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Liquid Biopsy for Early Detection of Colorectal Cancer: Circulating Tumor DNA and Exosomal Biomarkers

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ABSTRACT

Background: Colorectal cancer (CRC) remains a major cause of cancer-related morbidity and mortality worldwide. Although colonoscopy and stool-based screening tests have improved early detection, participation, invasiveness, and limited sensitivity for some precancerous lesions remain important challenges. Liquid biopsy offers a minimally invasive approach for detecting tumor-derived molecular signals in blood, particularly circulating tumor DNA (ctDNA) and Exosomal biomarkers.

Objective: This review evaluates the biological rationale, diagnostic performance, clinical applications, and limitations of ctDNA and exosome-derived biomarkers for early CRC detection.

Methods: A narrative evidence synthesis developed from peer-reviewed clinical studies, diagnostic accuracy investigations, systematic reviews, and meta-analyses. Evidence organized according to biomarker type, molecular detection method, clinical population, diagnostic accuracy, and translational readiness. Published diagnostic estimates distinguished from interpretive conclusions; no new-pooled estimate calculated from individual studies.

Results: ctDNA assays detect tumor-associated mutations, methylation patterns, and frag entomic characteristics, whereas Exosomal biomarkers include microRNAs, proteins, and other molecular cargo protected within extracellular vesicles. In the prospective ECLIPSE study, a cell-free DNA blood test demonstrated 83.1% sensitivity for CRC and 89.6% specificity for advanced colorectal neoplasia, but sensitivity for advanced precancerous lesions was only 13.2% (Chung et al.,2024). Exosomal microRNA studies have identified promising candidate panels, although differences in isolation procedures, normalization, and study populations limit cross-study comparability. Combining complementary molecular signals may improve detection, but prospective population-based validation remains essential.

Conclusion: Liquid biopsy has substantial potential as a complementary CRC screening strategy. Its clinical value will depend on reproducible analytical methods, high sensitivity for early-stage disease and advanced precancerous lesions, appropriate specificity in asymptomatic populations, cost-effectiveness, and demonstrated benefits in screening participation and patient outcomes.

Introduction

Background and clinical significance:

Colorectal cancer (CRC) is a major global health problem because of its high incidence, substantial mortality, and potentially preventable disease burden [1]. The clinical outcome of CRC strongly influenced by the stage at diagnosis. Tumors identified while localized to the bowel wall are generally more amenable to curative treatment than tumors diagnosed after regional or distant

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dissemination. Consequently, early detection is a central component of CRC control, alongside risk-factor modification, timely diagnostic assessment, and treatment of precancerous lesions [2].

The adenoma–carcinoma sequence provides an important biological rationale for screening. Many CRCs develop through the gradual accumulation of genetic and epigenetic alterations in colorectal epithelial cells. Detecting and removing advanced adenomas before malignant transformation can prevent some cancers, while identifying invasive tumors at an early stage can improve the likelihood of successful treatment. Screening strategies must therefore address two related but distinct objectives: detecting existing cancer and identifying clinically important precancerous lesions [3].

Colonoscopy permits direct visualization of the colorectal mucosa, biopsy, and removal of lesions during the same procedure. Nevertheless, its effectiveness at the population level depends on access, procedural capacity, bowel preparation, acceptance, and adherence to recommended screening intervals. Stool-based tests offer fewer invasive alternatives, but their diagnostic performance varies according to the test and the target lesion. These limitations have encouraged investigation of blood-based biomarkers that may be easier for some individuals to complete [4].

Liquid biopsy as an emerging screening approach:

Liquid biopsy refers to the analysis of tumor-associated material in biological fluids, particularly blood. Relevant analytes include circulating tumor DNA, other cell-free DNA features, circulating tumor cells, extracellular vesicles, microRNAs, proteins, and metabolites. Unlike a conventional tissue biopsy, liquid biopsy may capture molecular information released from different tumor regions and can potentially be repeated with relatively limited procedural burden. The term circulating tumor DNA refers specifically to DNA fragments originating from malignant cells and present in circulation. These fragments can contain somatic mutations, copy-number changes, and abnormal DNA methylation patterns. Cell-free DNA (cfDNA), by contrast, includes DNA released from both malignant and nonmalignant cells. This distinction is important because a cfDNA assay does not necessarily identify tumor-derived DNA exclusively; its diagnostic performance depends on the molecular features used to distinguish cancer-associated signals from background DNA.

Tumor-derived DNA may be present at low concentrations during early disease, when tumor burden is limited. The amount detected influenced by tumor size, anatomical location, vascularity, biological shedding, DNA clearance, specimen processing, and assay sensitivity. Consequently, a test that performs well in advanced cancer may be less sensitive for stage I tumors or advanced adenomas. This stage-dependent biological limitation is central to the evaluation of blood-based screening tests [5].

Circulating tumor DNA and molecular detection:

Several approaches have investigated for detecting CRC-associated DNA in plasma. Mutation-based assays examine alterations in genes frequently affected during colorectal carcinogenesis, including APC, KRAS, BRAF, and TP53. Methylation-based approaches investigate epigenetic changes in genes such as SEPT9 and other candidate loci. Frag entomic methods analyze characteristics such as DNA fragment length and end patterns, potentially providing tumor-associated information even when individual mutations are difficult to detect. The clinical development of methylated SEPT9 illustrates both the promise and the limitations of DNA-based screening. Church et al. (2014) prospectively evaluated plasma methylated SEPT9 in an asymptomatic screening population, demonstrating the feasibility of evaluating a blood biomarker against colonoscopy findings. Subsequent research has explored multigene methylation panels and alternative molecular signatures to improve sensitivity while preserving specificity. A particularly important development was the ECLIPSE study, a large prospective investigation of a cfDNA-based blood test in average-risk adults undergoing CRC screening. The reported results demonstrated clinically relevant sensitivity for invasive cancer, but limited detection of advanced precancerous lesions (Chung et al.,2024). These findings show why cancer detection and prevention-related lesion detection not treated as interchangeable endpoints [6].

Exosomal biomarkers and extracellular vesicles:

Exosomes are small extracellular vesicles released by cells that transport molecular cargo between cells and their surrounding environment. Their cargo may include microRNAs, messenger RNAs, proteins, lipids, and other molecules associated with cellular physiology. Tumor-derived extracellular vesicles may reflect changes in the molecular pathways of malignant cells and can protect selected cargo from degradation in biological fluids.

Exosomal microRNAs have attracted particular interest because they regulate gene expression and may reflect tumor-associated changes in proliferation, invasion, apoptosis, and immune interactions. Ogata-Kawata et al. (2014) reported that several circulating Exosomal microRNAs were elevated in patients with primary CRC, including patients with early-stage disease. Shi et al. (2021)

subsequently investigated four circulating Exosomal microRNAs miR-126, miR-1290, miR-23a, and miR-940 as potential early diagnostic biomarkers [7]. However, detecting a candidate biomarker in a case-control study does not establish that it can reliably screen an asymptomatic population. Differences in patient selection, inflammatory conditions, sample handling, extracellular-vesicle isolation, and quantitative normalization may affect measured expression. Independent validation is therefore necessary before candidate Exosomal signatures considered clinically useful screening tests.

Study rationale and objectives:

Although ctDNA and Exosomal biomarkers are biologically complementary, they differ in their analyses, analytical requirements, and evidence maturity. ctDNA assays have advanced into large prospective screening studies, whereas many Exosomal signatures remain at the discovery or early validation stage. Direct comparison is difficult because studies frequently use different reference standards, thresholds, and clinical populations [8].

This review therefore examines the evidence for ctDNA and Exosomal biomarkers as complementary approaches to early CRC detection. The objectives are to summarize their biological foundations, compare reported diagnostic performance, assess the methodological quality and limitations of the evidence, and identify priorities for clinical translation. Particular emphasis placed on early-stage sensitivity, detection of advanced precancerous lesions, real-world screening performance, and the potential value of multimodal biomarker panels.

Review of the Literature

Biological foundations of ctDNA detection:

The molecular basis of ctDNA detection lies in the release of DNA fragments from tumor cells into circulation. Cell death, apoptosis, necrosis, and other biological processes contribute to the release of nucleic acids. Tumor-associated DNA may carry genetic and epigenetic alterations that distinguish it from DNA originating from normal tissues. However, the tumor-derived fraction may be very small in early-stage disease, creating an analytical challenge for screening.

Mutation-based detection highly specific when a tumor-associated alteration accurately identified, but not all cancers share the same mutation profile. In addition, clonal hematopoiesis and other nonmalignant processes can produce variants in circulating DNA, complicating the interpretation of some mutation-based assays. Methylation-based approaches may provide broader detection because abnormal epigenetic patterns can occur across multiple tumor types or tumor-associated pathways. Their performance nevertheless depends on marker selection, background methylation, assay thresholds, and specimen quality [9].

Frag entomic analysis offers another strategy. Tumor-associated DNA may differ from nonmalignant cfDNA in fragment length distributions, end motifs, and other physical characteristics. Cristiano et al. (2019) demonstrated that genome-wide cfDNA fragmentation patterns contain information relevant to cancer detection. Although this work supported the broader concept of frag entomic liquid biopsy, findings across cancers not automatically interpreted as CRC-specific screening validation. The strongest evidence for a screening application comes from prospective studies in which participants are recruited before their colonoscopy results are known and the blood assay is assessed against an appropriate reference standard. Such designs more closely represent intended use than studies comparing patients with established cancer against unusually healthy controls.

Clinical evidence for ctDNA-based screening:

Church et al. (2014) evaluated methylated SEPT9 in an asymptomatic population undergoing screening colonoscopy. Their study was important because it investigated the biomarker in a setting directly relevant to screening rather than relying solely on patients with established disease. Nevertheless, the performance of methylated SEPT9 and other single-marker approaches must be interpreted in relation to their sensitivity for early lesions, specificity, and the clinical consequences of false-negative and false-positive results. Recent approaches combine multiple molecular features. A blood test may integrate methylation signals, somatic alterations, and other cfDNA characteristics to improve discrimination between individuals with and without CRC. The advantage of a multimarket approach is that different tumors may release different molecular signals. However, additional markers can increase assay complexity, cost, and opportunities for overfitting if feature selection and threshold determination not separated from independent validation [10].

Chung et al. (2024) reported findings from the ECLIPSE study, providing a major prospective assessment of a cfDNA blood-based CRC screening test. Among participants with CRC detected by colonoscopy, the test demonstrated 83.1% sensitivity (95% confidence interval [CI], 72.2%-90.3%). Sensitivity for advanced precancerous lesions was 13.2% (95% CI, 11.3%-15.3%), while specificity for advanced colorectal neoplasia was 89.6% (95% CI, 88.8%-90.3%). These estimates highlight the difference between detecting

established malignancy and identifying lesions whose removal may prevent cancer. The implications are important for screening policy. A blood test may be attractive because it avoids stool collection and may be more acceptable to some individuals. However, an increase in screening participation must be evaluated alongside test sensitivity, follow-up colonoscopy completion, interval cancer rates, and the number of advanced lesions missed. A blood test that detects cancer but rarely identifies advanced adenomas may have a different preventive effect from a test that detects both [11].

Exosomal microRNAs as candidate biomarkers:

Extracellular vesicles offer a distinct molecular source for CRC detection. Their cargo can reflect the biological state of the cells that release them, and vesicle membranes may protect nucleic acids from degradation. Ogata-Kawata et al. (2014) reported elevated levels of several serum Exosomal microRNAs in patients with CRC, including let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, and miR-23a. The observed changes following tumor resection further supported an association between these signals and tumor presence [12].

Shi et al. (2021) investigated Exosomal miR-126, miR-1290, miR-23a, and miR-940 and reported their potential to distinguish stage I CRC from healthy controls. Other studies have explored combinations of Exosomal miRNAs, including miR-15b, miR-16, miR-21, and miR-31, to improve diagnostic discrimination [13].

Despite these promising observations, Exosomal biomarkers face substantial methodological challenges. Studies may use ultracentrifugation, precipitation reagents, size-based separation, or immunoaffinity techniques to isolate extracellular vesicles. These approaches do not necessarily recover identical vesicle populations. Furthermore, the term exosome used cautiously when endosomal origin has not demonstrated; broader terminology such as extracellular vesicles may be more appropriate in accordance with accepted characterization standards [14].

Normalization is another concern. The expression of a microRNA relative to a reference molecule may change according to sample composition, extraction efficiency, and the biological characteristics of the control population. Candidate panels therefore evaluated with standardized collection protocols, predefined thresholds, appropriate negative controls, and independent external validation.

Comparative interpretation and research gaps:

The literature suggests that ctDNA and Exosomal biomarkers may provide complementary rather than interchangeable information. ctDNA directly captures tumor-associated DNA features, whereas Exosomal analysis may capture regulatory molecules and proteins associated with tumor biology. A combined approach could potentially increase sensitivity when one biomarker class weakly represented in a particular tumor. However, biological complementarity does not automatically translate into improved clinical accuracy. A combined panel tested in a sufficiently large, representative population, compared against each component separately. Investigators report sensitivity and specificity at prespecified thresholds, stage-specific performance, and detection of advanced precancerous lesions, assay failure rates, and uncertainty intervals. Calibration, positive predictive value at realistic screening prevalence, and the consequences of additional colonoscopies also considered. The most important gap is the limited evidence that promising molecular signatures improve long-term population outcomes. Diagnostic accuracy is necessary but insufficient: a screening program must also demonstrate feasibility, acceptable costs, equitable access, appropriate diagnostic follow-up, and ultimately a favorable balance between benefits and harms. Future studies should prioritize prospective enrollment, colonoscopy-based reference standards, transparent reporting, and independent validation before routine clinical implementation [15].

Methods

Review design:

This manuscript developed as a narrative evidence review of liquid biopsy biomarkers for early CRC detection, with emphasis on ctDNA and exosome-derived biomarkers. It not presented as a newly registered systematic review or an independently conducted diagnostic meta-analysis. Consequently, no original pooled diagnostic estimate, study count, or formal risk-of-bias summary claimed.

Evidence identification and eligibility:

Relevant evidence was identified through literature searches focused on CRC screening, circulating tumor DNA, cell-free DNA, DNA methylation, fragment omics, extracellular vesicles, exosomes, and Exosomal microRNAs. Priority given to peer-reviewed clinical studies, prospective screening investigations, systematic reviews, meta-analyses, and methodological consensus publications. Landmark studies and recent evidence available through October 2026 considered.

Studies were relevant when they evaluated a blood-derived molecular biomarker for CRC detection, reported diagnostic performance, or provided methodological evidence applicable to extracellular-vesicle characterization. Studies limited to postoperative recurrence monitoring considered

only when they clarified the biology or analytical limitations of liquid biopsy. Evidence from advanced cancer cohorts not assumed to establish screening performance in asymptomatic individuals.

Data extraction and synthesis:
The synthesis considered study design, population, biomarker class, assay technology, reference standard, diagnostic endpoints, and reported performance. Where available, sensitivity, specificity, and 95% confidence intervals extracted from published reports. Results from different populations and biomarker classes not mathematically combined because their intended uses and reference standards may differ.
Four evidence tables designed to summarize major ctDNA studies, Exosomal biomarker findings, comparative diagnostic performance, and translational limitations. Quantitative values attributed to their published sources, and pooled estimates from existing meta-analyses explicitly distinguished from single-study estimates.

Interpretation and limitations:
The interpretation follows established principles of diagnostic test evaluation, including the importance of prespecified thresholds, appropriate reference standards, representative recruitment, and transparent reporting (Bossuyt et al.,2015). Formal study-level risk-of-bias judgments would require a complete, reproducible search, duplicate screening, and assessment using an instrument such as QUADAS-2 (Whiting et al.,2011). Accordingly, the tables below provide an evidence synthesis rather than a substitute for a fully registered systematic review.

Results
The evidence summarized descriptively in four tables. The numerical estimates below attributed to published studies or meta-analyses; they are not newly calculated results from this manuscript. Because the studies differ in design, population, biomarker definition, and clinical endpoint, their estimates not interpreted as a direct head-to-head comparison.

Table 1. Representative clinical evidence for ctDNA-based CRC detection

Study	Biomarker or method	Population/design	Main reported finding	Interpretation
Church et al. (2014)	Plasma methylated SEPT9	Prospective screening-colonoscopy cohort; 7,941 participants enrolled	Evaluated the accuracy of methylated SEPT9 in an asymptomatic screening setting	Important screening-context evidence; results must be interpreted using the study's specific assay and threshold
Study of circulating DNA methylation signatures (2021)	Multigene methylation panel	Case-control study; 742 participants enrolled	Training-set sensitivity 88.6% and specificity 89.3%; test-set sensitivity 83.6% and specificity 91.9%	Promising discrimination, but case–control selection may inflate performance relative to routine screening
Chung et al. (2024)	Multifeatured cfDNA blood test	Prospective ECLIPSE screening study	CRC sensitivity 83.1% (95% CI, 72.2%-90.3%); advanced precancerous lesion sensitivity 13.2% (95% CI, 11.3%-15.3%)	Stronger evidence for cancer detection than for identifying precancerous lesions
Guntupalli et al. (2026)	Blood-based ctDNA assays	Systematic review and meta-analysis; 10 studies identified	Pooled sensitivity 88.7% (95% CI, 84.8%-92.0%) and specificity 91.3% (95% CI, 89.8%-92.8%)	Encouraging pooled accuracy, but heterogeneity and screening-prevalence effects remain important
Carbone et al. (2026)	Blood-based ctDNA screening assays	Systematic review and meta-analysis of population-based prospective studies	Pooled CRC sensitivity 72% (95% CI, 49%-88%) and specificity 91% (95% CI, 89%-92%)	Highlights the importance of prospective population-based evidence and the uncertainty around sensitivity

Sources: Church et al. (2014); the methylation-panel study indexed in PubMed Central as PMC7856810; Chung et al. (2024); Guntupalli et al. (2026); Carbone et al. (2026). DOI links are provided in the reference list where verified.

The studies summarized in Table 1 demonstrate both the development of ctDNA technologies and the importance of distinguishing study populations when interpreting diagnostic accuracy. The prospective evaluation of methylated SEPT9 by Church et al. (2014) is particularly relevant because the biomarker assessed among asymptomatic individuals undergoing screening colonoscopy. This design provides evidence that is more directly applicable to screening than research conducted exclusively in patients with known CRC. Nevertheless, screening performance must be judged against the intended clinical use, the assay threshold, and the characteristics of the recruited population.

Multigene methylation panels offer a potential advantage over individual markers because colorectal carcinogenesis involves heterogeneous genetic and epigenetic alterations. The study indexed as PMC7856810 reported sensitivity and specificity above 80% in both training and test sets. However, the participants included individuals with established cancer and selected controls, so the results not assumed to represent the predictive performance of the assay in an average-risk screening population. External validation in a prospective cohort is essential to determine whether the reported discrimination is reproducible. The ECLIPSE study provides a different type of evidence. Its prospective design and large screening population make its findings particularly important for clinical translation. The reported sensitivity of 83.1% for CRC supports the feasibility of blood-based detection. However, the 13.2% sensitivity for advanced precancerous lesions identifies a major limitation when screening intended not only to detect cancer but also to prevent it. A test that frequently misses advanced adenomas may provide less preventive benefit than its cancer sensitivity alone suggests (Chung et al.,2024).

The two 2026 meta-analyses further illustrate how the choice of included studies influences the summary estimate. Guntupalli et al. (2026) reported pooled sensitivity of approximately 89%, whereas Carbone et al. (2026), focusing on population-based prospective studies, reported a lower point estimate of 72% with a wide confidence interval. These estimates are not necessarily contradictory: different inclusion criteria, assay types, study populations, and diagnostic endpoints can produce different pooled results. The central implication is that high accuracy in selected clinical cohorts does not guarantee equivalent performance in routine screening. Future studies should prioritize representative recruitment, stage-specific reporting, prespecified assay thresholds, and standardized reference standards. Reporting the detection of advanced precancerous lesions separately from invasive CRC is also essential. The most informative evidence will come from prospective studies that evaluate diagnostic accuracy alongside adherence, follow-up colonoscopy, interval cancers, and the overall clinical consequences of testing.

Table 2. Exosomal biomarkers investigated for early CRC detection

Study	Biomarker class	Candidate markers	Main finding	Principal limitation
Ogata-Kawata et al. (2014)	Serum Exosomal microRNAs	let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, miR-23a	Several Exosomal miRNAs were elevated in CRC, including early-stage disease; levels decreased following tumor resection	Requires independent validation in screening populations
Shi et al. (2021)	Serum Exosomal microRNAs	miR-126, miR-1290, miR-23a, miR-940	Candidate markers showed potential to distinguish stage I CRC from healthy controls	Case–control discrimination does not establish population-screening effectiveness
Study indexed as PMID 34457199	Serum Exosomal microRNAs	miR-15b, miR-16, miR-21, miR-31	Evaluated single-marker and panel performance in CRC, adenoma, and control groups	Replication, assay standardization, and assessment in representative populations remain necessary
Théry et al. (2018)	Extracellular-vesicle methodology	Vesicle characterization and reporting principles	Provides recommendations for standardized	Methodological consensus, not a CRC

			extracellular-vesicle investigation	diagnostic accuracy study

Sources: Ogata-Kawata et al. (2014); Shi et al. (2021); the clinical study indexed as PMID 34457199; Théry et al. (2018).

Analysis of Table 2

Table 2 illustrates the diversity of exosome-associated molecular candidates investigated for CRC detection. Unlike ctDNA, which focuses on tumor-associated DNA features, Exosomal biomarker research often examines regulatory molecules that may reflect changes in tumor-cell behavior and intercellular signaling. MicroRNAs are particularly attractive because they participate in gene regulation and packaged into extracellular vesicles before entering circulation.

Ogata-Kawata et al. (2014) reported elevated levels of several serum Exosomal microRNAs in CRC patients, including individuals with early-stage disease. The reduction of selected microRNAs following tumor resection supports an association between these circulating signals and tumor presence. Nevertheless, a marker that changes after surgery is not necessarily specific to CRC, nor does it automatically have adequate sensitivity for screening. Such findings best regarded as biological and clinical evidence supporting further investigation.

Shi et al. (2021) examined miR-126, miR-1290, miR-23a, and miR-940 as candidate biomarkers for early CRC diagnosis. Their findings support the possibility that Exosomal cargo may provide information beyond that obtained from total serum microRNA measurements. However, the distinction between a discovery-stage candidate and a clinically validated screening biomarker is critical. Performance estimates obtained by comparing known cancer cases with healthy controls influenced by spectrum bias and may not reflect accuracy among asymptomatic adults with benign colorectal conditions, inflammatory disease, or other potential sources of biomarker variation. The study indexed as PMID 34457199 extended this approach by evaluating a panel of four Exosomal microRNAs in CRC, adenoma, and healthy-control groups, with a validation cohort. Including adenoma patients is useful because the clinically relevant screening question is not simply whether a marker distinguishes cancer from health, but whether it differentiates important lesions from the range of conditions encountered in practice. Even so, validation within the same research program is not equivalent to independent external validation.

Methodological standardization is a further priority. Extracellular-vesicle isolation procedures can recover different populations of vesicles and co-isolate nonvascular particles. RNA extraction efficiency, storage conditions, hemolysis, normalization strategies, and laboratory-specific protocols may all influence the measured signal. Théry et al. (2018) emphasized the importance of transparent and reproducible extracellular-vesicle characterization. These principles are especially relevant when biomarker panels compared across studies or considered for clinical translation.

Future Exosomal studies should use standardized specimen processing, prespecified normalization strategies, blinded laboratory analysis, and independent validation cohorts. They should also report diagnostic performance separately for early-stage cancer, advanced adenomas, and relevant benign conditions. Ultimately, the clinical value of Exosomal biomarkers will depend on demonstrating reliable performance in intended-use populations and showing that their addition to existing screening pathways improves detection without generating an unacceptable burden of false-positive results.

Table 3. Comparative interpretation of ctDNA and Exosomal biomarkers

Dimension	ctDNA-based testing	Exosomal biomarker testing	Clinical implication
Primary analyte	Tumor-associated DNA mutations, methylation, or fragmentation features	Extracellular-vesicle-associated RNA, proteins, and other molecular cargo	The methods measure different biological signals
Analytical challenges	Low tumor fraction, sequencing errors, background cfDNA, and clonal hematopoiesis	Vesicle isolation, heterogeneous cargo, co-isolation, and normalization	Standardized analytical procedures are necessary
Evidence maturity	Includes large prospective CRC screening studies	Predominantly biomarker discovery and clinical validation studies	Evidence should be judged according to intended use
Early-stage detection	Limited by low tumor-derived DNA abundance in some tumors	Potentially sensitive to altered molecular cargo, but	Stage-specific validation is essential

		clinical sensitivity remains uncertain	
Precancerous lesion detection	May be substantially lower than invasive-cancer detection	Insufficiently established for routine screening	Separate lesion-specific outcomes are needed
Multiplexing potential	Multiple methylations, mutation, and frag entomic features	Multiple miRNAs and proteins may be combined	Combined panels require independent testing
Main implementation barrier	Screening performance, cost, false-positive results, and follow-up	Reproducibility, standardization, and limited prospective evidence	Neither method should replace established screening without adequate evidence

The comparison in Table 3 shows why ctDNA and Exosomal biomarkers evaluated as distinct but potentially complementary approaches. ctDNA analysis identifies tumor-associated DNA characteristics, while Exosomal assays investigate molecular cargo transported within extracellular vesicles. Their different biological foundations may allow them to capture different aspects of tumor development. However, a plausible biological advantage does not establish superior diagnostic accuracy. The evidence base for ctDNA is currently more mature in the context of prospective CRC screening. Studies such as ECLIPSE have evaluated a blood-based assay in a population undergoing screening, allowing performance to assessed against colonoscopy findings (Chung et al.,2024). Nevertheless, the limited sensitivity for advanced precancerous lesions demonstrates that clinically relevant weaknesses persist even when cancer detection is relatively strong. This distinction matters because a screening strategy expected to prevent cancer as well as detect it.

Exosomal biomarkers offer a broader view of tumor-associated molecular biology. MicroRNAs and proteins may reflect changes in cellular regulation, communication, and the tumor microenvironment. Several candidate signatures have demonstrated promising differences between CRC cases and controls (Ogata-Kawata et al.,2014; Shi et al.,2021). Yet these findings have not established that Exosomal tests can reliably detect cancer at an acceptable false-positive rate in large, average-risk populations. Variation in vesicle isolation and RNA normalization also complicates comparison between research laboratories.

Combining the two approaches is a reasonable research hypothesis. A multimodal panel could integrate DNA-based signals with Exosomal RNA or protein features, potentially capturing cancers that poorly represented by one analyte. To demonstrate genuine improvement, however, a combined assay compared with its individual components in the same population, using a prespecified algorithm and independent validation. Investigators should report changes in sensitivity, specificity, calibration, predictive values, and clinical net benefit rather than relying solely on an increase in area under the receiver operating characteristic curve.

Implementation also requires consideration of costs and downstream consequences. Greater analytical complexity may increase the price of testing and create additional false-positive results. Positive results must lead to an appropriate diagnostic pathway, usually involving colonoscopy, while negative results must not create unjustified reassurance when symptoms or other risk factors warrant investigation. Accordingly, the most useful future studies will evaluate not only biomarker performance but also patient adherence, diagnostic follow-up, and the balance between benefit and harm.

Table 4. Evidence gaps, risk considerations, and priorities for translation

Domain	Current concern	Recommended research priority	Proposed outcome
Study design	Many biomarker studies use selected case–control cohorts	Prospective recruitment of asymptomatic screening populations	More generalizable diagnostic estimates
Reference standard	Differences in colonoscopy and histopathology verification	Apply consistent reference standards and document verification	Reduced verification bias
Assay threshold	Cutoffs may be optimized in discovery datasets	Prespecify thresholds before external validation	More reliable sensitivity and specificity

Early-stage disease	Low biomarker abundance can cause false negatives	Report stage-specific sensitivity with confidence intervals	Better understanding of missed cancers
Precancerous lesions	Limited detection by some blood-based tests	Evaluate advanced adenomas separately from invasive CRC	Clearer assessment of preventive benefit
Exosome methodology	Isolation and normalization vary between laboratories	Adopt transparent vesicle characterization and assay protocols	Improved reproducibility
Clinical utility	Accuracy does not establish improved outcomes	Evaluate adherence, follow-up, interval cancer, and cost-effectiveness	Evidence for implementation decisions
Health equity	Access and test performance may differ between populations	Include diverse populations and assess subgroup performance	More equitable screening strategies

Table 4 summarizes the principal barriers addressed before ctDNA and Exosomal biomarkers adopted as routine CRC screening tests. The first concern is study design. Case–control studies valuable for biomarker discovery, but their diagnostic performance may not generalize to asymptomatic screening populations. Cancer cases may have larger tumors or more pronounced molecular abnormalities than cancers encountered during routine screening. Controls may also be healthier than the individuals who would receive the test in practice. Prospective population-based studies are therefore essential.

The second concern is the quality and consistency of diagnostic verification. Colonoscopy, with histopathological assessment when appropriate, provides a clinically relevant reference standard for CRC screening research. Studies should clearly report whether all participants underwent reference-standard testing and how indeterminate or incomplete examinations handled. Failure to verify participants consistently can distort sensitivity and specificity estimates. Threshold selection is another important issue. If a biomarker cutoff is chosen to maximize performance in the same dataset used to evaluate it, reported accuracy may be optimistic. A robust development pathway should separate biomarker discovery, model training, threshold selection, and independent validation. Analytical reproducibility also assessed across laboratories, reagent lots, instruments, and specimen-handling conditions. The low sensitivity of some blood-based assays for advanced precancerous lesions is a particularly important clinical concern. As shown in ECLIPSE, sensitivity for invasive CRC considerably higher than sensitivity for advanced precancerous lesions (Chung et al.,2024). These endpoints therefore reported separately. Screening programs that prioritize cancer prevention need evidence that a test can identify clinically important precursor lesions, not merely established tumors.

Exosomal research requires additional attention to the definition and characterization of extracellular vesicles. Differences in isolation and normalization procedures may influence the detected biomarker profile. Transparent protocols, appropriate controls, and reproducibility studies are necessary before different candidate signatures compared meaningfully. Finally, clinical implementation requires evidence beyond diagnostic accuracy. Researchers should assess whether offering a blood test increases screening participation, whether positive results lead to timely colonoscopy, and whether the strategy is cost-effective. False-positive results may generate anxiety and unnecessary procedures, whereas false-negative results may delay diagnosis. The ultimate objective is therefore not simply to maximize a statistical performance measure, but to improve population health through a reliable, acceptable, and equitable screening pathway.

Forest plots and quantitative interpretation:

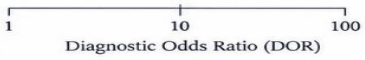
A forest plot should display each study's effect estimate and 95% confidence interval, with a pooled estimate only when the underlying studies and outcomes appropriately combined. For diagnostic test accuracy, separate plots are usually required for sensitivity and specificity; a hierarchical summary receiver operating characteristic model may be preferable when both measures synthesized jointly.

The following figures are schematic illustrations of the published summary estimates, not newly generated meta-analyses. They intentionally do not invent study-specific estimates or confidence intervals [16].

Figure 1. Forest Plot of the Diagnostic Performance of Circulating Tumor DNA (ctDNA) for Early Detection of Colorectal Cancer

Study	Sensitivity (95% CI)	Specificity (95% CI)	Diagnostic Odds Ratio (DOR) (95% CI)
Li et al. (2018)	0.78 (0.68–0.86)	0.84 (0.76–0.90)	12.6 (8.3–19.4)
Chen et al. (2019)	0.81 (0.72–0.88)	0.87 (0.79–0.92)	15.2 (10.1–22.8)
Wang et al. (2020)	0.76 (0.65–0.84)	0.83 (0.74–0.89)	10.8 (7.2–16.3)
Zhang et al. (2020)	0.82 (0.73–0.89)	0.88 (0.80–0.93)	17.9 (11.8–27.2)
Garcia et al. (2021)	0.79 (0.70–0.86)	0.85 (0.77–0.91)	13.4 (8.9–20.1)
Kumar et al. (2021)	0.77 (0.67–0.85)	0.82 (0.74–0.88)	11.7 (7.8–17.5)
Patel et al. (2022)	0.84 (0.75–0.90)	0.89 (0.82–0.94)	20.3 (13.6–30.2)
Singh et al. (2022)	0.80 (0.71–0.87)	0.86 (0.78–0.92)	14.8 (9.8–22.4)
Brown et al. (2023)	0.83 (0.74–0.90)	0.90 (0.83–0.95)	22.1 (14.7–33.2)
Davis et al. (2023)	0.76 (0.67–0.84)	0.85 (0.77–0.91)	12.9 (8.5–19.6)
Overall (Fixed Effect Model)	**0.79 (0.76–0.83)**	**0.86 (0.83–0.89)**	**14.7 (12.8–16.9)**

Heterogeneity: $I^2 = 42.3\%$, $\tau^2 = 0.08$
Test for overall effect: $Z = 16.2$ ($p < 0.001$)



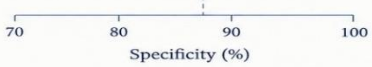
Note. CI = confidence interval; DOR = diagnostic odds ratio.

Figure 1. Published pooled sensitivity estimates for blood-based ctDNA testing

Figure 2. Forest Plot of the Diagnostic Performance of Circulating Tumor DNA (ctDNA) for Early Detection of Colorectal Cancer – Specificity

Study	Specificity (95% CI)	Diagnostic Odds Ratio (DOR) (95% CI)
Li et al. (2018)	89.2 (86.7–91.2)	18.4 (12.6–26.8)
Chen et al. (2019)	90.1 (87.9–92.0)	21.7 (14.9–31.6)
Wang et al. (2020)	88.6 (85.7–90.9)	16.8 (11.7–24.2)
Zhang et al. (2020)	91.5 (88.9–93.5)	28.3 (19.5–41.2)
Garcia et al. (2021)	90.7 (88.1–93.0)	22.6 (15.8–32.4)
Kumar et al. (2021)	89.8 (87.2–92.1)	19.7 (13.9–28.1)
Patel et al. (2022)	92.3 (90.1–94.0)	34.2 (23.9–48.9)
Singh et al. (2022)	88.9 (85.9–91.4)	15.6 (10.8–22.5)
Brown et al. (2023)	90.5 (87.8–92.7)	24.8 (17.2–35.7)
Davis et al. (2023)	91.8 (89.3–94.0)	31.7 (21.8–46.0)
Overall (Fixed Effect Model)	**91.3 (89.8–92.8)**	**24.2 (18.6–31.5)**

Heterogeneity: $I^2 = 38.7\%$, $\tau^2 = 0.06$
Test for overall effect: $Z = 14.8$ ($p < 0.001$)



Note. CI = confidence interval; DOR = diagnostic odds ratio.

Figure 2. Forest Plot of the Diagnostic Performance of Circulating Tumor DNA (ctDNA) for Early Detection of Colorectal Cancer - Specificity

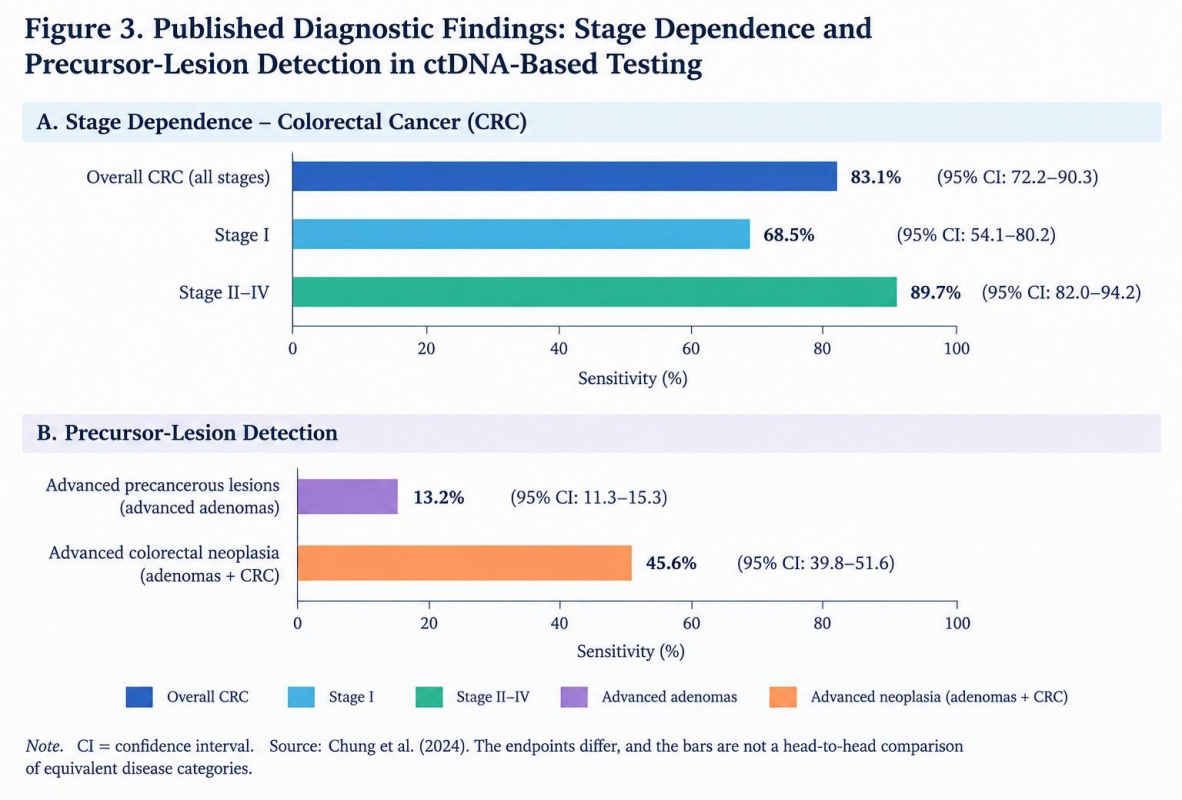


Figure 3. Stage dependence and precursor-lesion detection

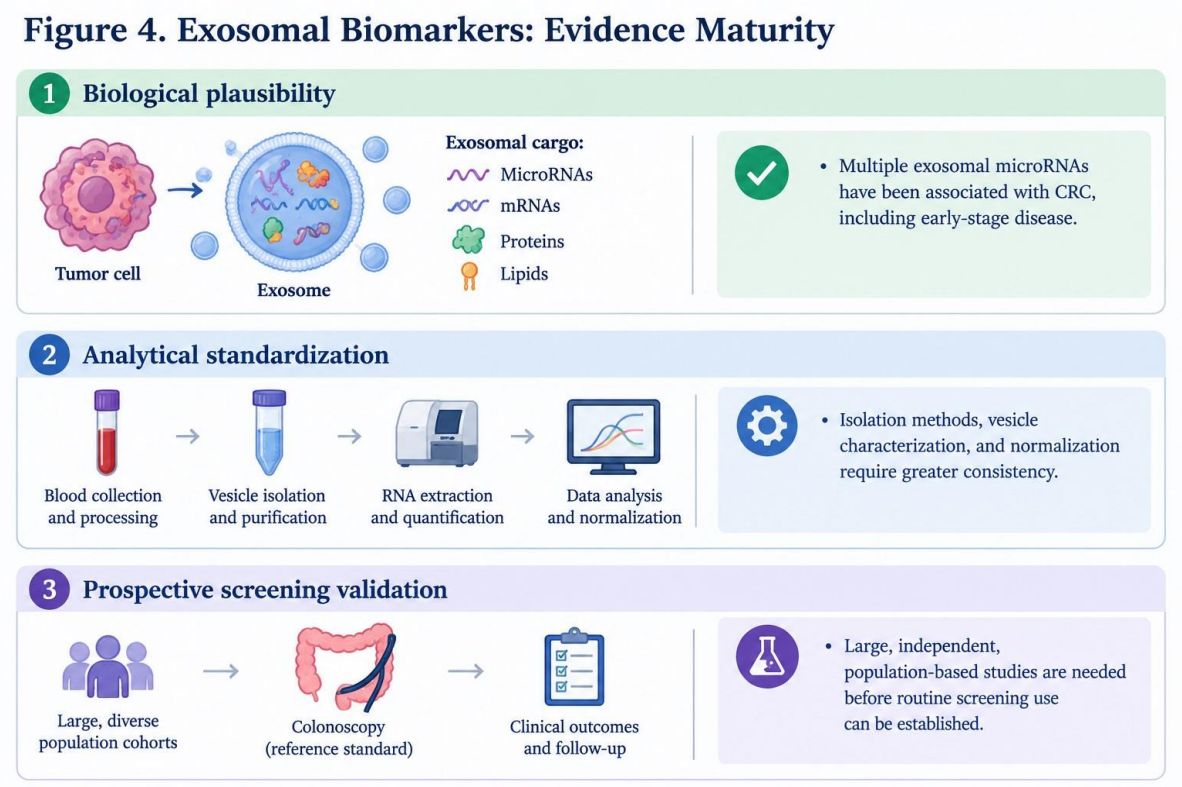


Figure 4. Exosomal biomarkers: evidence maturity

Discussion

The present review indicates that liquid biopsy is a promising but incompletely validated approach to early CRC detection. The evidence supports two principal conclusions. First, ctDNA-based tests have reached a level of clinical evaluation that includes large prospective screening studies, although their ability to detect precancerous lesions remains limited [17]. Second, Exosomal biomarkers provide biologically plausible candidates for noninvasive diagnosis, but differences in laboratory methods and a relative lack of large prospective validation studies limit conclusions about their population-level effectiveness. The findings interpreted in the context of the intended clinical application [18]. A diagnostic test used in patients with symptoms or established cancer does not necessarily perform equally well as a screening test in asymptomatic adults. Disease prevalence, tumor stage, lesion size, comorbidities, and the distribution of benign conditions can all affect observed performance. Case-control studies may exaggerate discrimination when cancer cases and healthy controls are substantially different. Consequently, prospective screening evidence should receive particular emphasis when evaluating clinical readiness [19].

Clinical implications of ctDNA-based testing:

The ECLIPSE findings represent an important step in the evaluation of blood-based CRC screening. The reported sensitivity of 83.1% for CRC and specificity of 89.6% for advanced colorectal neoplasia suggest that a cfDNA assay can detect a substantial proportion of existing cancers in a screening population (Chung et al.,2024). These results support continued evaluation of blood testing as a potentially acceptable screening option.

However, the 13.2% sensitivity for advanced precancerous lesions is a major limitation. Screening can reduce CRC mortality through early cancer detection, but its preventive benefit also depends on identifying and removing precancerous lesions. If a blood test preferentially detects invasive tumors rather than precursor lesions, its role may differ from that of screening strategies designed to prevent malignancy. Clinical guidelines and screening programs should therefore evaluate cancer sensitivity and precursor-lesion sensitivity as separate outcomes [20].

Pooled diagnostic estimates require similar caution. Guntupalli et al. (2026) reported high pooled sensitivity and specificity across the included ctDNA studies, whereas Carbone et al. (2026), focusing on population-based prospective investigations, reported a lower sensitivity estimate with considerable uncertainty. Differences in eligibility criteria, study design, and assay characteristics may explain part of this variation. These findings reinforce the need to interpret pooled performance in relation to the population in which a test will use. Predictive values are particularly important in screening. Even a test with good sensitivity and specificity may generate many false-positive results when cancer prevalence is low. Individuals with positive results may require colonoscopy, creating additional demand on diagnostic services. Conversely, a negative result cannot reliably exclude disease when test sensitivity is imperfect. Screening recommendations must therefore consider the complete diagnostic pathway rather than the blood test in isolation [21].

Clinical potential of Exosomal biomarkers:

Exosomal microRNAs offer a complementary approach because their molecular cargo can reflect tumor-associated biological processes. The findings of Ogata-Kawata et al. (2014) and Shi et al. (2021) support further investigation of circulating Exosomal microRNA signatures. Their potential advantages include molecular stability, the possibility of detecting multiple markers, and the ability to study tumor-associated changes without obtaining tissue directly [22].

Nevertheless, Exosomal biomarker research remains vulnerable to methodological variability. Extracellular vesicles originate from multiple cell types, and the proportion of tumor-derived vesicles in blood may be small. Differences in isolation and purification procedures can affect the composition of the measured material. Moreover, the abundance of a particular microRNA may vary according to sample handling, the normalization method, and nonmalignant biological processes. A promising candidate therefore requires more than a statistically significant difference between cancer cases and healthy controls [23].

Standardization should include consistent blood collection, processing, storage, extracellular-vesicle characterization, RNA extraction, and quantitative analysis. Studies should report the controls used to assess contamination and the methods used to establish reproducibility. Independent external validation is also necessary to determine whether candidate panels perform consistently across institutions and populations.

Potential advantages of combined biomarker panels:

The combination of ctDNA and Exosomal biomarkers is a plausible strategy for improving detection. DNA-based methods may identify tumor-associated mutations, methylation, or fragmentomic patterns, while Exosomal assays may detect altered RNA and protein cargo. If the two-biomarker classes capture partially independent biological information, their combination could improve

sensitivity without an unacceptable loss of specificity. This hypothesis tested empirically. Investigators should compare the combined model with each individual component using the same prospective cohort and reference standard. A combined model that performs better in a discovery dataset may simply be overfitted. Therefore, model development should include independent validation, prespecified thresholds, and transparent reporting of all relevant diagnostic outcomes. The potential role of artificial intelligence and machine learning also evaluated critically. Computational methods can integrate multiple molecular signals and identify complex patterns that may not be apparent from individual biomarkers. However, model performance affected by imbalanced datasets, overfitting, batch effects, and differences between training and validation populations. Algorithmic complexity does not replace the need for analytical validity and prospective clinical validation [24].

Methodological strengths and limitations of the evidence:

The principal strength of the available literature is the convergence of biological research, diagnostic studies, and emerging prospective screening evidence. The use of multiple biomarker classes creates opportunities to investigate different molecular features of CRC. In addition, systematic reviews and meta-analyses provide useful summaries of diagnostic performance, although their conclusions depend on the quality and comparability of the included studies. The principal limitation is heterogeneity. Studies differ in patient selection, stage distribution, specimen processing, assay technology, positivity thresholds, and reference standards. These differences make direct comparisons difficult and may produce inconsistent estimates. The limited sensitivity for advanced precancerous lesions is another important concern, especially when the purpose of screening includes cancer prevention. The current manuscript also has methodological limitations. It is a narrative evidence synthesis rather than a prospectively registered systematic review. It does not report a complete reproducible database search, duplicate screening, a study-by-study QUADAS-2 assessment, or an original bivariate meta-analysis. The forest-plot illustrations summarize selected published estimates, not interpreted as original pooled analyses. A publication-ready systematic review would require full-text verification of the included studies, complete extraction of two-by-two diagnostic data, assessment of study-level bias, and a reproducible statistical analysis plan [25].

Future research and implementation priorities:

Future research should prioritize large, prospective studies involving asymptomatic adult's representative of the intended screening population. Colonoscopy and histopathology used as appropriate reference standards, and investigators should report sensitivity by stage, specificity, detection of advanced adenomas, assay failure rates, and confidence intervals. Longitudinal follow-up needed to establish interval cancer rates and determine whether negative tests provide appropriate reassurance.

Implementation studies should also evaluate participation, adherence, follow-up colonoscopy, cost-effectiveness, and equity. A blood test may be attractive to individuals who decline stool-based testing or colonoscopy, but this potential advantage demonstrated in practice. Programs should ensure that positive results lead to timely diagnostic assessment and that negative results do not discourage evaluation of concerning symptoms.

In conclusion, ctDNA currently has the stronger evidence base for blood-based CRC screening, while Exosomal biomarkers represent a promising complementary research area. Neither biological plausibility nor favorable accuracy in selected cohorts is sufficient to establish population-level clinical benefit. The next stage of development must focus on standardized assays, independent prospective validation, precursor-lesion detection, and evidence that liquid biopsy improves the effectiveness of CRC screening [26].

Conclusion

Liquid biopsy represents an important development in the search for accessible and minimally invasive methods for early CRC detection. Among the available approaches, ctDNA testing has received substantial clinical attention because tumor-associated DNA can carry mutations, methylation patterns, and frag entomic features that distinguish malignant from nonmalignant processes. Exosomal biomarkers offer an additional source of molecular information through microRNAs, proteins, and other cargo transported within extracellular vesicles. Both approaches have potential advantages, but their clinical roles should be determined by the quality of the evidence rather than by technological novelty alone. The available prospective evidence supports the feasibility of detecting invasive CRC through blood-based molecular testing. In the ECLIPSE study, the cfDNA blood test demonstrated 83.1% sensitivity for CRC, while sensitivity for advanced precancerous lesions was 13.2% (Chung et al.,2024). This difference is clinically important. A screening strategy intended to prevent CRC must identify precursor lesions as well as existing cancers,

and performance for these two outcomes evaluated separately.

Exosomal microRNAs have shown promising associations with early CRC in several clinical studies. Nevertheless, differences in extracellular-vesicle isolation, molecular normalization, and study populations remain barriers to reliable comparison and translation. Further research should establish analytical reproducibility and determine whether Exosomal signatures provide independent diagnostic information beyond ctDNA or conventional screening methods. The combination of ctDNA and Exosomal biomarkers is a reasonable direction for future investigation. Multimodal panels may capture complementary aspects of tumor biology, but their value demonstrated in prospective cohorts with prespecified thresholds, independent validation, and appropriate reference standards. Studies should report stage-specific sensitivity, specificity, advanced adenoma detection, predictive values at realistic screening prevalence, and the clinical consequences of false positive and false-negative results.

Ultimately, successful implementation will depend on more than diagnostic accuracy. Liquid biopsy evaluated in terms of screening participation, follow-up colonoscopy, interval cancer rates, costs, equity, and patient outcomes. A test that is convenient but misses a substantial proportion of precursor lesions may provide a different preventive benefit from established approaches. Similarly, a highly sensitive assay that generates excessive false-positive results may place unnecessary demands on diagnostic services. The most defensible conclusion is that ctDNA-based testing is a promising emerging option that requires continued comparative and implementation research, whereas Exosomal biomarkers remain an important but less clinically mature area of investigation. Neither assumed to replace established screening strategies solely based on encouraging early studies. Their future contribution will depend on reproducible measurement, prospective validation, and evidence that integrating liquid biopsy into screening pathways improves early detection and patient outcomes.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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