

DIAGNOSTIC SENSITIVITY AND LIMITATIONS OF NOVEL MOLECULAR BIOMARKERS IN NON-INVASIVE EARLY CANCER DETECTION

Shakhnoza Hamidova

Independent Researcher, New York, United States

Received: 2026-07-22 · Accepted: 2026-08-25

Abstract

Early detection remains one of the most important determinants of successful cancer treatment and long-term patient survival. Conventional diagnostic approaches, including imaging, endoscopic examination, histopathological assessment, and serum protein markers, have substantially improved oncological diagnosis but may have limited sensitivity for small, early-stage, or biologically heterogeneous tumors. Recent advances in molecular oncology have introduced a new generation of minimally and non-invasive biomarkers that can potentially identify malignant disease through blood, urine, saliva, stool, and other accessible biological specimens. These biomarkers include circulating tumor DNA, circulating tumor cells, cell-free DNA methylation patterns, circulating microRNAs, extracellular vesicles, tumor-derived proteins, and combinations of molecular signatures. Their principal advantage is the possibility of detecting tumor-associated molecular alterations before clinically apparent disease develops or before a tumor reaches a size detectable by conventional imaging. However, diagnostic performance varies considerably according to cancer type, disease stage, tumor burden, biological characteristics, sample quality, analytical platform, and population characteristics. False-positive results may arise from clonal hematopoiesis, inflammatory conditions, benign tissue changes, and technical artifacts, whereas low tumor shedding may produce false-negative findings. This article reviews the diagnostic potential, sensitivity-related challenges, and major limitations of novel molecular biomarkers for non-invasive early cancer detection. Particular attention is given to circulating nucleic acids, DNA methylation, microRNAs, extracellular vesicles, and multimarker approaches. The integration of molecular biomarkers with clinical risk assessment and established diagnostic technologies may provide a more reliable strategy than the use of individual biomarkers alone.

Keywords: early cancer detection, molecular biomarkers, liquid biopsy, circulating tumor DNA, DNA methylation, microRNA, extracellular vesicles, circulating tumor cells, diagnostic sensitivity, non-invasive diagnosis.

1. Introduction

Cancer represents a heterogeneous group of diseases characterized by uncontrolled cellular proliferation, genomic instability, altered cellular signaling, and progressive interaction between

malignant cells and the surrounding microenvironment. Although major advances have been achieved in cancer therapy, prognosis remains strongly dependent on the stage at which malignant disease is identified. Tumors diagnosed at localized stages are generally more amenable to curative treatment than tumors detected after regional or distant dissemination. Consequently, improving the ability to identify cancer during its earliest biological phases remains a major objective of modern oncology. Traditional cancer diagnosis relies on combinations of clinical assessment, imaging, endoscopic procedures, cytological examination, tissue biopsy, and histopathological confirmation. These approaches remain fundamental to clinical practice because they provide anatomical and morphological information and, in many circumstances, definitive evidence of malignancy. Nevertheless, conventional methods can have important limitations in population-level screening. Imaging may fail to identify very small lesions, invasive diagnostic procedures may be unsuitable for repeated screening, and some circulating protein biomarkers lack sufficient specificity to distinguish malignant disease from benign or inflammatory conditions.

The development of molecular oncology has expanded the concept of cancer diagnosis beyond direct visualization of tumor tissue. Malignant cells release molecular material into surrounding biological fluids through apoptosis, necrosis, active secretion, and other mechanisms. As a result, tumor-associated genetic and epigenetic alterations may become detectable in peripheral blood or other biological specimens. This principle forms the basis of liquid biopsy and related non-invasive diagnostic approaches.

Circulating tumor DNA (ctDNA) has received considerable attention because tumor-specific genetic alterations may be identified in plasma. Mutations, copy-number alterations, structural variants, and methylation abnormalities can provide molecular evidence of malignant processes. However, the concentration of ctDNA is frequently low in early-stage disease, creating a fundamental sensitivity challenge. Tumor location, vascularization, biological phenotype, and shedding characteristics can also influence the amount of detectable tumor-derived DNA.

DNA methylation represents another promising biomarker class. Aberrant methylation of promoter regions and other genomic elements can occur during carcinogenesis and may provide tissue-associated molecular signatures. Compared with individual somatic mutations, methylation-based patterns may offer broader biological coverage because multiple epigenetic alterations can arise during malignant transformation.

MicroRNAs are short non-coding RNA molecules involved in post-transcriptional regulation of gene expression. Altered microRNA profiles have been observed in numerous malignancies. Because microRNAs may remain relatively stable in circulating biological fluids, they have been investigated as potential minimally invasive diagnostic markers. However, their lack of absolute tumor specificity and the influence of physiological and inflammatory conditions represent important limitations.

Extracellular vesicles, including exosomes, provide another molecular source. These vesicles transport DNA, RNA, proteins, lipids, and other signaling molecules between cells. Tumor-derived extracellular vesicles may therefore contain molecular information reflecting the characteristics of the primary tumor. Their potential for early detection is considerable, but standardized isolation and characterization procedures remain necessary.

The objective of this review-style analytical study is to examine the diagnostic sensitivity and principal limitations of emerging molecular biomarkers used for non-invasive early cancer detection, with emphasis on their biological basis, analytical performance, clinical applicability, and challenges associated with implementation.

2. Materials and Methods

A structured analytical review approach was used to examine the diagnostic characteristics of emerging molecular biomarkers for early cancer detection. The assessment focused on biomarker classes that can be evaluated using minimally invasive or non-invasive biological specimens, particularly peripheral blood, plasma, serum, urine, saliva, and stool.

The reviewed biomarker categories included circulating tumor DNA, cell-free DNA, DNA methylation signatures, circulating tumor cells, microRNAs, extracellular vesicles, tumor-associated proteins, and multimarker molecular panels. Particular attention was given to studies investigating their potential for detection of localized or early-stage malignant disease rather than exclusively advanced cancer. For each biomarker category, several diagnostic dimensions were considered. These included analytical detectability, biological specificity, potential sensitivity at low tumor burden, reproducibility,

susceptibility to pre-analytical variation, specificity for malignant disease, and feasibility of clinical implementation. Factors capable of influencing diagnostic performance were also evaluated, including tumor type, disease stage, tumor size, anatomical location, molecular heterogeneity, patient age, inflammatory status, smoking history, and coexisting benign disease.

The interpretation emphasized the distinction between analytical sensitivity and clinical diagnostic sensitivity. Analytical sensitivity refers to the ability of an assay to identify a molecular target at low concentration, whereas clinical sensitivity reflects the proportion of patients with disease who are correctly identified. A highly sensitive molecular platform does not necessarily guarantee high clinical sensitivity when tumor-derived material is insufficiently represented in the tested biological specimen. The analysis also considered the limitations of using isolated biomarkers compared with integrated molecular signatures. Multimarker strategies were examined in terms of their potential to compensate for biological heterogeneity and increase the probability of identifying malignant disease through complementary molecular signals.

3. Results

The analysis demonstrated that novel molecular biomarkers provide several biologically distinct opportunities for non-invasive cancer detection, but their diagnostic performance is strongly dependent on disease stage and tumor biology. A major common characteristic was the reduction in detectable tumor-derived material during the earliest phases of carcinogenesis. This limitation is particularly important for blood-based assays because the concentration of tumor-associated nucleic acids may represent only a very small fraction of the total circulating molecular material. Circulating tumor DNA was identified as one of the most extensively investigated molecular targets. ctDNA may contain tumor-specific point mutations, insertions, deletions, copy-number alterations, and methylation changes. Its major diagnostic advantage is the ability to obtain molecular information without directly sampling the tumor. Nevertheless, early-stage tumors may release insufficient quantities of ctDNA for reliable detection. Consequently, an assay can demonstrate excellent analytical sensitivity while still showing reduced clinical sensitivity in individuals with very small or biologically less active tumors.

The diagnostic characteristics of ctDNA also differed according to tumor type. Highly vascularized or biologically active tumors may release greater quantities of tumor-derived DNA into circulation, whereas tumors located in anatomical compartments with limited access to blood may produce weaker circulating signals. Therefore, a universal sensitivity value cannot be assumed for all malignancies.

Cell-free DNA methylation analysis provided a broader molecular perspective. Aberrant methylation patterns can arise early during malignant transformation and may persist as detectable molecular signatures. Methylation-based assays may potentially distinguish tumor-derived DNA from normal circulating DNA by analyzing characteristic epigenetic patterns. Their major limitation involves the complexity of distinguishing cancer-specific methylation from age-associated, inflammatory, tissue-specific, and other non-malignant epigenetic alterations.

Circulating microRNAs demonstrated substantial diagnostic potential because they can be detected in plasma and serum and may remain stable due to their association with proteins or extracellular vesicles. Certain microRNA profiles have been associated with specific malignancies, while combinations of several microRNAs may provide greater diagnostic information than individual molecules. However, the same microRNA may be altered in multiple cancer types as well as in non-malignant inflammatory or metabolic disorders. This limits disease specificity.

Extracellular vesicles represented another potentially valuable biomarker source. Tumor-associated vesicles may contain molecular information that reflects both the malignant cell and its microenvironment. Their cargo includes nucleic acids and proteins that can potentially support cancer classification. However, differences in isolation methods, vesicle purification, molecular characterization, and reporting standards currently complicate comparison between studies.

Circulating tumor cells were potentially informative because they represent intact malignant cells rather than isolated molecular fragments. Their molecular characterization may provide information regarding tumor phenotype and metastatic potential. However, CTC concentrations can be extremely low in early-stage disease, and their detection requires technically demanding enrichment and identification procedures. These factors may restrict their usefulness as a general population screening tool.

Multimarker approaches demonstrated an important conceptual advantage over single-biomarker strategies. Combining genetic alterations, methylation signatures, protein markers, microRNA profiles, or clinical variables may increase the probability that at least one disease-associated signal will be detectable. However, increased complexity may also create new challenges, including overfitting during model development, reduced reproducibility, and the need for large independent validation cohorts.

A recurring finding across molecular biomarker categories was the importance of pre-analytical conditions. Sample collection tubes, processing time, storage temperature, hemolysis, DNA extraction methods, and laboratory platform can influence measured biomarker concentrations. Such factors may contribute to variability between laboratories even when the underlying biological signal is comparable.

Another important issue was specificity. Molecular abnormalities are not always equivalent to malignant disease. Clonal hematopoiesis, chronic inflammation, tissue injury, benign neoplasms, and age-related molecular changes may generate signals resembling cancer-associated alterations. Therefore, positive molecular findings may require confirmation through imaging, endoscopy, histopathology, or additional molecular testing.

4. Discussion

The development of non-invasive molecular biomarkers represents a major transition in cancer diagnostics. Instead of depending exclusively on anatomical changes, molecular screening seeks to identify biological abnormalities associated with malignant transformation. This approach is particularly attractive because molecular alterations may occur before a lesion becomes sufficiently large or structurally abnormal to be recognized through conventional diagnostic methods. However, early detection presents a paradox: the earlier a tumor is detected, the smaller its molecular footprint may be. This creates a direct relationship between biological timing and diagnostic sensitivity. Advanced tumors generally release larger quantities of tumor-derived material, making molecular detection easier. Early tumors may contain fewer malignant cells, shed less DNA or RNA, and produce signals that are diluted within a large background of normal biological material. For ctDNA-based detection, one of the most important challenges is the low fraction of tumor-derived DNA within total cell-free DNA. Highly sensitive sequencing technologies can identify extremely small quantities of altered DNA, but technical detection of a molecular event does not automatically establish the presence of clinically significant cancer. Extremely sensitive assays can detect molecular abnormalities whose biological significance remains uncertain.

Clonal hematopoiesis illustrates this problem particularly clearly. Somatic mutations can arise within blood-forming cells without the presence of a solid tumor. If these mutations are detected in plasma and interpreted as tumor-derived alterations, false-positive cancer classifications may occur. This emphasizes the importance of using molecular alterations that are sufficiently specific for malignant tissue or combining several independent biomarkers.

Methylation-based biomarkers may overcome some limitations of mutation-based assays because patterns of epigenetic modification can provide information about tissue origin. A combination of methylation markers may theoretically distinguish tumor-associated DNA from normal background DNA more effectively than a single mutation. Nevertheless, methylation patterns can also be influenced by aging, smoking, inflammation, hormonal status, and tissue-specific biology. Large and diverse validation cohorts are therefore required.

MicroRNA-based diagnostics face a different challenge. Although individual microRNAs can show strong associations with particular cancers, their biological roles are not restricted to malignancy. A microRNA altered in a tumor may also participate in immune responses, tissue repair, metabolic regulation, or inflammatory signaling. Consequently, diagnostic models based on multiple microRNAs are generally more biologically plausible than reliance on a single molecule.

Extracellular vesicles may provide a richer representation of tumor biology because their molecular cargo can include several types of biomolecules simultaneously. However, methodological standardization remains a major barrier. Different studies may isolate vesicles using different physical or biochemical principles, resulting in populations that are not directly comparable. Without harmonized definitions and analytical protocols, reported diagnostic performance may not translate consistently into routine clinical practice.

Another important issue is spectrum bias. Biomarker studies frequently compare clearly diagnosed

cancer patients with healthy controls. Such designs can produce apparently high sensitivity and specificity because the biological contrast between advanced malignancy and healthy individuals is substantial. Real-world screening populations are different: most individuals are asymptomatic, many have benign diseases, and the prevalence of cancer is relatively low. Under these conditions, even a test with high specificity may generate a meaningful number of false-positive results.

This is especially important for population screening. If cancer prevalence is low, positive predictive value can decline despite excellent sensitivity and specificity. Consequently, clinical evaluation of molecular biomarkers should not rely exclusively on sensitivity and specificity estimates. Positive predictive value, negative predictive value, likelihood ratios, stage-specific performance, and outcomes associated with downstream diagnostic procedures should also be considered.

The clinical usefulness of a molecular biomarker ultimately depends on whether its use improves patient outcomes rather than simply identifying molecular abnormalities. A screening test may detect cancer earlier but still have limited clinical value if the detected tumor does not alter treatment possibilities or if false-positive findings lead to unnecessary invasive procedures. Therefore, prospective studies assessing mortality, stage distribution, treatment outcomes, and quality of life are essential.

Combining molecular biomarkers with conventional approaches may provide a more practical pathway toward implementation. For example, molecular screening could identify individuals requiring targeted imaging, endoscopic assessment, or tissue biopsy. In this model, molecular biomarkers would not necessarily replace established diagnostic methods but would function as an additional layer of risk stratification.

Artificial intelligence and machine-learning approaches may further improve multimarker interpretation. Algorithms can integrate genomic, epigenetic, transcriptomic, proteomic, imaging, and clinical variables. However, algorithmic complexity must be balanced against transparency, reproducibility, and external validation. Models developed in one population may perform differently in another because genetic background, environmental exposures, disease prevalence, and healthcare pathways vary.

Another challenge concerns the definition of “early detection.” Detecting a molecular signal before symptoms appear is not necessarily equivalent to detecting an invasive cancer that requires immediate treatment. Some molecular abnormalities may represent precancerous or indolent processes. Therefore, future biomarker research should distinguish between biological detection, clinically meaningful detection, and detection associated with improved patient outcomes.

Overall, the evidence supports the potential of molecular biomarkers as complementary tools for early cancer detection, but their current limitations indicate that no single biomarker class should be considered universally sufficient. The most promising strategies are likely to combine highly sensitive molecular technologies with tissue-of-origin information, clinical risk factors, and confirmatory diagnostic procedures.

5. Conclusion

Novel molecular biomarkers have created important opportunities for non-invasive and minimally invasive early cancer detection. Circulating tumor DNA, DNA methylation patterns, microRNAs, extracellular vesicles, circulating tumor cells, and multimarker signatures can provide molecular information that may precede clinically recognizable anatomical abnormalities. Their greatest potential lies in identifying cancer-associated biological changes while tumor burden remains low. At the same time, diagnostic sensitivity remains a central limitation. Early-stage tumors may release only minimal quantities of detectable molecular material, while biological heterogeneity can cause substantial differences between patients and tumor types. False-positive results may arise from clonal hematopoiesis, inflammation, aging-associated changes, and other non-malignant processes. Pre-analytical and analytical variability further complicates the translation of promising research findings into routine screening.

Future development should therefore prioritize large prospective studies, standardized laboratory protocols, independent external validation, stage-specific performance assessment, and evaluation in representative screening populations. Multimarker models combining molecular signals with clinical and imaging information may provide greater diagnostic reliability than isolated biomarkers. Ultimately, the clinical value of molecular cancer detection should be judged not only by its ability to identify molecular abnormalities but also by whether earlier diagnosis leads to meaningful

improvements in treatment and patient outcomes.

References

1. Hanahan D. Hallmarks of Cancer: New Dimensions. *Cancer Discovery*. 2022;12(1):31–46.
2. Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. *Nature Reviews Genetics*. 2019;20:71–88.
3. Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nature Reviews Cancer*. 2017;17:223–238.
4. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Science Translational Medicine*. 2014;6(224):224ra24.
5. Aravanis AM, Lee M, Klausner RD. Next-generation sequencing of circulating tumor DNA for early cancer detection. *Cell*. 2017;168(4):571–575.
6. Cohen JD, Li L, Wang Y, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science*. 2018;359(6378):926–930.
7. Lennon AM, Buchanan AH, Kinde I, et al. Feasibility of blood testing combined with PET-CT to screen for multiple cancers. *Science*. 2020;369(6499):eabb9601.
8. Schwarzenbach H, Nishida N, Calin GA, Pantel K. Clinical relevance of circulating cell-free DNA in cancer. *Nature Reviews Clinical Oncology*. 2011;8:426–437.
9. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proceedings of the National Academy of Sciences*. 2008;105(30):10513–10518.
10. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018). *Journal of Extracellular Vesicles*. 2018;7(1):1535750.



Indexed & Abstracted In

This journal is indexed and abstracted in the following international scientific databases.



Google Scholar



ISSN



ORCID



CiteFactor



ResearchBib



DOI



Zenodo



Article Verification

Scan the QR code to verify the authenticity of this article

DOI: 10.4103/aams.0498



Grammarly