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Review

Technological Advances and Methodologies in Liquid Biopsy: An Updated Review

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Abstract: Purpose: Liquid biopsy enables the non-invasive assessment of cancer by enabling real-time monitoring of tumor biology through particular biomarkers. Advancements in this fast-evolving approach are mainly dependent on the developments in the technology used to procure, obtain, and analyze key liquid biopsy markers. Methods: We conduct a comprehensive review of literature from 2018 to 2023 using PubMed and Google Scholar. Studies focused on advancements in liquid biopsy technologies, including ctDNA, CTC analysis, extracellular vesicles, methylation pattern detection, RNA biomarkers, and the integration of artificial intelligence. Key challenges and merging methodologies to overcome existing limitations were identified and discussed. Results: Next-generation sequencing (NGS) and digital droplet PCR have stronger precision in detecting ctDNA at lower concentrations, improving early cancer detection and monitoring of minimal residual disease (MRD). Newer techniques like targeted error correction sequencing (TEC-seq) and RNA biomarker profiling improve the cost-effectiveness while maintaining fidelity in detecting rare mutations. Microfluidic platforms provide a structured platform for isolating CTCs and extracellular vesicles, which can be integrated into AI platforms to improve diagnostic precision and treatment management. Conclusion: Newer technologies are more effective in capturing tumor heterogeneity and provide better, earlier accuracy. Future innovations are being shaped by artificial intelligence-integrated platforms to enhance the granularity of liquid biopsy.

Keywords: liquid biopsy; personalized medicine; sequencing; microfluidics

1. Introduction

Cancer diagnosis and management are shifting paradigms with the growth of non-invasive technologies that enable real-time monitoring of tumor biology. The push for novel approaches to evaluate cancer progression has led to the development of liquid biopsy—a non-invasive technique for cancer assessment.

The growth of metastatic tumors increases with the assimilation of their biofluids, cells, and other components into the blood [1]. Liquid Biopsy, involving analysis of these blood cells and fluids, assists in understanding the etiology, pathophysiology, and prognosis of tumor cell lines [2,3]. This procedure is performed by collecting blood samples and analyzing them to investigate minimal residual disease (MRD), resistance pathways, and treatment responses [4]. The diagnostic evaluation of the proteomic and genomic data of these bodily fluids has improved the understanding of several tumor characteristics, including clonal evolution, gene mutations, heterogeneity, tumor staging, and tumor progression [2]. This method provides a platform for personalized treatment methods and early detection of MRD or metastatic relapse.

Particularly, compared to traditional biopsy, liquid biopsy is minimally invasive, allows for the constant assessment of tumor evolution, and can capture tumor heterogeneity, even in the context of multiple metastatic sites. As liquid biopsy continues to be explored as a potential alternative to traditional tissue biopsies in specific contexts, challenges such as the potential for false positives nonetheless necessitate further refinement of its methodologies to improve diagnostic accuracy.

Newer technology focuses on enhancing the sensitivity, specificity, and reliability of current methods. Techniques such as next-generation sequencing (NGS), digital droplet PCR, and microfluidic platforms have enabled the identification of tumor-derived signals even at lower concentrations. Other emerging methods like targeted error correction sequencing (TEC-seq) and microfluidic-based isolation have improved the ability to catch rare circulating biomarkers among CTCs and extracellular vesicles. Artificial Intelligence (AI) is also being integrated with these technologies to position liquid biopsy as a versatile tool for clinicians to improve early diagnosis, treatment monitoring, and treatment resistance evaluation.

Accordingly, this review aims to discuss current challenges and provide an update on the latest technological advancements and methodologies in liquid biopsy. We discuss various approaches, including ctDNA and CTC analysis, microfluidic isolation techniques, methylation pattern detection, and RNA biomarker profiling, as well as emerging technologies that can overcome existing limitations in sensitivity, sensitivity, and reliability.

2. Methods

The authors reviewed the literature published from 2018 to 2023 to understand existing challenges and upcoming advances in liquid biopsy technology. Literature searches were performed using PubMed and Google Scholar, using keywords such as “Liquid Biopsy”, “extracellular vesicles”, “methylation analysis”, and “ctDNA.” Studies were selected based on relevance to advancements in sensitivity, accuracy, and applicability for cancer monitoring, and the data was subsequently organized to highlight key technologies and their approaches. Particularly, we included peer-reviewed oncology studies (or methods papers with clear translational evidence) that reported new or improved liquid-biopsy technologies (e.g., limit of detection, sensitivity/specificity, throughput, or cost/operational characteristics). Editorials, letters without data, and animal-only studies that lack a human link were excluded. The authors independently screened and reached consensus on articles to include. Findings were synthesized thematically, with a goal to compare technologies by their use case, the limitations they address, and clinical readiness. This review provides a concise overview of upcoming liquid biopsy methods that carry the potential to overcome existing limitations in the field

3. Existing Limitations

3.1. Low Shedding of cfDNA/cTDNA

The low shedding of cfDNA/ctDNA is a potential limitation that restricts the use of liquid biopsy techniques in patients with a reduced tumor bulk [5]. Consequentially, the absence of detectable levels of tumor DNA increases the risk of false negative outcomes. Of note, it is difficult to differentiate intermediate potential clonal hematopoiesis-related mutations from cfDNA changes, leading to biased results [6].

3.2. Tumor Heterogeneity

Furthermore, tumor heterogeneity evaluation via cfDNA and ctDNA levels assessment is increasingly difficult since the metastasis and the primary tumor do not shed them in equal amounts [5]. Not all disease locations release ctDNAs, and the ongoing treatments may also minimize their concentration, leading to false positive outcomes. CTC isolation, especially in such heterogeneous tumors, is another potential challenge that restricts the use of liquid biopsy for cancer assessment [7]. Different methods employed for CTC quantification result in variable outcomes that lack consistency. The CTC detection in specific solid tumors is further limited by the downregulation of surface markers [8]. Like cfDNA and ctDNA, the tumor heterogeneity assessment by CTC quantification is not possible since metastasis and primary tumors do not uniformly release CTCs.

3.3. Copy Number Variations and Analytical Challenges

Difficulties in obtaining copy number variations from liquid biopsy samples challenge the analysis of cancer prognosis and treatment outcomes. Other potential challenges impacting the sensitivity/specificity of liquid biopsy/biofluid sample assessments and their false positive/negative outcomes include difficulties in extracting disease-specific analytes, selection of study population, biases in environmental and biological variables, biobanking, prolonged sample storage protocols, and controls based on study design [9].

3.4. Biofluid Sample Integrity

The difficulty in isolating extracellular vesicles, ctDNAs, and CTCs from the liquid biopsy specimens is due to the absence of standardized algorithms and inconsistent use of biofluid extraction kits/methods [10]. Of note, selecting mutation detection assays via convenience sampling adds to the risk of selection bias. Furthermore, several pre-sampling factors, including lactation, pregnancy, hypertension, metabolic disorders, fasting, and circadian rhythm, deteriorate the quality of the liquid biopsy specimen, including its analytes [10]. Variations in the sample storage techniques lead to inconsistency in decay rates that further impact the diagnostic outcomes. The integrity and concentration of the target nucleic acid of the analytes are impacted by the thawing procedure and the freeze-thaw cycles. Nevertheless, unknown biological variations in the liquid biopsy samples/biofluids at different time points impact the accuracy of the overall results.

3.5. Cost Considerations

The high cost of liquid biopsy-based diagnostic investigation is another potential limitation that restricts the use of this technique for cancer analysis [11]. This elevated cost is attributed to high-value medical equipment, costly diagnostic algorithms/procedures, consultation prices, and additional costs incurred in quantifying mutation panels. Multiple screenings of the liquid biopsy samples or biofluids at different time points during treatment further increase the overall cost of diagnosing solid tumors and MRDs [12].

4. Techniques and Methodologies

The predominant liquid biopsy techniques include the assessment of tumor-derived extracellular vesicles, circulating miRNAs/ctRNAs, ctDNAs, methylation patterns, and other CTCs [13].

4.1. Tumor-Derived Extracellular Vesicles

Extracellular vesicles (EVs) are membrane-bound structures solid tumors produce due to apoptotic signals, stress inducers, biochemical shear, growth factors, inflammatory cytokines, thrombin, ATP, and proteases. These vesicles themselves perpetuate tumor biology by increasing chemotherapy resistance, angiogenesis, and metastasis [14]. EVs also trigger the development of pre-metastatic niches in distantly located organs and cells, disrupt the healthy cells adjacent to the tumor microenvironment, and promote the process of metastasis. Although their biogenesis has not been fully described, EVs have often been linked to signaling mediators that modulate actin cytoskeletal dynamics [15]. They further strengthen the tumor microenvironment by inducting the extracellular matrix remodeling, suppressing the immune system responses, and extending the blood supply to the tumor cells [16]. They also contribute to drug resistance, while their pathological effects are potentiated by several metabolites, mRNAs, microRNAs, and tumor-specific proteins. That is why the analysis of extracellular vesicles in the liquid biopsy samples assists in determining the prognosis of MRD and solid tumors.

Several potential techniques are employed to evaluate liquid biopsy samples for EVs, as described in Table 1. Each approach has its unique benefits and shortcomings, making them suitable for specific research or clinical applications. For example, ultracentrifugation is widely used given its established methodology and cost-effectiveness, but it can be time-consuming and may result in samples with impurities. In contrast, immunoaffinity-based approaches sort and quantify exosomes by tracking a color change induced by CD81, CD63, and CD9 proteins via ELISA, which is useful for specific exosomal markers [17]. However, its reliance on specific antibodies and potential for lower yield are limitations to keep in mind. In other words, the balance between yield and purity is critical; techniques like size-exclusion chromatography and polymer precipitation offer high yields but may co-isolate contaminants, whereas high-purity approaches like immunoaffinity-based separation sacrifice yield for specificity, making them better suited for precise molecular characterization.

Table 1. Upcoming techniques to evaluate extracellular vesicles (EVs) for liquid biopsy.

Technique	Principle	Advantages	Limitations
Ultracentrifugation [18]	Density gradient centrifugation to separate proteins and impurities from EVs	Cost-effective, widely used	Time-consuming, may contain impurities
Size Exclusion Chromatography [18]	Physical separation based on vesicle size	High EV yield, simple implementation	Potential co-isolation of contaminants
Immunoaffinity-based separation [13,19]	Isolation of exosomes using antibody-antigen interaction (e.g., magnetic beads or ELISA)	High purity, specificity to desired exosomes	Expensive, requires specific antibodies

Table 1. *Cont.*

Technique	Principle	Advantages	Limitations
Polymer Precipitation [13]	Use of polyethylene glycol to reduce solubility and precipitate EVs	Simple, cost-effective, fast	May result in co-precipitation of non-vesicular proteins
Electric Field-based Approach [13]	In-situ immunofluorescence via alternating-current electrokinetic chips	Rapid isolation, non-invasive analysis	Requires specialized equipment, lower throughput
Microfluidics [20]	Spiral inertial flow, antibody-functionalized channels for precise EV isolation	High efficiency allows nanoscale sorting	Complexity of fabrication and scaling issues
Nanoparticle Tracking/Fluorescence Activated Sorting [21]	Visualization-based quantification of EVs using fluorescent tags	Provides quantification and EV visualization	Requires complex instrumentation

4.2. Methylation Pattern Analysis

Liquid biopsies can also be evaluated for DNA methylation patterns, which can provide prognostic insights. The analysis of hypomethylation profiles, such as those observed in CTC clusters, as well as hypomethylated regions of regulatory genes (e.g., SIN3A, NANOG, SOX2, and OCT4), can indicate prognosis in cancers of the lung, colorectal, and breast [22,23]. These methylation-sensitive analyses assist in the early detection and characterization of tumor aggressiveness. Of note, there are two key technical domains to assess methylation status through liquid biopsy: PCR-based methods and Sequencing-based methods.

PCR-based methods, like digital PCR and Multiplex Methylation-specific PCR (MMSPA) are appropriate for targeted analysis, focusing on specific methylation sites or genes. These techniques are noted to be cost-effective and allow for rapid amplification and quantification of methylation markers of interest. MMSPA makes use of bead arrays and allows for synchronously identifying the methylation status on multiple tumor genes, enhancing the sensitivity and specificity in early cancer detection [24]. Droplet digital PCR is especially known for its sensitivity in detecting low concentrations of methylated DNA, making it promising in assessing tumor burden or scanning for MRD [25].

Sequencing-based methods such as Next-Generation Sequencing (NGS) and Oxford Nanopore Sequencing are used for comprehensive analysis of cfDNA methylation, providing a detailed view of methylation changes across the genome. NGS specifically allows for broad coverage, enabling the detection of rare epiallelic variants with high sensitivity, which is crucial for early-stage cancer detection [26]. Bisulfite Sequencing is another commonly used sequencing-based method that involves treating DNA with bisulfite to convert unmethylated cytosines to uracil and thereby allow for the precise identification of methylation sites at a single-base resolution [27]. However, bisulfite also degrades DNA samples, reducing the quality and yield of cfDNA. As a result, Oxford Nanopore Sequencing has emerged as a method of similarly detecting methylation at a single-based resolution without using bisulfite, therefore preserving cfDNA samples and fragmentation information that may be lost with conventional bisulfite sequencing [27]. Methylation-Sensitive Restriction Enzyme Sequencing (MRE-Seq) has also gained ground in sequencing approaches [25]. It involves using methylation-sensitive restriction enzymes to selectively excise unmethylated portions of DNA followed by sequencing, allowing for global hypomethylation patterns in cfDNA.

The REM-DREAMing Assay (Radiometric-Encoded Multiplex Discrimination of Rare EpiAlleles by Melt) is a unique approach that is different from either PCR or sequencing methodologies [28]. Unlike conventional innovations that primarily focus on overall methylation status, REM-DREAMing is a digital microfluidic platform designed to detect variability in methylation patterns across DNA molecules at single-molecule sensitivity. Considering the diverse subpopulations of cells within a tumor, methylation heterogeneity is a concern, as each cell can display different behaviors, such as treatment resistance or varied metastatic potential [28]. REM-DREAMing uses high-resolution melt (HRM) analysis to identify specific methylation changes by differentiation epialleles in a radiometric fluorescence approach, therefore detecting rare methylation events that can provide insights into tumor evolution.

4.3. Circulating Tumor DNA (ctDNA) and Circulating Tumor Cells (CTCs) Analysis

Circulating tumor DNA (ctDNA) is released by tumor cells into the bloodstream, especially during cell death, and the genetic identity of ctDNA often closely mirrors that of the original tumor, making it a useful biomarker for tumor progression, therapy response, and overall burden. One of the key techniques for ctDNA analysis is BEAMing (Beads, Emulsion, Amplification, and Magnetics) and Digital Droplet PCR, both of which are notable for their sensitive detection of specific mutations present in ctDNA. These methods are futile even if ctDNA

comprises a very minuscule fraction—approximately 0.01%—of the total circulating cfDNA, making them effective for the detection of low-frequency mutations [5].

Tumor-specific ctDNA-based tests have been recently approved to detect unique genetic alterations, such as mutations in PIK3CA, EGFR, and KRAS genes, as well as ALK rearrangement, microsatellite instability, and tumor mutation burden [5]. Another notable development in ctDNA analysis is Targeted Error Correction Sequencing (TEC-seq), which is an NGS-based sequencing technique designed to identify cfDNA and ctDNA with high accuracy [29]. By detecting rare mutations and minimizing sequencing errors, TEC-seq leverages reliability and precision over traditional methods. Deep neural network-based approaches have also found a niche in the interpretation of ctDNA, tracing the cancer's origin by analyzing sequencing patterns and inferring the tumor source.

Meanwhile, Circulating Tumor Cells (CTCs) are cells that detach from the primary tumor or other metastases and enter the bloodstream. Notably, the assessment of disseminated tumor cells (DTCs) and CTCs helps to determine the stage of metastasis, although the assessment of CTCs has also been used in predicting 60–80% of breast cancers in their early stages [30]. Regardless, the approaches for detecting CTCs aim to classify them based on their biological properties, such as electric charges, deformability, density, size or protein markers, and their differential expression. Techniques like filtration and density-gradient centrifugation exploit these differences to isolate CTCs from other blood components. Additionally, immunoaffinity-based techniques have been used to improve CTC detection. For example, markers like epithelial cell adhesion molecule (EpCAM) are used to capture CTCs via magnetic-activated cell sorting (MACS) or fluorescence-activated cell sorting (FACS) [30].

A newly developed liquid biopsy assessment non-contact technique, known as acoustophoresis, facilitates label-free segregation of CTCs by considering the compressibility, density, and size of the tumor cells [31]. The cell separation is activated by using acoustic standing waves to target the suspected particles. The potential benefits of these diagnostic approaches include their non-invasiveness, reduced consumption of reagents, and swift fabrication of the device.

Both ctDNA and CTCs are pillars in the realm of liquid biopsy that provide distinct insights into tumor behavior but complement each other. Particularly, while ctDNA is useful for real-time monitoring of mutations during treatment, CTC analysis may be useful in indicating metastasis. ctDNA represents a snapshot of tumor heterogeneity, including clonal evolution and resistance mechanisms, and is easy to isolate from a range of biofluids. On the other hand, CTCs capture both the phenotype and genotype of intact cells and provide a comprehensive view of tumor biology. Table 2 summarizes a comparative analysis of ctDNA and CTC techniques.

Table 2. Comparison between ctDNA and CTC for liquid biopsy.

Parameter	Circulating Tumor DNA (ctDNA)	Circulating Tumor Cells (CTCs)
Source	DNA fragments released from dead tumor cells	Intact cells shed from primary/metastatic tumors
Analysis Methods	PCR-based techniques, NGS, TEC-seq	Immunoaffinity capture, size-based separation
Clinical Use	Monitoring mutation status, MRD, and tumor burden	Predicting metastasis, drug resistance, and overall prognosis
Advantages	Easy isolation, represents whole tumor heterogeneity	Provides complete biological information, including tumor phenotype and genotype
Limitations	May produce false negatives in low-burden disease	Low abundance, technical difficulty in isolation

4.4. RNA Biomarkers in Liquid Biopsy

Biomarkers, including long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and messenger RNAs (mRNAs), are derived from circulating tumor RNA (ctRNA) and are conventionally evaluated using techniques like digital PCR (dPCR) or quantitative reverse transcription PCR (qRT-PCR) [32]. These RNA molecules garner interest as their stability in biofluids makes them suitable for liquid biopsy. Novel RNA biomarker analysis approaches have expanded beyond conventional PCR methods.

Table 3 summarizes the most recent and significant techniques used for the analysis of RNA biomarkers, which are each suitable in different diagnostic scenarios depending on the needs. Of note, techniques such SERS-PEF and nanoplasmonic biosensors allow detection at remarkably high sensitivity, making them particularly beneficial for detecting rarer RNA biomarkers at early stages of cancer when concentrations are significantly low [33,34]. Other developments have focused primarily on throughput. Microfluidic platforms and digital microfluidic techniques like MER-idPCR are designed to balance sensitivity with scalability, offering reduced processing times, automation, and cost-effectiveness, making them relevant for high-throughput applications during routine diagnostics [35,36]. Likewise, NGS and methods for detecting circular RNAs (circRNAs) enable a broader analysis of several RNA types and are also considered high-throughput technologies [32,37].

Table 3. Summary of upcoming techniques to analyze RNA for liquid biopsy.

Technique	Description	Quantitative Details
Surface-Enhanced Raman Scattering (SERS) & Plasmon-Enhanced Fluorescence (PEF) [33].	Combines SERS and PEF to detect miRNAs using gold triangular nanoprisms, enabling femtogram/microliter level sensitivity.	Femtogram/microliter sensitivity; dual-mode sensing enhances specificity.
Microfluidic Platforms for On-Chip Analysis [35].	Uses microfluidic devices for precise isolation and analysis of miRNAs, lncRNAs, and mRNAs in a high-throughput manner, with reduced processing time.	High purity, reduced sample volume requirements, high throughput.
Nanoplasmonic Biosensors for miRNA Detection [34].	Plasmonic nanoantenna-based biosensors using LSPR for multiplexed detection of multiple miRNAs directly from plasma at attomolar levels.	Attomolar detection level; capable of differentiating metastatic vs. non-metastatic cancers.
Digital Microfluidic Techniques (MER-idPCR) [36].	MER-idPCR uses structured oligonucleotide adapters with digital PCR for distinguishing methylated and unmethylated small RNA species.	High-resolution analysis of methylated vs. unmethylated small RNAs; sequence-specific detection.
Next-Generation Sequencing (NGS) [37].	Next-Generation Sequencing platforms for comprehensive analysis of multiple RNA classes, identifying indicators of tumor origin, progression, and treatment response.	Broad transcriptome analysis of mRNAs, lncRNAs, circRNAs, and piRNAs.
Circular RNA (circRNA) Detection in Liquid Biopsy [32].	Detection of circRNAs in body fluids using high-throughput transcriptome techniques for cancer development and progression analysis.	High stability and specificity in detection; identified from plasma, serum, exosomes.

4.5. Integration of Artificial Intelligence

Artificial intelligence (AI) techniques have gained attention by employing a data-driven approach that enhances the capabilities of newer advancements in liquid biopsy. AI-based methods help manage the immense amount of data with the analysis of circulating biomarkers and, therefore, improve the precision and sensitivity of traditional liquid biopsy technologies while offering a platform for obtaining additional insights [38]. The integration of AI allows for automated analysis through high-throughput data, reducing the manual workload and variability [39]. In addition to its capabilities of tracing cancer origins by detecting ctDNA patterns, AI-enabled platforms provide greater spatial-temporal resolution [38,39]. For example, a study discussed the role of AI, particularly in optimizing on-chip microfluidic platforms for the live analysis of biomarkers, making the process more scalable [39]. AI has also been considered for isolating and classifying CTCs by developing image-processing algorithms and boosting the sensitivity and specificity of liquid biopsy technology [35]. Finally, machine learning models can use time-series analysis to corroborate temporal changes in ctDNA levels and predict treatment resistance or cancer progression in real-time [40].

Ultimately, while several developments have worked towards advancing sensitivity and fidelity of liquid biopsy, their comparative advantages can be leveraged depending on the clinical or research objective. Figure 1 summarizes how the emerging technologies attempt to address the existing limitations in liquid biopsy discussed earlier. Ultimately, these platforms have different characteristics and are therefore designed to be positioned for different tasks (Figure 2). For example, ddPCR may be more relevant in quantifying known mutations in the context of minimal residual disease (MRD) monitoring, while NGS provides broader coverage for a comprehensive genomic profiling. Likewise, immunoaffinity-based microfluidic systems can be applied when high-purity exosome or CTC isolation is needed for downstream analyses. Ultracentrifugation is practical for large-scale studies where cost and simplicity are key. Complementing these nucleic-acid centric approaches, plasma and extracellular vesicle (EV) mass spectrometry/proteomics have also shown feasibility in longitudinal MRD assessment and stratification in leukemia; future technology is working towards integrating these alongside ctDNA and microfluidic workflows to capture dynamics if the ctDNA signal is limited [41,42]. We compare the characteristics of major liquid biopsy technologies discussed in Figure 1.

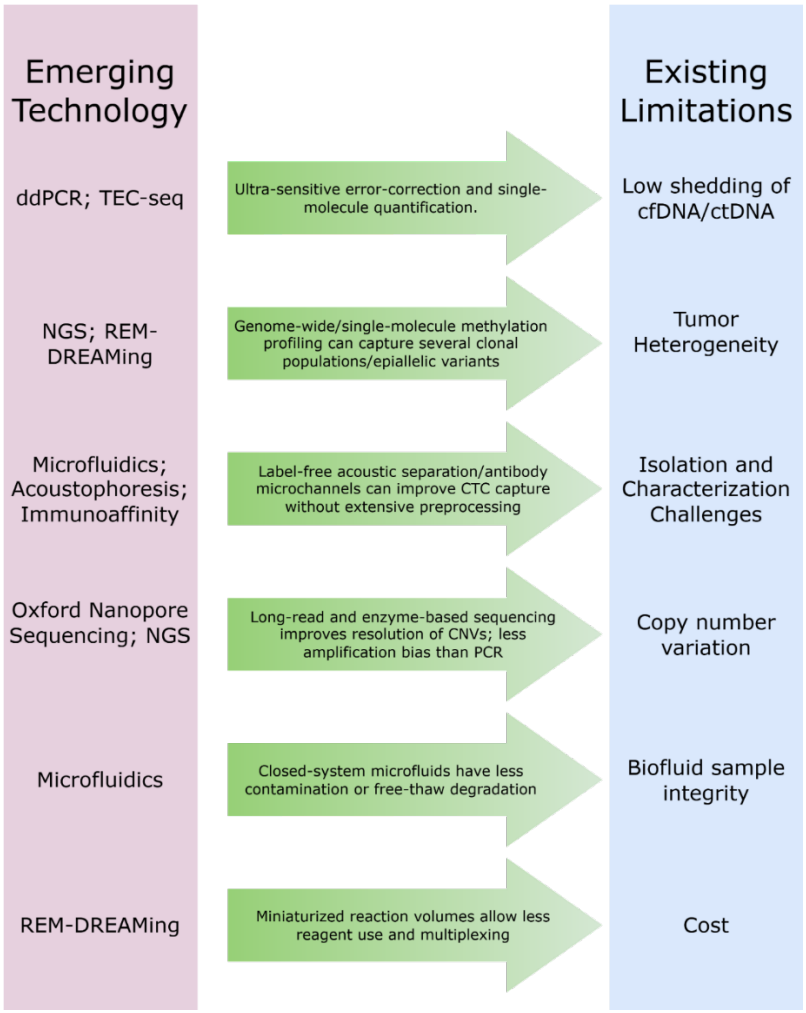

Figure 1. Mapping existing limitations in liquid biopsy (right) to emerging technological solutions (left). Each arrow describes how an innovation aims to address the constraint.

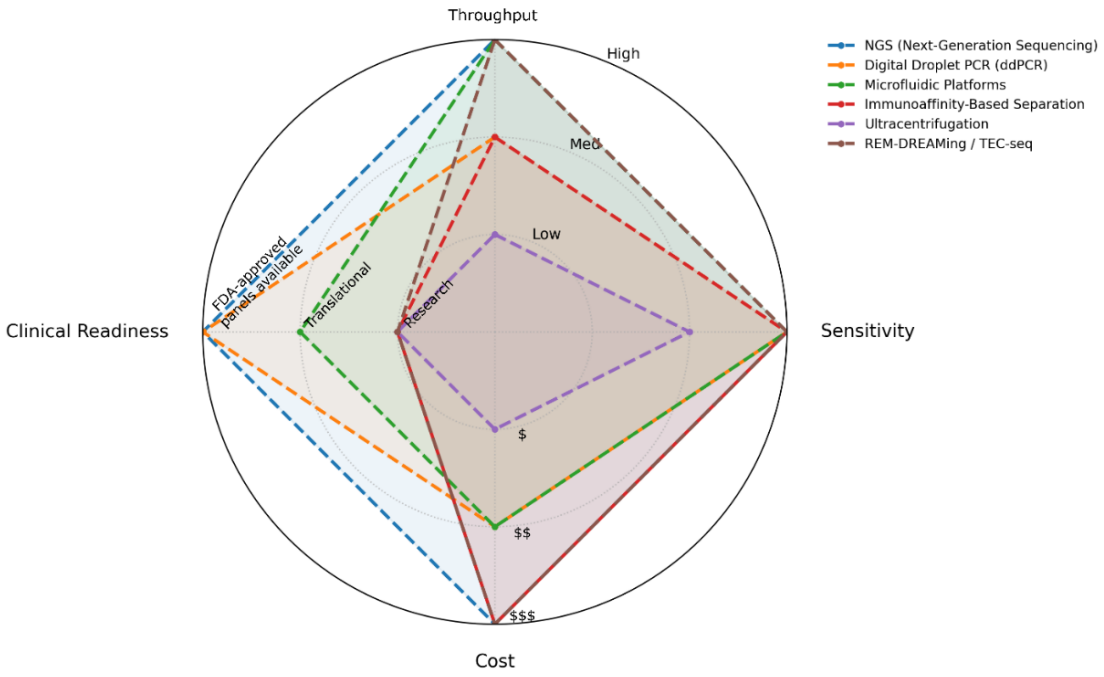

Figure 2. Radar plot comparing sensitivity, throughput, cost, and clinical readiness among key discussed emerging liquid biopsy techniques.

5. Conclusions

Liquid biopsy has emerged as a transformative tool in solid tumor management as it offers a less invasive and more comprehensive overview of tumor biology. While the field is rapidly advancing, the risk of false positives, variability in biomarker concentrations, and limitations in isolating rare circulating components push the need for further refinement in liquid biopsy techniques. Emerging technologies, including BEAMing/digital droplet PCR, TEC-seq, and biosensor-based quantitative assessments, have expanded the ability to rarer biomarkers at lower concentrations and show promise in addressing existing challenges. Despite the progress thus far, more steps are needed before full clinical translation. Standardizing pre-analytical variables (e.g., sample processing and data normalization), cost reduction, and validation is necessary or these technologies to make headway in precision oncology. These methods allow for the early diagnosis and profiling of tumors to illustrate tumor heterogeneity, therapeutic resistance, and treatment trajectories. Application of SERS, ELISA, microfluidics, immunoaffinity-based separation, and AI have further improved the accuracy and precision of liquid biopsy. As newer high-fidelity detections are developed, AI represents a new step forward in improving the scalability, reliability, and cost-effectiveness of liquid biopsy while offering insights into spatial-temporal resolution. The future of liquid biopsy lies in the integration of several approaches, combining technological innovations with additional biological insights to address current challenges.

Author Contributions

S.B.: data curation, formal analysis, investigation, methodology, resources, visualization, writing—original draft; R.S.C.: conceptualization, investigation, supervision, validation, writing—review and editing, project administration. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used Grammarly for editing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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