Circulating tumor DNA testing and liquid biopsy: the future for precision medicine and guided targeted therapy for breast cancer?

The current standard for breast cancer screening (for non–high-risk patients) is an annual or semi-annual mammogram for women aged 40 and older.[1] However, mammography-based screening can give false-positive or false-negative results. This can lead to excessive use of invasive tissue biopsies, and unnecessary exposure to ionizing radiation—which can also become expensive and time-consuming for patients.[2]

Both normal and cancerous cells shed cell-free DNA (cfDNA) into blood circulation.[3] Circulating tumor DNA (ctDNA) are fragments of DNA derived from tumor cells that circulate in the blood together with cfDNA. The ctDNA originates directly from a tumor or from circulating tumor cells (and carries information from the tumor cell genome), while cfDNA enters the bloodstream after apoptosis or necrosis and carries genome-wide DNA information. The amount of ctDNA in the blood has been shown to be elevated in patients with cancer.[3] Different cancers release varying levels of ctDNA; the amount of ctDNA released is depends on the number of tumor cells that are in senescence vs undergoing apoptosis.[4]

The possibility of incorporating this biomarker obtained from a “liquid biopsy” is currently being studied and will hopefully become a standard of care for breast cancer screening and monitoring. The liquid biopsy detects ctDNA that has been released into the bloodstream from tumor regions and helps identify intratumoral heterogeneity and clonal evolution.[5] Sequencing tumor DNA has opened new possibilities for precision oncology.[6] Detection of somatic gene mutations, amplifications, and gene fusions helps to deliver targeted therapies.[6] Analysis of potential somatic mutations in ctDNA in combination with cfDNA levels can help capture clinically-relevant information beyond single genetic alterations and tumor fraction, potentially improving the accuracy of early detection and screening for breast cancer.

Recent advances in ctDNA testing technology have made it more accurate and reliable than ever. ctDNA testing has several benefits, including previously mentioned early detection of cancer (detecting ctDNA at low levels)[7]; monitoring of tumor dynamics, therapeutic response, and residual disease[8]; as well as analysis of the evolution of genetic or epigenetic alterations characterizing the tumor.[9] Its noninvasiveness, rapidity, and low-cost allow for longitudinal monitoring of cancer in real-time, potentially capturing tumor heterogeneity.[10,11]

In breast cancer, ctDNA testing can be used to detect the presence of cancer cells in the breast tissue before a tumor has even formed. This can help identify patients who are at high risk of developing breast cancer and allow the initiation of preventive measures—such as enhanced surveillance or prophylactic surgery. Early-stage breast cancer is generally more curable; early detection and diagnosis remain crucial for reducing cancer-related mortality. The detection of early-stage breast cancer can pose challenges because it is characteristically asymptomatic, and detection with standard-of-care screening methods is limited.

The liquid biopsy is creating more options for non-invasive diagnosis and therapeutic monitoring for breast cancer, and these liquid biopsies may mirror clinically relevant genetic alterations that occur in all tumor tissues. Liquid biopsy offers many notable advantages in comparison with traditional tissue biopsy. It allows for the detection of small tumors, minimal residual disease (MRD), and micrometastatic disease that is difficult to detect with a traditional tissue biopsy.[12] Liquid biopsy detects ctDNA that has been released into the bloodstream from multiple tumor regions and opens the possibility of identifying intratumoral heterogeneity and clonal evolution.[13] It can also detect small quantitative variations within the blood, enabling real-time surveillance.

The liquid biopsy can offer earlier and easier access to some tumor-based genetic information at any given timepoint and can replace a tumor tissue biopsy in some cases, helping to avoid delays and complications of a solid tumor invasive biopsy procedure. This is especially relevant in the metastatic setting, where ctDNA might be

the only available genetic material from tumors.[14] Tissue biopsy can only provide a static and spatially limited view of the disease at the time of sampling. Targetable mutations may be detected easier by noninvasive ctDNA testing over tissue testing, as it provides a more sensitive way of detecting malignancies than physical exams and imaging techniques that require exposure to ionizing radiation. ctDNA analysis could potentially reflect the genetic alterations that occur in all breast cancer tissues.[15,16] Further, it has been demonstrated that machine learning multi-gene signatures, obtained from ctDNA, identify complex biological features, including measures of tumor proliferation and estrogen receptor signaling, like what is accomplished using direct tumor tissue DNA or RNA profiling.[17]

By analyzing the amount of ctDNA in a patient's blood, it is becoming possible to track the progression of the disease and identify when it is becoming more aggressive or resistant to treatment. It shows treatment efficacy for targeted therapies by capturing broader molecular alterations that could affect the efficacy of targeted therapies. This information can assist in adjusting the patient's treatment plan and improve their survivability. Therefore, biomarkers from liquid biopsy become valuable in predicting the efficacy of several immunotherapies and can help monitor the dynamics of treatment response. ctDNA also offers the ability to identify acquired treatment resistance mutations as well.

Another advantage of ctDNA testing is it can also be used to monitor patients who have already been diagnosed with breast cancer. Retrospective studies have shown that detection of ctDNA in plasma, after patients have completed therapy for early-stage breast cancer, is associated with a very high risk of relapse.[18] Ongoing studies are helping with the tailoring of adjuvant treatment based on ctDNA. The intensity of adjuvant treatment, such as adjuvant radiation, chemotherapy, or immunotherapy, could be de-intensified, or omitted, for patients who have undetectable ctDNA—or intensified for patients who have detectable ctDNA after definitive treatments. This personalizes treatment specifically to the patient, reducing unnecessary, potentially harmful, and expensive therapies.

The detection and persistence of ctDNA in the middle of neoadjuvant systemic therapy (NST) may have the potential to negatively predict response to treatment and identify patients who will not achieve pathological complete response (pCR) or be classified with residual cancer burden (RCB) II/III. A noninvasive identification of RCB may have the potential to aid in clinical decision-making for treatment escalation in non-responders who are known to benefit from additional adjuvant therapy, or de-escalation by identifying patients who might not derive benefit from breast surgery after NST.[19]

Despite these distinct characteristics, the low levels of ctDNA found in early-stage disease, along with the lack of ctDNA shedding from some tumors, can further complicate or impede detecting early-stage breast cancer. Testing is further complicated by hematologic genetic alterations.[20] The limitation of PCR approaches is that these techniques only detect known mutations in certain genes, so patients without these mutations could be overlooked, limiting the application of this technology as a generic diagnostic technique for ctDNA analysis.[21]

The consistency of peripheral blood biomarker assays with tissue assays is controversial due to limitations in assay technology and analytical methods. There is a need to develop more accurate and sensitive ctDNA assays to navigate the patients who are responsive to immunotherapy. The challenge now is to develop new methods to help identify patients who are at high risk of relapse after treatment completion, and who may benefit from further treatment therapies.

Overall, ctDNA testing represents a promising area of research for the diagnosis, treatment, and monitoring of breast cancer. While more research is needed to fully understand its potential, the advances in this technology are certainly exciting and could lead to significant improvements in patient outcomes. It is hopeful that in the near future, ctDNA testing from liquid biopsy will become the standard of care in breast cancer screening, ultimately helping clinicians to better personalize treatment therapies and improve patient outcomes when treating patients with breast cancer.

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