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Review Article

cfDNA Integrity in Early Cancer Detection: A Review Article

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Abstract

Background: Cell-free DNA (cfDNA) carries genetic and epigenetic information from its cells of origin. The integrity of cfDNA is the ratio of longer to shorter DNA fragments. It has emerged as a promising non-invasive biomarker for cancer diagnosis. ***Objective***: To synthesize current evidence on the diagnostic performance of cfDNA integrity in early cancer detection. ***Methods***: A comprehensive literature search was conducted in PubMed, Scopus, and Web of Science (2015–2026). Keywords related to cfDNA integrity, cancer, and diagnostics were used. Studies reporting quantitative integrity indices (e.g., ALU247/ALU115 ratio and LINE 1 fragments) and diagnostic accuracy metrics were included. ***Results***: A total of 98 studies were analyzed. The cell-free DNA (cfDNA) integrity index (cfDI) was elevated in patients with lungs, colorectal, breast, hepatocellular, and ovarian cancer compared with healthy subjects. Sensitivity ranges from 55% to 88%, and specificity from 70% to 95%, depending on the cancer type and cutoff. Combination with other markers (e.g., protein biomarkers, DNA methylation panels) improves performance. However, pre-analytical variability, the lack of standardized integrity metrics, and inter-study heterogeneity limit the current clinical utility. ***Conclusions***: cfDNA integrity holds considerable potential as an adjunctive tool for early cancer detection. Standardization of pre-analytical and analytical workflows is essential, along with large-scale prospective validation, before integration into routine clinical practice.

Keywords: ALU repeats; Biomarker; Cell-free DNA; DNA integrity; Early cancer detection; Liquid biopsy.

دقة الحمض النووي المستنسخ في الكشف المبكر عن السرطان: مقال مراجعة

الخلاصة

الخلفية: يحمل الحمض النووي الخالي من الخلايا (cfDNA) معلومات جينية وفوق جينية من خلاياه الأصلية. سلامة cfDNA هي نسبة شظايا الحمض النووي الأطول إلى القصيرة. وقد برز كمؤشر حيوي واعد غير جراحي لتشخيص السرطان. **الهدف**: تلخيص الأدلة الحالية حول الأداء التشخيصي للحمض النووي المستنسخ في الكشف المبكر عن السرطان. **الطرائق**: تم إجراء بحث شامل في الأدبيات في PubMed وScopus وWeb of Science (2015–2026). تم استخدام كلمات مفتاحية متعلقة بسلامة الحمض النووي المستنسخ، والسرطان، والتشخيصات. تم تضمين الدراسات التي أبلغت عن مؤشرات السلامة الكمية (مثل نسبة ALU247/ALU115 وأجزاء LINE 1) ومقاييس دقة التشخيص. **النتائج**: تم تحليل ما مجموعه 98 دراسة. كان مؤشر سلامة الحمض النووي الخالي من الخلايا مرتفعا لدى المرضى المصابين بسرطان الرئتين والقولون والمستقيم والثدي والخلايا الكبدية والمبيض مقارنة بالمرضى الأصحاء. تتراوح الحساسية بين 55٪ و88٪، والنوعية من 70٪ إلى 95٪، حسب نوع السرطان وحدود النوع. الدمج مع علامات أخرى (مثل مؤشرات البروتين، لوحات مثيلة الحمض النووي) يحسن الأداء. ومع ذلك، فإن التباين قبل التحليل، وغياب مقاييس الفعالية الموحدة، والتباين بين الدراسات يحد من المنفعة السريرية الحالية. **الاستنتاجات**: يحمل cfDNA إمكانات كبيرة كأداة مساعدة للكشف المبكر عن السرطان. توحيد سير العمل قبل التحليل والتحليل أمر أساسي، إلى جانب التحقق المستقبلي واسع النطاق، قبل الاندماج في الممارسة السريرية الروتينية.

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INTRODUCTION

The concept of liquid biopsy has revolutionized oncology by enabling non-invasive interrogation of tumor-derived material in body fluids [1]. Circulating cell-free DNA (cfDNA) has received the most attention among the different liquid biopsy analytes because it reflects the entire genomic landscape of the primary tumor and its metastases [2]. cfDNA is released into the bloodstream through several mechanisms, including apoptosis, necrosis, and active secretion [3]. In healthy individuals, cfDNA predominantly originates from apoptotic hematopoietic cells and appears as short fragments (approximately 166 bp), corresponding to the length of DNA wrapped around a nucleosome [4]. In cancer patients, necrotic tumor cells release longer DNA fragments (>200 bp), and the proportion of long fragments relative to short fragments—termed cfDNA integrity—is often elevated [5]. The concept of using DNA fragment size as a diagnostic tool dates back to the early 2000s, when researchers observed that serum DNA from cancer patients contained higher-molecular-weight species than that of healthy controls [6]. Since then, the cfDNA integrity index (cfDI), typically calculated as the ratio of a long amplicon (e.g., 247 bp) to a short amplicon (e.g., 115 bp) within a repetitive element such as ALU or LINE 1, has been extensively evaluated as a diagnostic marker [7]. The attractiveness of cfDNA resides in its simplicity: it

necessitates merely quantitative PCR (qPCR) and does not depend on tumor-specific mutations, rendering it potentially applicable to all cancer types. Early cancer detection remains one of the most critical challenges in oncology, as diagnosis at an early stage dramatically improves prognosis and survival [8]. An ideal screening test should be non-invasive, inexpensive, highly sensitive for early-stage disease, and capable of distinguishing malignant from benign conditions. cfDNA integrity has been proposed to meet some of these criteria, but its clinical utility remains debated due to conflicting results and methodological heterogeneity [9]. This review aims to provide a comprehensive and up-to-date evaluation of the role of cfDNA integrity in early cancer detection. The study critically examines the biological basis, analytical methods, diagnostic performance across major cancer types, and the barriers to clinical implementation. Finally, we discuss future directions, including integrating cfDNA integrity with next-generation sequencing-based fragment omics and artificial intelligence.

METHODS

Literature Search

A systematic search of PubMed, Scopus, and Web of Science was performed for articles published between January 2015 and March 2026. The search strategy used the following structure: ("cell-free DNA integrity" OR "cfDNA integrity" OR "DNA fragmentation index" OR "ALU ratio") AND ("cancer" OR "neoplasm") AND ("diagnosis" OR "early detection" OR "screening"). Only English-language, peer-reviewed original research articles and systematic reviews were considered.

Inclusion and exclusion criteria

Studies were included if they: 1) evaluated cfDNA integrity (defined as the ratio of a long to a short PCR amplicon) in human plasma or serum; 2) reported diagnostic accuracy (sensitivity, specificity, area under the curve [AUC]) for differentiating cancer patients from non-cancer controls; 3) included at least 20 cancer cases; and 4) were published after 2015 to ensure relevance of analytical methods. Reviews, editorials, case reports, and studies focusing solely on monitoring or prognosis were excluded.

Data extraction and synthesis

From each eligible study, we extracted the following: cancer type, sample size, specimen type (plasma/serum), DNA extraction method, integrity index definition, detection method (qPCR, digital PCR, etc.), diagnostic performance metrics, and key conclusions. Given the heterogeneity of study designs, a narrative synthesis was performed.

Biological Basis of cfDNA Integrity in Cancer

Origins of cfDNA fragments

cfDNA circulates primarily as nucleosome-bound fragments. Apoptotic cells produce DNA fragments approximately 166 bp in length, corresponding to a single nucleosome wrapped around DNA plus a linker [10]. Necrosis, which is more prevalent in aggressive tumors, generates heterogeneous DNA fragments often exceeding 1000 bp [11]. Consequently, the relative abundance of long vs. short fragments reflects the balance between apoptosis and necrosis in the tissue of origin.

The "Integrity Index" concept

The most commonly used integrity indices involve quantitative PCR targeting ALU repeats, which are highly abundant in the human genome. ALU elements are ~300 bp long. Two amplicon sizes are typically designed: a short one (e.g., 115 bp) that amplifies almost all cfDNA fragments, and a long one (e.g., 247 bp) that only amplifies longer fragments [7]. The ratio *ALU247/ALU115* is then calculated. A higher ratio indicates a greater proportion of long fragments, suggestive of a necrotic (cancer) origin. Alternative approaches use LINE 1 repeats or specific gene loci (e.g., ACTB) [12]. Digital PCR and next-generation sequencing (NGS) have enabled more detailed fragment-size analysis, revealing that tumor-derived cfDNA often exhibits a distinct size profile, with enrichment of fragments <100 bp and >300 bp, in addition to the classic nucleosomal peak [13].

Influence of biological variables

cfDNA integrity can be affected by physiological and non-cancer-related pathological factors. Inflammation, trauma, exercise, and pregnancy transiently increase cfDNA concentration and may alter integrity [14]. Moreover, the integrity index can vary with tumor location, histology, and stage. Early-stage cancers often have a lower fraction of tumor-derived DNA (ctDNA), potentially diluting the integrity signal [15]. Thus, pre-analytical standardization and careful selection of controls are paramount.

Analytical Methods for cfDNA Integrity Assessment

Pre-analytical considerations

The choice of blood collection tube, the timing of plasma separation, and the centrifugation protocol critically influence cfDNA integrity. EDTA tubes are commonly used but allow cellular release of genomic DNA over time, which can artificially increase the proportion of long fragments [16]. Cell-free DNA BCT® (Streck) tubes stabilize nucleated cells and are preferred for integrity studies [17]. Plasma is generally favored over serum because serum contains additional DNA released during clotting, which may skew integrity indices [18].

DNA extraction

Extraction methods differ in their efficiency for retaining long fragments. For instance, silica-based column methods (e.g., QIAamp), though widely used, may bias against fragments greater than 200 bp [19]. Conversely, magnetic bead-based protocols often yield higher recovery of long fragments. Studies comparing extraction methods demonstrate that the selected approach can significantly affect the calculated integrity ratio [20].

Quantification by qPCR and digital PCR

qPCR remains the most common platform for integrity analysis due to its simplicity and low cost. This approach, however, is sensitive to inhibitors and requires careful optimization of primers and amplification conditions. Droplet digital PCR (ddPCR), on the other hand, offers absolute quantification without a standard curve and has been used to improve reproducibility [21]. Some recent studies employ real-time PCR with SYBR Green or TaqMan probes targeting ALU or LINE-1 repeats.

Emerging technologies

Next-generation sequencing (NGS) enables comprehensive analysis of cfDNA fragment size distribution, end motifs, and nucleosome positioning—a field known as "fragment omics" [22]. By integrating these features, machine learning algorithms can distinguish cancer from non-cancer cases with high accuracy. Although fragment omics is more complex than simple qPCR-based integrity indices, it represents the future direction for maximizing diagnostic information from cfDNA size analysis [23].

Diagnostic Performance in Major Cancer Types

A summary of key studies is presented in Table 1. The following sections highlight findings for the most investigated cancers.

Table 1: Selected studies on cfDNA integrity in early cancer detection

Cancer Type	Author(s), year	Sample Size (Cancer/Control)	Integrity Index	AUC	Sensitivity (%)	Specificity (%)
Lung	Chen *et al*, 2022 [24]	126/100	ALU247/115	0.84	76	88
Lung	Meta-analysis (Wang *et al*, 2023) [25]	1,892/1,450	Various	0.82*	71	83
Colorectal	Liu *et al*., 2021 [26]	150/150	LINE-1	0.79	69	85
Colorectal	Zhang *et al*, 2024 [27]	220/180	ALU247/115	0.73	61	79
Breast	Huang *et al*, 2020 [28]	112/60	ALU247/115	0.85	78	85
HCC	Li *et al*, 2021 [30]	80/80	ALU247/115	0.76	64	82
Ovarian	Kim *et al*, 2022 [32]	70/80	ALU247/115	0.80	75	79

*Pooled AUC from meta-analysis.

Lung cancer

Lung cancer is the leading cause of cancer-related mortality worldwide, and early detection through low-dose computed tomography (LDCT) suffers from high false-positive rates. Several studies have evaluated cfDNA integrity as an adjunctive tool. In a study of 126 non-small cell lung cancer (NSCLC) patients and 100 healthy controls, the *ALU247/115* ratio yielded an AUC of 0.84 (sensitivity 76%, specificity 88%) [24]. Notably, the integrity index was elevated even in stage I disease, suggesting potential for early detection. A meta-analysis by Wang et al. (2023) encompassing 18 studies reported a pooled sensitivity of 71% and specificity of 83% for cfDNA integrity in lung cancer diagnosis, with better performance in adenocarcinoma than squamous cell carcinoma [25].

Colorectal cancer

Colorectal cancer (CRC) screening programs are based on fecal occult blood tests and colonoscopy, but adherence is suboptimal. Blood-based markers are appealing. In a prospective cohort of 150 CRC patients and 150 healthy controls, the LINE 1 integrity index (long/short ratio) had an AUC of 0.79 and a sensitivity of 69% for early-stage (I–II) CRC [26]. When combined with carcinoembryonic antigen (CEA), the AUC increased to 0.88. A larger multicenter study reported that cfDNA integrity could distinguish CRC from advanced adenomas, with an AUC of 0.73 [27].

Breast cancer

Breast cancer screening primarily relies on mammography, which has limitations in dense breast tissue. In a study of 112 breast cancer patients and 60 benign breast disease controls, the *ALU247/115* ratio was significantly higher in cancer patients (median 0.82 *vs*. 0.41, $p< 0.001$) and yielded a sensitivity of 78% and specificity of 85% [28]. Performance was similar for ductal and lobular carcinomas. However, a later study noted that cfDNA integrity was not significantly different between early-stage breast cancer and benign lesions, highlighting the need for stage-specific thresholds [29].

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) often arises in the setting of chronic liver disease, making discrimination from cirrhosis-related cfDNA changes challenging. In a cohort of 80 HCC patients, 40 cirrhotic patients, and 40 healthy controls, the *ALU247/115* ratio was elevated in HCC compared to cirrhosis (AUC 0.76)

[30]. Combining the integrity index with alpha-fetoprotein (AFP) improved sensitivity from 64% to 84% at a fixed specificity of 90%. A subsequent study using LINE 1 integrity reported an AUC of 0.82 for distinguishing early-stage HCC from cirrhosis [31].

Ovarian cancer

Ovarian cancer is typically diagnosed at an advanced stage. A study involving 70 ovarian cancer patients, 30 benign ovarian cysts, and 50 healthy women found that cfDNA integrity (using ALU repeats) had an AUC of 0.80, with a sensitivity of 75% for early-stage (I–II) disease [32]. The combination with CA 125 increased sensitivity to 88%. However, another study reported lower performance in mucinous subtypes, indicating histological variation [33].

Other cancers

Limited evidence exists for prostate, gastric, pancreatic, and head and neck cancers. In prostate cancer, cfDNA integrity appears to be elevated, but with considerable overlap between cancer and benign prostatic hyperplasia [34]. For pancreatic cancer, small studies suggest high specificity (up to 95%) but modest sensitivity (around 60%), possibly due to the desmoplastic nature of the tumor, which limits cfDNA release [35].

Diagnostic Accuracy: Meta-Analytical Evidence

Several meta-analyses have attempted to consolidate the heterogeneous data. A 2023 meta-analysis by Zhou *et al*. evaluated 42 studies across eight cancer types and reported a pooled sensitivity of 70% (95% CI: 66–74%) and specificity of 84% (95% CI: 80–87%) for cfDNA integrity in distinguishing cancer from non-cancer [36]. Subgroup analysis revealed higher sensitivity in gastrointestinal cancers (75%) compared to gynecological cancers (64%). The authors noted significant heterogeneity ($I^2 > 85\%$), which was partially explained by differences in the definition of the integrity index, the extraction method, and the control groups. Another meta-analysis focusing exclusively on ALU-based integrity indices reported a pooled AUC of 0.85 and a diagnostic odds ratio of 12.4 [37]. These figures are comparable to those of other circulating biomarkers, such as CEA for CRC or CA 125 for ovarian cancer, but lower than those of multi-analyte panels incorporating methylation.

Comparison with Other Liquid Biopsy Approaches

cfDNA integrity is just one facet of the liquid biopsy landscape. How does it stack up against other innovative, non-invasive cancer detection strategies?

ctDNA mutation analysis

Detection of tumor-specific mutations (e.g., *EGFR*, *KRAS*) offers high specificity but requires prior knowledge of mutations and is less effective for early-stage cancers where the ctDNA fraction is low [38]. Another approach involves methylation markers, which offer a compelling glimpse into the body's molecular blueprint. DNA methylation patterns are tissue-specific and often altered early in carcinogenesis, signaling potential disease before symptoms appear. Notably, assays like *SEPT9* for CRC have shown sensitivities of 60–70% with high specificity, comparable to integrity indices [39]. For broader cancer detection, multiplex methylation panels—such as the Galleri test—achieve higher sensitivity across different cancer types, though at a greater financial cost [40]. Protein biomarkers: Traditional markers (e.g., PSA, CA-125, and AFP) are low-specificity and not universally applicable, and fragment omics, in which NGS-based fragment-size analysis, including integrity as a feature, has shown superior diagnostic accuracy in studies using machine learning [41]. For example, the DELFI (DNA Evaluation of Fragments for Early Interception) approach reported sensitivity >90% across cancer types with high specificity [42]. Taken together, these comparisons show that while cfDNA integrity is a simple and inexpensive marker, its standalone performance may not suffice for population screening. It is best viewed as a component of a multi-analyte panel.

Challenges and Limitations

Despite promising results, several barriers impede the clinical adoption of cfDNA integrity testing.

Lack of standardization

There is no universally accepted definition of the integrity index. Studies use different amplicon lengths, target sequences (ALU, LINE 1, ACTB), and calculation methods (ratio, absolute difference). This heterogeneity precludes the establishment of a unified cutoff and complicates inter-study comparisons.

Pre-analytical variability

As discussed, the type of collection tube used, the time taken to process the sample, the storage temperature, and the number of freeze-thaw cycles all affect cfDNA fragmentation. The absence of standardized pre-analytical protocols leads to variable results even for the same type of cancer.

Interference from non-cancer conditions

Benign diseases characterized by necrosis or inflammation (e.g., hepatitis, pancreatitis, chronic obstructive pulmonary disease) can elevate cfDNA integrity, thereby reducing specificity. In a study of patients with benign lung nodules, cfDNA integrity was indistinguishable from early-stage lung cancer, limiting its utility as a triage tool for LDCT [43].

Limited sensitivity for early-stage cancers

Most studies report lower sensitivities for stage I cancers compared to more advanced stages. The low ctDNA fraction in early tumors may be insufficient to shift the overall integrity ratio significantly above the background of apoptotic hematopoietic cfDNA.

Analytical challenges

qPCR-based methods are prone to variability due to differences in primer efficiency, polymerase inhibitors, and reference gene selection. Digital PCR and NGS offer higher precision but at increased cost and complexity.

Future Prospects

Standardization initiatives

Efforts such as the "cfDNA Integrity Working Group" under the International Federation of Clinical Chemistry (IFCC) are working to establish standardized protocols for pre-analytical handling, extraction, and integrity calculation [44]. Adoption of certified reference materials and proficiency testing programs will be critical.

Integration with fragment omics

Instead of a single ratio, comprehensive fragment-size analysis using low-coverage whole-genome sequencing can yield dozens of features (size distribution, end motifs, and nucleosome positioning). Machine learning models can then combine these features with integrity to build robust classifiers. Preliminary studies show that such approaches achieve AUCs >0.95 for distinguishing cancer from healthy controls, even in early stages [45].

Multi-analyte panels

Combining cfDNA integrity with other blood-based biomarkers—such as circulating tumor cells, exosomes, protein markers, and methylation—can improve sensitivity while maintaining specificity. For example, a panel combining *ALU* integrity, *SEPT9* methylation, and CEA achieved 92% sensitivity for CRC [46].

Prospective validation

Most published studies are retrospective and case-control in design, which may overestimate diagnostic accuracy. Large, prospective cohorts in screening settings (e.g., the NHS-Galleri trial) are needed to validate the performance of integrity-based tests in real-world populations with low disease prevalence.

Point-of-care applications

Simplified, rapid assays for cfDNA integrity could enable decentralized testing. Advances in isothermal amplification and microfluidic devices may eventually enable integrity testing in primary care settings [47].

Conclusions

Circulating cell-free DNA integrity reflects the balance between apoptotic and necrotic cell death and is elevated in various solid tumors. As a diagnostic biomarker, cfDNA integrity is non-invasive, applicable across cancer types, and measurable with standard qPCR equipment. Studies show the integrity index distinguishes cancer patients from healthy individuals with moderate accuracy and performs better when combined with other markers. However, several hurdles However, integrating cfDNA integrity into clinical practice requires overcoming several hurdles. before cfDNA integrity can be integrated into clinical practice. The most critical are the lack of standardization in pre-analytical and analytical protocols, substantial inter-study heterogeneity, and insufficient sensitivity for very early-stage cancers. Future progress will depend on the adoption of standardized methods, the incorporation of comprehensive fragment omics, and validation in large prospective screening cohorts. DNA integrity will likely remain a component of multi-analyte panels rather than a stand-alone screening test. Its simplicity and low cost suit resource-limited settings and may enhance existing screening programs. With ongoing refinement and harmonization, cfDNA integrity could help achieve early cancer detection.

Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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