Research Article

Clinical Applications of Liquid Biopsy in Women with High Risk of Developing Breast Cancer

Nitya Sarvesha

Mission San Jose High School, 41717 Palm Ave, Fremont, CA 94539, USA

ABSTRACT

In recent years, advancements in breast cancer detection and treatment have been significant. However, critical gaps remain, highlighting the need for more accurate diagnostic tools. Liquid biopsies are emerging as a promising solution for cancer detection. These noninvasive tests can be performed more frequently than traditional biopsies and provide a comprehensive assessment of the tumor through a single blood sample. To ensure their clinical application, improvements in sensitivity and accuracy are essential. We reviewed recent studies on the use of liquid biopsies in women with both early-stage and metastatic breast cancer.

Keywords: Breast cancer; liquid biopsy; PCR; biomarkers; high risk

INTRODUCTION

Breast cancer is among the most common cancers globally, with around 240,000 new cases and 42,000 deaths annually in the U.S. (2). Given its prevalence, women aged 50–74 are advised to undergo biennial mammograms (2). Screening has significantly improved early detection, catching many cases before metastasis. However, mammograms carry risks like overdiagnosis, which can lead to unnecessary treatments and affect reporting accuracy (1). Recent studies indicate that early screening and advanced therapies improve early breast cancer prognosis (5). Treatment varies by cancer type, with options like neoadjuvant therapy, endocrine therapy, anti-HER2 targeting, and chemotherapy (5). Emerging immunotherapies also show promise for advanced breast cancer.

Breast cancer's complexity makes comprehensive diagnosis challenging, as tissue biopsies often fail to capture the tumor's heterogeneity. Liquid biopsy is a promising alternative, analyzing blood biomarkers for genetic information on tumors and their therapy response (7). This method involves sampling bodily fluids like blood, urine, or spinal fluid (Figure 1) to detect cancer cells or tumor-derived DNA/RNA (8). Techniques include circulating tumor DNA (ctDNA) analysis, derived from dying tumor cells, and circulating tumor cells (CTCs), analyzed via next-generation sequencing or PCR (9). Liquid biopsies are noninvasive and can be conducted more frequently than tissue biopsies, providing a fuller tumor profile through a single blood test (11). Already established in lung cancer diagnostics, liquid biopsy may soon see broader application in breast cancer. Complementary tools, like the Therascreen PIK3CA PCR assay, aid in detecting mutations such as PIK3CA, enhancing liquid biopsy's role in treatment (10). This technology currently

Corresponding author: Nitya Sarvesha, E-mail: nityasarvesha@gmail.com.

Received August 29, 2024**; Accepted** November 13, 2024
https://doi.org/10.70251/HYJR2348.247281

achieves 60–80% accuracy and is driving a projected 16% market growth from 2020 to 2030 (3).

Liquid biopsies hold great potential for cancer detection and management, though certain limitations must be addressed to optimize their use. Currently, liquid biopsies lack sensitivity for early-stage cancer detection, limiting their reliability and widespread adoption (3). Another challenge is their precision, as they may not accurately distinguish cancer types or detect gene alterations in early stages due to low ctDNA levels and insufficient DNA shed from small tumors (6). Additionally, the absence of standardized assays creates inconsistencies in results, highlighting a need for standardization to improve accuracy and reproducibility (7). Despite these challenges, the potential benefits of liquid biopsies justify efforts to overcome these limitations. This review explores the clinical applications of liquid biopsy for women at high risk of developing breast cancer.

METHODS

To find reliable, officially published journals, well-established databases like PubMed and ScienceDirect were used, with articles selected based on criteria including publication within the last five years and availability in English. Only studies focused on human samples and original research were chosen, ensuring each paper presented outcomes relevant to the topic. Keywords such as "liquid biopsy," "breast cancer," and "high risk" guided the search to identify pertinent studies. Articles were then narrowed to those most closely aligned with the research topic, specifically the application of liquid biopsies for women at high risk of developing cancer.

RESULTS

Methods

The research data revealed several patterns (Table 1). Primarily, most samples were from patients with metastatic or advanced breast cancer, with fewer from early-stage cases. While methods varied across studies, PCR (polymerase chain reaction) was a commonly used technique, particularly droplet digital PCR and qPCR. The studies focused on assessing and enhancing the efficiency of current liquid biopsy technology for breast cancer detection, with experiments testing various aspects of its effectiveness and potential improvements in its application.

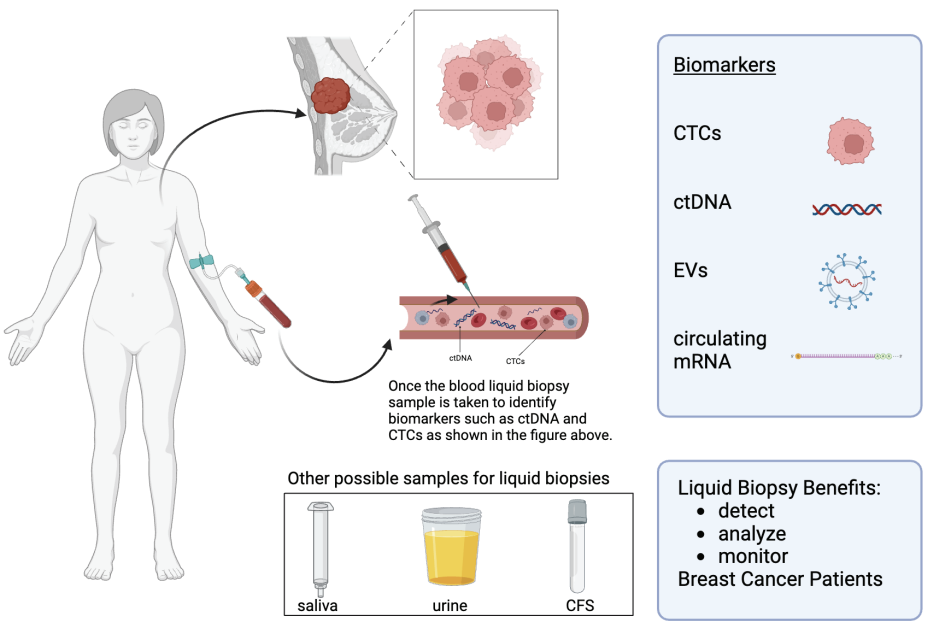

Figure 1. The figure shows the different possible samples that can be taken for a liquid biopsy. These samples include saliva, urine, and CFS. There are also multiple possible biomarkers such as CTCs, ctDNA, EVs, and circulating mRNA. These biomarkers are detected and used after being found from the samples taken. The liquid biopsy was then able to detect, analyze, and monitor breast cancer patients.

Table 1. Data from Original Research Done on Liquid Biopsy and Breast Cancer

Reference	Purpose	Marker	Target Genes	Method	Cancer Stage	Sample size
12	Earlier detection of triple-negative breast cancer (TNBC) by noninvasive detection of aberrant DNA methylation	cfDNA	SPAG6, LINC10606 and TBCD/ZNF750 genes	Differentially methylated regions of plasma-derived circulating cell-free DNA	Stage 1	
13	Molecular characterization of CTCs aids in understanding therapy resistance and enables targeted treatments over time.	CTCs	EpCAM and ESR1	comprehensive liquid biopsy analysis	Stage 1	13
14	Characterize the tumor evolutionary landscape and identify potential vulnerabilities in the relapsed setting	.	ESR1, NF1, RB1, ERBB2, PIK3CA, HRAS/NRAS/KRAS, FGFR1/2/3, BRAF, FGFR1/2, ERBB2, RET	examine patients profiled with tissue (TBx) and liquid biopsy (LBx)	Stage 3/4	
15	COGNITION empowers new therapy options for high-risk early breast cancer patients in a biomarker-driven, post-neoadjuvant trial, aiming to test precision oncology's impact on cure rates.	tumor tissue	breast cancer genes	Whole-genome/ exome and transcriptome sequencing	Stage 1	255
16	High levels of circulating methylated DNA are associated with subsequent disease progression in women with metastatic breast cancer	CM	cumulative methylation (CM) levels	A novel LBx-BCM assay detects breast cancer methylation via the GeneXpert system.	all stages	144
17	The effectiveness of highly sensitive and affordable digital PCR and SafeSEQ sequencing	ctDNA	PIK3CA , ESR1, TP53, and KRAS	digital PCR and SafeSEQ sequencing	Stage 3/4 or relapsed	180
18	The effective of liquid biopsy to detect PIK3CA mutations in patients with breast cancer.	ctDNA	PIK3CA	droplet digital PCR	PIK3CA mutation	52
19	To clarify the detection effect of liquid biopsy in breast cancer.	CTC	HER2 expression on CTC	peripheral blood of HER2-positive patients. CTCs were isolated and analyzed.	HER-2 positive patients	198

Continued Table 1. Data from Original Research Done on Liquid Biopsy and Breast Cancer

Reference	Purpose	Marker	Target Genes	Method	Cancer Stage	Sample size
20	To examine the clinical relevance of using multiple LBAs.	CTC mRNA, CTC gDNA, EV mRNA, cfDNA	ERBB2	qPCR	hormone receptor-positive, HER2-negative MBC patients	26
21	The aim is to assess cyclooxygenase-2 (COX-2) gene expression in breast cancer patients treated at the CUS-ABC/ FMABC outpatient clinic.	extracellular RNA	COX-2	PCR	all stages	34
22	This study examines nipple secretion protein composition and its potential as a liquid biopsy.	NAF	breast cancer genes	semiquantitative proteomic profiling and trypsin-digested peptides analyzed using 2D-LC Orbitrap Fusion MS	all stages	15
23	The effectiveness of palbociclib and bazedoxifene on heavily pretreated patient population with advanced HR+/HER2- breast cancer.	PFS	PIK3CA and ESR1	phase Ib/II study of bazedoxifene plus palbociclib	Stage 3/4	36
24	The effectiveness of sTKa in patients with HR+/ HER2- ABC treated with ribociclib plus letrozole as first-line therapy.	sTKa and PFS	HER2	sera from postmenopausal HR+, HER2- ABC patients treated with ribociclib and letrozole at baseline,	Stage 4	287
25	Is the screening assay developed suited for monitoring ESR1 mutations in the plasma of MBC patients?	cfDNA	ESR1	naica® three-color digital PCR platform	all stages	109
26	Is the addition of miR-923 and CA 15-3 information helpful?	microRNA	breast cancer genes	droplet digital polymerase chain reaction	all stages	30
27	What are new biomarkers that enable detection of systemic recurrences at the molecular level?	CTC, ctDNA, miRNA	breast cancer genes	liquid biopsies	Stage 1	1455

Continued Table 1. Data from Original Research Done on Liquid Biopsy and Breast Cancer

Reference	Purpose	Marker	Target Genes	Method	Cancer Stage	Sample size
28	Can LBx-BCM detect sensitivity and monitor therapeutic response in advanced breast cancer?	cMethDNA	breast cancer genes	DNA methylation detection cartridge assay	Stage 4	11
29	To characterize the size, stability, and cftDNA content of cfDNA in ascites.	cfDNA	IFFO1 and HOXA9	qPCR	ovarian cancer	18
30	Is molecular characterization of CTCs and miR profiling of serial samples useful in locally advanced breast cancer during neoadjuvant chemotherapy?	CTC, miRNA	ALDH1	NGS	Stage 3	
31	Is CTC count a promising modality in monitoring palbociclib response?	CTC	RB1 and GAPDH	ddPCR	Stage 4	46
32	The efficacy of trastuzumab-emtansine (T-DM1) in HER2-negative metastatic breast cancer (MBC) patients with HER2-positive CTC	CTC	HER2/CEP17	T-DM1 monotherapy	Stage 4	154
33	To test if CTCs from metastatic breast cancer patients treated with eribulin can predict eribulin efficacy by suppressing EMT.	CTC	EPCAM	Microfluidic Chip device	Stage 4	22
34	To test the feasibility of unbiased quantitative and reproducible assessment of treatment targets on CTCs.	CTC for liquid biopsy	HER2	FITC fluorescence of leukocytes	all stages	191
35	Pilot study on breast cancer detection using blood plasma analyzed by Fourier-transform infrared (FTIR) spectroscopy	blood plasma	breast cancer genes	FTIR spectroscopy	all stages	26
36	How can liquid biopsies be useful for personalized medicine?	cfDNA	TP53, PIK3CA	liquid biopsies	multiple cancers	99

Continued Table 1. Data from Original Research Done on Liquid Biopsy and Breast Cancer

Reference	Purpose	Marker	Target Genes	Method	Cancer Stage	Sample size
37	To clarify the prognostic significance of baseline cCSCs for metastatic breast cancer in terms of first-line chemotherapy.	CTC, cCSC	EPCAM, CD133	negative selection, flow cytometry, univariate and multivariate analyses	Stage 4	48
38	To determine the tissue distribution of EGCG and its effects on cell proliferation and biomarkers in breast cancer patients.	EGCG	breast cancer genes	GSP 300 mg, equivalent to 44.9 mg of EGCG, daily for 4 weeks prior to surgery.	Stage 1/2	12
39	Early changes in CTCs and ctDNA were explored as noninvasive tools, alongside tumor biopsies, to assess pharmacodynamics and early efficacy.	ctDNA, CTC	ESR1	droplet digital PCR	Stage 4	43
40	Clinical-risk stratification with the 21-gene recurrence score identifies premenopausal women who may benefit from more effective therapy.	HR+, HER2-, axillary node-negative breast cancer	21 genes	assay of 21 genes	all stages	9427
41	In the current study, we developed a cell-free DNA (cfDNA) methylation liquid biopsy for the risk assessment of early-stage HGSOC	cfDNA	HGSOC genes	OvaPrint	ovarian cancer	372

Breast Cancer Subtypes

Liquid biopsy results varied across breast cancer subtypes, making it a valuable tool for detecting unique mutations and biomarkers. In luminal A breast cancer, liquid biopsies target gene mutations linked to hormone signaling due to ER and PR expression and low HER2 levels. For luminal B, which has higher proliferation, they may detect alterations tied to faster tumor growth. In HER2+ subtypes, liquid biopsies focus on HER2 gene amplification, while triple-negative subtypes are monitored for specific mutations due to the lack of ER, PR, and HER2. Data showed that ER+, luminal B, and HER2+ subtypes were most frequently tested using liquid biopsies.

Breast Cancer Drugs

Breast cancer treatment includes multiple FDA-approved drugs that block hormones from signaling cancer cells to grow and divide. Additionally, several FDA-approved assays, especially those using next-generation sequencing (NGS), assist in breast cancer diagnosis, as shown in the Table 2. Most assays target metastatic or advanced breast cancer. The samples used are largely prospective, observing current cancer patients to evaluate outcomes, though some studies also use retrospective samples.

Table 2. Current Breast Cancer Drugs that are FDA-Approved

Test	Company	Cancer Type	Biomarkers	Intended Use	Technology	Year	Matrix
CT enumeration CellSearch®	Menarini Silicon Biosystems	Metastatic Breast	ÇTC detection (CK*/DAPI*/ CO45)	Prognostic significance	Ep-CAM based CIC enrichment & CIC detection with IF	2004	Whole blood
therascreen® PIK3CA RGQ PCR Kit	Qiagen	Advanced or Metastatic	PIK3CA mutations	CDx for alpelisib (PIQRAY, Novartis)	real time PCR	2019	Plasma
FoundationOne* Liquid CDx	Foundation One	Advanced or Metastatic	PIK3CA mutations	CDx for alpelisib (PIQRAY, Novartis)	NGS	2020	Plasma
Guardant360 CDx assay	Guardant Health	All Solid Cancers (including breast)	SNVs. Indels, amplifications, and fusions in 55 genes	Comprehensive genomic profiling (CGP)	NGS	2020	Plasma
CTC isolation / enrichment	ANGLE	Metastatic	Different biomarkers	CTC isolation	size-based enrichment microfluidics	2022	Whole blood
Guardant360 CDx assay	Guardant Health	Advanced or Metastatic	ESR1 mutations	CDx for elacestrant (ORSERDU, Menarini)	NGS	2023	Plasma

DISCUSSION

The study aimed to evaluate the current use of liquid biopsies for women with breast cancer, focusing on potential improvements for detecting and analyzing those at high risk. Findings suggest that increased sensitivity could be achieved by using multi-modal assays with multiple biomarkers. Most research emphasizes improving liquid biopsy efficiency, and combining biomarkers could advance early-stage breast cancer detection. The importance of preanalytical factors—such as blood tube collection, DNA extraction, and library preparation—was also highlighted. Research indicates that late-stage cancers have higher tumor fractions, while early-stage tumors often show less than 1% ctDNA, underscoring the need for greater sensitivity. The field has shifted from PCR-based approaches to next-generation sequencing, which offers higher sensitivity by sequencing multiple samples simultaneously. In the coming years, advancements in technologies like DNA sequencing and metabolomics should improve the sensitivity of circulating tumor cells (CTCs), increasing liquid biopsy reliability for detecting breast cancer in high-risk women.

FUNDING SOURCES

No funds were received to conduct the research or to prepare the paper.

DECLARATION OF CONFLICT OF INTERESTS

The author(s) declare that there are no conflicts of interest regarding the publication of this article

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