Modern Applied Medical Research

Volume 5,Issue 7, July, 2025,Page 06-12
ISSN: 2582-9181

Review Article Received: 25-06-2025| Accepted: 15-07-2025 | Published: 28-07-2025

Liquid Biopsy in Oncology: A Comprehensive Review of Circulating Biomarkers for Early Cancer Detection and Clinical Management

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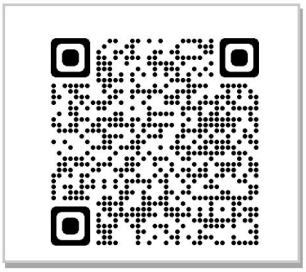


Abstract: The global impact of cancer calls for the creation of quick, precise, and non-invasive methods of diagnosis. Liquid biopsy, which involves analyzing tumor-related components in bodily fluids, has become a groundbreaking asset in cancer treatment, enhancing and occasionally exceeding the capabilities of traditional tissue biopsies. This review compiles current insights on the key elements of liquid biopsy—namely, circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and extracellular vesicles (EVs)/exosomes—and their role in early cancer detection. We explore advanced analytical methods, such as next-generation sequencing (NGS) and digital PCR, which enable the sensitive identification of these rare biomarkers. The review also evaluates the clinical application of liquid biopsy in major cancer types, including lung, breast, colorectal, prostate, and gastric cancers, emphasizing its significance not just in diagnosis but also in monitoring treatment response, tracking minimal residual disease, and understanding tumor heterogeneity. Despite its groundbreaking potential, challenges such as standardizing tests, low analyte levels in early-stage diseases, and the need for thorough clinical validation continue to present obstacles. This review concludes that liquid biopsy is set to become a vital component of precision oncology. Future initiatives must prioritize integrating multiple omics, conducting extensive prospective trials, and establishing standardized protocols to fully exploit its potential in advancing earlier cancer detection and personalized healthcare.

Keywords: *liquid biopsy, circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), exosomes, extracellular vesicles, early cancer detection, precision medicine, next-generation sequencing.*

1. Introduction

Cancer continues to be a major cause of death worldwide, with approximately 20 million new cases and 9.7 million fatalities recorded in 2022 [1]. The stage at which cancer is discovered is crucial for patient survival. Diagnosing cancer at an early stage (Stages I and II) is consistently linked with more effective treatments, reduced healthcare expenses, and notably higher survival rates than diagnosing it at a late stage (Stages III and IV) [2]. Nevertheless, the subtle and non-specific nature of early-stage tumor symptoms, along with the limitations of existing screening methods—which can be invasive, expensive, or insufficiently sensitive—results in many cancers being identified at an advanced stage [3].

The tissue biopsy has been the clinical gold standard for diagnosing cancer for many years. Despite its informative nature, this procedure is invasive and comes with potential risks like infection and bleeding. Additionally, it offers only a temporary and spatial glimpse into a tumor's complex and changing genetic composition, missing its complete heterogeneity [4]. Thus, there is an urgent need for a diagnostic method that is less invasive, more dynamic, and more comprehensive.

Liquid biopsy has emerged to meet this need. This minimally invasive technique involves the isolation and analysis of tumor-derived material shed into bodily fluids, most commonly blood [5]. The three primary analytes are:

1. **Circulating tumor DNA (ctDNA):** Short fragments of DNA released into the bloodstream via apoptosis, necrosis, or active secretion from tumor cells.
2. **Circulating tumor cells (CTCs):** Intact cancer cells that detach from the primary tumor and enter the circulation, representing a potential source of metastasis.
3. **Extracellular vesicles (EVs) and exosomes:** Nano-sized lipid bilayer vesicles secreted by cells that carry proteins, lipids, and nucleic acids (DNA, RNA, miRNAs), facilitating cell-to-cell communication.

1) The examination of these biomarkers offers an immediate "molecular portrait" of a tumor, facilitating uses in early detection, prognosis evaluation, treatment response monitoring, and pinpointing resistance mechanisms [6]. This review aims to deliver an in-depth summary of the biological basics, analytical technologies, and clinical uses of liquid biopsy, highlighting its growing importance in the early detection of cancer. Additionally, it will address the present obstacles and future steps needed for its complete adoption in regular clinical practice.

2. Core Components of Liquid Biopsy

2.1 Circulating Tumor DNA (ctDNA) and Cell-Free DNA (cfDNA)

Cell-free DNA (cfDNA) refers to all DNA fragments circulating freely in the bloodstream, originating from both

healthy and malignant cells. Circulating tumor DNA (ctDNA) is the fraction of cfDNA that is specifically derived from tumor cells [7]. In healthy individuals, cfDNA concentrations are typically low (<10 ng/mL of plasma), whereas cancer patients often exhibit elevated levels. ctDNA fragments are characterized by their shorter length (~143 bp) compared to non-malignant cfDNA (~167 bp) and harbor tumor-specific genetic and epigenetic alterations, such as mutations, copy number variations, and methylation patterns [8, 9]. This tumor-specific signature makes ctDNA an exceptionally powerful biomarker for cancer detection and monitoring. Its short half-life (minutes to hours) allows for real-time assessment of tumor dynamics [10].

Table 1: Key Characteristics of cfDNA and ctDNA

Characteristic	cfDNA	ctDNA
Source	Apoptosis/necrosis of all nucleated cells	Apoptosis/necrosis of tumor cells
Composition	Majority of circulating DNA	Small fraction of total cfDNA
Fragment Length	~167 bp (mononucleosomal)	Shorter, ~143 bp
Content	Germline DNA; no tumor-specific changes	Somatic mutations, methylation changes, CNVs
Clinical Utility	Non-specific marker of cellular turnover	Cancer diagnosis, monitoring, resistance detection

2.2 Circulating Tumor Cells (CTCs)

Circulating Tumor Cells (CTCs) are infrequent, whole cancer cells present in the peripheral blood of individuals with solid tumors. Their detection signifies a crucial stage in the process of metastasis. Although the immune system swiftly eliminates the majority of CTCs within the span of 1 to 2.5 hours, a minority has the capability to initiate distant metastases [11]. The extreme scarcity of CTCs—often numbering just 1-10 cells per milliliter of blood among billions of blood cells—poses a substantial technological challenge for their isolation and detection [12]. CTCs are highly diverse, with variable expression of epithelial markers (such as EpCAM) and mesenchymal markers, the latter due to epithelial-to-mesenchymal transition (EMT), which increases their ability to invade [13]. Exploring the molecular characteristics of CTCs offers invaluable understanding of the metastatic process and may serve as a prognostic marker.

2.3 Extracellular Vesicles and Exosomes

Exosomes, a type of extracellular vesicles measuring between 40–160 nm, are actively released by nearly all cell types, including cancerous ones. They are essential for cell-to-cell communication by transferring proteins, lipids, mRNA, and miRNA to target cells, thus affecting tumor growth, helping immune evasion, and establishing pre-metastatic niches [14]. For liquid biopsy purposes, exosomes present significant benefits: they are found in high concentrations in bodily fluids (~10^9 particles/mL), are stable due to their lipid bilayer, and contain a rich molecular cargo indicative of the condition of their originating cells [15]. Exosomes from tumors possess distinct surface proteins and nucleic acids that can act as highly specific indicators for early cancer detection [16].

Figure 1: Sources of Biofluids for Liquid Biopsy

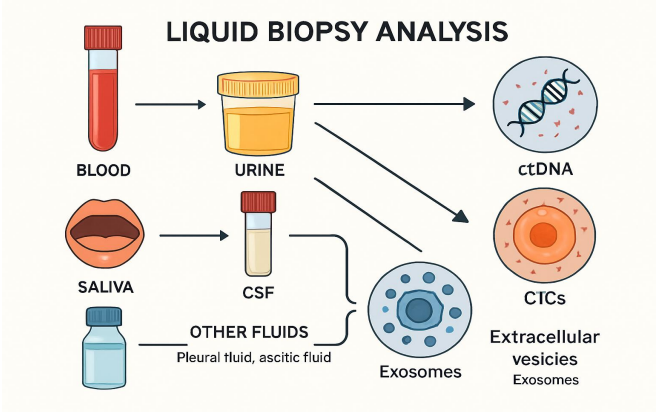


Fig. 1 in the Liang et al. paper, illustrating the various bodily fluids—blood, urine, CSF, saliva, etc.—that can be sampled for liquid biopsy analysis.)

3. Analytical Techniques

The effective clinical application of liquid biopsy hinges on sophisticated technologies capable of detecting extremely rare analytes against a high background of normal cellular material.

3.1 ctDNA Analysis

- **PCR-Based Methods:** Digital PCR (dPCR) and its derivative, droplet digital PCR (ddPCR), allow for the absolute quantification of nucleic acids by partitioning a sample into thousands of individual reactions. This enables the highly sensitive detection

of known, low-frequency mutations (e.g., EGFR in NSCLC) without the need for standard curves [17]. Techniques like BEAMing (Beads, Emulsion, Amplification, and Magnetics) combine dPCR with flow cytometry for ultra-sensitive detection [18].

- **Next-Generation Sequencing (NGS):** NGS offers a broader, hypothesis-free approach. Targeted panels focus on known cancer-related genes, allowing for deep sequencing to identify low-frequency variants. Whole-genome or whole-exome sequencing provides a comprehensive view but requires higher input DNA and greater computational resources. Key to NGS-based ctDNA analysis are molecular barcoding techniques that tag original DNA molecules, allowing bioinformatic correction of PCR and sequencing errors, thereby achieving sensitivities rivaling dPCR [19].
- **Methylation Analysis:** The disruption of DNA methylation patterns, specifically the addition of a methyl group to cytosine in CpG islands, is frequently observed in cancer. Analyzing the methylation patterns of circulating tumor DNA (ctDNA) presents a highly promising strategy for Multi-Cancer Early Detection (MCED) tests. Techniques such as cell-free methylated DNA immunoprecipitation and sequencing (cfMeDIP-seq) are capable of detecting cancer-specific methylation signatures and can even predict the tissue of origin (TOO) of the cancer [20, 21].

3.2 CTC Isolation and Detection

CTC enrichment strategies exploit either physical (size, density, deformability, electrical charge) or biological (surface antigen expression) properties.

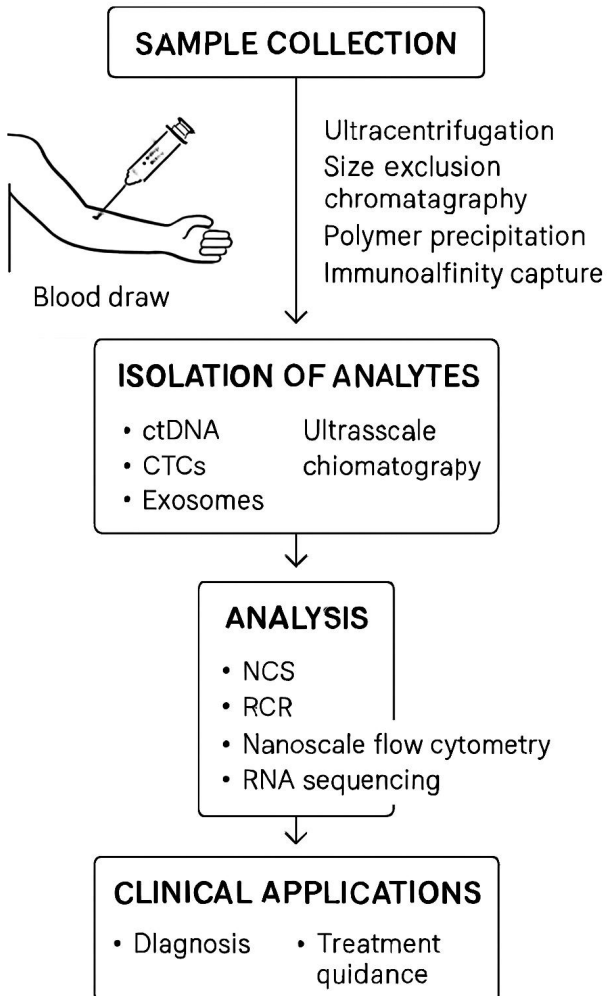
- **Label-Dependent Enrichment:** The FDA-approved CellSearch® system uses antibody-coated magnetic beads targeting EpCAM to positively select epithelial CTCs. However, this method may miss CTCs that have undergone EMT and downregulated EpCAM [12].
- **Label-Free Enrichment:** Microfluidic technologies (e.g., CTC-chip, ISET - Isolation by Size of Epithelial Tumor Cells) filter blood cells based on size or deformability differences, preserving cell viability and capturing EpCAM-low CTCs [22, 23]. Advanced platforms like the NP-HB CTC-Chip use nanotechnology for efficient capture and gentle release of viable CTCs for downstream culture and analysis [24].

Following enrichment, CTCs are typically identified using immunocytochemistry (ICC) for cytokeratins (positive marker) and CD45 (negative leukocyte marker). Molecular characterization via single-cell RNA sequencing or RT-PCR provides profound insights into CTC heterogeneity and resistance mechanisms [25].

3.3 Exosome Isolation and Analysis

Achieving high-purity isolation of exosomes is difficult because of their minuscule size and the presence of contaminants of similar dimensions. Popular techniques for this purpose involve ultracentrifugation, size-exclusion chromatography, polymer-based precipitation, and immunoaffinity capture, which utilizes antibodies targeting exosome surface markers such as CD9, CD63, and CD81 [26]. After isolation, the contents of exosomes, including proteins and miRNAs, can be examined using a range of methods like ELISA, western blot, Nanoscale Flow Cytometry, and RNA sequencing.

Figure 2: Overview of Liquid Biopsy Techniques and Workflow



illustrating the journey from sample collection (blood draw) to the analysis of different analytes (ctDNA, CTCs, Exosomes) via various technologies (NGS, PCR, Microfluidics, Sequencing) and culminating in clinical applications like diagnosis and monitoring.)

4. Clinical Applications in Early Cancer Detection

4.1 Lung Cancer

Lung cancer, the foremost cause of cancer-related deaths, could significantly benefit from early detection through liquid biopsy techniques. Research indicates that circulating tumor DNA (ctDNA) can be found in 50% of patients with Stage I and all patients with Stage II-IV non-small cell lung cancer (NSCLC) [27]. In addition to identifying mutations, the methylation patterns of ctDNA and particular microRNA (miRNA) signatures in exosomes (such as miR-30c-3p for adenocarcinoma and miR-15b-5p for squamous cell carcinoma) exhibit high specificity in differentiating cancer from non-cancerous conditions and predicting histology [28]. Most notably, circulating tumor cells (CTCs) have been identified in high-risk smokers even before lung nodules are visible on CT scans, with almost perfect predictive accuracy for future cancer development. This points to a remarkable potential for extremely early intervention [29].

4.2 Breast Cancer

In breast cancer research, liquid biopsy examines ctDNA methylation patterns (such as the hypermethylation of the EGFR and PPM1E promoters), ctDNA mutations, and serum miRNA profiles. An important study discovered a set of five serum miRNAs (miR-1246, miR-1307-3p, miR-4634, miR-6861-5p, and miR-6875-5p) that identified breast cancer with a sensitivity of 97.3% and a specificity of 82.9%, including for early-stage and in-situ cases. Detecting CTCs in early breast cancer stages is difficult; however, innovative technologies like nuclease-activated probes are enhancing sensitivity.

4.3 Colorectal Cancer (CRC)

Liquid biopsy presents an alternative approach to colonoscopy for screening colorectal cancer (CRC). The Epi proColon® test, approved by the FDA, identifies methylated SEPT9 in circulating tumor DNA (ctDNA) and is intended for average-risk adults who choose not to undergo first-line screening. Platforms for detecting circulating tumor cells (CTCs), such as the CellMax assay, have shown sensitivities of 86.9% and specificities of 97.3% for CRC, with the number of CTCs aligning with the stage of the disease [32]. Additionally, multi-marker panels for ctDNA methylation (for instance, those based on 11 biomarkers) and exosomal microRNAs (like miR-125a-3p, miR-320c) are proving highly effective in enhancing early detection rates [33, 34].

4.4 Prostate Cancer

The pursuit of enhancing the restricted specificity of Prostate-Specific Antigen (PSA) testing has fueled research into liquid biopsies for prostate cancer. Urine has proven to be a useful biofluid, with tests such as ExoDx® Prostate Intelliscore focusing on exosomal RNA analysis. Methylation in genes like GSTP1 and RARB2 within ctDNA, along with particular serum miRNA signatures (for instance, miR-24, miR-223, miR-375), can aid in distinguishing between significant and indolent prostate cancers, thereby minimizing unnecessary biopsies [35, 36].

4.5 Gastric Cancer

Timely identification of gastric cancer is vital, given its grim outlook at later stages. The difficulty lies in the scarcity of CTCs and ctDNA during the initial phases of the disease. Research indicates that utilizing EpCAM in conjunction with other markers, such as Protein Tyrosine Kinase 7 (PTK7), can enhance the sensitivity of CTC capture [37]. Moreover, methylated ctDNA markers (for example, PCDH10, RASSF1A, RUNX3) show superior diagnostic precision compared to conventional serum biomarkers like CA199 or CEA, presenting a promising non-invasive approach for screening populations at high risk [38, 39].

Table 2: Summary of Liquid Biopsy Applications in Selected Cancers

Cancer Type	Key Analyte(s)	Example Biomarker/Technology	Reported Performance
Lung (NSCLC)	ctDNA methylation, exo-miRNA	miR-30c-3p (adenocarcinoma)	High specificity for histologic subtype [28]
Breast	Serum miRNA	5-miRNA panel	Sens: 97.3%, Spec: 82.9% [30]
Colorectal	Methylated ctDNA	Epi proColon (*SEPT9*)	FDA-approved blood test [32]
Prostate	Urinary exosome RNA, ctDNA methylation	ExoDx Prostate test, *GSTP1* methylation	Improves specificity over PSA [35, 36]

5. Challenges and Future Perspectives

Despite its transformative potential, the widespread clinical implementation of liquid biopsy faces several hurdles:

1. **Technical Sensitivity:** The very low concentration of tumor-derived material in early-stage disease pushes the limits of current technologies.
2. **Standardization:** A lack of standardized protocols for sample collection, processing, storage, and analysis makes it difficult to compare results across studies and labs.
3. **Specificity and Interpretation:** Distinguishing true tumor-derived signals from clonal hematopoiesis (CHIP), background noise, or pre-malignant lesions is complex.
4. **Clinical Validation:** Large-scale, prospective, multi-center trials are needed to validate the clinical utility and cost-effectiveness of liquid biopsy for population-level screening.
5. **Integration into Clinical Pathways:** How liquid biopsy will complement or replace existing standards of care (e.g., colonoscopy, CT scans) requires careful definition.

Future directions are focused on overcoming these challenges:

- **Multi-Analyte and Multi-Omics Approaches:** Combining ctDNA, CTC, and exosome analysis with proteomics and metabolomics will likely yield higher sensitivity and specificity than any single analyte.
- **MCED Tests:** Large efforts are underway to develop blood tests that can screen for multiple cancer types simultaneously and predict the tissue of origin.
- **Ultra-Sensitive Technologies:** Advances in sequencing chemistry, single-molecule detection, and bioinformatics will continue to lower the limits of detection.
- **Longitudinal Monitoring:** The true power of liquid biopsy may lie in serial monitoring for cancer recurrence (MRD detection) and real-time assessment of treatment efficacy.

6. Conclusion

Liquid biopsy marks a revolutionary change in the field of oncology, shifting diagnosis from invasive, static tissue sampling to less invasive, dynamic molecular monitoring. By analyzing ctDNA, CTCs, and exosomes, it offers a comprehensive view of tumor biology, providing unmatched opportunities for early cancer detection, personalized therapy selection, and monitoring of treatment responses and resistance. Although there are still significant challenges in terms of sensitivity, standardization, and validation, the rapid advancement of technology and increasing clinical evidence strongly indicate that liquid biopsy is poised to become a crucial part of cancer care. As we continue to refine these methods and prove their value in large populations, liquid biopsy promises to make cancer more detectable and manageable, ultimately saving lives through earlier intervention.

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