

Circulating Tumour DNA for Early Diagnosis of Pancreatic Ductal Adenocarcinoma

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ABSTRACT

Background: Pancreatic ductal adenocarcinoma (PDAC) has been deathly cancer arising from pancreatic cells. Despite the improvement in the standard of diagnosis, most patients seek medical care in the late stage. Due to the aggressiveness of the disease, it is therefore imperative to detect the early lesion for a better outcome.

Methods: We identified 416 articles relevant to circulating tumor DNA (ctDNA) and PDAC using predefined keywords in PubMed, PubMed Central, and Cochrane Library from January 1, 2011, to January 1, 2021 (10 years). Firstly, we screened the titles and abstracts, and 63 articles were included. Then, we screened those articles for the full-text version and included only 8 articles fulfilling our inclusion criteria. All steps were reviewed by the author.

Results: The presence of ctDNA in the blood reflects the occurrence of the pancreatic cancer-specific mutation in the primary tumor. The detection of KRAS mutation in ctDNA and tumor samples is highly consistent. The number of positive findings in early-stage patients is low, in line with the low ctDNA concentration measured. However, the combination of KRAS detected in ctDNA and other biomarkers showed prominent results with higher sensitivity and specificity.

Conclusions: ctDNA is a promising tool for early detection of PDAC. Despite its low positivity rate in certain studies, it is considerably concordant with the primary tumor. Future improvement in the technique application is required to overcome the issue of low DNA concentration in circulation.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a malignancy that occurs in cells lining the pancreatic duct. It is the seventh most common cause of cancer-related deaths globally, with a survival rate of only 5% [1,2]. Generally, the standard diagnosis approach uses tissue biopsy following non-invasive tests such as imaging and serum markers. Unfortunately, 50% of PDAC present with metastasis [2], providing less probability for surgery as the primary treatment. Also, diagnostic modality such as imaging is unable to detect small tumor lesion. Given the aggressiveness and frequent late presentation, it is necessary to develop a robust tool to capture early lesions in PDAC. This review will argue that circulating tumor DNA (ctDNA) carrying specific gene mutations can be deployed to early diagnose PDAC.

METHODS

Search Strategies

The researcher used several medical subject headings (MeSH) to search the appropriate articles. These include “liquid biopsy”, “cell-free tumor DNA”, “cell-free DNA”, “circulating tumor DNA”, and “carcinoma, pancreatic ductal” from three databases (Pubmed, Pubmed Central, Cochrane Library) from January 1, 2011, and January 1, 2021 (**Table 1**). The articles were selected when all the inclusion criteria had been met. They are pancreatic ductal adenocarcinoma (population), patients with stage I and/or II (interest), patients with stage III/IV cancer (comparison), detection rate (outcome), and interventional and cohort observational study (study design).

Data Extraction

Details that were included from each article were the author’s name, the country of the study population, sample size, the method of mutation detection, gene

mutation identified, and the outcomes. We performed the Newcastle-Ottawa Validity test to appraise the articles, and the result is shown below (Table 2).

Quality Assessment and Evidence

We used the Newcastle-Ottawa Quality Assessment Scale to assess the methodological quality of the selected nonrandomized studies with three aspects to rate, including selection, comparability, and outcome. The result of the assessment (good, fair, and poor) was based on the number of stars in each aspect. A “good” study scored 3 or 4 stars in the selection, 1 or 2 stars in comparability, and 2 or 3 stars in outcomes; a “fair” study scored 2 stars in the selection, 1 or 2 stars in comparability, and 2 or 3 stars in outcomes; a “poor” study scored 0 or 1 star in the selection, or 0 stars in comparability, or 0 or 1 star in outcomes.

RESULTS

Literature Search Results

We identified a total of 416 articles from three big databases (Pubmed, Pubmed Central, and Cochrane Library). All articles were screened using the predefined criteria, and 20 duplicate articles were removed. We excluded 388 articles due to the unavailability of early-stage cases. For the final stage, only 8 articles were included in the review. The search workflow is illustrated in Figure 1.

Circulating Tumor DNA: Opportunity and Challenges

It is a natural phenomenon that tumor cells release DNA known as ctDNA during necrosis or apoptosis (Figure 2). This short fragment DNA represents the primary tumor genetic landscape, as shown in its high concordance rate. Above all, ctDNA serves as a promising diagnostic tool for PDAC. Despite the practicability in the sampling method and the high accuracy, some potential challenges need to be addressed before its implementation into the clinical setting.

High Concordance Rate

There is a high concordance between ctDNA and tissue DNA in early-stage disease (Table 3). Concordance is defined as the same mutation found in both tumor tissue and plasma. Four ctDNA studies reported a 74.3–82% concordance in detecting KRAS mutation [4–6]. Their findings are extremely relevant as KRAS is the most frequent mutation in PDAC. Furthermore, the second most common mutation in PDAC, TP53, was also reported to be abundantly found in ctDNA, with a concordance rate even higher than that of KRAS (61%) [7]. Conversely, several reports also outlined a low concordance rate (20%–35%) [5,8,9]. Of note, high concordance studies involved more metastatic cases, which may explain another source of KRAS mutant ctDNA. This is evident in Patel et al. [7] study, showing that metastatic cases’ concordance rate was higher than that with localized tumors (72% vs. 39%). However,

Table 1. Literature search strategy

Database	Keyword	Result
Pubmed	“liquid biopsy” OR “cell free tumor dna” OR “cell free dna” OR “circulating tumor dna”) AND (“carcinoma, pancreatic ductal”[MeSH Terms]	63
Pubmed Central	“liquid biopsy” OR “cell-free tumor DNA” OR “cell-free DNA” OR “circulating tumor DNA”) AND (“carcinoma, pancreatic ductal”[MeSH Terms]	71
Cochrane Library	“liquid biopsy” OR “cell-free tumor DNA” OR “cell-free DNA” OR “circulating tumor DNA” AND carcinoma, pancreatic ductal	282

Table 2. The Newcastle-Ottawa quality assessment result

Study	Selection	Comparability	Outcome	Results
Cohen 2017 [5]	3	1	2	Good
Nakano 2018 [11]	2	1	3	Fair
Hadano 2016 [4]	2	1	2	Fair
Patel 2019 [7]	3	1	2	Good
Groot 2019 [22]	3	1	2	Good
Kim 2018 [6]	2	1	2	Fair
Takai 2015 [10]	3	1	3	Good
Shiwei 2020 [23]	3	1	2	Good

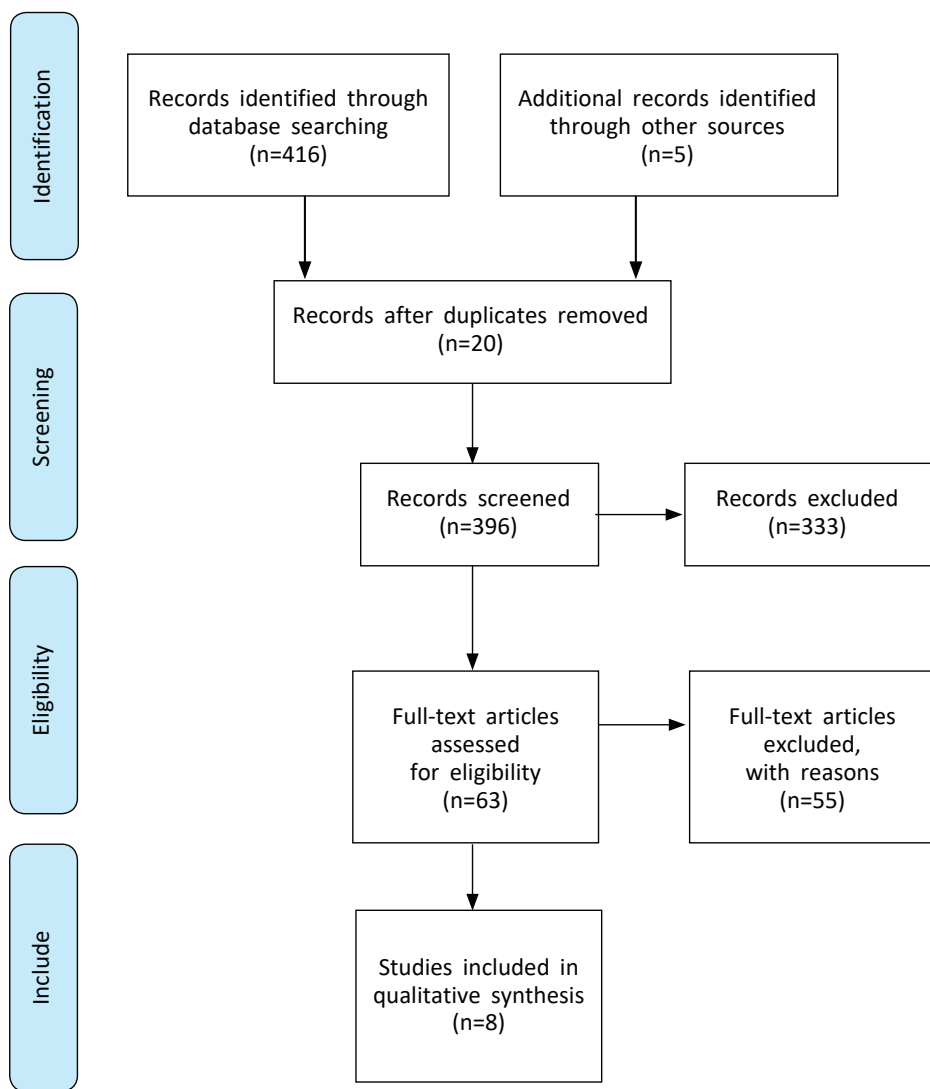


Figure 1. PRISMA literature searched flow diagram

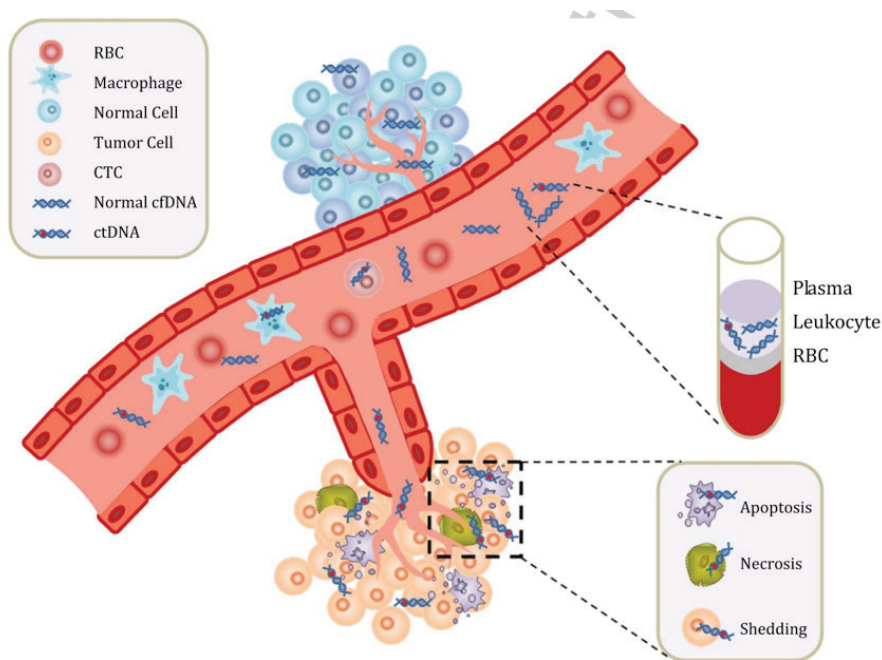


Figure 2. Tumor microenvironment. Tumor-associated materials, such as circulating tumor cells and circulating tumor DNA (as indicated), can be released into circulation. These components carry genetic information about primary tumors from apoptosis, necrosis, and shedding process [3].

Table 3. Characteristics of the studies

Author, years, country	Subjects	Method of Detection	Mutation	NOQ	Outcome of interest
Shiwei, 2020, China [23]	130 patients in the discovery cohort and 47 patients in the validation cohort: 150 patients with stage I-II disease.	Firefly NGS-based assessment ddPCR.	50-panel genes Six KRAS mutations G12D G12V G12R G12C G12A G12S	Good	33 PDAC associated gene mutations were found in 43 patients (38.03%). KRAS is the most frequent mutation (23%) with the KRAS G12D found to be the most common finding. Firefly NGS has a comparable concordance rate with ddPCR, but it provided more KRAS mutation landscape. 25 patients with KRAS mutation detected in ctDNA, also harbored KRAS mutation in a tissue sample (96.1%)
Groot, 2019, USA [22]	59 patients with localized PDAC.	ddPCR	Four KRAS mutations G12V G12D G12R Q61H	Good	49% of patients had KRAS mutation in ctDNA. 45% of patients were positive for G12V mutation. 29 out of 59 patients (49%) with detected KRAS mutation in tumor, also had KRAS mutation in ctDNA.
Patel, 2019, USA [7]	112 patients with 18 patients with surgically resectable disease.	NGS	54-72 panel genes	Good	5 out of 10 preoperative cases had detectable ctDNA, with median ctDNA range from 0 to 0.62. The most common alteration is TP53 (46%), followed by KRAs (44%). The concordance rate for KRAs between tissue and ctDNA is 52%.
Nakano, 2018, Japan [11]	45 stage I-II patients	qRT-PCR	KRAS mutation	Fair	KRAS mutation was detected in 11/45 preoperative patients (24.45%). KRAS mutations were detected in 35/42 of primary tumors (83.3%).
Kim, 2018, South Korea [6]	106 PDAC patients: 16 resectable cases	ddPCR	KRAS mutations G12A G12C G12D G12S G12V G12D	Fair	The concordance rate of KRAS mutation between tumor and cfDNA was 76.6%. The positivity rate of KRAS mutation in resectable cases was 68.6%.
Cohen, 2017, USA	221 resectable PDAC patients 182 control	PCR based assay is known as Safe Sequencing System (Safe-SeqS)	KRAS and TP53	Good	32% of patients harbor KRAS mutation in ctDNA with a 100 % concordance rate with primary tumor. 60% sensitivity from a combination of ctDNA and CA 19-9
Hadano, 2016, Japan [4]	105 PDAC patients: 84 stage I-II patients	ddPCR	KRAS mutations G12D G12V G12R	Fair	25/84 patients had KRAS mutation in ctDNA. 100% concordance rate between ctDNA and primary tumor.
Takai, 2015, Japan [10]	259 patients: 78 stage I-II patients	ddPCR	KRAS mutations G12D G12V G12R G13D	Good	8 patients had detectable KRAS mutation in ctDNA.

Abbreviations: NOQ: Newcastle-Ottawa validity test; NGS: next-generation sequencing; ddPCR: digital droplet polymerase chain reaction; ctDNA: circulating tumor DNA; CA 19-9: carbohydrate antigen 19-9.

despite the limited number of KRAS mutant positive ctDNA in the early stage [5,10,11], Hadano et al. [4] revealed that all localized cases with KRAS mutated ctDNA also harbor KRAS mutation in their primary tumors (100% concordance), supporting the notion that at a certain level, primary tumor releases DNA containing a specific mutation. It is also worth mentioning that variability in detection technology across studies, low DNA concentration in the plasma, and tumor heterogeneity-different molecular properties across tumor region-may foster the inconsistency in concordance rate. Therefore, a validation study using more stringent criteria involving early-stage PDAC cases with more advanced techniques targeting KRAS mutation is required.

High Accuracy

Circulating tumor DNA provides relatively high accuracy. Many reports have drawn this conclusion with varied numbers [8,12,13]. Firstly, KRAS detection via ctDNA achieved sensitivity and specificity of 95.7% and 100%, respectively [8]. Further investigation within the same study showed a correlation between KRAS mutation and different clinical stages ($p < 0.05$). Subsequently, combining KRAS mutation with a standard marker such as carbohydrate antigen 19-9 (CA19-9) showed a consistent detection rate in all stages [13]. With a sensitivity of 64%–78% and a specificity of 91%–99.5%, this combination was also superior to a single marker assay [5,14]. Furthermore, another strong point of ctDNA was its ability to distinguish the pre-invasive lesion (IPMN) from healthy control with comparable sensitivity and specificity (80.95% and 84.21%, respectively) [12]. However, it should be interpreted cautiously as stage I and II cases in the abovementioned studies showed less or undetectable KRAS mutation in their ctDNA [13]. Also, one study enrolled only two early-stage patients [8]. Nevertheless, these findings support the relevance of ctDNA measurement at any stage of the disease and establish the fundamental reasoning to promote the clinical translation of ctDNA into practice.

Easy to Obtain

Liquid biopsy is a popular name for blood collection and ctDNA testing. In the clinical setting, the liquid biopsy provides benefit since routine biopsy procedures, such as endoscopic ultrasound fine-needle aspiration (EUS-FNA), impose certain limitations. EUS-FNA requires sedation, which can put a patient in anesthesia-related adverse events [14]. At some conditions, a tumor might be inaccessible and, thus, incapable of being extracted and examined [15]. Even so, the risk of tumor seeding

might be increased during the procedure [16]. In contrast, drawing simple blood work is a routine procedure in clinical practice. Since it is an easy to access way, a serial measurement for an inconclusive result is achievable. Nevertheless, one aspect to date for ctDNA measurement is the demand for high technical expertise and higher cost. Eventually, one study already covered the issue of high cost by proposing digital droplet polymerase chain reaction (ddPCR), which has the lowest cost than any other ctDNA detection platform (€39–€298 per sample) [17]. However, this finding should be validated in non-metastatic cases, which inevitably pose more technical difficulties.

Challenges to be Addressed

Measurement of ctDNA in early-stage PDAC has become a major challenge for researchers. Firstly, the early-stage disease has a lower quantity of DNA in circulation than the metastatic disease (15 ng/ml vs. 22.6 ng/ml) [18]. In other words, a larger amount of ctDNA is associated with a greater tumor burden in the advanced stage. Most studies overcome this problem using ddPCR, which provides more wells to improve detection [4,8,13]. Secondly, the actual level of ctDNA might be confounded by another circulating free DNA (cfDNA) from normal cells. For instance, in ovarian cancer, cfDNA from normal cells makes up about 80–90% of all DNA in the blood [19]. Furthermore, a certain condition such as high-intensity exercise significantly increases cfDNA due to reactive oxygen species (ROS) production [20]. Fortunately, the current method, such as fragment analysis, allowed the separation of ctDNA from normal cfDNA based on the fragmentation pattern [21]. Finally, despite the promising accuracy, researchers have not reached a consensus on the target mutation of ctDNA. Specific to PDAC, the two most mutated genes, KRAS and TP53, have been identified in ctDNA [7]. Nevertheless, other works of literature also detected multiple genetic alterations with comparable accuracies [8,17]. Therefore, it is reasonable to measure the KRAS and TP53 genes even though they still require a cost-benefit analysis and validation in larger samples.

CONCLUSIONS

ctDNA undoubtedly promotes a feasible method for the early detection of PDAC. ctDNA, as a naturally released genetic information from the tumor in the blood, has a high concordance rate, reflecting the primary tumor. It shows high accuracies and easy sample collection. Nonetheless, improvements are still required to circumvent technical issues for the near future implementation.

DECLARATIONS

Ethics approval and consent to participate

Ethics approval for this review is not required.

Competing of Interest

The authors declare no competing interest in this study.

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