AMERICAN JOURNAL OF BIOMEDICINE AND PHARMACY
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Article

Liquid Biopsy and Circulating Biomarkers in Clinical Chemistry of Colorectal Cancer

Bayadir Abdul Hussein Mahameed [1] , Saja Ahmed Abdullah Al-Shajalee [2], Enas Adnan Khazaali [3]
1. Al-Nahrain University, College of Medicine, Department of Chemistry and Biochemistry, Iraq
2. College of Education for Pure Science (Ibn Al-Haitham), Department of Chemistry, Iraq
3. Al-Nahrain University, College of Medicine, Department of Obstetrics and Gynecology, Iraq
Corresponding Author: Bayadir Abdul Hussein Mahameed
Al-Nahrain University, College of Medicine, Department of Chemistry and Biochemistry, Iraq
Email: bayaderb79@gmail.com
ORCID: 0009-0008-9205-3690

Citation: Mahameed, B. A. H, Al-Shajalee, S. A. A., & Khazaali, E. A. Liquid Biopsy and Circulating Biomarkers in Clinical Chemistry of Colorectal Cancer. American Journal of Biomedicine and Pharmacy 2026, 3(6), 53-65

Received: 10th Mar 2026
Revised: 21th Apr 2026
Accepted: 08th May 2026
Published: 11th June 2026

Abstract: Colorectal cancer (CRC) is one of the most prevalent types of cancer worldwide and early detection and prompt therapeutic interventions are crucial for survival. While effective, traditional diagnostic tools like colonoscopes and tissue biopsies are invasive and are not always practical to use for ongoing surveillance. Liquid biopsy has become a promising minimally invasive method allowing to real-time assess tumor dynamics by analysing circulating biomarkers in recent years. Of these, circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), exosomes and microRNAs (miRNAs) have great promise in clinical chemistry applications. The analysis of ctDNA can detect tumor-specific mutations, microsatellite instability and minimal residual disease, which enhances the accuracy of diagnosis and enables personalized therapy. CTCs offer information on tumor heterogeneity, metastatic potential, and resistance to treatment, whereas exosomes and miRNAs are important mediators of cell-cell communication and can be used as stable biomarkers of disease progression. Several benefits of the routine use of liquid biopsy in CRC cases are its ability to detect recurrence, predict prognosis, to monitor therapeutic response and to provide early diagnosis. Moreover, improvements of analytical methods (e.g., digital PCR, next-generation sequencing) have increased the sensitivity and specificity of the detection of biomarkers in liquid biopsy, making it a valuable tool for precision oncology. Although there are issues of standardization, cost, and validation in large populations, liquid biopsy is a game-changing paradigm in the clinical care of colorectal cancer. Future studies should yield better panels of biomarkers and enhance their clinical utility, and increase the utility of liquid biopsy for personalized cancer treatment

Keywords: Colorectal Cancer; Liquid Biopsy; Circulating Tumor DNA; Circulating Tumor Cells; Exosomes; Biomarkers

Introduction

Worldwide, colorectal cancer (CRC) is a major health problem and is often one of the most deadly cancers. The treatment methods that are used at this time include endoscopic and surgical removal (resection), chemotherapy, radiation therapy, targeted therapy, and immunotherapy [1]. Chemoresistance is a significant clinical problem for the management of CRC. Despite the advent of the immune checkpoint inhibitors, this has led to improvement in survival, but only for a small proportion of CRC patients, highlighting the need for a precise characterization of the molecular mechanisms underlying drug resistance [2]. Therapeutic resistance is the result of multiple interactions between intrinsic and acquired tumor features, microenvironment, and host factors [3].

Direct molecular characterization provides information that directly guides clinical treatment decisions. However, single biopsies will not accurately characterize the tumor and multi-tumoral analyses are required to aid the development of personalized therapeutic regimens [4]. Additionally, tissue biopsy is invasive, labor intensive and not repeatable. Such restrictions lead to the search for other molecular marker assays which allow accurate diagnosis and monitoring. Therefore, researchers have proposed the use of the term liquid biopsy to allow the analysis of tumor samples from a distance in a minimally invasive approach [5].

2. Overview of Colorectal Cancer

Colorectal cancer (CRC) ranks as the second in incidence and mortality worldwide. Although the therapeutic options, such as surgical resection, chemotherapy, radiation, targeted therapy, and immunotherapy, have improved over the past few decades, 3–5-year survival rates are still not satisfactory. One of the major ones is that people are resistant to existing treatments, and this leads to the recurrence of tumors. Tumour heterogeneity is a challenge, as it refers to molecular, cellular, gene expression and tumour microenvironment (TME) differences within the tumour as well as between the primary and metastatic tumours [6]. This is the reason why there is no simple therapy for all of these types of problems. Therefore, the identification of several reliable biomarkers and the development of sensitive, minimally invasive methods to efficiently detect these biomarkers is a critical need for the efficient characterization of CRC and monitoring of the disease.

In CRC, there is a typical adenoma-carcinoma sequence with the involvement of three molecular carcinogenesis pathways: chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP). There are three main pathways for CRC: the CIN pathway, which occurs in approximately 85% of cases, characterized by the loss of gene function, including APC, P53, KRAS, SMAD4 and DCC; the MSI pathway, which is related to faulty function of genes of the DNA mismatch repair system, such as MLH1 and MSH2, and occurs in approximately 15% of cases; the CIMP pathway, in which the promoter regions of genes are hypermethylated. EGFR/MAPK, Notch, PI3K, TGF-β, and NF-κB pathways are the most important signaling pathways involved in CRC, which are involved in regulating cellular proliferation, differentiation, angiogenesis, migration and survival. Together, these pathways, regulated by multiple oncogenic molecules, contribute to the CRC heterogeneity [7][8][9].

2.1. Epidemiology

Colorectal cancer (CRC) is the third most prevalent cancer in the world, and on the rise, especially in developing countries. It is a major cause of death in the world. Existing treatments for CRC involve surgery, radiotherapy, chemotherapy, targeted therapy and immunotherapy. Tumor recurrence is due to chemoresistance and tumor heterogeneity, which are important factors influencing patient survival and patient care. Early diagnosis of CRC is therefore an important part to improve survival [10].

Tissue biopsy is the established method widely used to identify the primary tumor site for cancer diagnosis. However, the technique suffers from poor patient compliance, restricted representation of the whole tumor bulk due to intra-tumor heterogeneity, and high procedural and economic costs associated with the process. Hence, routine biopsy is often not feasible and cannot reflect relevant changes in tumor heterogeneity at multiple time points during treatment. Liquid biopsy, an emerging approach for routine monitoring of cancer therapy, consists of the analysis of blood, saliva, stool, urine, cerebro-spinal fluid, and other biological fluids to collect information about tumor-related molecular markers such as circulating tumor cells, DNA, exosomes, non-coding RNAs,

and protein molecules [11]. Compared with tissue biopsy, liquid biopsy is better suited to overcome the issue of tumor heterogeneity. It allows repeated and real-time detection of the molecular landscape of the tumor. Liquid biopsy also guides the use of standard chemotherapy, targeted therapy, and immune checkpoint blockades and facilitates personalized therapies for metastatic CRC patients. In particular, circulating tumor DNA and circulating tumor cells have demonstrated prognostic value in early diagnosis, monitoring of recurrence, and guidance of therapies. Numerous ongoing clinical trials are investigating the future role and utility of liquid biopsy in the management and prognosis of CRC. A brief presentation of the epidemiology, key pathophysiological mechanisms, and therapeutic options of CRC is provided to establish the clinical context for the rest of this publication [12].

2.2. Pathophysiology

Colorectal cancer (CRC) arises from an adenomatous lesion in the colonic or rectal mucosa and develops into carcinoma in situ and metastatic colon carcinoma through progressive accumulation of both epigenetic and genetic alterations [13]. Early detection and therapeutics are pivotal since the treatment strategy and prognosis closely depend on the disease stage at diagnosis. The significance of screening modalities is manifested in increasing numbers of patients who detected tumors at early stages and survived tumor resection [14].

2.3. Current Treatment Modalities

Treatment available for colorectal cancer includes systemic chemotherapy, surgery, radiation therapy, endoscopy and targeted therapy, as well as immunotherapy [15]. The key factors contributing to tumour recurrence are chemoresistance and tumour heterogeneity. A much needed development is early prediction of therapeutic response. Although tissue biopsy remains the gold standard for tumor diagnosis, it has some shortcomings, including invasiveness, poor patient tolerability, and inability to capture intra- and inter-tumoral heterogeneity over time. Liquid biopsy comes to the rescue as a less invasive technology that bypasses these limitations [16]. It includes the collection of different types of biofluids, including blood, saliva, stool and urine, for the study of tumour markers like circulating tumour cells, circulating tumour DNA, proteins and exosomes. This can provide a better way to treat tumor heterogeneity, allow for repeated real-time monitoring, and help guide treatment planning. Continuing clinical trials investigate the value of liquid biopsy in colorectal cancer treatment, in prognosis and follow-up [17].

Materials and Methods

A comprehensive narrative review across the literature was undertaken to assess the clinical significance of liquid biopsy and/or circulating biomarkers in selecting patients for treatment, predicting disease-free survival (DFS) or recurrence-free survival (RFS), monitoring therapeutic response, and detecting recurrence of colorectal cancer (CRC). Data from relevant peer-reviewed articles, clinical trials, systematic reviews and scientific reports were searched for in PubMed, Scopus, Web of Science, Google Scholar and different reputable scientific repositories. A literature search was conducted for studies examining liquid biopsy technology and associated circulating tumor factors, with an emphasis on the most promising circulating biomarkers for colorectal cancer (such as circulating tumor DNA [ctDNA], circulating tumor cells [CTCs], cell-free DNA [cfDNA] exosomes, microvesicles, microRNAs and other extracellular vesicles/endosomal-derived molecular markers). We focused on ways to extract the data, biomarker characteristics, mechanisms of release, analytical detection methods and clinical applications, and sub-classifications in terms of possible interactions (i.e. diagnostic performance; prognostic value; treatment-response monitoring; independent monitoring for recurrence). Read More The report takes a closer look at emerging analytical technologies and provides detailed insights into advanced analytical approaches, with specific focus on digital polymerase chain reaction (dPCR), next-generation sequencing (NGS), immunoaffinity-based cell separation from blood cells and exosome characterization technologies. We systematically synthesized the common evidence to compare liquid biopsy with traditional tissue biopsy approaches in response to the invasiveness, tumor heterogeneity assessment, real-time monitoring ability and personalized treatment guidance. Moreover, we critically evaluate challenges pertaining to sensitivity, specificity, standardization, analytical reproducibility and clinical implementation. An overview draft was prepared with the aim

to identify emerging trends, technology developments and research gaps in the use of circulating biomarkers for precision oncology. This review illustrates the interdisciplinary insights into the evolving role of liquid biopsy in colorectal cancer management, and develops evidence-based recommendations for future research and clinical translation to enhance early detection, therapeutic decision-making and long-term patient outcomes.

Results and Discussion

3. Liquid Biopsy: Definition and Importance

Extracellular components which are released in body fluids are analyzed by liquid biopsy. It has become very important in cancer screening, patient stratification and monitoring of treatment [18]. The need for comprehensive molecular characterizations and the heterogenicity of colorectal cancer (CRC) highlights the significance of liquid-biopsy techniques for prognosis. The major benefits are: Identification of a genetic and epigenetic landscape, genomic evolution and acquired resistance tracking to aid early diagnosis, progression and therapeutic response evaluation. Compared with the traditional tissue biopsy, liquid biopsy has the advantages of fast detection, can be used for long-term or real-time monitoring of heterogeneity during the disease course, and it requires less technical skills and infrastructure [19].

Liquid biopsy is an upcoming diagnostic approach that circumvents the spatial and temporal limiting nature of tissue biopsy, and yields holistic information of tumor spatial and temporal heterogeneity and progression dynamics [20]. Biological fluids (blood, urine or cerebrospinal fluid) are collected for the isolation of tumor-related biomarkers such as circulating tumor cells (CTC), circulating nucleic acids and extracellular vesicles. The range of tumor components that can then be analyzed for DNA, RNA, or protein is therefore significantly increased. Liquid biopsy therefore facilitates the possibility to perform a comprehensive analysis of the plasma, obtaining somatic mutations that are shed from primary tumours and metastases, to perform detailed genomic and phenotypic characterisation. Tumours may release cells into the blood which can implant themselves in other parts of the body and lead to a recurrence. Specialized methodologies for capture and quantification are necessary as CTCs are present in low numbers, usually less than 1 per 107 of blood cells, and are found in only one to ten cells per 10 mL of blood in early cancer stages [21].

3.1. Liquid Biopsy

Liquid biopsy is a non-invasive technique that uses biological fluids such as blood and urine to identify biological markers related to tumors such as circulating tumor cells (circulating cell-free DNA or ctDNA). This technique can be used to overcome tumour heterogeneity and to monitor disease progression in real-time, to help guide treatment. Its function in colorectal cancer (CRC) therapy is being evaluated in clinical trials to determine how it can be used to aid diagnosis and prognosis. CRC is the third most common cancer and the second most deadly cancer worldwide, with more than 150,000 new cases expected in the U.S. this year. The majority of CRCs develop from adenomatous polyps and typically grow in an insidious manner over many years. The changes occur in oncogenes such as KRAS and MYC and tumour suppressor genes such as APC and TP53. Surgery has been the mainstay of treatment in the early stages, and systemic chemotherapy for advanced CRC can increase survival, while chemotherapy and targeted drugs such as anti-EGFR, anti-VEGF can be used for metastatic CRC [22].

3.2. Advantages Over Traditional Biopsies

Liquid biopsy is a minimally invasive technique with a promising future as an alternative to tissue biopsy, with the ability to provide important molecular information about tumor tissues [23]. Often, it is performed by taking a blood sample and allowing the tumour to release blood-borne tumour cells (circulating tumour cells or CTCs), tumour DNA, RNA or extracellular vesicles into the blood, which may be isolated and analysed. Compared to tissue biopsies, which can be more invasive and may not be repeatable, liquid biopsy has some benefits, such as short turnaround time, the ability to be repeated longitudinally and in real-time to monitor the genetic heterogeneity, less technical expertise and cost [24].

4. Circulating Biomarkers in Colorectal Cancer

Liquid biopsy has great potential as a novel clinical tool to efficiently manage colorectal cancer. Sensitive analytical methods can measure molecules from the blood and provide useful information on tumor characteristics and evolution. The development of technologies for isolating and analyzing cancer-derived materials has permitted the detection of somatic mutations, epigenetic alterations, and gene expression profiles as cancer biomarkers in clinical chemistry. Cancer cells actively secrete circulating tumor DNA (ctDNA), RNA (ctRNA), and proteins into the blood. The amount of ctDNA also increases with cell necrosis. Circulating tumor cells (CTCs) invade the bloodstream through angiogenesis, hemorrhage, and endothelial gaps. Additional circulating materials include tumor-educated platelets (TEPs). Circulating exosomes, shed from cancer cells or normal stromal cells, also influence tumor progression and responses to adverse conditions [25].

The release mechanisms underscore the value of liquid biopsy for uncovering clinical clues about colorectal cancers. Once in circulation, ctDNA undergoes clearance through nuclease degradation, renal excretion, and uptake by the liver and spleen. ctDNA contains tumor-specific DNA mutations, methylations, and structural features. Tissue biopsy not only provides DNA but also captures RNA expression and post-translational modifications obtained from the microenvironment. Hence, liquid biopsy can potentially yield comprehensive phenotypic and functional information [26].

4.1. Types of Circulating Biomarkers

Among circulating biomarkers, cell-free DNA (cfDNA) is the first liquid biopsy biomarker described in CRC patients. Elevated levels are often observed in metastatic cancer, however there is no correlation between cfDNA concentration and primary tumor characteristics. High concordance (≥80%) is observed at the molecular level between cfDNA and primary tumors, but timing of sample collection, clonal evolution and heterogeneity can influence correlation with specific mutations, so analyses should be conducted prospectively. Only a small fraction of cfDNA originates from tumor cells and has been named circulating tumor DNA (ctDNA). Thus, ctDNA can be considered a "real liquid biopsy" which is less invasive and more easily accessible than conventional biopsies [27].

Liquid biopsies can additionally give details of metastasis by determining and characterizing circulating cells, such as circulating tumour cells (CTCs), in body fluids. They are either from primary tumors or metastases or tumor recurrences and enter the bloodstream as single cells or as clusters of similar cells. This phenotype offers CTCs greater survival potential in comparison to single cells as it suppresses apoptosis and shields them from interactions with the immune system. CTCs are able to spread to secondary organs and be responsible for the metastatic progression and recurrence of disease. CTCs can undergo EMT developing greater plasticity and resistance to anoikis. The short half-life of the ctDNA (~1-2 hours) is an advantage as compared to other markers because it gives a real-time picture of the tumor burden. Their number is usually very low (1–10 CTCs/10ml of blood), unless they are present in metastatic disease, in which case they require special enrichment procedures [28].

4.2. Mechanisms of Biomarker Release

The release of circulating biomarkers into the bloodstream is crucial for translating tumor-specific alterations into the surrogate markers of liquid biopsy . These biomarkers—circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and extracellular vesicles (EVs)—originate from primary and distal tumor sites via various mechanisms. The predominant mode of ctDNA entry is passive release after cell death, whereas CTCs detach and enter blood circulation to seed new lesions through active migration. Viable cancer cells also secrete EVs, which, due to their cargo (DNA, mRNA, miRNA, proteins), mediate intercellular communication and contribute to tumor evolution, epithelial-to-mesenchymal transition, and metastasis. The bloodstream may also contain other circulating factors of tumor origin such as RNAs, long non-coding RNAs (lncRNAs), and proteins. The varying abundance of these components depends on the stage and location of the disease and the intrinsic properties of the primary tumor and its microenvironmental niche [29].

5. Techniques for Liquid Biopsy Analysis

Colorectal cancer is the second most common cause of death due to cancer in the world. The associated cytogenetic and epigenetic modifications frequently result in changes to circulatory biomarkers, thus facilitating diagnosis and progression monitoring. Liquid biopsy is a non-invasive diagnostic technique that collects liquid markers of primary tumor(s), metastases and circulating tumor cells. It is extremely useful for monitoring the progression of the tumor and the therapeutic response

and could be repeated during treatment and would allow to gain insights into tumor biology and the tumor heterogeneity [30].

Circulatory biomarkers enter circulation by various pathways, such as apoptotic, necrotic, and secretory pathways. During the process of apoptosis, short fragments of DNA (185-200 base pairs in length) are released into the circulation, bound to histones that are also rapidly cleared from circulation. Necrosis generates long (heterogeneous) fragments of DNA that stay longer in circulation. In the secretory route, exosomes or microparticles, and paradoxical release of mitochondrial DNA, are found mainly in the circulating cell-free DNA pool. Circulating tumor cells that carry DNA, messenger RNA, micro RNA and proteins from the tumor. In addition, the analyses of circulating cell-free DNA and messenger RNA, circulating tumour cells (CTC) and exosomes represent the mainstream applications of liquid biopsy, and have been established as a comprehensive framework for current research [31].

5.1. Cell-Free DNA (cfDNA) Analysis

Both the occurrence rate and death rate of colorectal cancer (CRC) one of the most common malignant tumors have been increasing continuously in recent years. Although the 5-year survival rate of patients with CRC has increased significantly, the high rate of early metastasis and recurrence seriously limits the improvement of the survival rate of CRC patients. The tissue biopsy-based pathology is the gold standard for cancer diagnosis and postoperative monitoring, but the invasive nature and sample-harvesting difficulties have limited its application [32]. Hence, liquid biopsy especially in blood—acquires comprehensive clinical data of patients to provide information on early detection, location, and treatment evaluation of the tumor, to quickly adapt therapeutic strategies and combat drug resistance. Circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and exosomal microRNAs (miRNAs) in blood are the main targets in a wide range of circulating complementary DNA (cfDNA) analysis, immunoaffinity of CTC isolation, and exosomes' surface and contents analysis, respectively [33]. cfDNA generally refers to extracellular DNA existing in body fluids and mainly arises from apoptosis, necrosis, and active secretion of cells with a short half-life in circulation. Excessive amounts of cfDNA are released into the peripheral blood and other body fluids in various pathological conditions such as inflammation, cancer, and autoimmune diseases. Moreover, the concentration of cfDNA in plasma varies from several nanograms per milliliter to over 1000 ng/ml a relatively broad range for patients with cancer or severe sepsis. cfDNA analysis is a simple, noninvasive, inexpensive, and reproducible method that represents a promising avenue [34].

5.2. Circulating Tumor Cells (CTCs) Isolation

The circulating tumour cells (CTCs) are a rare population of blood cells derived from solid tumors, and occur at a rate of around one per million white blood cells. They originate within the primary tumor, migrate through blood circulation and play a role in the metastatic process in colorectal cancer (CRC) [35]. They were originally termed 'CTCs' by Ashworth in the late 19th century to refer to tumor cells which travel through the bloodstream and form 'distant metastases'. CTCs have been found in blood circulation during all stages of cancer progression and the number of CTCs is directly proportional to the advancement of the disease. The isolation and detection of CTCs from the blood stream has therefore become a very important noninvasive approach for early diagnosis, therapeutic monitoring and prognosis estimation in CRC [36] [37].

CTCs have distinct biological features which set them apart from other blood cells, such as a larger diameter, higher elasticity, and specific biomarkers. Isolation of CTCs is difficult, however, because they are rare in advanced cancer (only 100 cells per 10mL of blood) and unstable in the circulatory microenvironment. Several approaches have been developed to efficiently and rapidly separate and enrich CTCs, often based on biophysical characteristics (size and deformability) or molecular characteristics (surface antigens or nucleic acid expression) of the CTCs.

Biophysical separation techniques take advantage of the differences in the physical characteristics of the CTs compared to the hematopoietic cells. The size-based filtration methods rely on the porous membranes having selectively different diameters, so as to be able to trap larger tumor cells, while microfluidic chips incorporate size-based separation with a designed channel. In addition to this, the lower specific gravity of CTCs as compared with white blood cells, and their different

dielectric properties, can be utilized in the effective isolation of CTCs using density-gradient centrifugation and dielectrophoresis, respectively [38]. Immunoaffinity methods rely on the use of antibodies that recognize specific surface markers that are greatly expressed on CTCs but not on normal haematopoietic cells, such as epithelial cell-adhesion molecule (EpCAM), cytokeratins (CK8, CK18, CK19), and E-cadherin. Immunomagnetic separation uses magnetic beads coated with these antibodies to isolate and enrich CTCs for high throughput and specificity [39].

5.3. Exosomes and Microvesicles

A specific type of extracellular vesicles, exosomes (which are between 40 and 160 nm in size and are shed from cells via the endocytosis pathway) are of particular importance to the liquid biopsy due to their role in tumor progression. They serve as vehicles in humoral communication and transport molecular cargoes like proteins, metabolites, nucleic acids and lipids from donor to recipient cells. In the oncogenic setting, exosomes promote drug resistance, premetastatic niche formation, and progression, and their levels are increased in a cancer-dependent manner [40]. Their dynamic nature allows exosomal patterns to hinge on both therapeutic intervention and tumor grade, thus holding promise to support cancer diagnosis and real-time patient monitoring [41].

Microvesicles, larger than exosomes with diameters of 100−1000 nm, originate through the budding of plasma membranes. Their contents overlap with those of exosomes; however, their diagnostic utilization is still under investigation [42].

6. Clinical Applications of Liquid Biopsy

Liquid biopsy represents a promising tool for early detection, treatment response monitoring, and recurrence detection in colorectal cancer. Circulating tumor DNA (ctDNA) analysis can distinguish colorectal cancer patients from healthy individuals and patients with benign gastrointestinal disorders with high specificity and sensitivity. Blood-based biomarkers reflected pathological stages across early stages and were associated with the risk of progression to metastatic colorectal cancer, suggesting that circulating biomarkers could assist in early detection [43].

Assessment of the mutational status of genes such as KRAS and BRAF may facilitate the choice of targeted therapy and the prediction of treatment outcome. Monitoring of circulating tumor cells (CTCs) and ctDNA reliably reflects treatment response, corroborating the potential of liquid biopsy approaches in the assessment of therapy efficacy. Detection of minimal residual disease by liquid biopsy represents a valuable tool for the early identification of patients at risk of disease progression [44].

The estimated median lead time between molecular recurrence and radiologic recurrence is several months to more than a year, indicating the possibility of stratifying patients for adjuvant therapy. Combining the post-surgical assessment of clinical variables with ctDNA status can identify patients with ultra-low risk of colorectal cancer recurrence, who might be spared toxic and unnecessary treatment. Liquid biopsy can monitor ctDNA longitudinally before treatment, at the end of treatment, and during follow-up for residual disease, recurrence, and progressive disease [45].

6.1. Early Detection and Screening

Colorectal cancer (CRC) is a widespread digestive tract malignancy that builds up over several years by changes in genes and other factors. It is the third most common cancer worldwide and the second biggest killer of cancer deaths. Survival rates are higher if CRC is detected early as stage I and II have a 5-year survival rate of 90% and screening programmes are effective at reducing CRC incidence and mortality [46].

Colonoscopy is the invasive gold standard screening test for CRC, but has adverse events and poor use adherence. Other options include sigmoidoscopy, computed tomography colonoscopy, and stool tests like guaiac-based fecal occult blood test, fecal immunochemical test and multitarget stool DNA test. These are widely used clinically, but are either invasive, or less sensitive than colonoscopy, and have encouraged research for less invasive, more sensitive modalities [47].

The analysis and characterization of circulating biomarkers released from CRC cells into body fluids (liquid biopsy) is a promising technology to diagnose and monitor CRC. It's a less invasive option that could overcome the current limitations. Liquid biopsy, through the detection of circulating tumor DNA, RNA and rare circulating tumor cells, could have a role in early detection, staging, prognosis, and detection of cancer-related mutations associated with treatment resistance [48].

6.2. Monitoring Treatment Response

Treatment response monitoring in CRC poses a major problem in oncology. Current practice is based on surveillance using serial radiological investigations, and a single blood marker, carcinoembryonic antigen (CEA), to monitor colorectal cancer after surgery; CEA is the only guideline-recommended blood marker. If the CEA test is positive, the next step is usually to have a computed tomography (CT) scan sooner, usually. Early detection of lesions that can be cured with surgery is a critical factor in decreasing mortality [49]. However, there are currently limited options available for the monitoring of systemic therapy responses. Radiological imaging can be used to measure tumour changes in size and CEA level is of limited value [50]. This is complicated further by common mutations that are found in genes within the RAS pathway that render patients resistant to the targeting treatments they receive (e.g. the monoclonal antibodies cetuximab and panitumumab) [51]. Therefore, mutation profiling of tumour tissue biopsies is essential, but can be complicated by sampling errors. The limitations have led to more interest in the measurement of cell-free circulating tumour DNA in serum or plasma, called a liquid biopsy, that allows for the assessment of tumour-specific DNA modifications without any interference from normal cell DNA [52].

6.3. Recurrence Detection

Circulating biomarkers can also be assayed to detect recurrence. About 50% of patients with newly diagnosed colorectal cancer at stages I-III develop recurrence after surgery, and clinical or radiographic detection usually occurs months after relapse. Plasma can be separated from a blood draw to evaluate circulating tumor-derived DNA (ctDNA), which reflects minimal residual disease (MRD) after surgery. Patients with detectable ctDNA after treatment show higher rates of recurrence and a worse prognosis, yet there is not yet conclusive evidence that modifying therapy improves outcomes to support clinical practice. ctDNA is not able to replace imaging for detection of recurrence or serum markers for monitoring because of a lack of convincing demonstration of clinical utility with simultaneous or consecutive testing [53].

The sensitivity of ctDNA testing depends on identifying cutaneous or epigenetic alterations. Current platforms are tumour-informed or tumour-agnostic. Epigenetic alterations, especially DNA methylation, have key roles in early cancer development and metastasis in colorectal cancer. Plasma Assay of Cell-Free Methylated DNA Markers of Colorectal Cancer provides an innovative liquid biopsy assay to monitor colorectal cancer recurrence and response to therapy. The methylated DNA marker (MDM) panel was validated for colorectal cancer detection in independent case–control studies and was assessed for prognostication in three independent, prospective population-based studies of stage I to III colorectal cancer [54]. A nested longitudinal case–control study of stage III and IV colorectal cancer patients was designed to monitor radiographic disease status during chemotherapy at three time points. Furthermore, 333 plasma samples were assayed from 111 non-cancer controls, 173 patients with stage I–III colorectal cancer taken before surgery, and 61 patients with potentially operable stage IV colorectal cancer. MDMs can detect recurrence prior to radiographic verification, assisting cancer surveillance and monitoring [55].

7. Challenges in Liquid Biopsy Implementation

Despite the promising clinical applications of liquid biopsy in colorectal cancer (CRC), significant challenges hinder its routine implementation. Sensitivity and specificity remain critical issues, especially in early-stage disease where circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA) are present at very low concentrations and often below the detection threshold of commonly used tools. CTCs, due to their size, physical properties and heterogeneous surface protein expression, are difficult to enrich selectively [56]. Immuno-based antigen-dependent technologies rely on markers such as epithelial cell adhesion molecule, which are downregulated during epithelial-to-mesenchymal transition indicating potential metastasis while antigen-independent enrichments lack standardization. Exosomal isolation through ultracentrifugation is hindered by co-purification of contaminants, and immune methods targeting surface markers involve non-exclusive expression on other extracellular vesicles such as microvesicles and apoptotic bodies [57]. ctDNA represents only a small tumour-derived fraction of cell-free DNA (cfDNA); targeted approaches (PCR-based) and untargeted sequencing (next-generation sequencing) struggle with low mutation prevalence, high background of non-tumour cfDNA, and reproducibility issues. Unlike mutations following tumour-

specific clonal evolution, DNA methylation sites can be less heterogenous and might provide higher detection sensitivity, but a high degree of standardization is required. Analysis of circulating RNA (cRNA) suffers from substantial pre-analytical variability, with factors such as red blood cell haemolysis and contamination by residual platelets or microparticles impacting circulating microRNA levels and thus compromising quantitative results interpretation. Although liquid biopsy is increasingly considered an excellent adjunct in CRC management, enabling broader tumour characterization and monitoring of treatment response, tumour evolution and minimal residual disease, comprehensive validation of techniques and clinical protocols is still required before widespread adoption [58].

Conclusion

Colorectal cancer (CRC) is a major cause of cancer mortality, and is a multi-step disease with genetic and epigenetic changes. Although there has been a significant increase in knowledge and treatment options, early detection is still difficult and screening is inadequate. The development of lesions may be through an adenoma-carcinoma pathway or the serrated pathway, and metastasis requires additional modification in the clonal and sub-clonal evolution. Circulating tumour DNA (ctDNA) is proving to be the most promising method of diagnosis and monitoring in CRC. The revolution of liquid biopsy in clinical chemistry. It also includes the analysis of tumour cells or molecules from the tumour in the blood, or in other body fluids, thereby enabling non-invasive genomic profiling of a tumour. The abundance of biomarkers released in the system comes from various cell types of the tumour and tumour microenvironment. Circulating tumour cells (CTCs), cell-free DNA (cfDNA) and exosomes are examples of biomarkers. Multiple solutions are approved by national agencies and the FDA, and new tests are becoming available constantly that yield tumour heterogeneity data which aid in more timely and individualised therapeutic decision making.

Funding

There is no funding

Declaration of Competing Interest

The authors say they don't have any known personal or financial relationships or financial interests that could have seemed to affect the work in this study..

REFERENCES

[1] S. Mirza, K. Bhadresha, M. Jameel Mughal, M. McCabe et al., "Liquid biopsy approaches and immunotherapy in colorectal cancer for precision medicine: Are we there yet?," 2023.

[2] H. Zhou, L. Zhu, J. Song, G. Wang et al., "Liquid biopsy at the frontier of detection, prognosis and progression monitoring in colorectal cancer," 2022.

[3] A. Raza, A. Q. Khan, V. Philipose Inchakalody, S. Mestiri et al., "Dynamic liquid biopsy components as predictive and prognostic biomarkers in colorectal cancer," 2022.

[4] S. N. Lone, S. Nisar, T. Masoodi, M. Singh, A. Rizwan, et al., "Liquid biopsy: a step closer to transform diagnosis, prognosis and future of cancer treatments," Molecular Cancer, vol. 21, no. 1, 2022.

[5] N. Honoré, R. Galot, C. van Marcke, N. Limaye, et al., "Liquid biopsy to detect minimal residual disease: methodology and impact," Cancers, vol. 13, no. 21, 2021.

[6] M. Nikanjam, S. Kato, and R. Kurzrock, "Liquid biopsy: current technology and clinical applications," Journal of hematology & oncology, 2022.

[7] Lateef, Diyar, Nesreen Nasser, and Osama Mohsein. "The relationships between Aplein, Vaspin and thyroid hormone levels in obese diabetic and non-diabetic women." Journal of Experimental and Clinical Medicine 41.2 (2024): 239-245.

[8] E. Kazemi, F. Zayeri, A. Baghestani, "Trends of colorectal cancer incidence, prevalence and mortality in worldwide from 1990 to 2017," Iranian Journal of ..., 2023.

[9] Y. Jiang, H. Yuan, Z. Li, X. Ji, Q. Shen, and J. Tuo, "Global pattern and trends of colorectal cancer survival: a systematic review of population-based registration data," *Cancer Biology & Medicine*, vol. 19, no. 1, pp. 1-12, 2022.

[10] Nasser, N. A., et al. "The levels of prostate specific antigen and leptin hormone in patients with prostate cancer." Asian J. Life Sci 13 (2025): 1-7.

[11] Y. Xi and P. Xu, "Global colorectal cancer burden in 2020 and projections to 2040," Translational oncology, 2021.

[12] F. Baidoun, K. Elshiwy, Y. Elkeraie, "Colorectal cancer epidemiology: recent trends and impact on outcomes," *Current Drug*, 2021.

[13] S. M. Alzahrani and H. A. Al Doghaither, "General insight into cancer: An overview of colorectal cancer," Molecular and Clinical Oncology, vol. 2021.

[14] B. A. Sullivan and M. Noujaim, "Cause, epidemiology, and histology of polyps and pathways to colorectal cancer," Gastrointestinal Endoscopy, 2022.

[15] S. W. Fu, C. Tang, X. Tan, and S. Srivastava, "Liquid biopsy for early cancer detection: technological revolutions and clinical dilemma," Expert Review of Molecular Diagnostics, vol. 24, no. 1, pp. 1-10, 2024.

[16] Adnan, Azher, Zainab Mohammed Farhan, and Osama A. Mohsein. "Understanding the link between diabetes mellitus and fatty liver disease: A narrative review." Int J Chem Stud 12.4 (2024): 40-48.

[17] O. H. Abusara, A. S. A. A. Agha, and S. K. Bardaweel, "Advancements and innovations in liquid biopsy through microfluidic technology for cancer diagnosis," Analyst, 2025.

[18] D. Galoș, A. Gorzo, O. Balacescu, and D. Sur, "Clinical Applications of Liquid Biopsy in Colorectal Cancer Screening: Current Challenges and Future Perspectives," 2022.

[19] D. Galoș, A. Gorzo, O. Balacescu, and D. Sur, "Clinical applications of liquid biopsy in colorectal cancer screening: current challenges and future perspectives," Cells, 2022.

[20] H. Zhou, L. Zhu, J. Song, G. Wang, P. Li, W. Li, and P. Luo, "Liquid biopsy at the frontier of detection, prognosis and progression monitoring in colorectal cancer," Molecular Cancer, vol. 21, no. 1, 2022.

[21] Mustafa, Hamssa Majid. "Assessment of Immune and Oxidative Stress Biomarkers in Toxoplasmosis Patients: A Comparative Study with Healthy Controls: Penilaian Biomarker Kekebalan Tubuh dan Stres Oksidatif pada Pasien Toksoplasmosis: Sebuah Studi Perbandingan dengan Kontrol yang Sehat." Indonesian Journal on Health Science and Medicine 1.3 (2024): 10-21070.

[22] M. S. Hossain, H. Karuniawati, A. A. Jairoun, Z. Urbi, and D. J. Ooi, "Colorectal cancer: a review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies," Cancers, vol. 14, no. 12, 2022.

[23] X. Y. Tao, Q. Q. Li, and Y. Zeng, "Clinical application of liquid biopsy in colorectal cancer: detection, prediction, and treatment monitoring," Molecular cancer, 2024.

[24] A. Raza, A. Q. Khan, V. P. Inchakalody, S. Mestiri, "Dynamic liquid biopsy components as predictive and prognostic biomarkers in colorectal cancer," *Clinical Cancer Research*, vol. 2022, Springer.

[25] M. Nakamura, S. I. Kageyama, M. Seki, A. Suzuki, et al., "Liquid biopsy cell-free DNA biomarkers in patients with oligometastatic colorectal cancer treated by ablative radiotherapy," *Anticancer Research*, vol. 41, no. 7, pp. 3453-3461, 2021.

[26] I. Heidrich, T. S. A. Abdalla, M. Reeh, and K. Pantel, "Clinical applications of circulating tumor cells and circulating tumor DNA as a liquid biopsy marker in colorectal cancer," Cancers, 2021.

[27] M. Hu, Z. Wang, Z. Wu, P. Ding et al., "Circulating tumor cells in colorectal cancer in the era of precision medicine," 2022.

[28] G. Gattuso, S. Crimi, A. Lavoro, R. Rizzo, G. Musumarra, "Liquid biopsy and circulating biomarkers for the diagnosis of precancerous and cancerous oral lesions," Non-coding RNA, vol. 2022.

[29] Abdulhussien, Adeeb Abdulally, Zainab M. Farhan, and Osama A. Mohsein. "The Clinico-Pathological Role of Horizontal Gene Transfer in the Ability of Pseudomonas Aeruginosa to Cause Disease in Lung Tissue." European Journal of Medical and Health Research 3.1 (2025): 27-34.

[30] S. Alsakarneh, F. Jaber, A. Beran, M. Aldiabat, Y. Abboud, et al., "The national burden of colorectal cancer in the United States from 1990 to 2019," Cancers, 2024.

[31] A. F. Awedew, Z. Asefa, and W. B. Belay, "Burden and trend of colorectal cancer in 54 countries of Africa 2010–2019: a systematic examination for Global Burden of Disease," BMC gastroenterology, 2022.

[32] V. Vymetalkova, K. Cervena, L. Bartu, and P. Vodicka, "Circulating Cell-Free DNA and Colorectal Cancer: A Systematic Review," 2018.

[33] S. Connal, J. M. Cameron, A. Sala, P. M. Brennan, "Liquid biopsies: the future of cancer early detection," Journal of Translational Medicine, vol. 21, no. 1, 2023.

[34] S. M. Batool, A. Yekula, P. Khanna, T. Hsia, et al., "The Liquid Biopsy Consortium: Challenges and opportunities for early cancer detection and monitoring," Cell Reports, vol. 2023.

[35] Ghazi, Mustafa H., and Osama A. Mohsein. "Metabolic biomarkers in cancer: Linking metabolism to tumor progression and therapeutic targets." Central Asian Journal of Medical and Natural Science 6.1 (2025): 364-375.

[36] A. Yadav, A. Kumar, and M. Haris Siddiqui, "Detection of circulating tumour cells in colorectal cancer: Emerging techniques and clinical implications," 2021.

[37] D. Lin, L. Shen, M. Luo, K. Zhang, J. Li, and Q. Yang, "Circulating tumor cells: biology and clinical significance," Signal Transduction and Targeted Therapy, vol. 6, no. 1, 2021.

[38] Mohammad, Sarmad Qassim, Abdullah Khazal Mohsen, and Idriss Saleh Jalil. "Assessing the prevalence of bacterial vaginosis among infertile women in Thi-Qar Province, Iraq." (2024): 287-296.

[39] Y. P. Yang, T. M. Giret, and R. J. Cote, "Circulating tumor cells from enumeration to analysis: Current challenges and future opportunities," Cancers, 2021.

[40] Z. Deng, S. Wu, Y. Wang, and D. Shi, "Circulating tumor cell isolation for cancer diagnosis and prognosis," EBioMedicine, 2022.

[41] D. Lucchetti, I. Valeria Zurlo, F. Colella, C. Ricciardi-Tenore et al., "Mutational status of plasma exosomal KRAS predicts outcome in patients with metastatic colorectal cancer," 2021.

[42] S. Li, M. Yi, B. Dong, X. Tan, and S. Luo, "The role of exosomes in liquid biopsy for cancer diagnosis and prognosis prediction," Journal of Cancer, vol. 2021, Wiley Online Library.

[43] W. Yu, J. Hurley, D. Roberts, S. K. Chakrabortty, "Exosome-based liquid biopsies in cancer: opportunities and challenges," Annals of, vol. 2021, Elsevier.

[44] Hassan, Zina Lafta, et al. "Metabolic Syndrome and Its Biochemical Implications: Insights into Early Detection." Central Asian Journal of Medical and Natural Science 6.3 (2025): 1101-1126.

[45] O. Mazouji, A. Ouhajjou, R. Incitti, et al., "Updates on clinical use of liquid biopsy in colorectal cancer screening, diagnosis, follow-up, and treatment guidance," Frontiers in Cell and, vol. 2021.

[46] P. Ziranu, A. Pretta, G. Saba, D. Spanu, C. Donisi, "Navigating the Landscape of Liquid Biopsy in Colorectal Cancer: Current Insights and Future Directions," *International Journal , 2025.

[47] O. Ginghina, A. Hudita, M. Zamfir, A. Spanu, "Liquid biopsy and artificial intelligence as tools to detect signatures of colorectal malignancies: a modern approach in patient's stratification," Frontiers in, 2022.

[48] M. Reece, H. Saluja, P. Hollington, C. S. Karapetis et al., "The Use of Circulating Tumor DNA to Monitor and Predict Response to Treatment in Colorectal Cancer," 2019.

[49] L. Ma, W. Li, N. Liu, Z. Ding et al., "Prothrombin time (PT) and CEA as prognostic predictive biomarkers for postoperative recurrence after curative resection in patients with stage I–III colorectal cancer: ," Updates in surgery, 2022.

[50] R. Wang, Q. Wang, and P. Li, "Significance of carcinoembryonic antigen detection in the early diagnosis of colorectal cancer: A systematic review and meta-analysis," World Journal of Gastrointestinal, vol. 2023.

[51] S. Harlid, M. J. Gunter, and B. Van Guelpen, "Risk-predictive and diagnostic biomarkers for colorectal cancer; a systematic review of studies using pre-diagnostic blood samples collected in prospective …," Cancers, 2021.

[52] J. V. Machado Carvalho, V. Dutoit, C. Corrò, and T. Koessler, "Promises and challenges of predictive blood biomarkers for locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy," Cells, 2023.

[53] M. Zhu, W. R. Taylor, D. W. Mahoney, S. S. Then et al., "Plasma Assay of Cell-Free Methylated DNA Markers of Colorectal Cancer: A Tumor-Agnostic Approach to Monitor Recurrence and Response to Anticancer Therapies," 2023.

[54] A. Grancher, L. Beaussire, S. Manfredi, et al., "Postoperative circulating tumor DNA detection is associated with the risk of recurrence in patients resected for a stage II colorectal cancer," Frontiers , 2022.

[55] J. Nors, K. A. Gotschalck, R. Erichsen, "Incidence of late recurrence and second primary cancers 5–10 years after non-metastatic colorectal cancer," Journal of Cancer, 2024.

[56] S. Prabhakaran, J. C. Kong, M. Chin, P. Carne, "Predictive factors for distant recurrence of colorectal cancer in patients after curative resection for stage I–III colorectal cancer in Australia," Archives of Surgery, vol. 2021, pp. 1-10, Springer.

[57] G. Mauri, P. P. Vitiello, A. Sogari, G. Crisafulli, "Liquid biopsies to monitor and direct cancer treatment in colorectal cancer," *Journal of Cancer*, vol. 2022.

[58] U. Ladabaum, A. Mannalithara, Y. Weng, R. E. Schoen, "Comparative effectiveness and cost-effectiveness of colorectal cancer screening with blood-based biomarkers (liquid biopsy) vs fecal tests or colonoscopy," Gastroenterology, vol. 2024, Elsevier.