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REVIEW ARTICLE

Advances and challenges of liquid biopsy in oncology for breast and ovarian cancers: a narrative review

Avanços e desafios da biópsia líquida em oncologia nos cânceres de mama e de ovário: uma revisão narrativa

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ABSTRACT

Objective: To review the role of liquid biopsy in the diagnosis and management of breast and ovarian cancers, highlighting the main biomarkers, analytical approaches, clinical utility, and current limitations affecting its implementation. **Methods:** Literature searches were primarily conducted using the PubMed/Medline database and were complemented by direct consultation of international scientific publishers' platforms. Original articles, reviews, clinical guidelines, and translational studies were included based on thematic relevance. A total of 41 publications comprised the qualitative synthesis. **Results:** The literature indicates that biomarkers such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), extracellular vesicles, and microRNAs contribute to tumor monitoring, detection of actionable genetic alterations, and assessment of therapeutic response. Molecular techniques such as PCR, quantitative PCR, digital PCR, RT-PCR, and next-generation sequencing (NGS) are widely employed. Despite its advantages, liquid biopsy remains a complementary tool due to limitations such as low sensitivity in early-stage tumors, variability in ctDNA shedding, interference from clonal hematopoiesis, and the lack of standardized analytical protocols. Furthermore, most validation studies are conducted in high-resource settings, highlighting gaps in feasibility, cost-effectiveness, and implementation in public healthcare systems, such as the Brazilian Unified Health System (SUS). **Conclusion:** Liquid biopsy represents a promising complementary tool in oncology, with more established clinical applications in specific settings such as tumor genotyping and monitoring in advanced disease. However, its broader incorporation into clinical practice depends on the consolidation of prospective evidence, methodological standardization, and demonstration of impact on relevant clinical outcomes.

Keywords: Liquid Biopsy; Public Health; Oncology; Ovarian Cancer; Breast Cancer.

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RESUMO

Objetivo: Revisar o papel da biópsia líquida no diagnóstico e manejo dos cânceres de mama e ovário, destacando os principais biomarcadores, abordagens analíticas, utilidade clínica e as limitações atuais que afetam sua implementação. **Métodos:** As buscas foram realizadas tendo o PubMed/Medline como base de dados principal, complementadas por consultas diretas às plataformas de editoras científicas internacionais. Foram incluídos artigos originais, revisões, diretrizes clínicas e estudos translacionais, selecionados com base na relevância temática. Ao todo, 41 publicações compuseram a síntese qualitativa. **Resultados:** A literatura indica que biomarcadores como células tumorais circulantes (CTCs), DNA tumoral circulante (ctDNA), vesículas extracelulares e microRNAs contribuem para o monitoramento tumoral, a detecção de alterações genéticas acionáveis e a avaliação da resposta terapêutica. Técnicas moleculares como PCR, PCR quantitativa, PCR digital, RT-PCR e sequenciamento de nova geração (NGS) são amplamente empregadas. Apesar de suas vantagens, a biópsia líquida permanece como uma ferramenta complementar, devido a limitações como baixa sensibilidade em tumores em estágio inicial, variabilidade na liberação de ctDNA, interferência da hematopoiese clonal e ausência de protocolos analíticos padronizados. Além disso, a maioria dos estudos de validação é conduzida em ambientes com altos recursos, evidenciando lacunas quanto à viabilidade, custo-efetividade e implementação em sistemas públicos de saúde, como o Sistema Único de Saúde (SUS). **Conclusão:** A biópsia líquida representa uma ferramenta complementar promissora na oncologia, com aplicações clínicas mais consolidadas em cenários específicos, como genotipagem tumoral e monitoramento em doença avançada. No entanto, sua incorporação mais ampla na prática clínica depende da consolidação de evidências prospectivas, padronização metodológica e demonstração de impacto em desfechos clínicos relevantes.

Palavras-chave: Biópsia líquida; Saúde pública; Oncologia; Câncer de ovário; Câncer de mama.

Introduction

Cancer remains one of the leading global public health challenges due to its high incidence, mortality, and growing inequalities in access to diagnosis and healthcare. Recent global estimates project a substantial increase in cancer cases worldwide, with more than 35 million new cases expected by 2050, disproportionately affecting low- and middle-income countries[1,2]. These data highlight the urgent need for innovative diagnostic and monitoring strategies capable of improving early detection and supporting more effective therapeutic decision-making.

Breast cancer is the most commonly diagnosed malignancy among women and one of the leading causes of cancer-related mortality. In 2022, approximately 2.3 million new cases and 670,000 deaths were reported globally, with projections indicating a 38% increase in incidence and a 68% increase in mortality by 2050. Although incidence is higher among women over 50 years of age, increasing trends have been observed across different age groups[3].

Ovarian cancer, in turn, is the eighth most common cancer among women and represents one of the most lethal gynecological malignancies worldwide. It predominantly affects middle-aged and older women, with most cases diagnosed at advanced stages of the disease (FIGO III–IV), when five-year survival rates remain below 30%. Despite a decline in incidence

in some high-income regions, its global burden is expected to increase, mainly driven by population aging[4,5].

Although breast cancer benefits from established screening strategies such as mammography, which has been shown to reduce mortality in selected populations, these approaches still present important limitations, including reduced sensitivity in dense breasts and the occurrence of false-negative results. In contrast, ovarian cancer lacks effective early screening methods. In this context, such limitations hinder early-stage diagnosis, restrict therapeutic options, and reduce the likelihood of cure, reinforcing the need for molecular approaches that enable earlier detection, real-time disease monitoring, and more personalized therapeutic strategies[6,7].

Tumor genomic complexity, resulting from genetic heterogeneity and clonal evolution, poses additional challenges to conventional diagnostic tools[8]. Static tissue biopsies often fail to capture tumor dynamics over time, which complicates both diagnosis and therapeutic management[9].

In this context, liquid biopsy (Figure 1) has emerged as a promising complementary tool, allowing the detection of tumor-derived biomarkers in body fluids, particularly blood. Its minimally invasive nature enables repeated sampling and real-time disease monitoring. However, despite its potential, clinical implementation remains limited due to analytical challenges, variable sensitivity, and insufficient validation, particularly in public healthcare systems[9-11].

Therefore, the present study provides a narrative review of the literature on the application of liquid biopsy in the diagnosis and management of breast and ovarian cancers, focusing on key biomarkers, molecular techniques, clinical applicability, and the limitations that hinder its widespread adoption.

Method

This study is characterized as a narrative literature review aimed at analyzing the application of liquid biopsy in oncological diagnosis, with a specific focus on breast and ovarian cancers.

The literature search was conducted in the PubMed/MEDLINE database, covering publications up to March 2026, and was complemented by manual searches on scientific publishers' platforms, including Nature Portfolio, Springer Nature, MDPI, and Wiley. Articles published in English were prioritized, with an emphasis on recent studies and those of higher scientific relevance.

The search strategy was based on a combination of controlled descriptors (Medical Subject Headings – MeSH) and free-text terms, including: "*liquid biopsy*", "*circulating tumor DNA*", "*circulating tumor cells*", "*exosomes*", "*breast cancer*", and "*ovarian cancer*", combined using Boolean operators (AND/OR), as appropriate. Search combinations were adapted according to the database to enhance sensitivity.

Eligible studies included original articles, narrative and systematic reviews, clinical guidelines, and translational

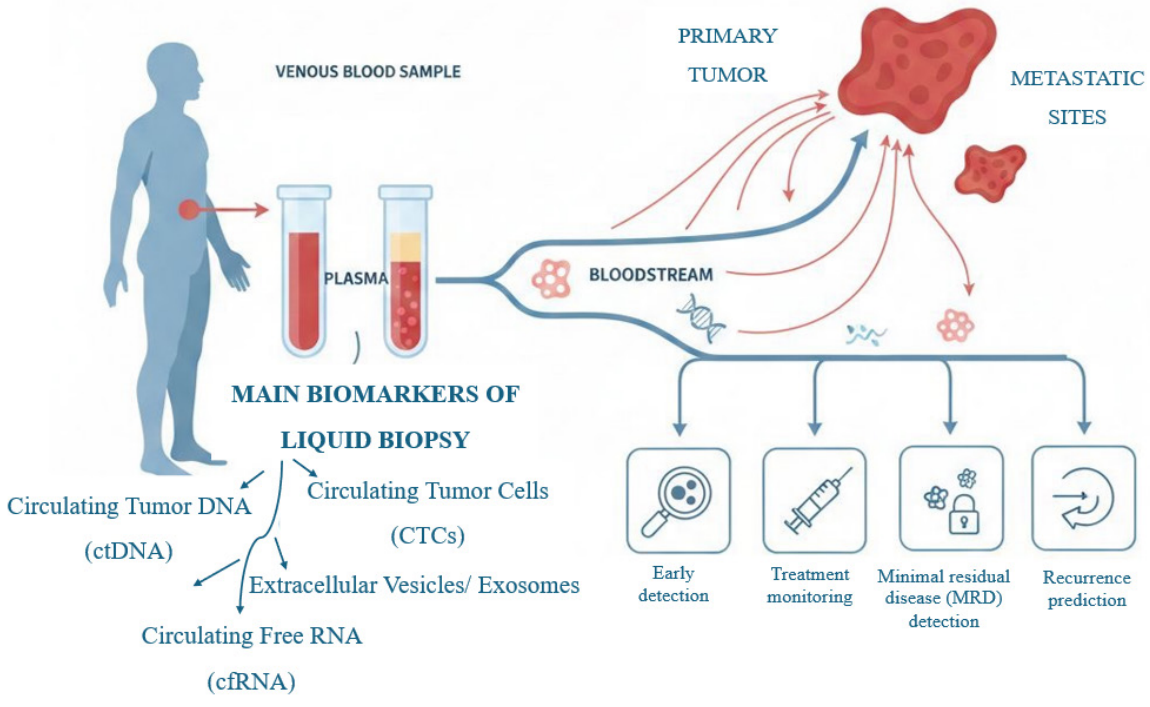

Figure 1. Schematic representation of liquid biopsy in oncology. Tumor-derived components released from primary and metastatic lesions enter the bloodstream and can be detected in peripheral blood samples. Key biomarkers include circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), cell-free RNA (cfRNA), and extracellular vesicles. These analytes support early detection, treatment monitoring, minimal residual disease assessment, and recurrence risk prediction, highlighting the role of liquid biopsy as a complementary tool in cancer management.

studies addressing biological mechanisms, analytical validity, clinical utility, or challenges related to the implementation of liquid biopsy. Exclusion criteria comprised duplicate studies, publications not directly related to the central topic, articles without full-text access, and studies considered to have limited evidence or low relevance to the objectives of this review.

Study selection was conducted based on thematic relevance, scientific consistency, and contribution to the current understanding of the topic.

A total of 41 publications were included in the qualitative synthesis. The selected literature was critically analyzed and organized into thematic sections, covering clinical applications, biomarkers, analytical methodologies, limitations, and perspectives of liquid biopsy in oncology.

Results

Tissue Biopsy and Liquid Biopsy in Oncological Diagnosis

Tissue biopsy remains a fundamental tool for cancer diagnosis, providing histopathological confirmation and initial molecular characterization to guide therapeutic decision-making. However, as an invasive procedure, tissue biopsy often causes patient discomfort and presents important limitations, particularly in clinical scenarios that require continuous monitoring of tumor progression, recurrence, or therapeutic response. Repeated tissue sampling is associated with risks such as infection, bleeding, and increased costs, making its application impractical in several clinical contexts[12].

In this scenario, liquid biopsy emerges as a complementary approach capable of overcoming part of these limitations by enabling minimally invasive and repeatable molecular profiling through the analysis of tumor-derived alterations in biological fluids, including blood, urine, cerebrospinal fluid, pleural and ascitic fluids, as well as saliva[11-13].

Among these biological materials, peripheral blood is the most widely used source of tumor biomarkers due to its accessibility and significant reproducibility. Processes such as neoangiogenesis and increased cellular turnover favor the release of tumor-derived genetic material into the circulation, enabling molecular characterization and real-time assessment of tumor burden and disease dynamics[9].

Clinical studies comparing liquid biopsy with tissue biopsy have shown that liquid biopsy can improve diagnostic efficiency, reduce procedural burden, and expand the detection of actionable genomic alterations, whether used alone or in combination with tissue biopsy[14-16]. However, these benefits are more evident in advanced disease settings, where higher tumor burden enhances the detectability of circulating biomarkers, whereas its application in early-stage disease remains limited by the sensitivity of currently available methods.

Xie et al. (2022)[14] evaluated the detection of HER2 gene amplification using digital polymerase chain reaction (dPCR) in plasma samples from patients with advanced and recurrent breast cancer, comparing liquid biopsy results with immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) performed on tissue samples. Although overall sensitivity was moderate, a significant increase in sensitivity was observed in advanced and recurrent cases, reflecting higher tumor burden and increased release of circulating tumor DNA (ctDNA). Notably, liquid biopsy identified HER2 amplification in some cases classified as negative by tissue biopsy, possibly due to spatial tumor heterogeneity. These findings suggest that liquid biopsy may reduce false-negative results and support therapeutic refinement, particularly in advanced or treatment-resistant breast cancer.

Evidence from lung cancer studies further supports the complementary role of liquid biopsy in clinical practice. Maity et al. (2023)[15] demonstrated that, in patients with stage IV lung adenocarcinoma, simultaneous genotyping using liquid and tissue biopsy significantly reduced the time to definitive molecular diagnosis compared with tissue analysis alone, in addition to decreasing the need for repeat biopsies in cases of insufficient tumor material. Each modality was able to identify actionable genomic alterations not detected by the other, highlighting the diagnostic value of a combined approach. Similarly, Raez et al. (2023)[16] reported high concordance between plasma-based next-generation sequencing (NGS) and tissue biopsy for guideline-recommended biomarkers in patients with metastatic non-small cell lung cancer (NSCLC). Liquid biopsy showed a shorter turnaround time and higher analytical success rate. No significant differences were observed in overall survival or progression-free survival between patients whose treatment decisions were primarily guided by liquid biopsy or tissue biopsy, supporting the clinical reliability of liquid biopsy-based genotyping in lung cancer.

From a clinical perspective, the most consistent evidence is concentrated in tumor genotyping in advanced disease, particularly in lung cancer, where liquid biopsy already has established applicability in clinical guidelines. This scenario is supported by recent position statements from specialized societies such as the European Society for Medical Oncology (ESMO), which recognize the clinical validity of ctDNA-based assays and their use in practice for the identification of actionable variants and therapeutic guidance. Furthermore, its application has been progressively expanded to other tumor types, especially in the context of disease monitoring and detection of therapeutic resistance[17].

Despite its recognized prognostic and predictive potential and the growing evidence of clinical utility in therapy selection, data demonstrating a direct impact on robust outcomes such as overall survival remain limited. This limitation is largely due to the predominance of retrospective or non-randomized studies, although prospective studies have already shown promising results across different malignancies. In this context, the conduct of well-designed prospective clinical trials remains essential to comprehensively evaluate clinically meaningful outcomes, thereby contributing to a more precise definition of the clinical value of liquid biopsy.

Main Biomarkers Used in Liquid Biopsy

The main targets of liquid biopsy include circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and cell-free DNA (cfDNA), as well as extracellular vesicles such as exosomes, in addition to proteins, metabolites, and circulating RNAs, including messenger RNA (mRNA) and microRNAs[18].

CTCs were the first non-invasive tumor biomarkers identified in peripheral blood and originate from primary or metastatic lesions. Despite their well-established prognostic value, including insights into tumor heterogeneity, mechanisms of therapeutic resistance, metastatic potential, and minimal residual disease, their routine clinical application remains limited due to technical challenges related to their low abundance and the lack of standardization across detection methods, which restricts their broader incorporation into clinical practice[19].

In several solid tumors, lower CTC counts, commonly defined as ≤5 CTCs per 7.5 mL of blood in assays based on the CellSearch platform, have been associated with improved clinical outcomes[20].

Ongoing studies aim to elucidate the biological factors influencing CTC circulation, as well as the role of immune surveillance in controlling these cells[19].

Other key biomarkers include cfDNA and ctDNA, which are nucleic acid fragments released into the circulation primarily through processes such as apoptosis and cellular necrosis. The analysis of ctDNA and cfDNA is recommended for molecular profiling, disease monitoring, detection of mutations associated with therapeutic resistance, and prognostic stratification[21].

Although ctDNA was first described in 1948, its clinical relevance has expanded substantially in recent decades due to the development of highly sensitive molecular techniques that have enabled its detection and quantification[20]. Plasma ctDNA levels are influenced by multiple factors, including tumor type, disease stage, tumor burden, patient clinical status, and ongoing treatment. In general, ctDNA concentrations are lower in early-stage disease and increase with tumor progression[21].

Despite this variability, ctDNA accurately reflects the tumor genomic landscape and intratumoral heterogeneity, particularly in metastatic settings, where repeated tissue biopsies are often impractical. Due to its short half-life, ctDNA enables real-time assessment of tumor dynamics and therapeutic response[18]. Currently, ctDNA represents the most consolidated biomarker in liquid biopsy for clinical application. The European Society for Medical Oncology (ESMO) recommends the use of ctDNA-based assays as a complementary tool and, in selected tumor-specific indications, as an alternative to conventional tissue genotyping, reinforcing its role in contemporary oncological practice[17].

Circulating cell-free RNA (cfRNA), including messenger RNA and microRNAs (miRNAs), has also gained relevance as a real-time indicator of gene expression patterns and regulatory pathways, expanding the molecular information obtained through liquid biopsy. miRNAs play a central role in post-transcriptional gene regulation and have been widely investigated as diagnostic, prognostic, and predictive biomarkers. Dysregulation of these small RNAs is associated with the activation of oncogenic pathways, therapeutic resistance, and tumor progression[13,22]. Despite these advances, the clinical application of cfRNA and miRNAs remains largely investigational, limited by methodological variability and the lack of robust clinical validation.

Similarly, exosomes have been described as one of the most promising sources of biomarkers in liquid biopsy; however, their clinical application is still considered experimental, being limited by technical challenges and the lack of standardized protocols for isolation and analysis. These nanometric vesicles (30–200 nm) are released by most cell types and carry a molecular cargo composed of DNA, RNA, proteins, and lipids, reflecting the physiological and pathological state of their cells of origin. The presence of a lipid bilayer confers high stability to exosomes, allowing preservation of their molecular content and detection in various biological fluids[18,23]. Studies demonstrate particular relevance of exosomes in epithelial ovarian cancer, in which exosomal miRNAs have been associated with improved prognostic accuracy[22].

Beyond their diagnostic potential, exosomes have also been investigated as therapeutic vehicles. Exosome-based strategies, particularly those derived from immune system cells, show potential for targeted drug delivery, modulation of immune responses, induction of apoptosis in tumor cells, and enhancement of antitumor immunity[24]. However, challenges such as vesicular heterogeneity, small size, and technical difficulties related to isolation and characterization still limit their routine clinical application[9].

Table 1 provides a comparative overview of the main liquid biopsy biomarkers, highlighting their biological sources, clinical applications, key limitations, and level of clinical evidence.

Overall, liquid biopsy biomarkers present different levels of clinical maturity. ctDNA stands out as the biomarker with the greatest clinical applicability, especially in advanced disease settings, where it has already been incorporated into guidelines for tumor genotyping and therapeutic monitoring. CTCs, in turn, have well-established prognostic value, although their routine clinical use remains limited by technical and standardization challenges. In contrast, emerging biomarkers such as cfRNA, microRNAs, and exosomes remain largely in the investigational phase, showing promising potential but still requiring robust clinical validation and methodological standardization.

In this context, the integration of multiple biomarkers through multi-omics approaches represents a promising strategy to enhance the sensitivity and specificity of liquid biopsy, although its clinical implementation still requires technological advances and additional evidence.

Biochemical Tests in Liquid Biopsy

Liquid biopsy analyses rely on a range of biochemical and molecular techniques, among which the most

commonly employed are polymerase chain reaction (PCR), quantitative PCR (qPCR), digital PCR (dPCR), reverse transcription PCR (RT-PCR), and next-generation sequencing (NGS). Each of these methodologies presents specific analytical characteristics and is applied according to distinct clinical and research objectives, such as mutation detection, quantification of low-frequency variants, and comprehensive genomic profiling[9,10,13,18].

Conventional PCR is a targeted approach that enables the amplification of specific genomic regions for the detection of genetic mutations or chromosomal rearrangements. It is a widely used technique in oncological diagnostics, particularly for tumor staging, disease monitoring, and identification of previously known genetic variants. RT-PCR, although capable of providing rapid and highly specific results, has limited sensitivity, often requiring confirmation through conventional tissue biopsy[25].

Recent technological advances have driven the application of qPCR and NGS, particularly due to their ability to detect low concentrations of ctDNA in peripheral blood. NGS allows the simultaneous analysis of multiple genes of interest, providing a more comprehensive genetic profile of the tumor. However, this technique is associated with higher costs, longer processing times, and, in certain clinical contexts, lower sensitivity compared to PCR-based methods[26].

One of the major challenges in ctDNA detection is that early-stage tumors release significantly lower amounts of genetic material into the circulation compared to metastatic tumors, thereby limiting the sensitivity of currently available assays[18,20,21]. As a consequence of these technical limitations, the European Society for Medical Oncology (ESMO) does not recommend ctDNA monitoring for recurrence detection in early-stage tumors, due to the inability of current technologies to reliably detect such low ctDNA levels[17].

This scenario underscores the need for continuous innovation in liquid biopsy methodologies, with a focus on the development of more sensitive and accurate assays capable of expanding their applicability in early cancer detection and oncological monitoring.

Liquid Biopsy and Breast Cancer

Liquid biopsy has emerged as a clinically relevant tool for breast cancer management, particularly in therapeutic monitoring and prognostic stratification. Compared with traditional serum markers such as CA 15-3, ctDNA demonstrates superior correlation with tumor burden and disease dynamics, often anticipating radiological progression or recurrence[17]. In metastatic breast cancer, ctDNA analysis has shown high clinical value for early detection of relapse and real-time assessment of treatment response[27,28].

A recent systematic review and meta-analysis including 37 studies and more than 4,200 patients with metastatic breast cancer demonstrated that the detection of ctDNA was significantly associated with worse overall, progression-free, and disease-free survival. Subgroup analyses identified *TP53* and *ESR1* alterations as being particularly associated with poorer outcomes, reinforcing the role of ctDNA as a biomarker of aggressive disease biology[29].

In early-stage breast cancer, liquid biopsy has also shown promise for minimal residual disease (MRD) detection.

Table 1. Comparative overview of liquid biopsy biomarkers: clinical applicability, level of evidence, and limitations

Biomarker	Biological source	Main clinical applications	Level of evidence	Main limitations
ctDNA (circulating tumor DNA)	Tumor-derived DNA fragments in plasma	Tumor genotyping, detection of actionable mutations, treatment monitoring, resistance detection	Established (especially in advanced disease)	Low sensitivity in early-stage disease; pre-analytical variability; interference from clonal hematopoiesis (CHIP)
cfDNA (cell-free DNA)	DNA released from normal and tumor cells	Global quantification of circulating DNA; support for ctDNA analysis	Intermediate	Low tumor specificity; requires complementary techniques
CTCs (circulating tumor cells)	Intact tumor cells in blood	Prognostic assessment, tumor heterogeneity, minimal residual disease	Intermediate	Low abundance; technical challenges in isolation; lack of standardization
cfRNA / miRNAs	Circulating RNA in plasma	Gene expression profiling; diagnostic and prognostic biomarkers	Investigational	Molecular instability; methodological variability; lack of robust clinical validation
Exosomes	Extracellular vesicles containing DNA, RNA, and proteins	Diagnostic/prognostic biomarkers; potential drug delivery systems	Investigational	Isolation challenges; heterogeneity; lack of standardized protocols
Proteins and circulating metabolites	Plasma/serum	Complementary biomarkers (e.g., CA-125)	Established (limited)	Low specificity; influenced by non-cancer conditions

Janni et al. (2025)[30] reported that a plasma-only, multiomic ctDNA assay detected ctDNA at or before distant recurrence in 79% of patients, with a lead time ranging from 3.4 to 18.5 months prior to clinical relapse.These findings highlight the potential of liquid biopsy to identify patients at high risk of recurrence, even in the absence of available tumor tissue, and to guide closer surveillance or therapeutic escalation.

From a predictive standpoint, ctDNA enables the noninvasive detection of actionable mutations, including alterations in *HER2*, *BRCA1,* and *BRCA2*. Studies indicates that *HER2* amplification detected through ctDNA is associated with aggressive disease phenotypes and tumor heterogeneity and may inform therapeutic decision-making regarding *HER2*-directed therapies, particularly in metastatic settings or when tissue biopsy is not feasible[31,32].

CTCs further complement ctDNA-based approaches. Elevated CTC counts are associated with reduced overall survival in both early and advanced breast cancer, underscoring their prognostic significance[10]. Advanced phenotypic and molecular characterization of CTCs, using epithelial markers (EpCAM), stemness-associated markers (CD44), and immune-evasion markers (CD47) has provided insights into metastatic potential and therapy resistance. Studies have linked epigenetic alterations, including promoter methylation of tumor suppressor genes such as *SOX17, BRMS1*, and *CST6*, to increased dissemination and resistance to endocrine therapies[33].

Liquid Biopsy and Ovarian Cancer

Liquid biopsy has gained increasing relevance in ovarian cancer due to the limitations of conventional diagnostic and monitoring strategies. CA-125 remains the most widely used serum biomarker; however, its low specificity compromises its accuracy, as elevated levels may also be observed in benign gynecological conditions, inflammatory processes, and other malignancies[34,35]. Therefore, its use as a standalone marker is insufficient for the early detection of recurrence and for the precise assessment of therapeutic response.

In this context, the analysis of circulating biomarkers, particularly ctDNA and cfDNA, has emerged as a promising approach. Evidence indicates that longitudinal assessment of these markers shows significant correlation with clinical and radiological parameters. Llueca et al. (2024)[36] evaluated women with advanced high-grade serous ovarian cancer and demonstrated that dynamic changes in ctDNA and cfDNA levels preceded disease recurrence when compared with conventional methods, including imaging and CA-125. Similarly, Rios-Doria et al. (2025)[37] observed that serial ctDNA analysis using tumor-informed next-generation sequencing reflects disease progression and therapeutic response in most cases. In one patient, an increase in ctDNA preceded clinical recurrence by approximately four months, even in the absence of imaging changes and CA-125 elevation. Nevertheless, discordant cases, such as clinical progression with undetectable ctDNA, highlight biological variability and the sensitivity limitations of current assays.

Complementarily, the review by Martinelli et al. (2025)[38] emphasizes that this approach can be applied across multiple stages of clinical management, including screening, early diagnosis, staging, therapeutic response assessment, and follow-up. In the context of early detection, biomarkers such as cfDNA, miRNAs, and protein signatures have demonstrated improved performance when used in combination, particularly alongside CA-125. Additionally, elevated cfDNA levels and increased ctDNA detection have been associated with more advanced stages, reflecting tumor burden and heterogeneity. Among clinical applications, those related to therapeutic monitoring and follow-up are particularly noteworthy, as ctDNA enables the identification of resistance and earlier detection of recurrence compared with conventional methods. However, pre-analytical and biological factors, such as sample type, extraction protocols, and interindividual variability, still impact reproducibility, reinforcing the need for methodological standardization and clinical validation.

In addition to ctDNA, exosomes have emerged as an additional source of biomarkers. These extracellular vesicles exhibit high stability and the ability to preserve their molecular cargo, enabling subsequent analyses[39]. In ovarian cancer, proteins such as TSG101, CD9, CD81, CD63, and claudin-4 have been extensively investigated, along with other markers of potential diagnostic and prognostic relevance, including EpCAM, PCNA, TUBB3, EGFR, APOE, CLDN3, FASN, ERBB2, and L1CAM[34]. Despite this potential, their clinical application remains largely investigational, limited by technical challenges related to isolation, standardization, and data interpretation.

Overall, liquid biopsy shows potential applicability across different stages of ovarian cancer management, ranging from early diagnosis to post-treatment monitoring[38]. However, most of the available evidence still derives from studies with small sample sizes, retrospective designs, and significant methodological heterogeneity, which limits its incorporation into clinical practice. Furthermore, the sensitivity of ctDNA-based assays remains low in early-stage disease, and their use is therefore recommended in combination with other diagnostic methods. Thus, in the current scenario, liquid biopsy should be considered a complementary tool, with greater applicability in advanced disease and longitudinal monitoring.

Main Challenges in Liquid Biopsy

Despite its growing relevance in oncology, the practical implementation of liquid biopsy faces significant economic, technical, and biological challenges (Figure 2). High assay costs, the need for sophisticated laboratory infrastructure, and reliance on advanced molecular platforms remain major barriers to widespread clinical adoption, particularly in low- and middle-income countries[40]. Consequently, most validation studies are conducted in high-resource settings, limiting the generalizability of their findings to resource-constrained health systems.

In Brazil, these challenges are further intensified by restricted reimbursement pathways within the Unified Health System (SUS), complex regulatory processes overseen

by ANVISA, and the absence of nationally standardized clinical guidelines and analytically validated panels, which collectively hinder routine adoption and inter-laboratory comparability. Similar implementation barriers have been consistently reported in the literature, underscoring persistent gaps in standardization, regulatory frameworks, and equitable access to liquid biopsy technologies[40,41].

Beyond structural barriers, pre-analytical and analytical limitations also affect test performance. Inadequate sample collection, handling, and processing can lead to both false-negative and false-positive results. False negatives are frequently associated with low ctDNA concentrations, especially in early-stage tumors, where tumor burden is limited. This represents a key limitation of current technologies and highlights a major knowledge gap regarding standardized thresholds for ctDNA detection across different cancer types and disease stages[21].

False-positive results, on the other hand, may occur due to clonal hematopoiesis of indeterminate potential (CHIP), which involves clonal expansions that release significant amounts of DNA into the bloodstream. CHIP-related somatic mutations are more common in smokers, elderly individuals, and patients who have undergone systemic antineoplastic therapy. Distinguishing CHIP-derived mutations from tumor-specific alterations remains a significant analytical challenge. For instance, mutations in genes associated with kidney (*VHL*), prostate (*SPOP*), lung (*EGFR*), breast (*ESR1*) cancers are specific to tumors, not CHIP. Similarly, mutations in *KRAS* are uncommon in the context of CHIP and are linked to cancers such as lung and colorectal. Although sequencing of matched white blood cell DNA has been proposed as a mitigation strategy, its routine implementation is still limited, and standardized protocols are lacking[17].

Another significant obstacle is that healthy cells, including hematopoietic cells, also release DNA into the bloodstream, which can increase the likelihood of false positives in ctDNA-based cancer diagnostics. Tumor heterogeneity further complicates interpretation, as different tumor sites may release variable amounts of genetic material into circulation[10].

Collectively, these challenges reveal gaps in methodological standardization, external biomarker validation, and clinical applicability. Addressing these limitations is essential to enhance the sensitivity, specificity, and reproducibility of liquid biopsy, particularly in cancer screening and early diagnosis[26]. Furthermore, successful integration into public healthcare systems will require not only technological advances but also coordinated policy development, cost-effectiveness analyses, and equity-oriented implementation strategies to prevent the amplification of existing disparities in cancer care[40].

Conclusion

This review analyzed the role of liquid biopsy in breast and ovarian cancers, highlighting its value as a complementary tool to conventional tissue biopsy and imaging-based diagnostic methods. Liquid biopsy offers relevant clinical advantages, including its minimally invasive nature, the possibility of dynamic monitoring of tumor evolution, and the potential for early detection of therapeutic resistance.

However, its clinical implementation remains limited by factors such as reduced sensitivity in early-stage disease, tumor heterogeneity, pre-analytical variability, and high costs. Therefore, the broader clinical impact of liquid biopsy will depend on the development of additional prospective studies, methodological standardization, and the establishment of cost-effective platforms compatible with public healthcare systems.

In this context, liquid biopsy holds significant potential to contribute to the advancement of precision oncology; however, its wider incorporation into clinical practice will depend on the consolidation of robust evidence demonstrating its benefit in clinically meaningful outcomes.

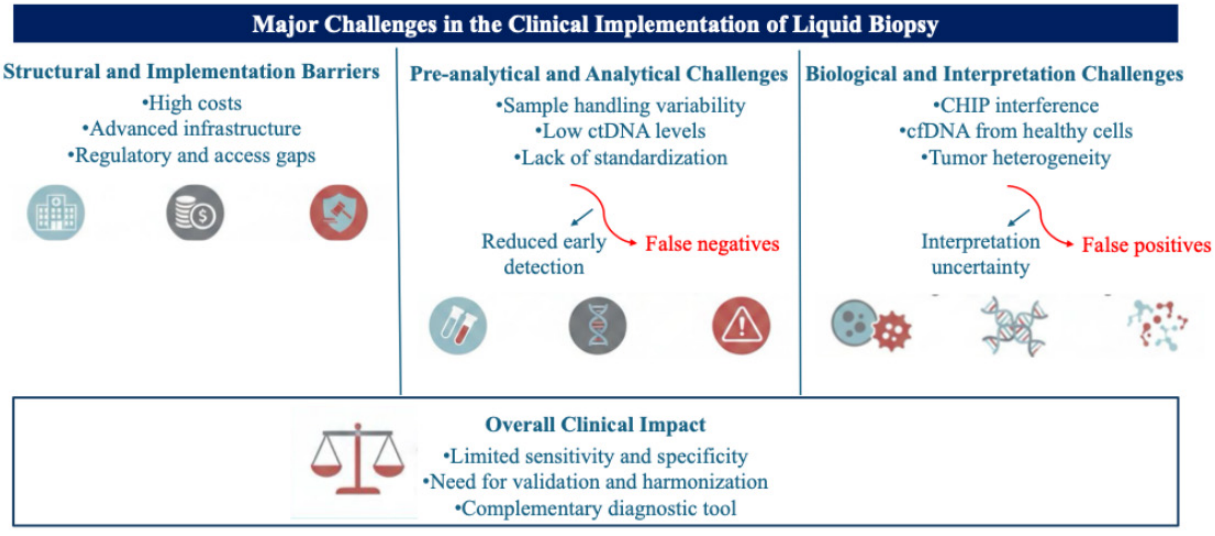

Figure 2. Major challenges limiting the clinical implementation of liquid biopsy in oncology

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Authors' contribution

We describe contributions to the papers using the taxanomy (CRediT) provide above:

Conceptualization, Investigation, Methodology & Writing–original draft: DC Nasorry; EVN da Silva; LC Passoni; MFF de Souza. *Visualization & Writing–analysis and editing*: VP Franco; YR Coutinho. *Validation, Data Curation & Formal Analysis*: LG Nogueira. *Project Administration, Resources, Supervision, Formal Analysis & Writing–analysis and editing*: FA Resende.

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