

Review

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Utility of circulating tumor DNA for predicting prognosis in the management of resectable pancreatic cancer

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Abstract

Background: The measurement of circulating tumor DNA (ctDNA) has been studied in several malignancies, including metastatic pancreatic cancer, but less is known about its utility in monitoring treatment response and recurrence in resectable pancreatic cancer. **Methods:** We conducted a systematic review of the literature examining the association of ctDNA with overall survival (OS) and disease-free survival. **Results:** Five articles met our exclusion criteria. Baseline and/or postoperative ctDNA was found to be associated with decreased OS and recurrence-free survival. **Discussion:** ctDNA has the potential to be used as a prognostic biomarker and to guide therapy in resectable pancreatic cancer.

Keywords: Pancreatic cancer, circulating tumor DNA, prognosis, survival

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related death in the United



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States, with a 5-year survival rate of approximately 10%^[1,2]. While surgical resection is the only curative treatment for pancreatic cancer, up to 80% of diagnosed patients are not candidates for resection due to aggressive spread and advanced-stage diagnosis^[3]. For the 20% who undergo curative resection, recurrence occurs in up to 80% of cases^[4], likely due to micro-metastatic disease present at the time of diagnosis^[5,6].

Current methods for diagnosis, treatment planning, and surveillance of pancreatic cancer are insufficient. The only serum biomarker recommended by the National Comprehensive Cancer Network guidelines is serum carbohydrate antigen 19-9 (CA 19-9), which can be used as an adjunct to guide treatment plans for patients, informing the diagnosis, resectability, response to therapy, recurrence, and overall prognosis of the disease^[7]. However, testing CA 19-9 levels can result in false positives due to the presence of certain medical conditions^[8-11], and false negatives in those who are Lewis genotype negative and do not express CA 19-9^[12]. More accurate biomarkers like circulating tumor DNA (ctDNA) could be valuable for recurrence risk stratification, therapeutic decision making, and detecting and monitoring disease burden in pancreatic cancer.

In 1946, Mandel and Metais described DNA in noncellular blood, which was referred to as cell-free DNA (cfDNA)^[13]. Further studies demonstrated that patients with cancer had higher circulating levels of cfDNA^[14]. In patients with cancer, DNA released from tumor cells, ctDNA, makes up a fraction of the overall cfDNA. Detection of tumor-specific ctDNA is a highly specialized process^[15,16]. There are two main methods of ctDNA detection and quantification, digital droplet polymerase chain reaction (ddPCR), and next-generation sequencing (NGS). ddPCR involves the detection of preselected genes (usually KRAS for pancreatic cancer) in each sample. Compared to traditional PCR, a single sample is partitioned into tens of thousands of nanoliter-sized droplets, allowing the measurement of thousands of independent amplification events in a single sample^[17]. However, for multiple gene targets, the use of ddPCR can be cumbersome, which is where NGS demonstrates its utility. With NGS, mutated gene sequences do not need to be preselected. NGS platforms perform sequencing of millions of small fragments of DNA in parallel and compare them against a human reference genome. This allows NGS to sequence entire exomes or genomes and identify novel sequences at an extremely high resolution. However, because the sequencing is more extensive, it is more time-consuming and expensive than ddPCR, especially for low numbers of desired gene targets^[18]. Though ctDNA levels often parallel tumor burden within individual patients, variability has been observed in patients with the same form of cancer, possibly reflecting individual tumor biology and rate of cell turnover^[19,20]. Unlike currently utilized serological biomarkers such as carcinoembryonic antigen (CEA) and cancer antigen 125 (CA-125), ctDNA has a short half-life (approximately 1 h)^[11,21]. Because of this, ctDNA may be used to more accurately monitor the time-related response of an individual patient to therapy^[22]. There is also potential for improved surveillance after treatment, where, in some studies, increased ctDNA can precede radiographic disease progression by months^[22-25].

ctDNA has gained traction in gastrointestinal malignancies^[26-28]; however, its use in pancreatic cancer remains investigational. Measuring ctDNA in pancreatic cancer has shown promise in monitoring locally advanced and metastatic tumors^[29-31], but less is known about how ctDNA can inform the management of localized, non-metastatic pancreatic cancer. This report reviews the literature on the prognostic value of ctDNA in resectable pancreatic cancer and the potential value of assessment during treatment.

METHODS

A computerized search was completed up to July 2020 on PubMed with no date restrictions. The search strategy included (pancreatic cancer OR PDAC OR pancreatic ductal adenocarcinoma) AND (cell-free DNA OR cfDNA OR circulating tumor DNA OR ctDNA) AND (resect OR local OR surgical OR operative

OR early-stage OR early). This search generated 316 articles.

Titles and abstracts were screened by three authors, and all articles not pertaining to ctDNA in patients with pancreatic cancer were excluded, as were reviews and meta-analyses. Disagreements between the first two authors were decided by a third author. A full-text analysis of the remaining articles was then completed with a focus on overall survival (OS) and disease-free survival. Articles with a study population containing only unresectable or metastatic pancreatic cancer cases or a sample size of fewer than five patients were excluded, as were retrospective studies. The study selection process resulted in the collection of four studies meeting our eligibility criteria, including prospective nature and pancreatic cancer patients that had undergone surgical resection [Figure 1].

RESULTS

ctDNA may be useful as a prognostic biomarker in patients with resectable pancreatic cancer. Baseline and/or postoperative ctDNA has been associated with decreased OS and recurrence-free survival (RFS) in several prospective studies, as summarized in Table 1.

In a prospective multi-institutional study, Lee *et al.* evaluated 81 pancreatic cancer patients undergoing curative resection^[32]. Plasma was tested for ctDNA pre- and postoperatively. Preoperative ctDNA detection (ctDNA positive disease [ctDNA+]) was associated with inferior RFS compared with ctDNA negative (ctDNA-) patients who had undetectable ctDNA levels. (ctDNA+: 10.3 months *vs.* ctDNA-: median not reached [NR]; HR 4.1; 95%CI: 1.8-9.0, $P = 0.002$). OS was also significantly lower [OS 13.6 months *vs.* NR (HR 4.1, 95%CI: 1.6-10.5; $P = 0.015$)]. Based on multivariate analysis adjusted for preoperative and pathologic factors, preoperative detection of ctDNA had a statistically significant association with recurrence (HR 4.1; 95%CI: 1.4-12.1; $P = 0.018$) and OS (HR 4.1; 95%CI: 1.0-16.6; $P = 0.049$).

After curative intent surgical resection, patients with persistent ctDNA detection postoperatively had a shorter interval of recurrence compared to those who remained undetectable (5.4 *vs.* 17.1 months; HR 5.4, CI 1.9-15.2; $P < 0.001$) and shorter OS (10.6 months *vs.* NR; HR 4.0, CI 1.2-13.6; $P = 0.003$). Of note, in the 13 patients who were ctDNA+ postoperatively, 100% had cancer recurrence during the median follow-up period of 38.4 months from the time of diagnosis. In comparison, in the 22 patients who were ctDNA- postoperatively, 10 (45%) had disease recurrence during the study period. Detectable ctDNA following curative resection was independently associated with recurrence, with a 100% positive predictive value, with a specificity and sensitivity of 100% and 57%, respectively. The authors concluded that postoperative ctDNA detection has the best prognostic value in predicting recurrence compared with preoperative ctDNA and other clinical and pathologic factors^[32].

In a similar study, Pietrasz *et al.* found postoperative ctDNA detection was prognostic of OS and RFS^[33]. In the subgroup of 31 patients who underwent curative intent resection for pancreatic cancer, patients with detectable ctDNA postoperatively had a median OS of 19.3 months, compared to 32.3 months for patients with undetectable ctDNA ($P = 0.027$). Similarly, ctDNA+ patients had an RFS of 4.6 months *vs.* 17.6 months in ctDNA- patients. Of note, in the subgroup of patients who underwent resection, preoperative ctDNA studies were not collected^[33].

In a prospective study of 59 patients with resectable PDAC, Groot and colleagues collected preoperative and longitudinal postoperative plasma samples for the detection of ctDNA^[34]. Subjects had a median follow-up of 16 months from the time of surgical resection. Patients with detectable preoperative ctDNA had a decreased median RFS and incidence of recurrence (8 months; 26 out of 29 [90%] recurred), compared with

Table 1. Prospective studies of ctDNA in resectable pancreatic cancer

Year	Journal	Author	No. of subjects	No. underwent resection	ctDNA detected preoperatively (%)	ctDNA detected postoperatively (%)	Stage	Target candidate	Detection method	Detection time point	Median follow-up (months)	Median overall survival (months)		Median recurrence free survival (months)	
												ctDNA+	ctDNA-	ctDNA+	ctDNA-
2019	<i>Ann Oncol</i>	Lee et al. ^[32]	112	81	62 (23/37)	37 (13/35)	Resectable	KRAS (Codons 12,13,61)	PCR-based SafeSeqS	Preoperatively, postoperatively	38.4	Preop 13.6; Postop 10.6	Preop NR; Postop NR	Preop 10.3; Postop 5.4	Preop NR; Postop 17.1
2019	<i>Clin Cancer Res</i>	Groot et al. ^[34]	59	59	49 (29/59)	26 (11/41)	Resectable	KRAS (G12D, G12V, G12R, Q61H)	ddPCR	Preoperatively, postoperatively	16	Preop 14; Postop 17	Preop NR; Postop NR	Preop 8; Postop 5	Preop 19; Postop 15
2018	<i>Clin Chem</i>	Kim et al. ^[35]	106	41	85 (35/41)	NA	Resectable, locally advanced, metastatic	KRAS (G12A, G12C, G12D, G12R, G12S, G12V, G13D)	ddPCR	At Diagnosis	10.3	High MC and MA : -7 Low MC and MA: NR	High MC and MA:NR Low MC and MA: NR*		
2017	<i>Clin Cancer Res</i>	Pietrasz et al. ^[33]	135	31	NA	19 (6/31)	All	KRAS (G12D, G12V, G12R)	dPCR	Postoperatively	33.3	19.3	32.2	4.6	17.6

ctDNA: Circulating tumor DNA; KRAS: Kirsten rat sarcoma viral oncogene; PCR: polymerase chain reaction. ddPCR: digital droplet PCR; dPCR: digital PCR; MC: mutational concentration; MA: mutational abundance. *No statistically significant difference in OS between low and high ctDNA concentration and fractional abundance.

ctDNA- patients (19 months; 15 out of 30 [50%] recurred; $P < 0.001$). Similarly, preoperatively ctDNA+ patients had a lower median OS of 14 months (18 out of 29 [62%] died), while median OS was not reached in the ctDNA- group (8 out of 30 [27%] died; $P < 0.001$). In multivariable Cox regression models adjusted for preoperative factors, preoperative ctDNA detection was an independent predictor of RFS (HR = 2.67; $P = 0.011$) and OS (HR = 2.37; $P = 0.048$).

Postoperative ctDNA+ status was also associated with poor RFS and OS. In the 11 patients who were ctDNA+ postoperatively (median follow-up for this subgroup was 15 months from the time of surgery), the median RFS was five months (95%CI: 3-8) compared with 15 months (95%CI: 8-22) in the 30 ctDNA- patients ($P < 0.001$). Similarly, the median OS was 17 months in ctDNA+ patients (95%CI: 10-24) vs. not reached in the ctDNA- group ($P = 0.011$). Notably, 24 of the 59 patients in this study underwent neoadjuvant chemotherapy prior to surgical resection. Those patients were significantly less likely to be ctDNA+ in their preoperative sample compared to chemotherapy naïve patients (21% vs. 69%; $P < 0.001$). The five patients who underwent neoadjuvant chemotherapy and were ctDNA+ preoperatively had poor outcomes; all five patients had disease recurrence with a median RFS of five months, and 2 out of 5 patients died after a 12-month median follow-up period. There were no statistically significant differences in ctDNA positivity based on chemotherapy regimen or duration

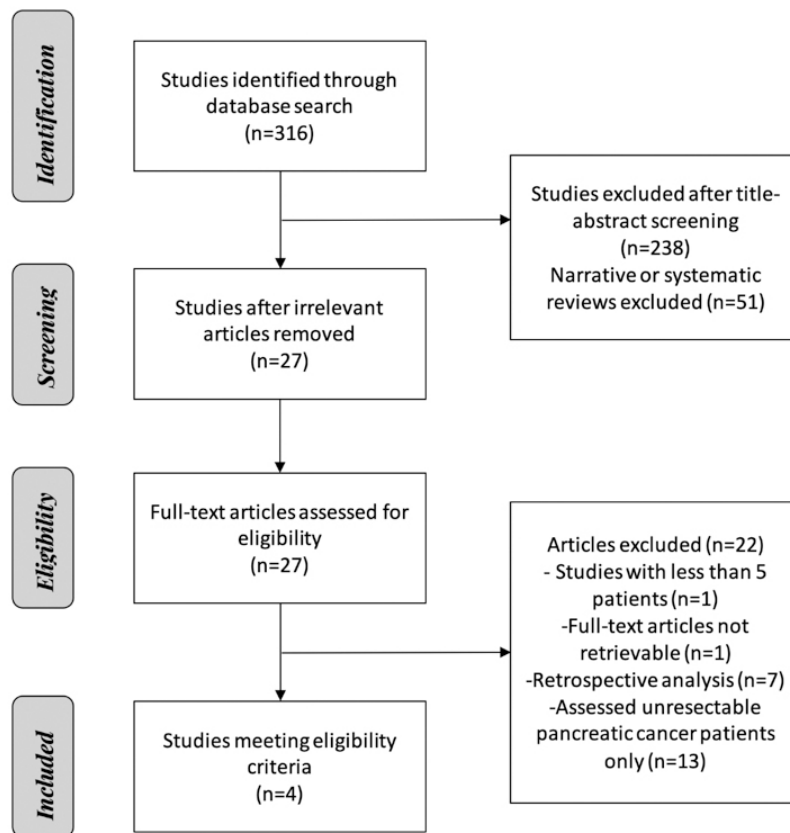


Figure 1. Flow chart of study selection constructed with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.

of treatment^[34].

Kim *et al.* examined the association with concentration and fractional abundance of ctDNA KRAS mutations at the time of diagnosis with outcomes in a study of 106 patients with PDAC^[35]. Of 41 patients with resectable tumors, 35 (85%) were ctDNA+ at diagnosis. The KRAS mutation concentration of 0.165 copies/ μ L and a median fractional abundance of 0.415% were utilized as the cutoff to dichotomize “high” vs. “low” in their dataset. Among those with resectable disease, patients with high KRAS mutation concentration experienced significantly lower median progression-free survival (PFS, defined as the interval from the day of diagnosis to the day of progression or death) compared to those in the low group (7 months vs. not reached, $P = 0.016$); but no statistically significant difference in OS (8 months vs. not reached $P = 0.072$). Similarly, patients with a high fractional abundance had a significantly shorter median PFS (7 months vs. not reached, $P = 0.02$) and similar OS (both did not reach 50% survival during the follow-up period, $P = 0.408$)^[35].

DISCUSSION

This systematic review highlights the utility of ctDNA as a prognostic indicator and biomarker in resectable or potentially resectable pancreatic cancer in the pre- and postoperative setting. As high-quality prospective studies continue to deliver additional data on the utility of this technology in pancreatic cancer, ctDNA has the potential to become a standard of care biomarker, which may help to stratify risk for recurrence and inform therapy decisions. Detection of ctDNA is accomplished within the normal clinical workflow. This

blood test can be added to routine blood work during pre- and postoperative visits.

As its detection may identify patients who are at higher risk for recurrence, ctDNA is a potential tool for neoadjuvant, surgical, and adjuvant therapy decision making. In Lee *et al.*'s study, all 13 patients with postoperatively detectable ctDNA had disease recurrence, despite all receiving adjuvant therapy^[32], suggesting that current adjuvant therapy regimens may not be as beneficial in patients who are ctDNA+ postoperatively, as they may be at higher risk for recurrence. However, it is still unclear if clearance of ctDNA during adjuvant therapy portends a better outcome. Persistent ctDNA detectability during adjuvant therapy may indicate the need for an alternative therapy. In line with this, 83% of patients in this study with detectable ctDNA at diagnosis had disease recurrence, despite 81% undergoing standard adjuvant therapy^[32], suggesting that preoperative ctDNA detection may be a factor in determining who may benefit from neoadjuvant therapy, rather than proceeding directly to surgery.

Groot and colleagues demonstrated that patients who underwent neoadjuvant therapy were significantly less likely to have ctDNA detected preoperatively (21% vs. 69% $P < 0.001$)^[34]. Notably, the five patients with persistent ctDNA after neoadjuvant therapy had a particularly poor prognosis, with 100% recurrence and a median RFS of five months; two of whom had an overall survival shorter than 12 months^[34]. Although this is a small sample size, these results favor avoiding surgery or prolonging therapy in patients with persistent ctDNA after neoadjuvant chemotherapy. Current prospective clinical trials are underway to better define the utility and predictive value of ctDNA to monitor for treatment response in pancreatic cancer. For example, Chawla and colleagues are assessing ctDNA detectability in a phase II prospective observational trial in patients undergoing neoadjuvant chemotherapy for potentially resectable pancreatic cancer. The study aims to determine if clearance of ctDNA during treatment correlates with traditionally accepted treatment response criteria like serum CA 19-9, histologic regression grade, and histologic margin assessment. (NCT04616131). Several other studies examining longitudinal changes in ctDNA levels in response to treatment modalities are ongoing. Heyd and colleagues are conducting a 3-year longitudinal study of ctDNA in patients with resectable and potentially resectable pancreatic cancer to determine its utility in monitoring treatment response and predicting RFS. Serum samples are analyzed at diagnosis, after neoadjuvant chemotherapy, preoperatively, at the time of surgery, 1 month postoperatively, and 1 month after the last round of adjuvant chemotherapy (NCT02818907). In a similar study, Woo and colleagues aim to generate a ctDNA gene panel and collect longitudinal serum samples to verify the applicability of predictive serum-based biomarkers for pancreatic cancer. Their patient population includes patients with both resectable and metastatic pancreatic cancers, from whom they are collecting serum samples every 1-3 months for 24 months (NCT04241367).

Though ctDNA currently shows promise as a prognostic indicator and a tool to measure treatment response, it also has potential as a diagnostic biomarker and screening test, especially when used alongside other serum biomarkers and imaging. CA 19-9 has a sensitivity of 58%-87% and a specificity of 70%-80%, which is inadequate in diagnosing early-stage tumors^[36-38]. Dabritz and colleagues found that a combination of testing CA 19-9 levels and KRAS ctDNA led to a sensitivity of 91% in detecting pancreatic cancer^[36]. Similarly, Wang *et al.* found that testing CA 19-9 in combination with ctDNA led to an increased sensitivity of 82% with an 81% specificity in their study of 100 pancreatic cancer patients^[39]. ctDNA, as a screening test for pancreatic cancer, may have further utility in high-risk individuals. In a current prospective trial, patients with a strong family history of pancreatic cancer are evaluated with MRIs and plasma ctDNA assessment every 6 months for 3 years to correlate biomarker changes with radiological abnormalities as they arise on imaging (NCT03250078). Ongoing prospective studies evaluating ctDNA in pancreatic cancer are summarized in [Table 2](#).

Table 2. Current trials exploring utility of ctDNA in pancreatic cancer

Trial number	Trial name	ctDNA focus and aims	Study type	Estimated completion	Recruitment status
NCT04616131	Evaluating the response to neoadjuvant chemotherapy with circulating tumor DNA in pancreatic cancer	Detection of ctDNA in patients with resectable pancreatic cancer before, during and after treatment and comparison with Ca-19-9, histologic regression, and margin assessment	Prospective observational cohort	October 2021	Recruiting
NCT02818907	Evaluation of survival prognostic factors for patients with exocrine pancreatic cancer resectable or potentially resectable (Pancreas-CGE)	Collection of clinical, biological, and quality of life data from patients with a borderline or resectable pancreatic cancer to identify new biomarkers evaluation of treatment response and surveillance post-treatment	Prospective observational cohort	May 2023	Recruiting
NCT04241367	Verification of predictive biomarkers for pancreatic cancer treatment using multicenter liquid biopsy	Quantification and monitoring of KRAS mutations in ctDNA in pancreatic cancer pretreatment and at multiple timepoints thereafter; Discovery of biomarkers through ctDNA panel using genomic DNA and ctDNA	Prospective observational cohort	December 2025	Recruiting
NCT03250078	A pancreatic cancer screening study in hereditary high risk individuals	Screening patients at high risk for pancreatic cancer with MRI/MRCP and collection of serum to correlate biomarkers (including ctDNA) with early pancreatic cancer	Prospective observational cohort	November 2026	Recruiting

ctDNA: Circulating tumor DNA; Ca-19-9: cancer antigen 19-9; KRAS: Kirsten rat sarcoma viral oncogene; MRI: magnetic resonance imaging; MRCP: magnetic resonance cholangiopancreatography.

ctDNA may also enhance pancreatic cancer post-treatment surveillance. In the previously described prospective study, Pietrasz and colleagues collected longitudinal plasma samples at various time points (every 10-30 days) in a subgroup of 8 patients. They noted that ctDNA levels correlated with radiologic change, and progression of disease was detected at a median of 2.4 months using ctDNA compared to 4 months with CT scan surveillance^[33]. Similarly, in a study containing nine patients with postoperative cancer recurrence, ctDNA levels were detectable at an average of 3.1 months, compared to 9.6 months for detectable change on CT scan ($n = 9$, $P = 0.0004$, paired t -test)^[40].

CONCLUSION

Despite advances in surgical management and chemotherapy, pancreatic cancer remains a difficult disease to treat. Given its often late presentation and high rates of distant recurrence, biomarkers are needed to enhance surveillance and treatment decision making. ctDNA may be a key biomarker to better understand the burden of disease and guide neoadjuvant and adjuvant therapies in pancreatic cancer. Utilizing ctDNA assessment to identify early-stage disease, guide therapy based on risk profile, and monitor for early recurrence may also have an impact on survival. Refinement of ctDNA detection techniques to make this biomarker more scalable and affordable to test, and more experience with its interpretation during the natural history of the disease, may affirm ctDNA as a useful biomarker in pancreatic cancer.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and performed article analysis and interpretation: Tyerman Z, Ambler E, Chawla A

Provided administrative, technical, and editorial support: Schlick CJR, Nwajei F

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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