limited. Although recent studies have explored the relationship between STARD3 and cancer, there are no studies that have simultaneously evaluated STARD3 in both breast and prostate cancer cases. In this context, our research provides unique data and may serve as an important resource for future investigations. Future studies with larger patient cohorts, inclusion of tissue-based analyses, and long-term follow-up data will help to better elucidate the role of STARD3 in cancer biology.

## Ethics

**Ethics Committee Approval:** The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Yozgat Bozok University Ethics Committee (protocol code: 2024-GOKAEK-247_2024.07.17_99, date: 17.07.2024).

**Informed Consent:** Informed consent was obtained from all subjects involved in the study.

## Footnotes

### Authorship Contributions

Concept: A.N.K., D.Ş.A., N.İ.; Design: A.N.K., D.Ş.A., N.İ.; Data Collection or Processing: A.N.K., M.B.B., D.Ş.A., A.N.K., Ş.B., A.Ö., N.İ.; Analysis or Interpretation: A.N.K., M.B.B., D.Ş.A., A.Ö., N.İ.; Literature Search: A.N.K., D.Ş.A., N.İ.; Writing: A.N.K., M.B.B., D.Ş.A., A.N.K., Ş.B., A.Ö., N.İ.

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

**Financial Disclosure:** This project was supported by the TÜBİTAK 2209/A (project number: 1919B012327057).

## References

1. Asif K, Memeo L, Palazzolo S, Frión-Herrera Y, Parisi S, Caligiuri I, et al. STARD3: a prospective target for cancer therapy. Cancers (Basel). 2021; 13: 4693. (PMID: 34572920) [Crossref]
2. Stoykova GE, Schlaepfer IR. Lipid metabolism and endocrine resistance in prostate cancer, and new opportunities for therapy. Int J Mol Sci. 2019; 20: 2626. (PMID: 31142021) [Crossref]
3. Röhrig F, Schulze A. The multifaceted roles of fatty acid synthesis in cancer. Nat Rev Cancer. 2016; 16: 732-749. (PMID: 27658529) [Crossref]
4. Luo X, Zhao X, Cheng C, Li N, Liu Y, Cao Y. The implications of signaling lipids in cancer metastasis. Exp Mol Med. 2018; 50: 1-10. (PMID: 30242145) [Crossref]
5. Hisano Y, Hla T. Bioactive lysolipids in cancer and angiogenesis. Pharmacol Ther. 2019; 193: 91-98. (PMID: 30048709) [Crossref]
6. Buch K, Gunmalm V, Andersson M, Schwarz P, Brøns C. Effect of chemotherapy and aromatase inhibitors in the adjuvant treatment of breast cancer on glucose and insulin metabolism-a systematic review. Cancer Med. 2019; 8: 238-245. (PMID: 30561133) [Crossref]
7. Sánchez-Martínez R, Cruz-Gil S, Gómez de Cedrón M, Álvarez-Fernández M, Vargas T, Molina S, et al. A link between lipid metabolism and epithelial-mesenchymal transition provides a target for colon cancer therapy. Oncotarget. 2015; 6: 38719-38736. (PMID: 26451612) [Crossref]
8. Lodi M, Kiehl A, Qu FL, Gabriele V, Tomasetto C, Mathelin C. Lipid intake and breast cancer risk: is there a link? A new focus and meta-analysis. Eur J Breast Health. 2022; 18: 108-126. (PMID: 35445180) [Crossref]
9. Ossoli A, Giorgio E, Cetti F, Ruscica M, Rabacchi C, Tarugi P, et al. HDL-mediated reduction of cholesterol content inhibits the proliferation of prostate cancer cells induced by LDL: role of ABCA1 and proteasome inhibition. Biofactors. 2022; 48: 707-717. (PMID: 35579277) [Crossref]
10. Mostaghel EA. Steroid hormone synthetic pathways in prostate cancer. Transl Androl Urol. 2013; 2: 212-227. (PMID: 25379460) [Crossref]
11. Montero J, Morales A, Llacuna L, Lluis JM, Terrones O, Basañez G, et al. Mitochondrial cholesterol contributes to chemotherapy resistance in hepatocellular carcinoma. Cancer Res. 2008; 68: 5246-5256. (PMID: 18593925) [Crossref]
12. Nara A, Komiya T. STARD3/MLN64 is striving at membrane contact sites: intracellular cholesterol trafficking for steroidogenesis in human placental cells. Am J Life Sci. 2015; 3: 48-52. [Crossref]
13. Korucu AN, Inandiklioglu N. Is STARD3 a new biomarker for breast cancer? Eur J Breast Health. 2024; 20: 89-93. (PMID: 38571685) [Crossref]
14. Nguyen MKL, Jose J, Wahba M, Bernaus-Esqué M, Hoy AJ, Enrich C, et al. Linking late endosomal cholesterol with cancer progression and anticancer drug resistance. Int J Mol Sci. 2022; 23: 7206. (PMID: 35806209) [Crossref]
15. Jung SM, Kang D, Guallar E, Yu J, Lee JE, Kim SW, et al. Impact of serum lipid on breast cancer recurrence. J Clin Med. 2020; 9: 2846. (PMID: 32887448) [Crossref]
16. Johnson KE, Siewert KM, Klarin D, Damrauer SM; VA Million Veteran Program; Chang KM, et al. The relationship between circulating lipids and breast cancer risk: a Mendelian randomization study. PLoS Med. 2020; 17: e1003302. (PMID: 32915777) [Crossref]
17. Jamnagerwalla J, Howard LE, Allott EH, Vidal AC, Moreira DM, Castro-Santamaria R, et al. Serum cholesterol and risk of high-grade prostate cancer: results from the REDUCE study. Prostate Cancer Prostatic Dis. 2018; 21: 252-259. (PMID: 29282360) [Crossref]
18. Giaquinto AN, Sung H, Miller KD, Kramer JL, Newman LA, Minihan A, et al. Breast cancer statistics, 2022. CA Cancer J Clin. 2022; 72: 524-541. (PMID: 36190501) [Crossref]
19. Dieci MV, Miglietta F, Griguolo G, Guarneri V. Biomarkers for HER2-positive metastatic breast cancer: beyond hormone receptors. Cancer Treat Rev. 2020; 88: 102064. (PMID: 32622272) [Crossref]
20. Fararjeh AFS, Al Khader A, Kaddumi E, Obeidat M, Al-Fawares O. Differential expression and prognostic significance of STARD3 gene in breast carcinoma. Int J Mol Cell Med. 2021; 10: 34-41. (PMID: 34268252) [Crossref]
21. Lodi M, Voilquin L, Alpy F, Molière S, Reix N, Mathelin C, et al. STARD3: a new biomarker in HER2-positive breast cancer. Cancers (Basel). 2023; 15: 362. (PMID: 36672312) [Crossref]
22. Vassilev B, Sihto H, Li S, Hölttä-Vuori M, Ilola J, Lundin J, et al. Elevated levels of StAR-related lipid transfer protein 3 alter cholesterol balance and adhesiveness of breast cancer cells: potential mechanisms contributing to progression of HER2-positive breast cancers. Am J Pathol. 2015; 185: 987-1000. (PMID: 25681734) [Crossref]
23. Vinatzer U, Dampier B, Streubel B, Pacher M, Seewald MJ, Stratowa C, et al. Expression of HER2 and the coamplified genes GRB7 and MLN64 in human breast cancer: quantitative real-time reverse transcription-PCR as a diagnostic alternative to immunohistochemistry and fluorescence in situ hybridization. Clin Cancer Res. 2005; 11: 8348-8357. (PMID: 16322295) [Crossref]
24. Fararjeh A, Kaddumi E, Al-Khader A, Aburayyan W. The significance of StAR-related lipid transfer protein-3 expression in breast cancer. Pol J Pathol. 2022; 73: 215-222. (PMID: 36734436) [Crossref]
25. Dong JT. Prevalent mutations in prostate cancer. J Cell Biochem. 2006; 97: 433-447. (PMID: 16267836) [Crossref]
26. Stigliano A, Gandini O, Cerquetti L, Gazzaniga P, Misiti S, Monti S, et al. Increased metastatic lymph node 64 and CYP17 expression are associated with high stage prostate cancer. J Endocrinol. 2007; 194: 55-61. Erratum in: J Endocrinol. 2007; 194: 458. (PMID: 17592021) [Crossref]

**Original Article**

Eur J Breast Health 2025; 21(4): 345-355
**DOI:** 10.4274/ejbh.galenos.2025.2025-6-2

# *ROPN1* Gene Expression as a Prognostic and Predictive Biomarker in Aggressive Breast Cancer: Clinical Implications and Survival Association

Ricardo Cesar Cintra[1], Andrés Galindo Céspedes[2,3,4], Olinda Maria Gamarra[5], Carlos Javier Melgarejo[5], Daniel Rodrigues de Bastos[5]

[1]Center for Translational Research in Oncology, Instituto do Cancer do Estado de São Paulo ICESP, Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo FMUSP HC, São Paulo, Brazil
[2]Facultad de Ciencias Biológicas, Universidad Nacional Pedro Ruiz Gallo, Lambayeque, Perú
[3]Hospital Nacional "Almanzor Aguinaga Asenjo", Chiclayo, Perú
[4]Universidad Católica Santo Toribio de Mogrovejo, Chiclayo, Perú
[5]Facultad de Ciencias Médicas, Universidad Privada del Este filial Ciudad del Este, Paraguay

## ABSTRACT

**Objective:** The *ropporin-1* (*ROPN1*) gene, initially linked to sperm motility, is differentially expressed in triple negative breast cancer (TNBC), suggesting a role in tumor progression and therapy resistance. To characterize *ROPN1* expression in breast cancer and evaluate its association with clinicopathological features, survival, and treatment response as a translational biomarker.

**Materials and Methods:** Data from The Cancer Genome Atlas (1,087 patients), Sweden Cancerome Analysis Network-Breast (3,273 patients), and geodatabases were analyzed. *ROPN1* transcriptional levels were assessed in relation to clinical variables and survival. Chemotherapy agents and epigenetic modulators were tested in cell lines to evaluate *ROPN1* regulation.

**Results:** Transcriptional overexpression of *ROPN1* was significantly enriched in TNBC/basal-like tumors ($p<0.0001$) and correlated with reduced overall survival, particularly in basal cases [hazard ratio (HR) = 1.85; 95% confidence interval (CI): 1.02–3.33; $p = 0.041$]. Patients treated with chemotherapy and exhibiting high *ROPN1* levels had unfavorable prognosis, with an even poorer profile in untreated cohorts (HR = 4.55; 95% CI: 1.33–14.29; $p = 0.01$). Hypomethylation at cg00101712 (HR = 0.59; $p = 0.016$) and cg09298623 (HR = 0.49; $p = 0.0014$) CpG sites were associated with worse survival at 5 years follow-up, underscoring epigenetic regulation of this pathway as a key driver of poor outcomes. Furthermore, *in vitro* treatment with cisplatin, doxorubicin, and paclitaxel resulted in variable responses, with a significant reduction of *ROPN1* in HCC70 and HS578T cell lines, while BT549 and MDA-MB-231 cell lines showed notable increases.

**Conclusion:** *ROPN1* overexpression in TNBC/basal-like tumors suggests a role as a prognostic biomarker and predictor of post-chemotherapy resistance. Investigation of *ROPN1* expression in breast tumors may lead to alternative strategies targeting pro-metastatic pathways and improve precision treatment for aggressive breast cancer.

**Keywords:** *ROPN1*; triple negative breast cancer; basal-like; aggressive breast cancer

**Cite this article as:** Cintra RC, Céspedes AG, Gamarra OM, Melgarejo CJ, de Bastos DR. *ROPN1* gene expression as a prognostic and predictive biomarker in aggressive breast cancer: clinical implications and survival association. Eur J Breast Health. 2025; 21(4): 345-355

## Key Points

- The *ropporin-1* gene (*ROPN1)* overexpression is linked to worse overall survival, especially in triple negative or basal-like breast cancer.
- High *ROPN1* levels predict poor prognosis in both chemotherapy-treated and untreated patients.
- *ROPN1* expression inversely correlates with DNA methylation and it is known that hypomethylation is associated with adverse outcomes.
- *In vitro*, cisplatin, doxorubicin, and paclitaxel variably modulated *ROPN1* expression in different cancer cell lines with increased expression levels in some cell lines, suggesting therapy resistance.
- Treatment with 5-aza-2′-deoxycytidine or trichostatin-A led to increased *ROPN1* expression.

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

**Received:** 11.06.2025
**Accepted:** 04.08.2025
**Epub:** 04.09.2025
**Available Online Date:** 25.09.2025

## Introduction

Breast cancer remains the most commonly diagnosed malignancy in women, comprising 24.5% of cases with over 2.3 million new diagnoses annually, and is the leading cause of female cancer death (685,000 deaths in 2020) (1). The incidence of breast cancer is higher in developed countries, reflecting both lifestyle factors and advanced screening, whereas delayed diagnosis and limited therapy access drive elevated mortality in low- and middle-income regions (2, 3). By 2040, cases are projected to exceed 3 million, with over 1 million deaths (4).

From a molecular perspective, breast cancer is a heterogeneous disease classified into distinct subtypes based on gene expression profiles and biomarker presence. The main molecular subtypes include luminal A and B tumors, which express estrogen receptor (ER) and progesterone receptor (PR) in various combinations; human epidermal growth factor receptor 2 (HER2)-enriched tumors, which are characterized by the overexpression of HER2; and triple-negative breast cancer (TNBC), which lacks ER/PR/HER2 expression (5). Molecular classification has been shown to be critical for therapeutic guidance and prognosis since HER2-positive and TNBC tumors typically exhibit more aggressive behavior and differential treatment responses (6). Owing to the heterogeneity of TNBC, Lehmann et al. (7) proposed a TNBC sub-classification system comprising six subtypes: basal-like 1/2, mesenchymal/mesenchymal-like, luminal androgen receptor (LAR), and an immunomodulatory group, each displaying unique molecular profiles with variability in prognosis/treatment sensitivity; some show increased chemotherapy responsiveness, whereas others are correlated with increased relapse risk. Another widely used classification, prediction analysis of microarray 50 (PAM50), categorizes breast cancers into luminal A/B, HER2-enriched, basal-like or normal-like subtypes, using a 50-gene transcriptional panel. This framework is clinically relevant because it provides insights into tumor behavior and aids in therapeutic decision-making (8). Although TNBC is frequently considered synonymous with the basal subtype, studies indicate an approximately 80% overlap between these two categories. This high correlation suggests that different classification techniques may lead to distinct interpretations of tumor behavior. However, given their significant molecular and clinical similarities, it is common for findings from TNBC-focused studies to be extrapolated to basal subtypes and *vice versa*. This widely adopted practice arises because researchers often access distinct datasets, some of which are based on immunohistochemical classification (TNBC) and others on transcriptional profiling (basal). Thus, extrapolation serves as a pragmatic tool to broaden the applicability of results despite limitations in standardized classification methods (9).

The TNBC/basal subtype represents the most challenging entity in oncological management and is characterized by complex molecular heterogeneity, the absence of specific therapeutic targets, high aggressiveness, elevated recurrence rates and reduced five-year overall survival (OS). Despite these therapeutic hurdles, recent advances in targeted immunotherapy with poly (ADP-ribose) polymerase (PARP) inhibitors and combination approaches have emerged as promising strategies (10). In this context, bioprospecting through "omics" data analysis has become an important auxiliary strategy, contributing to novel diagnostic/prognostic biomarker identification, mapping underexplored oncogenic pathways and uncovering potential molecular targets, thereby offering innovative perspectives for understanding this tumor subtype (11).

The *ropporin-1* (*ROPN1*) gene was initially identified as a regulator of sperm motility and was first described in human and murine testicular tissues. Current annotations indicate that it encodes a protein predominantly expressed in male reproductive tissues, with functional roles linked to sperm flagellum axoneme formation (12). Subsequent studies revealed *ROPN1* expression in diverse reproductive tissues at relatively low levels (13, 14). In the context of breast cancer, transcriptomic data suggest differential *ROPN1* expression patterns between normal and tumor tissues, with overexpression associated with aggressive malignancies, particularly TNBC subtypes. While the exact mechanistic contribution of *ROPN1* to tumorigenesis remains incompletely understood, its established roles in cellular motility pathways may facilitate invasive/metastatic processes (13).

## Materials and Methods

### Identification of *ROPN1*

GSE76275 was interrogated via GEO2R (15, 16) to identify DEGs between TNBC and non-TNBC using Benjamini-Hochberg-adjusted $p<0.05$ and $|\log_2 FC| \geq 1.5$. *ROPN1*, among the most dysregulated, was selected for focused TNBC expression and functional analyses.

### The Cancer Genome Atlas (TCGA) Data Analysis

TCGA Firehose Legacy data ($n$ = 1108) were retrieved via cBioPortal, excluding 12 male patients, five without age and four without *ROPN1* expression data, yielding 1087 female cases. *ROPN1* levels were merged with clinicopathological data and PAM50 calls from Xena by barcode (17). Cases were dichotomized at the median into low (≤median) and high (>median) groups. Categorical associations used $\chi^2$; continuous data were log-transformed to z-scores (RNA-Seq V2 RSEM), tested for normality (Shapiro-Wilk) and analyzed by Student's t/ANOVA or Mann-Whitney/Kruskal-Wallis. HM450 β-values at the *ROPN1* locus were compared across PAM50 subtypes and correlated with expression via Spearman's test.

### Study Methodology: Sweden Cancerome Analysis Network-Breast (SCAN-B) Analysis

RNA-seq from SCAN-B [GSE96058; n = 3,678; n = 3,273 tumors after excluding replicates/NAs; median follow-up 52 months (18)] were processed in R (gtsummary) (19). *ROPN1* expression was again dichotomized at the median; Wilcoxon rank-sum and $\chi^2$ tests assessed clinicopathological associations. Optimal cut-offs were derived via survminer residual-minimization (20) to generate Kaplan-Meier curves and Cox models (survival package; $p<0.05$), with analyses stratified by basal subtype and chemotherapy status.

### GEO Database Analysis

Five GEO datasets (GSE76275, GSE21653, GSE32646, GSE18864, GSE43358) were retrieved and analyzed via GEO2R to compare *ROPN1* probe-specific expression (224191_x_at, 231535_x_at, 233203_at) between groups. Post-analysis outputs were exported and plotted in GraphPad Prism 9 boxplots with consistent scaling and outlier thresholds to visualize cohort-wise expression differences.

### Functional Enrichment Analysis and Protein-Protein Interaction Network

To elucidate *ROPN1's* molecular interactions, we performed comprehensive correlation analyses via cBioPortal followed by gene selection for subsequent protein-protein interaction (PPI) network modeling, using STRING-db (https://www.string-db.org/). The selected genes were subjected to rigorous PPI network construction and

pathway enrichment analysis with a stringent interaction confidence threshold (≥0.4) to ensure biological relevance (21). Using STRING-db, potential PPI interaction networks were mapped followed by visualization and computational refinement through Cytoscape 3.10.1 (www.cytoscape.org/).

### MethSurv

MethSurv is an R Shiny web portal that uses TCGA CpG beta values (0–1) to perform univariate and multivariate survival analyses with built-in visualization and clustering, with no coding or extra software required (22). In the present study, MethSurv was used to assess survival outcomes using the invasive breast cancer dataset from TCGA, incorporating *ROPN1* as a focal point of our analyses.

### Cell Line Culture and Treatment

MCF10A (ATCC CRL-10317) cells were maintained in 1:1 DMEM/F12 supplemented with 5% horse serum, 0.5 µg/mL hydrocortisone, 10 µg/mL insulin, 20 ng/mL EGF and 100 ng/mL cholera toxin. Hs578T (ATCC HTB-126), MDA-MB-231 (ATCC HTB-26), SK-BR-3 (ATCC HTB-30), HCC70 (ATCC CRL-2315), MCF7 (ATCC HTB-22), BT-474 (ATCC HTB-20) and BT-549 (ATCC HTB-122) were cultured in DMEM or RPMI 1640 with 10% FBS and 1% penicillin-streptomycin. All lines were incubated at 37 °C, 5% $CO_2$, with medium renewed every 48 h until 50–60% confluence. Mycoplasma testing was performed before and after experiments; subcultures used 0.25% trypsin-EDTA.

Concentrations of 5-aza-2′-deoxycytidine (5-aza) and trichostatin A (TSA) were set by Alamar Blue assays to avoid morphological or growth alterations. Cisplatin, doxorubicin and paclitaxel were applied at ½ $IC_{50}$ [CancerRxGene (23)]; SK-BR-3 dosing was performed as described by Hai et al. A single 6 Gy fraction was delivered via an RS2000 irradiator with Gafchromic dosimetry, followed by 48 h recovery and RNA isolation (SV Total RNA, Promega).

cDNA was synthesized from 2 µg RNA (High-Capacity Kit, Thermo Fisher) and quantitative polymerase chain reaction (qPCR) performed (SYBR Green, Applied Biosystems 7500) using primers for *ROPN1* (NM_001394219.1; F: 5′-CCAAAGCCGCCATTAGGGT-3′, R: 5′-GGCTGCCCACTGGATGAG-3′) and GAPDH (NR_152150.2; F: 5′-GACTGTGGTCATGAGTCCTCCC-3′, R: 5′-CAAGATCATCAGCAATGCCTCC-3′). Relative expression was normalized to GAPDH and calculated by $\Delta\Delta C_t$ in triplicate.

### Statistical Analysis

Statistical analysis was performed using specialized software. This included SPSS, version 25.0 (IBM Inc., Armonk, NY, USA) and GraphPad, version 7 (California, USA). Data normality was assessed by Shapiro-Wilk test. Categorical variables were compared using $\chi^2$ test; continuous variables employed Student's t-test or ANOVA for normally distributed data and Mann-Whitney or Kruskal-Wallis tests otherwise. Associations were evaluated by Spearman's (non-parametric) rank correlation. Survival outcomes were estimated via Kaplan-Meier curves with log-rank testing and multivariate hazard ratios (HRs) calculated by Cox proportional hazards regression. All tests were two-tailed with significance defined as $p<0.05$.

## Results

### Differences Observed in TCGA Data

It was observed that high *ROPN1* expression was significantly associated with key clinical and pathological characteristics. Patients in the high *ROPN1* subgroup were more frequently premenopausal ($p = 0.0027$) and exhibited a predominance of hormone receptor-negative tumors (ER-/PR-, $p<0.0001$) and HER2-negative status ($p<0.0001$). Furthermore, high *ROPN1* expression levels were strongly correlated with basal-like and normal-like subtypes based on the PAM50 classification ($p<0.0001$). Among patients with TNBC, the majority exhibited high *ROPN1* expression ($p<0.0001$). Differences in histological type were significant ($p = 0.0013$), whereas TNM staging did not show significant variation between groups ($p = 0.2276$) (Table 1).

**Table 1. Clinicopathological characteristics of patients with breast cancer derived from the TCGA database and their associations with *ROPN1* expression levels**

| Variables | Low | | High | | *p* |
|---|---|---|---|---|---|
| | *n* | | *n* | | |
| **Age** | | | | | |
| ≤50 | 132 | 24.30 | 201 | 37.00 | <0.0001 |
| >50 | 412 | 75.70 | 342 | 63.00 | |
| **Menopause status** | | | | | |
| Pre | 101 | 20.60 | 129 | 26.70 | 0.0027 |
| Peri | 13 | 2.60 | 27 | 5.60 | |
| Post | 377 | 76.80 | 327 | 67.70 | |
| **Cancer type detailed** | | | | | |
| IDC | 418 | 77.00 | 383 | 70.50 | 0.0013 |
| ILC | 80 | 14.70 | 126 | 23.20 | |
| Other | 45 | 8.30 | 34 | 6.30 | |
| **TNM stage** | | | | | |
| Stage 1 | 80 | 15.00 | 100 | 18.80 | 0.2276 |
| Stage 2 | 309 | 58.10 | 307 | 57.80 | |
| Stage 3 | 131 | 24.60 | 117 | 22.00 | |
| Stage 4 | 12 | 2.30 | 7 | 1.30 | |
| **ER status by IHC** | | | | | |
| Negative | 44 | 8.60 | 194 | 37.00 | <0.0001 |
| Positive | 469 | 91.40 | 330 | 63.00 | |
| PR status by IHC | | | | | |
| Negative | 113 | 22.10 | 228 | 43.60 | <0.0001 |
| Positive | 398 | 77.90 | 295 | 56.40 | |
| **HER2 status by IHC** | | | | | |
| Negative | 250 | 70.00 | 308 | 85.10 | <0.0001 |
| Positive | 107 | 30.00 | 54 | 14.90 | |
| **PAM50 classification** | | | | | |
| Basal | 5 | 1.10 | 135 | 35.20 | <0.0001 |
| HER2 | 45 | 9.90 | 22 | 5.70 | |
| Luminal A | 243 | 53.60 | 173 | 45.20 | |
| Luminal B | 158 | 34.90 | 31 | 8.10 | |
| Normal-like | 2 | 0.40 | 22 | 5.70 | |

**Table 1. Continued**

| Variables | Low | | High | | *p* |
|---|---|---|---|---|---|
| | *n* | | *n* | | |
| **TNBC status** | | | | | |
| nTNBC | 496 | 98.2 | 356 | 76.9 | <0.0001 |
| TNBC | 9 | 1.8 | 107 | 23.1 | |

ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; IDC: Invasive ductal carcinoma; IHC: Immunohistochemistry; ILC: Invasive lobular carcinoma; PAM50: Prediction analysis of microarray 50 (50-gene panel used for molecular classification); Peri: Perimenopausal; Post: Postmenopausal; PR: Progesterone receptor; Pre: Premenopausal; TNBC: Triple-negative breast cancer; TNM: Tumor, node, metastasis (staging system for tumor size, lymph node involvement, and metastasis); TCGA: The Cancer Genome Atlas

The pattern of *ROPN1* expression was analyzed in different clinicopathological contexts, as illustrated in the graphs (Figure 1A-F). In each graph, the expression of *ROPN1* was compared among subgroups with distinct clinical characteristics and significant variations emerged. *ROPN1* mRNA expression was higher in ER-negative, PR-negative, and HER2-positive tumors compared with their opposite counterparts (all $p<0.0001$; Figure 1A-C). Expression varied by menopausal status ($p = 0.0171$; Figure 1D) and was elevated in invasive ductal carcinoma versus other histological types ($p = 0.0126$) (Figure 1E). Basal-like tumors showed the highest *ROPN1* levels among molecular subtypes ($p<0.0001$) (Figure 1F). Inverse correlation between *ROPN1* mRNA levels and promoter methylation was observed in unstratified TCGA breast tumors (Figure 2A; r = –0.41; $p<0.0001$) and was stronger in basal-like tumors (Figure 2B; r = –0.55; $p<0.0001$). Stratification by clinical subtype highlighted significantly lower methylation in basal-like versus HER2-enriched, luminal A/B and normal-like tumors (Figure 2C, D). Survival analysis via MethSurv showed that hypermethylation at cg00101712 and cg09298623 correlated with improved prognosis [Figure 2E; HR = 0.59; 95% confidence interval (CI): 0.38–0.92; $p = 0.016$; Figure 2F; HR = 0.49; 95% CI: 0.31–0.78; $p = 0.0014$].

Global correlation via cBioPortal identified 14,462 *ROPN1*-associated genes; the top 20 positive and 20 negative correlates were input into STRING to generate a PPI network (Figure 2G). Positively linked nodes included *ROPN1B, SOX10, FABP7, SOSTDC1, SFRP1, BCL11A, FOXC1* and *MIA*; negatively linked nodes comprised *BCAS1, GATA3, AR, ARMT1, FOXA1, XBP1, WWP1, ESR1* and *TMBIM6*. STRING enrichment highlighted glandular morphogenesis and hormonal response pathways, prostate gland epithelium and glandular acinus development; branched and epithelial tube morphogenesis; and cellular response to estrogenic stimuli, underscoring the interplay of gland architecture and hormone signaling in breast cancer progression (Figure 2H).

### Prognostic Insights from the SCAN-B Study on *ROPN1* Expression in Breast Cancer

SCAN-B RNA-seq data recapitulated TCGA associations: High ($n$ = 1,636) versus low ($n$ = 1,637) *ROPN1* expression groups differed in age ≤55 y (36%; $p<0.001$), tumor size ≤17 cm (56%; $p<0.001$), ER+ (89% *versus* 96% and PR+ 84% *versus* 90%; $p<0.001$), luminal A enrichment in high expression (55%) and luminal B/HER2 in low expression ($p<0.001$), endocrine therapy use in high *ROPN1* expression (71%; $p<0.001$) and chemotherapy in low expression (62%; $p = 0.031$) groups; nodal status was comparable (63% negative; $p>0.9$) (Supplementary Table1).

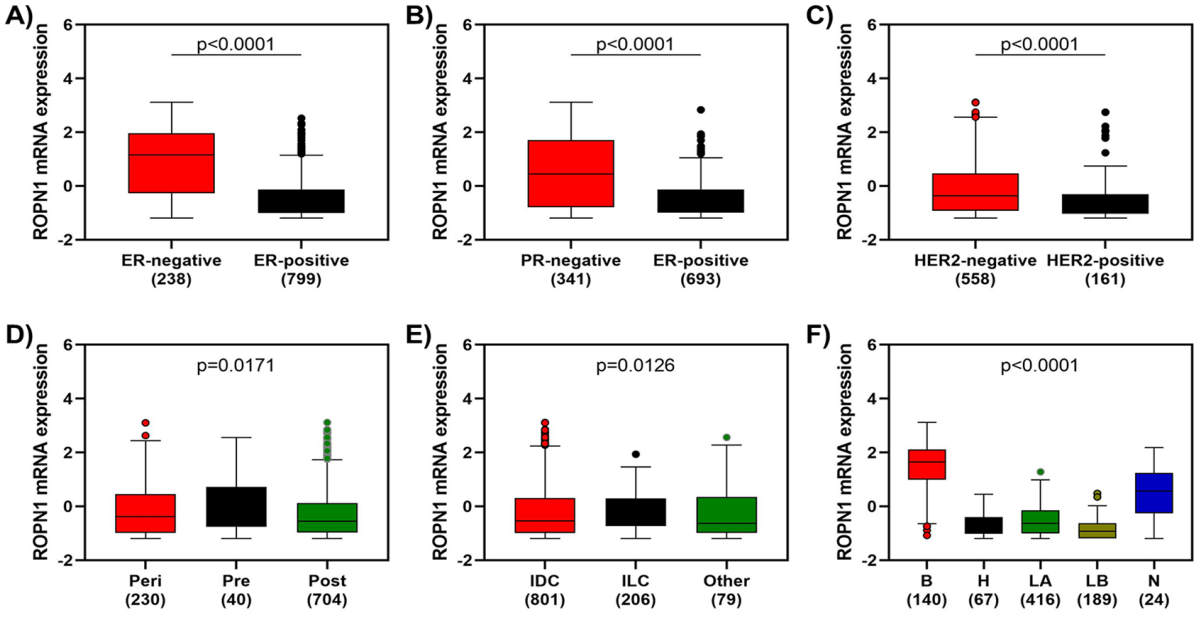



**Figure 1.** *ROPN1* expression patterns in different clinical-pathological contexts of breast cancer. The graphs show comparisons of *ROPN1* expression among subgroups for (A) estrogen receptor, (B) progesterone receptor, (C) HER2, (D) menopausal status, (E) histological type, and (F) molecular subtypes according to the PAM50 classification. Dunn's multiple comparisons test was applied, and differences between the basal and HER2, luminal A or luminal B subtypes were significant ($p<0.0001$), whereas only the difference between the basal and normal-like subtypes was not significant ($p = 0.2751$)

*ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor type II; Peri: Perimenopausal; Pre: Premenopausal; Post: Postmenopausal; IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; B: Basal; H: HER2; LA: Luminal A; LB: Luminal B; N: Normal-like*

*ROPN1* mRNA was elevated in ER–, PR– and HER2– tumors (all $p<0.0001$), and in tumors with high Ki-67 staining ($p = 0.0191$), as well as in tumors in which endocrine therapy was not used ($p<0.0001$). Expression was highest in basal and HER2-enriched subtypes, decreasing through luminal A and B and normal-like ($p<0.0001$), indicating an association with aggressive phenotypes (Supplementary Figure 1).

High *ROPN1* expression predicted poorer OS in SCAN-B (Figure 3A; HR = 2.17; 95% CI: 1.61–2.94; $p<0.0001$), with a pronounced effect in basal tumors (Figure 3B; HR = 1.85; 95% CI: 1.02–3.33; $p = 0.041$). In chemotherapy-treated patients, high *ROPN1* remained adverse (Figure 3C; HR = 2.86; 95% CI: 1.28–6.25; $p = 0.01$), and in untreated patients the mortality risk was even higher (Figure 3D; HR = 4.55; 95% CI: 1.33–14.29; $p = 0.01$).

Probes 224191_x_at (Figure 3E), 231535_x_at (Figure 3F) and 233203_at (Figure 3G) showed consistent *ROPN1* overexpression in TNBC versus non-triple negative subtypes across GSE76275, GSE21653, GSE32646, GSE18864 and GSE43358, underscoring its association with invasive tumor phenotypes.

### Transcriptional Modulation of *ROPN1* in Breast Cancer Cell Lines

After culturing non-tumoral mammary tissue cell lines and other representative malignant phenotypes were cultured, total RNA extraction was performed for reverse transcription-qPCR analysis. When MCF10A was used as a reference, SKBR3 and MCF7 cells exhibited *ROPN1* expression levels that were more than 2,000 times greater. The BT474, HS-578T, BT549, and MDA-MB-231 lines expressed increases ranging from 0.5 to approximately 60 times. Notably, among those analyzed, HCC70 demonstrated a *ROPN1* expression level over 7,000 times greater than that of MCF10A,

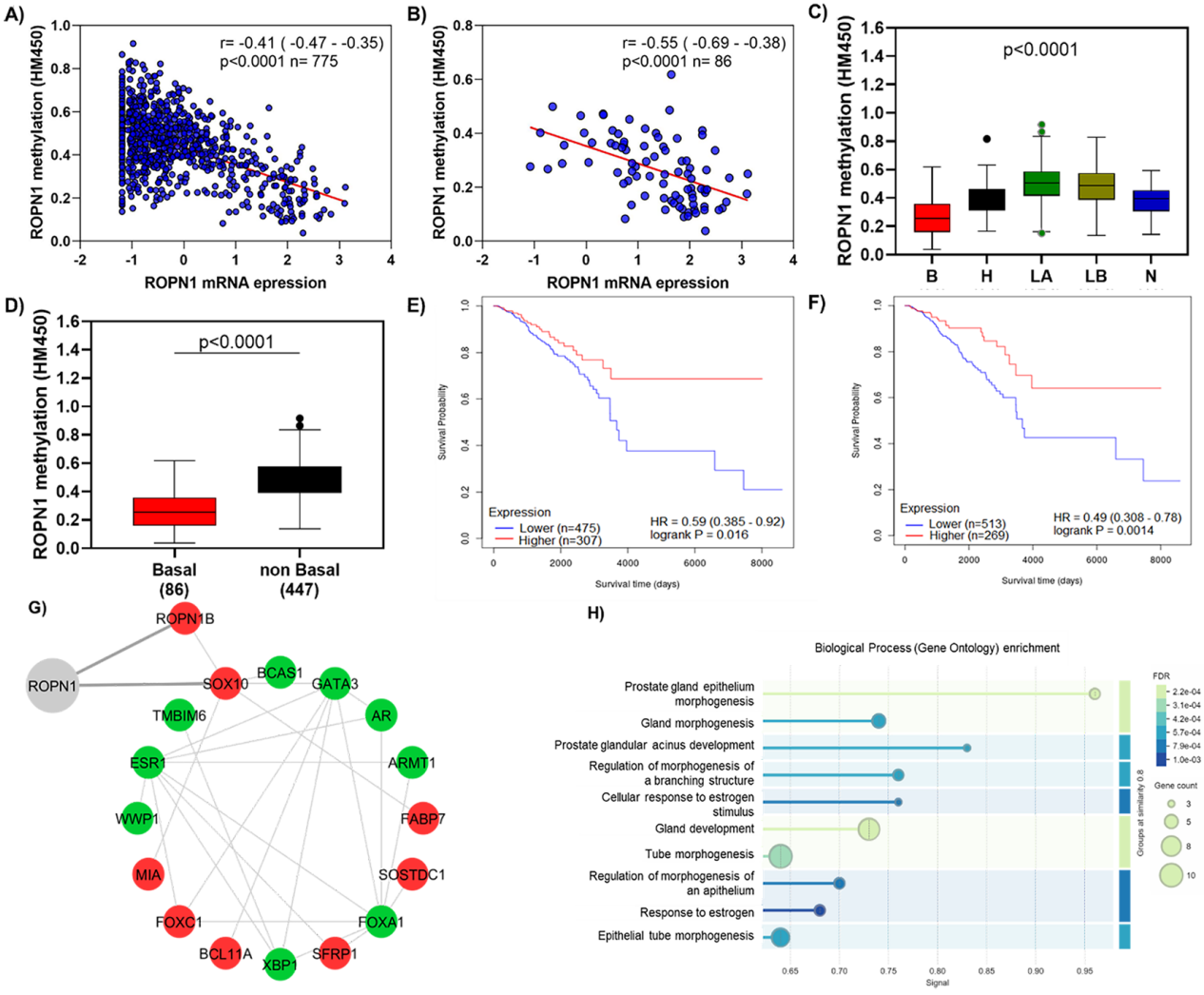



**Figure 2.** Methylation profile of *ROPN1* in breast cancer patients. (A) Correlation between *ROPN1* mRNA levels and methylation in a population of breast cancer patients. (B) Specific correlation for the basal subtype of breast cancer, where the negative correlation is more pronounced. (C) Methylation pattern of *ROPN1* according to the PAM50 classification. (D) Comparison of *ROPN1* methylation in the clinical profiles of basal and non-basal breast cancer patients. (E) OS analysis based on the methylation pattern of the *ROPN1* body opening site cg00101712. (F) OS analysis based on the methylation profile of *ROPN1* in TSS1500 N-shore cg09298623. (G) The PPI network was constructed using the STRING database, employing the top 40 genes correlated with *ROPN1* from the TCGA Firehose Legacy database. Red represents genes positively correlated with *ROPN1*, whereas green indicates genes negatively correlated with *ROPN1*. (H) Pathway enrichment analysis

*B: Basal; H: HER2; LA: Luminal A; LB: Luminal B; N: Normal-like; PPI: Protein-protein interaction; PAM50: Prediction analysis of microarray 50; HER2: Human epidermal growth factor receptor 2*

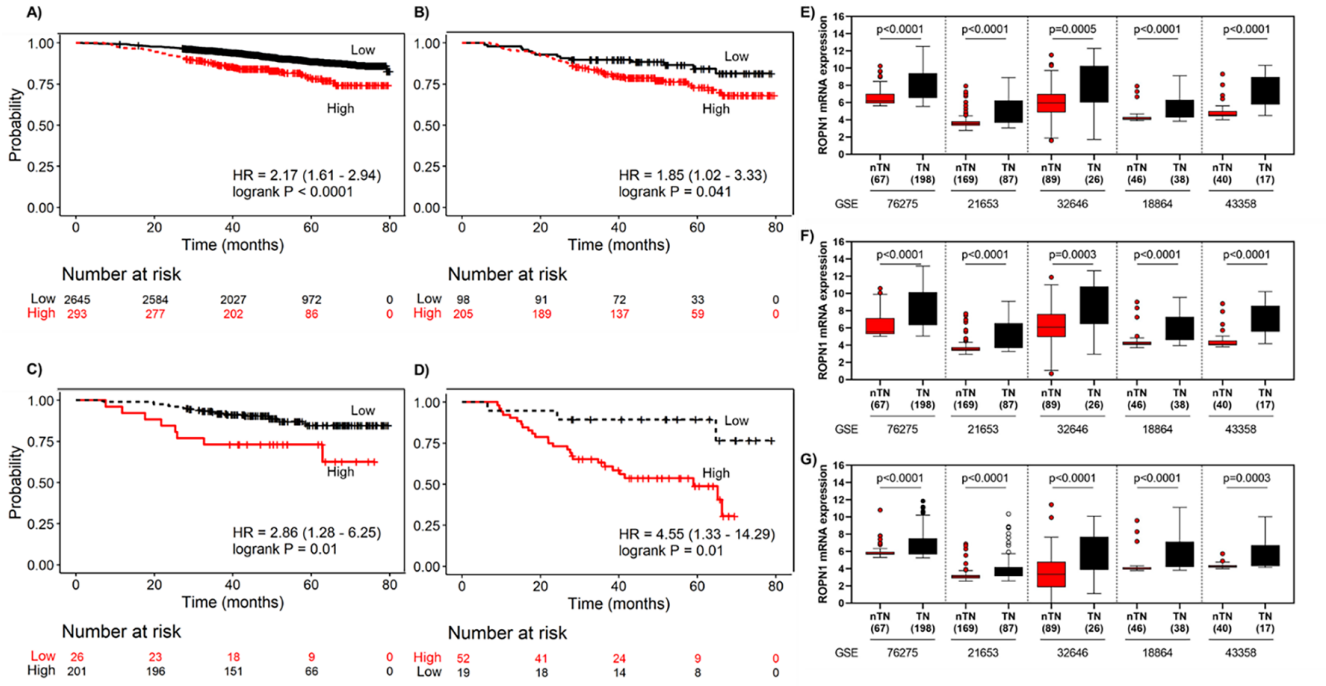



**Figure 3.** Associations between *ROPN1* expression and survival in breast cancer patients. (A) Overall survival analysis of all breast cancer patients. (B) Stratified survival of patients with the basal subtype of breast cancer. (C) Impact of *ROPN1* expression on the survival of basal breast cancer patients undergoing chemotherapy. (D) Comparative survival analysis of basal breast cancer patients who did not receive chemotherapy. Data were obtained from the GSE96058 study and analyzed via Kaplan-Meier curves. The "Low" and "High" categories refer to patient classification based on *ROPN1* gene expression levels, with "Low" indicating expression below the established cut-off value, and "High" indicating expression above this threshold. Expression pattern of *ROPN1* in different cohorts. The probes 224191_x_at (E), 231535_x_at (F), and 233203_at (G) were evaluated using datasets available in the GEO database, identified as GSE76275, GSE21653, GSE32646, GSE18864, and GSE43358, respectively

highlighting the increased expression of this gene in certain tumor contexts (Figure 4A).

The cell lines were subsequently treated with chemotherapeutics and radiotherapy to evaluate any transcriptional modifications. MCF7 cells presented a greater fold change than did wild-type cells (untreated), with increases of 23%, 3%, and 26% after exposure to cisplatin, doxorubicin, and paclitaxel, respectively. In contrast, irradiation with 6 Grays resulted in a drastic decrease in *ROPN1* (Figure 4B). For the SKBR3 line, there was a decrease of approximately 40% after both cisplatin and paclitaxel treatment, although doxorubicin and radiation did not reduce *ROPN1* expression levels in this cell line (Figure 4C). In BT474 cells, cisplatin and doxorubicin induced decreases of 48% and 38%, respectively. Conversely, irradiation led to a 25% increase in the transcript level (Figure 4D). For the HCC70 line, which, among the lines used, was the one that expressed *ROPN1* at the highest level, all the treatments induced a decrease in the level of this transcript, which was most evident with cisplatin, with which a 63% reduction was achieved (Figure 4E). Similarly, HS578T cells also exhibited a reduction of approximately 50% in response to cisplatin, doxorubicin, and irradiation and a 30% decrease after paclitaxel treatment (Figure 4F). BT549 cell expression of *ROPN1* was were reduced by 21% only after cisplatin treatment and significantly increased by 197% and 293% after doxorubicin and paclitaxel treatment, respectively (Figure 4G). Finally, for MDA-MB231 cells, in which the expression levels were close to those found in the MCF10A reference cells, all the treatments induced a significant increase in *ROPN1* (Figure 4H). The HS578T line was also treated with 5-aza and TSA, which resulted in increases in the expression of *ROPN1* by 9.5-fold and 4.9-fold, respectively (Figure 4I).

## Discussion and Conclusion

Breast cancer remains a leading cause of female cancer mortality, with TNBC/basal tumors exhibiting high recurrence and chemoresistance. Biomarker discovery is therefore essential for enhanced risk stratification and therapeutic decision-making. *ROPN1* emerged as a candidate, showing elevated expression in TNBC/basal cases and consistent association with poor survival. Our data indicate that high *ROPN1* expression is consistently associated with poor OS in breast cancer patients, especially those with basal-like subtype, which is known for its high degree of aggressiveness and poor prognosis. Risk analysis showed that patients with high *ROPN1* expression who were not treated with chemotherapy had an HR of 4.55 (Figure 3D), indicating a robust association between *ROPN1* expression and unfavorable outcomes. Among patients who received chemotherapy, high levels of *ROPN1* also correlated with poorer prognosis (HR = 2.53). These findings position *ROPN1* as a potent prognostic marker of aggressive breast cancer and a potential predictor of limited chemotherapy response, underscoring the need for personalized treatment strategies.

Previous findings by Liu et al. (13) showed that *ROPN1* is overexpressed in TNBC, enhancing migration, invasion and metastasis via RhoA activation. Overexpression increased actin stress fibers and contractility, while *ROPN1* silencing reduced invasiveness and metastasis *in vitro* and *in vivo*. Kortleve et al. (24) validated

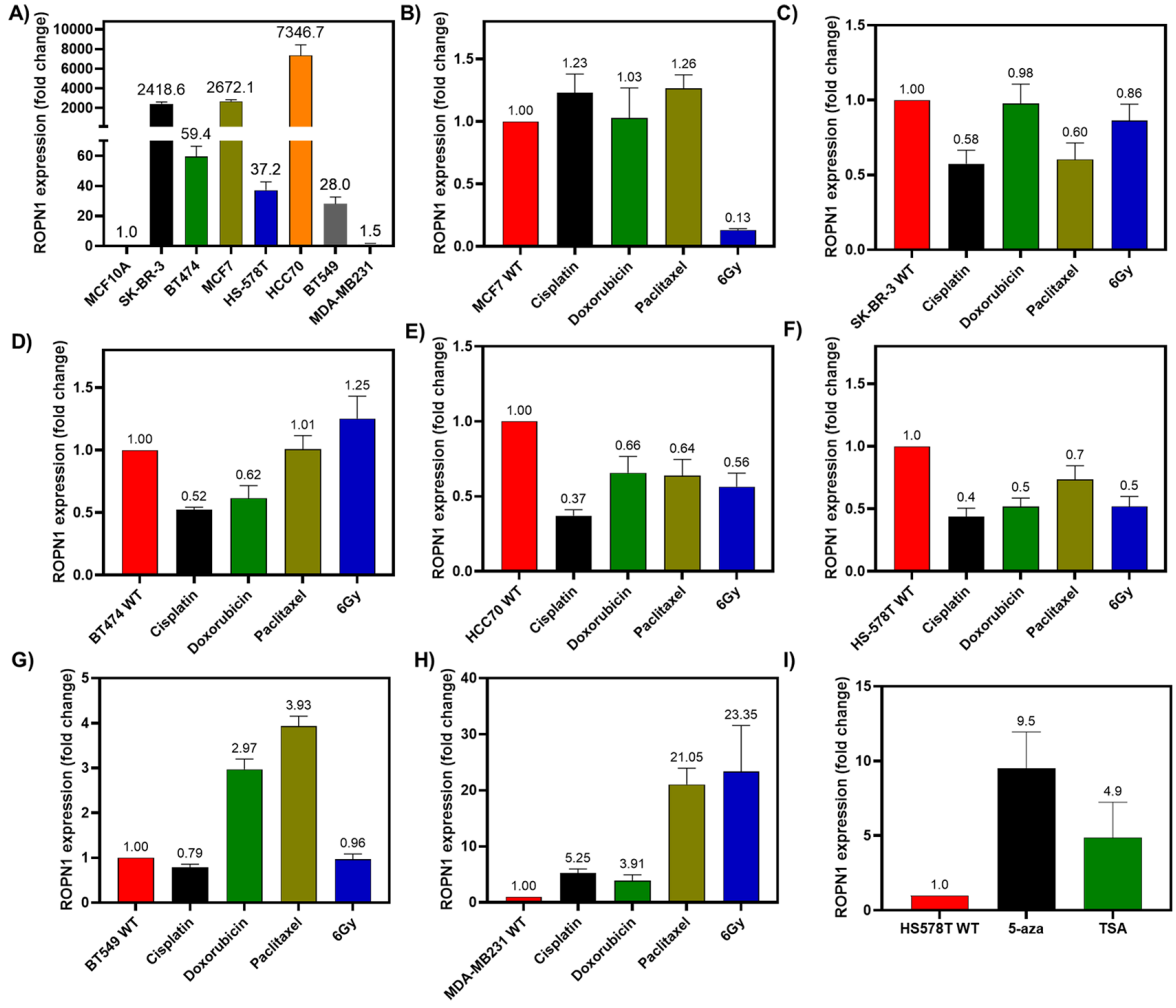



**Figure 4.** Expression of *ROPN1* in breast cancer cell lines and the effects of different treatments. (A) Basal transcription levels of *ROPN1* in a panel of mammary cell lines, including distinct tumor subtypes. (B-H) Variation in *ROPN1* expression after treatment with cisplatin, doxorubicin, paclitaxel, or radiotherapy. (I) Modulation of *ROPN1* expression in response to the epigenetic-acting drugs 5-aza and trichostatin A

*ROPN1* as a prognostic and therapeutic target in TNBC, showing strong expression in >90% of primary and metastatic tumors, with minimal expression in normal tissues, except testis. In patient-derived and murine models, anti-*ROPN1* TCR-T cells effectively eliminated *ROPN1*+ tumors, outperforming cisplatin and sacituzumab govitecan. *ROPN1* expression correlated with metastasis and poor prognosis. These findings align with our results, supporting the translational value of *ROPN1* as a biomarker and therapeutic target in TNBC.

In our PPI analysis, *ROPN1B* and *SOX10* emerged as key genes positively correlated with *ROPN1*. *ROPN1B*, a cytoskeleton-related protein sharing 96% sequence homology with *ROPN1*, may act synergistically to promote invasiveness. Da Gama Duarte et al. (25) reported a strong correlation between *ROPN1* and *ROPN1B* in melanoma ($r = 0.86$, $p = 8.71\times10^{-4}$), associating both with motility, chemoresistance and immune modulation. *SOX10*, which also correlated with *ROPN1*, drives mesenchymal traits and drug resistance in TNBC (26-28), suggesting that their co-expression may enhance tumor plasticity and therapeutic evasion.

The network also included clinically relevant genes, such as *ESR1*, *AR* and *FOXA1*, all downregulated or absent in TNBC/basal tumors. *ESR1*, a key marker in luminal subtypes, serves as both a prognostic indicator and therapeutic target. *AR*, though expressed in a subset of TNBCs, lacks the favorable impact seen with *ESR1* but may serve as a target in LAR tumors (10). *FOXA1*, a transcriptional cofactor for both *ESR1* and *AR*, promotes epithelial identity in luminal cancers but is minimally expressed in TNBC, supporting its undifferentiated and aggressive profile (29, 30).

Chemotherapeutic treatments and radiotherapy have heterogeneous effects on *ROPN1* levels, depending on the cell line and the therapeutic agent used. Among the parental lines analyzed, HCC70 exhibited an increase in *ROPN1* expression of more than 7,000 times, highlighting the unique behavior of the TNBC subtype, which is highly aggressive and has a poor prognosis (7). This overexpression may be associated with the role of *ROPN1* in fundamental biological processes, such as cell motility, which directly contributes to tumor migration and invasion. Indeed, previous studies have demonstrated that *ROPN1* plays an active role in tumor progression, especially in TNBC. Wu and collaborators reported that the overexpression of *ROPN1* was associated with a significant increase in cell migration and invasion, which is mediated by the activation of RhoA, a GTPase essential for cytoskeletal organization (24). This activation leads to actin reorganization, promoting the formation of cellular protrusions and facilitating tumor spread. Furthermore, *in vivo* models have shown

that *ROPN1* not only enhances metastasis but also that its suppression significantly reduces tumor dissemination (24).

By analyzing the effects of chemotherapeutic agents on a selection of cell lines, we propose a relevant translational hypothesis. We observed that, under certain conditions, treatments did not reduce or even increased the levels of *ROPN1*, as noted in BT549 cells after exposure to doxorubicin and paclitaxel. Considering the findings of Wu et al. on the prometastatic role of *ROPN1*, these results suggest that the persistence or elevation of this transcript following chemotherapy may represent an adaptive mechanism of tumor cells, promoting therapeutic resistance and enhancing their migratory and survival capabilities. This hypothesis becomes even more pertinent when the clinical survival data of patients treated with chemotherapy are reviewed (Figure 3C), where high expression of *ROPN1* was associated with a poorer prognosis, even after treatment.

The observed relationship between *ROPN1* expression and its methylation in breast cancer suggests that epigenetic mechanisms may be involved in the regulation of this gene, particularly in the context of more aggressive subtypes, such as HER2-positive breast cancer and TNBC/basal cancer. This pattern of hypomethylation associated with *ROPN1* overexpression indicates that DNA methylation might act as a modulator of gene expression in highly proliferative cancers with increased invasive capacity. To date, only the study by Atanackovic and colleagues has evaluated possible associated epigenetic mechanisms through pharmacological treatment in cell lines derived from acute myeloid leukemia (AML) (31). In their study, treatment with TSA and decitabine or their combination did not result in positive modulation of *ROPN1*, which is poorly expressed in AML, suggesting that other regulatory mechanisms may occur in these lines, such as post-transcriptional regulation mediated by microRNAs or interactions with inhibitory transcription factors. Of note, our *in vitro* experiments revealed a significant increase in *ROPN1* expression after treatment with 5-aza or TSA, with elevated levels of 9.5- and 4.9-fold, respectively. These results reinforce our *in silico* findings and suggest a distinct epigenetic role of *ROPN1* in breast cancer, especially considering the contrast with the AML model. These data highlight the importance of investigating how the epigenetic deregulation of *ROPN1* influences tumor behavior in different biological contexts, especially in more aggressive breast subtypes, and exploring whether hypomethylation and overexpression of this gene have direct functional impacts on tumor progression and therapeutic response. Moreover, we observed that low methylation in the regions cg09298623 and cg00101712 was associated with worse OS in patients with this phenomenon. The increased expression of *ROPN1*, which is mediated by a reduction in methylation, may represent a phenotypic adaptation of the tumor that facilitates cell invasion and metastasis, which can lead to a worsened clinical outcome for these patients.

Further studies are needed to clarify how epigenetic changes drive tumor aggressiveness and to define the functional role of *ROPN1* in breast cancer progression and therapy response. While our multicohort analysis offers strong associative evidence, functional validation (e.g., CRISPR/Cas9) is required to confirm causality in chemoresistance. Prospective cohorts and mechanistic studies will be key to validating *ROPN1* expression as a predictive biomarker, potentially guiding patient stratification and improving therapeutic outcomes in high-risk cases.

### Study Limitations

This study has limitations, including the absence of validation in patient-derived tumor samples and reliance on public gene expression datasets. In addition, the interactions between *ROPN1*, tumor subtypes, and therapeutic contexts, especially drug combinations, remain incompletely characterized. Further studies are needed to validate these findings and clarify the role of *ROPN1* in breast cancer biology.

*ROPN1* is markedly overexpressed in hormone receptor–negative and triple-negative/basal-like breast cancers, where it predicts significantly poorer OS. Its prognostic value persists regardless of chemotherapy status, high *ROPN1* expression doubles mortality risk in treated patients and quadruples it when treatment is absent, underscoring its utility for risk stratification. An inverse relationship between DNA methylation and *ROPN1* expression further links hypomethylation to adverse outcomes. *In vitro*, *ROPN1* expression following chemotherapeutics and radiotherapy was variable in different cell lines, with some agents inducing upregulation suggesting adaptive resistance mechanisms. Collectively, these findings position *ROPN1* and its protein product as both a robust prognostic and predictive biomarker and a candidate therapeutic target for high-risk breast cancer subgroups.

**Acknowledgements**

We sincerely thank Prof. Dr. Luisa Villa for graciously providing access to her laboratory facilities, which made the execution of this study possible. We express our sincere gratitude to Prof. Dr. Sandra Gonzalez and Prof. MSc. Nancy Ramona Segovia Coronel for their invaluable support to scientific research.

### Ethics

**Ethics Committee Approval:** Not necessary.

**Informed Consent:** Not necessary.

### Footnotes

### Authorship Contributions

Concept: R.C.C., D.R.d.B.; Design: R.C.C., D.R.d.B.; Data Collection or Processing: R.C.C., A.G.C., D.R.d.B.; Analysis or Interpretation: R.C.C., D.R.d.B.; Literature Search: R.C.C., O.M.G., C.J.M., D.R.d.B.; Writing: R.C.C., A.G.C., O.M.G., C.J.M., D.R.d.B

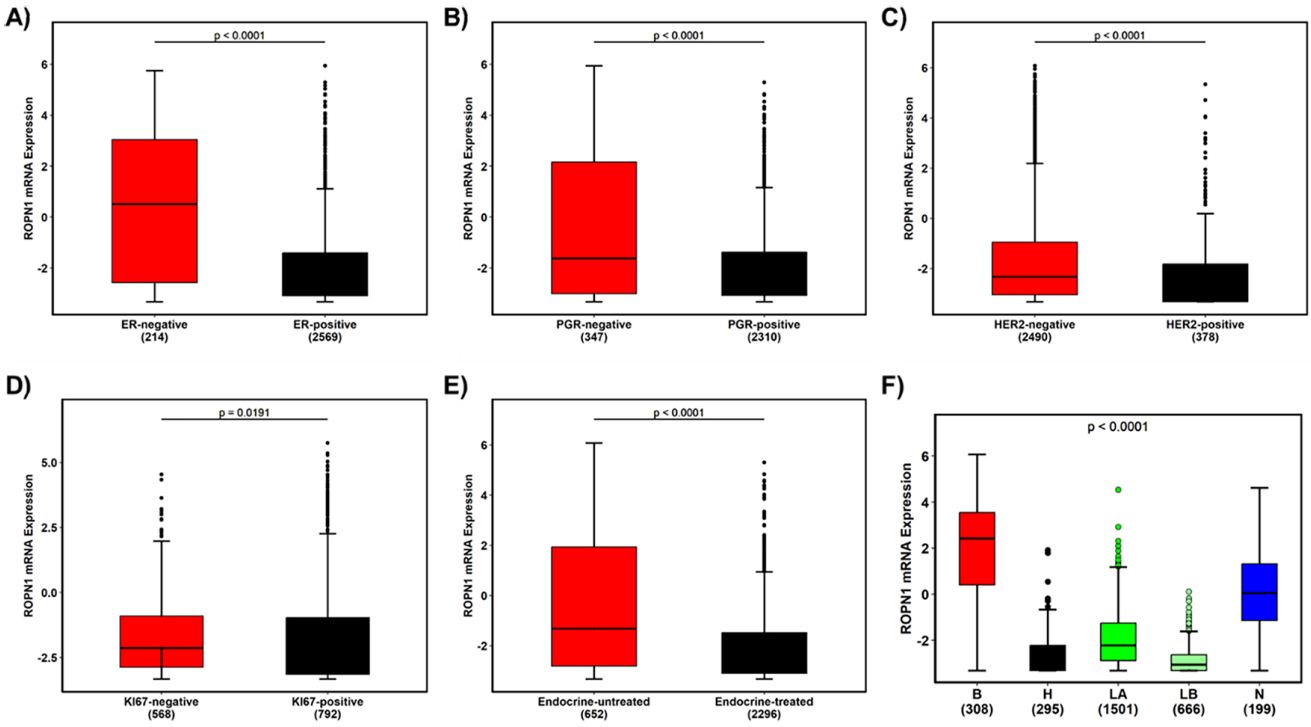
**Conflict of Interest:** No conflict of interest was declared by the authors.

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

## References

1. 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. DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, et al. Breast cancer statistics, 2019. CA Cancer J Clin. 2019; 69: 438-451. (PMID: 31577379) [Crossref]

3. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends--an update. Cancer Epidemiol Biomarkers Prev. 2016; 25: 16-27. (PMID: 26667886) [Crossref]
4. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, et al. Current and future burden of breast cancer: global statistics for 2020 and 2040. Breast. 2022; 66: 15-23. (PMID: 36084384) [Crossref]
5. Popa M-T, Noditi A, Peleaşa T-M, Stoleru S, Blidaru A. Breast cancer: a heterogeneous pathology. prognostic and predictive factors - a narrative review. Chirurgia (Bucur). 2025; 120: 32. [Crossref]
6. Zhang X. Molecular classification of breast cancer: relevance and challenges. Arch Pathol Lab Med. 2023; 147: 46-51. (PMID: 36136295) [Crossref]
7. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011; 121: 2750-2767. (PMID: 21633166) [Crossref]
8. Kensler KH, Sankar VN, Wang J, Zhang X, Rubadue CA, Baker GM, et al. PAM50 molecular intrinsic subtypes in the Nurses' Health Study Cohorts. Cancer Epidemiol Biomarkers Prev. 2019; 28: 798-806. (PMID: 30591591) [Crossref]
9. Zagami P, Carey LA. Triple negative breast cancer: pitfalls and progress. NPJ Breast Cancer. 2022; 8: 95. (PMID: 35987766) [Crossref]
10. Xiong N, Wu H, Yu Z. Advancements and challenges in triple-negative breast cancer: a comprehensive review of therapeutic and diagnostic strategies. Front Oncol. 2024; 14: 1405491. (PMID: 38863622) [Crossref]
11. Jiang P, Sinha S, Aldape K, Hannenhalli S, Sahinalp C, Ruppin E. Big data in basic and translational cancer research. Nat Rev Cancer. 2022; 22: 625-639. (PMID: 36064595) [Crossref]
12. Gao S, Chen Z, Shi J, Chen Z, Yun D, Li X, et al. Sperm immotility is associated with epididymis metabolism disorder in mice under obstructive azoospermia. FASEB J. 2023; 37: e23081. (PMID: 37410071) [Crossref]
13. Liu Q, Huang X, Li Q, He L, Li S, Chen X, et al. Rhophilin-associated tail protein 1 promotes migration and metastasis in triple negative breast cancer via activation of RhoA. FASEB J. 2020; 34: 9959-9971. (PMID: 32427399) [Crossref]
14. Adeola HA, Smith M, Kaestner L, Blackburn JM, Zerbini LF. Novel potential serological prostate cancer biomarkers using CT100+ cancer antigen microarray platform in a multi-cultural South African cohort. Oncotarget. 2016; 7: 13945-13964. (PMID: 26885621) [Crossref]
15. de Bastos DR, Conceição MPF, Michelli APP, Leite JMRS, da Silva RA, Cintra RC, et al. An *in silico* analysis identified FZD9 as a potential prognostic biomarker in triple-negative breast cancer patients. Eur J Breast Health. 2020; 17: 42-52. (PMID: 33796830) [Crossref]
16. Céspedes AG, Conceição MPF, de Bastos DR, de Grazia GÁ, Leite JMRS, do Nascimento RG, et al. Altered expression of CYSLTR1 is associated with adverse clinical outcome in triple negative breast tumors: an *in silico* approach. Eur J Breast Health. 2023; 19: 148-158. (PMID: 37025579) [Crossref]
17. Goldman MJ, Craft B, Hastie M, Repečka K, McDade F, Kamath A, et al. Visualizing and interpreting cancer genomics data via the Xena platform. Nat Biotechnol. 2020; 38: 675-678. (PMID: 32444850) [Crossref]
18. Brueffer C, Vallon-Christersson J, Grabau D, Ehinger A, Häkkinen J, Hegardt C, et al. Clinical value of RNA sequencing-based classifiers for prediction of the five conventional breast cancer biomarkers: a report from the population-based multicenter sweden cancerome analysis network-breast initiative. JCO Precis Oncol. 2018; 2: PO.17.00135. (PMID: 32913985) [Crossref]
19. Sjoberg DD, Whiting K, Curry M, Lavery JA, Larmarange J. Reproducible summary tables with the gtsummary package. R J. 2021; 13: 570. [Crossref]
20. Kassambara A. Survminer: drawing survival curves using "ggplot2." 2023. Accessed in December 2024. [Crossref]
21. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023; 51: D638-D646. (PMID: 36370105) [Crossref]
22. Modhukur V, Iljasenko T, Metsalu T, Lokk K, Laisk-Podar T, Vilo J. MethSurv: a web tool to perform multivariable survival analysis using DNA methylation data. Epigenomics. 2018; 10: 277-288. (PMID: 29264942) [Crossref]
23. Cokelaer T, Chen E, Iorio F, Menden MP, Lightfoot H, Saez-Rodriguez J, et al. GDSCTools for mining pharmacogenomic interactions in cancer. Bioinformatics. 2018; 34: 1226-1228. (PMID: 29186349) [Crossref]
24. Kortleve D, Hammerl D, van Brakel M, Wijers R, Roelofs D, Kroese K, et al. TCR-engineered T cells directed against ropporin-1 constitute a safe and effective treatment for triple-negative breast cancer. Cancer Discov. 2024; 14: 2450-2470. (PMID: 39172012) [Crossref]
25. Da Gama Duarte J, Woods K, Quigley LT, Deceneux C, Tutuka C, Witkowski T, et al. Ropporin-1 and 1B are widely expressed in human melanoma and evoke strong humoral immune responses. Cancers (Basel). 2021; 13: 1805. (PMID: 33918976) [Crossref]
26. Man K-H, Wu Y, Gao Z, Spreng A-S, Keding J, Mangei J, et al. SOX10 mediates glioblastoma cell-state plasticity. EMBO Rep. 2024 ;25: 5113-5140. [Crossref]
27. Yang K, Yun F, Shi L, Liu X, Jia YF. SOX10 promotes the malignant biological behavior of basal-like breast cancer cells by regulating EMT process. Heliyon. 2023; 9: e23162. [Crossref]
28. Feng W, Liu S, Zhu R, Li B, Zhu Z, Yang J, et al. SOX10 induced Nestin expression regulates cancer stem cell properties of TNBC cells. Biochem Biophys Res Commun. 2017; 485: 522-528. (PMID: 28189679) [Crossref]
29. Glont SE, Chernukhin I, Carroll JS. Comprehensive genomic analysis reveals that the pioneering function of FOXA1 is independent of hormonal signaling. Cell Rep. 2019; 26: 2558-2565.e3. (PMID: 30840881) [Crossref]
30. Dai X, Cheng H, Chen X, Li T, Zhang J, Jin G, et al. FOXA1 is prognostic of triple negative breast cancers by transcriptionally suppressing *SOD2* and *IL6*. Int J Biol Sci. 2019; 15: 1030-1041. (PMID: 31182923) [Crossref]
31. Atanackovic D, Luetkens T, Kloth B, Fuchs G, Cao Y, Hildebrandt Y, et al. Cancer-testis antigen expression and its epigenetic modulation in acute myeloid leukemia. Am J Hematol. 2011; 86: 918-922. (PMID: 21898529) [Crossref]




**Supplementary Figure 1.** Expression pattern of *ROPN1* in patients with breast cancer from the Sweden Cancerome Analysis Network-Breast study. Transcriptional levels of *ROPN1* based on (A) estrogen receptor, (B) progesterone receptor, (C) human epidermal growth factor receptor 2, (D) the proliferation marker Ki-67, (E) endocrine treatment, and (F) Prediction analysis of microarray 50 classification

**Supplementary Table 1. Clinicopathological characteristics of patients with breast cancer derived from the Sweden Cancerome Analysis Network-Breast study and their associations with *ROPN1* expression**

| Variables | High | | Low | | p |
|---|---|---|---|---|---|
| | *n* = 1.636 | % | *n* = 1.637 | % | |
| **Age** | | | | | <0.001 |
| ≤55 | 527 | 36 | 372 | 25 | |
| >55 | 955 | 64 | 1.115 | 75 | |
| **Tumor size group** | | | | | <0.001 |
| ≤17 cm | 813 | 56 | 705 | 48 | |
| >17 cm | 651 | 44 | 768 | 52 | |
| **Lymph node status** | | | | | >0.9 |
| Negative | 906 | 63 | 910 | 63 | |
| Positive | 533 | 37 | 531 | 37 | |
| **ER status** | | | | | <0.001 |
| No | 152 | 11 | 62 | 4 | |
| Yes | 1.198 | 89 | 1.371 | 96 | |
| **PR status** | | | | | <0.001 |
| No | 205 | 16 | 142 | 10 | |
| Yes | 1.091 | 84 | 1.219 | 90 | |
| **HER2 status** | | | | | 0.009 |
| No | 1.261 | 88 | 1.229 | 85 | |
| Yes | 164 | 12 | 214 | 15 | |
| **Ki-67 status** | | | | | 0.001 |
| No | 318 | 46 | 250 | 37 | |
| Yes | 372 | 54 | 420 | 63 | |
| **NHG** | | | | | <0.001 |
| G1 | 259 | 18 | 190 | 13 | |
| G2 | 680 | 47 | 711 | 48 | |
| G3 | 505 | 35 | 565 | 39 | |
| **PAM50 subtype** | | | | | <0.001 |
| Basal | 279 | 19 | 29 | 2 | |
| HER2 | 91 | 6 | 204 | 14 | |
| Luminal A | 821 | 55 | 680 | 46 | |
| Luminal B | 103 | 7 | 563 | 38 | |
| Normal-like | 188 | 13 | 11 | 0.7 | |
| **Chemo treated** | | | | | 0.031 |
| No | 848 | 58 | 911 | 62 | |
| Yes | 622 | 42 | 568 | 38 | |
| **Endocrine treated** | | | | | <0.001 |
| No | 424 | 29 | 228 | 15 | |
| Yes | 1.045 | 71 | 1.251 | 85 | |

Pearson's chi-squared test; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; PAM50: Prediction analysis of microarray 50 (50-gene panel used for molecular classification); NHG: Nottingham histologic grade

**Original Article**

Eur J Breast Health 2025; 21(4): 356-366
**DOI:** 10.4274/ejbh.galenos.2025.2025-5-3

# Relationship Between [$^{18}$F]FDG PET/CT Texture Analysis and Progression-Free Survival in Patients Diagnosed With Invasive Breast Carcinoma

Ogün Bülbül[1], Hande Melike Bülbül[2], Sibel Göksel[3]

[1]Department of Nuclear Medicine, Recep Tayyip Erdogan University Faculty of Medicine, Rize, Türkiye
[2]Department of Radiology, Recep Tayyip Erdogan University Faculty of Medicine, Rize, Türkiye
[3]Department of Nuclear Medicine, Adnan Menderes University Faculty of Medicine, Aydın, Türkiye

**ABSTRACT**

**Objective:** Breast cancer is the most common cancer and the leading cause of cancer-related deaths in women. Texture analysis provides crucial prognostic information about many types of cancer, including breast cancer. The aim was to examine the relationship between texture features (TFs) of 2-deoxy-2[$^{18}$F] fluoro-D-glucose positron emission tomography (PET)/computed tomography and disease progression in patients with invasive breast cancer.

**Materials and Methods:** TFs of the primary malignant lesion were extracted from PET images of 112 patients. TFs that showed significant differences between patients who achieved one-, three-, and five-year progression-free survival (PFS) and those who did not were selected and subjected to the least absolute shrinkage and selection operator regression method to reduce features and prevent overfitting. Machine learning (ML) was used to predict PFS using TFs and selected clinicopathological parameters.

**Results:** In models using only TFs, random forest predicted one-, three-, and five-year PFS with area under the curve (AUC) values of 0.730, 0.758, and 0.797, respectively. Naive Bayes predicted one-, three-, and five-year PFS with AUC values of 0.857, 0.804, and 0.843, respectively. The neural network predicted one-, three-, and five-year PFS with AUC values of 0.782, 0.828, and 0.780, respectively. These findings indicated increased AUC values when the models combined TFs with clinicopathological parameters. The lowest AUC values of the models combining TFs and clinicopathological parameters when predicting one-year, three-year, and five-year PFS were 0.867, 0.898, and 0.867, respectively.

**Conclusion:** ML models incorporating PET-derived TFs and clinical parameters may assist in predicting progression during the pre-treatment period in patients with invasive breast carcinoma.

**Keywords:** Texture analysis; machine learning; breast carcinoma; PET/CT; progression-free survival

**Cite this article as:** Bülbül O, Bülbül HM, Göksel S. Relationship between [$^{18}$F]FDG PET/CT texture analysis and progression-free survival in patients diagnosed with invasive breast carcinoma. Eur J Breast Health. 2025; 21(4): 356-366

**Key Points**

- Invasive breast carcinoma may progress after initial treatment.
- Positron emission tomography/computed tomography (PET/CT) parameters obtained before initial treatment can predict disease progression.
- Combining PET/CT texture features with clinicopathological parameters improves prediction of progression.

## Introduction

Breast cancer is the most common cancer and the leading cause of cancer-related deaths in women (1). Accurate staging of the disease is essential for successful treatment. 2-deoxy-2[$^{18}$F]fluoro-D-glucose ([$^{18}$F]FDG) positron emission tomography/computed tomography (PET/CT) is frequently used in oncology for purposes, such as staging various cancer types, evaluating response to treatment, determining radiotherapy fields, and detecting recurrence (2). Routine use of PET/CT is not recommended for patients with stage I-II or operable stage III breast cancer (3-5). However, PET/CT may be helpful when findings on other imaging modalities used for staging are uncertain. In addition, PET/CT is able to delineate many clinicopathological prognostic parameters in breast cancer (6).

Texture analysis (TA) of medical images, also known as radiomics, has recently become one of the most popular topics in research. TA allows medical images to provide more information than the human eye can detect (7). TA of PET/CT offers prognostic information about various malignancies, including breast cancer (8-12). In breast cancer, PET/

**Corresponding Author:**
Ogün Bülbül MD; ogun.bulbul@erdogan.edu.tr

**Received:** 06.05.2025
**Accepted:** 07.08.2025
**Epub:** 22.08.2025
**Available Online Date:** 25.09.2025

CT-based TA has been used to characterize lesions, evaluate tumor biology, including grade and immunohistochemical marker expression, predict response to neoadjuvant chemotherapy, and predict disease-free survival (DFS) (9). Currently, TA is primarily used for preclinical and research purposes because improvements and standardization of methodology are needed before TA can be integrated into clinical workflow. Early studies in the field of TA in breast cancer focused on predicting histopathological and immunohistochemical parameters, as well as treatment responses. Nevertheless, there are only a limited number of studies on TA and breast cancer survival. The aim of this study was to examine the relationship between [$^{18}$F]FDG PET/CT-derived TA and progression-free survival (PFS) in invasive breast carcinoma using machine learning (ML)-based analysis.

## Materials and Methods

### Patients

This study retrospectively identified and included 290 female patients diagnosed with invasive breast carcinoma who underwent PET/CT for staging at a single center between 2019 and 2022. During this period, PET/CT scans were routinely performed for staging purposes in female patients with invasive breast cancer whose primary tumor was larger than one centimeter. The exclusion criteria were: Inability to determine disease progression due to inaccessible medical records; inability to perform TA due to the metabolic volume of the primary tumor being less than 64 voxels on PET/CT images; and presence of a second malignancy. After applying these criteria, a total of 112 patients were included in the study (Figure 1).

### PET/CT Imaging Protocol

Following six hours of fasting, patients with a blood glucose level below 200 mg/dL received an intravenous injection of 0.1 mCi/kg [$^{18}$F]FDG. The patients were then asked to rest in a quiet, darkened room for approximately 60 minutes. PET/CT imaging was performed from the vertex to the mid-thigh using a Siemens Biograph mCT 20 PET/CT system (Siemens, Germany). First, nondiagnostic CT images were obtained using the following parameters: 120 kVp, 50 mAs, and 5-mm slice thickness. PET imaging was then performed for 2 minutes per bed position. PET images were corrected for attenuation using the corresponding nondiagnostic CT images. The ordered-subset expectation maximization method was used for image reconstruction.

### Texture Analysis

TA was performed using LIFEx software version 7.4.0 (lifexsoft.org) by two nuclear medicine physicians with six and nine years of experience in oncological PET/CT interpretation. LIFEx is a freely available software tool widely used for TA in the medical imaging literature (13). Attenuation-corrected PET images were imported into the LIFEx program. The primary breast lesions were manually segmented using a three-dimensional region of interest (ROI), defined to correspond with radiological findings. A threshold of 40% of the maximum standardized uptake value ($SUV_{max}$) was used to delineate the ROI (Figure 2). Segmentation was independently performed by both nuclear medicine physicians. For spatial resampling of the ROI, a voxel spacing of 4×4×4 mm was applied along the x, y, and z axes. Image intensity was discretized into 64 gray levels with a bin width of 0.3. Intensity rescaling was conducted using an absolute scale range of 0–20. Texture features (TFs) extracted from the three-dimensional ROI included first-order features, such as morphological, intensity-based, local intensity-based, intensity histogram, and local intensity histogram, as well as second-order features such as intensity-based rim, intensity histogram rim, gray-level co-occurrence matrix, neighboring gray-tone difference matrix, gray-level run-length matrix, and gray-level size zone matrix (Supplementary Table 1).

### Determination of PFS

To determine progression, imaging findings defined from molecular imaging methods (PET/CT and bone scintigraphy) and morphological imaging methods [breast ultrasound (US), breast magnetic resonance imaging (MRI), and thoracic-abdominopelvic CT or MRI] obtained during follow-up were compared with baseline staging images. PET/CT images were evaluated according to the PERCIST criteria, while CT and MRI findings were assessed according to the RECIST 1.1 criteria (14). The appearance of new bone metastases at previously uninvolved non-metastatic locations on bone scintigraphy and signs of recurrence or progression at the primary tumor site identified through

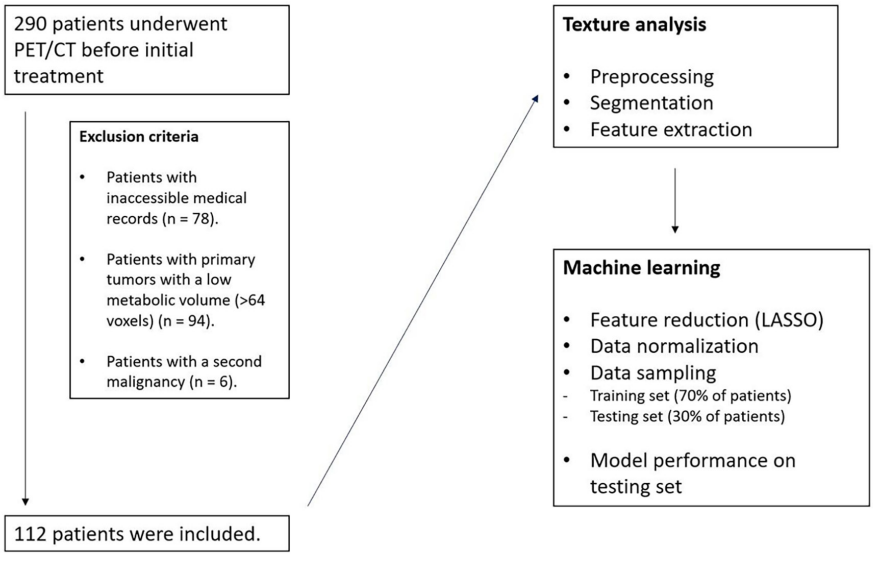



**Figure 1.** Workflow of the study

*PET/CT: Positron emission tomography/computed tomography; LASSO: Least absolute shrinkage and selection operator*

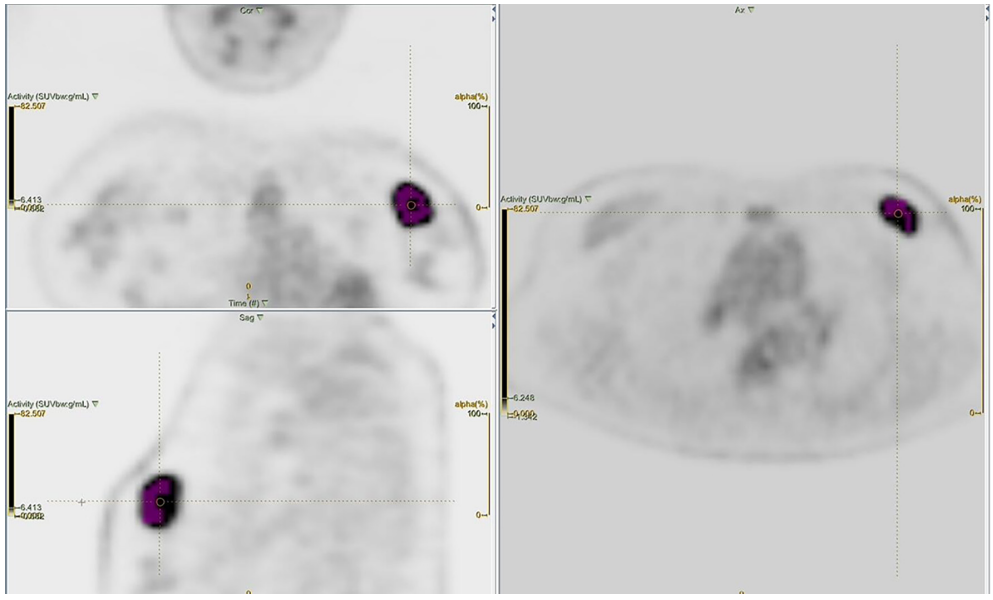


**Figure 2.** Three-dimensional segmentation of the primary breast lesion using a 40% $SUV_{max}$ in the LIFEx program

*$SUV_{max}$: Maximum standardized uptake value*

mammography and/or breast US were also accepted as indicators of progression. PFS was defined as the time interval between the date of breast cancer diagnosis and the first radiological evidence of progression, based on the criteria outlined above. For patients without progression, PFS was calculated as the time between the date of diagnosis and the date of last follow-up. The number of patients who achieved one-, three-, and five-year PFS was recorded.

The Recep Tayyip Erdogan University Ethics Committee approved this study (approval no: 2022/228, date: 22.12.2022). The ethical committee waived the requirement for informed consent as the study was retrospective. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments.

### Statistical Analysis and ML

All statistical analyses were performed using SPSS, version 24 (IBM Corp., Armonk, NY, USA). A *p*-value of <0.05 was considered statistically significant. The Mann-Whitney U test was used to compare the TFs of patients who achieved one-, three-, and five-year PFS with those who did not. TFs with a *p*-value of <0.05 were subjected to feature reduction using the least absolute shrinkage and selection operator (LASSO) regression method to prevent model overfitting (15). To predict PFS, three ML algorithms commonly used in medical imaging research, specifically random forest, naive Bayes, and neural network, were employed using both TFs and selected clinicopathological parameters. ML models were developed using the Orange data mining toolbox (version 3.34.0).

The dataset was randomly divided into training (70% of patients) and testing (30% of patients) sets. The mean ages of patients with and without progression were compared using the independent-samples t-test. Categorical variables, such as estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2) status, and cancer stage were compared between these groups using the chi-square test.

All data were normalized to a 0–1 scale prior to model training. Each ML model was trained on the training set using 10-fold cross-validation and subsequently evaluated on the testing set for internal validation. After this initial evaluation, selected clinical parameters were added to the models, and their predictive performance for achieving PFS was re-examined.

## Results

The mean age of the patients was 59±14 years. The median follow-up period was 112 (30–311) weeks. During follow-up, progression occurred in 21 patients (19%) within the first year, 43 (38%) within three years, and 46 (41%) within five years. One-, three-, and five-year PFS rates were 81%, 62%, and 59%, respectively. The five-year PFS rate was 88% in non-metastatic patients and 47% in metastatic patients. The majority of patients had invasive ductal carcinoma. Most cases were ER (+), PR (+), and HER2 (-) and had locally advanced breast cancer (LABC) (Table 1) (16).

The median primary tumor size was larger in patients who did not achieve one-, three-, and five-year PFS compared to those who did. ER (+) and PR (+) rates were higher among patients who achieved three- and five-year PFS than in those who did not. HER2 receptor status was similar between patients with and without progression at all time points. Lastly, distant and axillary metastases at diagnosis were more common in patients who did not achieve one-, three-, and five-year PFS (Tables 2, 3, and 4).

A total of 25, 58, and 57 of the TFs showed significant differences between patients who achieved one-, three-, and five-year PFS, respectively, and those who did not. These TFs were subjected to LASSO regression. Selected TFs (Figure 3) and relevant clinicopathological parameters that differed between the two patient groups (primary tumor size, ER and PR status, and axillary and distant metastases) were then used in ML models to predict PFS at one, three, and five-years.

The higher incidence of distant metastases among patients who did not achieve PFS at one, three, and five years suggested that the lower rates of surgery and radiotherapy in these patients were a consequence

rather than a cause of their poor prognosis. Therefore, the history of surgery and radiotherapy was not included in the ML models. Among the models using only TFs, random forest predicted one-, three-, and five-year PFS with area under the curve (AUC) values of 0.730, 0.758, and 0.797, respectively. Naive Bayes predicted one-, three-, and five-year PFS with AUC values of 0.857, 0.804, and 0.843, respectively. The neural network predicted one-, three-, and five-year PFS with AUC values of 0.782, 0.828, and 0.780, respectively. AUC values improved when clinicopathological parameters were added to the TFs (Figure 4 and Tables 5, 6, and 7).

**Table 1. Detailed characteristics of patients**

| Variables | | |
|---|---|---|
| **Age (years), mean ± SD** | 59±14 | |
| **Primary tumor size (mm), median (minimum-maximum)** | 28 (13–100) | |
| **Histopathological subtype, n (%)** | **IDC** | 64 (57) |
| | **ILC** | 4 (3) |
| | **NST** | 30 (27) |
| | **Others** | 14 (13) |
| **ER status, n (%)** | **ER (+)** | 79 (71) |
| | **ER (-)** | 27 (24) |
| | **N/A** | 6 (5) |
| **PR status, n (%)** | **PR (+)** | 73 (65) |
| | **PR (-)** | 33 (30) |
| | **N/A** | 6 (5) |
| **HER2 status, n (%)** | **HER2 (+)** | 33 (30) |
| | **HER2 (-)** | 73 (65) |
| | **N/A** | 6 (5) |
| **TNM stage, n (%)** | **Early *** | 33 (30) |
| | **Locally advanced**** | 49 (44) |
| | **Distant metastatic** | 30 (26) |
| **Surgery history, n (%)** | **No surgery** | 32 (29) |
| | **Mastectomy** | 56 (50) |
| | **Breast-conserving surgery** | 24 (21) |
| **Chemotherapy, n (%)** | **Yes** | 87 (78) |
| | **No** | 25 (22) |
| **Radiotherapy, n (%)** | **Yes** | 70 (63) |
| | **No** | 42 (37) |
| **Hormonal therapy, n (%)** | **Yes** | 79 (74) |
| | **No** | 27 (21) |
| | **N/A** | 6 (5) |
| **Anti-HER2 therapy, n (%)** | **Yes** | 33 (30) |
| | **No** | 73 (65) |
| | **N/A** | 6 (5) |

SD: Standard deviation; IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; NST: No special type; ER: Estrogen receptor; PR: Progesterone receptor; N/A: Not available; HER2: Human epidermal growth factor receptor 2; TNM: Tumor, node, and metastasis

*: Includes TNM stages I, IIA, IIB, and IIIA

**: Includes TNM stages IIIB and IIIC

**Table 2. Patient characteristics according to one-year PFS**

| Variables | Patients who achieved one-year PFS (*n* = 91) | Patients who did not achieve one-year PFS (*n* = 21) | *p*-value |
|---|---|---|---|
| **Age, mean ± SD** | 58±13 | 62±14 | 0.253 |
| **Primary tumor size (mm), median (minimum-maximum)** | 25 (13–80) | 40 (18–100) | **0.005** |
| **Histopathological subtype, n (%)** | | | |
| IDC | 55 (60) | 9 (43) | 0.222 |
| Others | 36 (40) | 12 (57) | |
| **ER status, n (%)** | | | |
| ER (+) | 67 (74) | 6 (29) | |
| ER (-) | 21 (23) | 12 (57) | 0.095 |
| N/A | 3 (3) | 3 (14) | |
| **PR status, n (%)** | | | |
| PR (+) | 62 (68) | 11 (53) | |
| PR (-) | 26 (29) | 7 (33) | 0.099 |
| N/A | 3 (3) | 3 (14) | |
| **HER2 status, n (%)** | | | |
| HER2 (+) | 27 (30) | 6 (29) | |
| HER2 (-) | 61 (67) | 12 (57) | 0.825 |
| N/A | 3 (3) | 3 (14) | |
| **Axillary metastasis, n (%)** | | | |
| Present | 53 (58) | 19 (90) | **0.005** |
| Absent | 38 (42) | 2 (10) | |
| **TNM stage, n (%)** | | | |
| Early* | 32 (35) | 1 (5) | |
| Locally advanced** | 44 (48) | 7 (33) | **<0.001** |
| Distant metastatic | 15 (17) | 13 (62) | |
| **Surgery history, n (%)** | | | |
| Yes | 75 (66) | 5 (4) | **<0.001** |
| No | 16 (15) | 16 (15) | |
| **Chemotherapy, n (%)** | | | |
| Yes | 68 (61) | 19 (17) | 0.152 |
| No | 23 (20) | 2 (2) | |
| **Radiotherapy, n (%)** | | | |
| Yes | 62 (54) | 8 (7) | **0.012** |
| No | 28 (26) | 14 (13) | |

PFS: Progression-free survival; SD: Standard deviation; IDC: Invasive ductal carcinoma; ER: Estrogen receptor; N/A: Not available; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; TNM: Tumor, node, and metastasis

*: Includes TNM stages I, IIA, IIB, and IIIA

**: Includes TNM stages IIIB and IIIC

**Table 3. Patient characteristics according to three-year PFS**

| Variables | Patients who achieved three-year PFS (*n* = 69) | Patients who did not achieve three-year PFS (*n* = 43) | *p*-value |
|---|---|---|---|
| **Age, mean ± SD** | 57±13 | 60±14 | 0.373 |
| **Primary tumor size (mm), median (minimum-maximum)** | 25 (13–62) | 39 (18–100) | **<0.001** |
| **Histopathological subtype, n (%)** | | | |
| IDC | 42 (61) | 22 (51) | 0.380 |
| Others | 27 (39) | 21 (49) | |
| **ER status, n (%)** | | | |
| ER (+) | 54 (79) | 25 (58) | |
| ER (-) | 14 (20) | 13 (30) | **0.021** |
| N/A | 1 (1) | 5 (12) | |
| **PR status, n (%)** | | | |
| PR (+) | 51 (74) | 22 (51) | |
| PR (-) | 17 (25) | 16 (37) | 0.013 |
| N/A | 1 (1) | 5 (12) | |
| **HER2 status, n (%)** | | | |
| HER2 (+) | 21 (31) | 12 (28) | |
| HER2 (-) | 47 (68) | 26 (60) | 0.941 |
| N/A | 1 (1) | 5 (12) | |
| **Axillary metastasis, n (%)** | | | |
| Present | 35 (51) | 37 (86) | **<0.001** |
| Absent | 34 (49) | 6 (14) | |
| **TNM stage, n (%)** | | | |
| Early* | 29 (42) | 4 (9) | |
| Locally advanced** | 36 (52) | 15 (35) | **<0.001** |
| Distant metastatic | 4 (6) | 24 (56) | |
| **Surgery history, n (%)** | | | |
| Yes | 64 (57) | 16 (14) | **<0.001** |
| No | 5 (4) | 27 (25) | |
| **Chemotherapy, n (%)** | | | |
| Yes | 52 (47) | 35 (31) | 0.494 |
| No | 17 (15) | 8 (7) | |
| **Radiotherapy, n (%)** | | | |
| Yes | 48 (44) | 22 (20) | **0.045** |
| No | 21 (18) | 21 (18) | |

PFS: Progression-free survival; SD: Standard deviation; IDC: Invasive ductal carcinoma; ER: Estrogen receptor; N/A: Not available; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2, TNM: Tumor, node, and metastasis

*: Includes TNM stages I, IIA, IIB, and IIIA

**: Includes TNM stages IIIB and IIIC

**Table 4. Patient characteristics according to five-year PFS**

| Variables | Patients who achieved five-year PFS (*n* = 66) | Patients who did not achieve five-year PFS (*n* = 46) | *p*-value |
|---|---|---|---|
| **Age, mean ± SD** | 58±13 | 60±13 | 0.475 |
| **Primary tumor size (mm), median (minimum-maximum)** | 25 (13–62) | 38 (15–100) | **<0.001** |
| **Histopathological subtype, n (%)** | | | |
| IDC | 40 (60) | 24 (52) | 0.285 |
| Others | 26 (40) | 22 (48) | |
| **ER status, n (%)** | | | |
| ER (+) | 51 (78) | 28 (61) | |
| ER (-) | 14 (21) | 13 (28) | **0.046** |
| N/A | 1 (1) | 5 (11) | |
| **PR status, n (%)** | | | |
| PR (+) | 48 (73) | 25 (54) | |
| PR (-) | 17 (26) | 16 (35) | **0.037** |
| N/A | 1 (1) | 5 (11) | |
| **HER2 status, n (%)** | | | |
| HER2 (+) | 20 (30) | 13 (28) | |
| HER2 (-) | 45 (69) | 28 (61) | 0.919 |
| N/A | 1 (1) | 5 (11) | |
| **Axillary metastasis, n (%)** | | | |
| Present | 32 (48) | 40 (87) | **<0.001** |
| Absent | 34 (52) | 6 (13) | |
| **TNM stage, n (%)** | | | |
| Early* | 29 (44) | 4 (9) | |
| Locally advanced** | 34 (51) | 17 (37) | **<0.001** |
| Distant metastatic | 3 (5) | 25 (54) | |
| **Surgery history, n (%)** | | | |
| Yes | 61 (54) | 19 (17) | **<0.001** |
| No | 5 (4) | 27 (25) | |
| **Chemotherapy, n (%)** | | | |
| Yes | 49 (44) | 38 (34) | 0.360 |
| No | 17 (15) | 8 (7) | |
| **Radiotherapy, n (%)** | | | |
| Yes | 48 (44) | 22 (20) | **0.045** |
| No | 21 (18) | 21 (18) | |

PFS: Progression-free survival; SD: Standard deviation; IDC: Invasive ductal carcinoma; ER: Estrogen receptor; N/A: Not available; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; TNM: Tumor, node, metastasis

*: Includes TNM stages I, IIA, IIB, and IIIA

**: Includes TNM stages IIIB and IIIC

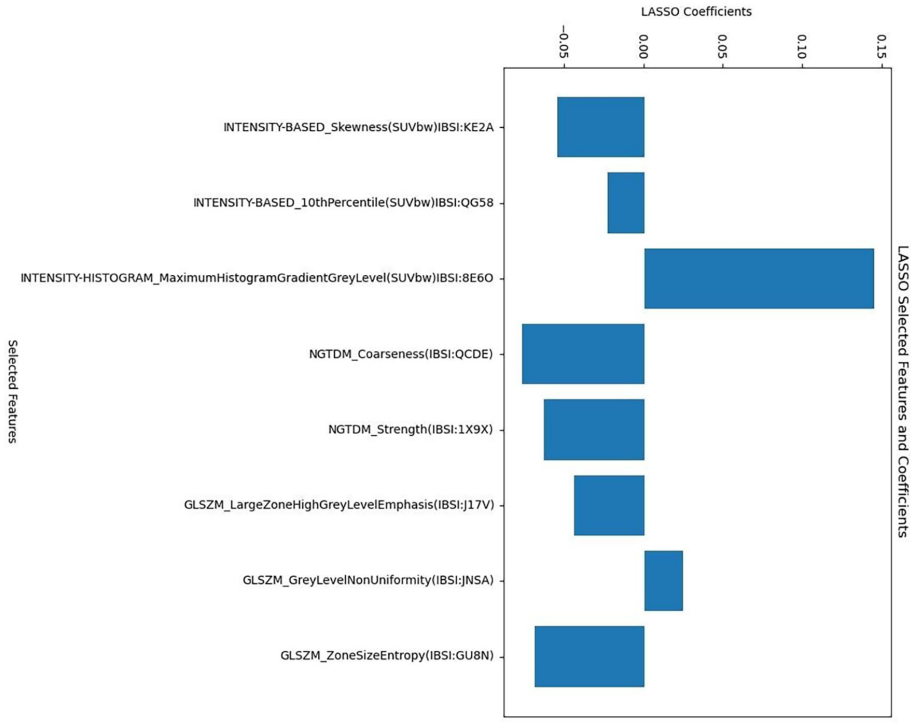



**Figure 3.** LASSO regression coefficients of the features used to predict disease progression

*LASSO: Least absolute shrinkage and selection operator*

**Table 5. Performance of different machine learning methods in predicting one-year progression in the testing set**

| | Using texture features alone | | | | | | Combining texture features with clinicopathological parameters | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | AUC | Accuracy (%) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | AUC | Accuracy (%) |
| Random forest | 90 | 69 | 84 | 55 | 0.739 | 90 | 90 | 69 | 83 | 80 | 0.923 | 83 |
| Naive Bayes | 83 | 75 | 93 | 52 | 0.857 | 81 | 82 | 83 | 89 | 73 | 0.907 | 83 |
| Neural network | 90 | 44 | 86 | 54 | 0.782 | 81 | 84 | 59 | 78 | 68 | 0.867 | 75 |

PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under the curve

**Table 6. Performance of different machine learning methods in predicting three-year progression in the testing set**

| | Using texture features alone | | | | | | Combining texture features with clinicopathological parameters | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | AUC | Accuracy (%) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | AUC | Accuracy (%) |
| Random forest | 82 | 46 | 74 | 59 | 0.758 | 70 | 86 | 75 | 86 | 75 | 0.933 | 83 |
| Naive Bayes | 78 | 61 | 78 | 61 | 0.804 | 72 | 80 | 82 | 89 | 70 | 0.917 | 81 |
| Neural network | 82 | 64 | 81 | 67 | 0.828 | 76 | 80 | 61 | 79 | 63 | 0.898 | 73 |

PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under the curve

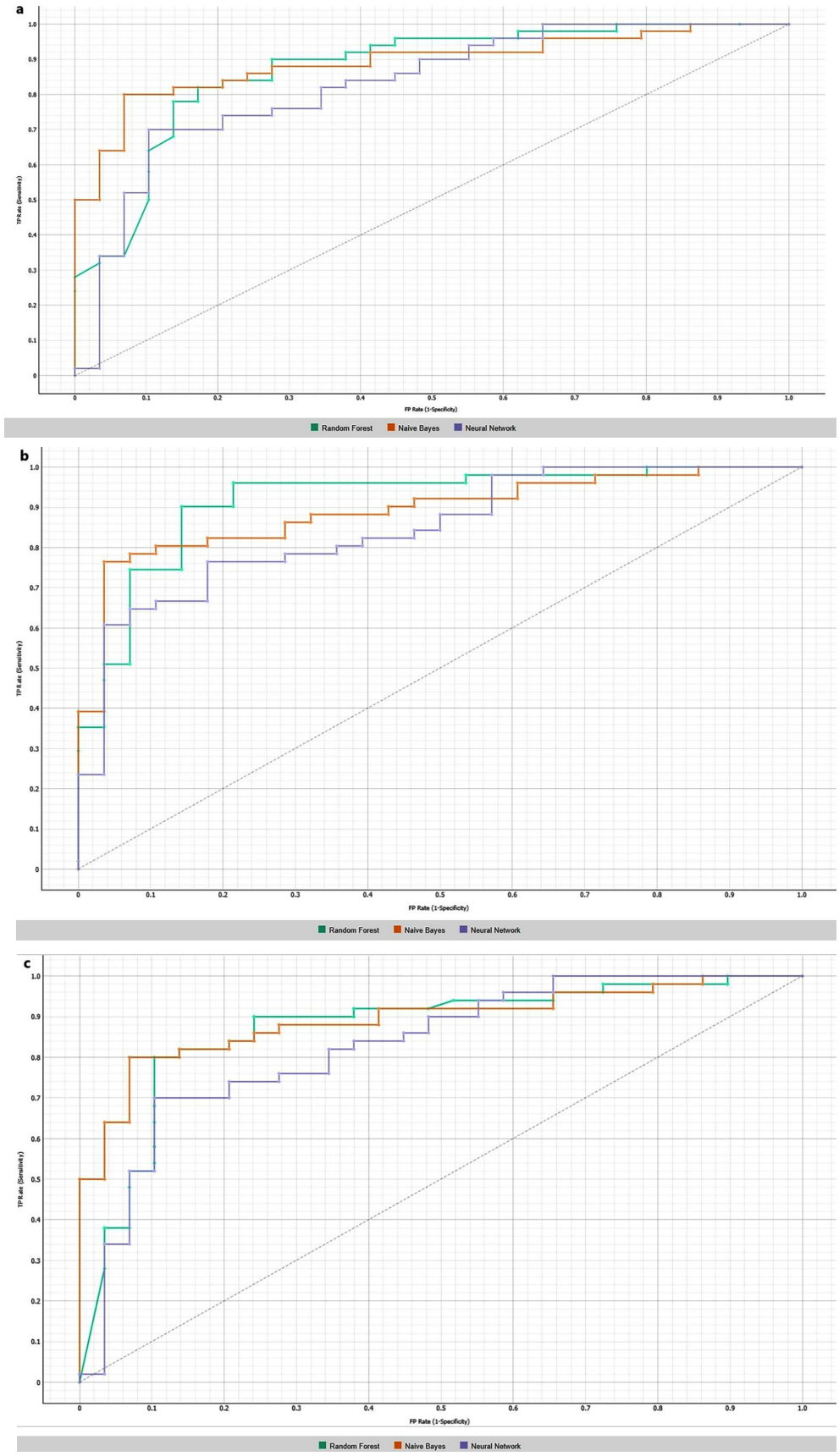



**Figure 4.** Receiver-operating characteristic curves of models incorporating texture features and clinicopathological parameters for predicting **(a)** one-year, **(b)** three-year, and **(c)** five-year progression-free survival

**Table 7. Performance of different machine learning methods in predicting five-year progression in the testing set**

| | Using texture features alone | | | | | | Combining texture features with clinicopathological parameters | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | AUC | Accuracy (%) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | AUC | Accuracy (%) |
| Random forest | 78 | 62 | 78 | 62 | 0.797 | 72 | 90 | 69 | 83 | 80 | 0.870 | 82 |
| Naive Bayes | 84 | 72 | 84 | 75 | 0.843 | 81 | 82 | 83 | 89 | 73 | 0.907 | 83 |
| Neural network | 82 | 66 | 80 | 68 | 0.780 | 76 | 84 | 59 | 78 | 68 | 0.867 | 75 |

PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under the curve

## Discussion and Conclusion

It is well established that conventional PET/CT parameters provide valuable prognostic information in breast cancer. Qu et al. (6) followed 125 patients with breast cancer for five years and demonstrated that higher $SUV_{max}$, metabolic tumor volume, and total lesion glycolysis values measured from the primary lesion were associated with increased rates of local recurrence and/or distant metastasis (8). Similarly, in a meta-analysis, Diao et al. (17) reported that higher $SUV_{max}$ values in the primary tumor were associated with an elevated risk of recurrence or progression but $SUV_{max}$ had no significant effect on overall survival (OS).

PET/CT TA combined with ML has been used to predict PFS or OS in various malignancies (18-21). TA reflects tumor heterogeneity, which is influenced by multiple factors beyond a single tumor characteristic, including tumor microenvironment, grade, genetic profile, and immunohistochemical expression. Given that the smallest volumetric unit in imaging is a voxel, TA essentially analyzes how neighboring voxels relate to each other, which may reveal underlying prognostic features of the tumor. Nevertheless, despite its potential, TA is not yet widely adopted in routine clinical practice. Existing studies, most of which are retrospective, suggest that TA could help identify patients at high or low risk of recurrence or metastasis. However, results from prospective studies with standardized methodologies are still necessary. Previous research has also examined the relationship between PET/CT TA and survival in breast cancer (10, 22, 23), although most investigations have focused on the association between TA and histological or immunohistochemical parameters or on predicting the response to neoadjuvant therapy (9, 11, 24).

In the current study, we focused on the relationship between PET-derived TFs and PFS in patients with breast cancer. Among the 148 TFs listed in Supplementary Table 1, many showed statistically significant differences between patients who achieved one-, three-, and five-year PFS and those who did not. ML models incorporating TFs and clinicopathological parameters successfully predicted one-, three-, and five-year PFS. Xu et al. (25) also attempted to predict PFS in breast cancer using TFs and clinical parameters and showed that the model combining TFs and clinical data outperformed models that used TFs or clinical variables alone. Importantly, their model remained successful in an external validation group. In our study, the addition of clinicopathological parameters to ML models similarly improved their predictive performance. Notably, model specificity increased, which significantly contributed to performance enhancement. However, we did not conduct external validation.

Yoon et al. (22) found that values above the threshold value for high-intensity zone emphasis and high-intensity short-zone emphasis among PET/CT-derived TFs were associated with shorter PFS in patients with LABC. However, that study did not investigate the effects of clinicopathological prognostic parameters or use ML, and it had a shorter median follow-up (17.3 months) than our study (112 weeks). In contrast, our study predicted PFS using both TFs alone and combined with clinicopathological parameters through ML.

In a prospective study investigating PET/CT-derived TFs in patients with LABC, TFs were associated with more aggressive tumor phenotypes. Cox regression analysis showed that certain features could predict longer DFS and OS (10). However, this study, like Yoon et al. (22), did not use ML or assess the effects of clinicopathological prognostic variables. In another study examining the relationship between PET/CT-derived TFs and clinicopathological parameters in patients with ER (+) and HER2 (-) breast cancer, high entropy values were linked to shorter event-free survival (23). The authors evaluated the effects of only two TFs (entropy and homogeneity) on event-free survival and did not use ML. TFs offer a mathematical representation of tumor heterogeneity via imaging. Given that heterogeneity may result in treatment resistance or failure, TA could reasonably be expected to predict outcomes such as PFS, supported by both the previous study and the present one.

Zheng et al. (26) predicted DFS in patients who did not achieve pathological complete response after neoadjuvant chemotherapy by integrating clinical, radiomic, and deep learning features. Their combined model outperformed those based on single feature sets, with AUC values of 0.889 and 0.938 for three- and five-year DFS, respectively. Similarly, our study demonstrated that combining TFs with clinicopathological parameters improved the prediction of PFS compared to using either alone.

Classical prognostic factors in breast cancer, such as tumor size, axillary lymph node metastasis, tumor, node, and metastasis stage, histopathological subtype, and hormone receptor status, have long been validated in the literature (27). Although our study primarily focused on imaging features, we observed that larger primary tumor size, presence of axillary and distant metastases, and ER (-) and PR (-) status were associated with disease progression.

### Study Limitations

Our study has several limitations. First, it was a retrospective, single-center study with a relatively small sample size. This limited our ability to analyze specific subgroups, such as patients with a particular histopathological subtype or hormone receptor profile (e.g., triple-negative). Second, for technical reasons, patients with primary tumors having a metabolic volume of less than 64 voxels on PET were excluded; therefore, our findings may not be generalizable to tumors with a low metabolic volume. Third, the median follow-up period may have been insufficient, as breast cancer can recur even five to 10 years after treatment. Fourth, mammography and/or breast US were used to detect local recurrence during follow-up, with breast MRI reserved for equivocal cases. Lastly, the ML models were trained on 70% and tested on 30% of the data from the same patient cohort. While internal validation was performed, external datasets were not available for independent validation due to the single-center nature of the study.

ML models incorporating PET/CT-derived TFs and clinicopathological parameters may assist in predicting progression during the pre-treatment period in patients with invasive breast carcinoma. Predicting disease progression may allow clinicians to manage neoadjuvant and adjuvant treatment more effectively for patients who are at high risk of disease progression. If technical challenges, such as harmonizing PET/CT images from different centers and standardizing segmentation methods, can be resolved, TA may then be integrated into routine PET/CT workflows.

### Ethics

**Ethics Committee Approval:** The Recep Tayyip Erdogan University Ethics Committee approved this study (approval no: 2022/228, date: 22.12.2022).

**Informed Consent:** The ethical committee waived the requirement for informed consent as the study was retrospective.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: O.B., H.M.B., S.G.; Concept: O.B., H.M.B., S.G.; Design: O.B., H.M.B., S.G.; Data Collection or Processing: O.B.; Analysis or Interpretation: O.B., H.M.B., S.G.; Literature Search: O.B., H.M.B., S.G.; Writing: O.B.

**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. 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]
2. Endo K, Oriuchi N, Higuchi T, Iida Y, Hanaoka H, Miyakubo M, et al. PET and PET/CT using 18F-FDG in the diagnosis and management of cancer patients. Int J Clin Oncol. 2006; 11: 286-296. (PMID: 16937302) [Crossref]
3. Groheux D, Cochet A, Humbert O, Alberini JL, Hindié E, Mankoff D. $^{18}$F-FDG PET/CT for staging and restaging of breast cancer. J Nucl Med. 2016; 57(Suppl 1): 17S-26S. (PMID: 26834096) [Crossref]
4. Çelik B, Boge M, Dilege E. Does F-18 FDG-PET/CT have an additional impact on axillary approach in early-stage breast cancer? Eur J Breast Health. 2023; 20: 45-51. (PMID: 38187104) [Crossref]
5. Aktaş A, Gürleyik MG, Aydın Aksu S, Aker F, Güngör S. Diagnostic value of axillary ultrasound, MRI, and 18F-FDG-PET/ CT in determining axillary lymph node status in breast cancer patients. Eur J Breast Health. 2021; 18: 37-47. (PMID: 35059590) [Crossref]
6. Qu YH, Long N, Ran C, Sun J. The correlation of 18F-FDG PET/CT metabolic parameters, clinicopathological factors, and prognosis in breast cancer. Clin Transl Oncol. 2021; 23: 620-627. (PMID: 32683540) [Crossref]
7. Lambin P, Leijenaar RTH, Deist TM, Peerlings J, de Jong EEC, van Timmeren J, et al. Radiomics: the bridge between medical imaging and personalized medicine. Nat Rev Clin Oncol. 2017; 14: 749-762. (PMID: 28975929) [Crossref]
8. Chicklore S, Goh V, Siddique M, Roy A, Marsden PK, Cook GJ. Quantifying tumour heterogeneity in 18F-FDG PET/CT imaging by texture analysis. Eur J Nucl Med Mol Imaging. 2013; 40: 133-140. (PMID: 23064544) [Crossref]
9. Sollini M, Cozzi L, Ninatti G, Antunovic L, Cavinato L, Chiti A, et al. PET/CT radiomics in breast cancer: mind the step. Methods. 2021; 188: 122-132. (PMID: 31978538) [Crossref]
10. Molina-García D, García-Vicente AM, Pérez-Beteta J, Amo-Salas M, Martínez-González A, Tello-Galán MJ, et al. Intratumoral heterogeneity in 18F-FDG PET/CT by textural analysis in breast cancer as a predictive and prognostic subrogate. Ann Nucl Med. 2018; 32: 379-388. (PMID: 29869770) [Crossref]
11. Li P, Wang X, Xu C, Liu C, Zheng C, Fulham MJ, et al. 18F-FDG PET/CT radiomic predictors of pathologic complete response (pCR) to neoadjuvant chemotherapy in breast cancer patients. Eur J Nucl Med Mol Imaging. 2020; 47: 1116-1126. (PMID: 31982990) [Crossref]
12. Bouron C, Mathie C, Seegers V, Morel O, Jézéquel P, Lasla H, et al. Prognostic value of metabolic, volumetric and textural parameters of baseline [18F]FDG PET/CT in early triple-negative breast cancer. Cancers (Basel). 2022; 14: 637. (PMID: 35158904) [Crossref]
13. Nioche C, Orlhac F, Boughdad S, Reuzé S, Goya-Outi J, Robert C, et al. LIFEx: a freeware for radiomic feature calculation in multimodality imaging to accelerate advances in the characterization of tumor heterogeneity. Cancer Res. 2018; 78: 4786-4789. (PMID: 29959149) [Crossref]
14. Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. J Nucl Med. 2009; 50(Suppl 1): 122S-150S. (PMID: 19403881) [Crossref]
15. Tibshiranit R. Regression shrinkage and selection via the lasso. J R Statist Soc B. 1996; 58: 267-288. [Crossref]
16. Aebi S, Karlsson P, Wapnir IL. Locally advanced breast cancer. Breast. 2022; 62(Suppl 1): S58-S62. (PMID: 34930650) [Crossref]
17. Diao W, Tian F, Jia Z. The prognostic value of SUVmax measuring on primary lesion and ALN by 18F-FDG PET or PET/CT in patients with breast cancer. Eur J Radiol. 2018; 105: 1-7. (PMID: 30017264) [Crossref]
18. Hotta M, Minamimoto R, Gohda Y, Miwa K, Otani K, Kiyomatsu T, et al. Prognostic value of 18F-FDG PET/CT with texture analysis in patients with rectal cancer treated by surgery. Ann Nucl Med. 2021; 35: 843-852. (PMID: 33948903) [Crossref]
19. Marr L, Haller B, Pyka T, Peeken JC, Jesinghaus M, Scheidhauer, et al. Predictive value of clinical and 18F-FDG-PET/CT derived imaging parameters in patients undergoing neoadjuvant chemoradiation for esophageal squamous cell carcinoma. Sci Rep 2022; 12: 1-9. [Crossref]
20. Morvan L, Carlier T, Jamet B, Bailly C, Bodet-Milin C, Moreau P, et al. Leveraging RSF and PET images for prognosis of multiple myeloma at diagnosis. Int J Comput Assist Radiol Surg. 2020; 15: 129-139. (PMID: 31256359) [Crossref]

21. Liu W, Sun X, Qi Y, Jia X, Huang Y, Liu N, et al. Integrated texture parameter of 18F-FDG PET may be a stratification factor for the survival of nonoperative patients with locally advanced non-small-cell lung cancer. Nucl Med Commun. 2018; 39: 732-740. (PMID: 30001264) [Crossref]

22. Yoon HJ, Kim Y, Chung J, Kim BS. Predicting neo-adjuvant chemotherapy response and progression-free survival of locally advanced breast cancer using textural features of intratumoral heterogeneity on F-18 FDG PET/CT and diffusion-weighted MR imaging. Breast J. 2019; 25: 373-380. (PMID: 29602210) [Crossref]

23. Groheux D, Martineau A, Teixeira L, Espié M, de Cremoux P, Bertheau P, et al. 18FDG-PET/CT for predicting the outcome in ER+/HER2- breast cancer patients: comparison of clinicopathological parameters and PET image-derived indices including tumor texture analysis. Breast Cancer Res. 2017; 19: 3. (PMID: 28057031) [Crossref]

24. Moscoso A, Ruibal Á, Domínguez-Prado I, Fernández-Ferreiro A, Herranz M, Albaina L, et al. Texture analysis of high-resolution dedicated breast 18 F-FDG PET images correlates with immunohistochemical factors and subtype of breast cancer. Eur J Nucl Med Mol Imaging. 2018; 45: 196-206. (PMID: 28936601) [Crossref]

25. Xu X, Sun X, Ma L, Zhang H, Ji W, Xia X, et al. 18F-FDG PET/CT radiomics signature and clinical parameters predict progression-free survival in breast cancer patients: a preliminary study. Front Oncol. 2023; 13: 1149791. (PMID: 36969043) [Crossref]

26. Zheng X, Huang Y, Lin Y, Zhu T, Zou J, Wang S, et al. 18F-FDG PET/CT-based deep learning radiomics predicts 5-years disease-free survival after failure to achieve pathologic complete response to neoadjuvant chemotherapy in breast cancer. EJNMMI Res. 2023; 13: 105. (PMID: 38052965) [Crossref]

27. Subramaniam DS, Isaacs C. Utilizing prognostic and predictive factors in breast cancer. Curr Treat Options Oncol. 2005; 6: 147-159. (PMID: 15717996) [Crossref]

**Supplementary Table 1. First order and second order PET texture features that extracted from the three-dimensional range of interest**

| First order texture features | Name of the texture feature |
|---|---|
| Morphological | Volume, approximate volume, voxels counting, surface area, surface to volume ratio, compacity, compactness 1, compactness 2, spherical disproportion, sphericity, aspehicirty, max value coordinates, center of mass, weighted center of mass, hoc max, hoc max normalized with radius ROI, hoc max normalized with radius sphere, hocpeak 0.5 mL, hocpeak 0.5 mL normalized with radius ROI, hocpeak 0.5 mL normalized with radius sphere, hocpeak 1 mL, hocpeak 1 mL normalized with radius ROI, hocpeak 1 mL normalized with radius sphere, centre of mass shift, centre of mass shiftmax normalized with radius ROI, centre of mass shiftmax normalized with radius sphere, maximum 3d diameter, sphere diameter, integrated intensity. |
| Intensity-based | Mean, variance, skewness, kurtosis, median, minimum gray level, 10$^{th}$ percentile, 25$^{th}$ percentile, 50$^{th}$ percentile, 75$^{th}$ percentile, 90$^{th}$ percentile, standard deviation, maximum grey level, interquartile range, range, mean absolute deviation, robust mean absolute deviation, median absolute deviation, coefficient of variation, quartile coefficient of dispersion, are under curve csh, energy, root mean square, total lesion glycolisis, |
| Local intensity-based | Intensity peak discretized volume sought (0.5 mL), global intensity peak (0.5 mL), intensity peak discretized volume sought (1 mL), global intensity peak (1 mL), local intensity peak. |
| Intensity-histogram | Intensity histogram mean, intensity histogram variance, intensity histogram skewness, intensity histogram kurtosis, intensity histogram median, intensity histogram minimum grey level, intensity histogram 10$^{th}$ percentile, intensity histogram 25$^{th}$ percentile, intensity histogram 50$^{th}$ percentile, intensity histogram 75$^{th}$ percentile, intensity histogram 90$^{th}$ percentile, intensity histogram standard deviation, intensity histogram maximum grey level, intensity histogram mode, intensity histogram interquartile range, intensity histogram range, intensity histogram mean absolute deviation, intensity histogram robust mean absolute deviation, intensity histogram median absolute deviation, intensity histogram coefficien of variation, intensity histogram quartile coefficien dispersion, intensity histogram entropy log10, intensity histogram entropy log2, area under curve csh, uniformity, root mean square, maximum histogram gradient, maximum histogram gradient grey level, minimum histogram gradient, minimum histogram gradient grey level. |
| Local intensity histogram | Intensity peak discretized volume sought (0.5 mL), global intensity peak (0.5 mL), intensity peak discretized volume sought (1 mL), global intensity peak (1 mL), local intensity peak. |
| **Second order texture features** | |
| Intensity-based rim | Min, mean, stdev, max, counting voxels, approximate volume, sum. |
| Intensity histogram rim | Min, mean, stdev, max, counting voxels, approximate volume, sum. |
| Gray-level co-occurrence matrix (GLCM) | Joint maximum, joint average, joint variance, joint entropy log2, joint entropy log10, difference average, difference variance, difference entropy, sum average, sum variance, sum entropy, angular second moment, contrast, dissimilarity, inverse difference, normalized inverse difference, inverse difference moment, normalized inverse difference moment, inverse variance, correlation, autocorrelation, cluster tendency, sluster shade, cluster prominence. |
| Neighboring gray tone difference matrix (NGTDM) | Coarseness, contrast, busyness, complexity, strength. |
| Gray-level run-length matrix (GLRM) | Short runs emphasis, long runs emphasis, low grey level run emphasis, high grey level run emphasis, short run low grey level emphasis, short run high grey level emphasis, long run low grey level emphasis, long run high grey level emphasis, grey level nonuniformity, run length nonuniformity, run percentage. |
| Gray-level size zone matrix (GLSZM) | Small zone emphasis, large zone emphasis, low grey level zone emphasis, high grey level zone emphasis, small zone low grey level emphasis, small zone high grey level emphasis, large zone low grey level emphasis, large zone high grey level emphasis. |

PET: Positron emission tomography; ROI: Region of interest

**Original Article**

Eur J Breast Health 2025; 21(4): 367-374
**DOI:** 10.4274/ejbh.galenos.2025.2025-7-1

# Hemochromatosis: A Risk Factor for Breast Cancer? Systematic Review and Meta-Analysis

Megane Buttignol[1], Caroline Bouche[1], Manon Chrétien[2], Nicolas Taris[2], Tolga Ozmen[3], Carole Mathelin[1,4,5]

[1]Institut de Cancérologie Strasbourg Europe (ICANS), Strasbourg, France
[2]Unité d'oncogénétique Clinique, ICANS, Strasbourg, France
[3]Massachusetts General Hospital, Department of Surgery, Division of Gastrointestinal and Oncologic Surgery, Massachusetts, USA
[4]National Academy of Surgery, Paris, France
[5]Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France

## ABSTRACT

**Objective:** Hereditary hemochromatosis and breast cancer are two major public health problems. The *HFE* gene variants C282Y and H63D, responsible for most cases of hemochromatosis, may contribute to carcinogenesis via iron overload, oxidative stress, and hormonal modulation. The aim of this study was to evaluate the association between *HFE* variants and breast cancer risk and propose a personalized surveillance strategy.

**Materials and Methods:** A systematic review and a meta-analysis were conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Eligible studies included case-control and cohort studies reporting breast cancer incidence in women with *HFE* gene C282Y and/or H63D variants. Data were pooled using a random-effects model. Subgroup analyses and meta-regressions explored sources of heterogeneity.

**Results:** Eight studies comprising 73,981 participants were included, published between 2000 and 2025. Among them, analysis of four revealed a link between hemochromatosis and breast cancer risk. In one study, a link was observed between the *HFE* C282Y allele and higher lymph node involvement, which may suggest an impact of hemochromatosis on tumor progression. By contrast, three studies did not find any link between the two diseases. Our meta-analysis showed a trend toward increased breast cancer risk in carriers of *HFE* variants, particularly C282Y homozygotes (odds ratio = 1.36, 95% confidence interval = 0.75–1.98). Substantial heterogeneity was present ($I^2$ >50%), but no tested covariates significantly explained this variation. Sensitivity analyses confirmed the robustness of the estimate.

**Conclusion:** In the absence of randomized trials with mortality endpoints, our findings do not yet justify changes in clinical practice. They nevertheless support prospective studies to assess whether women carrying these pathogenic variants, especially C282Y/C282Y homozygotes, could benefit from adapted breast cancer surveillance, potentially involving more frequent evaluations or advanced imaging to improve early detection.

**Keywords:** Breast cancer; genetic predisposition; hemochromatosis; iron overload; meta-analysis

**Cite this article as:** Buttignol M, Bouche C, Chrétien M, Taris N, Ozmen T, Mathelin C. Hemochromatosis: a risk factor for breast cancer? Systematic review and meta-analysis. Eur J Breast Health. 2025; 21(4): 367-374

## Key Points

- Hereditary hemochromatosis is a common genetic disorder in individuals of European descent, and its main pathogenic variants, C282Y and H63D, may contribute to carcinogenesis through iron overload and oxidative stress.
- This meta-analysis included eight studies and over 53,000 participants to assess the association between *HFE* gene mutations and breast cancer risk.
- Although the pooled analysis did not show a statistically significant association, a consistent trend toward increased breast cancer risk, particularly in C282Y homozygotes, was observed.
- The heterogeneity between studies could not be explained by genotype, zygosity, publication year, or methodological quality, but sensitivity analyses confirmed the robustness of the findings.
- An intensified screening protocols for individuals carrying *HFE* pathogenic variants is proposed, particularly homozygous C282Y carriers. Pending further data, we propose risk-adapted screening for these individuals, which may include more frequent clinical evaluations and imaging, potentially incorporating additional modalities such as ultrasound or breast magnetic resonance imaging.

**Corresponding Author:**
Carole Mathelin MD; c.mathelin@icans.eu

**Received:** 04.07.2025
**Accepted:** 13.08.2025
**Epub:** 05.09.2025
**Available Online Date:** 25.09.2025

## Introduction

Breast cancer and hereditary hemochromatosis are both relatively common diseases, especially among individuals of European ancestry. Emerging evidence suggests a potential association between the two, with iron overload implicated as a possible contributing factor in breast cancer development. Breast cancer is the most common cancer in women with an estimated 2,296,840 new cases worldwide in 2022. It also remains the leading cause of cancer-related death in women, accounting for roughly 666,103 deaths every year (1). Breast cancer incidence and mortality regularly rise, with over 3 million new cases and more than a million deaths expected by 2040. Hereditary hemochromatosis is one of the most common genetic diseases in populations of Northern European origin, with an estimated homozygous prevalence of between 1/200 and 1/400 (2).

Hereditary hemochromatosis is an autosomal recessive genetic disorder due to excessive iron absorption, resulting in a toxic accumulation of iron in the organs, particularly in the liver, heart, pancreas and joints (3). The most common form of hereditary hemochromatosis is associated with biallelic pathogenic variations of the *HFE* gene located on chromosome 6, especially the C282Y and H63D pathogenic variants (type 1), particularly in Northern Europeans with around 1 in 200 homozygous for C282Y, and 1 in 10 heterozygous for C282Y, but other genes are also associated to a minor extent, such as *HJV* or *HAMP* (type 2) in juvenile forms, *TfR2* (type 3) and *FPN* (type 4). The common characteristic of these pathogenic variants is that they interfere with the signal system responsible for hepcidin synthesis (2, 4), leading to increased intestinal iron absorption. Excess iron is then stored as ferritin in tissues, resulting in progressive toxicity, particularly in vital organs (3).

Diagnosis is based on elevated ferritin and transferrin saturation coefficient >45%, combined with a screening for homozygous or compound heterozygous pathogenic variants of the *HFE* gene, in particular C282Y and H63D (2, 5). Hereditary haemochromatosis is a disease with low clinical penetrance. Despite the frequency of pathogenic variants, only a minority of patients develop symptomatic hemochromatosis, with around 10–30% of homozygous men developing a significant overload. The rate is less significant in women due to menstrual loss and pregnancy.

Targeted screening is recommended, with systematic screening of first-degree relatives if a family member is homozygous for C282Y or has clinical haemochromatosis, with a genetic test carried out immediately in addition to the ferritin and transferrin saturation coefficient assays (6-8). Systematic screening of the general population is not justified, due to low clinical penetrance, the risk of overdiagnosis and anxiety in healthy carriers, and the presence of a simple and effective treatment.

The main treatment for hemochromatosis consists of regular phlebotomies to reduce iron levels. Other approaches include the use of iron chelators in certain cases, particularly in patients who cannot undergo frequent phlebotomies (9).

Iron is indispensable, yet overload is carcinogenic. Excess, particularly the readily absorbed heme iron in red meat, drives oxidative stress, free-radical DNA damage and higher serum-ferritin levels, all linked to increased cancer risk (10). In breast tissue, iron synergizes with estrogens metabolites to generate reactive species, a process intensified in post-menopause when iron stores rise (11, 12). Moreover, hemochromatosis often coexists with diabetes and obesity, that are both additional breast cancer risk factors. Hemochromatosis is known to increase the risk of liver carcinomas and other cancers. However, to date, scientific data remain contradictory in terms of breast carcinoma (11).

The objective of this literature review and meta-analysis was to assess the link between hemochromatosis and breast cancer and to propose a personalized surveillance for these patients.

## Materials and Methods

This review was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (prismastatement.org).

### Study Identification and Selection

Eligible publications were retrieved by searching for the PubMed database, published between 2000 and 2025, using the terms "hemochromatosis", "*HFE* pathogenic variants" and "breast cancer".

### Inclusion Criteria and Exclusion Criteria

Eligible studies were included in this review according to following criteria: publications that evaluated the association between *HFE* gene pathogenic variants and cancer risk; these publications were designed as prospective and retrospective cohort studies, case-control studies, meta-analysis and systematic reviews.

Studies were excluded when they were a case-only study, case-report, or abstract; without sufficient data; and publications concerning animals.

Only articles published in English or French were considered.

We have included a flow chart to describe the publications selection process (Figure 1). Previously two published meta-analyses were not included in our meta-analysis to avoid potential data duplication.

A meta-analysis was conducted to estimate the association between hereditary hemochromatosis-related pathogenic variants (notably C282Y and H63D) and breast cancer risk. Effect sizes extracted from the included studies were primarily expressed as odds ratios (OR) with 95% confidence intervals (CIs). The primary analysis used a random-effects model to account for between-study heterogeneity and to compute a pooled OR with a corresponding 95% CI.

Heterogeneity was assessed using Cochran's Q statistic and the $I^2$ index. In cases of substantial heterogeneity ($I^2$ >75%), subgroup analyses were conducted according to pathogenic variant**s** type (C282Y, H63D, or combined) and zygosity (homozygous, heterozygous, compound heterozygous). Meta-regressions weighted by the inverse of the variance were performed to evaluate the effect of potential moderators (year of publication, pathogenic variants type, zygosity, and methodological quality). A leave-one-out sensitivity analysis was performed to assess the robustness of the pooled estimate.

### Statistical Analysis

Potential publication bias was explored using visual inspection of a funnel plot and formally tested using Egger's regression test. Statistical analyses were performed using the software JASP (version 0.19.3 for Apple Silicon, www.jasp-stats.org). Assistance with the construction of data tables, supplementary calculations, and the generation of figures (annotated forest plots, meta-regressions, sensitivity analysis, Egger's test) was provided by ChatGPT (OpenAI, GPT-4) under supervised use, without automation of methodological decisions or unvalidated interpretations.

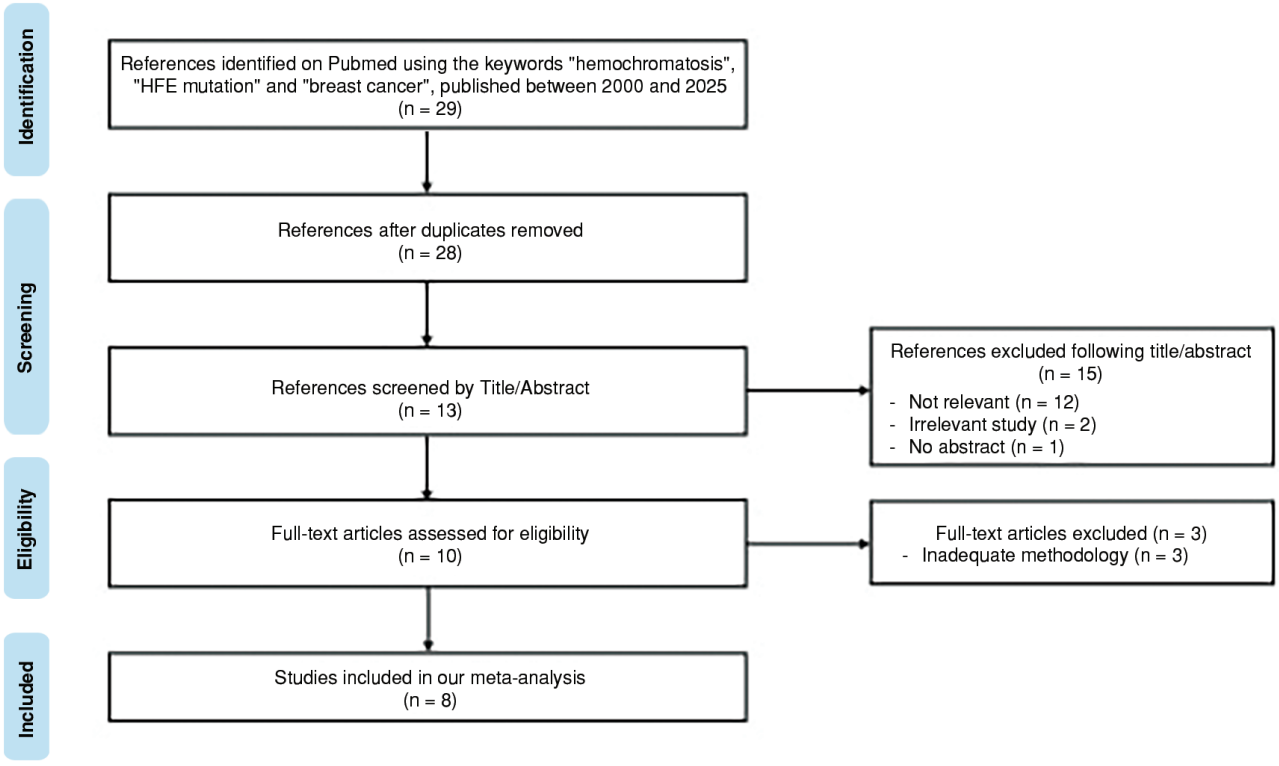



**Figure 1.** Flow chart showing the studies selection

## Results

The various studies included in the meta-analysis are summarized in Table 1.

### 1. Studies Reporting No Significant Association Between Hemochromatosis and Breast Cancer Risk

A multicenter retrospective cohort study was conducted in eight university hospitals in Sweden from 1997 to 2017 with 3,645 persons carrying homozygous or compound heterozygous *HFE* pathogenic variants, matched by age, sex and country of residence to 36,423 population-based reference individuals. This study showed no significant difference compared to reference individuals for the risk for breast cancer [hazard ratio (HR) = 1.08, 95% CI = 0.73–1.60] (12).

Similarly, a prospective European study of 451,143 participants from the UK Biobank, aged 40 to 70 years, over an average duration of 11.6 years, evaluated the risks of non-hepatic cancers in carriers of *HFE* pathogenic variants. The study found an increased risk of prostate cancer in men homozygous for C282Y pathogenic variants, but no increased risk for other types of cancer, including breast cancer in women for either the *HFE* pathogenic variant C282Y and/or H63D (H63D+/+ HR = 1.09 95% CI = 0.96-1.25, $p$ = 0.19; C282Y+/+ HR = 0.90, 95% CI = 0.69–1.18, $p$ = 0.45; C282Y+/H63D+ HR = 0.99, 95% CI = 0.87–1.14, $p$ = 0.93) (13).

This is also the case for a Brazilian case-control study, which evaluated 68 patients with operable breast cancer, with a mean age of 54.2 years, compared with a control population of 85 women with no family history of cancer and no use of hormonal therapies. There was no association between H63D and C282Y pathogenic variants in the *HFE* gene and breast cancer risk (C282Y+/+ OR = 0.34, 95% CI = 0.14–1.91; H63D+/+: OR = 0.53, 95% CI = 0.40–1.58) (14).

A German study involving 688 women under the age of 80 years, of Caucasian origin, and all diagnosed with breast cancer within the previous six months, analyzed 19 polymorphisms in genes involved in iron metabolism, including the *HFE* gene. The results showed no significant differences in allele or genotype frequencies between breast cancer patients and controls, suggesting that these variants do not have a direct effect on breast cancer incidence (C282Y +/- OR = 0.92, 95% CI = 0.65–1.37; H63D +/- OR = 0.87, 95% CI = 0.67–1.13; H63D +/+ OR = 0.79, 95% CI = 0.37–1.71). However, a possible association was observed between the *HFE* C282Y allele and higher lymph node involvement in patients, which may suggest a link with tumor progression. This observation, however, was limited by the small sample size (15).

### 2. Studies Reporting a Significant Association Between hemochromatosis and Breast Cancer Risk

In contrast to the studies described above, Kallianpur et al. (16) found a strong association between C282Y pathogenic variants in the *HFE* gene and an increased risk of breast cancer, with significant public health implications, particularly for hemochromatosis genetic screening and breast cancer prevention. They compared the frequency of C282Y pathogenic variants in a population of 168 patients who underwent chemotherapy or blood cell transplants for cancer treatment between 1995 and 1998 at the Vanderbilt University Medical Center in Tennessee. The study compared breast cancer patients with those treated for non-breast cancers, including hematologic cancers, as well as a sample of cancer-free individuals from a Tennessee clinic and national population data. The frequency of at least one C282Y allele in breast cancer cases was higher (36.6%, 5 homozygotes/10 heterozygotes) than in the Tennessee clinic population (12.7%, $p$<0.001), the general population (12.4%, $p$<0.001), and similarly selected non-breast cancer cases (17.0%,

**Table 1. Studies evaluating the association between *HFE* gene mutations and breast cancer risk**

| | Author (reference) | Study type (*n* = 73,981) | Year | Average age (years) | Country | *HFE* mutation | Results |
|---|---|---|---|---|---|---|---|
| *No link between HC and breast cancer* | Abraham et al. (15) | Case-control (*n* = 1,412) | 2005 | 58.7 | Germany | C282Y, H63D | C282Y: OR = 0.92, 95% CI = 0.65–1.37 H63D: OR = 0.79, 95% CI = 0.37–1.71 |
| | Batschauer et al. (14) | Case-control (*n* = 153) | 2011 | 54.12 | Brazil | C282Y, H63D | C282Y: OR = 0.34, 95% CI = 0.14–1.91<br>H63D: OR = 0.53, 95% CI = 0.40–1.58 |
| | Hagström et al. (12) | Retrospective cohort (*n* = 40,057) | 2021 | 52.6 | Sweden | C282Y, H63D | HR = 1.08, 95% CI = 0.73–1.60 |
| | Atkins et al. (13) | Prospective cohort (*n* = 9,238) | 2022 | 56.8 | United-Kingdom | C282Y, H63D | H63D: HR = 1.09, 95% CI = 0.96–1.25<br>C282Y: HR = 0.90, 95% CI = 0.69–1.18<br>C282Y/H63D: HR = 0.99, 95% CI = 0.87–1.14 |
| *Link between HC and breast cancer* | Kallianpur et al. (16) | Case-control (*n* = 5,510) | 2004 | 53.0 | United States of America | C282Y | OR = 2.55, 95% CI = 1.35–4.81 |
| | Gunel-Ozcan et al. (19) | Case-control (*n* = 188) | 2006 | 41.0 | Türkiye | H63D | HR = 2.05, 95% CI = 1.12–3.75 |
| | Kondrashova et al. (20) | Case-control (*n* = 360) | 2006 | 53.2 | Russia | H63D | HR = 4.4, 95% CI = 1.4–14.1 |
| | Osborne et al. (17) | Prospective cohort (*n* = 17,063) | 2010 | 48.3 | Australia | C282Y | C282Y: HR = 2.39, 95% CI = 1.24–4.61 |

HC: Hemochromatosis; HR: Hazard ratio; OR: Odds ratio; CI: Confidence interval

$p$ = 0.008). The probability of developing breast cancer increased with the number of C282Y alleles ($p$ = 0.010), and serum iron analysis confirmed higher levels in breast cancer patients carrying these pathogenic variants (16).

Similarly, a prospective cohort study using data from the Melbourne Collaborative Cohort Study followed 28,509 participants aged between 27 and 75 years, enrolled from 1990 to 1994, for an average of 14 years to examine the link between pathogenic C282Y in *HFE* and cancer risk, particularly for breast, colon, and prostate cancer. The study demonstrated a significant 2.39-fold increase in breast cancer risk among individuals homozygous for C282Y pathogenic variant (HR = 2.39, 95% CI 1.24–4.61, $p$ = 0.01). However, compound heterozygous C282Y/H63D individuals did not show an increased breast cancer risk (HR = 1.16, 95% CI = 0.74–1.84) (17).

A meta-analysis published in 2016, including 36 studies with 87,028 participants (13,680 cancer cases and 73,348 controls), also found a trend towards an approximately two-fold increased breast cancer risk in patients with C282Y homozygous pathogenic variant of *HFE* (OR = 2.14, 95% CI 1.24–3.70, $p$ = 0.673). However, no increased breast cancer risk was demonstrated in patients with H63D pathogenic variants of the *HFE* gene (18), contrary to Gunel-Ozcan et al. (19), who found a significant difference in a retrospective study comparing the frequency of C282Y and H36D pathogenic variants in the *HFE* gene among Turkish women with breast cancer and healthy controls, suggesting that H63D pathogenic variants might be associated with a two-fold increased breast cancer risk (HR = 2.05, 95% CI 1.12–3.75, $p$ = 0.02).

Similarly, another case-control study was conducted to determine the frequency of C282Y and H63D pathogenic variants in the *HFE* gene in Russian women with hormone-dependent cancers, including breast, ovarian and endometrial cancer. There was a significant increase in the risk of breast cancer in women over 57 years with heterozygous or homozygous H63D pathogenic variants (HR = 4.4, 95% CI = 1.4–14.1, $p$ = 0.002). This association was not found for C282Y pathogenic variants (20).

A meta-analysis was conducted by Zhang et al. (21), including 20 studies in total published between 1999 and 2005, of which seven assessed the risk of breast cancer in patients with C282Y pathogenic variants of the *HFE* gene, with a total of 2,353 cases and 19,171 controls, as well as five studies concerning the risk of breast cancer in patients with H63D pathogenic variants of the *HFE* gene, with a total of 1,570 cases and 2,449 controls. An association was found only in patients who were homozygous for the C282Y pathogenic variant

(OR = 1.76, 95% CI = 1.05–2.94, $p$ = 0 .425), suggesting an increased risk of breast cancer in this population. No association was found between H63D pathogenic variants in *HFE* and an increased risk of breast cancer (21).

### 3. The Present Meta-Analysis

We conducted a meta-analysis to investigate the association between hereditary hemochromatosis-related pathogenic variants (primarily C282Y and H63D) and the risk of breast cancer. This included eight studies with a total of 73,981 patients. Our meta-analysis was carried out with two additional recent studies, dating from 2021 and 2022, with non-negligible sample sizes, which the meta-analyses by Lv et al. (18) and Zhang et al. (21) did not provide.

#### a. Pooled Effect

The random-effects model estimated a pooled OR of 1.36 (95% CI = 0.75–1.98) for breast cancer among carriers of hemochromatosis pathogenic variants. Although the point estimate suggested an increased risk, between-study heterogeneity was substantial (Q = 88.4, df = 11, $p$<0.001; $\tau^2$ = 0.96).

#### b. Subgroup Analyses

**• By Mutation**

Subgroup analysis by mutation type (C282Y, H63D, combination) did not reveal significant differences between groups (F = 0.183, $p$ = 0.835). The type of mutation did not explain the observed heterogeneity.

**• By Zygosity**

Similarly, zygosity (homozygous vs. heterozygous/compound heterozygous) was not a significant moderator (F = 0.009, $p$ = 0.927).

**• By Design and Quality**

Meta-regression incorporating study design (cohort vs. other) and methodological quality (high vs. low) showed no significant effect. Design (cohort); β = +0.06 (log OR), $p$ = 0.69; Quality (high); β = -0.43 (log OR), $p$ = 0.19. These variables did not account for heterogeneity (Adjusted $R^2$ = –0.005).

#### c. Meta-Regression

Additional meta-regression assessed the effects of mutation (C282Y), zygosity (homozygous), and year of publication. C282Y mutation; β = -0.10 (log OR), $p$ = 0.55; Homozygosity; β = +0.10 (log OR), $p$ = 0.53; year of publication; β = -0.006 (log OR), $p$ = 0.58. None of these factors significantly influenced effect size variability (adjusted $R^2$ = -0.19).

#### d. Publication Bias

Funnel plot inspection revealed no evident asymmetry. Egger's regression intercept was not significant ($p$ = 0.41), suggesting no publication bias.

#### e. Sensitivity Analysis

Leave-one-out sensitivity analysis showed that no single study significantly influenced the pooled estimate. The OR remained stable across exclusions (Figure 2).

## Discussion and Conclusion

The international literature reveals contrasting results regarding the association between hemochromatosis and breast cancer. On the one hand, some studies, such as those by Hagström et al. (12) and the UK Biobank (13), showed no significant increase in the risk of breast cancer in individuals who were homozygous for C282Y pathogenic variants in the *HFE* gene. In contrast, some studies such as those by Kallianpur et al. (16) and Osborne et al. (17) indicated a significant link between these pathogenic variants and an increased risk of developing breast cancer. Several methodological and biological factors may explain these discrepancies. Firstly, differences in study design, sample size and length of follow-up considerably influence the results. For example, the study by Kallianpur et al. (16) is based on a smaller cohort. In contrast, large-scale studies such as the UK Biobank study (13) benefit from greater statistical power but may not capture specific sub-populations at risk, such as menopausal women.

The variable clinical penetrance of hereditary hemochromatosis associated with *HFE* pathogenic variants, particularly C282Y homozygosity, has important implications for patient monitoring in the context of cancer risk. Although only 10% to 33% of individuals with this genotype develop clinically manifest hereditary hemochromatosis, a significantly larger proportion exhibit elevated biochemical markers of iron overload, including increased serum ferritin and transferrin saturation. This discrepancy between biochemical and clinical expression highlights the need for an individualized follow-up strategy, rather than a genotype-based approach alone. Compound heterozygotes and H63D homozygotes typically present with mild elevations in iron indices, but these are generally not associated with progressive iron-related organ damage unless additional risk factors are present (22).

From a pathophysiological point of view, the mechanisms linking excess iron to breast carcinogenesis remain complex and yet incompletely elucidated. The role of oxidative stress induced by excess iron, implicated in DNA damage and genomic instability, is an argument in favor of a carcinogenic influence. In addition, the interaction between iron and estrogens suggested by Marques et al (23) and Wyllie and Liehr (24) could explain why this risk is more marked in post-menopausal women, where the increase in iron stocks coincides with alterations in hormonal metabolism. Finally, contradictory results are also emerging concerning the involvement of the H63D pathogenic variant in the *HFE* gene. While the meta-analysis by Lv et al. (18) shows no significant association, the study by Gunel-Ozcan et al. (19) suggested an increased risk of breast cancer in women with these pathogenic variants. These disparities highlight the importance of continuing investigations with more specific studies incorporating more parameters, such as menopausal status, environmental factors and metabolic co-morbidities.

Monitoring iron status in *HFE* pathogenic variant carriers is particularly relevant in oncology, as emerging evidence suggests a link between iron excess and tumor progression. High circulating iron levels and transferrin saturation have been associated with increased risk of distant metastasis in breast cancer, possibly via promotion of oxidative stress, immune evasion, and pro-metastatic niche formation. In a retrospective monocentric study conducted at the University Hospitals of Leuven, De Troy et al. (25) examined the relationship between iron metabolism markers at the time of early-stage breast cancer diagnosis and the risk of developing distant metastases. Among

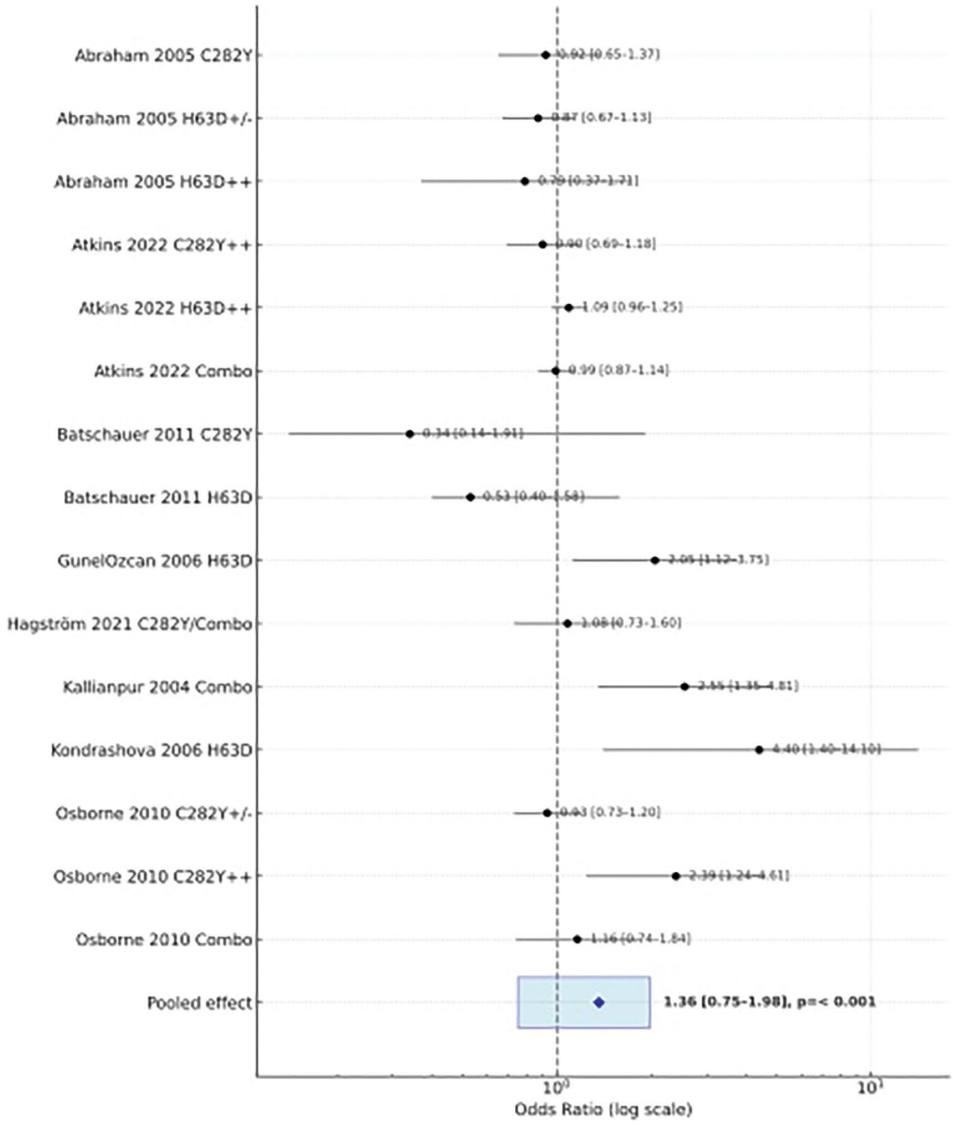



**Figure 2.** Meta-analysis of the association between hereditary hemochromatosis-related pathogenic variants and breast cancer risk

1,113 women with unilateral, unifocal breast cancer and available iron profiles (serum iron, ferritin, and transferrin saturation) measured within six weeks of diagnosis, 10% developed secondary metastases during a median follow-up of seven years. Multivariate Cox regression analysis showed that a 10% increase in transferrin saturation was associated with a 19% increase in metastatic risk (HR = 1.19, 95% CI = 1.02–1.38), and a 10 µg/dL increase in serum iron was associated with a 6% increase in metastatic risk (HR = 1.06, 95% CI = 1.01–1.12). In contrast, ferritin levels were not significantly associated with the occurrence of metastases. No associations were found between iron status and the metastatic site or tumor molecular subtype. The findings suggest that elevated circulating iron and iron saturation at diagnosis may contribute to the formation of a pro-metastatic microenvironment and support the potential benefit of targeting iron metabolism, for example through iron chelation or ferroptosis induction, as a therapeutic strategy in breast cancer.

In this context, iron overload may act not only as a metabolic comorbidity but also as a modifier of cancer behavior. Environmental and physiological factors, such as age, sex, alcohol intake, metabolic syndrome, viral hepatitis, and menopausal status, significantly influence the progression to clinical iron overload and may synergize with cancer-related pathways. Furthermore, secondary genetic modifiers (e.g., *HAMP*, *HJV*, or *BMP* variants), high dietary heme iron intake, or the use of acid suppression therapy can further modulate iron burden and therapeutic requirements (22).

Although several studies have investigated the association between *HFE* pathogenic variants and breast cancer risk, none have specifically reported on the histological subtypes of breast cancer in women with hereditary hemochromatosis. The current literature predominantly focuses on genetic associations, iron metabolism, and overall cancer incidence, without detailing tumor morphology or receptor status. Furthermore, while some studies suggest an increased prevalence of breast cancer in *HFE* pathogenic variant carriers, particularly those with H63D or C282Y variants, data regarding the age of onset remain limited. Notably, one study conducted in a Russian population found that the breast cancer risk associated with the H63D pathogenic variant increased significantly in women over the age of 57 years (20).

This meta-analysis offers several significant strengths compared to previous investigations on the association between hereditary

hemochromatosis and breast cancer risk. First, all included studies are recent, published in the last 20 years. Second, it integrates data from a geographically and ethnically diverse set of populations, including studies from Europe (Germany, Sweden, UK, Russia, Türkiye), North and South America (USA, Brazil), and Australia. This diversity enhances the generalizability of the findings across different genetic backgrounds and environmental contexts. Third, the included studies allow for stratified analysis by specific *HFE* genotypes (C282Y homozygotes, H63D carriers, compound heterozygotes), enabling a more nuanced assessment of genotype-specific cancer risks, particularly in women. This meta-analysis incorporates data from large-scale, prospective cohort studies such as the UK Biobank and the Melbourne Collaborative Cohort Study, which provide high-quality, population-based evidence with long-term follow-up. Moreover, age- and sex-stratified data enable the evaluation of potential effect modifiers, such as menopausal status, which may influence the penetrance of *HFE* mutations.

Our meta-analysis indicated a potential association between C282Y and H63D pathogenic variants in *HFE* and an increased risk of breast cancer. Although this trend did not reach statistical significance, the observed signal justifies further investigation through large-scale studies with reduced heterogeneity to better determine this relationship and potentially warrant adjusting breast surveillance in carriers. Indeed, the UK Age Trial, a randomized study of 160,921 women, showed that annual mammography from ages 40 to 48 years of age reduced breast cancer mortality by 25% in the first 10 years (risk ratio = 0.75, $p$ = 0.029), with one death prevented per 1000 women screened. No long-term increase in overdiagnosis or other mortality was observed (26).

Breast cancer and hereditary hemochromatosis are two major public health problems. While the present meta-analysis did not demonstrate a significant association between *HFE* pathogenic variants, especially C282Y and H63D, and breast cancer risk, a consistent trend toward increased risk, especially among C282Y homozygotes, was observed.

Given the absence of randomized trials with mortality endpoints, our findings cannot justify changes in clinical practice at this stage. However, they provide a rationale for future prospective studies to assess whether women carrying these pathogenic variants, especially C282Y/C282Y homozygotes, might benefit from adapted breast cancer surveillance strategies. Such research could help determine whether personalized screening approaches, potentially incorporating more frequent evaluations or advanced imaging modalities, would improve early detection in this probable genetically predisposed population.

## Ethics

**Ethics Committee Approval:** This meta-analysis is based on data extracted from previously published studies. No new human or animal subjects were involved, and no ethical approval was required.

**Informed Consent:** This meta-analysis is based on data extracted from previously published studies. No new human or animal subjects were involved, and no ethical approval was required.

## Footnotes

### Authorship Contributions

Surgical and Medical Practices: M.B., C.B., M.C., N.T., T.O., C.M.; Concept: M.B., C.M.; Design: M.B., C.M.; Data Collection or Processing: M.B.; Analysis or Interpretation M.B., C.B.; Literature Search: M.B., M.C., N.T.; Writing: M.B.

**Conflict of Interest:** Carole Mathelin MD is section editor in European Journal of Breast Health. She had no involvement in the peer-review of this article and had no access to information regarding its peer-review.

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

## References

1. Data visualization tools for exploring the global cancer burden in 2022. [Internet]. 2022. Available from: https://gco.iarc.fr/today/data/factscheets/cancers/20-Breast-fact-sheet.pdf [Crossref]
2. Brissot P, Pietrangelo A, Adams PC, de Graaff B, McLaren CE, Loréal O. Haemochromatosis. Nat Rev Dis Primers. 2018; 4: 18016. (PMID: 2962005) [Crossref]
3. Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med. 2008; 358: 221-230. (PMID: 18199861) [Crossref]
4. Pietrangelo A. Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. Gastroenterology. 2010; 139: 393-408, 408.e1-e2. (PMID: 20542038) [Crossref]
5. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996; 13: 399-408. (PMID: 8696333) [Crossref]
6. Bacon BR, Adams PC, Kowdley KV, Powell LW, Tavill AS; American Association for the Study of Liver Diseases. Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. Hepatology. 2011; 54: 328-343. (PMID: 21452290) [Crossref]
7. European Association For The Study Of The Liver. EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol. 2010; 53: 3-22. (PMID: 20471131) [Crossref]
8. Kowdley KV, Brown KE, Ahn J, Sundaram V. ACG Clinical Guideline: hereditary hemochromatosis. Am J Gastroenterol. 2019; 114: 1202-1218. Erratum in: Am J Gastroenterol. 2019; 114: 1927. (PMID: 31335359) [Crossref]
9. Kawabata H. The mechanisms of systemic iron homeostasis and etiology, diagnosis, and treatment of hereditary hemochromatosis. Int J Hematol. 2018; 107: 31-43. (PMID: 29134618) [Crossref]
10. Fonseca-Nunes A, Jakszyn P, Agudo A. Iron and cancer risk--a systematic review and meta-analysis of the epidemiological evidence. Cancer Epidemiol Biomarkers Prev. 2014; 23: 12-31. (PMID: 24243555) [Crossref]
11. Crownover BK, Covey CJ. Hereditary hemochromatosis. Am Fam Physician. 2013; 87: 183-190. (PMID: 23418762) [Crossref]
12. Hagström H, Ndegwa N, Jalmeus M, Ekstedt M, Posserud I, Rorsman F, et al; Swedish Hepatology Study Group (SweHep). Morbidity, risk of cancer and mortality in 3645 HFE mutations carriers. Liver Int. 2021; 41: 545-553. (PMID: 33450138) [Crossref]
13. Atkins JL, Pilling LC, Torti SV, Torti FM, Kuchel GA, Melzer D. Hereditary hemochromatosis variant associations with incident nonliver malignancies: 11-year follow-up in UK biobank. Cancer Epidemiol Biomarkers Prev. 2022; 31: 1780-1787. (PMID: 35709753) [Crossref]
14. Batschauer AP, Cruz NG, Oliveira VC, Coelho FF, Santos IR, Alves MT, et al. HFE, MTHFR, and FGFR4 genes polymorphisms and breast cancer in Brazilian women. Mol Cell Biochem. 2011; 357: 247-253. (PMID: 21625954) [Crossref]
15. Abraham BK, Justenhoven C, Pesch B, Harth V, Weirich G, Baisch C, et al; GENICA Network. Investigation of genetic variants of genes of the hemochromatosis pathway and their role in breast cancer. Cancer

Epidemiol Biomarkers Prev. 2005; 14: 1102-1107. (PMID: 15894659) [Crossref]

16. Kallianpur AR, Hall LD, Yadav M, Christman BW, Dittus RS, Haines JL, et al. Increased prevalence of the HFE C282Y hemochromatosis allele in women with breast cancer. Cancer Epidemiol Biomarkers Prev. 2004; 13: 205-212. (PMID: 14973098) [Crossref]
17. Osborne NJ, Gurrin LC, Allen KJ, Constantine CC, Delatycki MB, McLaren CE, et al. HFE C282Y homozygotes are at increased risk of breast and colorectal cancer. Hepatology. 2010; 51: 1311-1318. (PMID: 20099304) [Crossref]
18. Lv YF, Chang X, Hua RX, Yan GN, Meng G, Liao XY, et al. The risk of new-onset cancer associated with HFE C282Y and H63D mutations: evidence from 87,028 participants. J Cell Mol Med. 2016; 20: 1219-1233. (PMID: 26893171) [Crossref]
19. Gunel-Ozcan A, Alyilmaz-Bekmez S, Guler EN, Guc D. HFE H63D mutation frequency shows an increase in Turkish women with breast cancer. BMC Cancer. 2006; 6: 37. (PMID: 16503999) [Crossref]
20. Kondrashova TV, Neriishi K, Ban S, Ivanova TI, Krikunova LI, Shentereva NI, et al. Frequency of hemochromatosis gene (HFE) mutations in Russian healthy women and patients with estrogen-dependent cancers. Biochim Biophys Acta. 2006; 1762: 59-65. (PMID: 16216474) [Crossref]
21. Zhang M, Xiong H, Fang L, Lu W, Wu X, Wang YQ, et al. Meta-analysis of the association between H63D and C282Y polymorphisms in HFE and cancer risk. Asian Pac J Cancer Prev. 2015; 16: 4633-4639. (PMID: 26107216) [Crossref]
22. Barton JC, Parker CJ. HFE-related hemochromatosis. 2000 Apr 3 [updated 2024 Apr 11]. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2025. (PMID: 20301613) [Crossref]
23. Marques O, da Silva BM, Porto G, Lopes C. Iron homeostasis in breast cancer. Cancer Lett. 2014; 347: 1-14. (PMID: 24486738) [Crossref]
24. Wyllie S, Liehr JG. Release of iron from ferritin storage by redox cycling of stilbene and steroid estrogen metabolites: a mechanism of induction of free radical damage by estrogen. Arch Biochem Biophys. 1997; 346: 180-186. (PMID: 9343364) [Crossref]
25. De Troy J, Fendt SM, Hatse S, Neven P, Smeets A, Laenen A, et al. Plasma iron levels at early breast cancer diagnosis are associated with development of secondary metastases: a single-center retrospective cohort study. Breast Cancer (Auckl). 2025; 19: 11782234251317070. (PMID: 39927294) [Crossref]
26. Duffy SW, Vulkan D, Cuckle H, Parmar D, Sheikh S, Smith RA, et al. Effect of mammographic screening from age 40 years on breast cancer mortality (UK Age trial): final results of a randomised, controlled trial. Lancet Oncol. 2020; 21: 1165-1172. (PMID: 32800099) [Crossref]

Case Report

Eur J Breast Health 2025; 21(4): 375-378
DOI: 10.4274/ejbh.galenos.2025.2025-3-8

# Pseudoangiomatous Hyperplasia of Mammary Stroma: Insights from Two Cases, Data Update and Management Algorithm

Caroline Bouche[1,2], Thibaut Wolf [3], Mégane Buttignol[1,2], Carole Mathelin[1,2]

[1]Department of Surgery, Institut de Cancérologie Strasbourg Europe (ICANS), Strasbourg, France
[2]CHRU, Strasbourg University Hospitals, 1 place de l'Hôpital, Strasbourg, France
[3]Department of Pathology, University Hospital, Strasbourg, France

**ABSTRACT**

Pseudoangiomatous stromal hyperplasia (PASH) is a benign breast lesion frequently discovered incidentally during imaging or biopsy for other conditions. We present two cases of PASH associated with fibroadenomas in premenopausal women, both presenting as palpable, symptomatic breast masses. In the first case, a 26-year-old woman exhibited a 5.2 cm hypoechoic lesion, initially diagnosed as PASH on core biopsy, later confirmed as fibroadenoma with PASH components post-excision. The second case involved a 37-year-old woman with a painful 5.6 cm mass, diagnosed similarly via biopsy, and later confirmed as fibroadenoma fully colonized by PASH after surgical removal. Both cases highlight the diagnostic challenge in distinguishing PASH from fibroadenomas, given overlapping clinical and imaging features. Hormonal factors, particularly contraceptive use, may contribute to PASH development. Management remains controversial, with surgery indicated for symptomatic lesions, while conservative approaches may suffice for smaller, asymptomatic cases. Based on our findings and current literature, we propose a management algorithm to guide clinicians in differentiating cases warranting surgical intervention from those suitable for monitoring. Further studies are needed to validate this approach.

**Keywords:** Breast lesion; hormonal influence; pseudoangiomatous stromal hyperplasia; surgical management

**Cite this article as:** Bouche C, Wolf T, Buttignol M, Mathelin C. Pseudoangiomatous hyperplasia of mammary stroma: insights from two cases, data update and management algorithm. Eur J Breast Health. 2025; 21(4): 375-378

**Key Points**

- Pseudoangiomatous stromal hyperplasia (PASH) often coexists with other lesion, complicating preoperative diagnosis.
- Hormone therapy appears to influence PASH development.
- Symptomatic PASH lesions typically require surgical excision, while conservative management may be appropriate for asymptomatic cases.
- We propose an algorithm to optimize PASH management and avoid unnecessary surgeries.

## Introduction

Pseudoangiomatous stromal hyperplasia (PASH) is a benign breast condition identified in approximately 6% of biopsies performed for other benign lesions (1). First described by Vuitch et al. (2) in 1986, PASH is predominantly detected incidentally (3). A 23% incidence was reported in a series of 200 consecutive breast biopsies for benign and malignant lesions (4).

Histologically, PASH consists of a benign proliferation of myofibroblasts arranged in slit-like spaces mimicking vascular channels within the interlobular and intralobular connective tissue. PASH predominantly affects premenopausal women and is often associated with estrogen and progesterone receptor positivity (95%) (1, 3).

Immunohistochemistry typically shows positivity for fibroblast markers ($CD34^+$) and negativity for endothelial markers ($CD31^-$) (3).

Currently, there are no specific management guidelines for PASH. It can present as a microscopic finding, a palpable nodule, or in association with another lesion (4).

A retrospective study of 66 PASH cases observed progression, defined as an increase in lesion volume, in 16.6% of cases after a median follow-up of 26 months (5). PASH has been reported alongside other benign or malignant lesions, including apocrine metaplasia, fibroadenoma, hamartoma, intraductal papilloma, atypical ductal hyperplasia, and lobular carcinoma *in situ*, occurring in 1–26% of cases (5). Another study of 70 cases of PASH found that 60.4% were associated with

**Corresponding Author:**
Carole Mathelin MD; c.mathelin@icans.eu

**Received:** 28.04.2025
**Accepted:** 08.06.2025
**Epub:** 04.08.2025
**Available Online Date:** 25.09.2025

benign epithelial lesions, 25.6% with atypical hyperplasia, and 11% ($n$ = 9/79) with infiltrating carcinoma ($p$<0.001), including one case of non-invasive ductal carcinoma *in situ* (6).

Interestingly, Degnim et al. (1) found that women with PASH had a lower breast cancer risk compared to women without PASH ($p$ = 0.01), in a large cohort with a mean follow-up of 18.5 years. Esmer et al. (7) similarly reported no cases of malignant transformation after a mean follow-up of 55 months. Moreover, among 335 phyllodes tumors, PASH was present in 70% of cases and correlated with lower tumor grade, reduced malignancy risk, and lower recurrence rates (8). The mechanism behind the observed reduced breast cancer risk in PASH remains unclear.

The aim of this study was to better define which cases of PASH require surgical excision and to propose a decision-making algorithm, based on two cases managed at the Senology Department of the Institut de Cancérologie de Strasbourg, Europe.

**Case Presentations**

**Case 1 (Figure 1)**

A 26-year-old woman presented after detecting a lump in her right breast. She had no relevant medical history, was nulligravid, and had been on hormonal contraception for three years. On examination, a mobile, 4 cm mass was palpated in the inner quadrants of the right breast, with no skin abnormalities or palpable nodes.

Ultrasound revealed a hypoechoic, oval-shaped mass measuring 5.2 cm, with multilobulated contours, minimal vascularization, and benign elastographic features. No adenopathy was noted.

Core biopsy demonstrated a fibrous, hyaline collagenous lesion with multiple pseudo-vascular slit-like spaces, consistent with PASH, with no features suggestive of phyllodes tumor. The fibrotic architecture favored a diagnosis of PASH.

Due to the lesion's size and discomfort, a lumpectomy via a periareolar incision was performed. Intraoperative radiography confirmed complete excision. The pathological examination described a 4.8×3.7 cm nodular, fibrous, whitish lesion with multilobulated contours. Histology revealed a fibroadenoma with a low-cellularity stroma partially replaced by PASH, without malignancy.

Postoperative follow-up at 13 days showed good cosmetic results and complete wound healing. Clinical and imaging follow-up was scheduled at six months.

**Case 2 (Figure 2)**

A 37-year-old woman presented with a self-palpated, painful 5 cm mass in the upper outer quadrant of her right breast. Her history included two pregnancies, progestin-only contraception, and prior treatment of an Arnold nerve schwannoma with radiotherapy.

On clinical examination, a painful 5 cm mass was found. Mammography revealed a rounded central opacity measuring 5.6 cm, corresponding on ultrasound to a strongly hypoechoic mass. Imaging was classified as breast imaging reporting and data system (BI-RADS) 3 (right) and BI-RADS 1 (left).

Core biopsy showed a fibro-epithelial lesion with non-compressed glandular elements, without epithelial hyperplasia or atypia. The collagen-rich stroma contained a dense network of anastomotic cavities lined by fusiform cells without atypia or mitoses, highly suggestive of nodular PASH.

Surgical excision was performed for symptomatic relief. Intraoperative radiography confirmed complete removal. Histological analysis revised the diagnosis to a fibroadenoma entirely colonized by PASH. Histology demonstrated a hypercellular connective tissue component with abundant anastomotic, optically empty slits surrounded by hyalinized fibrocollagenous CD34⁺ stroma, without endothelial marker (e.g., ERG) expression.

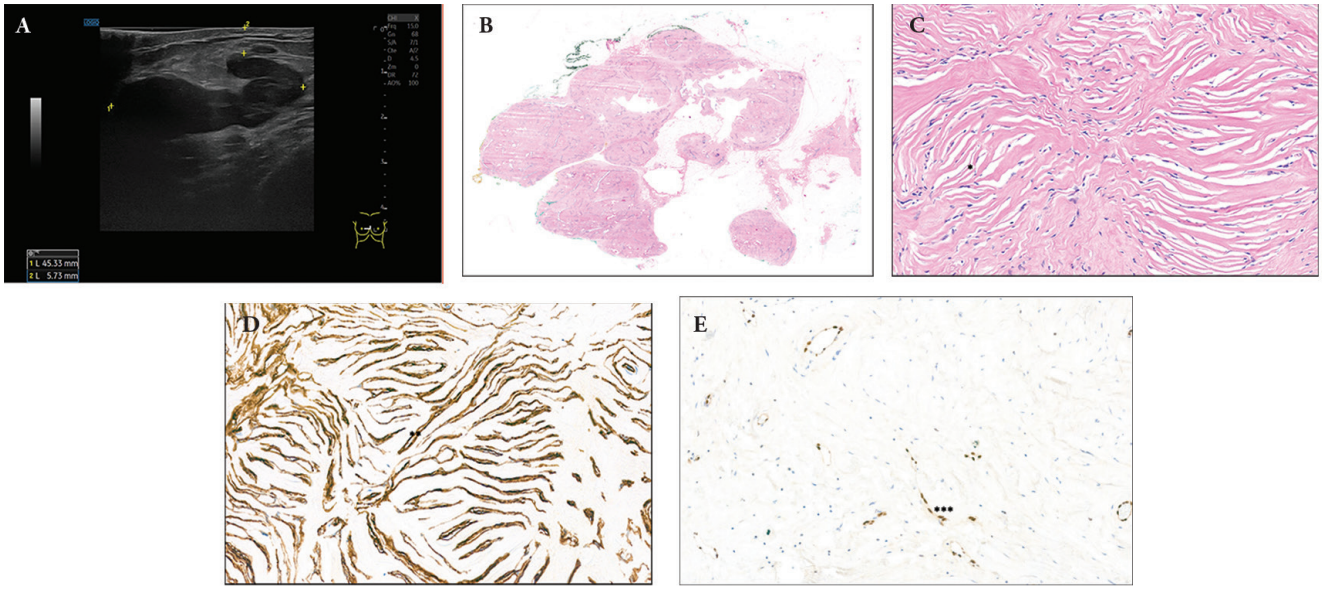



**Figure 1.** Case 1. Ultrasonography (A), low-power magnification showing a circumscribed but lobulated biphasic fibroepithelial lesion (B), with area exhibiting features of PASH at higher magnification (C), characterized by dense collagenic stroma shaping anastomosing slit-like channels (*) with CD34 immunoreactivity (**) (D) and without endothelial marker immunoreactivity (e.g., ERG) despite internal control positivity (***) (E)

*PASH: Pseudoangiomatous stromal hyperplasia*

At 12-day follow-up, wound healing and cosmetic outcomes were satisfactory. Surveillance was scheduled for six months.

## Discussion and Conclusion

These two cases highlight the frequent association of PASH with fibroadenomas. Despite biopsy results favoring a diagnosis of PASH, the palpable nature of the lesions was ultimately attributable to the fibroadenoma components.

Both patients were premenopausal and on hormonal contraception, consistent with known risk factors for PASH. Hormonal influence is supported by Vuitch et al. (2), who found similarities between PASH stroma and physiological changes during the luteal phase. In postmenopausal women, PASH is often linked to hormone replacement therapy (9), while in men, it is frequently associated with gynecomastia (10, 11). In addition, cytochrome P450-mediated drug metabolism may influence PASH development by altering estrogen and progesterone pathways (12). This is further supported by a retrospective study showing reduced PASH incidence among transgender individuals undergoing prolonged testosterone therapy (median follow-up: 17 months, $p<0.001$) (13).

Some reports suggest tamoxifen therapy (off-label) as a non-surgical treatment option for symptomatic PASH, given its presumed hormonal etiology. Two case reports described rapid symptom relief and breast volume reduction with tamoxifen, although efficacy waned in one case after three months (14, 15).

Currently, no formal PASH management guidelines exist. Based on our cases and literature review, we propose a management algorithm (Figure 3). According to this algorithm, symptomatic and palpable

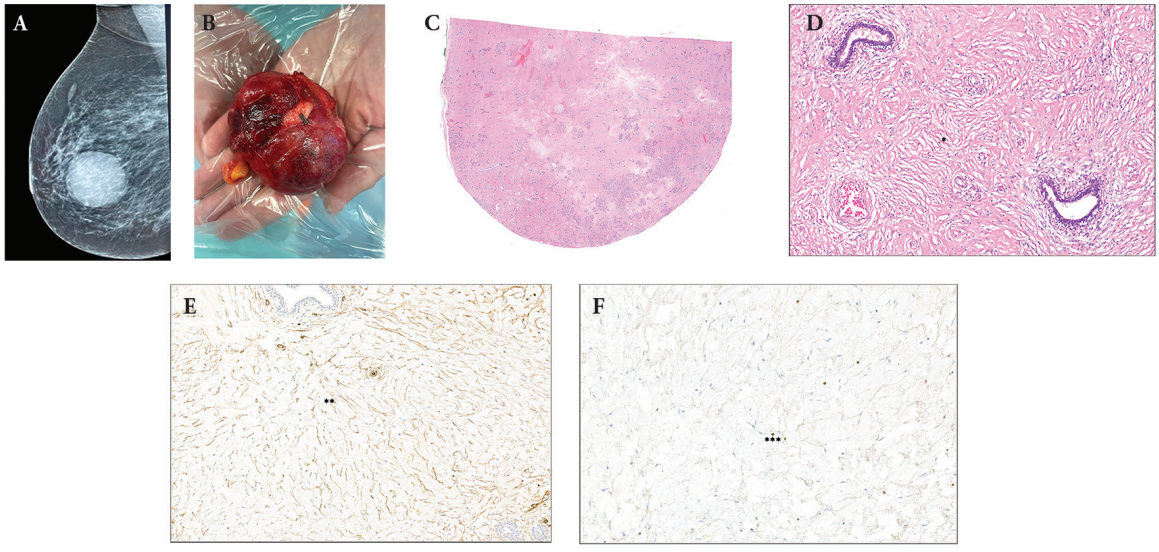



**Figure 2.** Case 2. Mammography (A), surgical specimen (B), low-power magnification showing a circumscribed biphasic fibroepithelial lesion with leaf-like pattern (C), comprising area exhibiting features of PASH at higher magnification (D), characterized by anastomosing slit-like channels (*) with weak CD34 immunoreactivity (**) (E) and without endothelial marker immunoreactivity (e.g., ERG) despite internal control (***) (F)

*PASH: Pseudoangiomatous stromal hyperplasia*

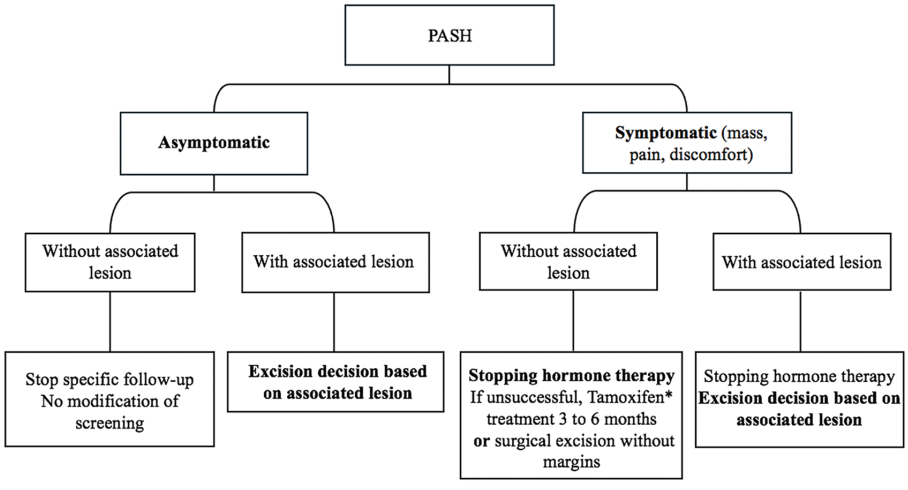



**Figure 3.** Algorithm for PASH management

*PASH: Pseudoangiomatous stromal hyperplasia; *: Off-label prescription*

lesions warrant surgical excision. In contrast, for smaller lesions, withdrawal of hormonal contraception followed by re-evaluation after six months may be considered. Validation of this approach in larger cohorts is necessary to avoid unnecessary surgeries in benign cases.

### Ethics

**Informed Consent:** Retrospective study.

### Footnotes

#### Authorship Contributions

Surgical and Medical Practices: C.B., T.W., M.B., C.M.; Concept: C.M.; Design: C.M.; Data Collection or Processing: C.B., T.W.; Analysis or Interpretation: C.B., T.W.; Literature Search: C.B.; Writing: C.B., T.W.

**Conflict of Interest:** Carole Mathelin MD is section editor in European Journal of Breast Health. She had no involvement in the peer-review of this article and had no access to information regarding its peer-review.

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

## References

1. Degnim AC, Frost MH, Radisky DC, Anderson SS, Vierkant RA, Boughey JC, et al. Pseudoangiomatous stromal hyperplasia and breast cancer risk. Ann Surg Oncol. 2010; 17: 3269-3277. (PMID: 20567920) [Crossref]
2. Vuitch MF, Rosen PP, Erlandson RA. Pseudoangiomatous hyperplasia of mammary stroma. Hum Pathol. 1986; 17: 185-191. (PMID: 3949338) [Crossref]
3. Gresik CM, Godellas C, Aranha GV, Rajan P, Shoup M. Pseudoangiomatous stromal hyperplasia of the breast: a contemporary approach to its clinical and radiologic features and ideal management. Surgery. 2010; 148: 752-757; discussion 757-758. (PMID: 20708765) [Crossref]
4. Ibrahim RE, Sciotto CG, Weidner N. Pseudoangiomatous hyperplasia of mammary stroma. Some observations regarding its clinicopathologic spectrum. Cancer. 1989; 63: 1154-1160. (PMID: 2917318) [Crossref]
5. Yoon KH, Koo B, Lee KB, Lee H, Lee J, Kim JY, et al. Optimal treatment of pseudoangiomatous stromal hyperplasia of the breast. Asian J Surg. 2020; 43: 735-741. (PMID: 31669037) [Crossref]
6. Drinka EK, Bargaje A, Erşahin ÇH, Patel P, Salhadar A, Sinacore J, et al. Pseudoangiomatous stromal hyperplasia (PASH) of the breast: a clinicopathological study of 79 cases. Int J Surg Pathol. 2012; 20: 54-58. (PMID: 21862488) [Crossref]
7. Esmer AC, Tazeoglu D, Dag A. Pseudoangiomatous stromal hyperplasia of the breast: clinical evaluation. Breast Dis. 2023; 42: 115-119. (PMID: 37066901) [Crossref]
8. Tan PH, Jayabaskar T, Chuah KL, Lee HY, Tan Y, Hilmy M, et al. Phyllodes tumors of the breast: the role of pathologic parameters. Am J Clin Pathol. 2005; 123: 529-540. (PMID: 15743740) [Crossref]
9. Anderson C, Ricci A Jr, Pedersen CA, Cartun RW. Immunocytochemical analysis of estrogen and progesterone receptors in benign stromal lesions of the breast. Evidence for hormonal etiology in pseudoangiomatous hyperplasia of mammary stroma. Am J Surg Pathol. 1991; 15: 145-149. (PMID: 1989462) [Crossref]
10. Badve S, Sloane JP. Pseudoangiomatous hyperplasia of male breast. Histopathology. 1995; 26: 463-466. (PMID: 7544764) [Crossref]
11. Milanezi MF, Saggioro FP, Zanati SG, Bazan R, Schmitt FC. Pseudoangiomatous hyperplasia of mammary stroma associated with gynaecomastia. J Clin Pathol. 1998; 51: 204-206. (PMID: 9659260) [Crossref]
12. Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett. 2005; 227: 115-124. (PMID: 16112414) [Crossref]
13. Baker GM, Guzman-Arocho YD, Bret-Mounet VC, Torous VF, Schnitt SJ, Tobias AM, et al. Testosterone therapy and breast histopathological features in transgender individuals. Mod Pathol. 2021; 34: 85-94. (PMID: 32939016) [Crossref]
14. Pruthi S, Reynolds C, Johnson RE, Gisvold JJ. Tamoxifen in the management of pseudoangiomatous stromal hyperplasia. Breast J. 2001; 7: 434-439. (PMID: 11843858) [Crossref]
15. Seltzer MH, Kintiroglou M. Pseudoangiomatous hyperplasia and response to tamoxifen therapy. Breast J. 2003; 9: 344. (PMID: 12846880) [Crossref]

Case Report

Eur J Breast Health 2025; 21(4): 379-383
DOI: 10.4274/ejbh.galenos.2025.2025-3-5

# Safe Delivery of Radiotherapy for Breast Cancer Patient With Left Ventricular Assist Device: Case Report and Review of the Literature

Senem Alanyalı[1], Emre Karaman[1], Murat Köylü[1], Ümit Kahraman[2], Sanem Nalbantgil[3]

[1]Department of Radiation Oncology, Ege University Hospital, İzmir, Türkiye
[2]Department of Cardiovascular Surgery, Ege University Hospital, İzmir, Türkiye
[3]Department of Cardiology, Ege University Hospital, İzmir, Türkiye

**ABSTRACT**

The increasing use of cardiac artificial devices, such as cardiac implantable electronic devices (CIED) and left ventricular assist devices (LVAD), results in longer life expectancy and thus may eventually coincide with a risk of cancer diagnosis and requirement for radiotherapy. Safe irradiation dose limits are better studied and reported for CIEDs, but data on LVAD irradiation are scarce. We present a case of a patient diagnosed with breast cancer who developed heart failure and was given an LVAD, received appropriate oncological care including chemotherapy, surgery, and, after careful multidisciplinary review, radiotherapy. The patient's right-sided initial stage II (T1N1) disease necessitated radiation treatment to the chest wall and regional lymphatic nodal areas. Meticulous radiotherapy planning and treatment delivery were performed, and daily LVAD performance checks were done. Maximum and mean doses received by the LVAD system were 767 cGy and 227 cGy, respectively, for the whole treatment period (5000 cGy/25 fractions). During radiotherapy and after 41 months of follow-up, no VLAD malfunction was observed. As this case shows, having an LVAD does not appear to be a contraindication for radiotherapy delivery. Possible risks and consequences should be evaluated in a multidisciplinary setting.

**Keywords:** Left ventricular assist device; breast cancer; radiotherapy; case report

**Cite this article as:** Alanyalı S, Karaman E, Köylü M, Kahraman Ü, Nalbantgil S. Safe delivery of radiotherapy for breast cancer patient with left ventricular assist device: case report and review of the literature. Eur J Breast Health. 2025; 21(4): 379-383

**Key Points**

- Increasing use of left ventricular assist devices (LVAD) raises the likelihood that these patients would develop cancer requiring radiotherapy.
- Safe irradiation dose limits are better studied for cardiac implantable electronic devices but data on LVAD are scarce.
- This case report involves a breast cancer patient, required LVAD placement during chemotherapy and later was indicated with radiotherapy.
- With maximum and mean doses to whole LVAD system being 767 cGy and 227 cGy, respectively, no LVAD malfunction occured throughout the follow-up period.
- LVAD does not necessarily contraindicate radiotherapy; risks and consequences should be evaluated in a multidisciplinary context.

## Introduction

Advances in cardiac device technologies have led to an increased use of cardiac implantable electronic devices (CIED) and left ventricular assist devices (LVAD) (1). With longer life expectancy in this patient population, the likelihood of cancer diagnosis, and thus the need for radiotherapy, also increases. While safe irradiation limits are more thoroughly studied for CIEDs, data regarding the safe irradiation of LVADs are scarce. Limited *in vitro* and *in vivo* studies indicate that radiotherapy doses up to 70 Gy, at the upper end of the therapeutic spectrum, may be administered safely. Herein, we report a case of breast cancer with a long follow-up for a patient with an *in situ* LVAD who was safely irradiated.

## Case Presentation

A 41-year-old premenopausal female with a history of non-Hodgkin lymphoma, treated 25 years ago with chemotherapy, the details of which were unavailable, and no history of radiotherapy and no known comorbidity, presented with a right axillary mass. With consideration of lymphoma relapse, the patient underwent excisional biopsy, which resulted in lymph node metastasis of "invasive ductal carcinoma of breast". Bilateral mammography and ultrasound revealed a right breast upper outer quadrant lesion with malignant features. Tru-cut biopsy from breast lesion was consistent with invasive ductal carcinoma of breast (estrogen receptor +++; progesterone receptor -, human epidermal growth factor receptor 2 +++). The patient was thus initially

**Corresponding Author:**
Senem Alanyalı MD; senem.alanyali@ege.edu.tr

**Received:** 19.03.2025
**Accepted:** 08.06.2025
**Epub:** 04.08.2025
**Available Online Date:** 25.09.2025

diagnosed as T1N1 (stage II) breast cancer, and the oncological team started treatment with neoadjuvant chemotherapy. The initial phase of neoadjuvant chemotherapy, including four cycles of doxorubicin (60 mg/m$^2$) + cyclophosphamide (600 mg/m$^2$), was completed with no adverse effects. After the first cycle of trastuzumab (8 mg/kg) + pertuzumab (840 mg) + docetaxel (75 mg/m$^2$), the patient developed chest pain, shortness of breath, abdominal and lower extremity swelling and was diagnosed with dilated cardiomyopathy with low left ventricular ejection fraction of only 25% due to cardiotoxic systemic therapy. With no clinical improvement with medical therapy, a HeartWare HVAD (HeartWare Inc., Miami Lakes, FL, USA) was implanted, stabilizing her hemodynamics. Subsequently, the patient underwent total mastectomy and axillary dissection, with pathology revealing complete response. After careful multidisciplinary evaluation of the cardiac and oncologic status of the patient, adjuvant radiotherapy was planned. Written informed consent was taken from the patient.

**Radiotherapy Simulation and Treatment Course**

External LVAD parts were carefully observed, and a 5 mm-thick lead shield box for the external system controller was produced. The patient was placed on a breast board with arms above the head (Figure 1). Radiotherapy was prescribed to the right chest wall and regional lymphatics (axillary levels 1–4) for 50 Gy in 25 fractions. Intracorporeal LVAD parts were delineated separately (Figure 2). An anterior supraclavicular field and opposed 6 MV photons to the right chest wall area (Figure 3) were used for treatment to minimize radiation exposure to LVAD subparts. Radiation doses received by separate parts of LVAD is shown in Table 1. A cardiology nurse and device specialist were available for each treatment and conducted daily pre- and post-treatment measurements of flow and power as a surrogate for LVAD performance, which showed no major measurable alterations. Treatment was completed without any device errors or malfunctions (Table 2). During cardiologic follow-up visits, no signs of LVAD malfunction were observed.

Oncological follow-up continued with no local or systemic breast cancer recurrence. Forty-nine months after breast cancer diagnosis and 41 months after LVAD placement, she had a non-traumatic intracranial hemorrhage and underwent decompression surgery, and unfortunately, died due to sepsis during post-op care.

**Figure 1.** Patient positioned on breast board and extracorporeal system control unit placed between the legs of the patient, covered with lead shield box

**Discussion and Conclusion**

Deciding whether to give radiotherapy for patients with LVAD may be challenging, considering the scarcity of high-quality data. Herein, we describe a breast cancer patient with LVAD who safely received adjuvant radiotherapy. Accumulated doses caused no disturbance to LVAD function during follow-up. To the best of our knowledge, this is the first patient who had a HeartWare LVAD and received adjuvant radiation therapy for primary breast cancer.

In terms of the safety of radiotherapy with LVADs, a review by Spano et al. (2), reported that LVAD performance was unaffected for doses of up to 70-75 Gy which are considered in therapeutic range for both photon and proton beams in several *in vitro* studies. However, Sindhu et al. (3) found, while the pump components were resilient to the 70 Gy of proton irradiation, the driveline part showed functional disturbance at 30 Gy of continuous proton irradiation, necessitating careful evaluation of various parts of the LVAD in dose-volume analysis.

To date, only twelve case reports, including eighteen cases, have been published (2, 5-13). These reports evaluated radiotherapy safety in various tumor sites and with different LVAD brands [HeartMate, Abbott, Chicago, IL, USA (HM II-III)]; HeartWare, HeartWare Inc., Miami Lakes, FL (HW); Thoratec, Thoratec, Pleasanton, CA; Novacor LVAD Atlas II VR SN, St. Jude Medical, Saint Paul, Minnesota, US. In most studies, patients were implanted with a HeartMate LVAD.

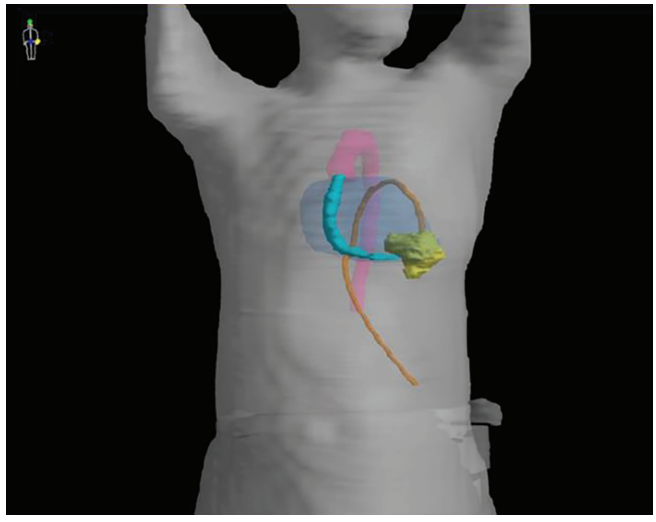


**Figure 2.** Delineation of heart (blue), aorta (pink), and LVAD parts [yellow: LVAD pump ($D_{max}$: 24 cGy, $D_{mean}$: 9 cGy); cyan: outflow graft ($D_{max}$: 767 cGy, Dmean: 227 cGy); orange: driveline ($D_{max}$: 199 cGy, $D_{mean}$: 52 cGy)]

*LVAD: Left ventricular assist devices*

**Table 1. Radiation doses received by different parts of LVAD**

| | Maximum dose (cGy) | Mean dose (cGy) |
|---|---|---|
| LVAD pump | 24 | 9 |
| Outflow graft | 767 | 227 |
| Driveline | 199 | 52 |

LVAD: Left ventricular assist devices

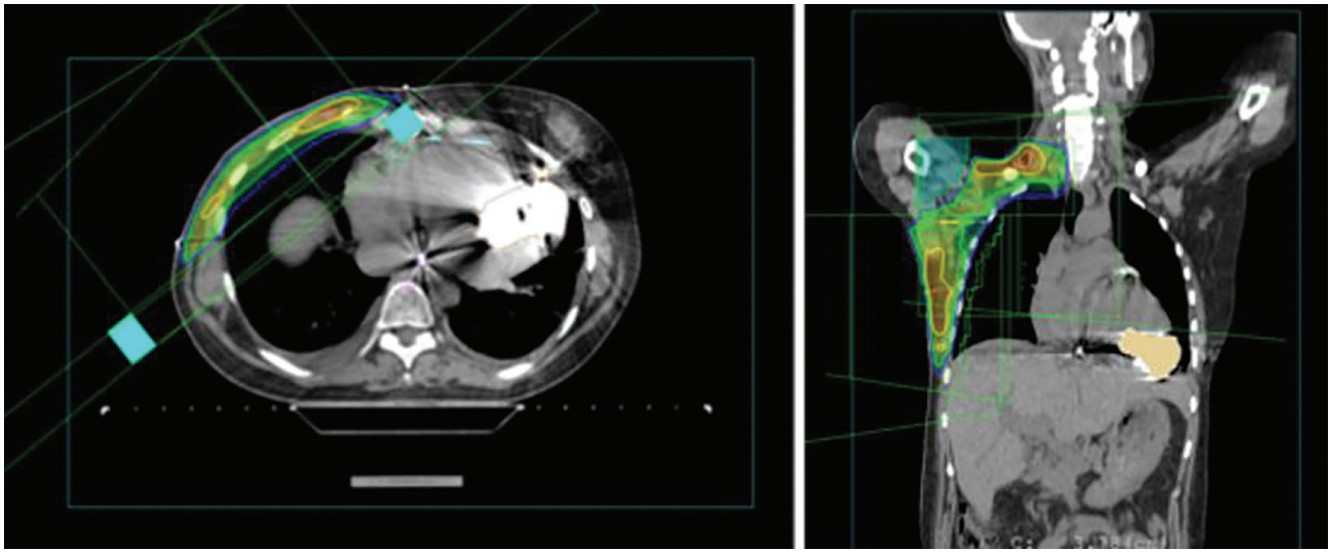


**Figure 3.** Radiotherapy dose distribution in axial and coronal views and LVAD placement (light orange) adequate target coverage was achieved (PTV_Chestwall D95%: 95.2%, PTV_Supraclavicular: D95%: 99.5%). Heart $D_{mean}$: 86 cGy

*LVAD: Left ventricular assist devices*

**Table 2. Measured device flow and power parameters during treatment**

| | Flow (L/min) | Power (watt) |
|---|---|---|
| 1st Fraction - beginning | 3.3 | 3.2 |
| 6th Fraction - week 2 | 3.9 | 3.4 |
| 11th Fraction - week 3 | 3.7 | 3.4 |
| 16th Fraction - week 4 | 3.9 | 3.4 |
| 21st Fraction - week 5 | 4.2 | 3.6 |
| 25th (Last) fraction | 3.3 | 3.2 |

Eight studies evaluated nine patients treated with conventionally fractionated radiotherapy, while six studies evaluated nine patients treated with stereotactic body radiotherapy (SBRT) (2, 8, 9, 13, 15). One study reported a lung cancer patient treated with proton beams (11). Studies reporting cardiac ablative therapies with SBRT for arrhythmias were reviewed by Benali et al. (4), with no LVAD-related complications after SBRT and are not included in this review. In fifteen cases, radiotherapy fields involved the thoracic region, in two cases, encompassed pelvic areas, and in one case, treatment was directed to the whole body.

In the studies reporting the variables that reflect the performance of the LVAD, such as power, flow, and rotational speed, all report insignificant changes between pre- and post-irradiation values (2,11,13). None of the studies reported any disturbance in device performance.

Previously published case reports are summarized in Table 3 (2, 5-15). Most of the studies used 6 to 15 MV photon beams with prescribed doses within the range of 20–66 Gy delivered in 3–33 fractions. Mean doses received by LVAD ranged between 8–1922 cGy, and maximum doses to LVAD and its subparts varied according to laterality of the tumor up to 6830 cGy (10). There was no reported device malfunctioning, with the longest reported follow-up being 29 months. We believe that this report will contribute to the current literature in several aspects, notably by reporting the successful delivery of radiotherapy in the first case with breast cancer primary and the second case with an implanted HeartWare LVAD. Furthermore, this report also reports the longest follow-up with 41 months and no device malformation.

With both *in vitro* and *in vivo* results stating the safe irradiation of LVAD and the review by Spano et al. (2) summarizing the recommendations, including ensuring a multidisciplinary approach, using beam energies <10 MV to minimize neutron contamination, ensuring a rapid response team is available, close monitoring of the patient, securing the extracorporeal parts, and interrogating the LVAD after each radiation session. In the presented case, contralaterality of the LVAD and primary tumor location made reducing radiation dose received by LVAD relatively simple, but in cases with close proximity between tumor site and the device, the radiotherapy becomes significantly more complex. Direct irradiation of the device may be unavoidable. In such situations, careful multidisciplinary planning is essential to balance optimal oncologic outcomes with device safety. Whenever feasible, attempts to reduce the dose received by the LVAD should be made, including advanced radiotherapy techniques such as intensity modulated radiotherapy or proton irradiation, or distancing the device from the radiotherapy field with techniques like deep inspiration breath hold.

Cancer patients with LVAD *in situ* pose a multifaceted challenge in radiotherapy in terms of treatment decision and technical considerations. The presented case described a patient who received adjuvant radiotherapy to the thoracic region, and yielded no evidence of device malfunction, thereby affirming its safe implementation in this case. There is limited published data regarding the safety of radiotherapy in patients with LVAD. More studies are needed in this area to ensure optimal patient safety and treatment decision-making.

**Table 3. List of the studies reporting patients irradiated with VAD for various sites and techniques**

| Study | Year | Device | Diagnosis | Beam | Prescription dose (cGy) | Fraction | Max dose (cGy) to the device | Mean dose (cGy) to the device |
|---|---|---|---|---|---|---|---|---|
| Lasher et al. (5) | 2008 | Thoratec | Rectal adenocarcinoma | 15 MV photon | 4500 | 25 | 425 | - |
| Netuka et al. (6) | 2013 | HM II | Hodgkin lymphoma | NR | NR | 12 | NR | NR |
| Scobioala et al. (7) | 2015 | Novacor LVAD Atlas II VR SN (St. Jude Medical, Saint Paul, Minnesota, US) | Lung squamous cell carcinoma | Conventional: 6/15 MV photon beams | 2520 | 14 | 538 | 231 |
| | | | | SBRT: 6 MV | 3500 | 5 | | |
| Emerson et al. (8) | 2016 | HM II | Gastroesophageal junction adenocarcinoma | 15 MV photon | 5040 | NR | 4900 | 1922 |
| | 2016 | HM II | Lung adenocarcinoma | SBRT: 6 MV photon | 5400 | 3 | 61 | 9.6 |
| | 2016 | HM II | Lung cancer/ vertebral mets | 6/15 MV photon | 2000/3000 | NR | 2450 | 1423 |
| Ostertag-Hill et al. (9) | 2018 | HeartWare | Lung nodule | SBRT: 6 MV photon | 5000 | 5 | 698 | 45 |
| Spano et al. (2) | 2019 | HM III | Lung adenocarcinoma | SBRT: 6 MV photon | 5000 | 5 | VAD: 29<br>Outflow graft: 991<br>Drive line: 34 | VAD: 8<br>Outflow graft: 147<br>Drive line: 11 |
| Sato et al. (10) | 2020 | NR | Thymic carcinoma | 6 MV photon | 6600 | 33 | Outflow graft: 6830<br>VAD: 0<br>CRT-D: 99 | -<br>-<br>- |
| Schumer et al. (11) | 2022 | HM II | Lung squamous cell carcinoma | Proton beams | 6000 | 30 | NR | NR |
| Yousafzai et al. (12) | 2022 | HM III | Sternal osteosarcoma | NR | 6660 | NR | 46.4 | |
| Butt and Sheikh (13) | 2023 | HM III | Lung adenocarcinoma | SBRT | 3000 | 5 | NR | NR |
| | 2023 | HMIII | Lung small cell carcinoma | SBRT | 5000 | 5 | NR | NR |
| Webster et al. (14) | 2024 | HM III | Acute myeloblastic leukemia | 16 MV photon | 400 | 2 | 120 | |
| Hayashi et al. (15) | 2024 | | Lung adenocarcinoma | SBRT | 42-52 Gy | 4 | <200 | |
| | | | Lung adenocarcinoma | SBRT | 42-52 Gy | 4 | <200 | |
| | | | Lung adenocarcinoma | SBRT | 42-52 Gy | 4 | <200 | |
| Alanyalı et al. | 2025 | HeartWare | Breast invasive ductal carcinoma | 6 MV photon | 5000 | 25 | Outflow graft: 767<br>VAD: 24 | Outflow graft: 227<br>VAD: 9 |

NR: Not reported; LVAD: Left ventricular assist devices

## Ethics

**Informed Consent:** Written informed consent was taken from the patient.

## Footnotes

### Authorship Contributions

Surgical and Medical Practices: S.A., E.K., M.K., Ü.K., S.N.; Concept: S.A., E.K., Ü.K., S.N.; Design: S.A., E.K., M.K., Ü.K., S.N.; Data Collection or Processing: S.A., E.K., S.N.; Analysis or Interpretation: S.A., E.K., S.N.; Literature Search: S.A., E.K.; Writing: S.A., E.K.

**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. Molina EJ, Shah P, Kiernan MS, Cornwell WK 3rd, Copeland H, Takeda K, et al. The Society of Thoracic Surgeons Intermacs 2020 annual report. Ann Thorac Surg. 2021; 111: 778-792. (PMID: 33465365) [Crossref]
2. Spano G, Stutz E, Elicin O, Hugi B, Henzen D, Fürholz M, et al. Is it safe to irradiate the newest generation of ventricular assist devices? A case report and systematic literature review. Artif Organs. 2020; 44: 449-456. (PMID: 31769042) [Crossref]
3. Sindhu KK, Shi C, Moss N, Lin H, Zhang J, Hu L, et al. The effects of pencil beam scanning proton beam therapy on a heartmate 3 left ventricular assist device: implications for patient safety. ASAIO J. 2022; 68: e145-e147. (PMID: 35537112) [Crossref]
4. Benali K, Lloyd MS, Petrosyan A, Rigal L, Quivrin M, Bessieres I, et al. Cardiac stereotactic radiation therapy for refractory ventricular arrhythmias in patients with left ventricular assist devices. J Cardiovasc Electrophysiol. 2024; 35: 206-213. (PMID: 38018417) [Crossref]
5. Lasher DE, Wojcicka JB, Malcom R, Shears LL. Case study of radiation therapy treatment of a patient with a cardiac ventricular assist device. J Appl Clin Med Phys. 2008; 9: 214-220. (PMID: 19020490) [Crossref]
6. Netuka I, Stepankova P, Urban M, Maly J, Szarszoi O, Dorazilova Z, et al. Is severe cardiac dysfunction a contraindication for complex combined oncotherapy of Hodgkin's lymphoma? Not any more. ASAIO J. 2013; 59: 320-321. (PMID: 23644622) [Crossref]
7. Scobioala S, Ernst I, Moustakis C, Haverkamp U, Martens S, Eich HT. A case of radiotherapy for an advanced bronchial carcinoma patient with implanted cardiac rhythm machines as well as heart assist device. Radiat Oncol. 2015; 10: 78. (PMID: 25885061) [Crossref]
8. Emerson LY, Deek MP, Almendral J, Jabbour SK. Radiation therapy in patients with left ventricular assist device: a case report and literature review. Pract Radiat Oncol. 2016; 6: e145-e147. (PMID: 27017262) [Crossref]
9. Ostertag-Hill CA, Mudd J, Werle DP, Tieu BH, Nabavizadeh N. Safe delivery of lung stereotactic body radiation therapy in a patient with a left ventricular assist device and implantable cardioverter defibrillator. Clin Case Rep. 2018; 6: 1704-1707. (PMID: 30214746) [Crossref]
10. Sato GE, Matsuo Y, Kanemitsu H, Minatoya K, Nakajima D, Date H, et al. Safe delivery of postoperative radiotherapy for thymic carcinoma located on the outflow graft of a left ventricular assist device. JTO Clin Res Rep. 2020; 2: 100101. (PMID: 34589971) [Crossref]
11. Schumer EM, Schettle S, Stulak JM, Rosenbaum AN. First report of proton beam therapy in a patient with a left ventricular assist device. ASAIO J. 2022; 68: e84-e86. (PMID: 35503645) [Crossref]
12. Yousafzai OK, Stewart SM, Zaman MO, Ingenito A, Kim B. Sternal radiation for osteosarcoma treatment in a patient with left ventricular assist device. JACC. 2022; 79 (9_Supplement): 2579. [Crossref]
13. Butt N, Sheikh FH. Feasibility of mediastinal radiation use in heartmate 3 LVAD recipients with cancer. Methodist Debakey Cardiovasc J. 2023; 19: 15-19. (PMID: 36742441) [Crossref]
14. Webster M, Dona Lemus OM, Zheng D, Wancura JN, Tanny S, Sakthivel G, et al. Case study with dosimetric analysis: total body irradiation to a patient with a left ventricular assist device. Clin Case Rep. 2024; 12: e8868. (PMID: 38756618) [Crossref]
15. Hayashi K, Ishikawa H, Fujiwara K, Nakai M, Ono H, Fumimoto Y, et al. Stereotactic ablative radiotherapy for early-stage lung cancer in patients with left ventricular assist device: a case series. Anticancer Res. 2024; 44: 4113-4117. (PMID: 39197891) [Crossref]

Case Report

Eur J Breast Health 2025; 21(4): 384-386
DOI: 10.4274/ejbh.galenos.2025.2024-12-4

# Air-Assisted Mastectomy Using LigaSure for a Breast Cancer Patient With a Cardiac Pacemaker

Mustafa Tukenmez, Baran Mollavelioglu, Selman Emiroglu, Neslihan Cabioglu, Mahmut Muslumanoglu, Vahit Ozmen

Department of General Surgery, İstanbul Faculty of Medicine, İstanbul University, İstanbul, Türkiye

**ABSTRACT**

Mastectomy is often performed using unipolar electrocautery. However, for patients with a pacemaker, alternative methods are necessary, as the use of unipolar cautery is not recommended. In the case presented herein, we made half-centimeter incisions on the skin to be removed. We then pumped air under the mastectomy flaps through these incisions using a hand pump and a lipoplasty cannula equipped with a filter. Following this, we made a Stewart incision and conducted the dissection using a LigaSure vessel-sealing device from the plane formed by the air. The surgery was successfully completed without any significant bleeding, and the patient was discharged without any complications. Notably, this innovative surgical technique was employed for the first time in a breast cancer patient. The cannula we developed has facilitated the creation of a dissection plane using air, similar to endoscopic mastectomy, without requiring additional ports or equipment. This technique has the potential to facilitate surgery for selected patients.

**Keywords:** Mastectomy; air-assisted mastectomy; minimally invasive surgical procedures; breast cancer; pacemaker; BRCA mutation

**Cite this article as:** Tukenmez M, Mollavelioglu B, Emiroglu S, Cabioglu N, Muslumanoglu M, Ozmen V. Air-assisted mastectomy using LigaSure for a breast cancer patient with a cardiac pacemaker. Eur J Breast Health. 2025; 21(4): 384-386

**Key Point**

- Novel surgical technique: Air-assisted mastectomy.

## Introduction

Breast-conserving surgery (BCS) is the preferred approach in the surgical management of breast cancer; however, mastectomy remains essential for selected patients with high-risk features. Unipolar electrocautery is commonly used during mastectomy, but it is not suitable for patients with pacemakers (1). The aim of this report is to describe an air-assisted mastectomy technique in a case where bilateral mastectomy was required, but unipolar electrocautery could not be used due to the patient's complete complete atrioventricular (AV) block.

## Case Presentation

In 1996, at the age of 31 years, the patient had BCS and axillary lymph node dissection (ALND) for a left breast tumor. The pathology report showed a T2N1 tumor (3.5 cm in size with 6 out of 28 metastatic lymph nodes). Adjuvant treatment included six cycles of endoxan + methotrexate + fluorouracil and radiotherapy to the left breast and regional lymphatics. In 2002, at the age of 37 years, a T1N0 medullary carcinoma was found in the right breast. The patient underwent BCS + ALND, and the pathological evaluation revealed a 1.5 cm triple-negative breast cancer with nine non-metastatic lymph nodes. Adjuvant treatment consisted of four cycles of adriamycin + cyclophosphamide and radiotherapy to the right breast. One year after chemotherapy, the patient developed complete AV block due to anthracycline toxicity, leading to the implantation of a cardiac pacemaker and an implantable cardioverter-defibrillator (ICD). In the course of the follow-up, a pathogenic mutation of *BRCA-1* was identified on genetic analysis. Subsequently, in the 27$^{th}$ year of follow-up, a 2 cm invasive carcinoma was detected in the retro-areolar region of the right breast. The multidisciplinary council advised upfront surgery in the form of a bilateral mastectomy. Given the patient's pre-existing comorbidities and the contraindication of unipolar electrocautery due to the presence of a pacemaker, a decision was made to employ the air-assisted technique, which had previously been described for the treatment of gynecomastia (2).

A standard horizontal elliptical incision encompassing the nipple was marked on both breasts. Half-centimeter incisions were made on the skin to be excised. Air was pumped under the mastectomy flaps through

Corresponding Author:
Baran Mollavelioglu MD; baran.mollavelioglu@istanbul.edu.tr

Received: 23.03.2025
Accepted: 04.08.2025
Epub: 18.08.2025
Available Online Date: 25.09.2025

these incisions with a hand pump and a lipoplasty cannula with a filter (Figure 1, Video 1). Air insufflation into the subcutaneous tissue causes separation between the breast parenchyma and the overlying subcutaneous layer, making Cooper's ligaments more prominent. This phenomenon is referred to as pneumocooper. The pneumocooper, achieved by introducing air beneath the skin facilitated the creation of a space between the mammary gland and subcutaneous tissue. Then a further incision was made, and the dissection was carried out with a vessel-sealing device (LigaSure) from the plane between the subdermal layer and the glandular tissue formed by the air. Furthermore, a dissection plan was formulated, delineating the space between the breast tissue and the fascia of the pectoralis major muscle. This was achieved through the introduction of air from the lateral border of the pectoralis major muscle via the hand cannula. Deep plane dissection was efficiently completed using the vessel-sealing device. The same procedure was performed bilaterally and a chemotherapy port was inserted with its catheter advanced into the right subclavian vein. The operation was completed in 150 minutes. As a vessel sealing device was used throughout the procedure, intraoperative bleeding was significantly less than in conventional mastectomy. The estimated blood loss was approximately 30 mL.

The patient was discharged on the second postoperative day (Figure 2). No complications occurred during the postoperative period or throughout the six months of follow-up. Histopathological evaluation revealed two foci of invasive ductal carcinoma measuring 2 cm (triple negative) and 0.5 cm (luminal A) in the right breast, and ductal carcinoma *in situ* in the left breast. The sentinel lymph node biopsy on the right side showed three non-metastatic lymph nodes. The recommended treatment plan included adjuvant chemotherapy (taxane-based) and hormonotherapy. Informed consent was obtained from the patient included in the study.

## Discussion and Conclusion

This is the first case report describing the use of air-assisted dissection with LigaSure in a breast cancer patient with a cardiac pacemaker, offering a unique alternative to unipolar cautery. The use of the Harmonic device in breast cancer patients with pacemakers has been reported previously (3, 4). In the present case LigaSure was used as a vessel-sealing device. In addition, the air-assisted technique was used to make the dissection faster, easier, and with less bleeding. The guidelines of the Heart Rhythm Society comment that unipolar electrocautery poses a significant risk of interference with pacemakers or defibrillators (1). While precautions such as magnet placement or reprogramming may be considered, the presence of an ICD in this patient rendered the use of unipolar cautery inadvisable, hence prompting the use of the LigaSure device.

The patient had a history of bilateral BCS for previous bilateral breast cancer. While recurrence was detected unilaterally on imaging, a bilateral mastectomy was deemed appropriate in light of the patient's

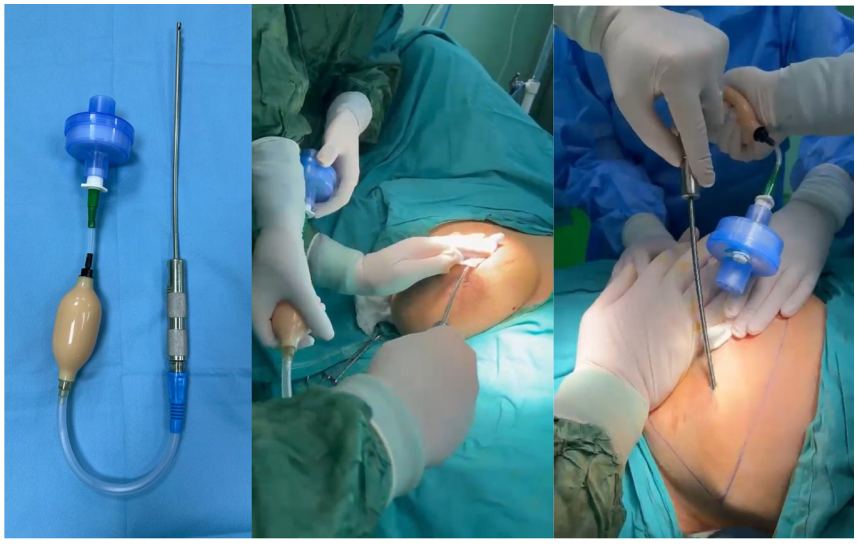


**Figure 1.** Hand pump and lipoplasty cannula with a filter and its application

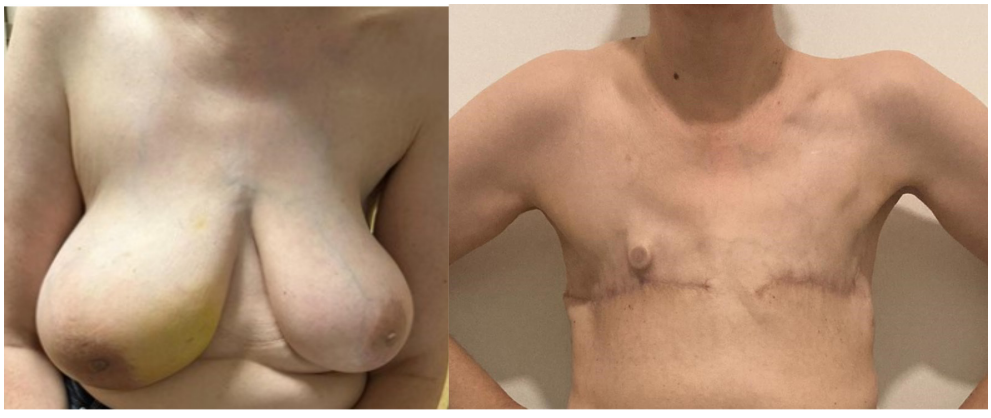


**Figure 2.** Preoperative and postoperative images

*BRCA1* mutation, high comorbidity burden, and limited tolerance for additional surgeries. While a second BCS may be considered in selected cases of recurrence (5), the patient's genetic background and comorbid profile made this option unsuitable.

Previous research suggested that in high-risk anesthesia patients, mastectomy may be performed using a subcutaneous tumescent solution (6). However, the tumescent technique can make dissection with bipolar cautery more challenging and there is evidence that skin necrosis rates may be higher (7). Unlike the tumescent solution, which may increase the risk of skin necrosis, an air-assisted dissection technique may offer a safer alternative by facilitating clearer dissection planes and reducing thermal injury, thereby potentially lowering skin-related morbidities. The Shaw scalpel has also been reported as a safe alternative in patients with pacemakers but it is not routinely used in our institution and would incur additional cost (8).

Our method involved using a hand pump to insufflate air into the subcutaneous area, creating a pneumocooper, which allowed for dissection without any bleeding through the use of the vessel-sealing device. Our team developed this specific cannula for this purpose, enabling us to expedite the dissection process in a similar fashion to the previously described endoscopic mastectomy technique without necessitating additional ports or equipment (9, 10). $CO_2$ insufflation has been shown to facilitate both dissection and the creation of a working space in minimally invasive breast surgery techniques, such as endoscopic and robotic mastectomy (11). In our technique, the key advantage of subcutaneous $CO_2$ insufflation was to enhance tissue separation and facilitate dissection with the vessel-sealing device. In our series of patients with gynecomastia, we used air-assisted surgery with the application of $CO_2$ subcutaneously using an insufflator. We observed that this technique makes dissection easier (2, 6). Although various approaches have been reported for patients with a pacemaker, this is the first report of using air-assisted dissection with LigaSure in a breast cancer patient with a pacemaker, providing a practical solution without requiring additional equipment (3, 8). We believe that this method will make surgery easier for selected patients. The disadvantages of our technique are that it is more costly than the standard surgical method and requires additional equipment.

The technique described in the present case report can be considered as an alternative, especially for patients for whom unipolar electrocautery is not a suitable option. Larger patient series are necessary to unequivocally demonstrate the safety and feasibility of this method.

**Video 1. Pumping air under the mastectomy flaps**

### Ethics

**Informed Consent:** Informed consent was obtained from the patient included in the study.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: M.T.; Concept: M.T., S.E., N.C., M.M., V.O.; Design: M.T., S.E., N.C., M.M., V.O.; Data Collection or Processing: B.M., S.E., N.C., M.M., V.O.; Analysis or Interpretation: M.T., B.M.; Literature Search: B.M.; Writing: M.T., B.M.

**Conflict of Interest:** Vahit Ozmen MD is editor-in-chief in European Journal of Breast Health. He had no involvement in the peer-review of this article and had no access to information regarding its peer-review.

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

## References

1. Crossley GH, Poole JE, Rozner MA, Asirvatham SJ, Cheng A, Chung MK, et al. The Heart Rhythm Society (HRS)/American Society of Anesthesiologists (ASA) expert consensus statement on the perioperative management of patients with implantable defibrillators, pacemakers and arrhythmia monitors: facilities and patient management this document was developed as a joint project with the American Society of Anesthesiologists (ASA), and in collaboration with the American Heart Association (AHA), and the Society of Thoracic Surgeons (STS). Heart Rhythm. 2011; 8: 1114-1154. (PMID: 21722856) [Crossref]
2. Tukenmez M, Mollavelioglu B, Kozanoglu E, Emiroglu S, Cabioglu N, Muslumanoglu M. A novel surgical technique for gynecomastia: air-assisted minimally invasive surgery with single axillary incision. Surg Innov. 2024; 31: 5-10. (PMID: 37995296) [Crossref]
3. Poole G, Biggar M, Moss D. Use of the harmonic scalpel for breast surgery in patients with a cardiac pacemaker--a tip. Breast J. 2010; 16: 108-109. (PMID: 19968657) [Crossref]
4. Sabaretnam M, Mishra A. Utility of ultrasonic scalpel for axillary dissection in a patient with permanent cardiac pacemakers. Indian J Cancer. 2015; 52: 209. (PMID: 26853407) [Crossref]
5. Hannoun-Levi JM, Gal J, Van Limbergen E, Chand ME, Schiappa R, Smanyko V, et al. Salvage mastectomy versus second conservative treatment for second ipsilateral breast tumor event: a propensity score-matched cohort analysis of the GEC-ESTRO breast cancer working group database. Int J Radiat Oncol Biol Phys. 2021; 110: 452-461. (PMID: 33383125) [Crossref]
6. Carlson GW. Total mastectomy under local anesthesia: the tumescent technique. Breast J. 2005; 11: 100-102. (PMID: 15730454) [Crossref]
7. Siotos C, Aston JW, Euhus DM, Seal SM, Manahan MA, Rosson GD. The use of tumescent technique in mastectomy and related complications: a meta-analysis. Plast Reconstr Surg. 2019; 143: 39-48. (PMID: 30589774) [Crossref]
8. Tokumine J, Sugahara K, Matsuyama T, Nitta K, Fuchigami T, Miyaguni T. Shaw scalpel for breast mastectomy in a pacemaker-implanted patient. J Anesth. 2005; 19: 349. (PMID: 16261480) [Crossref]
9. Soybir G, Fukuma E. Endoscopy assisted oncoplastic breast surgery (EAOBS). J Breast Health. 2015; 11: 52-58. (PMID: 28331692) [Crossref]
10. Tukenmez M, Ozden BC, Agcaoglu O, Kecer M, Ozmen V, Muslumanoglu M, et al. Videoendoscopic single-port nipple-sparing mastectomy and immediate reconstruction. J Laparoendosc Adv Surg Tech A. 2014; 24: 77-82. (PMID: 24401140) [Crossref]
11. Mok CW, Lai HW. Evolution of minimal access breast surgery. Gland Surg. 2019; 8: 784-793. (PMID: 32042687) [Crossref]

Case Report

Eur J Breast Health 2025; 21(4): 387-388
DOI: 10.4274/ejbh.galenos.2025.2025-2-9

# Primary Giant Cell Tumor of the Breast: Report of a Rare Case and Review of the Literature

Sangeeta Pradhan[1], Meenakshi Rao[1], Sudeep Khera[1], Mahendra Lodha[2], Parmod Kumar[3], Taruna Yadav[4], Vinay N Gowda[1]

[1]Department of Pathology and Laboratory Medicine, All India Institute of Medical Science, Jodhpur, Rajasthan, India
[2]Department of General Surgery, All India Institute of Medical Science, Jodhpur, Rajasthan, India
[3]Department of Medical Oncology, All India Institute of Medical Science, Jodhpur, Rajasthan, India
[4]Department of Diagnostic and Interventional Radiology, All India Institute of Medical Science, Jodhpur, Rajasthan, India

**ABSTRACT**

Primary giant cell tumors (GCTs) of soft tissue of the breast are extremely rare breast tumors, with only ten cases previously reported in the English literature. They are not suspected clinically, and clinically and histopathologically too, can mimic breast carcinoma or phyllodes tumor, and cause diagnostic dilemma. It is important to correctly recognize these tumors, due to management implications. Hereby, we present a case of 58 year old female with GCT of the breast presenting as a malignant breast tumor.

**Keywords:** Biopsy; breast; giant cell tumor; immunohistochemistry; soft tissue tumors

**Cite this article as:** Pradhan S, Rao M, Lodha M, Kumar P, Yadav T, Gowda VN. Primary giant cell tumor of the breast: report of a rare case and review of the literature. Eur J Breast Health. 2025; 21(4): 387-388

**Key Points**

- Giant cell tumor (GCT) of breast is rare entity. Complete tumor resection is essential not only for local control but also for achieving a definitive diagnosis. Given the rarity of GCT in the breast, awareness of this condition is crucial. Long-term follow-up is necessary for deeper understanding of its clinical behavior and outcomes.

## Introduction

Giant cell tumor (GCT) of soft tissue is a rare tumor arising primarily from the soft tissue of extremities. GCT is very rare in the breast, and the incidence of primary GCT of breast is not known due to its extreme rarity, with only ten published cases in the English literature to date (1). These tumours mimic breast carcinoma or phyllodes tumor, and often cause a diagnostic dilemma.

### Case Presentation

We recently encountered a case of a 58-year-old female who presented with a lump of size 8.5x7x6 cm in her right breast that had persisted for two months. On clinical examination, the differential diagnosis of phyllodes tumor and carcinoma of the breast were made. Imaging studies, including mammography and contrast-enhanced computed tomography of the chest, revealed a large, complex, cystic mass. An ultrasound-guided core needle biopsy from the lesion revealed a tumor comprising multinucleated giant cells admixed with mononuclear oval to plump, elongated cells. On immunohistochemistry, the multinucleated giant cells were positive for CD68, and were negative for estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2/neu, and pan-cytokeratin cocktail (AE1/AE3), suggestive of a GCT of the breast. Further imaging showed no metastatic lymphadenopathy or additional lesions. The patient underwent a modified radical mastectomy, which confirmed the initial diagnosis. Grossly, the tumor was well-circumscribed, with cystic areas. Histological examination revealed features consistent with GCT of the breast, emphasizing the importance of distinguishing this tumor from commoner breast tumors, like breast carcinoma with osteoclast-like giant cells and Phyllodes tumor.

**Corresponding Author:**
Meenakshi Rao MD; drmeenakshirao@gmail.com

**Received:** 17.02.2025
**Accepted:** 03.05.2025
**Epub:** 12.05.2025
**Available Online Date:** 25.09.2025

Primary GCT of the breast was first reported in 1981 by Lucas et al. (2), in a male patient. All the cases reported in the literature presented as well-circumscribed breast masses. The current case was clinically diagnosed as a malignant breast mass with differentials of phyllodes tumor and carcinoma of the breast. Primary GCT of the breast may be differentiated from carcinoma of the breast by the absence of epithelial immunostains. Similarly, malignant phyllodes is excluded by the absence of epithelial component or sarcomatous component. On microscopy, the other differentials considered were metastatic GCT, direct infiltration of breast by a primary bone tumor, reactive lesions, and granulomatosis. Metastatic GCT and direct infiltration of a primary bone tumor were excluded by the absence of bone lesions radiologically. The patient did not have any prior systemic diseases or history of any infection, which ruled out the possibilities of reactive lesions or granulomatosis. There were no epithelioid cell granulomas and the giant cells seen were osteoclastic type, so the possibility of a granulomatous lesion, such as tuberculosis, was also excluded. Cystic change can also be GCT of the breast (1). The current case also showed cystic change, which may suggest a differential diagnosis of papillary neoplasms. However, no papillae were seen grossly or on microscopy in the current case. In all cases, including the, presented case, initial concerns of malignancy were prompted by imaging findings. GCTs of the breast have a variable clinical course. Most of the cases reported in the literature did not recur. A few cases had local recurrence (3, 4) and a single case showed pulmonary metastases. (5) The current case is on regular follow-up and has been followed-up for eleven months to date, with no recurrence so far.

We report a primary GCT of the breast, which is a very rare breast lesion. The importance of immunohistochemistry in the differential diagnosis of similar lesions is highlighted.

### Ethics

**Informed Consent:** Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: S.P., M.L., P.K., T.Y., V.N.G.; Concept: S.P., M.R., S.K., M.L., V.N.G.; Design: S.P., M.R., M.L., V.N.G.; Data Collection or Processing: S.P., M.R., V.N.G.; Analysis or Interpretation: S.P., M.R., S.K., V.N.G.; Literature Search: S.P., M.R., S.K., V.N.G.; Writing: S.P., M.R., V.N.G.

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

**Financial Disclosure:** The authors declare that they received no financial support for this study.

## References

1. Zhang W, Kong X, Qi Y, Wang X, Liu Q, Fang Y, et al. Primary giant cell tumor of the breast with pulmonary metastasis: a case report and review of the literature. Front Oncol. 2021; 11: 638237. (PMID: 34804910) [Crossref]
2. Lucas JG, Sharma HM, O'Toole RV. Unusual giant cell tumor arising in a male breast. Hum Pathol. 1981; 12: 840-844. (PMID: 7309033) [Crossref]
3. Suleman FE, Vilakazi MN, Bida M, Edwards R. Primary giant cell tumour of the breast with recurrence: a rare case report. SA J Radiol. 2022; 26: 2393. (PMID: 35548707) [Crossref]
4. Luangxay T, Osako T, Yonekura R, Sugiura Y, Kikuchi M, Gomi N, et al. Giant cell tumor of soft tissue of the breast: case report with H3F3A mutation analysis and review of the literature. Pathol Res Pract. 2020; 216: 152750. (PMID: 31784095) [Crossref]
5. Sawa A, Ikeda T, Ichioka E, Tsushima Y, Iguchi-Manaka A, Bando H, et al. Preoperative diagnosis of a giant cell tumor of soft tissue arising from the breast by ultrasound-guided core needle biopsy. J Med Ultrason (2001). 2019; 46: 257-261. (PMID: 3006249) [Crossref]

Clinical Image

Eur J Breast Health 2025; 21(4): 389-391
DOI: 10.4274/ejbh.galenos.2025.2025-3-13

# Dermatofibrosarcoma Protuberans of the Breast

Ramita Mukherjee[1], Rachana Meena[2], Brijesh Kumar Singh[1]

[1]Department of Surgical Disciplines, All India Institute of Medical Sciences, New Delhi
[2]Department of Pathology, All India Institute of Medical Sciences, New Delhi, India

**ABSTRACT**

Dermatofibrosarcoma protuberans (DFSP) is a rare, low-grade, fibroblastic mesenchymal tumor derived from the dermis. Breast is an uncommon site with an incidence of only 0.8–4.5% and an overall population incidence at any site of 4.2–4.5 per million. Surgical excision with 2–3 cm margin is the gold standard treatment. Selected cases are subjected to radiotherapy or systemic therapy with Imatinib. Due to the rare presentation, we report a similar case of DFSP on the left breast in a 42-year-old woman, who was initially diagnosed with benign phyllodes tumor of the left breast and final histopathology report of the wide local excision specimen diagnosed DFSP of the breast.

**Keywords:** Breast; cutaneous lesion; dermatofibrosarcoma protuberans; keloid-like

**Cite this article as:** Mukherjee R, Meena R, Singh BK. Dermatofibrosarcoma protuberans of the breast. Eur J Breast Health. 2025; 21(4): 389-391

**Key Point**

- Dermatofibrosarcoma protuberans on breast is an unusual location of the tumor, which can be confused with phyllodes due to its rarity.

## Introduction

Dermatofibrosarcoma protuberans (DFSP) is a rare, low-grade, fibroblastic mesenchymal tumor originating from the dermis. It most commonly affects the trunk, followed by the proximal extremities (1). Involvement of the breast is uncommon, with an estimated incidence of 0.8–4.5% among DFSP cases, and an overall DFSP incidence of only 4.2–4.5 per million population (1-3). Surgical excision with 2-3 cm margin is the gold standard treatment (1, 2). Selected cases are subjected to radiotherapy or systemic therapy with Imatinib (2).

Due to its rare presentation, breast DFSPs are often misdiagnosed during clinical examination, commonly mistaken for dermatofibroma, hemangioma or fibroepithelial lesions, as clinicians are often not familiar with its occurrence in the breast (1).

Here, we report a case involving a 42-year-old woman with DFSP of the left breast, which was initially diagnosed as a benign phyllodes tumor based on core needle biopsy. However, the final histopathological evaluation of the wide local excision specimen diagnosed DFSP of the breast.

## Case Presentation

A 42-year-old woman presented with a discolored protruding lesion on the skin of the left breast which was insidious in onset and gradually progressive over the course of two years from approximately 2x1 cm to around 4x3 cm at presentation. The lesion was painless and non-itchy. She did not have any contributory family history. On clinical examination, a lobulated, exophytic, keloid-like lesion with pink to whitish discoloration was noted on the left breast, located just above the inframammary fold, extending from the 6 to 5 o'clock position. The lesion was well-defined, measured 4.2×3.0 cm, and was fixed to the overlying skin but free from the underlying breast tissue (Figure 1a). The right breast, the remaining left breast and bilateral axillae were unremarkable.

A mammogram with ultrasound correlation was performed. Mammogram (Figure 1b) demonstrated two high-density, lobulated lesions with well-circumscribed margins in the lower outer quadrant of the breast, 12 cm away from the nipple measuring 2.6x2.1 cm and 2x1.9 cm respectively. No intramammary lesion or microcalcification were observed. Ultrasound correlation revealed a few well defined, oval shaped, solid hypoechoic lesions, the largest measuring 3x1.3 cm located at 5 o'clock position in the left inframammary fold, about 10 cm away from the nipple/areolar complex. The lesion exhibited cleft-like cystic spaces with posterior acoustic enhancement and significant internal vascularity on color Doppler. Axillae were normal. The findings were suggestive of phyllodes tumor. For histopathological confirmation, a core needle biopsy was done which showed a mesenchymal tumor, favoring benign phyllodes. It had ovoid to spindled shaped cells with no epithelial component. So, as this was a breast lesion with such morphology, phyllodes is the usual diagnosis (Figure 2).

**Corresponding Author:**
Brijesh Kumar Singh MD; brijeshkumarsinghssmc04@gmail.com

**Received:** 15.04.2025
**Accepted:** 21.06.2025
**Epub:** 12.08.2025
**Available Online Date:** 25.09.2025

Hence, no immunohistochemistry (IHC) was planned initially. The patient subsequently underwent wide local excision with 2 cm margin.

The perioperative period was uneventful. On histology, the resected specimen showed dermal-based spindle cell tumor with infiltration into the subcutaneous fat, sparing the underlying breast parenchyma. This tumor was composed of uniform, bland, spindle cells arranged in a storiform pattern with areas of whorled fascicles and characteristic entrapment of adnexal structures. No significant mitosis, nuclear atypia or necrosis were seen. On IHC these cells were diffusely positive for CD34 (Figure 3a, b). Features were consistent with DFSP. For confirmation, fluorescence *in situ* hybridization was done on the paraffin embedded tissue block, which showed *PDGFB* gene rearrangement (Figure 3c).

It is important to note that diagnosing DFSP with small core biopsy samples can be challenging, particularly in unusual anatomical locations such as the breast. In such limited samples where the representation of tumor is not adequate, the potential misdiagnosis with more common entities like benign phyllodes, solitary fibrous tumor, fibromatosis and cellular fibrous histocytoma is common. Given these challenges, a carefully selected IHC panel including CD34, STAT6, Beta catenin,

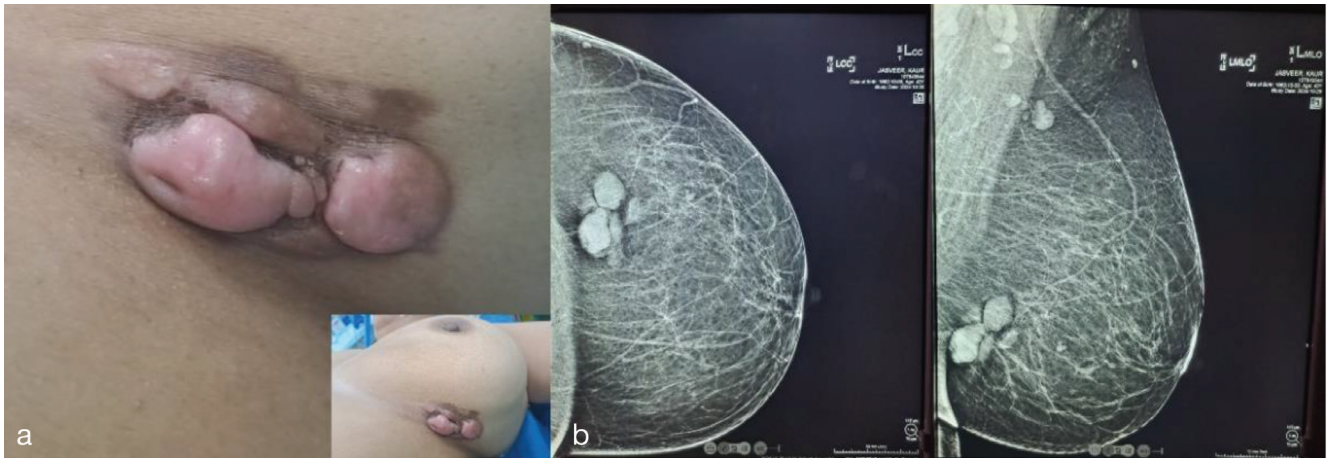



**Figure 1. a.** Keloid-like lobulated exophytic pink to whitish colored lesion on the left breast which increased from 2x1 cm to 4.2x3 cm over two years, **b.** Mammogram showing high-density lobulated well-circumscribed lesions without any intramammary lesion or microcalcification in both views (craniocaudal and medio-lateral oblique)

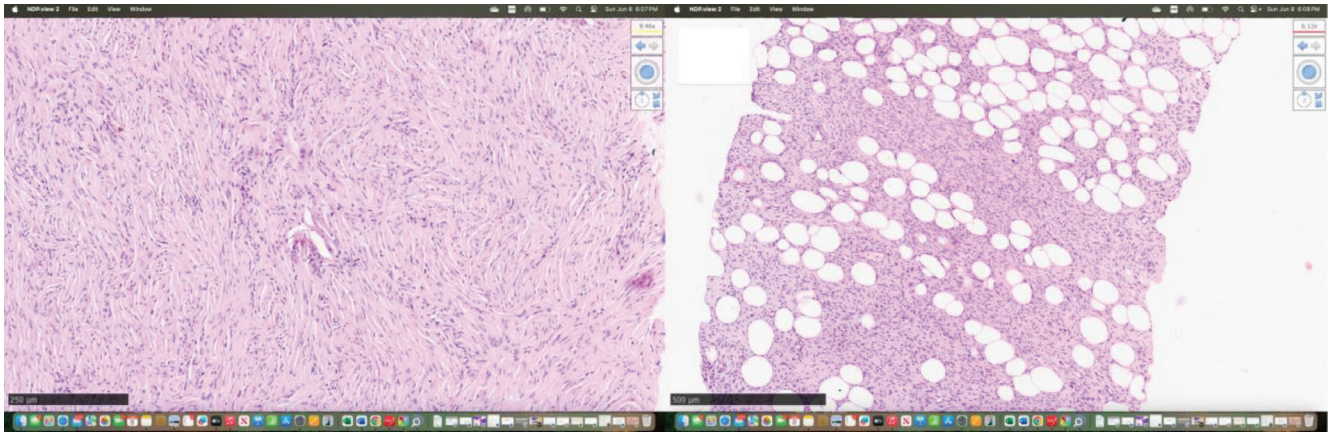


**Figure 2.** Mesenchymal tumor with round to spindled shaped tumor cells (H&E, 200x)

*H&E: Hematoxylin and eosin*

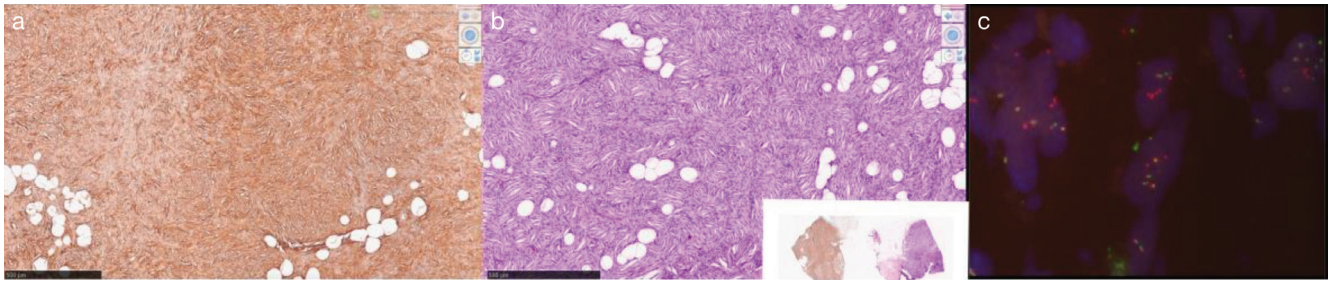



**Figure 3. a.** CD34 positivity in tumor cells (100x) – final histopathology specimen, **b.** Characteristic storiform pattern of tumor cells (H&E 200x) - final histopathology specimen, **c.** Fluorescence *in situ* hybridization on tissue block shows *PDGFB* gene rearrangement in tumor cells - final histopathology specimen

*H&E: Hematoxylin and eosin*

S100 and Ki-67 is essential to distinguish DFSP from its closest morphological mimics in small biopsy samples. Informed consent was taken from patient.

### Follow-up

The patient was planned for close follow-up post surgery. Clinical examination at three months of follow-up showed no evidence of recurrence and the surgical scar was healthy. A follow-up mammogram performed subsequently (post 3-months) showed no residual lesion or new abnormalities, indicating a favorable postoperative course.

### Ethics

**Informed Consent:** Informed consent was taken from patient.

### Footnotes

### Authorship Contributions

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

**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. Wang Y, Wang Y, Chen R, Tang Z, Liu S. A rare malignant disease, dermatofibrosarcoma protuberans of the breast: a retrospective analysis and review of literature. Biomed Res Int. 2020; 2020: 8852182. (PMID: 33224981) [Crossref]
2. Ramesh O, Leila Haji M, Sadaf A. Corrigendum to "dermatofibrosarcoma protuberance of the skin of the breast: a case study and review of the literature" [Int J Surg Open 50 (2023) 100583]. IJS Open 52: 100591, March 2023. [Crossref]
3. Dimas D, Boutas I, Potiris A, Koufopoulos N, Balalis D, Sitara K, et al. Dermatofibrosarcoma protuberans of the breast: a case study. Mol Clin Oncol. 2021; 14: 50. (PMID: 33604040) [Crossref]

**Letter to the Editor**

Eur J Breast Health 2025; 21(4): 392-393
**DOI:** 10.4274/ejbh.galenos.2025.2025-3-12

# Comment on the "Breast Imaging: Correlation Between Axillary Lymph Nodes Apparent Diffusion Coefficient and Pathological Lymphovascular Invasion in Patients With Invasive Breast Cancer"

Ahmet Bozer
Clinic of Radiology, İzmir City Hospital, İzmir, Türkiye

**Cite this article as:** Bozer A. Comment on the "breast imaging: correlation between axillary lymph nodes apparent diffusion coefficient and pathological lymphovascular invasion in patients with invasive breast cancer". Eur J Breast Health. 2025; 21(4): 392-393

**Dear Editor,**

I read with great interest the article entitled "Breast Imaging: Correlation Between Axillary Lymph Nodes Apparent Diffusion Coefficient and Pathological Lymphovascular Invasion in Patients With Invasive Breast Cancer" (1). The authors have addressed a clinically relevant and timely topic by investigating the relationship between the apparent diffusion coefficient (ADC) values of axillary lymph nodes and lymphovascular invasion (LVI) in patients with invasive breast cancer. Notably, their demonstration of the prognostic potential of magnetic resonance imaging-based ADC measurements in the preoperative setting represents a valuable contribution to the literature. The detailed evaluation of both radiological and histopathological correlations is also commendable.

However, certain aspects of the study could be further clarified or improved to enhance its scientific impact:

i. *Patient Selection:* The study population was limited to patients with single, unilateral breast tumors and ipsilateral lymph node positivity. This selective cohort limits the generalizability of the findings. Inclusion of a more heterogeneous patient population could improve the applicability of the results.

ii. *Unclear Methodology for ADC Measurements:* The type of regions of interest (ROI) used (e.g., elliptical, freehand) was not specified, and the figures suggest that only a single ROI was used. In addition, the criteria for identifying the "most suspicious" lymph node were not clearly defined. It is also unclear whether the three radiologists reached a consensus or made independent assessments. These methodological ambiguities undermine the reproducibility and transparency of the study.

*iii.* *LVI Evaluation:* LVI was assessed solely on postoperative histopathological examination, but the use of immunohistochemical markers to enhance detection sensitivity was not mentioned. This could affect the accuracy of LVI identification.

iv. *Neoadjuvant Treatment Status:* The study does not specify whether patients received neoadjuvant chemotherapy. As neoadjuvant therapy may influence both ADC values and Ki-67 expression levels, this missing information may limit the interpretation of the findings.

v. *Lack of Multivariate Analysis:* Although the study presents ROC analyses for ADC and Ki-67, multivariate regression analyses were not performed. Such analyses would be necessary to determine whether ADC and Ki-67 are independent predictors of LVI.

These constructive comments are intended to support the authors and guide future research, without detracting from the value of the current study. I commend the authors for their contribution to the field.

Sincerely,

**Corresponding Author:**
**Ahmet Bozer MD; drahmetbozer@gmail.com**

**Received:** 26.03.2025
**Accepted:** 01.04.2025
**Epub:** 27.05.2025
**Available Online Date:** 25.09.2025

**Response to the Letter**

**Dear Editor,**

We appreciate the constructive criticisms from the comment regarding our article "Breast Imaging: Correlation Between Axillary Lymph Nodes Apparent Diffusion Coefficient and Pathological Lymphovascular Invasion in Patients With Invasive Breast Cancer" (1).

We believe addressing the mentioned points in comments will improve upon clarity and impact of our research work.

i. Regarding the issue of patient selection, we acknowledge that the study's focus on patients with single, unilateral breast tumors and ipsilateral lymph node positivity limits the generalizability of our findings. However, this more selective protocol strengthens internal validity and help reducing confounding variables. Expanding the cohort might introduce more heterogeneity that complicates interpretation, with future studies encouraged to explore broader populations.

ii. The reader raised valid concerns regarding ADC measurements and ROI type. As mentioned in methodology, we would like to clarify that elliptical ROIs were manually drawn to measure the solid portions of the lymph nodes and exclude necrotic areas. While figures may show a single ROI, this is for illustrative purposes and to avoid confusion, at least three ROIs measurements were used within each lymph node, and the mean ADC value was calculated. The "most suspicious" lymph node was identified, as mentioned in the discussion segment of the article, based upon established radiological criteria, including size, cortical thickening, loss of fatty hilum, irregular margins, and heterogeneous cortex [references (22, 23) in the original manuscript provide further details]. Furthermore, conjoint interpretation of the magnetic resonance imaging was done by the three radiologists as mentioned to reach consensus-based final agreement regarding lymph node selection and measurements.

iii. We acknowledge the reader's point about LVI evaluation. We relied on standard histopathological markers examination as it remains the conventional method in clinical practice, the use of more immunohistochemical markers (e.g., CD31, D2-40) could indeed enhance the sensitivity of LVI detection. The study provides meaningful results within the scope of conventional diagnostic protocols, and further studies could explore the added value of immunohistochemistry markers in correlating with ADC values.

iv. We recognize that neoadjuvant chemotherapy can influence both ADC values and Ki-67 expression. The retrospective nature of our study made it challenging to avoid this confounding variable. Future prospective studies should incorporate neoadjuvant treatment as a factor in the analysis.

v. Finally, we agree that a multivariate regression analysis, as suggested by the reader, would provide more robust assessment of the ADC and Ki-67 as independent predictors for LVI. Our study primarily focused on the ROC analysis between these parameters using simpler statistical methods, and already provides valuable clinical insights. This study's primary goal was to establish a correlation, paving the way for further investigations incorporating multivariate approaches.

We appreciate the insightful feedback, which will undoubtedly contribute to the improvement of future research in this area.

Sincerely,

---

**Footnotes**

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

**Financial Disclosure:** The author declare that they received no financial support for this study.

## Reference

1. Mounir AM, Shokeir FA, Abd Elraouf GH. Breast ımaging: correlation between axillary lymph nodes apparent diffusion coefficient and pathological lymphovascular ınvasion in patients with ınvasive breast cancer. Eur J Breast Health. 2025; 21: 141-153. (PMID: 40079346) **[Crossref]**

# 2025 Reviewer Index

Abdullah Bahadır Öz
Abut Kebudi
Adnan Aydıner
Ahmet Umut Yuvacı
Alexander Mundinger
Alper Öztürk
Amiy Arnav
Arda Işık
Aslı Metin Mahmutoğlu
Atilla Soran
Ayça Taş
Ayfer Kamalı Polat
Ayşegül Akdoğan Gemici
Ayşegül Şahin
Banu Arun
Bekir Kuru
Berk Göktepe
Beyza Özçınar
Birol Topçu
Cem Yılmaz
Ceren Yalnız
Chitresh Kumar Sharma
Çetin Ordu
Çiğdem Irkkan
Daniel Rodrigues de Bastos
Didier Verhoeven
Duygu Has Şimşek
Efe Sezgin
Eli Avisar
Enver Özkurt
Esma Özmen
Esra Canan Talu
Esra Eren
Fatma Aktepe
Fien Neckebroeck
Filiz Çelebi
Gül Alço
Güldeniz Karadeniz Çakmak
Hande Koksal
Hasan Besim
Hasan Karanlık
Hassan Malekinejad
Hüseyin Abalı
Hüseyin Özgür Aytaç
Ismail Jatoi
İsmail Güzeliş
Kandace McGuire
Kazım Şenol
Lejla Hadzikadic-Gusic
Levent Yeniay
Mauricio Magalhaes Costa
Mehmet Ali Nazlı
Melda Apaydın
Melis Gültekin
Merve Tokocin
Mervi Siekkinen
Mustafa Ege Şeker
Mustafa Emiroğlu
Naziye Ak
Nazmiye Yıldırım
Neslihan Cabioğlu
Nilgün Kapucuoğlu
Osman Toktaş
Osman Zekioğlu
Ömür Karakoyun Çelik
Philip Poortmans
Safiye Aktaş
Seher Demirer
Semen Önder
Semra Günay
Serap Erel
Serdar Özbaş
Sevgi Canbaz
Sevgi Kurt Yazar
Sıtkı Tuzlalı
Takashi Kuwayama
Thorsten Heimann
Tibor Tot
Tuğba Akın Telli
Turkan İkizceli
Vildan Kocatepe
Yasemin Kayadibi
Yasemin Şanlı
Yeliz Emine Ersoy
Zekiye Altun
Zeynep Erdoğan İyigün

# 2025 Author Index

# 2025 Author Index

# 2025 Author Index

# 2025 Subject Index

# 2025 Subject Index