Review Article

Liquid biopsy in breast cancer: a practical guide for surgeons

Janhavi Venkataraman^, Timothy Crook, Kefah Mokbel

The London Breast Institute, Princess Grace Hospital, HCA Healthcare pvt Ltd., London, UK

Contributions: (I) Conception and design: K Mokbel, T Crook; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: J Venkataraman; (V) Data analysis and interpretation: K Mokbel, T Crook; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Janhavi Venkataraman, MBBS, MS (Gen Surgery), MCh (Plastic Surgery), MRCS. The London Breast Institute, Princess Grace Hospital, HCA Healthcare pvt Ltd., 42-52 Nottingham Place, London, W1U 5NY, UK. Email: Janhavi.Venkataraman@hcahealthcare.co.uk.

Abstract: Breast cancer remains a global health challenge, requiring innovative strategies for early detection, diagnosis, treatment monitoring, and recurrence detection. Liquid biopsy—leveraging circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), microRNAs (miRNAs), exosomes, immune-based biomarkers, and tumor-educated platelets (TEPs)—has emerged as a promising tool to address these needs. CTCs and ctDNA provide critical insights into tumor heterogeneity, therapeutic targets, and resistance mechanisms, while miRNAs, exosomes, and other non-CTC-based markers reflect the tumor microenvironment and offer potential biomarkers for disease progression. Importantly, liquid biopsy offers distinct advantages in early detection and precise diagnosis, as well as in identifying therapeutic resistance in real time, allowing clinicians to adapt treatment strategies effectively. The non-invasive nature of liquid biopsy further enables real-time tumor monitoring, paving the way for personalized treatment approaches. However, several challenges hinder its routine clinical adoption, including technical complexity, economic constraints, and variations in detection sensitivity due to low biomarker abundance. Additionally, a lack of standardization in methodology and interpretation limits its widespread application. Rigorous standardization and clinical validation are essential to address these barriers, ensuring equitable access across diverse healthcare settings and transforming breast cancer care for millions worldwide. Future directions include integrating artificial intelligence and multi-omic approaches to enhance diagnostic accuracy and clinical utility.

Keywords: Liquid biopsy; circulating tumor cells (CTCs); circulating tumor DNA (ctDNA); microRNAs (miRNAs); exosomes

Submitted Jan 10, 2025. Accepted for publication Apr 17, 2025. Published online Apr 25, 2025.
doi: 10.21037/gs-2025-11
View this article at: https://dx.doi.org/10.21037/gs-2025-11

Introduction

Liquid biopsy represents a transformative approach in oncology, offering a non-invasive method to analyze tumor dynamics through biomarkers present in bodily fluids. In breast cancer, its utility spans diagnosis, monitoring treatment response, guiding therapy, and detecting recurrence. This article explores the role of circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), microRNAs (miRNAs), exosomes, and other emerging biomarkers in advancing breast cancer management.

CTCs

CTCs are tumor cells that detach from the primary tumor at an early stage and enter the bloodstream, correlating with tumor stage, metastasis, and poor prognosis (1). Enumeration of CTCs is Food and Drug Administration (FDA)-approved as a prognostic tool in metastatic breast cancer. Beyond enumeration, molecular profiling of CTCs enables the identification of actionable targets and resistance mechanisms, facilitating personalized therapy (1-4).

^ ORCID: 0000-0002-4924-6680.

CTC clusters are groups of 2–50 tumor cells traveling together in the bloodstream, often accompanied by non-cancerous cells such as immune or stromal cells. CTC clusters composed solely of tumor cells are termed homotypic; those containing both tumor and non-tumor cells are heterotypic (5). While individual CTCs are more commonly used in clinical practice for diagnosis and monitoring due to their higher prevalence and established utility, particularly in metastatic breast cancer, CTC clusters provide additional prognostic information. They are particularly relevant in aggressive subtypes and resistant disease, making them a focus of research but less practical for routine monitoring.

Techniques for CTC detection

- Immunomagnetic enrichment (CellSearch System): FDA-approved for CTC enumeration with a cut-off level at 5 CTCs per 7.5 mL of whole blood in metastatic breast cancer. However, it may miss CTCs undergoing epithelial-to-mesenchymal transition (EMT) (1). The established cut-off values for CTC detection—one CTC per 7.5 mL of blood for early breast cancer and five CTCs for metastatic disease—are based on the biological and clinical significance of tumor cell dissemination. In early breast cancer, even a single detectable CTC suggests minimal residual disease (MRD) and a potential risk of recurrence, warranting closer monitoring or escalation of therapy. Conversely, in metastatic breast cancer, a higher threshold is used because the tumor burden is significantly higher, and ≥5 CTCs correlate with worse prognosis, faster disease progression, and reduced overall survival. These cut-offs are supported by large clinical studies demonstrating their prognostic value, making them critical markers for risk stratification and treatment decisions in breast cancer patient.
- Microfluidic platforms: capture CTCs based on size, deformability, or surface markers, offering high sensitivity and specificity (2).
- Molecular methods [e.g., reverse transcription polymerase chain reaction (RT-PCR)]: detect CTC-specific transcripts like CK19 but lack enumeration capability.
- Label-free techniques: use properties like electrical charge to isolate diverse CTC populations without marker bias (3).
- Multiplexed fluorescence immunocytochemistry (ICC) profiling: this method detects CTCs by simultaneously analyzing multiple markers, including GCDFP15, GATA3, EpCAM, PanCK, and CD45, to distinguish tumor cells from normal blood cells. It allows for comprehensive molecular characterization of CTCs, supporting both detection and profiling for therapy guidance.

In a prospective clinical study conducted by our team, the test (TruCheck) demonstrated 93.1% specificity and 94.64% overall sensitivity in differentiating early breast cancer cases from benign breast conditions (4). Notably, it was also effective in detecting ductal carcinoma in situ (DCIS).

While CellSearch remains the clinical standard, microfluidic platforms and label-free approaches show promise due to their sensitivity and versatility.

ctDNA

ctDNA comprises fragmented tumor DNA released into the bloodstream, reflecting tumor burden in real time. Its analysis can detect actionable mutations [e.g., Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA), estrogen receptor-1 (ESR1)], monitor treatment response, and, in the case of tumor-informed digital PCR, predict relapse earlier than imaging (6).

Tumor-informed versus tumor-agnostic ctDNA approaches

ctDNA assays can be broadly categorized into tumor-informed and tumor-agnostic approaches:

(I) Tumor-informed ctDNA
- These assays require prior knowledge of a patient's tumor-specific mutations, typically obtained through sequencing of the primary tumor tissue;
- They track a personalized set of mutations, improving sensitivity and specificity for detecting MRD and recurrence;
- Example: Signatera™ (by Natera) is a tumor-informed ctDNA assay used in oncology.

(II) Tumor-agnostic ctDNA
- These assays do not require prior tumor sequencing and instead detect commonly occurring mutations in a panel of cancer-associated genes;
- They can be used for early detection, treatment monitoring, and identifying actionable

mutations for targeted therapy;
- ❖ Example: Guardant360® is a tumor-agnostic ctDNA assay used for comprehensive genomic profiling.

Detection techniques

- ❖ Digital PCR (dPCR): offers high sensitivity for known mutations.
- ❖ Next-generation sequencing (NGS): provides comprehensive profiling of genomic alterations.
- ❖ BEAMing and epigenetic analysis: focus on rare mutations or tumor-specific methylation (7).

FDA-approved ctDNA-based assays, such as Guardant360 CDx and FoundationOne Liquid CDx, guide targeted therapies in cancers, including breast cancer (8).

Fragmentomics and methylation patterns

Recent advances in liquid biopsy have expanded beyond mutation detection to include fragmentomics and methylation analysis. Fragmentomics analyzes the fragmentation patterns of cell-free DNA, as tumor-derived DNA fragments often exhibit distinct size distributions and end motifs compared to normal cell-free DNA. These fragmentation signatures can serve as cancer biomarkers even in the absence of detectable mutations (9).

Methylation patterns in ctDNA offer another layer of cancer detection and classification. Tumor-specific DNA methylation alterations occur early in cancer development and remain stable during disease progression. Genome-wide methylation profiling of ctDNA can detect cancer-specific epigenetic signatures with high sensitivity and specificity, potentially improving early detection capabilities. Technologies like methylation-specific PCR, bisulfite sequencing, and array-based methods are being utilized to analyze these patterns (10).

Both fragmentomics and methylation analysis represent promising approaches that may complement conventional mutation-based liquid biopsy strategies, especially for early-stage cancer detection where mutational burden may be low.

miRNAs

miRNAs are small, non-coding RNAs that regulate gene expression and reflect tumor characteristics.

Detection techniques

- ❖ qRT-PCR: gold standard for sensitivity and specificity.
- ❖ NGS: comprehensive profiling of known and novel miRNAs.
- ❖ Droplet digital PCR (ddPCR): quantifies low-abundance miRNAs effectively.

Several miRNAs, including miR-103a-3p, miR-145, and miR-451, have shown promise in early breast cancer detection. However, an eight-miRNA panel demonstrated superior performance, with area under the curve (AUC), accuracy, sensitivity, and specificity of 0.915, 82.3%, 72.2%, and 91.5%, respectively. The model successfully detected breast cancer across Caucasian and Asian populations, achieving AUCs from 0.880 to 0.973, including pre-malignant lesions (stage 0; AUC 0.831) and early-stage cancers (stages I–II; AUC 0.916) (11).

Serum levels of miR-17-5p, miR-125a, miR-125b, miR-200a, let-7a, miR-34a, miR-21, miR-99a, and miR-497 have demonstrated predictive and prognostic significance in breast cancer (12).

Despite their diagnostic and prognostic potential, miRNA-based assays have yet to receive FDA approval.

Exosomes

Exosomes are small vesicles carrying tumor-derived molecular cargo, such as proteins and nucleic acids. They reflect the molecular profile of breast cancer and can aid in diagnosis, therapy monitoring, and recurrence detection (13).

Detection techniques

- ❖ Nanoparticle tracking analysis (NTA): measures exosome size and concentration.
- ❖ Flow cytometry: identifies exosome surface markers like CD63 and CD81.
- ❖ RNA sequencing: analyzes RNA cargo, including miRNAs. Exosomal miR-1910-3p has recently been identified as one of the most promising miRNAs for breast cancer diagnosis, demonstrating high sensitivity (88%) and specificity (76%) (14).

While certain exosomes show high sensitivity and specificity as cancer biomarkers, their clinical utility requires validation through large-scale studies. Moreover, the lack of standardized isolation and characterization methods hinders

research reproducibility and scalability, despite techniques like ultracentrifugation and size exclusion. Exosome-based liquid biopsy has yet to achieve FDA approval, but its potential for early detection and monitoring therapy response is significant.

Other blood-based biomarkers

Circulating immune-based biomarkers

The immune system's response to cancer provides valuable biomarkers for liquid biopsy. Circulating immune cells, particularly T-cells and natural killer cells, change in number and phenotype during cancer progression. Study has shown that specific T-cell receptor repertoires and immune checkpoint molecule expressions [such as programmed death protein-1 (PD-1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4)] in peripheral blood can indicate breast cancer presence and predict treatment response to immunotherapies (15).

Tumor-educated platelets (TEPs)

Platelets can sequester tumor-derived RNA and proteins, becoming "educated" by their interaction with tumor cells. These TEPs exhibit altered RNA profiles that can be detected through RNA sequencing and other molecular techniques. Research has demonstrated that TEP RNA signatures can distinguish cancer patients from healthy individuals with high accuracy and can even discriminate between different cancer types (16). In breast cancer, specific TEP signatures correlate with disease stage and molecular subtypes, offering a promising approach for non-invasive diagnostics.

Protein biomarkers

Beyond the traditional serum markers [carcinoma antigen 15-3 (CA 15-3), carcinoembryonic antigen (CEA)], novel proteomic approaches are identifying specific protein signatures in plasma that correlate with breast cancer. Mass spectrometry-based techniques and protein microarrays have identified panels of proteins with improved sensitivity and specificity compared to single markers. These protein signatures can reflect tumor burden, treatment response, and even resistance mechanisms (17).

Clinical applications in breast cancer

Diagnosis

Liquid biopsy detects CTCs and tumor-derived biomarkers like ctDNA or specific miRNAs, complementing imaging modalities for early cancer detection (4,11). This approach is particularly valuable for women with dense breasts, indeterminate mammographic findings or those reluctant to undergo screening mammography (4).

In a prospective clinical study conducted by our team, the test (TruCheck) demonstrated 93.1% specificity and 94.64% overall sensitivity in differentiating early breast cancer cases from benign breast conditions (4). Notably, it was also effective in detecting DCIS.

A key challenge emerges when a positive liquid biopsy is not corroborated by imaging findings. Evidence suggests that in such cases, breast cancer often becomes detectable within the following two years (4).

It should be noted that the GRAIL's Galleri introduced the pan-cancer screening test based on methylation profiling in ctDNA (4). However, the Galleri test has very low sensitivity (<10–16%) for stage I breast cancer with no data on its ability to detect DCIS (4). Similarly, the CancerSEEK test, based on simultaneous evaluation of serum proteins and gene variants, has ~40% cumulative sensitivity for early stage and overall ~30% sensitivity (4).

Prospective clinical trials are underway to further validate the clinical utility of ctDNA analysis in early-stage breast cancer, potentially integrating it into routine screening and monitoring protocols. Such tests must accurately detect small, early-stage tumors (stage 0 and stage Ia) with high specificity and sensitivity and at a low cost to serve as a viable alternative to digital mammography.

Currently, no liquid biopsy test is FDA-approved for breast cancer screening. While some liquid biopsies are authorized for detecting genetic mutations in other cancers, their use in early breast cancer detection remains under investigation. Multi-cancer early detection (MCED) tests, like GRAIL's Galleri, analyze ctDNA and may include breast cancer, but they are not yet standard for screening.

Monitoring therapy response

Declining ctDNA levels post-treatment correlate with positive outcomes. Similarly, miRNA profiles indicate

sensitivity or resistance to therapies.

Personalizing treatment

Liquid biopsy identifies actionable mutations in ctDNA, such as PIK3CA ESR1, serine-threonine protein kinase (AKT1) and phosphatase and tensin homolog (PTEN), guiding the selection of targeted therapies like alpelisib, elacestrant, and capivasertib, respectively. Both tumor-informed and tumor-agnostic approaches are utilized, with tumor-informed methods requiring prior knowledge of the primary tumor's genetic profile, while tumor-agnostic methods screen for common cancer-associated mutations without prior tumor information (18).

Moreover, consecutive comprehensive tumor profiling using ctDNA, as demonstrated by our team, can reveal ongoing tumor evolution, enabling the identification of novel, effective therapeutic strategies that may outperform empirical treatment options based on existing clinical guidelines in patients with metastatic breast cancer (19,20).

Detecting recurrence

Rising levels of ctDNA or tumor-specific miRNAs can signal relapse months before radiological evidence, enabling timely intervention (21). Clinical trials are needed to determine whether therapeutic intervention at this stage improves disease outcomes.

Clinical guidelines and current practice

ctDNA analysis of PIK3CA or ESR1 variants in the plasma of metastatic breast cancer patients is now part of clinical practice. FDA-approved tests are available, and their use to guide targeted therapy with alpelisib or elacestrant is recommended by American Society of Clinical Oncology (ASCO). The National Comprehensive Cancer Network (NCCN) and The European Society for Medical Oncology (ESMO) and recommend its use for detecting actionable mutations, such as PIK3CA, in metastatic breast cancer (22,23).

CTC enumeration is an established prognostic tool for metastatic disease, while ctDNA-based assays guide therapy selection. However, its use in early-stage breast cancer and recurrence detection remains investigational.

In the secondary analysis of a randomized clinical trial, the detection of ctDNA and CTCs in patients with early-stage TNBC (triple negative breast cancer) after neoadjuvant chemotherapy was independently associated with disease recurrence, highlighting their potential as important stratification factors for future post-neoadjuvant trials (24).

Limitations of liquid biopsy technology

Challenges include detecting low-abundance biomarkers, particularly in early-stage disease. Variability in test quality, sample handling, and analytical techniques hampers reproducibility. Tumor heterogeneity further complicates interpretation. Standardized protocols are needed to ensure reliable clinical application.

Cost-effectiveness

Despite its potential, liquid biopsy remains expensive, limiting accessibility. Lowering prices through technological advancements and increased demand is crucial to enable broader adoption and improve cancer care.

Future directions

Advancements in bioinformatics and multi-omic approaches, combined with artificial intelligence, promise to enhance the sensitivity and specificity of liquid biopsy. Continued validation in clinical trials will be essential to its integration into personalized oncology.

Future studies should explore the potential of machine learning models that integrate ctDNA and CTCs quantification and transcriptomic analysis, for a deeper understanding of tumor biology and treatment response (25).

Conclusions

Liquid biopsy represents a paradigm shift in breast cancer management. Addressing challenges in cost, sensitivity, and standardization is essential to realizing the full clinical potential of liquid biopsy.

Acknowledgments

The authors thank Breast Cancer Hope Charity for supporting their research program.

Footnote

Peer Review File: Available at https://gs.amegroups.com/

article/view/10.21037/gs-2025-11/prf

Funding: None.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://gs.amegroups.com/article/view/10.21037/gs-2025-11/coif). All authors report they are employees of HCA Healthcare pvt Ltd. T.C. and K.M. are fractional shareholders of Datar Genetics stock. K.M. has received honoraria for providing academic and clinical advice to Merit Medical and QMedical corporations. Additionally, he owns shares in HCA Healthcare UK. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are 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.

References

1. Cani AK, Hayes DF. Breast Cancer Circulating Tumor Cells: Current Clinical Applications and Future Prospects. Clin Chem 2024;70:68-80.
2. Yu M, Bardia A, Wittner BS, et al. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science 2013;339:580-4.
3. Alix-Panabières C, Pantel K. Challenges in circulating tumor cell research. Nat Rev Cancer 2014 Sep;14:623-31.
4. Crook T, Leonard R, Mokbel K, et al. Accurate Screening for Early-Stage Breast Cancer by Detection and Profiling of Circulating Tumor Cells. Cancers (Basel) 2022;14:3341.
5. Fabisiewicz A, Grzybowska E. CTC clusters in cancer progression and metastasis. Med Oncol 2017;34:12.
6. Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;368:1199-209.
7. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.
8. Razavi P, Li BT, Brown DN, et al. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. Nat Med 2019;25:1928-37.
9. Mouliere F, Chandrananda D, Piskorz AM, et al. Enhanced detection of circulating tumor DNA by fragment size analysis. Sci Transl Med 2018;10:eaat4921.
10. Moss J, Magenheim J, Neiman D, et al. Comprehensive human cell-type methylation atlas reveals origins of circulating cell-free DNA in health and disease. Nat Commun 2018;9:5068.
11. Zou R, Loke SY, Tang YC, et al. Development and validation of a circulating microRNA panel for the early detection of breast cancer. Br J Cancer 2022;126:472-81.
12. Turkoglu F, Calisir A, Ozturk B. Clinical importance of serum miRNA levels in breast cancer patients. Discov Oncol 2024;15:19.
13. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol 2002;2:569-79.
14. Zhu M, Gao Y, Zhu K, et al. Exosomal miRNA as biomarker in cancer diagnosis and prognosis: A review. Medicine (Baltimore) 2024;103:e40082.
15. Savas P, Virassamy B, Ye C, et al. Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis. Nat Med 2018;24:986-93.
16. Best MG, Sol N, Kooi I, et al. RNA-Seq of Tumor-Educated Platelets Enables Blood-Based Pan-Cancer, Multiclass, and Molecular Pathway Cancer Diagnostics. Cancer Cell 2015;28:666-76.
17. Geyer PE, Holdt LM, Teupser D, et al. Revisiting biomarker discovery by plasma proteomics. Mol Syst Biol 2017;13:942.
18. Coombes RC, Page K, Salari R, et al. Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence. Clin Cancer Res 2019;25:4255-63.
19. Hayes DF, Cristofanilli M, Budd GT, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. Clin Cancer Res 2006;12:4218-24.
20. Schaffrin-Nabe D, Josten-Nabe A, Tannapfel A, et al. Dynamic changes in tumor profiling reveal intra- and inter-tumoral heterogeneity focused on an uncharacterized HER2 mutation: a case report of a young breast cancer

patient. Front Oncol 2024;14:1395618.
21. Garcia-Murillas I, Schiavon G, Weigelt B, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med 2015;7:302ra133.
22. National Comprehensive Cancer Network (NCCN). NCCN clinical practice guidelines in oncology: breast cancer. Version 4.2024. Available online: www.nccn.org. Accessed 2025 Jan 1.
23. Pascual J, Attard G, Bidard FC, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2022;33:750-68.
24. Radovich M, Jiang G, Hancock BA, et al. Association of Circulating Tumor DNA and Circulating Tumor Cells After Neoadjuvant Chemotherapy With Disease Recurrence in Patients With Triple-Negative Breast Cancer: Preplanned Secondary Analysis of the BRE12-158 Randomized Clinical Trial. JAMA Oncol 2020;6:1410-5.
25. Gerratana L, Davis AA, Foffano L, et al. Integrating machine learning-predicted circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) in metastatic breast cancer: A proof of principle study on endocrine resistance profiling. Cancer Lett 2025;609:217325.

Cite this article as: Venkataraman J, Crook T, Mokbel K. Liquid biopsy in breast cancer: a practical guide for surgeons. Gland Surg 2025;14(4):754-760. doi: 10.21037/gs-2025-11