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The Potential of Liquid for Breast Cancer: A Review

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Abstract

Introduction. Tissue biopsy is the gold standard for cancer diagnosis, targeted treatment, and prognosis. However, a biopsy is an invasive procedure that could result in postoperative bleeding, pain, and infection. Such limitations may now be resolved by the clinical technique known as liquid biopsy, which enables a better representation of disease status.

Method. This literature review was conducted through online databases (PubMed, Ascopubs, EuroPMC) using the following keywords: "liquid biopsy", "ctDNA", "CTC", "breast cancer", "pathogenesis of breast cancer", "tumor microenvironment", "ctdna detection technologies", "early diagnosis", "targeted therapy" ,"monitoring disease progression", and "prognosis". The literature search was conducted using the PRISMA format (Figure 1). The appraised articles were further evaluated using the Joanna Briggs Institute (JBI).

Discussion. Liquid biopsy, also known as blood-based analysis of circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), has become more significant in breast cancer in recent years. There are several techniques for CTC and ctDNA detection that are continuously developing. PCR-based techniques are the initial approaches used to identify ctDNA. However, targeted deep sequencing is now superior. Instead of a tumor biopsy, a liquid biopsy might be beneficial for breast cancer diagnosis, therapy, and prognosis based on clinical trials. However, more clinical trial studies are still needed.

Conclusion. The advancement of technology has made genetic alteration detection via liquid biopsy feasible to detect genetic alterations that are very important not only for early detection of breast cancer but also targeted therapy and disease monitoring. Numerous studies have shown the potential of liquid biopsy as an alternative to tumor biopsy.

Keyword: breast cancer, ctDNA, CTC, liquid biopsy

Introduction

The World Health Organization reported that in 2021 breast cancer was the most prevalent cancer, contributing to 12% of all new cancer cases each year.[1] Studies showed that breast cancer is a multifactorial disease, and its etiology is often unclear. However, it has been demonstrated that genetic and environmental factors are the primary causes. In addition, early detection and treatment can significantly increase the chances of survival and recovery.[2]

Tissue biopsy is the gold standard for cancer diagnosis, molecular analysis, and prognosis. However, it is an invasive procedure that could result in postoperative bleeding, pain, and infection.[1,2] As biotechnology advanced along with the development of research in breast cancer biology, liquid biopsy has gradually turned into a promising minimally invasive tool focused on the analysis of circulating tumor cells (CTC) and cell–free circulating tumor DNA (ctDNA) of the plasma. The proportion of cell–free circulating DNA derived from tumor tissue is called circulating tumor DNA (ctDNA). Identification and measurement of ctDNA is the potential to detect the early stage of breast cancer, identify genomic mutation for personalized therapies, prediction of prognosis, minimal residual disease (MRD), serial sampling of disease progression, and therapy efficacy.[1] In addition, without the requirement for a further tumor biopsy, circulating tumor DNA (ctDNA) may offer a current evaluation of the genetic profile of advanced cancer.[2] In this review, we discuss a variety of tumor components applied in liquid biopsy that are already applied in clinical practice and are under investigation.

This review is based on sixteen eligible articles found in online databases, i.e., PubMed, Ascopubs, and EuroPMC, using the following keywords: "liquid biopsy", "ctDNA", "CTC", "breast cancer," "pathogenesis of breast cancer," "tumor microenvironment", "ctdna detection technologies", "early diagnosis", "targeted therapy", "monitoring disease progression" and "prognosis." These include (1) meta–analysis, systematic review, literature review, randomized controlled trial, clinical trials, or cohort study; (2) the subject is breast cancer that was assessed with liquid biopsy; (3) no limitation of publication year. In addition, the studies included in this review will be presented in a narrative format on the potential application of the liquid biopsy chapter.

Pathogenesis of breast cancer

Breast cancer is a complex process of cancer cells growing by progressive development through stages, commencing with epithelial hyperproliferation and advancing to in situ, invasive, and metastatic carcinomas.[3] Recent research has demonstrated that breast cancer comprises neoplastic cells and significantly alters the surrounding stroma or tumor microenvironment. Cancer cells in host tissues undergo significant genetic, cellular, and physical alterations to sustain tumor development and progression. One of the factors that influence the steps of cancer progression is the tumor microenvironment.[4] The tumor microenvironment comprises immune cells, stromal cells, blood vessels, and an extracellular matrix. Engaging in a low–oxygen and acidic environment, the tumor microenvironment promotes angiogenesis to gain oxygen and remove metabolic waste. Various studies have identified that the tumor microenvironment is a key factor in cancer development, progression, and response to treatment.[3,4] In selecting the most effective therapy and monitoring disease progression, a cancer diagnosis has revolutionized over the past decade.[3] Detecting and analyzing genomic alterations in cancer cells has led to improved prognosis prediction and treatment and the development of novel targeted therapies.[4] However, gaining information on tumor microenvironment (TME) characteristics needs biopsy and resection specimens which are invasive. The limitations of current diagnostic tools

have delayed technique development for decades.[4,5] Nowadays, personalized medicine and genetic profiling using liquid biopsy have recently emerged as less invasive tools for therapeutic, diagnosing, and monitoring breast cancer progression.[5]

Components in liquid biopsy

Liquid biopsy is a minimally invasive method for diagnosing a patient's illness and is a crucial technique for assessing cancer progression.[6] Nucleic acid, extracellular vesicles, proteins, or other biological components released into body fluids by cancer cells are the compounds of liquid biopsies. According to the findings of several research studies, among all the analytes, CTC and ctDNA are potential biomarkers in breast cancer.[6,7] In this review, we will focus on detecting plasma CTC and ctDNA.

Circulating nucleic acids (CTCs)

CTCs are primary or metastatic tumor cells that are released into circulation. Through epithelial–to–mesenchymal transition (EMT), these cells can adapt and survive in the bloodstream and may contribute to metastasis. The precise process responsible for their release remains unknown.[7] CTCs have a nucleus, are positive for cytokeratins, are identified by the antibodies C11 and A53–B/A2, do not stain for CD45, are larger than 4 x 4 μm, and have a cell–like shape. CTCs have a short peripheral circulatory system with a half–life of approximately 1 to 2.4 hours.[8,9] Active clearance of CTCs from the circulation or extravasation to secondary organs that contribute to metastasis. Thus, clusters of CTCs are associated with tremendous metastatic potential and a worse prognosis.[9]

Various studies have shown the ability to identify CTCs using immunologic, molecular, or functional techniques. Epithelial markers such as epithelial cell adhesion molecule (EPCAM) and cytokeratins are used to detect CTC. In contrast, the epithelial tumor may go through epithelial–to–mesenchymal transition (EMT), which reduces the expression of epithelial markers. Thus, several researchers are developing novel approaches for detecting CTC undergoing EMT.[10] Cristofanilli et al. conducted the first seminal study on the prognostic significance of CTC level in 2004 utilizing a highly automated immunomagnetic CTC screening technology called CellSearch. A threshold of > 5 CTC/7.5 mL measured at baseline and a few weeks after therapy was associated with short progression–free survival (PFS), which was linked with a poor prognosis. This study showed that the detection of CTC level in plasma outperformed plasma CEA and CA.15.3 in terms of accuracy. Multiple studies have demonstrated that CTC can accurately assess therapy effectiveness, early diagnosis, metastatic progression, recurrence, and prognosis.[11]

Cell–free DNA (cf–DNA)/circulating tumor DNA (ctDNA)

Cell–free DNA (cfDNA) consists of DNA from the normal host cells and circulating tumor DNA (ct–DNA). Normal host cells consistently produce fewer than 200 base pairs in length DNA fragments but circulating tumor cells have longer DNA fragments that can reach several kilobases. In cancer patients, tumor cells produce the majority of cfDNA, referred to as ctDNA. Depending on tumor size, location, vascularization, therapy, and clearance, the fraction of ctDNA ranges from 0.05% to 90%.[12] ctDNA is the double or single–stranded fragmented DNA released into body fluids by tumor–specific genetic alterations released from primary tumors, CTCs, micrometastasis, or metastatic tumors in cancer patients.[13] Tumor cells have the same inherent potential to shed DNA as normal cells but with larger quantities due to higher cellular turnover. cfDNA levels vary from 13 ng/mL in healthy people to 180 ng/mL in advanced malignancies.[14] Once ctDNA is detected in circulation, it is feasible to analyze these fragments' genetic and epigenetic profiles to identify specific cancer mutations generated by tumor cells. ctDNA is eliminated by the liver, spleen, and kidneys between 30 and 4 hours, enabling a real–time evaluation of tumor burden.[13,14] In addition, ctDNA may be transferred by other cells in the body and contribute to the genometastasis that occurs in cancer patients.[13]

Technologies for ctDNA analysis

PCR–based methods

Quantitative real–time PCR has been used to measure the amount of specific ctDNA fragments. However, traditional PCR–based liquid biopsy techniques have limited sensitivity to detect low mutant allele concentrations. Due to the limitation of conventional PCR, the researchers have developed the last generation of PCR, digital PCR (ddPCR), with higher sensitivity.[15] Other methods for quantifying ctDNA that are PCR based are BEAMing (Beads, Emulsion, Amplification, and Magnetics). BEAMing could use ctDNA to identify mutations in the *PIK3CA* gene. In addition, ctDNA may also assess epigenetic modifications such as promoter or enhancer methylation.[15,16] Methylation–specific PCR (MS–PCR) is one of the most frequent gene–specific DNA methylation detection methods. The detection of methylated CpG sites starts with bisulfite conversion. Methylated sequences are amplified using methylation–specific primers after the conversion.[16]

Targeted deep sequencing

Using next–generation sequencing (NGS) and a combination of PCR and NGS, targeted deep sequencing has been used to identify particular genomic regions or novel somatic mutations. However, the sensitivity of NGS for identifying targeted ctDNA mutations is highly sensitive. Therefore, tagged–Amplicon deep sequencing (TAm–seq), Safe–Sequencing System (Safe–SeqS), Cancer Personalized Profiling by deep sequencing (CAPP–Seq), and Ion Torrent were developed for applying NGS to target panels.[21,25]

Table 1. Various techniques using the platform to detect alterations and their targets

Technology	Platform	Type of alteration	Target
PCR–based methods	qPCR	Known point mutation	Rearrangements
	ddPCR	Known point mutation	Rearrangements, *PIK3CA* mutations
	BEAMing	Known point mutation	*PIK3CA* mutations
	MS–PR	Known point mutation	Gene–specific detection of specific cpG islands
	ARMS	Known point mutation	Hotspot *PIK3CA* mutations, SNPs
Targeted deep sequencing	TAm–seq	Known point mutation	SNVs
	Safe–SeqS	Known point mutation and copy number variations	SNVs
	CAPP–Seq	Known point mutation, copy number variations, and rearrangements	Rearrangements
	Ion Torrent	Somatic variant detection	Selected SNVs

qPCR: Quantitative polymerase chain reaction; ddPCR: Droplet digital polymerase chain reaction; BEAMing: Beads, emulsion, amplification, magnetics; MS–PCR: Methylation–specific polymerase chain reaction; Safe–SeqS: Safe–sequencing system; CAPP–Seq: Cancer personalized profiling by deep sequencing; SNV: Single–nucleotide variant

Epigenetic DNA alterations

Bisulfite conversion–based methods

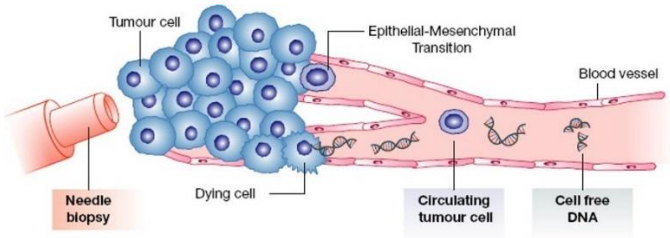
DNA methylation occurs early in carcinogenesis; hence, methylation ctDNA biomarkers have been analyzed in various tumor types. As a biomarker, methylation DNA has the advantage of not requiring the presence of particular mutations. In cancer patients, CpG islands that are typically unmethylated may have become methylated, resulting in gene repression.[17] Early DNA methylation research focused on the hypermethylation of CpG islands of important driver genes, such as estrogen receptors and BRCA1. A potential biomarker for early breast cancer identification has been plasma–circulating cfDNA methylation status.[18] The gold standard for DNA methylation study is bisulfite conversion–based methods. There are some bisulfite conversion–based methods such as whole–genome bisulfite sequencing (WGBS), Reduced–Representation Bisulfite Sequencing (RRBS), Methylation–specific PCR (MSP), Methylated CpG Tandems Amplification and Sequencing (MCTA–seq), Targeted Bisulfite Sequencing. The most comprehensive and reliable DNA methylation profiling technique is WGBS. WGBS can detect the methylation status of each cytosine, particularly low CpG density areas and non–CpG sites (CpA, CpT, and CpC).[17,18] However, the disadvantage of WGBS is its extremely high cost. Until now, no individual sample analysis has been performed due to the high cost of large–scale WGBS[17–19] RBS was developed by combining MspI digestion, bisulfite conversion, and NGS to analyze CpG–rich regions. Widschwendter et al. discovered that blood DNA methylation indicators using RRBS might predict breast cancer mortality up to one year after diagnosis.[19] Unfortunately, since RRBS requires a high amount of cfDNA, it is unsuitable to be applied, particularly in early–stage breast cancer. A solution to this issue is that DNA damage may be prevented by single–cell RRBS (scRRBS).[18,19] Other methods are methylation–specific PCR (MSP) which is the most common technique for gene–specific detection, methylated CpG Tandems Amplification and Sequencing (MCTA–seq), which is effective detection of hepatocellular carcinoma (HCC), and colorectal cancer.[17,18] The last technique is targeted bisulfite sequencing, which is not practicable for clinical applications since the primer and probe designs for bisulfite–converted sites are pretty complex.[17]

Potential application of liquid biopsy

Liquid biopsy has some advantages and disadvantages compared to tumor biopsy. Tumor biopsy is invasive; obtaining the sample in certain tumors is sometimes challenging. On the other hand, liquid biopsy only requires a simple blood sample to obtain several tumor materials like circulating tumor cells (CTC) and circulating–free DNA (cfDNA), which are needed for analysis.[2] In addition, not all patients are eligible for tumor biopsy; for example, to perform breast core biopsy, there are several relative contraindications: consuming anticoagulant, pregnancy, lactation, and the patient's clinical condition. Compared to tumor biopsy, liquid biopsy does not have specific contraindications and is eligible for all patients.[9]

Whole genome amplification (WGA) is a technique to analyze cancer heterogeneity mutation in CTC. The advantages of using WGA are that it is superior to detecting heterogeneity compared to cell–free DNA or exosome. For example, in breast cancer, PIK3CA mutations detected in CTC with WGA are linked with poor diagnosis. In addition, both collection of tumor samples and liquid biopsy can do RNA profiling except for cfDNA.[13] CTC is a cell shed from a primary or metastatic tumor containing DNA and RNA. Meanwhile, cfDNA are degraded DNA fragments (including ctDNA) with no RNA fragment. As a result, RNA profiling from cfdna is not achievable. Nevertheless, RNA profiling provides reliable indicators for disease status, implying that it could be used for early diagnosis and disease classification.[19]

The clinical application of tumor biopsy is more widely used in daily practice since tumor biopsy remains the gold standard for cancer diagnosis.[14] However, due to the expansion of liquid biopsy research, oncologists have started to utilize liquid biopsy as early cancer detection, treatment follow–up, and determining prognosis. On the other hand, the study of liquid biopsy may enhance genetic analyses of tumor heterogeneity and clonal evolution in solid tumors. In addition, identifying circulating biomarkers in liquid biopsy is effective, enhancing personalized medicine.[11]



	Biopsy	CTC	cfDNA
Invasive	+	–	–
All patients eligible	–	+	+
Instrumentation required	+	+	–
WGA required	–	+	+/–
RNA profiling	+	+	–
Research applicability	+++	++	+
Biomarker applicability	–	++	+++

Figure 1. Benefits of liquid biopsy to tumor biopsy

Early diagnosis of breast cancer

Circulating tumor DNA (ctDNA) provides a new generation of early breast cancer diagnostic tools. However, plasma ctDNA levels are assumed to be low in early–stage breast cancer due to the low tumor burden. A study of 640 cancer patients revealed that individuals with stage 4 disease had a 100–fold increase in median ctDNA concentration compared to those with stage 1. As a result of the low level of ctDNA in plasma, early detection of breast cancer with ctDNA is quite challenging, requiring highly sophisticated detection and quantification methods.[20] Aside from detecting ctDNA levels, genetic alteration can also be observed. Multiple technologies have been employed to detect ctDNA that are continuously being developed. The first popular method to detect plasma DNA mutations is digital–PCR (dPCR). However, dPCR has several limitations, including the inability to detect new mutations that have not yet been identified.[6] Tumor protein p53 (TP53) and phosphatidylinositol–4,5–bisphosphate 3–kinase catalytic subunit alpha (PIK3CA) are frequently mutated genes in primary breast cancer, according to Cancer Genome Atlas (TCGA).[21] A prospective study by Beaver et al. showed that ctDNA could detect PIK3CA mutation with the ddPCR technique during the early stage of breast cancer plasma before and after surgery.[22] Analysis for mutations in tumor tissues led to 93% concordant detection of those mutations in presurgical plasma samples. The potential of ctDNA in patients in the early stage was established by the high sensitivity (93.3%) and specificity (100%) using ddPCR. Another technique recently emerging as a novel instrument for enhancing the sensitivity of massively parallel sequencing systems for identifying rare variants in plasma DNA is a new targeted next–generation sequencing (NGS) like SafeSEQ.[15] A study designed by Rodriguez et al. showed that ctDNA analysis using SafeSEQ (Sysmex Inostics) technology and an NGS Truseq custom low input panel might be used as an alternative to tissue biopsy (Illumina). Based on the study, ctDNA sequencing could identify additional *TP53* and *PIK3CA* mutations which were not detected in tumor biopsy sequencing (21).

Another study found that 71% of patients with early–stage breast cancer had somatic mutations in the plasma using targeted error correction sequencing (TEC–seq) for identifying genetic abnormalities in ctDNA.[23]

Table 2. Clinical trials using ctDNA as an early diagnosis in breast cancer

Authors (Reference)	Type of Study	Number of Patients	Techniques	Mutation Detected	Result
Beaver et al. (22)	Prospective Study	30 patients	ddPCR	PIK3CA	The potential of ctDNA in patients with early–stage breast cancer was demonstrated by the high specificity (100%) and sensitivity (93.3%) of ddPCR.
Rodriguez et al. (21)	Pilot Study	29 patients	NGS	*TP3* and *PIK3CA*	PIK3CA mutations were discovered in the tumor biopsies of 79.3% (23/29) and 34.5% (10/29) of the patients with early breast cancer, respectively. At the same time, a plasma sample showed the same alterations in 34% of 10/29 of the patients.
Phallen et al. (23)	Cohort	200 patients (Multiple tumor types)	TEC–Seq	Not specified	ctDNA was found in 56% of stage I–III breast cancer patients.

Targeted Therapy

Mutations in tumor–related genes have led to important advances in cancer biology and the development of precision medicine. Early intervention in breast cancer patients may be advantageous by detecting CTCs and/or ctDNA. However, enumeration of CTC level is not often associated with metastatic breast cancer prognosis, especially in ER–positive breast cancer. At the same time, tumors are not always susceptible to endocrine therapy due to ER–negative CTCs resistant to the treatment. As a result, A CTC–Endocrine Therapy Index (CTC–ETI) was developed based on CTC quantification and the expression of estrogen receptors, *BCL–2, HER2,* and *Ki67*.[24] A study by Bidard et al. regarding CTC–driven selection therapy was randomly done in 764 metastatic breast cancer patients. Three hundred eighty–seven patients were treated according to clinicians–driven treatment options, while another 377 patients were treated with CTC–driven treatment options. Driven by the CTC, patients will receive either endocrine therapy or chemotherapy depending on the quantity of CTC: chemotherapy if CTC is high or endocrine therapy if CTC is low. Targeted therapy is particularly challenging in metastatic breast cancer due to unfeasible sites, especially when metastasis has untypical localization.[25] In addition to CTC, a phase 2a multi–cohort research discovered the potential use of ctDNA for targeted treatment. The samples were collected from patients who had received minimal one prior treatment for metastatic breast cancer or patients who relapsed within a year after receiving neoadjuvant or adjuvant chemotherapy. The patients were enrolled in one of four treatment cohorts based on their ctDNA mutation status: cohort A included those with ESR1 mutations who were given intramuscular extended–dose fulvestrant 500 mg; cohort B included those with HER2 mutations who were given oral neratinib 240 mg and estrogen receptor–positive, intramuscular standard–dose fulvestrant; and cohort C had those with AKT1 mutations and estrogen receptor which were treated with oral capivasertib 480 mg. The findings demonstrated that ctDNA detection is highly reliable, with excellent agreement between different ctDNA investigating methods and high sensitivity for mutations found in advanced breast cancer tissue biopsies. Additionally, ctDNA detection offers rapid genotyping that enables patients with breast cancer to determine mutation–directed therapy.[26]

Monitoring disease progression

Monitoring CTCs and ctDNA during cancer therapy can be less challenging than early detection of breast cancer. Liquid biopsies enable continuous sampling during the treatment. The non–invasive and dynamic properties may provide patients with a real–time indicator of the efficacy of the adjuvant treatment. It has been shown that monitoring disease relapse while receiving treatment significantly involves ctDNA and CTCs.[27] In a study by Rack et al., CTCs levels were compared before and after neoadjuvant chemotherapy. Before treatment, CTCs were found in 21.5% of patients, despite the tumor's size, grade, or hormone receptor status. After chemotherapy, 22.1% of patients were CTC–positive. Poor disease–free survival was associated with the presence of CTCs.[28]

In addition to monitoring treatment, early diagnosis of relapse after a complete primary breast cancer resection is also a priority for oncologists. Currently, laboratory evaluations and routine mammography are still advised for follow–up. Therefore, rapid innovations in sequencing technology and ctDNA analysis have made it potential to be monitored during follow–up. A study by McDonald et al. showed that the detection of ctDNA mutations consistently in two to four weeks after surgery was associated with a high risk of early relapse. Detection of ctDNA mutations has some benefits, including delivering precise or targeted therapy while keeping track of the disease progression, resistance to treatment, and relapse.[29] Another prospective cohort study demonstrates that monitoring ctDNA during treatment can predict cancer progression 4–6 months earlier than the traditional approaches. The study sample consists of 45 breast cancer patients receiving neoadjuvant chemotherapy, endocrine treatment, or palliative care for metastatic diseases. The plasma and tumor samples obtained before and after treatment were analyzed to determine the mutation status. Approximately two–thirds of patients had detectable mutations, and there were novel pathogenic changes in the follow–up plasma that was not present in the tumor sample or baseline plasma.[30]

Table 3. Clinical trials using ctDNA for monitoring disease progression in breast cancer.

Authors (Reference)	Study Design	Techniques	Outcome
Rack et al.[28]	CTCs before and following adjuvant chemotherapy	CellSearch System	CTCs following chemotherapy is associated with reduced disease–free and overall survival.
McDonald et al.[29]	ctDNA before, during, and following neoadjuvant chemotherapy	Targeted Digital Sequencing (TARDIS)	ctDNA levels reduced during therapy The presence of ctDNA mutations two to four weeks after surgery was associated with a high risk of early relapse.
Priskin et al.[30]	Detection of ctDNA mutations before, during, and after neoadjuvant chemotherapy, endocrine therapy, or palliative care for metastatic illnesses.	Next Generation Sequencing	Two–thirds of the patients exhibited detectable mutations, and the follow–up plasma had pathogenic changes that were not found in the tumor sample or baseline plasma.

Prognosis and minimal residual disease

Evidence shows that minimal residual disease (MRD) is strongly related to disease recurrence; hence, finding particular genetic and molecular

abnormalities as new minimal residual disease (MRD) detection targets utilizing ctDNA has become a research focus. ctDNA is a feasible biomarker as it contains genetic and epigenetic changes found in tumors. Therefore, it has the potential for prognosis prediction in breast cancer. Patients with early–stage breast cancer receive neoadjuvant therapy (NAT) as a standard in clinical practice. However, evaluating and predicting NAT response remains a significant challenge after completing NAT. Different biochemical biomarkers, such as proteins, enzymes, DNA, and RNA, could be used to predict breast cancer and monitor treatments.[31] Currently, CA15–3 and CEA are chemical biomarkers that are widely utilized. However, Due to low sensitivity and specificity, the American Society of Clinical Oncology (ASCO) stated that CEA, CA15–3, CA27,[29] lactate dehydrogenase, and others were not recommended for screening, diagnosis, or staging of breast cancer patients after primary therapy. In early–stage breast cancer, pathologic complete response (pCR) following neoadjuvant therapy has been linked to improved event–free survival (EFS) and overall survival (OS). However, some patients with pCR experienced recurrence or metastasis, and the absence of pCR does not necessarily correlate with recurrence.[32]

The innovation of circulating tumor DNA (ctDNA) has potential clinical applications. Some studies proved that ctDNA could predict the prognosis of the disease. A study by Lin et al. demonstrated that identifying ctDNA after NAT has significant clinical value potential as a predictive marker in stage II to III breast cancer patients. In the study, 95 patients were included, 60 showed ctDNA positivity before NAT, and 31 showed ctDNA positivity after NAT. In both patients who achieved and did not achieve pCR, the presence of ctDNA following NAT was a substantial risk factor for recurrence.[33] Another systematic review and meta–analysis by Papakonstantinou et al. demonstrated that identification of ctDNA at baseline and after NAT completion was substantially linked with reduced Relapse–Free Survival (RFS).[34] Moreover, the measurement of ctDNA levels is also statistically significant for predicting disease–free survival (DFS), according to Cullinane et al. This meta–analysis and systematic review discovered that patients with increased ctDNA levels had shorter disease–free survival (DFS). ctDNA was substantially related to a lower relapse–free survival rate, suggesting that it has the potential to identify preclinical disease recurrence in breast cancer patients following treatment.[35]

Conclusions

Liquid biopsies in breast cancer have shown promising results, particularly in early diagnosis, targeted therapy, evaluating therapy response, and predicting disease progression or recurrence. Liquid biopsies may play a larger role in the breast cancer clinic due to more research and, ideally, the continuous development of technologies that identify tumor–derived substances.

Disclosure

The authors declare no conflict of interest.

Role of authors

Conceptualization 123, data curation 3, formal analysis123, funding acquisition 3, investigation 123, methodology 123, project administration 123, resources 123, software 3, supervision 12, validation 12, visualization 3, writing – original draft preparation 3, writing – review and editing 123.

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