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Breast Cancer(BC) And The Role of Circulating Tumor DNA

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Abstract: Immunohistochemistry is presently used to classify breast cancer. Nevertheless, breakthroughs in the monitoring of flowing tumor DNA (ctDNA) have opened up new avenues for diagnosis, categorization, biological understanding, and therapy selection. Thus, ctDNA tool further enhance our understanding the treatment. BC is a diverse illness, and ctDNA properly reflects this heterogeneity, enabling us to diagnose, monitor, and comprehend the disease's progression. Patients have greater circulating DNA levels than healthy persons, and ctDNA can be employed for a variety of purposes at various stages of disease, from advance screening diagnosis for advance disease. ctDNA is present in higher patients and can be used to detect mutations with the disease. It can also be used to monitor the progression of the disease and to detect mutations in advanced cases. The use of genetic testing in cancer diagnosis has grown significantly. It may be used to identify genetic changes linked to certain forms of cancer, allowing clinicians better understand the condition and determine the best course of action for their patients. This enables earlier and more accurate diagnosis as well as more intelligent treatment choices. Better rates of a full pathological response after neoadjuvant therapy and fewer cases after radical treatments have been associated with ctDNA in early breast cancer.

Keywords: breast cancer, cancer diagnosis, ctDNA, liquid biopsy, personalized medicine

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1. INTRODUCTION

1.1. Circulating Tumor DNA (ctDNA) and Breast Cancer(BC)

Amidst advances in breast cancer diagnosis, protection, and therapy, more than few million new cases are diagnosed each year, resulting in more than 650 thoudands deaths. BC[1] is a diverse illness, irrespective of the source of malignant cells. Even with improved treatments and early detection, the sheer number of people affected by breast cancer can make it difficult to effectively treat. Additionally, the diversity of breast cancer means that no two cases are the same, making it difficult to develop a one-size-fits-all approach to treatment. Breast cancer is classified mostly primarily on immune histochemical markers found in tumor biopsies: oestrogen receptor (ER), progesterone receptor (PR), KI-67[2], and human epidermal increase component 2. (HER2). Breast cancer is a group of diseases that affects the cells of the breast and can spread to other parts of the body. It is the most common cancer in women, though it can also affect men. It is typically diagnosed via mammogram, MRI, ultrasound, and/or biopsy. Several subgroups have indeed been put forth in order to personalise therapy and prognosis, as well as certain organizations advocated utilizing gene expression patterns to describe five separate intrinsic molecular categories of breast cancer, each with a different result (luminal A, B, basal-like, HER-2, enriched and claudin-low). Understanding breast cancer is like trying to piece together a giant puzzle. Every piece of the puzzle, from the subgroups to the molecular categories, work together to build a better picture of how to diagnose and treat it effectively. This is significant since it allows for tailored treatments and prognoses, as well as a more accurate comprehension of the nature of the disease. By classifying the subtypes, doctors can better understand the biology of the tumours and the responses to treatments, which allows for more effective treatment protocols and more accurate outcomes[3,4].

1.2. ctDNA

Extracellular systemic lupus erythematosus patient's human plasma was found to contain nucleic acid in the late 1950s. This is possible because the ctDNA contains genetic information about the cancer cells, which can be used to track their evolution and monitor the progression of the disease. DNA from circulating tumours (ctDNA) is one kind of genetic material that is released by cancer cells into the bloodstream. It is a biomarker of cancer, and its presence has been linked to the development and progression of various cancers. Therefore, it is essential to consider CTCs when interpreting ctDNA results to ensure a more accurate diagnosis. The capacity to measure ctDNA is a significant factor, and the variable allele fraction (VAF) is a key statistic[5]. The VAF measures the number of sequence reads that were found to suit a particular DNA strand when the locus was completely covered.

2. METHODOLOGY

degree of vascularization means that the quantity of ctDNA in a tumor sample is very small and can be difficult to detect. Furthermore, the quantity of ctDNA can vary depending on the type and stage of the tumor, in addition where it is located and how vascularized it is.

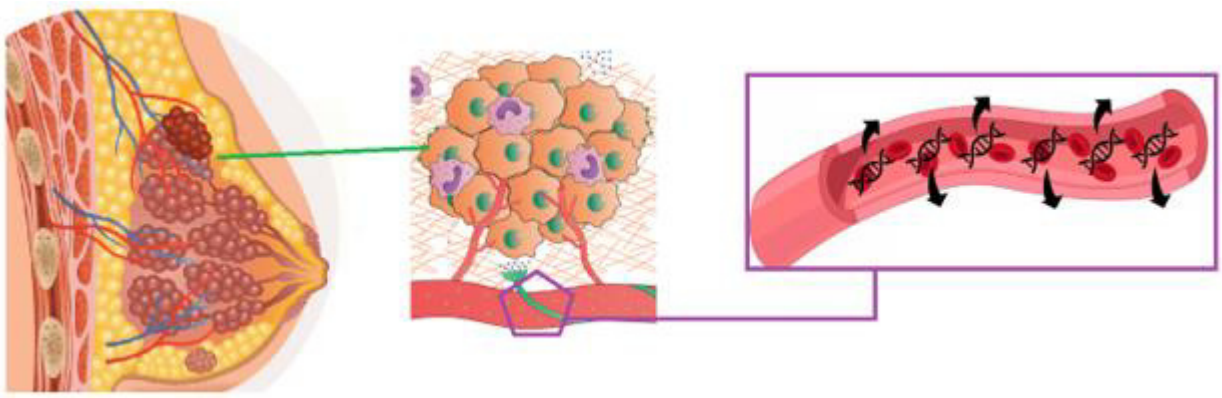

Fig 1: (a) Initial breast cancer and the connection seen between circulatory and breast cancer cells.[6] (b) Blood samples' primary components were, in order from left to right: cell lymphocyte, ctDNA, erythrocyte, ctDNA, platelet, CTC, exosome with ctDNA

The results showed that ctDNA levels vary between patients and change dynamically over time, which could be used to monitor the progression of the disease and to detect early metastases. The most pertinent articles were reviewed and chosen by the writers for evaluation. Plasma is used for ctDNA analysis is advised since this larger quantity is linked to DNA generated by blood cells during the coagulation process can cause contamination. A type of free-floating DNA in the body is known as circulating tumour DNA (ctDNA) bloodstream that originates from tumor cells. It can be utilised to track the development of cancer and to detect early metastases. It is evident that to identify, track, and characterise this ctDNA, high-throughput isolation procedures and extremely sensitive detection techniques are required. This is because ctDNA is present in very low concentrations within the blood, making it difficult to detect and quantify. Furthermore, ctDNA's existence can vary over time, making it even more challenging to track. Thus, via means of high-throughput isolation procedures and sensitive detection techniques is essential to accurately detect and quantify the ctDNA[7]. To obtain high isolation yields and discover more about how to accomplish this, it's important to comprehend how to extract and quantify ctDNA. Cancerous DNA in motion (ctDNA) is DNA that is shed from dying or dead tumor cells enters the blood. It is possible to identify the presence of ct DNA in the blood by using cancer, track the progression of the disease, and monitor the impact of treatment. Sanger

sequencing and qPCR were formerly quite helpful methods, but others have now supplanted them owing to their lack of sensitivity. Next-generation sequencing (NGS) is now the most commonly used method to detect ctDNA, due to its ability to find ctDNA even in small amounts. NGS also allows for the purpose of multiple mutations simultaneously, which is not possible with Sanger sequencing and qPCR[8]. Targeted methods have been created, including magnetics, emulsions, beads, ddPCR, or droplet digital polymerase chain reaction (BEAMing).

3. METHODOLOGIES THE CTDNA ANALYSIS AND DETECTION

ctDNA is typically identified in extremely small amounts (0.01-1.0%) of generally less than the entire ctDNA 1 ng/ L, in cancer patients[9]. The fact that serum contains 2–24 times more ctDNA than plasma. The reason for this is that ctDNA is released directly out of the tumour and into the circulation, so the concentration the ctDNA in serum is typically superior to plasma. Additionally, because ctDNA is quickly removed from the circulation, the concentration ctDNA in serum is further increased due to prolonged residence in the circulation. Plasma is used for ctDNA analysis is advised since this larger quantity is linked to DNA contamination is produced during the coagulation process by blood cells. This increased concentration ctDNA in the plasma yields a sample that is perfect for ctDNA analysis, as it is less likely to be affected by contaminating DNA from cellular sources. It is obvious that to identify, track, and characterise this ctDNA, high-throughput isolation procedures and extremely sensitive detection techniques are required. Cell-free DNA (ctDNA) is a type of DNA located in the bloodstream that is released from dead or dying cells. It is present in low concentrations and is highly fragmented, making it difficult to detect. Focused there have been methods created, including magnetics, emulsions, beads, digital polymerase chain reaction (ddPCR) using droplets, and ddPCR (BEAMing)[10]. ctDNA fragments are generally shorter than 500 bp, with the majority being shorter than 300 bp. In the initial emulsion stage of ddPCR, many lipid droplets are formed, allowing DNA fragments to be individually identified. In the second step, PCR is conducted within each drop using a solitary ctDNA particle. DdPCR enables the absolute measurement given a high sensitivity of the initial sample (0.01-0.1%), even if only known mutations can be tested for using this method, and particular methylation sites. BEAMing is founded the first stage of precise pre-amplification of areas of interest, then a PCR emulsion employing magnetic spheres covered in precise primers using cancer amplicons attach and are amplified once again within a droplet of lipid. Both technologies are used to detect circulating free DNA from fluids (ctDNA) biopsy samples, which provide an alternative biopsies of tissue for cancer screening. Amplicon sequencing, cancer personalized deep sequencing profiling using the CAPP-Seq, TAM-Seq, and safe sequencing system technologies (Safe-Seq) are further focused DNA sequencing methods (AmpliSeq). For the examination of a small panel of possible mutations in a first tumor or biopsy tissues, these approaches are particularly helpful. For instance, ctDNA from the Oncomine Breast test from Thermofisher in Waltham, Massachusetts, uses AmpliSeq technology and is utilised in clinical settings to identify a tiny fraction of breast cancer patients who have gene alterations samples. Yet, the ctDNA's representation of the tumor's heterogeneity might be lost since these focused approaches can only examine a certain amount of simultaneous mutations. This means that the test can only detect mutations that are already known and that are comprised of the test. As such, mutations that are unique to each patient might be overlooked and the full heterogeneity of the tumor likely not accurately captured. Moreover, previous individual mutational data is from the tumour necessary. Last but not least, and most significantly, a sizable amount of ctDNA originates originating from blood cells, as well as some somatic mutations discovered known to result from clonal hematopoiesis by NGS. For example, if a patient has a clonal hematopoiesis of indeterminate potential (CHIP) mutation, the mutation may be falsely considered to be a somatic mutation originating due to the tumour, leading to an incorrect interpretation of the patient's disease[11]. In sequencing investigations, the blood cell fraction should be included along with the ctDNA to prevent false positives because this problem is yet not fully understood. Furthermore, it is significant to remember that CHIP mutations can be inherited also may be present in the germline, potentially introducing a false positive bias if not considered.

4. EARLY BREAST CANCER AND CTDNA

4.1. ctDNA and preclinical prostate cancer

Self-examination currently, the only choices for screening and early detection are imaging tests including magnetic resonance imaging, echography, mammography and identification of localised breast cancer, which is treatable, possibility of curative therapy. These tests allow for the detection of very tumors that are small and localised and are frequently too small to be felt during a physical examination. Early breast cancer detection increases the chances of successful treatment and increases the likelihood of a positive outcome. Every two years, a sensitive test called a mammogram, is advised for women over 50. Nevertheless, there is no specialized test for individuals under 50, who make up around 20% of those with breast cancer diagnoses. Invasive ductal carcinoma and invasive lobular carcinoma are the two most prevalent kinds of breast cancer, which make up about 70-80% and 10-15% a total of breast cancers respectively. Regular self-exams and clinical breast exams should be performed to look for any changes in the breast tissue that may be indicative of breast cancer. Additionally, mammograms should be done regularly since they are the most effective approach to find breast cancer in its early stages.

4.2. Follow-Up Assessments Using ctDNA

Oncologists place a high premium on after complete removal of cancer, the early diagnosis of recurrence. During follow-up visits, it is advised to perform standard laboratory tests, anamnesis of symptoms, routine mammography, and clinical examination to look for distant relapses. This is because recurrences breast cancer cases are often identified before the patient is symptomatic, likewise, early detection can lead to more effective treatments and improved prognoses. Early detection also reduces the chance of complications, in addition the costs associated with treating advanced phases of the illness. Many studies have evaluated the ctDNA use during follow-up as a result of technological advancements. For instance, 45 individuals in the future research by Garcia-Murillas. harboured at least one of the55 patients' initial tumour biopsies revealed 14 breast cancer driver gene

alterations. [12] Patients with non-metastatic (stage I-III) breast cancer were included in two quite different analyses. They discovered with 100% specificity and 86-93% sensitivity for serial ctDNA monitoring, and could detect metastatic evolution 11 months on average prior to a clinical symptom, an imaging study, the CA 15-3 test, or a determination of liver function. CtDNA, or floating tumour DNA is a biomarker that can be used to detect the presence of tumour cells in the circulation. It might be useful in predicting cancer recurrence and detecting metastatic progression. Patient-specific ctDNA, sometimes referred to as customised DNA, which targets variations chosen from the main tumor exome, can be an useful option for individualsindividuals cannot be identified by common mutations to determine relapse after initial therapy. Cancer patients can benefit from CtDNA because it enables doctors to track changes in tumour DNA over time. This can be used to detect early signs of cancer recurrence and progression, allowing doctors to adjust treatment plans accordingly. Additionally, customised Patients with cancer can utilise DNA to identify tumour recurrence uncommon mutations, which could otherwise be difficult to detect. The drawback of this approach is that it is unable to detect an additional primary breast cancer. CtDNA can therefore offer helpful insight into the development of cancer in a patient, allowing doctors to make more informed decisions about treatment plans and potentially improve patient outcomes.

4.3. MRD detection

Reducing the quantity or length of therapy can help lowering the potential for both short- and long-term toxicities while still providing the same therapeutic effect. This can aid in raise the overall rate of survival by reducing the risk of serious side effects[13]. To assess therapy response, ctDNA maybe a more accurate way. Additionally, Identification of patients at high risk of developing future distant metastases and those with micro-metastases may be facilitated by ctDNA analysis, enhancing patient choice for specific treatments. Thus, ctDNA testing may be a valuable tool to optimize therapy strategies and better tailor treatment to individual needs. Also, it might aid in identifying people who won't benefit from adjuvant chemotherapy. Monitoring and detecting MRD may be essential for gauging therapy effectiveness and directing following therapeutic choices. CtDNA analysis can be used for therapy monitoring and predicting responses to treatment. It can provide insights about how well cancer treatments work and help determine which therapies are most likely to help individual patients. Indicating that individuals with these mutations may benefit from a more combative or specialised treatment[14]. Tumor size, aggressiveness, and subtype have all been linked to significant levels of ctDNA before to neoadjuvant therapy.

5. OUTLOOKS FOR THE FUTURE

There are a few requirements which must be satisfied before ctDNA's prospective clinical uses can be put into practise: a reliable and validated technology, a proven clinical value, and an economical strategy. These days, large assay platforms are being used to carry out research in central laboratories, which can support the standard pathological diagnostics carried out at each hospital with helpful information. This combination of reliable technology, proven clinical value, and cost-effective approach makes ctDNA a promising tool for clinical use. Moreover, the centralization of ctDNA is made possible by the utilisation of large assay platforms studies, which can provide additional data and insights to supplement traditional hospital-based pathological diagnoses. Being able to undertake multigene experiments, which call for specialist expertise and higher resources, is made possible by centralizing ctDNA analysis. For all produced data, appropriate in addition to the platforms for NGS, cloud storage is needed itself. ctDNA DNA from circulating tumors a type of DNA that is present in a person's bloodstream as a result of being present tumor cells. It is applicable to diagnose and monitor cancer, in addition to develop personalized treatments based on the tumor's genetic makeup. Also, workers for web-based laboratories duties, bio-informaticians for data processing, and specialists in data interpretation are required. For instance, compared to luminal breast cancer without these mutations, the prognosis of luminal breast cancer with PIK3CA and TP53 mutations may be completely different, and as a result, various treatment modalities may be required[15]. There may be a game changer for oncology research as it may allow for more precise and individualized treatments that target the specific mutations present in a patient. Additionally, it could provide a deeper comprehension of the disease and help to identify new therapies and treatments that are tailored to each specific patient.[16,17]

6. CONCLUSION

The utilisation of ctDNA analysis and fluid phase biopsy in the treatment of breast malignancy, in conclusion, provides a gateway for possibilities includes all potential disease scenarios, from early diagnosis to MRD recognition, initial recurrence identification, screening, and therapy management for chronic cancer. This technology has the potential to provide physicians with unprecedented details on the progression of a patient's disease, and to help them make more informed decisions about their treatment. It also allows for earlier detection and intervention, which can improve the chances of successful treatment and potentially decrease the chance of relapse. The development of early breast cancer detection, wherein the diagnosis of in situ carcinoma, for instance, is still unsure, is one of several issues it needs to be handled. Moreover, the inclusion of this fluid phase biopsy in active research initiatives and diagnostic tests ideally signals the acceptance of this method in medical care in the not-so-distant upcoming years for response monitoring, initial screening of progression, detection of MRD after neoadjuvant therapy, initial screening of hormonal resistance mechanisms, guiding subsequent treatments, and learning more about the malignancy whenever a biopsy is indeed not feasible. It is essential to step up technical efforts to advance fluid phase biopsy usage procedures and create uniform guidelines for its use in clinics. This could enable clinicians to better leverage the potential of liquid biopsy to improve patient care.

7. AUTHOR CONTRIBUTION STATEMENT

All authors have reviewed and approved the manuscript before submission.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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