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May Cancer Diagnostic Probe reduce re-operation rate in post-neoadjuvant breast cancer patients undergoing breast conserving surgery? A 5-year retrospective study

Mansour Nateghi
Shahid Beheshti University of Medical Sciences
Afsaneh Momtaz
Shahid Beheshti University of Medical Sciences
Zohreh Sadat Miripour
University of Tehran
Marzieh Kamarehee
Tarbiat Modares University
Mohammad Akbari
Shahid Beheshti University
Atieh Akbari
Shahid Beheshti University of Medical Sciences
Morteza Yousefi
Shahid Beheshti University of Medical Sciences
Mohammad Abdolahad
University of Tehran
Mohammad Esmaeil Akbari
me-akbari@sbmu.ac.ir

Shahid Beheshti University of Medical Sciences

Short Report

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Title: May Cancer Diagnostic Probe reduce re-operation rate in post-neoadjuvant breast cancer patients undergoing breast conserving surgery? A 5-year retrospective study

Authors List:

Mansour Nateghi [1], Afsaneh Momtaz [1], Zohreh Sadat Miripour [2,3], Marzieh Kamarehee [4], Mohammad Akbari [5], Atieh Akbari [1], Morteza Yousefi [1], Mohammad Abdolahad [2,3#], Mohammad Esmaeil Akbari [1#]

Post-Publication Corresponding authors.

Affiliations:

[1] Cancer Research Center (CRC), Shohadaye Tajrish Hospital, Shahib Beheshti University of Medical Sciences, Tehran, Iran.

[2] Nano Bio Electronic Devices Lab, School of Electrical and Computer Engineering, College of Engineering, University of Tehran, Tehran, Iran.

[3] UT&TUMS Cancer Electronics Research Center, Tehran University of Medical Sciences, Tehran, Iran.

[4] Department of Statistics, Faculty of mathematical Sciences, Tarbiat Modares University, Tehran, Iran.

[5] Department of Economics, Faculty of Economics and Political Sciences, Shahid Beheshti University, Tehran, Iran.

Corresponding Authors:

Mohammad Esmaeil Akbari, Cancer Research Center, Shahid Beheshti University of Medical Sciences, P.O. Box: 19835-3551, Tehran, Iran. me-akbari@sbmu.ac.ir

Mohammad Abdolahad, Nano Bio Electronic Devices Lab, School of Electrical and Computer Engineering, College of Engineering, University of Tehran, P.O. Box 14395/515, Tehran, Iran. m.abdolahad@ut.ac.ir; abdolahad@tums.ac.ir

Keywords

breast cancer; cancer diagnostic probe; CDP; neoadjuvant; breast conserving surgery; margin

Abstract

Background: Achieving negative margins in breast conserving surgery following neoadjuvant chemotherapy remains a clinical challenge, largely due to the limitations of intraoperative frozen section analysis in the post-neoadjuvant setting. Consequently, the adoption of novel intraoperative modalities aimed at reducing reoperation rates in post-neoadjuvant breast cancer patients undergoing breast conserving surgery is gaining momentum. This study evaluates the diagnostic accuracy of Cancer Diagnostic Probe (CDP®) in confirming tumor cavity margin negativity in 201 post-neoadjuvant female breast cancer patients undergoing breast conserving surgery.

Methods: Tumor cavity margins of 201 post-neoadjuvant female breast cancer patients undergoing breast conserving surgery were assessed using intraoperative CDP and the findings were compared with the current gold standard of margin evaluation by permanent pathology. Statistical analysis was performed using Monte Carlo simulation and beta distribution modeling to calculate negative predictive value and reoperation rate.

Results: CDP demonstrated an acceptable reoperation rate of 1.49% and a negative predictive value of 97.8%.

Conclusions: Compared with existing intraoperative margin assessment modalities, CDP® has demonstrated higher negative predictive value and lower reoperation rates, supporting its utility as a superior intra-operative margin assessment tool to address the aforementioned clinical challenge in post-neoadjuvant breast cancer patients undergoing breast conserving surgery. Moreover, its use is associated with substantial time and cost savings relative to those incurred with reoperation.

Implications for practice

Achieving negative surgical margins in post-neoadjuvant breast cancer remains a persistent challenge, largely because the accuracy of intraoperative frozen-section analysis declines in this setting. Consequently, the need for reliable intraoperative margin-assessment technologies has become an important area of investigation. This study reports the 5-year retrospective re-operation rate among 201 post-neoadjuvant breast cancer patients whose intraoperative margins were evaluated using the Cancer Diagnostic Probe (CDP®) between March 2020 and March 2025.

1 | Introduction

Local therapy followed by adjuvant systemic therapy has become the cornerstone of treatment for early-stage breast cancer.[1] Breast conserving surgery (BCS) has evolved from a purely anatomical resection to a function-preserving oncologic procedure. Evidence suggests that BCS combined with radiotherapy (RT) yields superior survival outcomes compared to mastectomy, regardless of RT administration.[2,3] Although BCS has become an appealing choice for most breast

cancer patients especially in early stages, achieving a pathologically confirmed negative surgical margin has long been a challenge especially in post-neoadjuvant setting.

Although tumors downsized by neoadjuvant chemotherapy (NACT) might have higher local recurrence after breast-conserving therapy than might tumors of the same dimensions in women who have not received neoadjuvant chemotherapy (5.5% higher 15 year local recurrence rate)[4], given the lack of significant differences in overall survival (OS) between pre- and postoperative chemotherapy in early-stage breast cancer, the observed increase in BCS rates and improved cosmetic outcomes, make neoadjuvant chemotherapy followed by BCS an attractive option for select patients.[5,6]

Intratumoral heterogeneity contributes to variable responses to neoadjuvant chemotherapy, leading to unpredictable patterns of tumor shrinkage. While concentric shrinkage may result in a solitary residual mass, other patterns can produce multifocal, patch-like, or satellite lesions.[7] Consequently, a higher incidence of positive margins following neoadjuvant chemotherapy is anticipated. Volders et al. reported that tumor-free margins were not achieved in one out of four patients undergoing BCS after neoadjuvant chemotherapy, particularly in those with invasive lobular carcinoma or poor pathological response. Importantly, excessive resection volume does not guarantee negative margins [8], and margin width has not been shown to correlate with locoregional recurrence-free survival, disease-free survival (DFS), or OS. [9]

Intraoperative frozen section analysis has been shown to significantly decrease reoperation rates for positive margins in breast-conserving surgery; however, reported false-negative rates remain substantial, ranging from 3% to 23%.[10,11] Gnant et al. demonstrated that, in the post neoadjuvant setting, the likelihood of requiring a second operation due to false frozen section analysis increases markedly, with an odds ratio of 10.621 (95% CI: 6.321–17.845). [12] This has underscored a substantial need for the development of alternative intraoperative margin assessment tools. Two commercially available devices, the Histolog® Scanner and MarginProbe®, utilize laser-based microscopic tissue evaluation and spectroscopy, respectively, and both have demonstrated superior performance through reduced rates of margin positivity compared with frozen section analysis.

Hypoxia is a hallmark of solid tumors, including breast cancer.[13] It correlates with carcinogenesis, metastasis, treatment resistance, and poor prognosis.[14] This local hypoxia within the tumor and its surrounding microenvironment primarily results from abnormal vascular architecture, excessive angiogenesis causing vascular compression or obstruction, and disrupted microcirculatory flow.[15] The development of a hypoxic milieu and subsequent activation of its key regulator, hypoxia-inducible factor 1 (HIF-1), are hallmark features of advanced malignancies.[16] Hypoxia may preserve cancer stem cells in an undifferentiated state, allowing malignant cells to selectively undergo differentiation and progressively accumulate genetic and epigenetic alterations over time.[17] Intra-tumoral hypoxia has been shown to increase the population of breast cancer stem cells (BCSCs), which play a pivotal role in tumor progression and recurrence.[14] Hypoxic breast tumors—and other solid malignancies—are consistently associated with more aggressive phenotypes, elevated metastatic potential, and resistance to conventional anti-cancer therapies.[18]

The Cancer Diagnostic Probe (CDP)® is a novel technology that detects pre-cancerous and cancerous cells at tumor cavity margins during BCS by electrically probing live cell metabolism. CDP identifies reactive oxygen species (ROS) generated via glycolysis and the reverse Warburg

effect in cancer cells.[19] In a recent clinical trial involving 188 BCS candidates without prior NACT, CDP improved intraoperative margin assessment accuracy from 88% (frozen section) to 97%.[20] Another study demonstrated a CDP sensitivity of 91% for cavity-side margin evaluation in post-NACT patients undergoing BCS.[21] CDP may address limitations posed by tissue distortion and shrinkage following NACT, offering a promising tool for intraoperative margin assessment and thus reducing re-operation rate.

2 | MATERIALS AND METHODS

2.1 | Study design

Following promising initial results from intraoperative application of the Cancer Diagnostic Probe (CDP) to the tumor cavity for detecting involved surgical margins, this modality replaced intraoperative frozen section analysis for post-neoadjuvant chemotherapy (NACT) breast cancer patients at the Cancer Research Center of Shahid Beheshti University of Medical Sciences, beginning in March 2020. Following excision of the tumor with grossly negative margins, surgeons employed the CDP probe within the tumor cavity, targeting areas that grossly appeared suspicious. A positive signal necessitated additional cavity shaving and replacement of the probe head.

Between March 2020 and March 2025, a total of 5,603 breast cancer surgeries were performed at the center. Among these, data from 201 post-NACT female patients who underwent BCS using intraoperative CDP for cavity-side margin assessment, were retrospectively analyzed to determine the rate of margin involvement and subsequent reoperation, based on final histopathological reports.

2.2 | Inclusion criteria

Female patients with histologically confirmed breast cancer who underwent breast-conserving surgery following completion of neoadjuvant chemotherapy

2.3 | Exclusion criteria

Patients who underwent mastectomy

Patients with incomplete clinical or pathological data

2.4 | Demographic table

The data extracted from the records of 201 patients are presented in Table 1. Overall, three patients had positive final pathology margins despite intraoperative CDP negativity (false negative CDPs), and the distribution of these three cases is also shown in the table.

Table 1

	No. (n=201)	%	No. of False Negative CDPs (n=3)
Age at diagnosis, y			
20-30	6	2.99	0
30-40	55	27.36	2
40-50	78	38.81	1
50-60	40	19.9	0
60-70	14	6.97	0
70<	7	3.48	0
Menopausal Status			
Pre-Menopausal	142	70.65	3
Post-Menopausal	59	29.35	0
Positive Family Hx for Breast Cancer			
1st Degree	15	7.46	1
2nd Degree	14	6.97	0
Pathologic Diagnosis			
IDC	190	94.53	1
ILC	3	1.49	0
Papillary	1	0.5	0
Micropapillary	4	1.99	1
Mucinous	1	0.5	0
Cribriform	1	0.5	1
Metaplastic	1	0.5	0
Concomitant DCIS			
DCIS Component	96	47.76	2
T Status			
T1	53	26.37	0
T2	116	57.71	3
T3	26	12.94	0
T4	5	2.49	0
N Status			
N0	44	21.89	0
N1	121	60.2	2
N2	26	12.94	0
N3	9	4.48	1
ER			
+	132	65.67	3
-	67	33.33	0
PR			
+	116	57.71	3
-	83	41.29	0
HER-2			
3+	49	24.38	1
2+	21	10.45	1

1	32	15.92	1
0	97	48.26	0
Ki-67			
<10%	6	2.99	0
10% - 20%	34	16.92	3
>20%	139	69.15	0
Triple Negative	35	17.41	0
Multifocal	11	5.74	1
Grade			
1	22	10.95	0
2	123	61.19	2
3	55	27.36	1
Stage ǂ ǂ			
1	19	9.45	0
2	127	63.18	2
3	55	27.36	1
Pathologic Response to NACT			
Complete	62	30.85	0
Partial	111	55.22	1
No Response	24	11.94	2
Progressive	3	1.49	0

CDP, cancer diagnosis probe; ER, estrogen receptor; Hx, history; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; NCAT, neoadjuvant chemotherapy; PR, progesterone receptor.

ǂ HER2-positive tumors with an immunohistochemistry (IHC) score of 2+ that demonstrate gene amplification by either fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH) are reclassified as HER2 3+

ǂ ǂ Based on 8th edition of AJCC TNM staging system

2.5 | Statistical analysis

Intraoperative CDP results of 201 patients (mean age, 45 years; SD, 10.2) were as follows:

- True positives: 7 patients had positive CDP findings confirmed by permanent pathology; subsequent cavity shaving yielded negative margins.
- False positives: 67 patients had positive CDP findings (hypoxia detected), but permanent pathology showed no malignant cells (negative margins)
- True negatives: 124 patients had negative CDP findings confirmed by permanent pathology.
- False negatives: 3 patients (1.49%) had negative CDP findings but positive margins on final pathology.

3 | RESULTS

Using Stata statistical software with Monte Carlo simulation and beta distribution modeling, the negative predictive value (NPV) was 97.8% (95% CI: 94.4–99.5) and the reoperation rate was (1.49%, 95% CI: 0.49–5.6). Based on these findings, intraoperative CDP demonstrates high NPV

and a low reoperation rate, supporting its utility as a superior margin assessment tool in post-NACT patients undergoing BCS.

4 | DISCUSSION

4.1 | Margins After Neoadjuvant Therapy: Clinical Obstacles and Practical Solutions

Achieving negative margins in post-NACT patients undergoing BCS presents a unique challenge. Evidence supports that a “no ink on tumor” margin is sufficient, and wider margins do not confer additional benefit in terms of local recurrence-free survival, disease-free survival, or overall survival.[9]

While frozen section analysis performs well in non-NACT patients, its false-negative rate increases significantly following neoadjuvant chemotherapy, leading to higher rates of positive surgical margins and subsequent reoperations.[6,10,11,12] Therefore, there was a substantial need for alternative intraoperative margin assessment tools. Histolog® Scanner (SamanTree Medical SA, Switzerland); a large field-of-view confocal laser scanning microscope designed to generate intraoperative histological images, enabling margin assessment directly within the operating room. In non-NACT patients, it has demonstrated a NPV of 86.2% ± 1.2%.[22] MarginProbe® (Dilon Technologies, USA); utilizes dielectric spectroscopy to assess excised tissue margins within 20 minutes of resection. It applies an electric field to the tissue through a sensor mounted at the tip of the probe and analyzes the reflection over a wide range of RF frequencies. In a study of 214 post-NACT patients undergoing BCS, it achieved a re-excision rate of 6%.[23]

Cancer Diagnostic Probe (CDP)® (Nano Hesgar Sazan Salamat Arya Lab, Iran); detects reactive oxygen species (ROS) generated via glycolysis and the reverse Warburg effect in hypoxic tumor cells.[19] CDP guides cavity shaving until hypoxic tissue is no longer detected. In this study, CDP achieved the highest NPV (97.8%) and lowest reoperation rate (1.49%) among currently available commercial intraoperative margin assessment tools for post-NACT patients undergoing BCS.

The tumor microenvironment (TME) comprises the cellular and structural milieu surrounding the tumor, including malignant cells, fibroblasts and cancer associated fibroblasts (CAFs), immune cells (such as T cells, B cells, natural killer cells, and tumor-associated macrophages), blood vessels, signaling molecules, and the extracellular matrix.[24] TME in solid tumors frequently exhibits hypoxic characteristics, which play a critical role in tumor cell metabolism and resistance to apoptosis.[25] CAFs contribute to extracellular matrix deposition and remodeling, leading to vascular compression and impaired perfusion—key drivers of intratumoral hypoxia. Through the combined induction of extracellular matrix stiffness, hypoxia, and epithelial-to-mesenchymal transition, CAFs facilitate tumor progression and therapeutic resistance.[26]

In our cohort, approximately one-third of patients had positive CDP findings without pathologic confirmation. This may reflect CDP’s detection of hypoxic TME rather than malignant cells, explaining its low specificity 64.9% (95% CI, 58.3-71.6) and positive predictive value 9.2% (95% CI, 4.1-16.7). CDP’s ROS-based detection encompasses both tumor and surrounding hypoxic stroma, potentially enabling more complete margin clearance. An important question that emerges is whether resection of the hypoxic TME, alongside the primary tumor mass, can lead to improvements in disease-free survival (DFS) or overall survival (OS)? A prospective study is

currently underway at our center to investigate the molecular and biological characteristics of CDP-positive, histologically negative tissue specimens.

Limitations of CDP include its random application within the tumor cavity and dependence on the operator's ability to visually identify abnormal tissue. Increasing the number of probe applications and directing them toward grossly suspicious areas may enhance diagnostic accuracy. Moreover, not all breast cancer subtypes exhibit hypoxia, which may affect CDP's sensitivity. Future studies should evaluate the prevalence and distribution of hypoxia across various histologic subtypes and tumor grades to better inform CDP utilization based on preoperative biopsy findings.

4.2 | Financial Burden

Population-based studies have consistently reported reoperation rates after breast-conserving surgery of approximately 19–21%, including nationwide analyses from England, the United States Medicare population, and provincial data from Alberta.[27,28,29] In a single-center cost study by Grant et al. within the UK NHS, patient-level PLICS data indicated a median re-excision cost of £4,511—approximately £2,136 higher than for patients whose initial BCS was definitive.[30] A decision-analytic model by Pataky and Baliski, conducted in the single-payer system of British Columbia, demonstrated that patients undergoing a single definitive BCS incurred an average pathway cost of CAD 8,225, whereas scenarios involving re-operation increased total costs to as much as CAD 26,026.[31] Using private insurance claims from the United States, Metcalfe et al. demonstrated that any repeat surgery within 90 days of BCS increased mean two-year healthcare expenditures by $16,072, raising total costs from $89,016 in patients without re-operation to $105,088 in those requiring at least one additional procedure. The cost increase was $11,621 for repeat BCS and $26,276 for conversion to mastectomy.[32] Wing et al. further showed that in a cohort of 8,869 patients, 25% required re-operation after initial lumpectomy. Median one-year costs increased from $55,985 for patients undergoing a single procedure to $63,416 for repeat lumpectomy and $87,961 for those converted to mastectomy.[33] Collectively, these findings indicate that re-operation after BCS typically incurs approximately $4,000–$5,000 in direct surgical costs and can add tens of thousands of dollars in pathway-level expenditures over one to two years. In contrast, intraoperative CDP application added only a few additional minutes to the index operation time and incurred an incremental expense of approximately $50–$100, depending on the institution. So, reducing reoperations not only alleviates patient anxiety, enhances quality of life, and improves cosmetic outcomes, but also yields direct cost savings per avoided reoperation.

5 | CONCLUSION

Intraoperative CDP demonstrates high negative predictive value and a remarkably low reoperation rate in post-neoadjuvant chemotherapy patients undergoing breast-conserving surgery, positioning it as a promising margin assessment tool. Its ROS-based detection mechanism enables identification of hypoxic tumor microenvironment components beyond malignant cells, potentially contributing to more comprehensive margin clearance. It also results in substantial time and cost savings, at the expense of only a few additional minutes added to the index operation and minimal costs when compared with those associated with a reoperation.

List of abbreviations

BCS: Breast Conserving Surgery

CAD: Canadian Dollar

CAF: Cancer Associated Fibroblasts

CDP: Cancer Diagnostic Probe

DFS: Disease-free Survival

NACT: Neoadjuvant Chemotherapy

OS: Overall Survival

ROS: Reactive Oxygen Species

TME: Tumor Microenvironment

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AUTHOR CONTRIBUTIONS

Mansour Nateghi: Formal analysis, Data curation, Writing, review & editing
Mohammad Esmaeil Akbari: Conceptualization, Formal analysis, Review & editing, Supervision
Mohammad Abdolahad: Review & editing
Afsaneh Momtaz: Data curation, Writing
Zohreh Sadat Miripour: Writing
Marzieh Kamarehee: Formal analysis
Mohammad Akbari: Writing
Atieh Akbari: Review & editing
Morteza Yousefi: Data curation

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest related to this study.

CONSENT TO PUBLISH DECLARATION:

Not applicable.

DATA AVAILABILITY STATEMENT

The original data generated in this study are available from the corresponding authors upon reasonable request and in accordance with patient protection laws.

ETHICS APPROVAL

The study protocol was reviewed and approved by the Ethics Committee of Cancer Research Center of Shahid Beheshti University of Medical Sciences (approval number: IR.SBMU.CRC. REC. 1403.026). This research was explained for followed-up patients. All of them agreed to use their information in this study and written informed consent was obtained from participants.

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