Malays J Pathol 2023; 45(2) : 175 – 186

REVIEW ARTICLE

Liquid biopsy in breast carcinoma: Are we there yet?

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Abstract

Breast carcinoma is the most common malignancy among women worldwide. Liquid biopsy is a method of obtaining tumour-derived material from blood and body fluid. This includes the assessment of circulating tumour cells (CTCs), circulating tumour deoxyribose nucleic acid (ctDNA), tumour educated platelets (TEPs) and exosomes. Detection of CTCs and ctDNA in liquid biopsy has been shown to have prognostic and predictive value in both early and metastatic breast carcinoma. The study of CTCs could also advance our understanding of aspects of tumour biology, including epithelial mesenchymal transition. ctDNA can be used to assess and monitor the molecular profile of breast carcinoma. It may help detect new genetic alterations in tumours and predict disease progression before the onset of clinical features or radiological evidence. TEPs and exosomes are also emerging as diagnostic, prognostic and predictive markers of breast carcinoma. Thus, liquid biopsy provides a non-invasive, repeatable method for the dynamic assessment of the tumour. Many methods have been used for the detection of CTCs and ctDNA. Most of these are still in the research stage and only the CellSearch method for the detection of CTCs and Therascreen PIK3CA RGQ polymerase chain reaction (PCR) assay for the detection of PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha) mutations in liquid biopsy have approval of the United States, Food and Drug Administration. However, their high costs, lack of standardized procedures, and a long and complicated detection process have limited their use. Despite its limitations, liquid biopsy is a useful tool in clinical decision making and has the potential to play an increasingly important role in the management of breast carcinoma in the future as we move toward more personalized cancer care.

Keywords: breast carcinoma, liquid biopsy, circulating tumour cells, circulation tumour DNA, predictive marker, prognostic marker

INTRODUCTION

Liquid biopsy is a method of obtaining tumour-derived materials such as tumour deoxyribose nucleic acid (DNA), ribose nucleic acid (RNA), circulating tumour cells (CTCs) and extracellular vesicles (exosomes) from body fluids. Assessment of tumour educated platelets (TEPs) is also emerging as a form of liquid biopsy (Fig. 1). Of these CTCs and circulating tumour DNA (ctDNA) are the most well studied.[1] Body fluids such as blood and urine can be obtained easily. Therefore, liquid biopsy is considered a fairly non-invasive and repeatable test. A tissue biopsy provides information related to a single tumour at a single point of time. In contrast, a liquid biopsy is reflective of both primary and metastatic tumours and allows real-time dynamic assessment of cancer profiles. Therefore, it is more representative of tumour heterogeneity. [2-4]

The different types of liquid biopsy

Circulating tumour cells

Circulating tumour cells (CTCs) are tumour cells that have split away from the main primary tumour and are present in the circulatory or lymphatic systems as single cells or clusters. CTCs detected in the patient's blood were first described in autopsy studies in the late 19th century.[5] Detection of CTCs has now been established as a reliable and valuable marker of metastasis and has been shown to have prognostic value in breast,[6] lung,[7] ovarian,[8] colorectal[9] and prostate[10] carcinoma. A test on a few millilitres of blood is sufficient to detect the presence of CTCs

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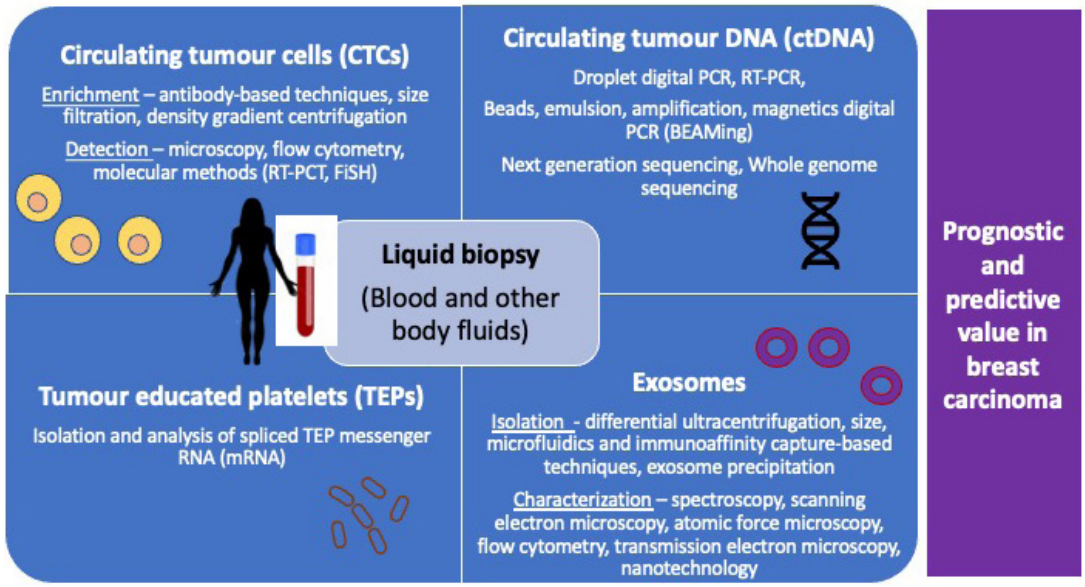

Fig. 1: Liquid biopsy in breast carcinoma.

and is predictive of disease stage and clinical outcome in patients with a malignant disease.[11]

Many methods have been described for CTC detection including immunomagnetic selection, reverse transcription polymerase chain reaction, flowcytometry and the CellSearch (Janssen Diagnostics, Raritan, NJ, USA) platform. The CellSearch system, which was developed in the early 2000s[12] is the first and only product that has been approved by the United States, Food and Drug Administration (FDA) to detect CTCs.

CTC detection techniques generally consist of isolating cells with epithelial markers against a background of mesenchymal-derived blood cells. As CTCs are rare, current CTC detection techniques usually combine several steps of capture and primary enrichment, CTC detection, counting, and analysis.[6,13,14] In the enrichment step, best results are obtained by combining the physical and biological properties of CTC enrichment.[15] Enrichment techniques include antibody-based techniques using positive or negative immunoselection by various membrane antigens e.g. epithelial-cell adhesion molecule [EpCAM], MUC1, CD45 or techniques based on physical properties of CTC such as size filtration and density gradient centrifugation.[14] CellSearch uses antibodies to EpCAM (a transmembrane glycoprotein that is specific to epithelial cells) coupled to magnetic beads to immunomagnetically enrich samples.

CTC detection can be achieved on a cellular level through microscopy or flow-cytometry, or on molecular level using RT-PCR.[14] The detection of CTCs usually involves techniques to visualize cells using markers by nuclear staining and epithelial antigen immune-cytolabelling. In most assays CTCs are defined as nucleated cells that are cytokeratin positive and CD45 negative. For example, CellSearch semiautomatically counts CTCs that are identified by immunofluorescent stains showing positivity for epithelial markers.

Flow cytometry can be used to quantify and characterise CTCs using antibodies against any chosen CTC-expressed marker.[15] Fluorescent in situ hybridisation (FISH) uses fluorescent probes to identify specific DNA sequences.[14] Molecular methods can be used to detect CTC mRNA markers, then be used to estimate the number of CTCs in the sample and their expression patterns. However, since these markers are often expressed in low levels in normal blood cells, identifying CTC numbers and expression using this technique is challenging.[14]

Cell free DNA

Cell-free DNA (cfDNA) was first described in 1948. Cell-free DNA (or cfDNA) refers to all non-encapsulated DNA in the blood stream. Cell-free DNA is seen in conditions where there is necrosis or apoptosis of cells in malignancies, stroke, trauma, myocardial infarction, and autoimmune disease.[15,16] cfDNA can also be released from neutrophils and therefore may be found in small quantities in healthy individuals.[17] Cancer patients usually have a high level of

cfDNA in their serum or plasma because of cellular necrosis or apoptosis, because tumour cells divide faster than normal cells, and cfDNAs are released in a high proportion. Circulating tumour DNA or ctDNA is the fraction of cfDNA that is released from CTCs and tumour cells from the primary or metastatic tumour.

Technologies used for detection of ctDNA include polymerase chain reaction (PCR)-based assays such as droplet digital PCR, Beads, Emulsion, Amplification, Magnetics digital PCR (BEAMing) and reverse transcriptase-PCR (RT-PCR); targeted deep sequencing using next generation sequences with or without PCR and whole genome sequencing methods.[3]

Tumour educated platelets

Tumour educated platelets (TEPs) are emerging as an important biomarker in cancer diagnostics. An association between platelets and cancer was first described by Trousseau in 1868 who observed venous thrombosis in cancer patients. Platelets play an important role in the systemic and local responses to tumour growth particularly in tumour metastasis.[18-21] During the process of metastasis they secrete factors such as transforming growth factor that promotes invasion of the tumour into the local vasculature, in the intravascular phase, platelets help to provide protection from shear stress and evade the host's immune response, once the tumour cells reach the site of metastasis the platelets help with the process extravasation of tumour cells from the circulation, building of tumour stroma and neoangiogenesis.[20] During the process of interacting with tumour cells, platelets become "educated" resulting in an altered RNA profile. In recent years, methods for isolation and analysis of spliced TEP messenger RNA (mRNA) have been developed to detect cancer with high accuracy.[19-21]

Exosomes

Exosomes are small extracellular vesicles with sizes between 30–150 nm. They are a subtype of extracellular vesicles (EVs), which are secreted by all cell types and are responsible for intercell communication.[22,23] Tumour-derived exosomes are critically related to tumour progression, metastasis, and immune evasion.[23,24] They have been found to be present in most body fluids and are emerging as an important form of liquid biopsy in the diagnosis and management of malignancy. Exosomes have many advantages compared to other forms of liquid biopsy. They can exist in almost all types of body fluid including serum, cerebrospinal fluid, plasma, saliva, breast milk and urine.[23] They also exhibit high biological stability that facilitates storage and transport of the biopsy sample.[23] In contrast to cfDNA which is released from necrotic or apoptotic cells, exosomes are secreted by living cells.[23,25]

Exosome isolation techniques include differential ultracentrifugation-based techniques, size-based techniques, immunoaffinity capture-based techniques, exosome precipitation and microfluidics-based techniques.[26,27] Of these differential ultracentrifugation is the most commonly used method. Alongside isolation techniques, the characterisation of the exosomes is also critical to developing exosome-based assays. There are three main methods of characterisation including biophysical techniques based on spectroscopy and other principles (e.g., scanning electron microscopy, atomic force microscopy), antibody-based molecular techniques including flow cytometry and transmission electron microscopy and nanotechnology-dependent exosome characterisation techniques.[28]

What are the benefits of liquid biopsy?

The gold standard for the diagnosis of malignancy and the retrieval of crucial prognostic and predictive information is a tumour tissue biopsy. However, liquid biopsy is a minimally invasive procedure by which information that is predictive of disease stage and clinical outcome can be obtained from a few millilitres of blood or body fluids collected from patients with malignancy.[14]

CTCs can be used as a biomarker for the detection of early cancer[29] for predicting survival[30] and determining response to treatment.[31] In this era of precision medicine, it also has the potential to play a key role in the personalised treatment of cancer patients.

CTCs have many advantages over other blood biomarkers. They are representative of active tumour sites and information of distant tumour sites can be obtained repeatedly over time from easily accessible blood samples.[32] Therefore, they provide us with a tool to monitor the evolution of the disease and the therapeutic response in real time.

ctDNA provides an opportunity to assess the molecular characteristics of a tumour. It can be used to detect and characterise early-stage disease and monitor the genomic profile of tumours over time. Thus, in advanced disease, ctDNA has the

potential to predict disease progression through the detection of new genetic alterations before the onset of clinical symptoms or the emergence of radiological evidence.[1]

TEPs have currently been harnessed for the assessment of a wide range of malignancies in adults including glioblastoma and lung, breast, prostate, colorectal, pancreatic, hepatobiliary and nasopharyngeal carcinoma. In 2015, Best *et al*. performed extensive RNA sequencing to determine differentially spliced RNA profiles in platelets from cancer patients with six different types of cancer and healthy individuals.[33] This resulted in a predictive "pan-cancer" test with 96% accuracy.[33]

Exosomes have been shown to be useful in the early diagnosis and prognostication of a wide range of malignancies including pancreatic, colorectal, lung and breast cancer. They have also been shown to be useful in the determination of treatment response in lung cancer, breast cancer and melanoma.[23]

Is there a role for liquid biopsy in breast carcinoma?

There are many established prognostic and predictive factors for breast carcinoma including age, stage of the disease (determined by tumour size, nodal status, and metastatic status), type/grade of tumour, surgical margin status, lymphovascular status,[34] tumour infiltrating lymphocytes,[35] hormone receptor and HER2 status[36] and Ki67 proliferation index.[37]

CTC as a prognostic marker in breast carcinoma

Detection of CTCs has been proven, with the highest level of evidence, to be of prognostic value in both early and metastatic breast carcinoma.[14,38,39] CTC counts before and after adjuvant therapy and before neoadjuvant chemotherapy have been found to be strong and independent prognostic indicators of survival and loco-regional relapse.[40,41]

In non-metastatic early breast carcinoma, the detection of CTC identifies cases with a worse prognosis and may be predictive of future metastatic events and relapse,[38] resulting in a potential for early therapeutic interventions in this group of patients.

The presence of detectable CTCs has been shown to be associated with worse disease-free survival and overall survival in prospective trials of non-metastatic breast carcinoma.[38] In the metastatic setting, CTC counts ≥5 both at baseline and various points of follow-up, have been associated with shorter progression-free survival and overall survival.[42, 43] (Table 1)

CTC as a predictive marker in breast carcinoma

Hormone receptor (HR) and HER2 status have prognostic and predictive value in breast carcinoma and guide anti-estrogen and anti-HER2 therapy in eligible patients.[44,45]

HR and HER2 status may change during treatment or as the disease progresses. The estrogen receptor (ER) and progesterone receptor (PR) status of CTCs shows a better correlation with the metastatic deposit than the primary tumour.[46,47] Therefore, evaluation of ER/PR and HER2 status in CTCs may provide additional prognostic information that can be useful in the management of patients with breast carcinoma. Studies have reported that a discordance in HR expression between the primary tumour and metastases can occur in up to 40% of matched cases.[48-52]

Studies have also compared the rates of discordance of HR expression between primary tumours and CTCs using various technologies with results ranging from 32% to 68%.[46,53,54] One of the studies showed a concordance of 68% in HR status between primary tumour and CTCs versus an 83% concordance between CTCs and the metastasis.[47] The higher concordance rate for ER/PR between CTCs and metastatic tumour tissue raises the possibility that CTCs could be used as a surrogate for evaluation of biomarkers, particularly if biopsy is not feasible from the metastatic site.

HER2 can also be tested in CTCs and a study using the OncoCEE™ platform showed a 93% rate of concordance between CTCs and tumour tissue with HER2 status.[55] However some trials have shown that HER2-positive CTCs are detected in patients with HER2 negative primary tumours. Therefore, correlation of HER2 status of CTCs to the clinical response maybe important in follow up of patients receiving HER2-targeted therapies.[56]

CTCs offer a minimally invasive real-time assessment of the biology of a patient's metastatic breast carcinoma. Testing for CTCs may also allow the detection of metastatic clones with biological profiles different from the primary tumour in patients of all stages.[57] They can be assessed to determine and serially monitor the HR and HER2 status. All this will pave the way to more effective monitoring and modification of therapy during treatment. (Table 1)

Table 1. Studies on the role of liquid biopsy in breast cancer

Role	Study	Study population	Type of study	Number of patients	Method of extraction/detection	Main findings
Circulating tumour cells						
Prognostic marker in breast cancer	Bidard *et al.*[39]	Early breast cancer patients treated with neoadvant chemotherapy. Stage I-III	IMENEO study Metanalysis	2156	CellSearch®	CTC counts before neoadjuvant chemotherapy were a strong and independent prognostic indicator for distant-metastasis-free survival, OS and locoregional relapses
	Bidard 2014[40]	Metastatic breast cancer	Pooled analysis of individual patient data	1944	CellSearch®	CTC count before the start of treatment had an independent prognostic effect on PFS and OS
	Rack *et al.*[41]	Early breast cancer before adjuvant chemotherapy and after chemotherapy	Large prospective study	2026 Before treatment 1492 (after treatment	CellSearch®	CTCs count before treatment were confirmed as independent prognostic markers for DFS and OS. The presence of persisting CTCs after chemotherapy showed a negative influence on DFS and OS
	Cristanofelli *et al.*[42] Hayes *et al.*[43]	Metastatic breast cancer	Multicentre prospective study	177	CellSearch®	Pre-treatment CTCs and at each follow-up time point during therapy are an independent predictor of PFS and OS
Predictive marker in breast cancer	Aktas B *et al.*[46]	Metastatic breast cancer	Participants of the German DETECT study	193	Immuno-magnetic enrichment using the AdnaTest BreastCancerSelect RNA isolation and gene expression analysis by reverse transcription and Multiplex-PCR in separated tumour cells using the AdnaTest Breast Cancer Detect.	Most of the CTCs were ER/PR-negative despite the presence of an ER/PR- positive primary tumour.
	Kalinsky *et al.*[47]	Metastatic breast cancer	Cohort study	36	CTCs were isolated using the microfluidic OncoCEE™ platform. Detection was with an expanded anti-cytokeratin cocktail mixture and anti-CD45.	The concordance of ER/PR status between metastatic tumour and CTCs was higher than between primary breast tumour and CTCs.
	Somlo *et al.*[53]	Locally advanced/ inflammatory breast cancer and metastatic breast cancer	Cohort study	36	A fiber-optic array laser-scanning technology was used to detect CTCs were identified based on presence of cytokeratin and nucleus staining with DAPI, and the absence of CD45.	There was a discordance between ER and HER2 status of CTCs and both primary and metastatic tumours.
	Mayer *et al.*[55]	Advanced breast cancer	Cohort study	54	OncoCEE™ platform	An overall concordance of 93% between CTCs and the primary tumour was observed with regard to HER2 status
	Fehm *et al.*[56]	Metastatic breast cancer	Prospective multicentre trial	254	CellSearch® assay and AdnaTest BreastCancer™	HER2-positive CTCs are detected in patients with HER2 negative primary tumours.

Circulating tumour DNA/Cell free DNA						
Prognostic marker in breast cancer	Rossi *et al*.[59]	Locally advanced and metastatic breast cancer	Cohort study	91	Next-generation sequencing	ctDNA is an independent prognostic factor of PFS
	Fujita *et al*. [60]	Stage I/II breast cancer	Cohort study	336	Polymerase chain reaction	Total DNA and methylated DNA in serum detected with the one step methylation-specific PCR assay are independent prognostic factors of OS.
	Oshiro *et al*. [61]	Stage I-III breast cancer	Cohort study	313	Digital polymerase chain reaction (dPCR)	dPCR is a highly sensitive and specific method for the detection of PIK3CA mutant ctDNA. ctDNA(PIK3CA mutant high) status is a significant and independent prognostic factor of OS and RFS.
	Stover *et al*. [62]	Metastatic triple negative breast carcinoma	Retrospective cohort study	164	Low-coverage genome-wide sequencing	cfDNA tumour fraction is an independent prognostic biomarker in metastatic triple-negative breast cancer
	Madic *et al*. [63]	Metastatic triple negative breast carcinoma	Cohort study	40	Next-generation sequencing	ctDNA levels had no prognostic impact on time to progression (TTP) or overall survival (OS)
	Garcia – Murrilias *et al*.[64]	Early breast cancer patients receiving neoadjuvant therapy	Prospective cohort study	55	Personalised digital polymerase chain reaction	Detection of ctDNA in plasma after completion of apparently curative treatment-either at a single postsurgical time point or with serial follow-up plasma samples-predicted metastatic relapse
	Olsson *et al*. [65]	Non-metastatic (stage I–III) breast cancer at initial diagnosis who received no neoadjuvant therapy	A cohort of patients selected from the Breast Cancer and Blood Study	20	Droplet digital PCR	ctDNA analysis can discriminate patients with eventual metastasis from those with long-term DFS with high sensitivity and specificity.
Predictive marker in breast cancer	Sporke *et al*. [54]	ER+/HER2– locally advanced or metastatic breast cancer	International, multicentre, randomised, double-blinded, placebo-controlled, phase 2 clinical trial	153	Digital PCR	ESR1 status of metastatic tumours shows a better concordance with ctDNA than the primary tumour
	Schiavon *et al*.[50]	Advanced breast cancer who relapsed or progressed after therapy	Cohort study	171	Ultra-high-sensitivity multiplex digital polymerase chain reaction	ESR1 mutations are detected in ctDNA of women with ER positive breast cancer and are predictive of resistance to subsequent aromatase inhibitor-based therapy

	O'Leary *et al.*[69]	Advanced, estrogen receptor-positive, HER2-negative breast cancer	PALOMA-3 study -international, multi-center, phase III, double-blind randomised controlled trial of Palbociclib and fulvestrant	73	Multiplex droplet digital PCR assay	Relative change in PIK3CA ctDNA level after 15 days treatment strongly predicts PFS on palbociclib and fulvestrant
	Vidula *et al.*[70]	Metastatic breast cancer	Cohort study	215	Next generation sequencing	Somatic BRCA1/2 mutations are readily detectable in metastatic breast cancer by cfDNA analysis
Tumour educated platelets						
Prognostic and predictive marker of breast cancer	Best *et al.*[33]	Localised and metastatic breast cat alncer	Proof-of-principle study	39	Platelet isolation followed by SMARTer mRNA amplification and sequencing	TEP profiles distinguished patients with HER2-amplified, PIK3CA mutant or triple-negative breast carcinoma versus random classifiers
Exosomes						
Prognostic and predictive marker of breast cancer	Rodriguez - Martinez *et al.*[73]	Localised and metastatic breast cancer receiving neoadjuvant therapy	Cohort study	53 patients and 8 healthy donors	Exosomal miRNA levels analysed by qPCR.	Exosomal miRNA expression levels were higher in metastatic versus non-metastatic patients and healthy donors. Higher levels of miRNA-222 were observed in basal-like and in luminal B versus luminal A tumour subtypes. Lower levels of EmiR-21 were seen in HER2-positive patients during neoadjuvant treatment with Trastuzumab

CTCs in understanding tumour biology

Epithelial-mesenchymal transition (EMT) is believed to play an important role in the metastasis of epithelial cancers, including breast carcinoma. Studies on CTCs from breast carcinoma patients found that mesenchymal markers were enriched in CTCs, while primary tumour cells rarely express both mesenchymal and epithelial markers simultaneously, supporting the role of EMT in the metastasis of breast carcinoma.[58]

ctDNA as a prognostic marker in breast carcinoma

Both static and temporal measurements of ctDNA have shown associations with survival outcomes. Levels of ctDNA and the number of mutations detected at a single point of time and changes in ctDNA levels and the frequency of mutations after treatment have been shown to have prognostic value in breast carcinoma.[1,59-62] Several studies have shown that higher levels of ctDNA and cfDNA in the blood were associated with poorer recurrence free survival and overall survival.[1,60-62] However, studies conducted specifically in metastatic triple negative breast cancer have yielded conflicting results.[33,62,63]

Early breast carcinoma may be associated with micrometastatic disease and ctDNA levels are usually undetectable, limiting its use. However, some studies have been carried out in early breast carcinoma using high-sensitivity digital PCR. These have shown that, detection of ctDNA prior to surgery in women who received neoadjuvant chemotherapy was predictive of a poor prognosis, distant recurrence, and risk of relapse. They also found that post-surgery monitoring of ctDNA can predict the development of metastasis preceding the development of symptoms by several months.[64,65] Sequencing of the ctDNA has also been shown to be more predictive of the molecular profile of the metastatic tumour than sequencing of the primary cancer.[64] Therefore, it may provide more useful information to guide treatment. (Table 1)

ctDNA as a predictive marker in breast carcinoma

Mutations in the estrogen receptor -1 (ESR1) gene have been found to appear in patients who received endocrine therapy.[1] These mutations are associated with an aggressive clinical phenotype and ER-positive breast carcinoma recurrence. The ESR1 status of metastatic tumours shows a better concordance with ctDNA than the primary tumour.[54] Therefore, sequencing ctDNA would be more useful than sequencing the primary tumour mutation of this gene. Metastatic breast carcinoma patients with ctDNA ESR1 mutations have shorter progression-free survival on aromatase inhibitor-based therapy.[66]

Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA) is a promising biomarker for the treatment of advanced and metastatic breast carcinoma.[61] Clinical trials have shown that patients with HR positive, HER2 receptor negative, PIK3CA mutated advanced or metastatic breast carcinoma have prolonged progression-free survival when treated with alpelisib in combination with fulvestrant endocrine therapy than fulvestrant alone.[67,68] Clinical trials of palbociclib and fulvestrant in advanced ER positive, HER2 negative breast cancer have shown that a relative change in PIK3CA ctDNA level after 15 days treatment on palbociclib and fulvestrant, strongly predicts progression free survival.[69] The FDA has now approved the Therascreen PIK3CA RGQ PCR assay as a companion diagnostic assay for use in tissue and liquid biopsies.

Studies have shown that ctDNA sequencing can be used to identify patients with HER2 mutated cancers for clinical trial participation,[69] suggesting that it has the potential to be a useful predictive marker. ctDNA may be of predictive benefit in tumours with breast cancer gene (BRCA) mutations, since a ctDNA-based evaluation of somatic BRCA mutations may identify a group of patients treatable with poly ADP-ribose polymerase (PARP) inhibitors and platinum salts.[70,71] (Table 1)

TEPs in breast carcinoma

The Pan Cancer panel using TEP-derived RNA profiles developed by Best *et al*. was able to successfully identify breast carcinoma in patients with an average accuracy of 62%. TEP profiles distinguished patients with HER2-amplified, PIK3CA mutant or triple-negative breast carcinoma versus random classifiers which has potential prognostic and predictive implications.[33] (Table 1)

Exosomes in breast carcinoma

Cohort studies have shown that exosomes detected in the serum or plasma are helpful in the early diagnosis, prediction of prognosis and assessment of treatment response in patients with breast carcinoma.[72] Studies have shown a correlation between exosomal microRNA and disease stage, suggesting that they will be helpful in distinguishing between patients with localised

and occult metastatic disease.[73] Lower levels of exosomal micro-RNA level were observed in HER2-positive patients during neoadjuvant treatment with Trastuzumab, suggesting a potential value of exosomes to predict the treatment response of patients to Trastuzumab. Associations were also observed between specific exosomal micro RNAs and different breast cancer subtypes, suggesting that these biomarkers could have value in improving the classification and diagnosis of different BC subtypes.[73] (Table 1)

Limitations in the use of liquid biopsy

There are many factors that have limited the use of liquid biopsy in routine clinical practice. The high cost and lack of standardised testing methods have limited its use. The use as a screening test in early disease is limited due to the low quantities of CTCs (usually less than 1 CTC/10mL of blood in non-metastatic cancers) and ctDNA in early stages, short half-life of CTCs ex vivo and lack of specificity.[74,75] Although highly sensitive assays may be able to detect minute amounts of ctDNA, a particular cancer does not always show the same genetic mutations and a single genetic mutation may be present in different cancers, limiting the ability to localise the cancer.

Although it has been used in most large studies and is the only method approved by the FDA for testing of CTCs, the CellSearch method has the disadvantages of having expensive equipment, high detection cost per sample, complicated enrichment steps, long detection time for each sample, low purity of the captured CTCs, inability to isolate CTCs for phenotype identification and molecular analysis, and low sensitivity and selectivity resulting in high false positive and false negative rates.[76]

Most of the studies on exosomes and TEPs have been small cohort studies and large clinical trials are required before they are used widely as diagnostic, prognostic and therapeutic biomarkers in breast carcinoma.

Additionally, the lack of standardised testing procedures for all forms of liquid biopsy has resulted in some inconsistency in results, limiting the use in a clinical setting.

CONCLUSION

Liquid biopsy has proven value in prognostication, prediction of treatment benefit, and monitoring of patients with breast carcinoma. Many methods have been used for the detection of CTCs, ctDNA, TEPs and exosomes by liquid biopsy. However, most of these are still in the research stage. The CellSearch method for CTC and Therascreen PIK3CA RGQ PCR assay for PIK3CA mutations have FDA approval.

The CellSearch method, with its many limitations, is probably not feasible for routine practice particularly in low-and middle-income countries. In addition to the high cost, the lack of trained specialized personnel and infrastructure in the fields of molecular biology, computational biology and bioinformatics is a limiting factor in these settings.[77] Therefore, it may be worthwhile to work towards developing a less costly methodology to detect and assess the biological profile of CTCs in women with breast carcinoma that can be used in various clinical settings. Such a methodology if developed, could also be applied to other malignancies such as lung,[7] ovarian,[8] colorectal[9] and prostate[10] carcinoma, in which detection of CTC may also have prognostic value. However, any method that is adopted would need to be standardized prior to its use in the routine clinical setting.

Most of the technologies used for detecting genetic mutations and alterations have been developed in the west. These may not always be directly applicable to Asian populations. Therefore, more research on the development of population-specific genomic, proteomic, and cell-based biomarkers is also needed.

Despite the above limitations, liquid biopsy is a useful tool in clinical decision making, particularly with respect to prognostication, prediction of treatment benefit and monitoring of patients with breast carcinoma, paving the way toward more personalised cancer care. Limited knowledge of signs and symptoms of cancer, limited screening facilities, and fear of surgery have been identified as barriers to the early diagnosis of cancer in low-income countries. In comparison to tissue biopsy, liquid biopsy is a fairly non-invasive procedure. Therefore, despite the initial high cost of introducing the technology, liquid biopsy could help in addressing these challenges.

Liquid biopsy is emerging as a rapid, reliable, and minimally invasive cancer screening solution, with high specificity and sensitivity for cancer diagnosis, monitoring and prognostication that is likely to play an increasingly important role in the future in the management of patients with breast carcinoma.

Acknowledgements: None

Authors' contribution: Both authors made contributions to the concept of the review article. The first author (HDW) conducted the literature review and prepared the initial drafts of the paper. The paper was reviewed and revised critically by the second author (MDSL). Both authors have approved the final version of the manuscript.

Conflict of interest: The authors declared no conflict of interest.

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