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The Value of Circulating Tumor DNA in Biliary Tract Cancer: Enhancing Cancer Management and Predicting Patient Outcomes

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Abstract

Circulating Tumor Cells (CTCs) refer to malignant cells that have separated from the main tumor and disseminated throughout the circulation. Nowadays, scientists are devising instruments and methodologies to capture and detect these malignant cells more precisely and delicately from the blood of individuals with cancer, through the implementation of both physical and immunoaffinity-based methodologies, as well as positive and negative enrichment approaches all through separation. Biliary Tract Cancer (BTC) is a malignant tumor that displays a high degree of ferocity and carries an unfavourable outcome. Despite the challenges posed by BTC, there have been significant advances in identifying genetic mutations that can be targeted in affected patients. These breakthroughs have led to the creation of novel targeted therapies, with promising results in recent studies. Utilizing liquid biopsy, a non-intrusive technique for detecting tumor biomarkers from samples, can provide valuable assistance in diagnosing and molecularly characterizing the tumor. The utilization of ctDNA analysis has the capability of providing timely identification of oncogenic mutations, timely detection, treatment surveillance, and identification of treatment resistance pathways in cancer management. This article presents a comprehensive review of the existing literature on the use of ctDNA in patients with BTC, with an emphasis on the latest innovative methodologies and future prospects for managing this extremely malignant disorder.

Keywords: Cholangiocarcinoma; Circulating tumor cells; Liquid biopsy; Occult metastases; Biomarker; Predictive biomarker

Introduction

Biliary Tract Cancer (BTCs) encompasses a wide range of malignant neoplasm's that are typically classified according to their anatomic location, including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer, and cancer of the ampulla of Vater.

While this classification may appear to be oversimplified, it effectively captures the diversity of BTC subtypes with regards to their clinical presentation, molecular features, etiology, epidemiology, and treatment strategies. Biliary tract cancers represent a small proportion of gastrointestinal malignancies, accounting for around 3% of all such cancers. Among primary liver cancers, BTCs are the second most frequent type after Hepatocellular Carcinoma (HCC) [1]. Even though BTC is infrequent in Western nations, its frequency is growing, especially in Asian regions. There has been a past record of geographical diversity in the epidemiology of BTC. Although surgery continues to be the main therapy for initial phases of BTC, a majority of patients receive a diagnosis of progressed illness, rendering surgical management infeasible. The standard primary treatment for progressed, non-operable BTC is a chemotherapy regimen consisting of cisplatin and gemcitabine. The advantage in survival offered by initial chemotherapy is limited, as nearly all patients undergo disease advancement subsequent to treatment. Despite significant strides in genomic sequencing, patients with BTC continue to exhibit a bleak prognosis, with a brief life expectancy [2]. Liquid biopsy has garnered mounting interest as a potential instrument for the diagnosis and treatment of cancer in recent times. Liquid biopsy utilizes circulating free DNA (cfDNA), Circulating Tumor Cells (CTCs), circulating cell-free RNA (ccfRNA), and circulating tumor DNA (ctDNA) to extract cancer-specific genetic and epigenetic characteristics, which can be identified through a direct analysis of the blood [3]. Circulating tumor DNA (ctDNA) is a type of DNA portion that is specifically derived from the tumor and discharged into the bloodstream. While the bulk of circulating free DNA (cfDNA) is generated by healthy cells, circulating tumor DNA (ctDNA) is produced by primary tumors, metastatic locations, or Circulating Tumor Cells (CTCs).

The capacity to identify circulating tumor-specific material in bodily fluids can hold noteworthy implications for the management of cancer, such as early detection, relapse monitoring, determining the targets for treatment, evaluating the effectiveness of treatment, and monitoring the emergence of resistance. The significance of CTCs as a prognostic factor has been established for various neoplasms, such as breast, colorectal, pancreatic, and small cell lung cancer. Additionally, in patients undergoing palliative treatment, CTCs have demonstrated prognostic relevance for overall survival and recurrence risk following tumor resection in HCC. There is limited knowledge regarding the capacity of CTC quantification to detect hidden metastases before surgery with curative intent in BTC.

The purpose of this review is to examine the current body of literature concerning the possibility of practical applications of ctDNA in the management of BTC, emphasizing the current status and potential future prospects with a particular emphasis on the current cutting-edge technology and feasible future prospects [4,5].

Current Constraints in BTC Diagnosis: Blood-based Biomarkers, Imaging Techniques, and Histopathology

Even with various diagnostic modalities at hand, the identification of BTC continues to be a formidable challenge. CA19-9 and Carcinoembryonic Antigen (CEA) are markers of tumors that are detectable in blood that are frequently used in clinical settings. According to the ESMO guidelines, CA19-9 is the sole biomarker that is suggested for clinical use [6]. Nonetheless, the sensitivity of CA19-9 is a matter of debate owing to its elevated levels being detected in different malignancies and liver injuries. Ultrasound and cross section imaging are central imaging techniques for diagnosis and staging, with MRI being the preferred modality due to its capability to identify the vascular, biliary, and parenchyma spread of the tumor with high contrast resolution [7]. The primary imaging modalities for the diagnosis and staging a disease are ultrasound and cross-sectional imaging. However, Magnetic Resonance Imaging (MRI) is the most preferred modality, this is because MRI has the ability to produce high-quality images with excellent

contrast resolution, and can accurately assess the extent of the neoplastic growth in terms of its impact on the biliary, parenchymal, and vascular structures. It is crucial to obtain a pathological confirmation of the diagnosis before commencing any treatment. Verifying a diagnosis of BTC can be difficult, especially for patients with primary sclerosing cholangitis and biliary strictures. The collection of biopsy samples often falls short of the necessary amount of material for conducting genomic analysis, and obtaining tissue samples has a low sensitivity in detecting malignant biliary strictures, which can pose challenges in confirming a diagnosis of BTC [8]. Moreover, the fibrous tissue response seen in BTC makes conventional cytological and pathological methods less precise. Due to the drawbacks of the current diagnostic approaches, it is crucial to devise novel tactics that can detect BTC at an initial phase, which can be surgically removed and can furnish sufficient material for genomic analysis.

Molecular Characterization of Biliary Tract Tumors

Progress in the field of Genetic sequencing and molecular typing has brought about a significant transformation in the way Biliary Tract Cancer (BTC) is treated and managed [9]. Extensive research on the molecular characteristics of BTC has allowed for the identification of biological marker with predictive and prognostic value, as well as mechanisms underlying resistance and pathogenesis. Approximately half of BTCs is observed to harbor at least one oncogenic mutation. Novel studies have demonstrated the potential of targeted therapies in treating BTC [10]. The genetic abnormalities in various BTC subtypes have been illuminated various clinical trials. Extrahepatic cholangiocarcinoma showed KRAS as the most frequent genetic abnormality, whereas GBC had a higher prevalence of ERBB2, and intrahepatic cholangiocarcinoma had IDH1 and FGFR alterations detected. Significantly, FGFR genetic alterations were correlated with a more favourable prognosis in cases of intrahepatic cholangiocarcinoma. The combination of mutations, copy-number alterations, genetic transcription, and Epigenetic modification data was used to multimodal clustering analysis, resulting in the identification of four unique genetic cluster of BTC [11]. These groupings exhibited diverse genetic and epigenetic characteristics and were correlated with distinct prognoses. The use of targeted therapies based on genetic anomalies in clinical research has produced encouraging outcomes. The MOSCATO-1 study is a case in point, where the genetic profiles of 1,035 tumor samples were examined, and 199 patients were assigned to particular targeted therapies based on their genetic makeup [12].

Out of the group of 18 advanced BTC patients who had received previous treatment, 33% showed a favourable response to targeted therapies, while the progression-free survival and overall survival rates stood at 5.2 months and 17 months, respectively. Earlier studies have combined BTC patients with varying anatomical and molecular subtypes, which has resulted in clinical trials that overlook the considerable heterogeneity of BTC [13]. As a result, the survival benefit seen with existing treatments is moderate, underscoring the requirement for novel and efficacious agents, as well as personalized clinical studies, which take into account the genetic profile and histological features of BTC [14].

A non-invasive and secure option for obtaining meaningful knowledge on BTC is provided by liquid biopsy. This approach includ

es identifying tumor biomarkers present in bodily fluids such as blood, urine, plasma, and bile [15]. Liquid biopsy enables a timely and comprehensive assessment of the tumor in an individual patient, serving various purposes such as early detection, detection of small amounts of residual disease, tracking treatment response, tracking genetic changes in the tumor, examining tumor diversity, recognizing potential molecularly targeted treatments, and identifying new mechanisms of chemotherapy resistance. Using liquid biopsy on plasma samples has numerous benefits, such as being minimally invasive, posing a low risk of complications, and a simple and easily attainable

approach. Nevertheless, the broad utilization of this method is restricted due to the high expenses associated with the analysis [16]. The present article investigates recent findings regarding the application of liquid biopsy in patients with BTC. It also examines current and potential future uses of this method, with particular attention given to the use of peripheral blood and bile, which are distinctive features of this disease [17].

Comparing ctDNA Assay to Tissue-based Assay for Cancer Diagnosis and Management

Tumor biopsies are recognized as the most dependable technique for Uncovering cancerous cells and an essential tool for conducting genetic testing, which assists in identifying suitable treatments [18]. However, procuring tissue samples can present difficulties, and traditional tissue biopsies may not always be feasible due to their invasive nature. Additionally, conducting the process repeatedly and acquiring an adequate amount of satisfactory material for genomic testing can be challenging [19]. On the other hand, examining ctDNA has the ability to overcome these constraints by more effectively detecting differences in tumor location and progression over time, creating possibilities for active surveillance [20]. The minimally invasive nature of liquid biopsy renders it a swiftly evolving molecular diagnostic method that exhibits tremendous potential [21]. In contrast to conventional tissue biopsies, analysing ctDNA is a fast and uncomplicated process with Low probability of procedural complications. Due to the fact that it is easier to obtain bodily fluids such as blood, saliva, and urine, as opposed to tissue biopsy [22,23].

In general, liquid biopsy is a suitable ally for personalized oncology, allowing for a more comprehensive understanding of the tumor and the detection of tumor location and progression over time (80-82). Nonetheless, the analysis of cfDNA/ctDNA does have some drawbacks, including the absence of tumor site specificity for crucial anatomical and clinical lesions, low release of ctDNA by some types of cancers, and the absence of future confirmation for most cancer types in clinical practice [24]. Furthermore, the existing ctDNA tests have limitations in identifying some genes in relation to tissue-based testing, which is an important problem that advanced technologies are attempting to tackle [25].

Exploring the Potential of Liquid Biopsy (Clinical Implications of ctDNA/cfDNA Analysis in Personalized Oncology)

Cell free DNA was identified in blood in 1948 and many years later, increased levels were seen in cancer patients compared to healthy ones [26]. Subsequent research has focused on examining cfDNA, ctDNA, and CTCs as new indicators for the early diagnosis and better prognosis of cancer patients. The reason for the presence of cfDNA in the bloodstream is due to apoptosis and necrosis. Low concentrations of cfDNA in plasma are typically observed under normal physiological conditions [27]. CtDNA is a fraction of cfDNA that is released exclusively by tumor cells. The uses of cfDNA/ctDNA include but are not limited to cancer diagnosis, detecting the extent of tumor growth, predicting the likely course of the disease, selecting the appropriate treatment, and monitoring for the effectiveness of the treatment or the recurrence of the disease. Detecting cancer in its early stages is difficult, and although cfDNA analysis is a promising method, it demands a highly sensitive approach to identify minute levels of cfDNA in the blood [28]. Liquid biopsy can identify new genetic changes that cause drug resistance and sequential liquid biopsies can help identify these changes and adjust treatment in real-time. This can eliminate the need for invasive tumor biopsies. In addition, liquid biopsy can recognize biomarkers that can predict the course of the disease, find remaining disease after surgery, monitor the response to treatment, and uncover the re-emergence of the tumor [29]. To make informed clinical decisions, it is important to have a comprehensive understanding of the benefits and limitations of liquid biopsy as an approach to cancer diagnosis and treatment.

Advancements in the Detection and Monitoring of Biliary Tract Cancer using ctDNA

The technological issues caused by reduced amount of ctDNA in patients with early-stage BTC have limited the potential of liquid biopsy and ctDNA for confirming the diagnosis and performing genetic analysis. This poses an obstacle to validate the diagnosis [30]. In cases of BTC, such as eCCA and GBC, where biopsy samples are usually insufficient for genetic analysis, ctDNA could be of significant value. Multiple research works have shown that there is a strong agreement between mutations identified in tumor biopsies and cfDNA. The use of liquid biopsy is gaining traction for genomic profiling of BTC due to the scanty tumorous cellular material in tissue samples that restricts sequencing. ctDNA sequencing can be utilized to keep track of the development of resistance and also track the effectiveness of chemotherapy and targeted therapy [31,32]. The occurrence of FGFR2 genomic mutations is common in iCCA, with a prevalence of 13% to 45% and incompatible. It is currently not known how sensitive cfDNA/ctDNA mutations are for detecting early-stage BTC.

Circulating Tumor Cells (CTCs) have been identified as markers for the potential for detecting, predicting outcomes, and monitoring treatment in various solid tumors. CTCs could be useful for early detection of cancer, and their number has been correlated with tumor stage and outcome. CTCs can also be utilized to track the effectiveness of cancer treatments [33]. The usefulness of CTCs as a proxy biomarker for cancer diagnosis, treatment assessment, and prognostication prediction is restricted due to the limited availability and isolation of CTCs. The use of imaging studies to assess treatment response can result in alterations in CTC counts due to radiation exposure. Although it is now feasible to genotype CTCs due to the latest advancements in tools for assessing CTCs, there is still a requirement for established recommendations on the clinical application of CTCs [34-37].

In a study involving 121 patients with cholangiocarcinoma a study conducted by Zill OA, et al. [35] examined 26 cases of pancreaticobiliary malignancies and noticed a substantial consistency between the mutations identified in tumor biopsies and cfDNA. The research included 18 patients with pancreatic cancer and 8 with BTC, and cfDNA detected 90.3% of the mutations detected in tissue biopsies. In a study involving patients with perihilar cholangiocarcinoma (pCCA) and a control group of 95 healthy individuals, a set of four genes (HOXA1, PRKCB, CYP26C1, and PTGDR) that exhibit Distinct Methylated Regions (DMRs) in patients with CCA was identified through the analysis of cfDNA. This set of four genes with Differentially Methylated Regions (DMRs) identified through cfDNA analysis had a high specificity of 93% and sensitivity of 83% for detecting cholangiocarcinoma. It is worth noting that the ctDNA panel with Differentially Methylated Regions (DMRs) was able to identify 80% of eligible surgery or transplantation cases of Cholangiocarcinoma (CCA), and 60% of non-eligible cases, according to a study. Currently, it is not known how effective cfDNA/ctDNA mutations are in detecting early-stage BTC. Mody and colleagues [38] examined 138 samples obtained from patients with Biliary Tract Cancer (BTC) using ctDNA analysis, and observed that a minimum of one genomic alteration was present in 89% of the cases. Nonetheless, the primary focus of this investigation was on iCCAs, which can be more readily sampled through liver biopsies, highlighting the principal constraint of this study. Though the most often identified genomic alterations were TP53, KRAS, and FGFR2, it is imperative to conduct more extensive studies to validate the agreement between ctDNA and molecular alterations in tissue [38-40]. It is worth noting that the exploration of genome analysis in BTC has now extended to the analysis of bile as a constituent of liquid biopsy. Shen and colleagues [41] examined the bile of 10 patients with BTC, which included four cases of GBC. The findings of their study indicated that bile cfDNA may consist of long stretches and show robust consistency in genomic traits with tissue analysis. Nevertheless, it is imperative to conduct more extensive cohort studies to

corroborate these results and assess the feasibility of using bile for cfDNA analysis.

The Potential of ctDNA/cfDNA Analysis in BTC: Towards a New Era in Cancer Management

The utilization of ctDNA/cfDNA for the identification, profiling, and genetic investigation of tumors holds the promise of transforming cancer management. This is particularly significant for Biliary Tract Cancer (BTC), which is a rapidly spreading and progressively prevalent illness. Although there is insufficient data on the application of liquid biopsy in BTC, its use has the potential to facilitate the adoption of accurate medicine and enhance patient outcomes. Nonetheless, the limited sample size and inconclusive outcomes restrict the practicality of liquid biopsy in clinical settings. Moreover, the high expenses associated with this technique pose a significant hurdle to its broad implementation. To overcome these constraints, there is a need for increased endeavours to integrate liquid biopsy into clinical protocols, specifically by incorporating the systematic application of this method in future Research trials for BTC. This can help to elucidate its role in monitoring treatment response and identifying the cellular process that led to treatment refractoriness.

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